

Cellular Transplantation as the Treatment of Alzheimer's Disease in Mouse Models

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Abstract

Acetylcholine (ACh) and N-methyl-D-aspartate (NMDA) have been two major therapeutic targets of Alzheimer's disease (AD) for decade. However, truly effective remedy for AD has not been successfully developed.

We previously transplanted neurons derived from human induced pluripotent stem (hiPS) cells into the hippocampus of human amyloid precursor protein transgenic AD model mice.

The cell transplantation significantly improved cognitive dysfunction in the dementia model mice. Human choline acetyl transferase (ChAT) positive cholinergic neurons located throughout the cortex of the grafted mice. Human and mouse ChAT positive neurons and alpha7 nicotinic acetylcholine receptor ($\alpha 7nAChR$) positive neurons significantly increased in the cortex and hippocampus of the grafted dementia mice compared with the vehicle injected dementia mice.

Human and mouse vesicular GABA transporter (VGAT) positive neurons distributed mainly in the hippocampus and, though the number was small, human VGAT positive neurons located in the cortex. In the grafted mouse cortex, the number of GABA receptor (GABAR) positive neurons of both hiPS origin and mouse origin increased significantly compared with those in the vehicle injected mouse cortex.

We suggested that positive feedback loops of neurotransmitter secretion of the cortex and hippocampus induced the characteristic distribution of the transplanted neurons. In this review, we summarized current advances in stem cell therapy for dementia model mice, especially to highlight the relationships between major neurotransmitters and host/transplanted neurons.

Keywords: Alzheimer's disease; Human iPS cells; Transplantation, Acetylcholine, GABA, Hippocampus

Introduction

Cholinergic neurons which secrete acetylcholine (ACh) play important roles in learning and memory functions. Cholinergic neuron activity and their acetylcholine production are down regulated in patients with Alzheimer's disease (AD) [1], and down modulation of alpha7 nicotinic acetylcholine receptors ($\alpha 7nAChRs$) has been reported as one of the hallmarks of AD [2].

Japanese government is trying to handle the rapidly aging society with several new technology using, for example, robotics and regenerative medicine. In Japan, people older than 64 years increased to one fourth of the whole population and the cost of treating people with dementia was estimated to be around US\$120 billion in 2014 [3].

We hardly modified the disease progression of AD using a single conventional medication [4]. Besides ACh and N-methyl-D-aspartate (NMDA) targeted conventional treatments, researchers tried to developed remedy using blocking molecules for production and accumulation of amyloid β ($A\beta$) [4]. Despite the increasing number of studies, no new drugs have been approved since 2003 for AD treatment. It was suggested that synaptic loss of brain by the accumulating $A\beta$ oligomers was strongly associated with AD cognitive dysfunction compared with $A\beta$ pathology, in which $A\beta$ plaques were thought to be causally implicated [5].

Human induced pluripotent stem (hiPS) cell transplantation therapy for a degenerative eye disease has been reported in Japan [6]. Thereafter, they have become to receive much attention for their potential to rescue the impaired cellular functions of various diseased conditions, including cognitive function in patients with AD.

This review focused on the usefulness of stem cell transplantation for the treatment of dementia models and we summarized their effects on the disease pathology and cognitive function in AD models.

Neural Induction from Pluripotent Stem Cells

In the first step of vertebrate development, cells which are committed to differentiate into neural cells are suggested to differentiate into anterior/rostral fate (forebrain cells) of central nervous system (CNS), mainly by bone morphogenetic protein (BMP) inhibitors [7].

After the cell fate acquisition, an appropriate regional patterning of CNS is provided by several organizing centers of the neural tube [8]. BMP and sonic hedgehog (SHH) are important molecules for the dorsalization and ventralization of neurons within the neural tube, respectively. Retinoic acid (RA) is a key molecule for the caudalization of the neurons towards midbrain, hindbrain and spinal cord neuron fate [7]. Because of the inherent limitation of experimental studies, underlying molecular mechanisms of human brain development remain largely unexplored.

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Recent advances in cell biology of pluripotent stem cells, including embryonic stem (ES) and iPS cells, provided us new approaches for the analysis of brain development with human cells. Similar to *in vivo* assays, mouse [8] and human [9] ES cells showed forebrain identity during early differentiation period in default condition without any morphogens. RA promotes caudate fates of the ES cell forebrain identity and ventralization of the cells is induced by SHH supplementation [10].

We previously demonstrated that mouse [11] and monkey [12,13] ES cells differentiated into motor neurons with RA and the neuron transplantation ameliorated motor dysfunction of hemiplegic model mice. We observed that addition of Noggin, a BMP inhibitor, promoted forebrain identity of culturing mouse ES cells and, on the contrary, basic fibroblast growth factor (bFGF) promoted caudal fates of the cells [14].

Using a combination of RA, SHH and Noggin, we induced rapid neurogenesis in hiPS cells and the neuron progenitor cells were applicable for the treatment of AD [15,16] and spinal cord injury model mice [17].

MASH1 is a basic helix-loop-helix transcription factor and essential for the neuron development [18]. We found that MASH1 gene transfection of mouse ES cells induced motor neuron differentiation and the cell transplantation improved the motor function of hemiplegic [19] and spinal cord injury models [20]. Furthermore, we observed that brain derived neurotrophic factor (BDNF) significantly enhanced neural differentiation of mouse ES cells [21]. It seems that we are able to observe brain development process in pluripotent stem cells, especially using several morphogens.

Humoral Factors in Cell Transplantation of AD models

Neurotransmitters

One of the major histopathological changes of human AD is massive neural degeneration of nucleus basalis [22]. The degeneration leads to ACh deficits in the cortex and hippocampus. The ACh expression was negatively correlated with AD disease severity [23]. Available medicines approved for AD treatment are used to inhibit acetylcholinesterase and increase cholinergic neurotransmission of the brain. Certainly, the molecules improved cognition, behavior and functional and global clinical state of patients with AD at the mild to moderate stage [4]. In addition, there have been little advances of cholinergic medications during this decade [4].

Neural stem cell derived cholinergic neurons [24] and choline acetyltransferase (ChAT) overexpressing neural stem cells [25] significantly shorten the escape latency of Morris water maze (MWM) test after the transplantation, suggesting that oversupply of cholinergic neurotransmitter improved cognitive function of AD models.

It was recently reported that down regulation of vesicular GABA transporter (VGAT) [26] and GABA receptor (GABAR) [27] was more severe than previously thought in AD patients. Decreases of GABA expression were observed in PDAPP mice [28], tau protein transgenic mice, and apolipoprotein (apo) E4 knock-in/APP mice [29], all of which exhibited an immature hippocampus with GABAergic neuronal dysfunction and memory deficits.

Carrying apoE4 gene is a strong risk factor of AD and apoE4 directly impair the GABAergic inhibitory neuron function [30]. GABAergic interneuron progenitors transplanted into the hippocampal hilus were functionally integrated into the host hippocampus and improved learning and memory function in apoE4 knock-in/APP mice [31].

Growth and neurotrophic factors

Simultaneous supplementation of BDNF enhanced therapeutic effects of stem cell transplantation on the learning and memory ability of AD model mice [32]. When genetically modified stem cells which produced nerve growth factor (NGF) were transplanted into the hippocampus of AD model mice, the learning and memory ability of the grafted mice were improved [33]. Transplantation of the vascular endothelial growth factor (VEGF) gene transfected cells ameliorated cognitive dysfunction of AD model mice, as well [34]. It has been shown that human neural stem cells expressed growth and trophic factors including BDNF, NGF and VEGF in the brain of AD model mice upon transplantation [35].

Cytokines, chemokines and toll-like receptors (TLR)

A β binds CD36, TLR4 and TLR6 and activates microglia [36]. Activated microglia secretes proinflammatory cytokines, such as interleukin (IL)1 β , IL6 and TNF α and the process is thought to be the beginning of neuroinflammation of AD [36]. Thus, it was suggested that immune system has close relationship with integrity of neural network in the brain and therefore, AD progression.

Bone marrow stem cells and adipose tissue derived stem cells produced anti-inflammatory cytokines, such as IL4 and IL10, in the brain after the transplantation [37,38]. The cell transplantation significantly reduced A β plaque formation of the mice and improved the cognitive dysfunction. These stem cells may be useful to reduce local inflammation of the brain when applied to those with pre symptomatic stages of AD.

Cellular factors in cell transplantation of AD models

Recently, several researchers observed that intrinsic factors in the brain induced neural differentiation of transplanted stem cells. Human adipose tissue derived mesenchymal stem cells were transplanted by the two approaches; intravenous administration and injection into the cerebral ventricles of aging mice [39]. Both of the cell transplantation significantly improved the cognitive function. The transplanted cells effectively differentiated into ChAT positive cells and brain concentration of ACh significantly increased by both of the transplantation protocols. The transplanted mouse neural stem cells differentiated into cholinergic neurons and exhibited similar positive effects on the ACh concentration of brain and cognitive function in AD model mice [24].

SHH and BMP9 successfully generated ChAT, ISL LIM homeobox (ISL)1 and NK2 homeobox1 (NKX2.1) positive basal forebrain cholinergic neurons from mouse and human ES cells. ISL1 and NKX2.1 expressions are essential for the maturation and maintenance of the cell type in the development [40,41]. The transplantation into basal forebrain restored the cholinergic projection system and cognitive function in two AD mouse models [41].

Furthermore, GABAergic neuron progenitors differentiated into mature cells with inhibitory interneuron phenotype after the transplantation and migrated into hippocampus of AD model mice. The cell transplantation restored learning and memory function in the AD mice [31]. The transplanted mouse neuron stem cells promoted synaptogenesis of the brain and significantly improved cognitive function in two AD mouse models without alteration of A β or tau pathology [42]. Intracerebral micro environment as well as host cell-to-grafted cell interactions seem to play a crucial role in histological restoration and improvement of cognitive dysfunction in AD model mice.

As mentioned above, we previously transplanted hiPS cell derived neural cells into the hippocampus of AD model mice [15,16]. The cell transplantation significantly improved cognitive dysfunction of the model mice. Human and mouse ChAT positive neurons and $\alpha 7nAChR$ positive neurons significantly increased in the cortex and hippocampus of the grafted mice compared with the vehicle injected mice [16].

In addition, human and mouse VGAT positive neurons were distributed mainly in the hippocampus and, though the number was small, human VGAT positive neurons were observed in the cortex [16]. In the grafted mouse cortex, the number of GABAR positive neurons of both human origin and mouse origin were significantly increased compared with those in the vehicle injected mouse cortex [16]. The $\alpha 7nAChR$ 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 [16].

We suggested that positive feedback loops of neurotransmitter secretion of the cortex and hippocampus induced the characteristic distribution of the transplanted neurons. Each neuron distribution may form distinctive neuron networks using various humoral and cellular factors.

Conclusions

Transplantation of hiPS cell derived neuron is a promising candidate for the treatment of advanced AD. The graft autonomous effects on the regeneration of damaged neuron circuits are attractive mechanisms for the clinical application. Further studies are needed to elucidate how the grafts select the migration routes, which signals are important to decide the cell destination, whether long axonal projection of grafts exist, and how long the grafts survive and prolong the host neuron survival. These themes are suggested to be equally important for the practical application.

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