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Research paper

Cellular and molecular mechanisms of the restoration of human APP transgenic mouse cognitive dysfunction after transplant of human iPS cell-derived neural cells

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ABSTRACT

Cholinergic neuronal loss is a common finding in patients with Alzheimer's disease (AD) and AD model mice. We previously transplanted neurons derived from human induced pluripotent stem (iPS) cells into the hippocampus of human amyloid precursor protein transgenic AD model mice. In the present study, we examined the cellular and molecular mechanisms involved in the alleviation of cognitive dysfunction in transplanted mice.

After transplant, mice showed improvement in cognitive function, confirming our previous findings. Human choline acetyltransferase (ChAT)-positive cholinergic neurons were distributed throughout the cortex of the grafted mice. Human and mouse ChAT-positive neurons and alpha7 nicotinic acetylcholine receptor (α7nAChR)-positive neurons were significantly increased in the cortex and hippocampus of the grafted mice compared with the vehicle-injected mice. In addition, human and mouse vesicular GABA transporter (VGAT)-positive neurons were located mainly in the hippocampus and, though the number was small, human VGAT-positive neurons were observed in the cortex. In the grafted mouse cortex, the number of GABA receptor (GABAR)-positive neurons of both human origin and mouse origin were significantly increased compared with those in the vehicle-injected mouse cortex. The α7nAChR-positive and GABAR-positive neurons expressed phosphorylated Akt and c-fos in the cortex, suggesting that these receptor-expressing neurons were possibly activated by the neurotransmitters secreted from the grafted neurons.

Collectively, the grafted and host neurons may form positive feedback loops via neurotransmitter secretion in both the cerebral cortex and hippocampus, leading to alleviation of cognitive dysfunction in dementia model mice.

1. Introduction

Cholinergic neurons and acetylcholine play important roles in learning and memory functions. Cholinergic neuron activity and acetylcholine production are downregulated in patients with Alzheimer's disease (AD) (Davis et al., 1999), and downregulation of alpha7 nicotinic acetylcholine receptors (α7nAChRs) has been reported as one of the hallmarks of AD (Court et al., 2001). Immunohistochemical studies demonstrated that GABAergic neuronal deficits were more severe in AD patients than previously estimated (Schwab et al., 2013). Furthermore, the expressions of several subunits of $GABA_A$ receptor (GABAR) were shown to be frequently altered in AD (Rissman and Mobley, 2011).

It has been shown that cell transplantation improved learning and memory function in AD models (Babaei et al., 2012; Bissonnette et al., 2011; Blurton-Jones et al., 2009; Ma et al., 2013; Wang et al., 2006). We have previously reported that transplantation of human induced pluripotent stem (hiPS) cell-derived neurons improved cognitive deficits of PDGF promoter-driven amyloid precursor protein (PDAPP) transgenic dementia model mice (Fujiwara et al., 2013). Choline acetyltransferase (ChAT)-positive cholinergic neurons derived from hiPS cells were shown to be able to survive in the PDAPP mouse hippocampus (Fujiwara et al., 2013).

Here, we transplanted hiPS cell-derived neurons into the hippocampus of PDAPP mice. Cognitive function of the mouse improved significantly by transplantation, confirming our previous finding. After transplantation, we investigated the localization of ChAT, vesicular GABA transporter (VGAT), α7nAChR, and GABAR-expressing cells as well as the activation status of their associated pathways in the brain of dementia model mice.

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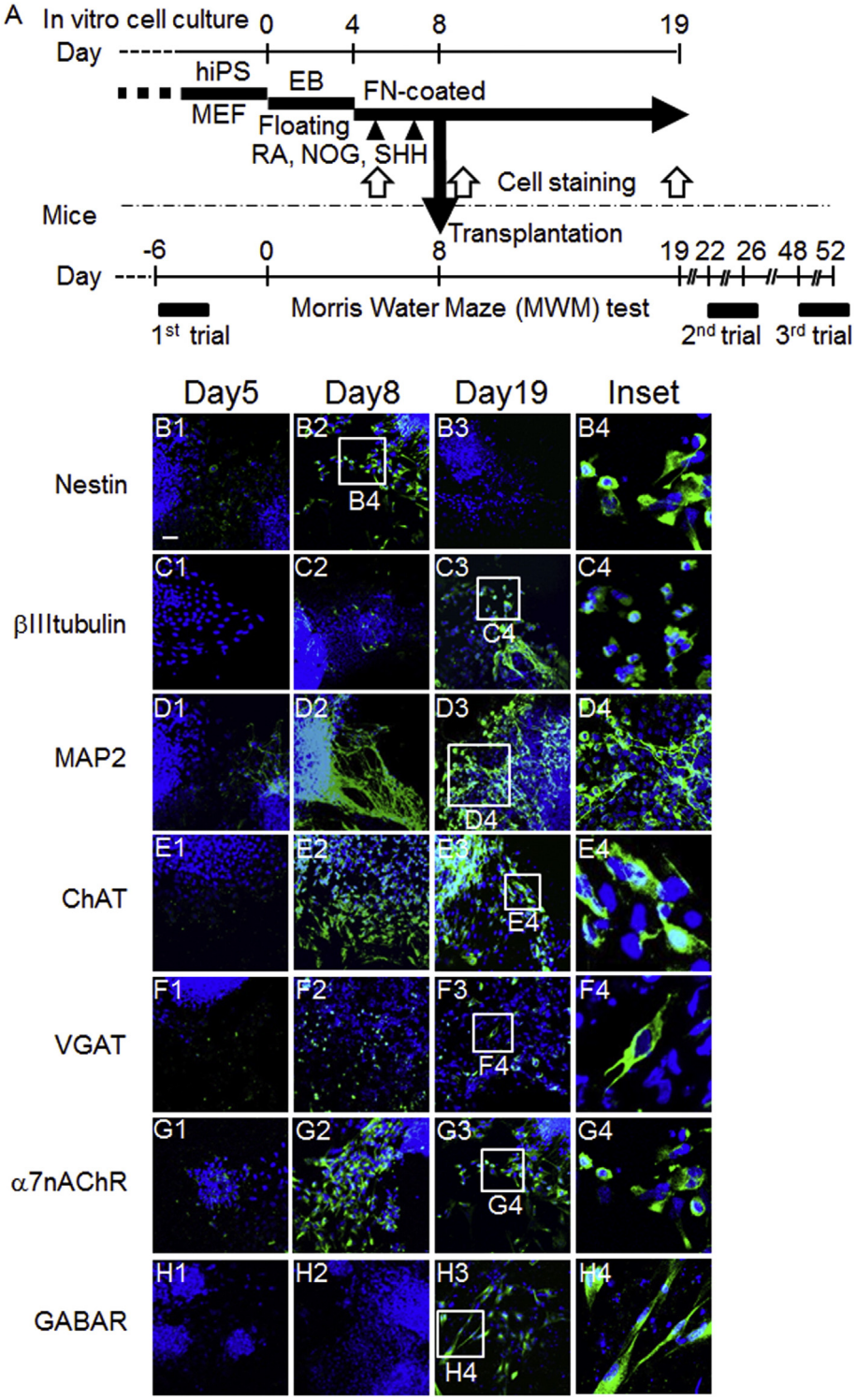

Fig. 1. Induction of hiPS cell differentiation into cholinergic and GABAergic neurons. A, Schematic representation of neural induction from hiPS cells and transplantation. We utilized retinoic acid (RA), noggin-Fc (NOG), and Sonic hedgehog (SHH) to induce neural cell differentiation from embryoid bodies (EB). FN, fibronectin; MEF, mouse embryonic fibroblasts. B–H, Immunocytochemistry for neural markers of the hiPS cell-derived cells. Neural cells derived from hiPS cells at day 8 (1 day after the second addition of RA, NOG, and SHH; B2, C2, D2, E2, F2, G2, H2) and day 19 (12 days after the second addition of RA, NOG, and SHH; B3, C3, D3, E3, F3, G3, H3), and control hiPS cells at day 5 (before the addition of RA, NOG, and SHH; B1, C1, D1, E1, F1, G1, H1) were stained with anti-nestin (B), anti-βIII-tubulin (C), MAP2 (D), ChAT (E), VGAT (F), α7nAChR (G), and GABAR (H) antibodies. Cells at day 5 showed very little expression of the neural markers. Neural cells by day 8 expressed βIII-tubulin (C2, 13.1 ± 0.7%: mean ± s.e.m. from three independent experiments), MAP2 (D2, 72.3 ± 5.0%), ChAT (E2, 82.0 ± 1.5%), VGAT (F2, 2.5 ± 0.2%), α7nAChR (G2, 79.0 ± 4.0%) and GABAR (H2, 7.3 ± 2.9%). Many cells expressed nestin by day 8 (B2, 93.6 ± 1.0%), which was decreased by day 19 (B3, 8.1 ± 0.7%). By day 19, βIII-tubulin-expressing cells were increased (C3, 86.7 ± 4.6%). MAP2 (D3, 48.3 ± 3.8%), ChAT (E3, 77.2 ± 7.8%), VGAT (F3, 10.0 ± 1.4%), α7nAChR (G3, 86.4 ± 3.7%), and GABAR (H3, 5.0 ± 0.5%)-positive cells were also observed. Scale bar represents 20 μm. Panels B4, C4, D4, E4, F4, G4 and H4 were under higher magnification of panels B2, C3, D3, E3, F3, G3 and H3, respectively.