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MESENCHYMAL STROMAL CELLS

Dopaminergic-primed fetal liver mesenchymal stromal–like cells can reverse parkinsonian symptoms in 6-hydroxydopamine-lesioned mice

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Abstract

Background aims. Cell replacement therapy is considered a promising alternative in the treatment of degenerative diseases, and in this context, mesenchymal stromal cells (MSCs) have been proposed for transplantation in Parkinson disease (PD). Thus far, the results of animal studies are found to be inconsistent and inconclusive regarding the therapeutic ability of the cells. This study investigated the efficacy of fetal liver (FL)-MSC-derived dopaminergic (DA) neuronal primed cells for correction of parkinsonian symptoms in mice. *Methods.* FL-MSCs were differentiated for 21 days in the presence of a combination of neurotropic factors. The extent of cellular reprogramming was analyzed by quantitative polymerase chain reaction for DA-specific neuronal gene expressions and protein expressions by immuno-cytochemistry. The functionality of the cells was determined by electrophysiology and dopamine release assays. Ten-day-primed neuron-like cells or unprimed MSCs were transplanted into the 6-hydroxydopamine (6-OHDA)-lesioned striatum using a stereotaxic device. Dopamine-secreting properties and behavioral studies were used to assess improvement of parkinsonian symptoms. *Results.* The differentiated cells expressed DA-specific genes and proteins, while exhibiting a high level of voltage-gated potassium current. Furthermore, neuronal primed cells differentiated into tyrosine hydroxylase immunoreactive and dopamine-secreting functional neuron-like cells. Symptomatic correction of PD in the recipient mice within 2 months of transplantation was also observed. *Discussion.* FL-MSC-derived primed neuron-like cells integrated into the striatum of PD mice, improving parkinsonian symptoms. This study demonstrates an effective cell-based therapy for PD.

Key Words: *Apomorphine-induced rotation, Differentiation, Dopaminergic neurons, Dopamine secretion, Fetal liver MSCs, 6-OHDA, Parkinson disease*

Introduction

Parkinson disease (PD) is a progressive neurodegenerative disorder afflicting the aged population. The pathogenesis of the disease is related to loss of dopaminergic (DA) neurons of the substantia nigra (SN) pars compacta and the subsequent decline in dopamine levels in the striatum of the brain [1]. The adult neurons do not regenerate, although there are reports of neurogenesis in distinct areas of human brain, such as the subventricular zone, olfactory bulb and hippocampus, for example. In neurodegenerative disorders such as Huntington disease [2] and PD [3], no natural neurogenesis has been observed. The standard practices for the treatment of PD are L-DOPA therapy and deep brain stimulation [4,5], which improve patients' characteristic motor symptoms but do not repair or regenerate DA neurons or prevent further degeneration. Thus, replacement of degenerating neurons with exogenous cells has been considered as a highly promising alternative for the treatment of PD [6]. In this regard, different sources of neuronal precursor cells have been tested in rodents and non-human primate disease models [7,8]. Transplantation of human fetal mesencephalic tissue showed partial improvement in PD patients [9,10]. However, the major limitations associated with such cell-based therapy are poor engraftability, limited differentiation ability and probable immune rejection. In adults, central nervous system precursor–derived

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dopaminergic neurons showed limited life span in rodents and exhibited no improvement in apomorphine-induced contralateral rotation [11]. Again, results of the clinical trials were inconsistent because of complications, such as graft-induced hyperkinesia [12].

Transplantation of mouse embryonic stem cell–derived DA neurons has shown encouraging results in 6-hydroxydopamine (6-OHDA)-treated parkinsonian rats [13]. Although the results seem promising, the immunological rejection of the transplanted cells and the formation of teratomas have been the major obstacles in clinical applications. To avoid immune rejection, patient-specific DA neurons of midbrain characteristics, derived from induced pluripotent stem (iPS) cells, have been used. These cells improved motor function and behavioral deficits in a rat model of PD [14]. The grafted neurons were found to be functionally integrated in the recipient brain, as determined by electrophysiological and morphological analysis [14]. iPS technology is expected to solve many therapeutic challenges, although treatment of PD using patient-specific cells has proven to be prohibitively expensive. To make this treatment affordable to the patients, transplantation of allogeneic iPS-derived DA neurons under immunosuppressive regimen has been proposed.

Thus far, adult mesenchymal stromal cells (MSCs) were found to be the most attractive choice for therapy because of their immunomodulatory properties and cellular plasticity. Mouse bone marrow (BM)-MSCs were engrafted and differentiated into DA neurons in the 6-OHDA-lesioned mice [15]. Furthermore, *in vitro* studies have demonstrated the formation of DA progenitor-like cells from human BM or umbilical cord–derived MSCs [16,17]. Recent studies have shown that adult MSCs not only differentiate into DA neurons in culture, they also engraft on neurotoxin-lesioned SN or striatum, depending on the site of delivery. The donor cells resembled DA neurons, both phenotypically and morphologically [18,19], and improved subjects' parkinsonian symptoms [19]. Despite this direct role of MSCs in the recovery of PD, a large body of literature has assumed that various neurotrophic factors, secreted by MSCs, stimulate endogenous neurogenesis and rejuvenate damaged DA axons [20–22]. In a recent study, human adipose–derived MSCs were found to induce significant neurogenesis in rat subventricular zone compared with the sham control [23].

In these studies, MSCs were isolated from adult tissue, which was either directly transplanted or, before that, differentiated into DA neuron–like cells. In this study, we have examined the potential of mouse fetal liver (FL)-MSCs, unprimed or primed to differentiate into DA-like neurons, for the recovery of 6-OHDA-lesioned PD mice. We transplanted primed DA neuron–like cells instead of mature neurons because they are expected to have longer life expectancy and to function better within the physiological niche. The recipient mice significantly improved from the parkinsonian symptoms when transplanted with primed MSCs. Thus the study emphasizes that cell-based treatment could be a better option for PD.

Methods

Isolation of MSCs and neuronal induction

Single cell suspension was prepared from 14.5 dpc FLs of pregnant females, expressing enhanced green fluorescence protein (eGFP), C57BL6/J [C57Bl6/J-Tg(UBCGFP) 30Scha/J] mice. MSCs were isolated using the two-step magnetic-activated cell sorting technique (Miltenyi Biotech): initial negative selection of $CD45^{-}Ter119^{-}$ cells was followed by positive selection of CD44-expressing cells. A fraction of sorted cells were used for characterization, and the remaining cells were used for expansion and subsequent experiments. Before differentiation, cells were cultured up to passage 3 (P3) in six-well plates (100 000 cells/well) in Dulbecco's Modified Eagle's Medium/F12 supplemented with 10% fetal bovine serum (Biological Industries). These cells were separately cultured in the presence of osteogenic [24], adipogenic [25] and neurogenic induction media. For differentiation into DA-like neurons, approximately 250 000 cells in six-well plates, coated with 50-μg/mL poly L-ornithine and 1 μg/mL of fibronectin, were cultured in the presence of neurobasal medium supplemented with B-27 (0.5%), vitamin C (200 μmol/L), fibroblast growth factor (FGF)-2 (20 ng/mL), Shh (200 ng/mL), FGF8 (100 ng/mL), transforming growth factor-β3 (10 ng/mL), brain-derived neurotrophic factor (20 ng/ml) and glial cell line–derived neurotrophic factor (20 ng/mL) for 10 or 21 days as per the scheme (see Figure 2A later in the article). All neurotropic factors were purchased from R&D Systems. The experiments using mice were conducted as per procedures approved by the Institutional Animal Ethics Committee at the National Institute of Immunology.

Characterization of DA neuron–like cells

After 21 days of differentiation, neurite outgrowth was measure using ImageJ software (National Institutes of Health) on non-overlapping images (200×); for this, a scale was set by tracing a line across the neurite length, measured [26] to compare with that of SH-SY5Y cells obtained after differentiation with all trans-retinoic acid (ATRA). The electrophysiological property of cells was analyzed by using the whole-cell patch clamp technique (Axopatch 200B amplifier, Axon Instruments). The dopamine released by the cells was