Cytotherapy, 2018; ■■: ■■–■■

International Society for Cellular Therapy
ISCT®

Intrastriatal transplantation of stem cells from human exfoliated deciduous teeth reduces motor defects in Parkinsonian rats

NAN ZHANG[1,*], XIANJIE LU[1,*], SHICHAO WU[1,*], XUELI LI[2,*], JING DUAN[1], CHAO CHEN[1], WEI WANG[1], HAO SONG[1], JIABEI TONG[1], SEN LI[1], YANMING LIU[3], XINJIANG KANG[4], XUEXIANG WANG[5] & FABIN HAN[1,5]

[1]*The Institute for Tissue Engineering and Regenerative Medicine, Liaocheng University/Liaocheng People's Hospital, Liaocheng, Shandong, China,* [2]*Department of Neurology, Liaocheng People's Hospital, Liaocheng, Shandong, China,* [3]*College of Biological Science, Qingdao University, Qingdao, Shandong, China,* [4]*College of Biological Science, Liaocheng University, Liaocheng, Shandong, China, and* [5]*The Institute for Translational Medicine, The Second Affiliated Hospital, Shandong University, Jinan, Shandong, China*

Abstract

Background. This study explored the neural differentiation and therapeutic effects of stem cells from human exfoliated deciduous teeth (SHED) in a rat model of Parkinson's disease (PD). *Methods.* The SHED were isolated from fresh dental pulp and were induced to differentiate to neurons and dopamine neurons by inhibiting similar mothers against dpp (SMAD) signaling with Noggin and increase conversion of dopamine neurons from SHED with CHIR99021, Sonic Hedgehog (SHH) and FGF8 *in vitro*. The neural-primed SHED were transplanted to the striatum of 6-hydroxydopamine (6-OHDA)–induced PD rats to evaluate their neural differentiation and functions *in vivo*. *Results.* These SHED were efficiently differentiated to neurons (62.7%) and dopamine neurons (42.3%) through a newly developed method. After transplantation, the neural-induced SHED significantly improved recovery of the motor deficits of the PD rats. The grafted SHED were differentiated into neurons (61%), including dopamine neurons (22.3%), and integrated into the host rat brain by forming synaptic connections. Patch clamp analysis showed that neurons derived from grafted SHED have the same membrane potential profile as dopamine neurons, indicating these cells are dopamine neuron-like cells. The potential molecular mechanism of SHED transplantation in alleviating motor deficits of the rats is likely to be mediated by neuronal replacement and immune-modulation as we detected the transplanted dopamine neurons and released immune cytokines from SHED. *Conclusion.* Using neural-primed SHED to treat PD showed significant restorations of motor deficits in 6-OHDA–induced rats. These observations provide further evidence that SHED can be used for cell-based therapy of PD.

Key Words: *dopamine neuron, mesenchymal stromal cell, Parkinson's disease, patch clamp, stem cells from human exfoliated deciduous teeth, transplantation*

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease and is mainly caused by loss of dopaminergic neurons in the substantia nigra of the middle brain leading to the movement dysfunction [1,2]. Even though levodopa treatment can relieve some symptoms in the early phase of the disease, it can not stop the progression of the disease. Because of the specific cell death of dopamine (DA) neurons, cell replacement has been suggested as the therapeutic hope for PD.

Stem cell transplantation studies showed different stem cells have relieved some of the motor deficits of animal PD models and improved the movement dysfunction of patients with PD [3–7]. Neural stem cells (NSCs) from fetal brains, human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) were shown to be effective in mouse, rat and monkey models of PD, but these cells have limitations of immune rejection, ethical issues and tumorigenicity, which may affect their clinical applications [8–10]. The mesenchymal stromal cells from bone marrow (BM-MSCs) and umbilical cords (hUC-MSCs) were also shown to differentiate to neurons and DA neurons and improve the functional deficits in PD animal models after transplantation [3,11–15].

*These authors equally contributed to the work.
Correspondence: **Fabin Han**, MD, The Institute for Tissue Engineering and Regenerative Medicine, Liaocheng University/Liaocheng People's Hospital, Shandong 252000, China. E-mail: fhan2013@126.com

(Received 30 October 2017; accepted 21 February 2018)

ISSN 1465-3249
https://doi.org/10.1016/j.jcyt.2018.02.371

Recent studies showed human dental pulp stem cells (DPSCs) and stem cells from human exfoliated deciduous teeth (SHED) have strong neural differentiation ability and were used for the treatment of neurological diseases [16,17]. SHED reside within the perivascular niche of the dental pulp, which is a derivative of neural crest, suggesting SHED have potential for treatment of neurological diseases. It is reported that human DPSCs and SHED have similar characteristics of BM-MSCs and were able to differentiate into different types of cells, including adipogenic, osteogenic and chondrogenic lineages, neurons and DA neurons [18,19]. In addition, several studies indicated that transplanted DPSCs in rodent brains differentiated to mature neuronal cells, suggesting their potential of neuronal differentiation ability [20,21]. Thus, they are suitable for repairing neural degeneration and traumatic damage of the central nervous system [3,20,22].

Previously we reported the isolation and characterization of the SHED and showed the multiple potential of SHED including neural differentiation [23]. The advantage of SHED is that they keep their strong proliferation ability for more than 30 passages, which are much more than the 6–7 passages of BM-MSCs as reported in other studies [23–25]. In this study we explored whether the SHED can differentiate into DA-like neurons and their therapeutic effect after the neural-primed SHED were engrafted to the 6-hydroxydopamine (6-OHDA)–induced rat model of PD.

Methods

Human subjects and ethical approval

All research involving human participants was performed with approval by the Human Research Ethics Committee of our hospital (LUHREC201428, Liaocheng University/Liaocheng People's Hospital, Liaocheng, China). All subjects or subjects' legal representatives filled out and signed a consent form before the biopsy was performed. Human dental pulp tissues for SHED isolation were obtained from the Department of Stomatology, the Liaocheng People's Hospital, with donors' written consent and approval by the institutional review board of the Liaocheng People's Hospital.

Animals

All animal experiments were carried out in accordance with local ethical guidelines and approval of the Animal Care and Use Committee in Liaocheng University/The Liaocheng People's Hospital (LUACC201428), Shandong, China. This study was carried out January 2014 to December 2016. All surgeries were performed under general anesthesia, and all effort was made to reduce the number of animals used. The study rats were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd., which is a collaborative company with Charles River Laboratories. In total, 24 Wistar male rats (catalog number 102; 180–200 g) were used in the study. The rats were kept in an animal experiment room under standard environmental conditions with free access to water and were fed twice per day.

Reagents, chemicals and antibodies

Dulbecco's modified Eagle's medium (DMEM), DMEM/F12, fetal bovine serum (FBS), non-essential amino acid (NEAA), glutamine, penicillin-streptomycin (P/S), fibroblast growth factor 2 (FGF2) (10 ng/mL), FGF-2 (10 ng/mL), TrypLE and N2 were purchased from Life Technologies (Invitrogen); Sonic Hedgehog (SHH; 100 ng/mL), Noggin (250 ng/mL) and CHIR99021 are from R&D Systems, Inc.; FGF-8 (10 ng/mL) is from PeproTech, Inc.; 200 mmol/L AAP and TGF-β are from Sigma; glial-derived neurotrophic factor (GDNF) and BDNF (50 ng/mL) are from R&D Systems. The primary antibodies were as follows: antibodies of βIII tubulin (Tuj1; mouse monoclonal, 1:500; Sigma-Aldrich); microtubule-associated protein 2 (MAP2) (mouse monoclonal), tyrosine hydroxylase (TH; rabbit poly-clonal, 1:250; Pel Reeze), dopamine active transporter (DAT) (rabbit poly-clonal, 1:250; Sigma), glial fibrillary acidic protein (GFAP; rabbit polyclonal, 1:250; Dako), synaptophysin (mouse monoclonal, 1:250; Sigma Aldrich) and Nestin (mouse monoclonal, 1:500; Sigma Aldrich).

Isolation, culturing and characterization of the SHED in vitro

After consent forms were filled out and signed by the donors, isolation and expansion of SHED *in vitro* were done according to the procedures described previously [13]. Briefly, human deciduous incisors were collected from 5- to 12-year-old children. Before extraction, each subject was examined to exclude for systemic and oral infections. Only disease-free subjects were selected for the study. Under aseptic conditions, the dental pulp cavity of the crown was opened by a dental surgeon and the pulp was extracted with a broach, immediately placed in DMEM/F12 medium and brought to the cell culture room. The dental pulps were then cut into small clumps with a diameter of 1–2 mm and digested with 0.075% collagenase II (Sigma) and 0.125% trypsin (Gibco) at 37°C. The digested mixture was then passed through a 100-μm filter to obtain cell suspensions. Cells were cultured in DMEM/F12 supplemented with 10% FBS,