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BRAIN RESEARCH

Research Report

Combined treatment of neurotrophin-3 gene and neural stem cells is ameliorative to behavior recovery of Parkinson's disease rat model

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ABSTRACT

Neural stem cell transplantation therapy was developed for replacing lost or damaged neural cells for the neurodegenerative disease, including Parkinson's disease (PD), in which dopaminergic neuron cells are lost. The growth factor, neurotrophin-3(NT-3), has been shown to promote neuroregeneration, differentiation and migration during brain development. In this report, we construct rat neural stem cells that express neurotrophin-3 endogenously (rNSC-NT3) and transplant them into 6-hydroxydopamine (6-OHDA)-treated Parkinsonian rats. Molecular approaches including quantitative real time PCR, Western blot and immunocytochemistry were used to identify the expression of NT-3 and the differentiation of planted cells. Behavioral recover was also tested. The result indicated that combined treatment of neurotrophin-3 gene and neural stem cells had a functional impact on reversing the main symptoms of the Parkinson's disease that significantly reduced apomorphine-induced rotational asymmetry and improved spatial learning ability. The rNSCs-NT3 is able to differentiate into dompaminergic neuron in the ventral tegmental area (VTA) and the medial forebrain bundle (MFB), and migrated around the lesion site. Endogenous expressed NT-3 exerts induction and trophic effects on neural stem cells. The rNSCs-NT3 showed higher activity than the rNSCs in regenerating tyrosine hydroxylase positive cell numbers and migrating distance, behavior improving in this dopa-deficit rat model. These findings suggest that the neural stem cells expressed NT-3 endogenously would be a better graft candidate for the treatment of Parkinson's disease.

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disease, which is the consequence of loss of the dopaminergic neurons in substantial nigral, leading to an over 80% depletion of dopamine (DA) in the striatum. It afflicts about 3% of people over 65 years (Haeri et al., 2005; Mayhall et al., 2004; Moore et al., 2005; Petroske et al., 2001). The incidence of Parkinson's disease (PD) increases in age with a prevalence of 0.1% of the global population. The rising proportion of PD patients will increase the healthcare burden to our society and calls for accelerated efforts to understand this disease better and treat it more effectively and to halt the progression of PD (Fiandaca et al., 2008; Wang et al., 2007).

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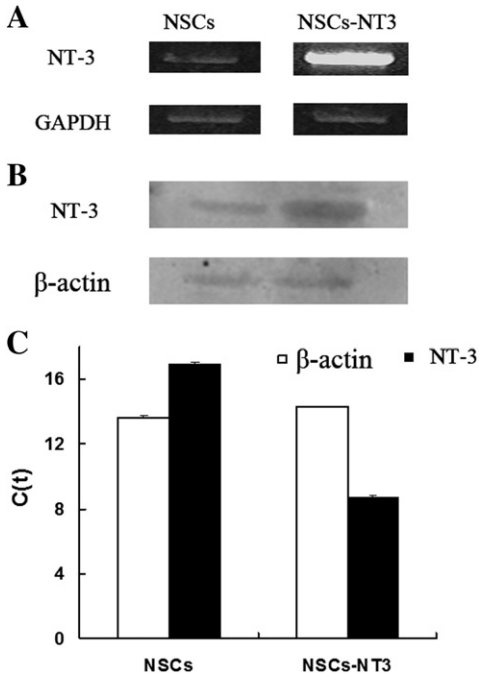

Fig. 1 – Exotic NT3 expression in NSCs. The expression of NT3 was evaluated through RT-PCR (A), Western blot (B) and realtime PCR (C). It is obvious that NT3 has a high expression in NSCs-NT3 at the transcriptional level. A significant difference was observed (C). Protein expression was also further detected by Western blot experiment and was indicated an increase expression (B).

The transplantation of new, exogenously generated neural cells in the central nervous system, is extensively studied with the initial experimental clinical studies in PD and is thought to be a prospective treatment for this disease (Bjorklund et al., 2002; Ebert et al., 2008; Mayhall et al., 2004; Zhang et al., 2007). It aims to replace the lost dopaminergic neuron in substantial nigral and to reconstruct the damaged circuitries to reverse, at least in part, the major symptom of PD. Neural stem cells (NSCs) have the potential of self-renewal and can only differentiate into three lineages of in the nerve system, namely neurons, astrocytes and oligodendrocytes. These properties make NSCs an attractive and presumably unlimited donor source for cell replacement therapy to treat neurological disorders.

NT3 is one of the neurotrophin family of secreted growth factors, which plays pivotal roles in the nervous system (Snider, 1994; Tessarollo, 1998). Neurotrophic factors are interesting candidates for neuroprotective therapy (Lu and Tuszynski, 2008), comprising nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT3) and neurotrophin 4/5 (NT4/5) (Bothwell, 1995; Lundblad et al., 2002, 2004). Neurotrophin-3 showed positive functions in promoting neuronal survival, differentiation and neurite growth (Coppola et al., 2001; Zhou et al., 2003). However, growth factors cannot cross the blood–brain barrier the administration route should be critically considered (Lundblad et al., 2002, 2004).

The aim of the present study was to determine that whether transplanted NSCs genetically modified to express endogenously NT-3 would promote NSCs survival, selectively directed towards dompaminergic neurons and gain functional recovery in PD rat model. We prepared NSCs isolated from neonatal rat hippocampus (Yu et al., 2007) and transfected with plasmid that expressed neurotrophin-3 (NT-3) endogenously, then transplanted into the rats that have treated with 6-OHDA at striatal (Cenci et al., 1998; Ungerstedt, 1971). The results obtained demonstrated that injection of NSCs with NT3 is capable of significantly reducing apomorphine-induced rotational asymmetry, improving spatial learning ability, and protecting dopamine neurons in the substantia nigra, when compared to untransfected NSCs nor a sham transplant. These findings suggest NT-3 may be a very interesting growth factor to explore further in animal models and might have clinical application in the treatment of PD in future.

2. Results

2.1. *Exotic NT-3 effective expression in NSCs*

The NT-3 cDNA was amplified by PCR and subcloned into the plasmid pEGFP-C1 to create an expression vector pEGFP-C1-NT3. pEGFP-C1-NT3 was transfected into rat neonatal NSCs by Lipofectamine 2000. The efficiency of the transfection was about 40%–50% by estimating the green fluorescent light in cells after 2 days.

To identify the NT-3 expression in NSCs-NT3, total RNA was extracted from NSCs and NSCs-NT3 after transfection for 2 days and analyzed using quantitative real-time PCR (Fig. 1C) and RT-PCR (Fig. 1A), an amount of NT-3 mRNA was increased by 481 times in NSCs-NT3 than in NSCs (Fig. 1B). The expression augment of the NT-3 in NSCs-NT3 was further confirmed to have an increase expression by Western blot (Fig. 1B).

2.2. *NSCs-NT3 inclines to differentiating into dopaminergic neurons in vitro*

To examine the effect of the endogenous expression of NT-3 on the differentiation of neural stem cell *in vitro*, cells were cultured in DMEM/F12, plus EGF, bFGF and B27 additives. The markers of neuron (NF) and astrocyte (GFAP) were checked by immunochemistry method. An increase cell numbers of NF positive in NSCs-NT3 was observed after 7 days (Fig. 2G) comparing with the control NSCs (Fig. 2C), and more GFAP positive cells were showed in NSCs group (Figs. 2B, F). The proportion of NF positive cells appearing is 39.9%±12.38% in NSCs-NT3 while 17.48%±3.48% in NSCs by calculation ($p<0.01$, Fig. 2I).

Furthermore, differentiated NSCs-NT3 were validated to be dopaminergic neurons staining with TH (Fig. 3D) in contrast to that of NSCs (Fig. 3B). Very strong TH activity was showed in NSCs-NT3 (Figs. 3F, G, I, J).

Through RT-PCR analysis, the expression of NT3 in NSCs-NT3 was obviously up-regulated in contrast to that in NSCs after transfection for 7 days *in vitro* (Fig. 3K). TH was also