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Human induced pluripotent stem cell–derived neurons improve motor asymmetry in a 6-hydroxydopamine–induced rat model of Parkinson's disease

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Abstract

Background aims. Since human embryonic stem cells and human fetal neural stem cells have immune rejection and ethical issues, recent advancements in induced pluripotent stem cells (iPS cells) provide new possibilities to study autologous cell therapy for Parkinson's disease (PD). *Methods.* We isolated human skin fibroblasts from normal individuals and patients with PD; we generated iPS cells by transfecting these human skin fibroblasts with retroviral reprogramming factors of OCT4, SOX2, KLF4 and c-MYC and induced iPS cells to differentiate neural stem cells (NSCs) and then into neurons and dopamine neurons *in vitro*. *Results.* We found that iPS cell–derived NSC transplant into the striatum of the 6-hydroxydopamine (OHDA)–induced PD rats improved their functional defects of rotational asymmetry at 4, 8, 12 and 16 weeks after transplantation. iPS cell–derived NSCs were found to survive and integrate into the brain of transplanted PD rats and differentiated into neurons, including dopamine neurons *in vivo*. *Conclusions.* Transplantation of iPS cell–derived NSCs has therapeutic potential for PD. Our study provided experimental proof for future clinical application of iPS cells in cell-based treatment of PD.

Key Words: *differentiation, dopamine neuron, iPS cell, neural stem cell, Parkinson's disease, transplantation*

Introduction

Parkinson disease (PD) is a neurodegenerative disease that affects 1% to 3% of the population over 60 years of age. Even though levo-dopa can improve the symptoms, it cannot stop the disease progress; the patient's condition becomes more and more severe as time progresses. Because the selective loss of dopamine neurons in the substantia nigra is the key pathological characteristic of PD, neuronal cell replacement therapy is most suitable for curing patients with PD [1,2]. The current human neural stem cell sources for experimental and clinical PD therapy include neural stem cells (NSCs) and dopamine neurons derived from human fetal brain and human embryonic stem cells (hES cells). Transplantation of NSCs and dopamine neurons from fetal brains has been shown to improve the functional deficits of dopamine neurons in PD animal models and in clinical trials for patients with PD [3–6]. hES cells are isolated from the inner cell mass of a blastocyst and are pluripotent; therefore they are able to differentiate into all kinds of cells, including neurons [7,8]. Dopamine neurons have been successfully derived from hES cells, and these differentiated dopamine neurons are shown to improve behavior abnormalities of rat PD models [9–11]. Because the hES cell–differentiated neurons may contain some undifferentiated hES cells that may produce a teratoma after transplantation, some studies have tried to purify the differentiated neurons and dopamine neurons through the use of fluorescence-activated cell sorting (FACS) [12,13]. However, these cell sources are allogenic and may incur immune rejection. In addition, the use of human embryos or fetal central nervous system tissue may give rise to ethical concerns when they are used in clinical practices for PD [14,15]. The generation of induced pluripotent

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stem cells (iPS cells) through somatic cell reprogramming provides great promise to patients with neurodegenerative diseases such as PD because the iPS cells can be generated from autologous cells and are able to overcome the barriers of allogenic cell transplantation [16–20].

Human iPS cells are produced by reprogramming somatic cells such as the skin fibroblasts, blood cells, lung cells and recent urine epithelial cells with pluripotent transcription factors of OCT4, SOX2, c-MYC, KLF4, LIN28, NANOG and so forth [19,21,22]. iPS cells have characteristics of hES cells and can differentiate into a variety of functional cells such as neurons, cardiomyocytes and blood cells for studying the molecular pathology of a disease, screening therapeutic drugs and small molecules and investigating cell transplantation therapy for PD and other degenerative diseases [23–25]. To study the neural differentiation ability and therapeutic efficacy of iPS cells for PD, we have generated iPS cells from normal individuals and patient with PD and transplanted the differentiated iPS cell–derived NSCs to the 6-OHDA–induced PD rat model. The transplanted iPS cell–derived NSCs differentiate into dopamine neurons, integrate into the rat brain and improve the movement defects of the PD rats.

Methods

Subjects and materials

Subjects were recruited to donate skin samples for isolation of skin fibroblasts. In this study, the two subject groups are normal control individuals and patients diagnosed with PD. All research involving human participants was performed with approval of the Human Research Ethics Committee of the hospital (Liaocheng People's Hospital/The Affiliated Liaocheng Hospital, Taishan Medical University, Liaocheng, Shandong, China). All subjects filled and signed a consent form before the biopsy was performed. We collected skin samples from three normal control individuals identified as NCF-1 (female, 58 years old), NCF-2 (female, 65 years old) and NCF-3 (female, 62 years old) as well as two PD patients identified as PD-1 (male, 68 years old) and PD-2 (male, 63 years old).

Dulbecco's modified Eagle's medium (DMEM), DMEM/F12, fetal bovine serum, non-essential amino acid (NEAA), glutamine, penicillin-streptomycin (P/S), mitomycin C, knockout serum replacement, mercaptoethanol, fibroblast growth factor 2 (FGF2), TrypLE, polybrene and N2 were purchased from Life Technologies; 0.25-μm filters were purchased from Corning Incorporated; Plat-A cells were from Cell Biolabs Inc; gelatin, Noggin, transforming growth factor, sonic hedgehog (SHH) and glial-derived neurotrophic factor (GDNF) were purchased from R&D Biosystems; brain-derived neurotrophic factor (BDNF) and FGF8b were purchased from Peprotech Inc; SB431542 was purchased from Tocris Bioscience; poly-ornithine/laminin, paraformaldehyde, goat serum, donkey serum, Triton X-100, Hoechst 33258, sucrose, isoflurane, ascorbic acid and apomorphine were purchased from Sigma-Aldrich. Sterile plastic 15-mL tubes, 50-mL tubes, 60-cm dishes, 25-cm flasks, 75-cm flasks and six-well plates and pipettes were purchased from Corning Incorporated.

Culturing and flow cytometry analysis of primary skin fibroblasts

After consent forms were filled and signed by the donors, a skin punch with a diameter of 3 to 5 mm was collected from each of the control individuals and PD patients. On the basis of the published protocol to obtain skin tissue [26], we used an electric dental drill (MD20, NOUVAG AG/SA/Ltd) to punch the skin tissues and found that this method was efficient. After the punch area was sterilized with alcohol and anesthetized with articaine hydrochloride and epinephrine tartrate injection (Produits Dentaires Pierre Rolland), a dental drill was applied to the anesthetized area, drilling to a depth of 2 mm to obtain a skin punch with a diameter of 3 to 5 mm. The skin tissue was immediately put into the sterile tube with DMEM medium and brought to the lab for culturing. The skin tissue was washed with DMEM once and washed with Dulbecco's phosphate-buffered saline twice; it was then cut into small clumps of 1- to 2-mm diameter and cultured in mouse embryonic fibroblasts (MEF) medium containing DMEM, 20% fetal bovine serum, 1% NEAA, 1% glutamine and 1% P/S for 2 to 4 weeks. After the fibroblast cells grew out from the clumps and reached a confluency of 70% to 80%, these cells were passaged every week with medium change every 3 to 4 days.

Flow cytometry analysis

The isolated human skin fibroblast cells were cultured and analyzed with the use of a BD FACSAria II flow cytometer (BD Biosciences). A standard procedure was performed to characterize the fibroblast lines through the cell surface markers of CD73, CD90 and CD34. These antibodies were fluoresced in isothiocyanate (FITC)-conjugated anti-human CD90, CD73 and R-phycoerythrin (PE)-conjugated anti-human CD34 (BD Biosciences). Our detailed procedure is as follows: The fibroblast cells were suspended at a concentration of 1×10^7/mL in phosphate-buffered saline (PBS); 100 μL of cell suspension (1×10^6 cells)