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Neural stem cell transplantation enhances mitochondrial biogenesis in a transgenic mouse model of Alzheimer's disease—like pathology

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ABSTRACT

Mitochondrial dysfunction, especially a defect in mitochondrial biogenesis, is an early and prominent feature of Alzheimer's disease (AD). Previous studies demonstrated that the number of mitochondria is significantly reduced in susceptible hippocampal neurons from AD patients. Neural stem cell (NSC) transplantation in AD-like mice can compensate for the neuronal loss resulting from amyloid-beta protein deposition. The effects of NSC transplantation on mitochondrial biogenesis and cognitive function in AD-like mice, however, are poorly understood. In this study, we injected NSCs or vehicle into 12-month-old amyloid precursor protein (APP)/PS1 transgenic mice, a mouse model of AD-like pathology. The effects of NSC transplantation on cognitive function, the amount of mitochondrial DNA, the expression of mitochondrial biogenesis factors and mitochondria-related proteins, and mitochondrial morphology were investigated. Our results show that in NSC-injected APP/PS1 (Tg-NSC) mice, the cognitive function, number of mitochondria, and expression of mitochondria-related proteins, specifically the mitochondrial fission factors (dynamin-related protein 1 [Drp1] and fission 1 [Fis1]) and the mitochondrial fusion factor optic atrophy 1 (OPA1), were significantly increased compared with those in age-matched vehicle-injected APP/PS1 (Tg-Veh) mice, whereas the expression of mitochondrial fusion factors mitofusion 1 (Mfn1) and Mfn2 was significantly decreased. These data indicate that NSC transplantation may enhance mitochondria biogenesis and further rescue cognitive deficits in AD-like mice.
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1. Introduction

Increasing evidence suggests that mitochondrial dysfunction plays a pivotal role early in the pathogenesis of Alzheimer's disease (AD), although this disease is not fully understood yet (Swerdlow, 2007). Mitochondrial dysfunction includes abnormalities in mitochondrial properties such as impaired biogenesis and energy metabolism, defects in key respiratory enzyme activity/function, accumulation/generation of mitochondrial reactive oxygen species, and formation of membrane permeability transition pores (Chen and Yan, 2010). These mitochondrial abnormalities are found to arise before amyloid-beta (Aβ) plaque deposition (Takuma et al., 2005; Yao et al., 2009) and are considered to be closely related to the Aβ or tau pathology in AD (Atamna and Frey, 2007; Manczak et al., 2006; Perez-Gracia et al., 2008). Aβ plaques accumulate in mitochondria in brains of human AD cases, and AD-like animals (Caspersen et al., 2005; Gillardon et al., 2007) may cause severe structural and functional abnormalities in mitochondria and contribute to neuronal network dysfunction in both transgenic mice overexpressing mutant amyloid precursor protein (APP) and in AD patients (Xie et al., 2013).

Antioxidant therapeutics such as vitamin E, curcumin, *Gingko biloba*, melatonin, and lipoic acid SS31 have demonstrated potential to reduce Aβ levels, improve mitochondrial function, restore mitochondrial transport and synaptic plasticity, protect mitochondria and synapses from Aβ toxicity, and attenuate cognitive deficits in animal models of AD (Calkins et al., 2011; Hardas et al.,

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2013; Matsubara et al., 2003; Stackman et al., 2003; Yang et al., 2011).

Clinical trials of these therapeutic approaches, however, have shown that they have only a modest or no effect in improving cognitive function in AD patients. The reason they may have only a modest effect could be because they do not successfully cross the blood-brain barrier (Reddy et al., 2012). To improve their effectiveness, many studies have attempted to develop mitochondria-targeted antioxidants, which enhance the protection of mitochondria from oxidative damage (Murphy and Smith, 2007). Other strategies for the induction of autophagy as a therapeutic strategy in AD have been tested in animal models of the disease (Lai and McLaurin, 2012; Steele and Gandy, 2013; Yang et al., 2011). These therapeutic approaches do not compensate for the massive and progressive mitochondria loss in AD. It is noteworthy that neurogenesis has been shown to be impaired in animal models of AD (Demars et al., 2010), and neural stem cell (NSC) transplantation has been found to rescue cognitive deficits in AD-like animals (Blurton-Jones et al., 2009; Zhang et al., 2014). Thus, supplementation with new mitochondria via engrafted NSCs may be a promising option to treat AD.

Unfortunately, little is known about the influence of NSC transplantation on mitochondrial biogenesis in AD-like transgenic mice. In this study, we first demonstrate that NSC transplantation significantly increased the cognitive function, the number of mitochondria with normal structure, and the expression of the mitochondria-related proteins, whereas it also altered the expression of mitochondrial fission and fusion factors and the mitochondrial length in APP/PS1 mice with AD-like pathology.

2. Materials and methods

2.1. Experimental animals

APP/PS1 double transgenic mice, B6C3-Tg (APPswe, PSEN1dE9) 85Dbo/J, were obtained from the Jackson Laboratory (USA). Mice were raised in separate cages in a 12-hour light-dark cycle at constant temperature with free access to food and water. Forty 12-month-old APP/PS1 Tg mice and 40 wild-type (Wt) littermate controls were used in our study. The study was approved by the Shanghai Ethics Committee, and all experiments were performed in accordance with the guidelines from the Chinese Animal Welfare Agency.

2.2. NSC culture and transplantation

NSCs were harvested from non-Tg B6C3 mouse embryos at embryonic day 14 (E14) and were cultured in NSC culture medium as previously described (Blurton-Jones et al., 2009; Zhang et al., 2014). NSC differentiation was induced by withdrawing growth factors (epidermal growth factor and basic fibroblast growth factor) and heparin from the proliferation medium. Passage 2 NSCs were transfected with adeno-associated virus–enhanced green fluorescent protein (EGFP), and then EGFP-positive (EGFP+) cells were sorted by flow cytometry. The EGFP+ cells were used for transplantation at passage 10. Before transplantation, cells were trypsinized, washed, triturated, and filtered through a 70-μm mesh. NSCs were then counted and resuspended at 5×10^5 to 1×10^6 cells/μL in 1× Hanks Balanced Salt Solution containing 20-ng/mL human epidermal growth factor (vehicle).

Stereotactic delivery of NSCs was performed as previously described (Zhang et al., 2014). Each mouse was stereotactically injected bilaterally with 5 μL of either vehicle (Tg-Veh mice) or NSCs (Tg-NSC mice) at a rate of 1 μL/minute (David Kopf Instruments, Tujunga, CA, USA). The injection coordinates, relative to Bregma, were anteroposterior, −2.06 mm; mediolateral, ±1.85 mm; and dorsoventral, −2.50 mm.

2.3. Immunohistochemistry

EGFP-labeled NSCs were cultured on a chamber coated with collagen (Sigma-Aldrich, USA). Immunohistochemistry was performed using specific antibodies to identify Nestin (rabbit polyclonal, 1:200; Sigma-Aldrich), Tju1 (mouse monoclonal, 1:200; Sigma-Aldrich), and glial-fibrillary acidic protein (GFAP; rabbit polyclonal, 1:200; Abcam, USA) as described (Zhang et al., 2014).

Mouse brains were collected, fixed in 4% paraformaldehyde, embedded in paraffin, and then serially sectioned into 20-μm-thick coronal sections using a sliding microtome (Leica CM1950, Germany). Immunohistochemistry was performed on these sections using specific primary antibodies against microtubule-associated protein 2 (Map-2; mouse monoclonal, 1:300; Sigma-Aldrich, USA), GFAP and galactosylceramidase (GalC; rabbit polyclonal, 1:200; Abcam), peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1α; mouse monoclonal, 1:300; Chemicon [Millipore]), and nuclear respiratory factor 1 (NRF-1) and cytochrome c oxidase subunit IV (COXIV; rabbit monoclonal, 1:100; Abcam) as previously described (Zhang et al., 2014). To analyze the differentiation of NSCs, sections were double labeled for EGFP and Map-2, GFAP, or GalC. Sections were double labeled for EGFP and PGC-1α, NRF-1, or COXIV for identifying mitochondrial biogenesis markers. The nuclei were counterstained with Hoechst 33,342 (Sigma-Aldrich). Images representing either a single confocal Z slice or Z stacks were acquired with a Zeiss 710 confocal system. Images were counted by 2 blind observers. Ten serial sections with a 10-μm interval of each 2 adjacent sections from each animal in each group ($n = 4$) were used in this study. A total of 1200 EGFP+ cells were analyzed, and the proportion of cells that were double labeled with neuronal or glial markers were counted. Three regions of interest were randomly defined in the hippocampus. Data from the 3 regions of interest were averaged for each section.

2.4. Behavioral assessment

Five and 10 weeks after NSC transplantation, behavioral studies were carried out to examine the spatial learning and memory using the Morris water maze (MWM) according to the standard procedures adapted to mice (Billings et al., 2005). The complete MWM apparatus used in our study was purchased from Shanghai Mobile Datum Information Technology Co, Ltd. Swimming paths were recorded by a computerized video imaging analysis system. MWM training comprised 2 procedures: the place navigation test and the spatial probe test. The place navigation test assessed the learning ability of the mice in the water maze. The place navigation test lasted for 6 days to record the escape latency while searching for the platform within 60 seconds. A spatial probe test was performed to evaluate memory retention of the mice. The spatial probe test was performed on the afternoon of the seventh day, and the frequency of crossing through the platform place and the time spent in the target quadrants (TQs) were recorded.

2.5. Long-fragment polymerase chain reaction and mitochondrial DNA quantification

DNA integrity was assessed using the long-range polymerase chain reaction (PCR)–mediated detection method as described previously (Chen et al., 2007). Long-fragment PCR was used to quantify the relative abundance of intact mitochondrial DNA (mtDNA) as previously described (Chen et al., 2007). The integrity of mtDNA was analyzed by long-range PCR, using primers

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