Original Paper

Effect of TNF- α Inhibition on Bone Marrow-Derived Mesenchymal Stem Cells in Neurological Function Recovery after Spinal Cord Injury via the Wnt Signaling Pathway in a Rat Model

Ren-Jun Peng Bing Jiang Xi-Ping Ding He Huang Yi-Wei Liao Gang Peng
Quan Cheng Jian Xi

Department of Neurosurgery, Xiangya Hospital Central South University, Changsha, China

Key Words

Bone marrow-derived mesenchymal stem cell • Neurological function • Recovery • Spinal cord injury • Tumor necrosis factor- α • Wnt signaling pathway

Abstract

Aim: The present study aimed to examine the effect of tumor necrosis factor- α (TNF- α) inhibition on bone marrow-derived mesenchymal stem cells (BMSCs) in neurological function recovery after spinal cord injury (SCI) via the Wnt signaling pathway in a rat model. **Methods:** The rat model of SCI was established using Allen's method. Seventy-two adult male Sprague Dawley (SD) rats were randomly assigned into 4 groups (18 rats in each group): the sham control group, saline control group, BMSCs group (injection with BMSCs at the injured site) and BMSCs + TNF- α group (injection with BMSCs under TNF- α treatment at the injured site). Immunohistochemistry was performed to characterize the culture media after TNF- α -induced differentiation. qRT-PCR and Western blotting analyses were performed to detect the mRNA and protein expression of β -catenin, Wnt3a, GSK-3 β and Axin. The Basso Beattie Bresnahan (BBB) locomotor score, neurological deficit score (NDS), and balance beam test (BBT) score were used to assess neurological functional recovery of SCI rats. **Results:** In the BMSC group, numerous spherical cell clusters grew in suspension, and the cells were nestin-, NF200- and GFAP-positive. Compared with the sham control and BMSC groups, the β -catenin and Wnt3a mRNA and protein expression was increased, but the GSK-3 β and Axin mRNA and protein expression was decreased in the BMSCs + TNF- α group. The SCI rats in the BMSCs + TNF- α group exhibited lower BBB scores, and higher NDSs and BBT scores compared to the BMSCs group. **Conclusion:** Our study provides evidence that TNF- α inhibition may weaken the ability of BMSCs in neurological functional recovery after SCI by activating the Wnt signaling pathway.

© 2017 The Author(s)
Published by S. Karger AG, Basel

Dr. Jian Xi

Department of Neurosurgery, Xiangya Hospital Central South University, No. 87,
Xiangya Road, Changsha 410008, Hunan Province (China)
Tel. +86-0731-84327133, E-Mail xijianxysw@163.com

KARGER

Introduction

Spinal cord injury (SCI) refers to traumatic insult occurring in the spinal cord, which not only causes motor or sensory dysfunctions, but permanent disability in autonomic function, resulting in irreversible symptoms [1, 2]. There are approximately 2.5 million patients with SCI worldwide, with an estimate of 130,000 new cases reported annually [3]. In the present study, several approaches have been shown to be effective in the treatment of SCI, although none of these approaches were sufficiently efficient to achieve axonal regeneration and to rewire the spinal cord after injury [4]. Although SCI treatment has always been a challenge for clinical practitioners and scientists, some progress has still been made, such as stem-cell-based therapies [5]. In addition, after SCI, the central nervous system fails to effectively reactivate developmental programs to rebuild novel circuitry to restore function [6]. Thus, it is vital to assess the functional state and to quantify the clinical neurological impairment of patients with traumatic SCI [7].

Tumor necrosis factor- α (TNF- α) is recognized as a biomarker to monitor and predict the pro-inflammatory state and plays an important role in the development and progression of chronic inflammatory diseases [8, 9]. TNF- α is secreted in response to stress and after injury, which indicates that the pathological situation of TNF- α may also affect synaptic transmission [10]. Interestingly, measurement of serum levels of TNF- α over time can be useful in tracking the course of SCI, and SCI patients with and without neurological improvement demonstrate differences in measured TNF- α [11]. Wnts are lipid-modified glycoproteins that play important roles in embryonic development and they are involved in a variety of cellular behaviors, such as proliferation, migration, differentiation and stem cell self-renewal [12]. TNF- α can activate the Wnt/ β -catenin signaling pathway, which in turn inhibits adipocytic differentiation [13]. According to Akihiko Hiyama et al., activation of the Wnt signaling pathway increases TNF- α expression and may contribute to degeneration of nuclear pulposus cells, which suggests that blockade of Wnt signaling pathway has the potential to reduce the degeneration of nuclear pulposus cells [14]. However, the effect of the Wnt signaling pathway on the recovery of SCI still requires further investigation. Thus, our study established the SCI rat model and aimed to investigate the effect of TNF- α inhibition on BMSCs in neurological function recovery after SCI via the Wnt signaling pathway, and we hope to identify a new therapeutic strategy for neurological improvement of SCI.

Materials and Methods

Animals and grouping

Seventy-two adult male Sprague Dawley (SD) rats provided by the Animal Research Institute of China Academy of Medical Science (Beijing, China) were used in this study. The rats were randomly assigned into 4 groups (18 rats in each group): the sham control group, saline control group (injection with saline at the injured site), BMSCs group (injection with BMSCs at the injured site) and BMSCs + TNF- α group (injection with BMSCs under 20 ng/mL TNF- α treatment at the injured site). All procedures performed on the animals were in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Establishment of a rat model of SCI

Rats were anesthetized by intraperitoneal injection of 2% pentobarbital sodium (40 mg/kg), and then fixed in the prone position under a dissecting microscope with the hair of the back and chest shaved. After conventional disinfection, the skin and subcutaneous tissues were dissected and the muscle was separated by a median incision along the back with T10 spines as the center. Next, the dura mater was exposed. After the spinal cord was fixed, the surrounding area of T10 was considered the injury region. The T9-T10 spinal cord was injured with modified Allen's method [15] in the saline control, BMSCs and BMSCs + TNF- α groups, and the rat model of SCI was successfully established. The wound was washed and dusted with an appropriate amount of penicillin powder on the surface. The muscle and skin were sutured layer-by-layer.

Rats in the sham control group were only operated with an incision of the spinal cord without any damage. After the operation, the rats were independent raised in a cage with natural light, and water was provided ad libitum.

Primary culture and purification of BMSCs

Adult male SD rats were sacrificed by cervical dislocation and placed in 75% ethanol. The bilateral lower limb bone was removed under sterile conditions, and the muscle and soft tissues were shaved. Next, the metaphysis of the collected lower limb bone was dissected to allow the exposure of the marrow cavity. The marrow cavity was washed with 10 mL phosphate buffered solution (PBS), and the eluate was made into a monoplast suspension. The cell concentration was adjusted to 6×10^6 /mL by the addition of Dulbecco's modified Eagle's medium (DMEM). The adjusted cell suspension was incubated in an incubator with 5% CO₂ at 37°C. The nutrient solution was replaced after 48 h of culture and the culture medium and non-adherent cells were aspirated. Cell growth was regularly observed, and the culture medium was replaced every 3 days. After reaching approximately 80% confluence, the cells were digested and subcultured with 0.25% trypsin and DMEM containing fetal bovine serum (FBS) was added to terminate the digestion. The cell suspension was centrifuged at 1500 rpm/min for 5 min at 4°C, and the supernatant was aspirated. The cell precipitation was obtained after resuspension by 10 mL culture medium. Cells were quantified under a microscope. The BMSCs cells were subcultured with the density of 8×10^3 /cm². BMSCs cells were purified through repeated passage. Changes in the morphology of the BMSCs cells were observed under a microscope. The second generation of BMSCs cells was preserved at -80°C.

Identification of BMSCs

The preserved BMSCs were inoculated into a six-well plate at a density of 1×10^5 /mL and DMEM/F1 containing 10% FBS and 10 ng/mL basic fibroblast growth factor (bFGF) were added for differentiation [16]. Subsequently, 20 ng/mL TNF- α was added into the BMSCs in the BMSCs + TNF- α group, and the incubation lasted for 4 h in an incubator with 5% CO₂ at 37°C. Changes in the morphology of cells in the BMSCs group and BMSCs + TNF- α group were observed under a microscope. The original medium was aspirated, and the cells were inoculated in fresh DMEM/F12 medium containing 10% FBS. After 3 days, immunohistochemistry was performed to detect neurocytic markers on the surface of the BMSCs. The expression of NF200 and GFAP was detected to identify differentiated neuron-like cells and differentiated as astrocyte-like cells. The primary antibodies were rabbit anti-rat GFAP (1: 200) and mouse anti-rat NF200 (1: 200) and the secondary antibodies were TRITC-labeled sheep anti-rabbit IgG (1: 200) and FITC-labeled sheep anti-mouse IgG (1:200). The immunohistochemistry results were images under a fluorescence microscope.

In vivo transplantation

The second surgery was operated at the original incision after 2 weeks. The original injured site was exposed, and a 10 μ L microsyringe was injected into the rostral and the caudal ends of the injury site (the distance between the two ends was 5 mm), and 5 μ L of BMSCs suspension was slowly administered (for 3 min). Rats in the saline control group were injected with the same amount of saline at the injury site of spinal cord in the same manner 2 weeks after surgery, while rats in the sham control group received no treatment.

Basso Beattie Bresnahan (BBB) locomotor score, neurological deficit score (NDS) and balance beam test (BBT)

The BBB locomotor score [17], NDS [18] and BBT [19] were used to assess the recovery of neurological function of rats in each group after 6 weeks of transplantation.

Quantitative real-time polymerase chain reaction (qRT-PCR)

The RNA of BMSCs in each group was extracted according to the manufacturer's instructions (Promega Corp., Madison, Wisconsin, USA). After the RNA concentration and optical density (OD260/280) values were measured using the Nano Drop2000 (Thermo Fisher Scientific, California, USA), the RNA was stored at -80°C. Primer 5.0 was applied to design the primers based on the gene sequences published in the Genbank primers and database. The designed primers are shown in Table 1 and were synthesized

by Invitrogen Inc., (Carlsbad, California, USA). PCR was performed according to the procedures of the Reverse Transcription System A3500 (Promega Corp., Madison, Wisconsin, USA). The reaction conditions were: initial denaturation for 15 min at 95°C, and 40 cycles of denaturation for 10 s at 95°C, annealing 30 s and extension for 30 s. The reaction volume was 20 μ l, including 12.5 μ l Premix Ex Taq or SYBR Green Mix, 1 μ l Forward Primer, 1 μ l Reverse Primer, 4 μ l DNA template and supplemented with ddH₂O. GAPDH served as the internal control, and the melting curve was used to evaluate the reliability of the PCR results. The relative expression of the target gene was calculated using the $2^{-\Delta\Delta Ct}$ method:

$\Delta\Delta Ct = [Ct(\text{target gene}) - Ct(\text{reference gene})]_{\text{experimental group}} - [Ct(\text{target gene}) - Ct(\text{reference gene})]_{\text{control group}}$. These experiments were repeated 3 times.

Western blotting

Total protein of the BMSCs was isolated and the concentration was measured using a bicinchoninic acid (BCA) kit (Wuhan Boster Biological Engineering Co., Ltd., Wuhan, Hubei, China) according to the manufacturer's instructions. Equal amounts of protein were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, Massachusetts, USA). The membranes were blocked in 5% non-fat dried milk at room temperature for 2 h. Next, the membranes were separately probed with the following primary antibodies: Axin (1: 1000, ab32197, Abcam Inc., Cambridge, UK), β -catenin (1: 500, ab16051, Abcam Inc., Cambridge, UK), GSK-3 β (1: 500, ab93926, Abcam Inc., Cambridge, UK) and Wnt3a (1:500, ab28472, Abcam Inc., Cambridge, UK) overnight at 4°C. The membranes were washed three times (each time for 10 min) using poly (butylene succinate-co-butylene terephthalate) (PBST), and then incubated with the corresponding secondary antibodies (1: 2000) for 1 h at room temperature. The membranes were washed three times (each time for 10 min), and visualized using an enhanced chemiluminescence (ECL) kit. β -actin served as the internal control, and the average intensities of the band area were measured using Image J software.

Statistical analysis

All data were analyzed using SPSS 21.0 statistical software (SPSS Inc., Chicago, Illinois, USA). Quantitative data were displayed as the mean $\pm$ standard deviation. Comparison between two groups was analyzed by the least significant difference-t test (LSD-t), and comparison between more than two groups was performed using one-way analysis of variance (ANOVA). $P < 0.05$ was regarded as a significant difference.

Results

Housing condition of the rats in each group

All rats behaved normally before modeling. At the initiation of the operation, the SCI rats in the saline control, BMSCs and BMSCs + TNF- α groups all presented different degrees of nerve injury symptoms. In total, there were 15 rats with the symptoms of hematuria or chyluria, and 12 rats with bedsores. During the observation period of 3 weeks, 8 rats in the saline control group, 3 rats in the BMSCs group, 4 rats in the BMSCs + TNF- α group and 2 rats in the sham control group died. To ensure sample size, the rats had already been supplemented. The living conditions of the rats in each group are shown in Table 2.

Identification of BMSCs with and without TNF- α treatment

In the BMSCs group, numerous spherical cell clusters grew in suspension, and the cells exhibited the characteristics of neural stem cells. Three days later, these cells gradually formed spherical cell clusters, which had gradually enlarged, with strong refraction

Table 1. The primer sequences for qRT-PCR. Note: qRT-PCR, quantitative real-time polymerase chain reaction; GSK-3 β , Glycogen synthase kinase-3 β

Primer	Sequence (5'-3')
Wnt3a	Forward: 5'-TGTTTCCACTGGTGCTGTTATG-3' Reverse: 5'-AATGAACCTGCTCCCTCTGT-3'
Axin	Forward: 5'-ACCTCACATTCCTCGCAC-3' Reverse: 5'-AGAAGGCATTTCCCATC-3'
β -catenin	Forward: 5'-AACGGCTTTCGGTTGAGCTG-3' Reverse: 5'-TGGCGATATCCAAGGGCTTC-3'
GSK-3 β	Forward: 5'-ATAGGTGACAGGCACAACGACA-3' Reverse: 5'-CGGGGTTAGGACAAAAGGTACTC-3'
β -actin	Forward: 5'-CACCCGCGAGTACAACCTTC-3' Reverse: 5'-CCCATACCCACCATCACACC-3'

Table 2. The living condition of rats in each group. Note: BMSCs, bone marrow derived mesenchymal stem cells; TNF- α , tumor necrosis factor- α

Group	Weight loss	Food intake reduced	Hematuria or chyluria	Bedsore	Death
Saline control	4	3	6	4	8
Sham control	0	1	0	1	2
BMSCs	2	1	5	3	3
BMSCs + TNF- α	1	2	4	4	4

Fig. 1. Identification of BMSCs without/with TNF- α treatment in vitro (A: Morphology of BMSCs without/with TNF- α treatment [400 $\times$]; B: Immunocytochemistry of Nestin in BMSCs without/with TNF- α treatment [400 $\times$]; C: NF200 and GFAP double labeling in BMSCs without/with TNF- α treatment [400 $\times$]) Note: TNF- α : tumor necrosis factor- α ; BMSCs: bone marrow-derived mesenchymal stem cells; NF200, neurofilament 200; GFAP, glial fibrillary acidic protein.

(Fig. 1A). BMSCs without TNF- α treatment were nestin-positive as assessed using immunocytochemistry (Fig. 1B). After several hours of incubation in DMEM/F12, the cells began to adhere to the wall of the culture dish. Some cells had migrated from the spherical cell clusters and elongated, and appeared similar to neurons. After some time, the projections of a small number of cells began to form connections to other neighboring cells. BMSCs without TNF- α treatment were NF200- and GFAP-positive (Fig. 1C). BMSCs with TNF- α treatment showed fibroblast-like morphology with multi-pseudopodia and adhered to the wall. BMSCs with TNF- α treatment showed no spherical cell clusters (Fig. 1D). Immunohistochemistry assays showed that BMSCs with TNF- α treatment were nestin-, NF200- and GFAP-negative (Fig. 1E-F).

The mRNA and protein expression of β -catenin, Wnt3a, GSK-3 β and Axin in BMSCs with and without TNF- α treatment in vitro

Compared with the BMSCs group, the mRNA and protein expression of β -catenin and Wnt3a were increased, while the mRNA and protein expression of GSK-3 β and Axin were decreased in the BMSCs + TNF- α group (all $P < 0.05$) (Fig. 2).

The mRNA and protein expression of β -catenin, Wnt3a, GSK-3 β and Axin in rats among the four groups in vivo

Compared with the sham control group, the mRNA and protein expressions of β -catenin and Wnt3a were increased, while the mRNA and protein expressions of GSK-3 β and Axin were decreased in the saline control, BMSCs and BMSCs + TNF- α groups (all $P < 0.05$). In comparison with the saline control group, the mRNA and protein expressions of Wnt3a and β -catenin were decreased while the mRNA and protein expressions of GSK-3 β and Axin were increased in the BMSCs group and the BMSCs + TNF- α group (all $P < 0.05$). Additionally, the mRNA and protein expressions of Wnt3a and β -catenin were dropped, while the mRNA and protein expressions of GSK-3 β and Axin were elevated in the BMSCs + TNF- α group compared with the BMSCs group (all $P < 0.05$). These results revealed that the Wnt signaling pathway was activated by TNF- α (Fig. 3).

Fig. 2. Comparisons of the mRNA and protein expression of β-catenin, Wnt3a, GSK-3β and Axin between the BMSCs group and BMSCs + TNF-α group (A: mRNA expression of β-catenin, Wnt3a, GSK-3β and Axin between the BMSCs group and BMSCs + TNF-α group; B: Protein expression of β-catenin, Wnt3a, GSK-3β and Axin between the BMSCs group and BMSCs + TNF-α group; C: histogram of β-catenin, Wnt3a, GSK-3β and Axin protein expression between the BMSCs group and BMSCs + TNF-α group). Note: *, compared with the BMSCs group, $P < 0.05$; TNF-α: tumor necrosis factor-α; BMSCs, bone marrow-derived mesenchymal stem cells; GSK-3β, Glycogen synthase kinase-3 beta.

Fig. 3. Comparisons of the mRNA and protein expression of β-catenin, Wnt3a, GSK-3β and Axin in the spinal cord cells of rats among the four groups (A: mRNA expression of β-catenin, Wnt3a, GSK-3β and Axin in each group; B: protein expression of β-catenin, Wnt3a, GSK-3β and Axin in each group; C: histogram of β-catenin, Wnt3a, GSK-3β and Axin protein expression in each group). Note: *, compared with the sham control group, $P < 0.05$; #, compared with the saline control group, $P < 0.05$; &, compared with the BMSCs group, $P < 0.05$; TNF-α: tumor necrosis factor-α; BMSCs, bone marrow-derived mesenchymal stem cells; GSK-3β, Glycogen synthase kinase-3 beta.

Fig. 4. Comparisons of BBB locomotor scores, NDS and BBT scores of rats at 6 weeks after transplantation among the four groups (A: BBB locomotor scores of rats at 6 weeks after transplantation among the four groups; B: NDS of rats at 6 weeks after transplantation among the four groups; C: BBT results of rats at 6 weeks after transplantation in the BMSCs + TNF-α group). Note: *, compared with the sham control group, $P < 0.05$; #, compared with the saline control group, $P < 0.05$; &, compared with the BMSCs group, $P < 0.05$; BBB: Basso Beattie Bresnahan; NDS, neurological deficit score; BBT, balance beam test; TNF-α, tumor necrosis factor-α; BMSCs, bone marrow-derived mesenchymal stem cells.

Comparisons of BBB locomotor score, NDS and BBT score of rats among the four groups

These results indicated that the BBB scores of the rats in the BMSCs + TNF-α group were lower than those in the BMSCs group at each time point after transplantation ($P < 0.05$) (Fig.

4A). Compared with the BMSCs group, the NDSs of rats were increased in the BMSCs + TNF- α group ($P < 0.05$) (Fig. 4B). The BBT may reflect the recovery of sensory-motor integration and motor coordination in the SCI rats. In the BMSCs + TNF- α group, the rats made missteps and even fell off the balance beam, while rats in the BMSCs group could successfully walk through the balance beam. After 6 weeks of transplantation, the BBT scores of the rats were increased in the BMSCs + TNF- α group compared with the saline control groups (all $P < 0.05$) (Fig. 4C), which revealed that the BMSCs + TNF- α group had a specific inhibitory effect on the recovery of motor function in SCI rats.

Discussion

In this study, we generated a rat model of SCI to investigate the effect of TNF- α inhibition on BMSCs in neurological functional recovery after SCI. According to the identification of BMSCs after TNF- α treatment, suspension growth of many spherical cells showed characteristics of neural stem cells in the BMSCs group and were nestin-positive. However, in the TNF- α group, there was no spherical cell-like structure and the immunohistochemistry studies showed negative expression of nestin, indicating that TNF- α inhibited the differentiation of BMSCs into neural stem cells. TNF- α is synthesized by a variety of cell types, including neurons, glia, T cells, astrocytes, activated macrophages and Schwann cells [20]. TNF- α is immediately increased after CNS-associated trauma, such as SCI, and it is derived from the damaged CNS and from damaged skin, muscles, and bones [21, 22]. According to the study of Oda et al., BMSCs are known as non-hematopoietic and adherent cells, and the most important practical advantages of using BMSCs are its potential in autologous transplantation, as it poses very low risk of teratoma formation and exhibits low cost in culturing [23]. In the study of Han et al., BMSCs significantly decreased the expression of TNF- α after transplantation, remarkably increased axonal regeneration and promoted motor functional recovery [24]. Thus, on the basis of our study, we proposed that the addition of TNF- α inhibited the differentiation ability of BMSCs into neural stem cells.

Our in vivo and in vitro studies indicated that after TNF- α induction, the mRNA and protein expression of β -catenin and Wnt3a were significantly up-regulated, while the mRNA and protein expression of GSK-3 β and Axin was downregulated, revealing that TNF- α activates the Wnt signaling pathway. The secretion of TNF- α as well as other cytokines had increased in response to stress and injury, indicating that these pathological situations may also have an effect on synaptic transmission [10]. Additionally, TNF- α induces overexpression of matrix metalloproteinase 9 (MMP9) in localized cancer, and MMP9 can be reduced via dexmedetomidine preconditioning in rats with ischemia reperfusion injury [25, 26]. The involvement of TNF- α in inflammatory pain is particularly relevant in SCI-induced pain [27]. And the treatment with glycine, a strychnine-sensitive inhibitory neurotransmitter in brainstem, spinal cord and retina, could reduce TNF- α level [28, 29]. Han et al. demonstrated that BMSCs might alleviate damage in spinal cord inflammation by suppressing TLR4-mediated signaling pathways and decreasing the levels of IL-1 β and TNF- α in tissues [30]. This finding is consistent with the role of TNF- α in a variety of pathologies ranging from inflammatory and peripheral injury-induced pain to neurological disorders of CNS, such as Alzheimer's disease and Parkinson's disease [31-33]. Takeshi et al. reported that Wnt signaling can inhibit oligodendrocyte development, and the Wnt/ β -catenin signaling pathway can prevent oligodendrocyte differentiation from progenitors to an immature state [34]. Moreover, the Wnt signaling pathway plays an important part in human embryonic stem cells (hESC) self-renewal and differentiation and can activate many intracellular signal transduction cascades [35]. Previous studies have analyzed the Wnt signaling pathway in nuclear pulposus cells and reported that activation of the Wnt signaling pathway inhibits the proliferation of nuclear pulposus cells and induces cell senescence, revealing that the Wnt signaling pathway promotes the progression of degeneration of intervertebral discs [36-38]. Largely consistent with our study, Hiyama et al. reported that

the Wnt signaling pathway modulates TNF- α , and the Wnt signaling pathway and TNF- α form a positive-feedback loop in nuclear pulposus cells [14].

In addition, the BBB scores of the rats were significantly lower, but the NDSs of the rats were higher in the TNF- α group compared to the BMSCs group. In the TNF- α group, the rats made missteps and even fell off the balance beam, while rats in the BMSCs group could successfully walk through the balance beam. As reported by Zhao et al., SCI induces neuronal death as well as axonal damage, resulting in functional motor loss, sensory loss and limited regeneration due to the adverse microenvironment, including neuroinflammation and glial scarring [39]. Ohta et al. suggested that BMSCs might secrete some trophic factors into the cerebrospinal fluid (CSF), contact with host spinal tissues to reduce cavities, and improve behavioral function in the rat [40]. Furthermore, in the study of Garraway et al., TNF- α has been proposed to contribute to numerous injury-induced processes, such as pain hypersensitivity and the increased expression of TNF- α is correlated with behavioral changes observed in rats [27]. Chi et al. advocated that TNF- α might play a dual role in SCI and its role is potentially dependent on when TNF- α is produced after SCI as well as in which cells TNF- α functions [41].

In conclusion, our study provides evidence that TNF- α inhibition may weaken the ability of BMSCs in neurological function recovery after SCI by activating the Wnt signaling pathway. However, the role of TNF- α in this process is still under debate due to conflicting evidence. Thus, further study is still needed to examine the precise mechanism of TNF- α in SCI and to develop an effective treatment for the neural functional recovery of SCI.

Acknowledgments

The authors wish to express their gratitude to the reviewers for their critical comments.

Disclosure Statement

The authors declare no conflict of interests.

References

- 1 Liao B, Zhang Y, Sun H, Ma B, Qian J: Ryanodine Receptor 2 Plays a Critical Role in Spinal Cord Injury via Induction of Oxidative Stress. *Cell Physiol Biochem* 2016;38:1129-1137.
- 2 Yang L, Ge Y, Tang J, Yuan J, Ge D, Chen H, Zhang H, Cao X: Schwann Cells Transplantation Improves Locomotor Recovery in Rat Models with Spinal Cord Injury: a Systematic Review and Meta-Analysis. *Cell Physiol Biochem* 2015;37:2171-2182.
- 3 Shi LB, Tang PF, Zhang W, Zhao YP, Zhang LC, Zhang H: Naringenin inhibits spinal cord injury-induced activation of neutrophils through mir-223. *Gene* 2016;592:128-133.
- 4 Kwon BK, Sekhon LH, Fehlings MG: Emerging repair, regeneration, and translational research advances for spinal cord injury. *Spine (Phila Pa 1976)* 2010;35:S263-270.
- 5 Muheremu A, Peng J, Ao Q: Stem cell based therapies for spinal cord injury. *Tissue Cell* 2016;48:328-333.
- 6 York EM, Petit A, Roskams AJ: Epigenetics of neural repair following spinal cord injury. *Neurotherapeutics* 2013;10:757-770.
- 7 Kalsi-Ryan S, Wilson J, Yang JM, Fehlings MG: Neurological grading in traumatic spinal cord injury. *World Neurosurg* 2014;82:509-518.
- 8 Campion J, Milagro FI, Goyenechea E, Martinez JA: Tnf-alpha promoter methylation as a predictive biomarker for weight-loss response. *Obesity (Silver Spring)* 2009;17:1293-1297.
- 9 Gonzalez-Gay MA, Gonzalez-Juanatey C, Vazquez-Rodriguez TR, Miranda-Filloo JA, Llorca J: Insulin resistance in rheumatoid arthritis: The impact of the anti-tnf-alpha therapy. *Ann N Y Acad Sci* 2010;1193:153-159.

- 10 Ferguson AR, Christensen RN, Gensel JC, Miller BA, Sun F, Beattie EC, Bresnahan JC, Beattie MS: Cell death after spinal cord injury is exacerbated by rapid tn timerelated trafficking of glur2-lacking ampars to the plasma membrane. *J Neurosci* 2008;28:11391-11400.
- 11 Biglari B, Swing T, Child C, Buchler A, Westhauser F, Bruckner T, Ferbert T, Jurgen Gerner H, Moghaddam A: A pilot study on temporal changes in il-1beta and tn timerelated serum levels after spinal cord injury: The serum level of tn timerelated alpha in acute sci patients as a possible marker for neurological remission. *Spinal Cord* 2015;53:510-514.
- 12 Xavier CP, Melikova M, Chuman Y, Uren A, Baljinnyam B, Rubin JS: Secreted frizzled-related protein potentiation versus inhibition of wnt3a/beta-catenin signaling. *Cell Signal* 2014;26:94-101.
- 13 Qadir AS, Lee HL, Baek KH, Park HJ, Woo KM, Ryoo HM, Baek JH: Msx2 is required for tn timerelated alpha-induced canonical wnt signaling in 3t3-l1 preadipocytes. *Biochem Biophys Res Commun* 2011;408:399-404.
- 14 Hiyama A, Yokoyama K, Nukaga T, Sakai D, Mochida J: A complex interaction between wnt signaling and tn timerelated alpha in nucleus pulposus cells. *Arthritis Res Ther* 2013;15:R189.
- 15 Zhang D, Ma G, Hou M, Zhang T, Chen L, Zhao C: The Neuroprotective Effect of Puerarin in Acute Spinal Cord Injury Rats. *Cell Physiol Biochem* 2016;39:1152-1164.
- 16 Yang H, Xia Y, Lu SQ, Soong TW, Feng ZW: Basic fibroblast growth factor-induced neuronal differentiation of mouse bone marrow stromal cells requires FGFR-1, MAPK/ERK, and transcription factor AP-1. *J Biol Chem* 2008;283:5287-5295.
- 17 Xu J, He J, He H, Peng R, Xi J: Comparison of rn timerelated ngr and nep1-40 in acting on axonal regeneration after spinal cord injury in rat models. *Mol Neurobiol* 2016;
- 18 Dean RL, 3rd, Scozzafava J, Goas JA, Regan B, Beer B, Bartus RT: Age-related differences in behavior across the life span of the c57bl/6j mouse. *Exp Aging Res* 1981;7:427-451.
- 19 Montoya CP, Campbell-Hope LJ, Pemberton KD, Dunnett SB: The "staircase test": A measure of independent forelimb reaching and grasping abilities in rats. *J Neurosci Methods* 1991;36:219-228.
- 20 Vidal PM, Lemmens E, Geboes L, Vanganswinkel T, Nelissen S, Hendrix S: Late blocking of peripheral tn timerelated alpha is ineffective after spinal cord injury in mice. *Immunobiology* 2013;218:281-284.
- 21 Oshima T, Lee S, Sato A, Oda S, Hirasawa H, Yamashita T: Tn timerelated alpha contributes to axonal sprouting and functional recovery following traumatic brain injury. *Brain Res* 2009;1290:102-110.
- 22 Pineau I, Lacroix S: Proinflammatory cytokine synthesis in the injured mouse spinal cord: Multiphasic expression pattern and identification of the cell types involved. *J Comp Neurol* 2007;500:267-285.
- 23 Oda Y, Tani K, Asari Y, Quintanilha LF, Haraguchi T, Momota Y, Katayama M, Itamoto K, Nakazawa H, Taura Y: Canine bone marrow stromal cells promote functional recovery in mice with spinal cord injury. *J Vet Med Sci* 2014;76:905-908.
- 24 Han S, Wang B, Li X, Xiao Z, Han J, Zhao Y, Fang Y, Yin Y, Chen B, Dai J: Bone marrow-derived mesenchymal stem cells in three-dimensional culture promote neuronal regeneration by neurotrophic protection and immunomodulation. *J Biomed Mater Res A* 2016;104:1759-1769.
- 25 Banzola I SG, Sulser T, et al: IFN- γ and TNF- α favor the progression of prostate cancer in distinct ways. *Cancer Research* 2012;72:4278-4278.
- 26 Fang B, Li XQ, Bi B, Tan WF, Liu G, Zhang Y, Ma H: Dexmedetomidine attenuates blood-spinal cord barrier disruption induced by spinal cord ischemia reperfusion injury in rats. *Cell Physiol Biochem* 2015;36:373-383.
- 27 Garraway SM, Woller SA, Huie JR, Hartman JJ, Hook MA, Miranda RC, Huang YJ, Ferguson AR, Grau JW: Peripheral noxious stimulation reduces withdrawal threshold to mechanical stimuli after spinal cord injury: Role of tumor necrosis factor alpha and apoptosis. *Pain* 2014;155:2344-2359.
- 28 Huang B, Xie Q, Lu X, Qian T, Li S, Zhu R, Yu W, Chen G, Chen Z, Xu X, Wang T, Li L: GlyT1 Inhibitor NFPS Exerts Neuroprotection via GlyR Alpha1 Subunit in the Rat Model of Transient Focal Cerebral Ischaemia and Reperfusion. *Cell Physiol Biochem* 2016;38:1952-1962.
- 29 Nosworthy MG, Brunton JA: Cysteinyglycine reduces mucosal proinflammatory cytokine response to fMLP in a parenterally-fed piglet model. *Pediatr Res* 2016;80:293-298.
- 30 Han D, Wu C, Xiong Q, Zhou L, Tian Y: Anti-inflammatory mechanism of bone marrow mesenchymal stem cell transplantation in rat model of spinal cord injury. *Cell Biochem Biophys* 2015;71:1341-1347.
- 31 Schafers M, Svensson CI, Sommer C, Sorkin LS: Tumor necrosis factor-alpha induces mechanical allodynia after spinal nerve ligation by activation of p38 mapk in primary sensory neurons. *J Neurosci* 2003;23:2517-2521.

- 32 McAlpine FE, Lee JK, Harms AS, Ruhn KA, Blurton-Jones M, Hong J, Das P, Golde TE, LaFerla FM, Oddo S, Blesch A, Tansey MG: Inhibition of soluble tn timer signaling in a mouse model of alzheimer's disease prevents pre-plaque amyloid-associated neuropathology. *Neurobiol Dis* 2009;34:163-177.
- 33 McCoy MK, Martinez TN, Ruhn KA, Szymkowski DE, Smith CG, Botterman BR, Tansey KE, Tansey MG: Blocking soluble tumor necrosis factor signaling with dominant-negative tumor necrosis factor inhibitor attenuates loss of dopaminergic neurons in models of parkinson's disease. *J Neurosci* 2006;26:9365-9375.
- 34 Shimizu T, Kagawa T, Wada T, Muroyama Y, Takada S, Ikenaka K: Wnt signaling controls the timing of oligodendrocyte development in the spinal cord. *Dev Biol* 2005;282:397-410.
- 35 Hay DC, Fletcher J, Payne C, Terrace JD, Gallagher RC, Snoeys J, Black JR, Wojtacha D, Samuel K, Hannoun Z, Pryde A, Filippi C, Currie IS, Forbes SJ, Ross JA, Newsome PN, Iredale JP: Highly efficient differentiation of hescs to functional hepatic endoderm requires activina and wnt3a signaling. *Proc Natl Acad Sci U S A* 2008;105:12301-12306.
- 36 Hiyama A, Sakai D, Risbud MV, Tanaka M, Arai F, Abe K, Mochida J: Enhancement of intervertebral disc cell senescence by wnt/beta-catenin signaling-induced matrix metalloproteinase expression. *Arthritis Rheum* 2010;62:3036-3047.
- 37 Hiyama A, Sakai D, Arai F, Nakajima D, Yokoyama K, Mochida J: Effects of a glycogen synthase kinase-3beta inhibitor (lcl) on c-myc protein in intervertebral disc cells. *J Cell Biochem* 2011;112:2974-2986.
- 38 Hiyama A, Sakai D, Tanaka M, Arai F, Nakajima D, Abe K, Mochida J: The relationship between the wnt/beta-catenin and tgfbeta/bmp signals in the intervertebral disc cell. *J Cell Physiol* 2011;226:1139-1148.
- 39 Zhao H, Cheng L, Du X, Hou Y, Liu Y, Cui Z, Nie L: Transplantation of cerebral dopamine neurotrophic factor transduced bmscs in contusion spinal cord injury of rats: Promotion of nerve regeneration by alleviating neuroinflammation. *Mol Neurobiol* 2016;53:187-199.
- 40 Ohta M, Suzuki Y, Noda T, Ejiri Y, Dezawa M, Kataoka K, Chou H, Ishikawa N, Matsumoto N, Iwashita Y, Mizuta E, Kuno S, Ide C: Bone marrow stromal cells infused into the cerebrospinal fluid promote functional recovery of the injured rat spinal cord with reduced cavity formation. *Exp Neurol* 2004;187:266-278.
- 41 Chi LY, Yu J, Zhu H, Li XG, Zhu SG, Kindy MS: The dual role of tumor necrosis factor-alpha in the pathophysiology of spinal cord injury. *Neurosci Lett* 2008;438:174-179.