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Protocol

Therapeutic benefit of TH-engineered mesenchymal stem cells for Parkinson's disease

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Abstract

The present study was designed to assess the potential of marrow stromal cells (MSCs) to deliver therapeutic genes to the brain and result in biologically significant functional recovery. The tyrosine hydroxylase (TH) gene was transfected to MSCs with an adeno-associated virus (AAV) vector. MSCs expressing *TH* gene were transplanted into the striatum of Parkinson's disease (PD) rat. The asymmetric rotation of these models after apomorphine administration was detected every week after transplantation. Six weeks after grafting, animals were sacrificed. Some brains were sectioned to do TH immunohistochemistry. The others were used to detect the dopamine levels by high-performance liquid chromatograph and electrochemical detection (HPLC-ECD). The results showed that MSCs multiply rapidly and formed fibroblast colony-forming units in primary culture. The gene expression efficiency was about 75%. The rounds of asymmetric rotation after apomorphine administration decreased after *TH*-engineered MSCs were grafted. Histological examination showed that *TH* gene was expressed around the transplantation points. The dopamine level in the lesioned striatum of rats injected with TH-MSCs was significantly greater than that in rats treated with LacZ-MSCs ($P < 0.05$). All the data demonstrated that MSCs could readily be genetically engineered. Therefore, MSCs could be useful gene delivery vehicles of gene therapy for Parkinson's disease.

Theme: Disorders of the nervous system
Topic: Degenerative disease: Parkinson's

Keywords: Mesenchymal stem cells; *TH* gene; Parkinson's disease; AAV; Gene transfection

1. Introduction

Accumulating evidence indicates that the differentiation potential of pluripotent stem cells is not restricted to lineages found in the tissue where they originate. For example, embryonic and adult neural stem cells can generate hematopoietic cells [2], and conversely, adult bone marrow stromal cells (BMSCs) can give rise to central nervous system cells [6,15,17,19,20,25]. Direct infusion of human mesenchymal stem cells (MSCs) into the rat striatum results in engraftment and differentiation into astrocytes [1]. Furthermore, MSCs have the capacity to migrate extensively throughout the adult animal. Following intravenous bone marrow transplantation in rodents, MSCs labeled with various methods have been detected in many non-hematopoietic tissues [17]. A study by Dr. Eglitis [7] showed that MSCs preferentially migrate to the site of a brain insult such as acute ischemia in rats. These features render MSCs an attractive candidate as vehicles to deliver therapeutic molecules to the brain in disease states.

Parkinson's disease (PD), a neurological disease suited to gene therapy, is characterized by tremors, rigidity, and akinesia due to the progressive loss of the dopaminergic neurons in the substantia nigra and a severe decrease in the dopamine content of the striatum. In the dopamine synthetic pathway, tyrosine is first hydroxylated and converted to L-dopa by tyrosine hydro-

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xylase (TH), which is the rate-limiting step in dopamine synthesis. One current strategy for gene therapy of PD involves local production of dopamine in the striatum achieved by indu cing the expression of rate-limiting enzymes involved in the biosynthetic pathway for dopamine. Therefore, gene therapy strategies for PD have focused on *TH* gene [5,11,13,21,24]. The present study was designed to assess the potential of marrow stromal cells to deliver therapeutic genes to the brain and result in biologically significant functional recovery.

2. Materials and methods

2.1. Isolation and primary culture of rat MSCs

MSCs were prepared from bone marrow as described previously [25]. Briefly, Sprague–Dawley rats (9 weeks old, male; animals were purchased from the Animal Science Department in Capital University of Medical Sciences) were sacrificed by cervical dislocation. Femurs and tibias were dissected free of soft tissue, and the epiphyses were removed with a rongeur. Femoral and tibial midshaft bone marrow tissue was flushed out into phosphate-buffered saline (pH 7.2). A single cell suspension was obtained by sequentially repeated pipetting. Nucleated cells were isolated with a density gradient (lyamphocytes separation, TBD) and seeded in complete culture medium (α-MEM supplemented with 20% FBS, GIBCO/BRL) with additional 100 units/ml of penicillin / 100 μg/ml of streptomycin at a density of 6×10^7 cells/100 mm tissue culture plate (Falcon). MSCs were incubated at 37 °C with 5% humidified CO_2. After 24 h, non-adherent cells were discarded and adherent cells were thoroughly washed twice with PBS.

2.2. Plasmid construction

pSP72-*TH* and pAAV-MCS (Stratagene) were both digested by restriction endonuclease of *Hin*dIII and *Bgl*II (Takara). The restriction fragment was purified by glass milk (BioDev-Tech). The purified vector and interesting gene were ligated with T4 DNA ligase (Promega) under 16 °C for 18 h. The ligated production was used to transduce the compenent cells XL1-Blue. Cells were selected on medium containing ampicillin. The recombinant plasmid was isolated from the positive clones by alkaline lysis. The orientation and sequence of the insert gene was verified by DNA sequencing. Therefore, *TH* gene was subcloned into the ITR/MCS-containing vectors, pAAV-MCS. The inverted terminal repeat (ITR) sequences present in this vector provide all of the cis-acting elements necessary for AAV-2 replication and packaging.

2.3. AAV mediated TH gene transfection

2.3.1. Transfecting HEK293 cells

Calcium phosphate-based protocol was used to transfect HEK293. HEK293 cells were plated at 3×10^6 per 100-mm tissue culture plate in 10 ml of DMEM growth medium (DMEM supplemented with 10% FBS, GIBCO/BRL) 48 h prior to transfection. The three plasmids to be co-transfected (the recombinant plasmid pAAV-TH or control plasmid pAAV-LacZ, pAAV-RC, and pHelper) were removed from storage at −40 °C. The concentration of each plasmid was adjusted to 1 mg/ml in TE buffer, pH 7.5. 10 μl of each of the three plasmid DNA solutions and 1 ml of 0.3 M $CaCl_2$ were added into a 15-ml conical tube and mixed gently. Then, 1 ml of 2 × HBS was pipetted into a second 15-ml conical tube. The 1.03-ml DNA/$CaCl_2$ mixture was added into the second conical tube dropwise and the solution was mixed gently by inversion or by repeated pipetting. The DNA/$CaCl_2$/HBS suspension was immediately applied to the plate of cells in a dropwise fashion, swirling gently to distribute the DNA suspension evenly in the medium. Then, the tissue culture plate was returned to the 37 °C incubator for 6 h. The suspension was replaced with 10 ml fresh DMEM growth medium and the plate was returned to the 37 °C incubator for an additional 72 h.

2.3.2. Preparing viral stocks

As viral production proceeds, the medium color changes from orange to yellow. Cells will round up and detach from the plate, and can be seen floating in the medium. This is the optimal time to prepare AAV stocks. The transfected cells plus DMEM medium were transfered to a 15-ml conical tube. The cell suspension was subjected to four rounds of freeze/thaw by alternating the tubes between the dry ice–ethanol bath and the 37 °C water bath. Cellular debris were collected by centrifugation at 10,000 × *g* for 10 min at room temperature. The supernatant (primary virus stock) was transferred to a fresh tube. Viral stocks were stored at −80 °C. At the same time, the package efficiency was detected by staining another plate of HEK293 transfected with LacZ.

2.3.3. Viral titer measurement

HT1080 cells were plated at a density of 1.5×10^5 per well in 1 ml of DMEM growth medium in 12-well tissue culture plates and incubated overnight at 37 °C. The medium was removed and cells were washed once with 1 ml of pre-warmed L-DMEM (DMEM supplemented with 2% FBS, GIBCO/BRL). 0.5 ml of each viral stock dilution (10^{-1} to 10^{-12}) was added to separate wells. Cells were incubated at 37 °C for 2 h. Plates were swirled gently at 30-min intervals during the incubation. 0.5 ml of pre-warmed H-DMEM (DMEM supplemented with 18% FBS, GIBCO/BRL) was added for 48 h. Infected cells were detected using β-gal staining kit (Roche). The blue-stained cells were counted and the viral particles (number of stained cells) per ml stock were calculated.

2.3.4. MSC transfection

The protocol is similar to step 3 (Viral titer measurement).