Cytotherapy, 2017; 19: 916–926

International Society for Cellular Therapy
ISCT®

Transplantation of bone marrow stromal stem cells overexpressing tropomyosin receptor kinase A for peripheral nerve repair

MEIGE ZHENG[1,2,★], JUNXIU DUAN[2,★], ZHENDAN HE[2], ZHIWEI WANG[3], SHUHUA MU[4], ZHIWEN ZENG[2], JUNLE QU[1], DONG WANG[5] & JIAN ZHANG[2]

[1]Key Laboratory of Optoelectronic Devices and Systems of Ministry of Education and Guangdong Province, College of Optoelectronic Engineering, Shenzhen University, Shenzhen, China, [2]School of Medicine, Shenzhen University, Shenzhen, China, [3]Department of Neurology, Shenzhen Shekou people's Hospital, Shenzhen, China, [4]Psychology & Social College of Shenzhen University, Shenzhen, China, and [5]Department of Orthopedic and Microsurgery, The First Affiliated Hospital of Sun Yat-Sen University, No. 58 Zhongshan Road 2, Guangzhou, China

Abstract

Background aims. Previously we reported that overexpression of tropomyosin receptor kinase A (TrkA) could improve the survival and Schwann-like cell differentiation of bone marrow stromal stem cells (BMSCs) in nerve grafts for bridging rat sciatic nerve defects. The aim of this study was to investigate how TrkA affects the efficacy of BMSCs transplantation on peripheral nerve regeneration and functional recovery. *Methods.* Rat BMSCs were infected with recombinant lentiviruses to construct TrkA-overexpressing BMSCs and TrkA-shRNA-expressing BMSCs, which were then seeded in acellular nerve allografts for bridging 10-mm rat sciatic nerve defects. *Results.* At 8 weeks post-transplantation, compared with Vector and Control BMSCs-laden groups, TrkA-overexpressing BMSCs-laden group demonstrated obviously improved axon growth, such as significantly higher expression of myelin basic protein and superior results of myelinated fiber density, axon diameter and myelin sheaths thickness. In accordance with this increased nerve regeneration, the animals of TrkA-overexpressing BMSCs-laden group showed significantly better restoration of sciatic nerve function, manifested as greater sciatic function index value and superior electrophysiological parameters including shorter onset latency and higher peak amplitude of compound motor action potentials and faster nerve conduction velocity. However, these beneficial effects could be reversed in TrkA-shRNA-expressing BMSCs-laden group, which showed much fewer and smaller axons with thinner myelin sheaths and correspondingly poor functional recovery. *Conclusions.* These results demonstrated that TrkA may regulate the regenerative potential of BMSCs in nerve grafts, and TrkA overexpression can enhance the efficacy of BMSCs on peripheral nerve regeneration and functional recovery, which may help establish novel strategies for repairing peripheral nerve injuries.

Key Words: *bone marrow stromal stem cells, lentiviral vector, peripheral nerve repair, shRNA interference, transplantation, tropomyosin receptor kinase A*

Introduction

Repair of peripheral nerve injury remains a major clinical challenge. Stem cell–based therapy is emerging as a novel treatment strategy for peripheral nerve regeneration. Bone marrow stromal stem cells (BMSCs) have been considered the ideal candidate seed cell source for repairing nerve injuries because of their easy accessibility, rapid proliferation, weak immunogenicity, production of trophic factors and neural cell differentiation plasticity [1]. Indeed, a considerable number of studies have revealed that BMSCs transplantation could effectively promote peripheral nerve regeneration and functional recovery [2–8]. However, several problems of the transplanted stem cells remain to be solved: their vitality, homing and low survival and differentiation rates, which would significantly hinder their clinical application [2,4,6,9,10].

Nerve growth factor (NGF) and its high affinity receptor tropomyosin receptor kinase A (TrkA) play a crucial role in neuronal survival and differentiation, axonal and dendritic growth and remodeling, and nerve regeneration [11]. The main signaling cascades required for axon elongation and branching during nerve

★These two authors contributed equally to this work.
Correspondence: **Jian Zhang**, MD, School of Medicine, Shenzhen University, Nanhai Ave 3688, Shenzhen 518060, China. E-mail: jzhanghappy@163.com;
Correspondence: **Dong Wang**, MD, Department of Orthopedic and Microsurgery, The First Affiliated Hospital of Sun Yat-Sen University, No. 58 Zhongshan Road 2, Guangzhou 510080, China. E-mail: david74429@126.com

(Received 15 December 2016; accepted 24 April 2017)

ISSN 1465-3249
http://dx.doi.org/10.1016/j.jcyt.2017.04.007

regeneration are the Ras/extracellular signal regulated kinase (ERK) and the phosphatidylinositol-3 kinase (PI3K)/Akt pathways, which could be activated by receptor TrkA upon NGF binding [12,13]. It has been reported that the expression levels of NGF and TrkA were significantly elevated in the process of BMSCs differentiating into neurons [14–16]. We propose that TrkA may regulate the beneficial effects of BMSCs on the repair of peripheral nerve injuries. To address this question, lentiviral vector mediated TrkA-overexpressing BMSCs and TrkA-shRNA-expressing BMSCs were constructed and then used in transplantation for rat sciatic nerve defects. Our previous studies have shown that overexpression of TrkA can promote the survival and Schwann-like cell differentiation of BMSCs and prevent cell death in nerve grafts [17]. In this study, the efficacy of the infected BMSCs on peripheral nerve regeneration and functional recovery was systematically evaluated using histological, electrophysiological and functional analyses.

Methods

Animals

Forty-two adult male Sprague-Dawley (SD) rats, weight 200–250 g, were used in the study. The animals were housed in a temperature- and humidity-controlled room with a 12:12 light:dark cycle and fed regular diet *ad libitum*. All experiments procedures were approved by the Institutional Animal Care and Use Committee of Shenzhen University. All surgical procedures were performed under general anesthesia via the weight-appropriate dose intraperitoneal injection of 2% pentobarbital sodium (0.4 mL/100 g body weight).

Preparation of TrkA-overexpressing BMSCs and TrkA-shRNA-expressing BMSCs

BMSCs were isolated from rat bone marrow, expanded, and identified as described previously [17]. The cells were cultured in low glucose Dulbecco's Modified Eagle's Medium (Gibco) containing 10% fetal bovine serum (Hyclone), 100 U/mL penicillin and 100 μg/mL streptomycin (Hyclone) at 37°C, 5% CO_2 under fully humidified condition. BMSCs at passage 3 were used for the subsequent experiments.

The lentivirus encoding rat TrkA cDNA (Sino Biological) and its empty lentiviral vector (pHBLV-CMVIE-T2A-Puro), the lentivirus encoding shRNA targeting rat TrkA at nucleotides 403 (TrkA-shRNA: 5′-AUUCAGGUGACUGAGCCGAGGG-3′) and its empty lentiviral control (pHBLV-U6-T2A-Puro) were produced by co-transfecting these lentiviruses individually with the packaging plasmids psPAX2 and pMD2G into 293T cells using Lipofiter (Hanbio). BMSCs were infected with the lentiviruses as the following procedures to obtain stable infected BMSCs including TrkA-overexpressing BMSCs (Over-TrkA BMSCs), TrkA-shRNA-expressing BMSCs (TrkA-shRNA BMSCs) and their respective empty vector infected controls (Vector BMSCs and Control BMSCs). Briefly, for lentiviral infection, the culture medium was removed when BMSCs reached nearly 60% confluence in six-well dishes (5×10^5 per well). After washed with 10 mmol/L phosphate-buffered solution (PBS), the cells were treated with the lentivirus-containing medium (multiplicity of infection = 15) combined with polybrene (5 μg/mL, Hanbio). After 24 h, the culture medium was discarded and replaced with fresh medium. After 48 h of infection, puromycin (Sigma) was added to the culture medium at a final concentration of 2 μg/mL for selecting the stable cell lines. Uninfected BMSCs were used as negative control. After antibiotic selection for 3 weeks, stable infected cells were obtained. The effects of TrkA and TrkA-shRNA transduction were detected as previously reported [17].

Preparation of the allogenic acellular nerves

Twelve adult male SD rats were used to prepare the allogenic acellular nerves. Under sterile conditions, bilateral sciatic nerves of anesthetized SD rats were exposed and 15-mm-long nerve segments were excised. Adipose and connective tissues on the surface of the nerves were removed. Upon harvest, the nerves were immediately placed in deionized distilled water (ddH_2O) and subjected to chemical acellularization as previously described [5,18]. Briefly, the nerve tissues were agitated in ddH_2O for 7 h and then exposed to 3% TritonX-100 (Sigma) in ddH_2O and digested for 12 h. After being rinsed in PBS for 10 min ×3, the nerve tissues were agitated in 4% sodium deoxycholate (Sigma) in ddH_2O for 24 h. These procedures were repeated once, followed by three final washes in PBS for 6 h. All the treatments were carried out at room temperature. Finally, the treated nerves were stored in PBS containing 100 U/mL penicillin and 100 μg/mL streptomycin at 4°C until use.

Hematoxylin and eosin staining was used to evaluate the effects of the chemical extraction treatments on the nerves. As shown in Figure 1, there was no evidence of visible cells, myelin sheaths or axons in the representative acellular nerve sections, and the basal lamina tubes remained structurally intact, demonstrating the high efficacy and specificity of the chemical extraction method.

In vitro construction of tissue-engineered nerves

The stable infected BMSCs were used for seeding in allogenic acellular nerves to construct the tissue-engineered nerve grafts. Single cell suspension was