Clinical Pharmacology & Biopharmaceutics
ISSN: 2167-065X
Gu, Clinic Pharmacol Biopharmaceut 2013, 2:1
DOI:10.4172/2167-065X.1000111
Review Article
Open Access

Stem Cell-Derived Neurons for the Treatment of Neurodegenerative Diseases

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Abstract

Neurodegenerative diseases are termed for the age-related chronic and progressive loss of structure and function of neurons. Most common neurodegenerative diseases include Alzheimer's disease (AD) and Parkinson's disease (PD). So far, no drugs can be used to reverse the neuronal degeneration. Recent progresses in stem cells biology and technology allow us to generate specific types of neurons, such as cholinergic and dopamine neurons from stem cells, with defined culture conditions. These stem cell-derived neurons have been used for cell replacement therapy in animal models of neurodegenerative neurons, such as AD and PD. Here, I summarized recent progresses of stem cell-derived cholinergic and dopamine neurons and their applications in AD and PD.

Keywords: Neurodegenerative diseases; Embryonic stem cells; Neural stem cells; Cholinergic neurons; Dopamine neurons

Introduction

Neurodegenerative diseases are termed for the age-related chronic and progressive loss of structure and function of neurons, such as Alzheimer's disease (AD) and Parkinson's disease (PD). One of the major pathological changes in AD is degeneration of basal forebrain cholinergic neurons which connected to the cerebral cortex and hippocampus [1]. The loss of structure and function of dopamine neurons in basal ganglia has been found in PD [2]. Damaging the neurons in basal forebrain and basal ganglia is widely used to make the animal models for the studies of AD and PD. In clinical, patients suffered from neurodegenerative diseases show progressive impaired memory, movement and cognitive function. In the end, patients loss the independent ability and need the help from other people for their daily life [3]. Although a lot of efforts have been made to ameliorate the clinical symptoms of neurodegenerative diseases, no drugs can be used to stop the neuronal degeneration process so far. Previous studies have shown that cell transplantation is a good strategy to replace the degenerative neuronal cells [4]. It is still challenge to obtain the appropriate cell donors for the cell replacement therapy of neurodegenerative diseases. Recent progress in stem cell biology and techniques allows scientists to use stem cell-derived neurons for the cell replacement therapy of neurodegenerative diseases.

Neurons have been generated from different types of stem cells including embryonic stem cells (ESCs), neural stem cells (NSCs), adipose-derived stem cells (ADSCs) and mesenchymal stem cells (MSCs) [5-11]. ESCs give rise to all derivatives of three primary germ layers: ectoderm, mesoderm and endoderm and can be induced differentiation into more than 200 cell types of the adult body when they are given sufficient and necessary stimulation with appropriate culture condition [12]. Furthermore, ESCs have the unlimited proliferation ability which allows scientists get enough cells for the basic and clinical studies. In 2009, Geron Inc. got the first license of stem cells clinical application from FDA using ESCs based product. NSCs have the tendency to differentiate into neurons, astrocytes and oligodendrocytes [13,14]. The advantages of ESCs and NSCs make them widely used to generate neurons in the past years. The functions of stem cell-derived neurons have been fully characterized in vitro and in vivo. On the other hand, the functional recovery in the animal model of neurodegenerative diseases has been observed after stem cell-derived neurons transplantation. Moreover, induced pluripotent stem cells (iPSCs) have similar properties with ESCs and make autologous transplantation possible to use stem cell-derived neurons from patient. But iPSCs have a large variability of differentiation ability and carry genetic memory. Here, we will focus on embryonic and neural stem cell-derived neurons used for the treatment of AD and PD.

Stem Cell-derived Cholinergic Neurons for the Treatment of AD

It is a long way to induce stem cells to differentiate into cholinergic neurons. The cholinergic neuronal differentiation efficiency of stem cells is far from satisfication. Nerve growth factor (NGF), leukemia inhibitory factor (LIF) and ciliary neurotrophic factor (CNTF) were used to induce cholinergic neurons from NT2 embryonal carcinoma cells (ECCs) [15]. In this enriched culture, choline acetyltransferase (ChAT) activity (40 pmol ACh/min/mg protein) can be detected for around 3 weeks. Further studies showed that retinoic acid treatment can increase ChAT expression. Manabe et al. have reported that L3/Lhx8 is an important factor for cholinergic neuronal differentiation from mouse ESCs (mESCs). Suppression of L3/Lhx8 in ESCs by siRNA cause dramatic decrease ChAT positive neuronal differentiation and overexpression of L3/Lhx8 could recover the suppression by siRNA [16,17]. Using this hint, cholinergic neurons were generated from human ESCs by overexpression of Lhx8 and Gbx1 [18]. For the functional studies of ESC-derived cholinergic neurons, ESC-derived neuronal precursor cells (ES-NPCs) were transplanted into ibotenic acid-lesioned rat model of AD. Morris water-maze and spatial-probe testing showed a significant functional recovery in memory deficits following ES-NPCs transplantation. Furthermore, ES-NPCs locally differentiated into cholinergic neurons and integrate with host tissue [19]. Recently, Dr. Zhang's group differentiated hESCs into medial ganglionic eminence (MGE)-like cells after applied sonic hedgehog (SHH). MGE progenitors could be further differentiation into cholinergic- and GABA- neurons in vitro and in vivo. Human ESC-derived MGE progenitors transplantation significantly increased the

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Received April 29, 2013; **Accepted** June 03, 2013; **Published** June 05, 2013

Citation: Gu H (2013) Stem Cell-Derived Neurons for the Treatment of Neurodegenerative Diseases. Clinic Pharmacol Biopharmaceut 2: 111. doi:10.4172/2167-065X.1000111

learning and memory of AD model by Morris water-maze testing analysis. This study also suggested that ESC-derived GABA neurons may have a beneficial effect on learning and memory of AD due to large proportion of transplanted MGE progenitors differentiated into GABA neurons [20].

NSCs have been harvested from subventricular zone (SVZ) and subgranular zone (SGZ) and induced to differentiate into neurons, astrocytes and oligodendrocytes. Especially, under defined culture condition, several groups previously reported that NSCs have been induced to differentiate into cholinergic neurons. After NSCs transplantation in adult mammal animal brains, NSCs differentiated into region-specific cholinergic neurons [9,21]. We have harvested NSCs from basal forebrain or SVZ of neonatal rats. NSCs formed typical neurospheres under floating-free culture protocol using serum-free medium supplement with epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) after 3-5 days plating. The proliferation ability was identified by 5-bromo-2-deoxyuridine (BrdU). The multipotential capability was characterized by differentiation assay [13,14,22]. Although few NSCs differentiated into cholinergic neurons in vitro, NSCs could locally differentiate into ChAT- and NGFR-positive neuronal cells in the adult forebrain after 3-4 weeks transplantation. Some of them differentiated into parvalbumin-positive neuronal cells. Most of transplanted NSCs differentiated into astrocytes. Y-maze testing showed that NSCs transplantation significantly increased the learning and memory in the rat model of AD [23,24]. These results suggested that NSC-derived astrocytes could improve neural plasticity of AD brain. Furthermore, we used NGF and BDNF to increase outcome of NSCs transplantation. The results showed that NGF microspheres or SVZ injection of BDNF can dramatically improve the learning and memory in the rat model of AD after NSCs transplantation [25-28]. To study endogenous NSCs response in AD model, Calza and his colleagues injected retinoic acid (RA), nerve growth factor (NGF), EGF and bFGF into immunolesioned rat brains by 192 IgG-saporin. RA, NGF and mitogens can stimulate endogenous NSCs proliferation and differentiate into cholinergic neurons. Water-maze task test showed that the learning and memory of treated group animals was better than that of untreated group [21].

Stem Cell-derived Copamine Neurons for the Treatment of PD

Embryonic stem cells have been widely used to generate dopamine neurons with different protocols. Actually, the protocols of the generation of dopamine neurons from stem cells are more efficient than that of the generation of other type neurons, such as basal forebrain cholinergic neurons, motorneurons and GABAergic neurons. Currently, most of dopamine neurons generation protocols are based on three previous protocols including co-culture with stromal cells, embryoid bodies (EBs) formation and monolayer culture. Dr. Sasai's lab developed a co-culture protocol to generate dopamine neurons from mESCs. They screened different types of cells and found co-culture ESCs with PA stromal cells could get neural differentiation. They named it with stromal cell- derived inducing activity (SDIA). When mESCs were plated on top of PA6 stromal cells, mESCs successfully were induced differentiation into dopoamine neurons [29]. After transplantation, SDIA-induced dopamine neurons could be integrated into mouse striatum with 6-hydroxydopamine (6-OHDA) treatment. Their following works showed that SDIA-induced dopamine neurons transplantation had a beneficial effects on the animal model of PD. Dr. Mckay's lab developed a five-stage EB formation protocol, which included (1) maintaining ESCs in undifferentiated status, (2) generation of EBs, (3) nestin-positive cells selection, (4) nestin-positive cells expansion, and (5) induction of neuronal precursor cells to differentiate into dopamine neurons. After transplantation, dopamine neurons generated from EB formation integrated into the striatum and showed functional properties characterized by clamp patch recording. Behavioral tests showed functional recovery in mouse model of PD after 3-8 weeks transplantation [30]. After that, a lot of groups used this protocol to generate dopamine neurons for functional and developmental studies [30,31]. Dr. Smith's lab developed a monolayer differentiation protocol. Mouse ESCs were seeded onto gelatin-coated tissue culture vessel at a density of $0.5\text{-}1.5\times10^4$ cells/cm^2 in N2B27 medium. Nestin-positive neural progenitor cells (NPCs) could be detected as early as 3 days after induction. Tau-GFP-positive cells could be observed after 4 days of monolayer differentiation [32]. Although dopamine neurons were observed adding sonic hedgehog (SHH) and FGF8 into the culture, the differentiation efficiency of mESCs was low. Cells in monolayer culture have more chance to get nutrients comparing with other protocols. Based on monolayer differentiation protocol, scientists have developed high differentiation efficiency of dopamine neurons from ESCs. Dopamine neurons related transcriptional factors were used to study the dopamine neuronal differentiation and improve the generation efficiency of dopamine neurons from stem cells [33]. Nurr1 was firstly used to increase the generation efficiency of ESC-derived dopamine neurons. Then, Lmx1a/b, Foxa1/2, Engrailed-1/2 (En-1/2), Msx1, Neurogenin 2 (Ngn2) and Pitx3 were used to generate ESC-derived dopamine neurons [34,35]. Furthermore, dopamine neurons have been generated from human ESCs (hESCs). Dopamine neurons generated from hESC are less efficient than that from mESCs. Pioneering works were done by Dr. Studer's group. BMP pathway inhibitors and TGF-β pathway inhibitors were used to increase dopamine neurons differentiation efficiency from hESCs [36]. Recently, GSK-3β inhibitor was also used to generate dopamine neurons from hESCs. Human ESC-derived dopamine neurons have been used for the treatment of PD and showed functional recovery in the animal model of PD [37].

NSC-derived dopamine neurons have been generated from mammal and human brain. In general, the efficiency of dopamine neurons differentiation from NSCs that isolated from brain is low compared with NPCs from ESCs. Although SVZ was widely used to harvest NSCs, most of NSCs that are used to generate dopamine neurons are harvested from ventral midbrain. Overexpression transcriptional factors involved in the development of midbrain dopamine neurons in NSCs, such as Lmx1a and Ngn2, have been used to promote dopamine neuronal differentiation [38]. Carvey et al. [39] have developed a hemaptopoietic cytokines protocol including interleukin-1 and 11, LIF and GDNF to induce rodent mesencephalic NSCs to differentiate into dopamine neurons and yield 20-25% TH-positive neuronal cells. NSCs transplantation is able to provide significant functional recovery in the rat model of PD. NSCs have been genetically modified to express growth or transcriptional factors, such as GDNF, IGF-1, SHH, Nurr1 and Bcl-XL [40]. This preparation also improves the survival of grafting into striatum, provides trophic effects on degenerating dopamine neurons and increases behavioral recovery output in the rat model of PD [41,42].

Furthermore, recent programming techniques have generated induced pluripotent stem cells (iPSCs), NSCs and neurons from mammal and human fibroblasts [34,43-46]. Induced PSCs, NSCs and neurons have been used to model neurodegenerative diseases, drug screen and cell replacement therapy. For example, fibroblast-derived

dopamine neurons and human iPSCs-derived NPCs transplantation could ameliorate the symptoms in the mouse model of PD [47,48]. These preparations make us more options to get appropriate cell donors for cell replacement therapy.

However, stem cells have a risk to form tumors after transplantation. ESCs and iPSCs transplantation have the potential capacity to form teratomas [49]. One possibility to decrease tumor and teratoma formation is to use specific neuronal precursor cells transplantation after differentiation in vitro. Nevertheless, efforts on stem cell therapy strategy should be encouraged due to embryonic/fetal neurons have provided the functional recovery in patients suffered from AD and PD. I believe that stem cell therapy will be used in the clinic for the treatment of neurodegenerative diseases in the future.

Acknowledgements

Due to page limitations, I am regret that a lot of excellent papers cannot be cited here.

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