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Overexpression of tropomyosin receptor kinase A improves the survival and Schwann-like cell differentiation of bone marrow stromal stem cells in nerve grafts for bridging rat sciatic nerve defects

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Abstract

Background aims. Bone marrow stromal stem cells (BMSCs) can differentiate into Schwann-like cells *in vivo* and effectively promote nerve regeneration and functional recovery as the seed cells for peripheral nerve repair. However, the survival rate and neural differentiation rate of the transplanted BMSCs are very low, which would limit their efficacy. *Methods.* In this work, rat BMSCs were infected by recombinant lentiviruses to construct tropomyosin receptor kinase A (TrkA)-overexpressing BMSCs and TrkA-shRNA-expressing BMSCs, which were then used in transplantation for rat sciatic nerve defects. *Results.* We showed that lentivirus-mediated overexpression of TrkA in BMSCs can promote cell survival and protect against serum-starve-induced apoptosis *in vitro*. At 8 weeks after transplantation, the Schwann-like differentiated ratio of the existing implanted cells had reached 74.8 ± 1.6% in TrkA-overexpressing BMSCs-laden nerve grafts, while 40.7 ± 2.3% and 42.3 ± 1.5% in vector and control BMSCs-laden nerve grafts, but only 8.2 ± 1.8% in TrkA-shRNA-expressing BMSCs-laden nerve grafts. The cell apoptosis ratio of the existing implanted cells in TrkA-overexpressing BMSCs-laden nerve grafts was 16.5 ± 1.2%, while 33.9 ± 1.9% and 42.6 ± 2.9% in vector and control BMSCs-laden nerve grafts, but 87.2 ± 2.5% in TrkA-shRNA-expressing BMSCs-laden nerve grafts. *Conclusions.* These results demonstrate that TrkA overexpression can improve the survival and Schwann-like cell differentiation of BMSCs and prevent cell death in nerve grafts, which may have potential implication in advancing cell transplantation for peripheral nerve repair.

Key Words: *BMSCs, nerve transplantation, Schwann-like cell differentiation, shRNA interfere, survival, TrkA overexpression*

Introduction

Bone marrow stromal stem cells (BMSCs) have become one of the most promising seed cells for peripheral nerve regeneration because of their accessibility, quick proliferation, weak immunogenicity and neural differentiation plasticity [1]. Considerable experimental studies have demonstrated the beneficial effect of BMSCs in regeneration and repair of peripheral nerve injuries [2–8]. Although the mechanism is still unclear, it should include at least two aspects: (i) BMSCs constitutively express trophic factors and supporting substances, such as nerve growth factor (NGF), brain-derived neurotrophic factor, glial cell line-derived neurotrophic factor, ciliary neurotrophic factor, collagen, fibronectin and laminin [3]. *In vivo* neurotrophic microenvironment facilitates repair of the injured nerve [2]. A portion of transplanted BMSCs differentiate into Schwann-like cells, which then promote peripheral nerve regeneration and functional recovery. However, the survival rate and Schwann-like cell differentiation rate of the transplanted BMSCs in nerve grafts are very low, which would limit their efficacy [2,4,9–11].

NGF and its specific high-affinity transmembrane receptor tropomyosin receptor kinase A (TrkA) signaling pathway plays an important role in neuronal survival and differentiation, axonal and dendritic

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growth and remodeling, synapse formation and function, and nerve regeneration [12]. Paradoxically, it has also been shown that TrkA could induce cell death in certain cell types [13]. NGF induces dramatic apoptosis in both TrkA transfected medulloblastoma cells and TrkA transfected PC12 cells, which could be blocked by anti-NGF antibodies or by K252a, an inhibitor of TrkA tyrosine phosphorylation [14–16]. Similarly, overexpression of TrkA followed by exposure to NGF induces p53-dependent apoptosis in neuroblastoma cells [17]. However, this TrkA-mediated apoptosis should be cell type–specific, because overexpression of TrkA in both the NIH-3T3 and $PC12^{nnr5}$ cells did not lead to apoptosis after treatment with NGF [15]. Furthermore, TrkA overexpression in newborn sympathetic neurons could enhance NGF-independent and -dependent survival [17]. TrkA therefore appears to have dual roles, either in promoting cell survival or in facilitating cell death in specific contexts [18].

Previous studies have shown that NGF was expressed in undifferentiated as well as neurally induced BMSCs, but the expression of TrkA was restricted to neurally differentiated BMSCs [19,20]. Compared with normal and neurally induced BMSCs, self-differentiating BMSCs expressed significantly higher levels of NGF and TrkA [21]. NGF expression was also increased in nerve grafts [3,9], and NGF-containing polymeric microspheres have been used to enhance peripheral nerve regeneration [22–24]. However, it is unclear whether NGF/TrkA signaling pathway regulates the survival, differentiation or apoptosis of BMSCs in nerve grafts. To answer this question, rat BMSCs were infected by recombinant lentiviruses to construct TrkA-overexpressing BMSCs and TrkA-shRNA expressing BMSCs, which were then used in transplantation for rat sciatic nerve defects in this study. The survival rate and Schwann-like cell differentiation efficiency of the transplanted BMSCs in nerve grafts were evaluated.

Methods

Animals

Forty adult male Sprague-Dawley (SD) rats weighing 200–250 g were used in the study. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Shenzhen University. The animals were housed in a temperature and humidity controlled room with a 12:12 light/dark cycle and *ad libitum* access to food and water.

Isolation, culture and identification of BMSCs

Adult male SD rats (200–250 g) were sacrificed by an intraperitoneal overdose injection of sodium pentobarbital (10 mg/100 g body weight, Sigma). Bilateral femurs and tibias were removed, and the marrow was flushed with 10 mL of Dulbecco's Modified Eagle's Medium (DMEM, Gibco) containing 10% fetal bovine serum (FBS, Hyclone), 100 U/mL penicillin and 100 μg/mL streptomycin (Hyclone). Whole marrow cells were extracted and cultured at a density of 2×10^5 cells/cm^2 in the medium on 100-mm plastic dishes. The cells were incubated at 37°C, 5% CO_2 under fully humidified condition, and the nonadherent cells were removed after 48 h by changing the medium. Thereafter, the fresh medium was replaced every 3 days. The adherent cells were labeled passage 0 (P0). When the cells reached 80–90% confluence, the cultures were treated with 0.25% trypsin-ethylenediaminetetraacetic acid (EDTA; Gibco), then harvested and diluted to 1:3 per passage for the further expansion. P3 cells were used for all experiments.

BMSCs at P3 were characterized by evaluation of cell marker profile. Cells in 10-mm plastic dishes were harvested using 0.25% trypsin-EDTA. The cell suspension was washed with 10 mmol/L phosphate-buffered solution (PBS) three times, and 1×10^5 cells/400 μL were aliquoted into flow cytometry tubes. The cells were incubated with fluorescence-labeled monoclonal antibodies against rat: CD29-FITC, CD90-FITC, CD45-FITC (eBioscience) and CD34-FITC (Santa Cruz) for 30 min at 4°C. After three washes, the labeled cells were resuspended in 300 μL PBS and analyzed by flow cytometry using FACS Calibur (BD Biosciences). The data were analyzed using Cell Quest software (BD Biosciences).

Lentivirus production and infection into BMSCs

The lentivectors encoding rat TrkA cDNA (Sino Biological), shRNA targeting rat TrkA at nucleotides 403 (TrkA-shRNA: 5′-AUUCAGGUGACUGAGCCGAGGG-3′) [25] and their respective controls were purchased from Hanbio. Lentiviruses were produced by co-transfecting these lentivectors individually with the packaging plasmids psPAX2 and pMD2G into 293T cells using Lipofiter (Hanbio).

For lentiviral infection, the culture medium was removed when the BMSCs were at 60% confluence in six-well dishes (5×10^5 per well). The cells were washed gently with PBS and treated with the lentivirus-containing medium (multiplicity of infection [MOI] = 15) combined with Polybrene (5 μg/mL, Hanbio). After 24 h, the culture medium was replaced with fresh medium. After 48 h of infection, puromycin (Sigma) was added to the medium at a concentration of 2 μg/mL for stable cell lines selection. The normal uninfected BMSCs were used as negative control. Stable infected cells were obtained after 3 weeks of antibiotic selection. After harvesting these