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Regenerative effect of neural-induced human mesenchymal stromal cells in rat models of Parkinson's disease

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Background

Human bone marrow multipotent mesenchymal stromal cells (hMSC), because of their capacity of multipotency, may provide an unlimited cell source for cell replacement therapy. The purpose of this study was to assess the developmental potential of hMSC to replace the midbrain dopamine neurons selectively lost in Parkinson's disease.

Methods

Cells were isolated and characterized, then induced to differentiate toward the neural lineage. In vitro *analysis of neural differentiation was achieved using various methods to evaluate the expression of neural and dopaminergic genes and proteins. Neural-induced cells were then transplanted into the striata of hemi-Parkinsonian rats; animals were tested for rotational behavior and, after killing, immunohistochemistry was performed*

Results

Following differentiation, cells displayed neuronal morphology and were found to express neural genes and proteins. Furthermore, some of the cells exhibited gene and protein profiles typical of dopaminergic precursors. Finally, transplantation of neural-induced cells into the striatum of hemi-Parkinsonian rats resulted in improvement of their behavioral deficits, as determined by apomorphine-induced rotational behavior. The transplanted induced cells proved to be of superior benefit compared with the transplantation of naive hMSC. Immunohistochemical analysis of grafted brains revealed that abundant induced cells survived the grafts and some displayed dopaminergic traits.

Discussion

Our results demonstrate that induced neural hMSC may serve as a new cell source for the treatment of neurodegenerative diseases and have potential for broad application. These results encourage further developments of the possible use of hMSC in the treatment of Parkinson's disease.

Keywords

dopaminergic cells, mesenchymal stromal cells, Parkinson's disease, rat model of Parkinson's disease, transplantation.

Introduction

Regenerative cell-based therapy aims at grafting therapeutically relevant cells to impaired tissues and has been proposed for future therapies of intractable neurodegenerative disorders. Parkinson's disease (PD), characterized by progressive and selective loss of dopamine (DA) neurons in the midbrain substantia nigra, is a prime target for cell replacement therapy, with more than a decade of successful clinical experiences with fetal ventral mesencephalic cell transplantation in PD patients [1,2]. However, fetal cell transplantation has significant technical, ethical and practical limitations, partly because of limited availability and variable outcomes [2,3]. Stem cells, because of their self-renewal capacity and multilineage developmental potential, could be an ideal cell source for cell replacement therapy.

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It has been demonstrated that adult human bone marrow multipotent mesenchymal stromal cells (hMSC) are a subset of multipotential precursor non-hemopoietic cells from the bone marrow. They are notable for their ability to self-proliferate and differentiate along multiple lineages, including bone, cartilage, adipose and muscle cells [4,5]. Other researchers have shown that the cultured hMSC also have the potential to differentiate into neural cells [6–8]. As neural stem cells, they can migrate and integrate into the damaged brain [9,10]. However, it has yet to be demonstrated that these hMSC-derived neural cells have the capacity to function as neurons. hMSC can be easily isolated, cultured and expanded *in vitro* and their usage bears no ethical restriction. Moreover, hMSC enable the use of autologous transplantation, thus avoiding the risk of immune rejection.

We have previously derived highly enriched cultures of neural cells from hMSC [8,11,12]. These neural cells may serve as a platform for generating DA neurons to treat Parkinsonism. The major aim of this work was to investigate the ability of hMSC to differentiate *in vitro* toward functional dopaminergic neuronal-like cells. Neural and dopaminergic differentiation were characterized by the detection of neural markers and analysis of some level of neuronal function both *in vitro* and *in vivo*. Following neural induction, the majority of hMSC adopted a neuron-like morphology, expressed various neural genes and proteins, and acquired some functions of the dopaminergic neuron, such as production and release of DA precursor molecules. Finally, transplantation of these cells resulted in behavioral improvement of a rat model of PD. Our results firmly demonstrate that hMSC can differentiate along the neural pathways toward a dopaminergic functional phenotype. Moreover, we also provide evidence for an enhanced clinical benefit in PD rat models of the differentiated cells in comparison with naive hMSC.

Methods

Adult hMSC

Adult hMSC were collected from the iliac crest of 20 healthy human donors ranging in age from 20 to 70 years. The cells employed in this study were not pooled from multiple donors. The study was approved by the Tel-Aviv University and the Ministry of Health Helsinki Ethical Committee, and individual informed consent was obtained. The isolation, culture conditions, propagation and characterization of hMSC have been described in detail previously [8,12].

Induction and differentiation of dopaminergic neurons

hMSC were proliferated for at least 30 days from bone marrow isolation. To induce neural differentiation, hMSC (passage 2–7) were incubated for 24–72 h with stage 1 media, consisting of Dulbecco's modified Eagle's medium (DMEM; Biological Industries, Beit Haemek, Israel) supplemented with 10% fetal bovine serum (FBS; HyClone, Logan, UT, USA), 2 mm glutamine, 100 μg/mL streptomycin, 100 U/mL penicillin, 12.5 U/mL nystatin (SPN; Biological Industries), basic fibroblast growth factor (bFGF; R&D Systems, Minneapolis, MN, USA), 10 ng/mL EGF, 30 μm docosahexaenoic acid (DHA; Sigma, St Louis, MO, USA) and N2 supplement (insulin 5 μg/mL, progesterone 20 nm, putrescine 100 μm, selenium 30 nm and transferrin 100 μg/mL; all from Sigma). Then the cells were incubated for 6–96 h with stage 2 media, composed of DMEM supplemented with 2 mm glutamine, SPN, N2 supplement, 200 μm butylated hydroxyanisole (BHA), 1 mm dibutyryl cyclic AMP (dbcAMP), 0.5mm 3-isobutyl-l-methyl-xanthine (IBMX), 100 μm ascorbic acid and 1–10 μm all-trans-retinoic acid (RA) (all from Sigma).

Transcript identification

Isolation and preparation of RNA

Total RNA was extracted from undifferentiated hMSC, hMSC incubated in neuronal differentiation medium for various lengths of time and human lymphocytes as a negative control, by using the guanidine isothiocyanate method [13]. Reverse transcription was carried out on 0.05-μg/μL mRNA samples using 5 U/μL enzyme SuperScript™ II RNase H–reverse transcriptase (RT) in a mixture containing 2 μm random primers (mostly hexamers), 10 mm dithiotheitol (DTT), 1 × buffer supplied by the manufacturer (Invitrogen Life Technologies, Carlsbad, CA, USA; http://www.invitrogen.com), 20 m dNTPs (TaKaRa Bio Europe, Gennevilliers, France; http://www.takarabioeurope.com) and 1 U/L RNase inhibitor (RNAguard; Amersham Biosciences, Amersham, UK; www.amersham.com). The reverse transcription reaction was performed at 25°C for 10 min, 42°C for 120 min, 70°C for 15 min and 95°C for 5 min.