RESEARCH ARTICLE

TrkA regulates the regenerative capacity of bone marrow stromal stem cells in nerve grafts

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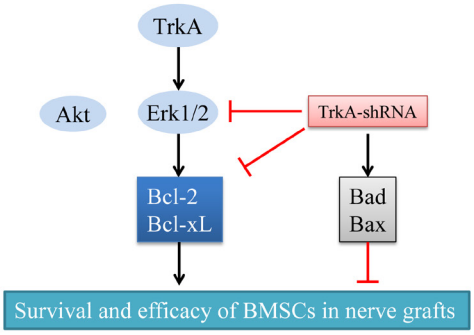
Graphical Abstract

TrkA regulates the regenerative capacity of bone marrow stromal stem cells (BMSCs) in nerve grafts *via* Erk/Bcl-2 pathway



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Abstract

We previously demonstrated that overexpression of tropomyosin receptor kinase A (TrkA) promotes the survival and Schwann cell-like differentiation of bone marrow stromal stem cells in nerve grafts, thereby enhancing the regeneration and functional recovery of the peripheral nerve. In the present study, we investigated the molecular mechanisms underlying the neuroprotective effects of TrkA in bone marrow stromal stem cells seeded into nerve grafts. Bone marrow stromal stem cells from Sprague-Dawley rats were infected with recombinant lentivirus vector expressing rat TrkA, TrkA-shRNA or the respective control. The cells were then seeded into allogeneic rat acellular nerve allografts for bridging a 1-cm right sciatic nerve defect. Then, 8 weeks after surgery, hematoxylin and eosin staining showed that compared with the control groups, the cells and fibers in the TrkA overexpressing group were more densely and uniformly arranged, whereas they were relatively sparse and arranged in a disordered manner in the TrkA-shRNA group. Western blot assay showed that compared with the control groups, the TrkA overexpressing group had higher expression of the myelin marker, myelin basic protein and the axonal marker neurofilament 200. The TrkA overexpressing group also had higher levels of various signaling molecules, including TrkA, pTrkA (Tyr490), extracellular signal-regulated kinases 1/2 (Erk1/2), pErk1/2 (Thr202/Tyr204), and the anti-apoptotic proteins Bcl-2 and Bcl-xL. In contrast, these proteins were downregulated, while the pro-apoptotic factors Bax and Bad were upregulated, in the TrkA-shRNA group. The levels of the TrkA effectors Akt and pAkt (Ser473) were not different among the groups. These results suggest that TrkA enhances the survival and regenerative capacity of bone marrow stromal stem cells through upregulation of the Erk/Bcl-2 pathway. All procedures were approved by the Animal Ethical and Welfare Committee of Shenzhen University, China in December 2014 (approval No. AEWC-2014-001219).

Key Words: *nerve regeneration; bone marrow stromal stem cells; tropomyosin receptor kinase A receptor; lentiviral vector; shRNA; extracellular signal-regulated protein kinases 1/2; Bcl-2; nerve grafts; peripheral nerve regeneration; survival; neural regeneration*

Chinese Library Classification No. *R4543; R364*

Introduction

Mesenchymal stem cells, such as bone marrow stromal stem cells (BMSCs) and adipose-derived stem cells, are an ideal autologous stem cell source for repairing nerve injuries because of their numerous attractive properties, including easy isolation, immunomodulatory ability, the production of trophic factors, and neural plasticity (Oliveira et al., 2013; Masgutov et al., 2016, 2018; Jiang et al., 2017). A recent study showed that neither BMSCs nor adipose-derived stem cells within venous grafts provide satisfactory results for peripheral nerve repair (Fernandes et al., 2018), suggesting that further optimization of tissue-engineered nerve grafts is needed. A number of studies have shown that BMSC-seeded acellular nerve grafts effectively improve neural regeneration and functional recovery after peripheral nerve injuries (Hu et al., 2007; Wang et al., 2008, 2010, 2012; Jia et al., 2012, 2017; Pang et al., 2013; Zhao et al., 2014; Zheng et al., 2017), depending on the density and viability of BMSCs in nerve grafts (Raheja et al., 2012; Zheng et al., 2016; Hou et al., 2018).

Tropomyosin receptor kinase A (TrkA) is the high affinity membrane-bound receptor for nerve growth factor (NGF), and mediates multiple effects, including neuronal survival and differentiation, and nerve growth and regeneration (Huang and Reichardt, 2001; Marlin and Li, 2015). Previous studies show that TrkA is markedly upregulated during the differentiation of BMSCs into neurons (Woodbury et al., 2000; Yaghoobi and Mowla, 2006; Li et al., 2007; Jian et al., 2015). Upon NGF binding to TrkA, the phosphatidylinositol-3 kinase/Akt and Ras/extracellular signal-regulated kinase (Erk) signaling pathways are activated and facilitate axonal outgrowth, branching, elongation and maintenance during the regeneration of peripheral nerves (Auer et al., 2012; Klimaschewski et al., 2013; Chan et al., 2014; Xing et al., 2016; Huang et al., 2017). Accordingly, we hypothesized that TrkA might be a key regulator of BMSC function in nerve grafts. To test this hypothesis, we overexpressed or knocked-down TrkA in BMSCs and then seeded these cells into rat sciatic nerve defects in a previous study (Zheng et al., 2016). We found that TrkA overexpression promoted the survival and Schwann cell-like differentiation of BMSCs in nerve grafts (Zheng et al., 2016), and stimulated the ability of these cells to enhance peripheral nerve regeneration and functional recovery (Zheng et al., 2017). In the present study, we investigated the molecular mechanisms underlying this process.

Materials and Methods

Animals

Thirty adult specific-pathogen-free male Sprague-Dawley rats, 7–8 weeks of age and weighing 200–250 g, were obtained from Guangdong Medical Laboratory Animal Center, China (license No. SCXK (Yue) 2013-0002). Rats were housed in temperature- and humidity-controlled specific-pathogen-free animal rooms with a 12/12-hour light/dark cycle and fed standard lab chow and water *ad libitum*. All surgical procedures were performed under general anesthesia with a dosage of 0.4 mL/100 g body weight *via* intraperitoneal injection of 2% pentobarbital sodium (Sigma-Aldrich, St. Louis, MO, USA). All procedures were approved by the Animal Ethical and Welfare Committee of Shenzhen University, China in December 2014 (approval No. AEWC-2014-001219).

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

BMSCs were harvested from the femurs and tibias of rats (n = 10), which were also used as the source of the allogeneic acellular nerves. The BMSCs were isolated, expanded and identified as previously described (Zheng et al., 2016). Lentiviruses encoding rat TrkA cDNA (Sino Biological, Beijing, China) and shRNA targeting rat TrkA (TrkA-shRNA sequence: 5′-ATT CAG GTG ACT GAG CCG AGG G-3′; product size: 22 bp; Hanbio, Shanghai, China), as well as their respective empty lentiviral controls (Hanbio), were used to infect rat BMSCs at passage 3, according to a previously published protocol (Zheng et al., 2016). Briefly, for lentiviral infection, BMSCs at approximately 60% confluence in 6-well dishes (5×10^5 per well) were treated with the lentivirus-containing medium (multiplicity of infection = 15) combined with polybrene (5 μg/mL; Hanbio). After 24 hours, the culture medium was replaced with fresh medium. Then, 24 hours later, puromycin (Sigma-Aldrich) was added to the medium at a final concentration of 2 μg/mL. Stably-infected BMSCs were obtained after 3 weeks of antibiotic selection. Uninfected BMSCs were used as negative controls. The following stably-infected BMSCs were obtained: TrkA-overexpressing BMSCs (Over-TrkA BMSCs), TrkA-shRNA expressing BMSCs (TrkA-shRNA BMSCs), and their respective empty vector controls (Vector BMSCs and Control BMSCs).

Preparation of allogeneic acellular nerves

Bilateral sciatic nerves of anesthetized rats (n = 10) were excised and dissected into 15-mm-long nerve segments under sterile conditions. Adipose and connective tissues were removed from the surface of the nerves with the help of a dissecting microscope. The acellular nerves were prepared as described previously (Zheng et al., 2017). Briefly, the nerve segments were sequentially rinsed twice in distilled water, 3% Triton X-100 (Sigma-Aldrich) and 4% sodium deoxycholate (Sigma-Aldrich). Each acellular nerve was trimmed to a 10-mm-long segment and stored in phosphate-buffered saline containing 100 U/mL penicillin and 100 μg/mL streptomycin (Hyclone, Thermo Fisher Scientific, Waltham, MA, USA) at 4°C. Storage buffer was replaced every week. Hematoxylin and eosin staining was used to assess the effects of the chemical extraction treatments on the nerves as described below.

In vitro construction of tissue-engineered nerves

Tissue-engineered nerve grafts were constructed by seed-

ing the stably-infected BMSCs into the allogeneic acellular nerves. The 10-mm-long acellular nerves were pre-incubated in cell culture medium at 37°C for 3 hours. BMSCs for graft seeding were labeled with PKH26 (Sigma-Aldrich) according to the manufacturer's instructions. A single-cell suspension of BMSCs in 2% gelatin (Sigma-Aldrich), a relatively inert material for preventing cell leakage (Chen et al., 2007; Jia et al., 2012) was prepared at 2×10^7 cells/mL. A total of 2×10^5 BMSCs in 10 μL cell suspension was injected into an acellular nerve graft in equal volumes at four evenly-spaced points using a microinjector. The nerve grafts implanted with the infected BMSCs were then incubated in low-glucose Dulbecco's modified Eagle's medium (Gibco, Thermo Fisher Scientific) containing 10% fetal bovine serum (Hyclone, Thermo Fisher Scientific), 100 U/mL penicillin and 100 μg/mL streptomycin at 37°C, 5% CO_2 under humidified conditions for 48 hours until the *in vivo* transplantation was performed. The fluorescent signals of PKH26-labeled BMSCs in the nerve grafts were detected on an inverted fluorescence microscope (IX71, Olympus, Tokyo, Japan) before transplantation.

***In vivo* transplantation of BMSC-containing nerve grafts**

Twenty adult male rats were randomly divided into the following four groups (n = 5 per group): Over-TrkA BMSC-seeded nerve grafts (over-TrkA group), vector BMSC-seeded nerve grafts (vector group), TrkA-shRNA BMSC-seeded nerve grafts (TrkA-shRNA group) and control BMSC-seeded nerve grafts (control group). As described previously (Zheng et al., 2017), the right sciatic nerve was exposed through an incision in the muscle under anesthesia. A 10-mm-long nerve segment distal to the sciatic notch was dissected. The tissue-engineered nerve graft was then attached with 10-0 nylon interrupted epineurial sutures to the proximal and distal stumps of the sciatic nerve to bridge the 10-mm gap. The incision was closed in layers with 3-0 nylon sutures, and the rats were left to convalesce for 8 weeks after surgery.

Hematoxylin and eosin staining

Eight weeks after the surgery, rats were anesthetized with pentobarbital sodium, and the 5-mm-long proximal segments of the nerve grafts were harvested and fixed in 4% paraformaldehyde in phosphate-buffered saline overnight at 4°C, as described before (Zheng et al., 2017). The segments were then submerged in 30% sucrose for 24 hours and mounted in optimal cutting temperature compound (Tissue-Tek, Sakura, Tokyo, Japan), and cut into 12-μm-thick frozen serial sections on a cryostat (CM1850; Leica, Wetzlar, Germany). The allogeneic acellular nerves were fixed and prepared in the same manner. Hematoxylin and eosin staining was performed for observing histological changes, and images were acquired with an Eclipse Ni-U microscope with NIS-Elements BR Imaging software (Nikon Instruments, Tokyo, Japan).

Western blot assay

Eight weeks after the surgery, rats were sacrificed under anesthesia. Nerve grafts were harvested and flash frozen in liquid nitrogen. Tissues were homogenized in RIPA buffer (Sigma-Aldrich) supplemented with protease and phosphatase inhibitors (Roche Applied Science, Mannheim, Germany). Protein extracts were centrifuged at 13,201 × g (12,000 rpm) for 30 minutes at 4°C. Protein quantification was performed using the Pierce BCA protein assay kit (Thermo Fisher Scientific). Equal amounts of protein were separated by 10–12% SDS-PAGE, and transferred to polyvinylidene fluoride membranes. The membranes were blocked with 5% non-fat milk or 10% bovine serum albumin in Tris-buffered saline, and immunoblotted with appropriate primary and secondary antibodies. The following antibodies were used: mouse monoclonal anti-glial fibrillary acidic protein (GFAP, Schwann cell marker; 1:1000; Millipore, Darmstadt, Germany), mouse monoclonal anti-neurofilament 200 (NF200, axonal marker; 1:500; Boster, Wuhan, China), rabbit polyclonal anti-myelin basic protein (MBP, myelin marker; 1:500; Boster), rabbit polyclonal anti-TrkA (1:500; Millipore), rabbit polyclonal anti-TrkA phosphorylated at Tyr490 (pTrkA; 1:1000; Cell Signaling Technology, Danvers, MA, USA), rabbit polyclonal anti-Akt (1:1000; Cell Signaling Technology), rabbit polyclonal anti-Akt phosphorylated at Ser473 (pAkt; 1:1000; Cell Signaling Technology), rabbit polyclonal anti-Erk1/2 (1:1000; Cell Signaling Technology), rabbit polyclonal anti-Erk1/2 phosphorylated at Thr202/Tyr204 (pErk1/2; 1:1000; Cell Signaling Technology), rabbit polyclonal anti-Bcl-2 (anti-apoptotic protein; 1:1000; Cell Signaling Technology), rabbit polyclonal anti-Bcl-xL (anti-apoptotic protein; 1:1000; Cell Signaling Technology), rabbit polyclonal anti-Bad (pro-apoptotic factor; 1:1000; Cell Signaling Technology), rabbit polyclonal anti-Bax (pro-apoptotic factor; 1:1000; Cell Signaling Technology), rabbit polyclonal anti-cleaved caspase-3 (apoptotic marker; 1:1000; Cell Signaling Technology) and mouse monoclonal anti-β-actin (1:5000; Sigma-Aldrich). Secondary antibodies included goat polyclonal anti-mouse antibody conjugated with horseradish peroxidase (1:10,000; Sigma-Aldrich) and goat polyclonal anti-rabbit conjugated with horseradish peroxidase (1:10,000; Sigma-Aldrich). Signals were visualized with ECL substrate (Thermo Fisher Scientific) and exposed to autoradiographic film (Eastman Kodak, Rochester, NY, USA). Protein bands were quantified by densitometric analysis and normalized to β-actin as internal control with ImageJ software (National Institutes of Health, Bethesda, MD, USA). Relative protein expression level was calculated as the ratio of the integrated density value of the target protein to that of β-actin.

Statistical analysis

All data are shown as the mean ± SEM. Statistical analyses were performed in GraphPad Prism 5 software (GraphPad, La Jolla, CA, USA). One-way analysis of variance was used to compare mean values, followed by the Student-Newman-Keuls *post hoc* test. A value of $P < 0.05$ was considered statistically significant.

Results

Histological examination of acellular nerves before and after BMSC seeding

To evaluate histological changes in the nerve grafts before and after BMSC seeding, hematoxylin and eosin staining was performed. As shown in **Figure 1**, there was no evidence of visible cells, axons or myelin sheaths in the acellular nerves, while the basal lamina tubes remained structurally intact. In contrast, many blue nuclei, axons and myelin sheaths were visible in the BMSC-containing nerve grafts, suggesting the seeded cells were viable and motile (**Figure 1**). Compared with the vector and control groups, the cells and fibers were more densely and uniformly arranged in the over-TrkA group, whereas they were distributed more sparsely and in a disorderly manner in the TrkA-shRNA group (**Figure 1**).

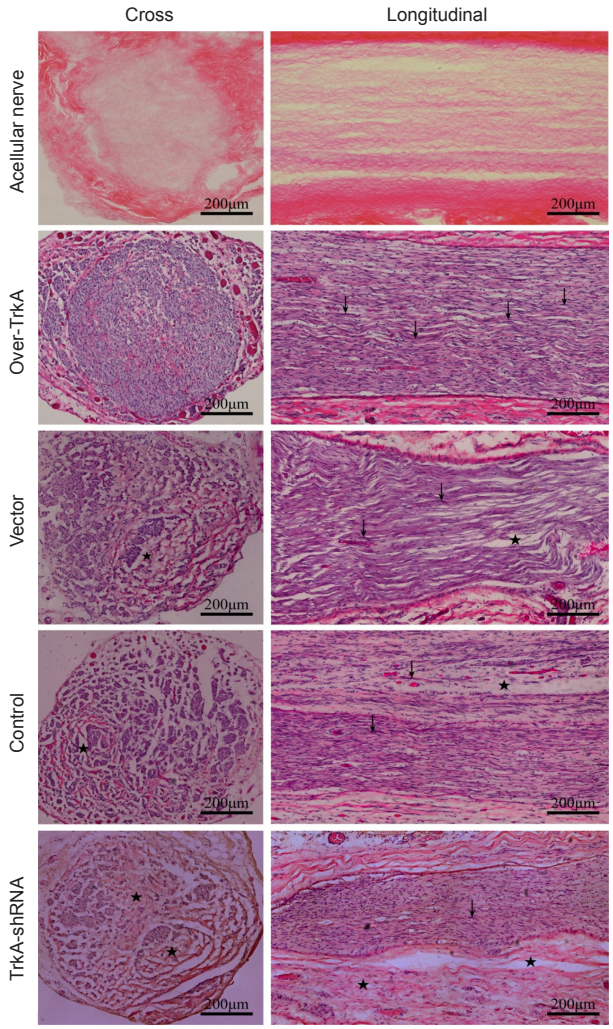

Figure 1 Images of transverse and longitudinal sections of hematoxylin and eosin-stained acellular nerve and nerve grafts from over-TrkA, vector, TrkA-shRNA and control groups 8 weeks after surgery.

The sparse areas devoid of blue nuclei and myelin sheaths are labeled with asterisks. Arrows designate myelinated nerve fibers. Scale bars: 200 μm.

TrkA overexpression increases GFAP, NF200 and MBP expression in BMSC-seeded nerve grafts

We previously showed that TrkA overexpression promotes the Schwann cell-like differentiation of BMSCs, as demonstrated by the colocalization of S100/GFAP and PKH26 (Zheng et al., 2016), and the regeneration of MBP-positive nerve fibers (Zheng et al., 2017). Here, we assessed the protein levels of GFAP, NF200 and MBP in nerve grafts by western blot assay. As shown in **Figure 2**, the expression levels of GFAP, NF200 and MBP were significantly upregulated in the over-TrkA group ($P < 0.001$ or $P < 0.01$), but significantly downregulated in the TrkA-shRNA group ($P < 0.01$ or $P < 0.05$), compared with the vector and control groups. These results are consistent with our previous findings (Zheng et al., 2016, 2017) and show that TrkA overexpression enhances the ability of BMSCs to promote peripheral nerve regeneration.

TrkA overexpression activates the Erk1/2 pathway in BMSC-seeded nerve grafts

To clarify the role of TrkA in the BMSC-containing nerve grafts, the levels of proteins involved in the TrkA pathway, including TrkA, pTrkA, Akt, pAkt, Erk1/2 and pErk1/2, were evaluated by western blot assay. Compared with the vector and control groups, TrkA and pTrkA levels were significantly higher in the over-TrkA group ($P < 0.001$ and $P < 0.05$; **Figure** 3), whereas they were significantly lower in the TrkA-shRNA group ($P < 0.05$; **Figure 3**). However, Akt and pAkt levels were similar among all four groups (**Figure 3**), suggesting that TrkA overexpression in BMSCs may not affect the Akt pathway. Erk1/2 and pErk1/2 levels were significantly higher in the over-TrkA group ($P < 0.01$ and $P < 0.05$; **Figure 3**), while they were significantly lower in the TrkA-shRNA group ($P < 0.01$; **Figure 3**), compared with the vector and control groups. These results suggest that TrkA functions through the Erk1/2 pathway in BMSC-seeded nerve grafts.

TrkA overexpression upregulates Bcl-2 and Bcl-xL in BMSC-containing nerve grafts

To identify downstream effectors in the TrkA–Erk1/2 signaling pathway in nerve grafts, we examined the expression of apoptosis-related proteins. As shown in **Figure 4**, compared with the vector and control groups, the anti-apoptotic proteins Bcl-2 and Bcl-xL were significantly upregulated in the over-TrkA group ($P < 0.001$ and $P < 0.01$). Conversely, their levels were much lower in the TrkA-shRNA group ($P < 0.01$ and $P < 0.001$). The expression levels of the pro-apoptotic proteins Bad and Bax and cleaved caspase-3 were significantly higher in the TrkA-shRNA group ($P < 0.001$), and significantly lower or undetectable in the other three groups. Taken together, these observations suggest that TrkA may induce anti-apoptotic signals and inhibit apoptotic signals, thereby enhancing the survival and function of BMSCs in nerve grafts.

Discussion

The transmembrane protein TrkA is a high-affinity func-

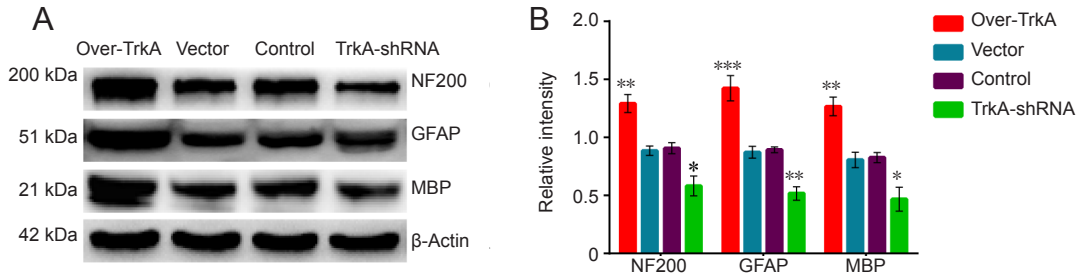

Figure 2 GFAP, NF200 and MBP protein expression in nerve grafts 8 weeks after surgery.
(A) Western blot assay for GFAP, NF200 and MBP in nerve grafts from over-TrkA, vector, TrkA-shRNA and control groups. (B) The bands were quantified by densitometry and normalized to β-actin. Data are expressed as the mean ± SEM (n = 5 per group; one-way analysis of variance followed by Student-Newman-Keuls *post hoc* test). *P < 0.05, **P < 0.01, ***P < 0.001, *vs.* vector and control groups. NF200: Neurofilament 200; GFAP: glial fibrillary acidic protein; MBP: myelin basic protein.

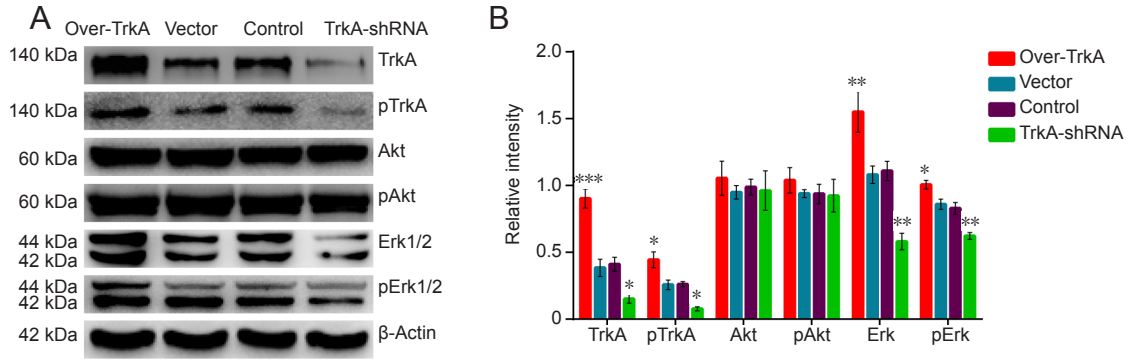

Figure 3 Effects of TrkA upregulation and downregulation on the Erk1/2 pathway 8 weeks after surgery.
(A) Western blot assay for TrkA, pTrkA, Akt, pAkt, Erk1/2 and pErk1/2 in nerve grafts from over-TrkA, vector, TrkA-shRNA and control groups. (B) The bands were quantified by densitometry and normalized to β-actin. Data are presented as the mean ± SEM (n = 5 per group; one-way analysis of variance followed by Student-Newman-Keuls *post hoc* test) *P < 0.05, **P < 0.01, ***P < 0.001, *vs.* vector and control groups. TrkA: Tropomyosin receptor kinase A; pTrkA: TrkA phosphorylated at Tyr490; pAkt: Akt phosphorylated at Ser473; Erk1/2: extracellular signal-regulated kinases 1/2; pErk1/2: Erk1/2 phosphorylated at Thr202/Tyr204.

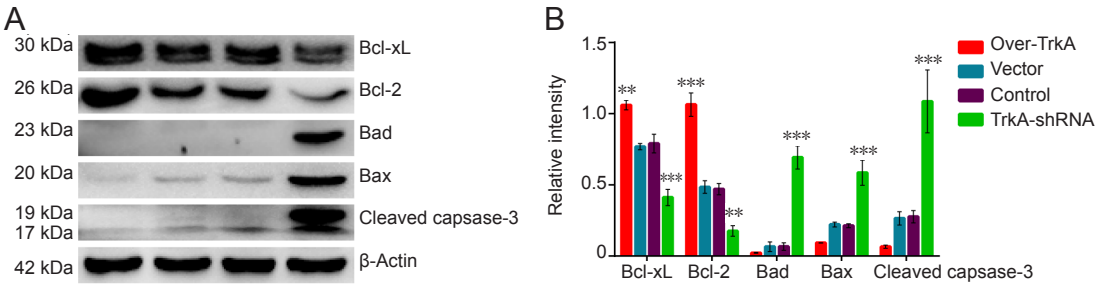

Figure 4 Expression of apoptosis-related proteins in nerve grafts 8 weeks after surgery.
(A) Western blot assay for Bcl-2, Bcl-xL, Bad, Bax and cleaved caspase-3 in nerve grafts from over-TrkA, vector, TrkA-shRNA and control groups. (B) The bands were quantified by densitometry and normalized to β-actin. Data are presented as the mean ± SEM (n = 5 per group; one-way analysis of variance followed by Student-Newman-Keuls *post hoc* test). **P < 0.01, ***P < 0.001, *vs.* vector and control groups. Bcl-xL: B-cell lymphoma-extra large; Bcl-2: B-cell lymphoma 2; Bad: Bcl-2-associated death promoter; Bax: Bcl-2-associated X protein; C-caspase-3: cleaved caspase-3.

tional receptor for NGF. The NGF homodimer binds to the extracellular domain of TrkA, causing receptor dimerization and autophosphorylation. Phosphorylation at Tyr490 in the juxtamembrane domain recruits the adapter proteins Shc and Frs2, which activate the phosphatidylinositol-3 kinase/Akt and Ras-Erk1/2 pathways. The signals are conveyed to the nucleus, where they regulate the expression of downstream targets, such as the Bcl-2 family, thereby promoting the survival and differentiation of neurons (Patapoutian and Reichardt, 2001; Marlin and Li, 2015). The Akt and Erk1/2 pathways are necessary for axon sprouting during peripheral nerve regeneration (Okada et al., 2010; Yu et al., 2015; Gaesser and Fyffe-Maricich, 2016). The Erk1/2 pathway regulates neuronal survival and axonal elongation and maintenance after injury (Waetzig and Herdegen, 2005; Seo et al., 2009; Tsuda et al., 2011), whereas the Akt pathway regulates cyto-

skeletal proteins and axon branching (Markus et al., 2002; Christie et al., 2010; Klimaschewski et al., 2013).

Our previous studies showed that TrkA regulates the survival and Schwann cell-like differentiation of BMSCs in nerve grafts and promotes the repair and regeneration of peripheral nerves (Zheng et al., 2016; Zheng et al., 2017). In the present study, we investigated the underlying molecular mechanisms. Our findings show that the expression levels of the Schwann cell marker GFAP and of axonal regrowth-related proteins NF200 and MBP paralleled TrkA expression. They increased when TrkA was overexpressed and decreased when TrkA expression was silenced. These results are in line with our previous findings, and show that TrkA overexpression robustly promotes the differentiation of BMSCs into Schwann-like cells in nerve grafts and promotes the regeneration of peripheral nerves.

We also found that TrkA overexpression and downregulation affects the levels of pTrkA, Erk1/2, pErk1/2 and other related proteins, while Akt and pAkt did not. Thus, TrkA overexpression in BMSCs seeded into nerve grafts mainly activates the Erk1/2 pathway without affecting the Akt pathway. Because the Erk1/2 pathway regulates neuronal survival and axonal regrowth, extension and maintenance after injury, TrkA overexpression may enhance regeneration by activating the Erk1/2 pathway. However, the functions of the Erk1/2 and Akt pathways in our model remain unclear. Inhibitors of Erk1/2 (U0126) and Akt (LY294002) phosphorylation may help clarify their roles, and will be considered for future studies.

The NGF/TrkA signaling pathway promotes neuronal survival and differentiation by regulating Bcl-2 expression (Orike et al., 2001; Patapoutian and Reichardt, 2001; Liang et al., 2003). During embryonic development, Bcl-2 is widely expressed in the nervous system (Abe-Dohmae et al., 1993; Opferman and Kothari, 2018), and plays a key role in axonal growth and regeneration (Chen et al., 1997). Bcl-2 expression in the adult central nervous system declines with age, but remains widely expressed in the peripheral nervous system (Merry et al., 1994). This suggests that in addition to promoting neuronal survival and differentiation, Bcl-2 may also play an important role in the maintenance of peripheral nerve function. Indeed, the repair and regeneration of peripheral nerves are severely impaired in the absence of Bcl-2 (Kotulska et al., 2003, 2005). This is in line with our current findings that overexpression or downregulation of TrkA in nerve grafts affects the expression of Bcl-2 and Bcl-xL. Previous reports that Bcl-2 overexpression enhances cellular survival but fails to promote axonal regeneration might be due to an unfavorable microenvironment and the specific cell types used (Inoue et al., 2002; Sole et al., 2004; Ghoumari et al., 2005; Leaver et al., 2006). Bcl-2 appears to promote axonal regeneration by enhancing intracellular Ca^{2+} signaling and by activating CREB and Erk. In contrast, Bcl-xL does not activate CREB and Erk or support axonal growth (Jiao et al., 2005). Therefore, Bcl-2 may be a key downstream effector of TrkA in BMSCs that stimulates peripheral nerve regeneration.

In summary, we show that TrkA activates the Erk1/2 pathway and upregulates Bcl-2 expression in BMSCs seeded in peripheral nerve grafts. Bcl-2 may promote the survival and Schwann cell-like differentiation of the BMSCs, thereby enhancing peripheral nerve regeneration. Our findings should help in the optimization of BMSCs as seed cells for the clinical treatment of peripheral nerve defects.

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