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Research Report

In vitro non-viral lipofectamine delivery of the gene for glial cell line-derived neurotrophic factor to human umbilical cord blood CD34+ cells

Guolong Yu[a,*], *Cesar V. Borlongan*[b,*], *Yali Ou*[a], *Christine E. Stahl*[c], *SeongJin Yu*[b], *EungKyung Bae*[b], *Yuji Kaneko*[b], *Tianlun Yang*[a], *Chunjun Yuan*[a], *Li Fang*[a]

[a]*Department of Cardiology, Xiangya Hospital, Southern Central University, Changsha, 410008, China*
[b]*Department of Neurosurgery and Brain Repair, University of South Florida College of Medicine, Tampa, FL 33612, USA*
[c]*Department of Flight Medicine, MacDill Air Force Base, Tampa, FL 33611, USA*

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ABSTRACT

Using a lipofection technique, we explored a non-viral delivery of plasmid DNA encoding a rat pGDNF (glial cell line-derived neurotrophic factor) to CD34+ cells derived from human umbilical cord blood (HUCB) cells in order to obtain cells stably expressing the GDNF gene. The target gene GDNF was amplified from cortex cells of newborn Sprague–Dawley rats by reverse transcriptase polymerase chain reaction (RT-PCR) and inserted into vector pEGFP-N1 to construct the eukaryotic expression vector pEGFP/GDNF. The positive clones were identified by sequencing and endonuclease digestion. The expression of pEGFP/GDNF-transfected HUCB cells CD34+ was examined by ELISA. Single fragment of 640 bp was obtained after the rat GDNF cDNA was amplified by RT-PCR. Two fragments of about 4.3 kb and 640 pb were obtained after digestion of recombinant plasmid pEGFP/GDNF with XhoI/KpnI. The nucleic acid fragment of 640 bp was confirmed to agree well with the sequence of GDNF gene published by GenBank. The expression of GDNF mRNA and the level of GDNF from pEGFP/GDNF-transfected CD34+ cells were increased substantially, compared with pEGFP control plasmid transfected CD34+ cells ($P<0.05$). Moreover, co-culture of primary rat cells with the pEGFP/GDNF-transfected CD34+ cells promoted enhanced neuroprotection against oxygen-glucose deprivation induced cell dysfunctions. The present results support the use of the non-viral plasmid liposome for therapeutic gene expression for stem cell therapy.

1. Introduction

Transplantation of stem cells has been proposed as a means of treating stroke. Stem or progenitor cells derived from human umbilical cord blood (HUCB) can be differentiated into all the major cellular phenotypes of the brain including neurons, oligodendrocytes, and glial cells (Sanchez-Ramos et al., 2001; Ha et al., 2001; Bicknese et al., 2002; Buzanska et al., 2002), but also secrete multiple growth factors (Borlongan et al., 2004a). These multipotent features of HUCB cells make them a potential source of transplantable cells capable of repairing the damaged brain, including stroke.

* *Corresponding authors*. Fax: +1 813 974 6352.
E-mail addresses: yuguolong123@yahoo.com.cn (G. Yu), cborlong@health.usf.edu (C.V. Borlongan).

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The growing evidence from studies on HUCB cells following either systemic or intracerebral transplantation suggests that these cells promote behavioral recovery in preclinical stroke (Borlongan et al., 2004b; Chen et al., 2001; Willing et al., 2000; Taguchi et al., 2004; Vendrame et al., 2004; Xiao et al., 2005; Boltze et al., 2005; Nystedt et al., 2006). However, histological analysis revealed only a few implanted cells that remain viable and retain their capabilities to different into functional neurons, which may not be sufficient for therapeutic purposes (Yu et al., 2009). Neurotrophic factors such as GDNF, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF) are likely to contribute to this neuroprotective mechanism during the early period after transplantation of stem cells in the ischemic brain (Borlongan et al., 2004a, Borlongan et al., 2004b; Horita et al., 2006; Nomura et al., 2005; Pisati et al., 2007; Zhu et al., 2005; Yasuhara et al., 2006; Cairns and Finklestein, 2003).

The participation of trophic factors in stroke recovery is further demonstrated by the observation that intravenous administration of stem cells increased the levels of trophic factors including VEGF, FGF, GDNF, NGF and BDNF in the ischemic brain (Borlongan et al., 2004a,b; Taguchi et al., 2004). This set of data suggests that tropic factors secreted by transplanted HUCB cells play a direct role in the reduction of stroke-induced histological and behavioral deficits.

Recently, a number of reports have shown that neural stem cells or bone marrow cells even taken from adults can be used as effective vehicles for therapeutic gene transfer to the brain with many advantages, including long-term activity and very mild host immune response (Nomura et al., 2005; Ali and Saver, 2007; Leng et al., 2008). GDNF engineered autologous marrow cells transplanted intravenously have been proven to produce more nigral neurons and greater striatal terminal density because marrow stromal cells have the ability to migrate from the periphery to the CNS, but also to localize preferentially at the site of brain injury in chronic inflammatory conditions (Park et al., 2001). Transplantation of adult neural stem and progenitor cells (NSPC)-GDNF cells appears to have suppressed microglial activation and host neuronal cell death in the ischemic boundary zone, and reduced infarct volume after middle cerebral artery occlusion (MCAo) in rats (Kameda et al., 2007). Based on this trophic factor-mediated action of grafted cells, these transplanted cells should be a means of supplying neurotrophic factors to the damaged CNS tissue. We thus envisioned that if the grafted cells can be transfected with genes of neurotrophic factors that are known to have neuroprotective effects, then such strategy should render the cells to stably secrete higher amounts of the therapeutic proteins, thereby affording improved neuroprotection.

To determine whether this is feasible, we used a lipofection technique via non-viral delivery of plasmid DNA encoding a rat pGDNF to CD34+ cells derived from HUCB in order to generate cells with enhanced expression of the GDNF gene and secretion of GDNF protein. The long-term aim is to improve the localized overexpression of GDNF, ultimately for reparative therapy in brain disorders.

2. Results

2.1. *Rat GDNF cDNA cloned*

The extraction from rats' fetal brain tissues with TRIzol reagent yielded total RNA in which ratio of A260 and A280 was 2.0. As shown in Fig. 1A, the total RNA was subjected to agarose electrophoresis to obtain 28S and 18S ribosome RNA, which suggested that the extracted RNA was not digested. Based on the full-length cDNA sequences of GDNF in GenBank, upstream and downstream primers were designed by primer software 5.0 to synthesize the rat GDNF and with its cDNA amplified by RT-PCR. Electrophoretic mobility shift assays obtained GDNF cDNA fragment with a molecular weight of 640 bp (Fig. 1B).

2.2. *Construction and identification of recombinant plasmid pEGFP/GDN*

The PCR product and pEGFP vectors were digested by both XhoI and KpnI, and then the DNA fragments were ligated into plasmid pEGFP with T4 ligase. The candidate recombinant plasmids (pEGFP/GDNF) were transfected into E.coliDH5a, which were extracted, and then digested with both XhoI and KpnI (Fig. 1C). Electrophoresis analysis showed two bands of 640 bp and 4.3 kb (Fig. 1D), which suggested that the target fragment was inserted into plasmid pEGFP correctly. In addition, the generated recombinant plasmids (pEGFP/GDNF) displayed the same sequence as t2PAcDNA in GenBank (Fig. 1E), indicating that the eukaryotic expression plasmid of pEGFP/GDNF was constructed successfully.

2.3. *Identification of isolated HUCB CD34+ cells by flow cytometry*

The majority of cells extracted from HUCB were mononuclear (Fig. 2A). Flow cytometry demonstrated that less than 1% of cells expressed CD34+ before the mononuclear cells were sorted (Fig. 2B), which was dramatically increased to over 95% after anti-CD34+ antibody sorting (Fig. 2C).

2.4. *Expression of GDNF mRNA and GDNF protein in transfected HUCB CD34+ cell*

Total RNAs were extracted from HUCB CD34+ cells transfected by the plasmid pEGFP/GDNF or pEGFP, with both amplified

Fig. 1 – Panels show total RNA examined with gel electrophoresis, which was performed in triplicates (A), GDNF cDNA amplified by PCR then examined with gel electrophoresis in duplicates (B) mass spectrogram of recombinant plasmid pEGFP-GDNF (C), double enzyme-digestion identification of recombinant plasmid pEGFP-GDNF (D), and the single fragment of 640 bp obtained after RT-PCR amplification of the rat GDNF cDNA (E), which reveals its sequence closely resembling the GDNF gene published by GenBank.