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BRAIN RESEARCH

Research Report

Transplantation of bone marrow stromal cells containing the neurturin gene in rat model of Parkinson's disease

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ABSTRACT

The experiment was to evaluate the therapeutic benefit of transplanted bone marrow stromal cells (BMSCs) transfected with a kind of neurotrophic factor gene, neurturin (NTN) gene, in treating the rat model of Parkinson's disease (PD). The 6-OHDA-lesioned rats were assigned to one of three groups, those receiving BMSCs transfected with NTN gene, those receiving untransfected BMSCs containing a void plasmid and those receiving phosphate buffer solution (PBS). Treatments were injected into the right striatum (6-OHDA-lesioned side). One to six months post-transplantation, apomorphine-induced rotational behavior was observed. One month after transplantation, green fluorescent protein (GFP)/NTN, GFP/glial fibrillary acidic protein (GFAP), GFP/neuron specific enolase (NSE) and GFP/tyrosine hydroxylase (TH) fluorescence determinations of brain sections were carried out. One to six months after transplantation, brain sections containing striatum and substantia nigra were stained for TH. *In situ* hybridization and Western blots were used to determine NTN mRNA and protein concentration, respectively, in affected brain regions. High performance liquid chromatography (HPLC) was used to measure the dopamine (DA) content in the lesioned striatum 1 and 3 month(s) post-transplantation. The results were shown that: in the first 3 months after transplantation, the number of rotations was lower in NTN-transplant group than the void vector group, and during 1–6 months post-transplantation, the number of rotations was lower in both transplant groups than that in the PBS group ($P<0.05$). One month after transplantation, we detected GFP/NTN-, GFP/GFAP- and GFP/NSE-labeled cells in the transplantation area of the NTN-transplanted group, but no obvious GFP/TH labeled cells were found. Quantitative analysis of TH-positive cells 1 to 6 months after transplantation indicated that there were no significant differences between groups in survival rates of TH-positive neurons in the lesioned substantia nigra ($P>0.05$). *In situ* hybridization and Western blot identified NTN mRNA and protein expression in the transplantation area of the NTN-transplanted group. After transplantation of NTN-expressing cells, DA content in the lesioned striatum was significantly higher in the transgenic group than that in the void vector group or the PBS group ($P<0.05$). The overall

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therapeutic effects of the NTN-transplanted group were superior to those of the void plasmid group and the PBS group. The mechanisms by which transgenic therapy treats PD might involve functional enhancement of residual dopaminergic neurons by NTN, which significantly reduces the number of rotations in animals, but not increase the numbers of existing dopaminergic neurons.

1. Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder that emerges when afflicted individuals are in their fifth to seventh decade of life. PD is characterized primarily by a progressive degeneration of dopaminergic neurons in the substantia nigra, a midbrain structure with extensive efferent projection targets. This degeneration significantly reduces striatal dopamine (DA) release from the nigrostriatal pathway, resulting in the hallmark PD "triad" that includes tremor, rigidity and bradykinesia. To date, the most widely used and effective treatment for PD is dopamine replacement therapy via oral supplementation of the DA precursor levodopa. However, long-term application of levodopa can result in severe toxic or debilitating side effects, including symptomatic fluctuation, dyskinesias and/or hallucinations. As the sole known cause of PD symptoms is the degeneration of DA neurons, transplantation of embryonic stem cells into the substantia nigra or striatum presents a rational treatment for PD. However, the availability of this treatment is restrained by several factors, including a limited availability of donors and the difficulty in promoting the long-term survival of transplanted embryonic stem cells. In recent years, research has begun to focus on gene therapy approaches to treat PD, and indeed PD is considered one of the best candidates for gene therapy because of its clear pathogenesis and focused alterations in DA levels. Recent studies have suggested that gene therapy for PD is likely to enter clinical trials in the near future (Eberhardt and Schulz, 2004; Chen et al., 2005). The most suitable candidate genes for PD gene therapy are members of the glial cell-derived neurotrophic factor (GDNF) family. A wide range of studies has demonstrated that GDNF is neuroprotective for the dopaminergic neurons in substantia nigra. NTN is a neurotrophic factor with actions similar to GDNF, and which exerts trophic, supportive, protective and repairing influences on dopaminergic neurons, thus being a potential therapeutic alternative for PD. Of the known trophic factors, GDNF and Neurturin (NTN) exhibit the most potent neurotrophic activity at dopaminergic neurons (Akerud et al., 1999; Horger et al., 1998; Oiwa et al., 2002). Nevertheless, previous studies have indicated that the therapeutic effect of direct injection of NTN into the substantia nigra in animal models of PD lasts less than 1 month. It is therefore imperative to increase the duration of the therapeutic effects of NTN in clinical applications of gene therapy. This may be accomplished by altering the vectors and cells through which the NTN gene is delivered.

The AdEasy adenovirus vector system (Ad) and bone marrow stromal cells (BMSCs) are widely considered to be promising vectors and carrying cells for gene therapy. The AdEasy adenovirus vector system can efficiently integrate exogenous genes into a wide spectrum of host genomes, without resulting in adjunct infection. This vector system can contain gene segments longer than 7.5 kb, and infect mammalian cells in either division or non-division phases, and thus is widely used in clinical gene therapy studies (He et al., 1998; Russell, 2000). BMSCs are non-hemopoietic stem cells found in adult mammals or in human bone marrow, which possess many stem cell properties and, under certain conditions, can be induced to differentiate into neuron- and glia-like cells. Like neural stem cells, they can migrate and integrate into the brain. Studies in animal models have revealed that BMSCs may present a promising vector for cellular gene engineering treatments for the neurological diseases (Sanchez-Ramos et al., 2000; Dezawa et al., 2004). In addition, the use of BMSCs avoids several problems plaguing studies with neural stem cells, including ethical issues, limited donor sources, and immunological rejection.

In the present study, we transfected primary rat BMSC cultures with recombinant adenovirus containing the NTN gene in order to obtain BMSCs stably expressing the NTN gene. For the first time to our knowledge, PD rat models were treated with transplanted BMSCs carrying the NTN gene and tested on rotational behavior to evaluate efficacy, and the mechanism involved in the treatment was also studied.

2. Results

2.1. *Identification of the expression vector Ad-NTN and the expression of Ad-NTN in BMSC*

Recombinant adenoviral plasmid DNA and pAdEasy-1 viral plasmid were identified by *Hin*dIII cleavage, and a specific 7 kb band was found in the former following separation by agarose gel electrophoresis. After digestion of recombinant adenoviral plasmids with PacI, a 4.5 kb specific fragment was obtained, but no fragment was obtained following PacI digestion of pAdEasy-1, thus confirming recombinant adenoviral plasmids (Figs. 1, 2). After plasmid identification, the gels were recovered and DNA sequencing was performed providing the predicted sequence which was then named Ad-NTN.

2.2. *Transfection of HEK293 cells*

Three days following the transfection of 293 cells by Ad-NTN, cell confluence was observed under a light microscope. Some cells exhibited a round morphology, indicative of a cytopathic effect (CPE). On day 7, cells showing CPE and their medium supernatants were harvested by repeated freeze-thawing to prepare a stock virus solution. One half of the virus stock solution was sufficient to infect the 293 cells, and the infection was easily reproduced, suggesting that the infective particles reduplicated successfully.