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Intranigral Transplantation of Epigenetically Induced BDNF-Secreting Human Mesenchymal Stem Cells: Implications for Cell-Based Therapies in Parkinson's Disease

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It is thought that the ability of human mesenchymal stem cells (hMSC) to deliver neurotrophic factors might be potentially useful for the treatment of neurodegenerative disorders. The aim of the present study was to characterize signals and/or molecules that regulate brain-derived neurotrophic factor (BDNF) protein expression/delivery in hMSC cultures and evaluate the effect of epigenetically generated BDNF-secreting hMSC on the intact and lesioned substantia nigra (SN). We tested 4 different culture media and found that the presence of fetal bovine serum (FBS) decreased the expression of BDNF, whereas exogenous addition of epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) to serum-free medium was required to induce BDNF release (125 ± 12 pg/day/10^6 cells). These cells were called hM(N)SC. Although the induction medium inhibited the expression of alpha smooth muscle actin (ASMA), an hMSC marker, and increased the nestin-positive subpopulation of hMSC cultures, the ability to express BDNF was restricted to the nestin-negative subpopulation. One week after transplantation into the SN, the human cells integrated into the surrounding tissue, and some showed a dopaminergic phenotype. We also observed the activation of Trk receptors for neurotrophic factors around the implant site, including the BDNF receptor TrkB. When we transplanted these cells into the unilateral lesioned SN induced by striatal injection of 6-hydroxydopamine (6-OHDA), a significant hypertrophy of nigral tyrosine hydroxylase $(TH)^+$ cells, an increase of striatal TH-staining and stabilization of amphetamine-induced motor symptoms were observed. Therefore, hMSC cultures exposed to the described induction medium might be highly useful as a vehicle for neurotrophic delivery to the brain and specifically are strong candidates for future therapeutic application in Parkinson's disease.

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INTRODUCTION

Bone marrow (BM) contains mesenchymal stem cells (MSCs), also termed multipotent stromal cells, with the ability to differentiate in vitro into mesenchymal and nonmesenchymal lineages, including neuronal and glial lineages [1-8]. MSCs are potentially useful for transplantation in several neurodegenerative diseases, but the mechanisms responsible for a beneficial outcome are not fully understood [9]. A possible mechanism involved in the neuroprotective effect of MSCs is neural transdifferentiation. However, it is currently thought that the therapeutic benefits of MSCs depend on their ability to produce soluble factors that can promote neuronal cell survival and neuritogenesis [10,11], stimulation of axonal growth [12,13], functional recovery [14,15], and modulation of microglial activation [16]. At present, the positive behavioral effects observed after MSC transplantation in the striatum, which is the usual target implantation region of parkinsonian animal models, cannot be explained by the low/insufficient number of dopaminergic donor neurons derived from the grafted cells [17-19], but it may depend on immunomodulation and/or growth factor delivery [9,11,17,20].

Parkinson's disease (PD) is characterized by the degeneration of nigrostriatal dopaminergic neurons

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of the lateral substantia nigra pars compacta (SNpc) [21] and is associated with reduced levels of neurotrophins such as brain-derived neurotrophic factor (BDNF) [22-24]. Dopaminergic neurons of the SNpc depend on BDNF [25]. BDNF is produced mainly in nigral neurons [26], which also express its receptor TrkB [27]. Infusion of BDNF into the SNpc region promotes survival of axotomized nigrostriatal dopaminergic neurons [28]. Additionally, intranigral antisense oligonucleotide infusion indicated that loss of BDNF expression leads to dopaminergic neuronal death [29]. Herein, we suggest that BDNF supplemented by cells implanted in a nigral location could be better suited for a neurotrophin-based therapy for PD than a striatal placement [30].

Some previous results indicate that hMSC in culture express BDNF using standard and induction conditions [10,11,20,31-34], but other reports observed that significant BDNF release is obtained only after BDNF gene transfer into hMSC [13,35-37]. To date, the variability of results published with regard to the levels and types of neurotrophins produced by hMSC could be explained by the age of the human donors or the method used to isolated hMSC from BM [38]. In addition, the presence of fetal bovine serum (FBS) in the medium is the most variable factor contributing to heterogeneous results because of its composition and endogenous levels of growth factors and cytokines, which can vary based on the batch and quantity used [39]. These factors could mildly modify the biology of the hMSC (eg, the proteins they express and/or secrete). We previously observed BDNF in conditioned media obtained from hMSC cultures isolated by plastic adhesion after exposure to a defined serum-free medium [34]. In the present study, we evaluated the effects of FBS supplemented with both epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) on gene expression, cell proliferation, and the ability to express and/or release BDNF. We also evaluated the in vivo effect of BDNF-secreting hMSC, called hM(N)SC, in the intact and lesioned rat SNpc. Induced human cells integrated perfectly into the rat SN, some of which differentiated into a dopaminergic phenotype and activated Trk receptors at the implant site. Our data suggest that epigenetically generated hM(N)SC exert neurotrophic effect on the impaired dopaminergic nigrostriatal system.

MATERIALS AND METHODS

Isolation and Expansion of hMSC Cultures

hMSC cultures were isolated by plastic adhesion and characterized after 1 passage as described previously [34]. After informed consent, BM aspirates were collected from 5 normal young individuals undergoing BM harvest for allogeneic transplantation, as part of a protocol approved by the Ethical Committee of the Hematology Department of Clinica Alemana (Santiago, Chile). Total cells were seeded at a density of 1×10^6 nucleated cells/cm^2 in α-minimal essential medium supplemented with 10% FBS and 0.8 mg/L gentamycin (MSC-medium). After 24 hours, nonadherent cells were removed by replacing the culture medium. When the foci of fibroblast-like cells were confluent, the cells were detached with 0.25% (p/v) trypsin and 2.65 mM EDTA and subcultured at 7×10^3 cells/cm^2 for further expansion. Isolated cells were characterized by real-time RT-PCR for CD34, CD105, and alpha smooth muscle actin (ASMA), and their capacity to differentiate in vitro into adipocytes, chondrocytes, and osteocytes was evaluated as described previously [34].

Culture Media Composition

To evaluate the effect of FBS and specific growth factors (EGF and bFGF), we cultured cells in MSC-medium, MSC + GF-medium (MSC-medium with human EGF plus human bFGF, both at 10 ng/mL [R&D Systems, Minneapolis, MN]), NSC-medium (DMEM/F12 [1:1] [Gibco, Grand Island, NY], 1% bovine serum albumin [BSA, Merck, Rahway, NJ], 6 g/L D[+]-glucose [Merck], 0.8 mg/L gentamicin supplemented with N2 and B27 supplements [Gibco]) and NSC + GF-medium (NSC-medium supplemented with EGF and bFGF, 10 ng/ml of each) for 3 and 8 days.

Cellular Viability and Proliferation

To examine cell viability and proliferation, we used a colorimetric assay using WST-1 reagent (Roche, Germany), a tetrazolium salt that is converted into formazan dye by mitochondrial dehydrogenases. Cells were seeded at a density of 4×10^3 cells/cm^2, and 2 to 4 hours later, WST-1 was diluted 1/10 (v/v) in the respective serum-free media and incubated for 4 hours at 37°C. The net absorbance at 450 nm was normalized with respect to day 0.

RT-PCR Analysis

Total RNA was isolated from cells using TRIZOL® reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions, and 1 μg of total RNA was used for reverse transcription (RT). PCR was performed in a capillary containing 100 ng of cDNA, PCR LightCycler-DNA Master SYBR Green reaction mix (Roche, Indianapolis, IN), 3-4 mM $MgCl_2$, and 0.5 mM of each specific primer, using a LightCycler® thermocycler (Roche). For primer sequences and control human cell lines, see the description from a previous article [34]. Negative controls without reverse transcriptase were performed. Negative PCR results were validated by amplification of the housekeeping gene GAPDH. PCR sensitivity for each neural gene was calculated previously [34].