Original Paper

Suppression of MicroRNA-383 Enhances Therapeutic Potential of Human Bone-Marrow-Derived Mesenchymal Stem Cells in Treating Spinal Cord Injury via GDNF

Guo-Jun Wei^a Gang An^a Zuo-Wei Shi^a Kai-Fu Wang^a Ying Guan^a
Yan-Song Wang^a Bo Han^a En-Ming Yu^a Peng-Fei Li^a Da-Ming Dong^a
Li-ping Wang^b Zhao-wei Teng^c De-lai Zhao^d

^aDepartment of Orthopaedics, the 1st Affiliated Hospital of Harbin Medical University, Harbin, China;

^bSansom Institute for Health Research, School of Pharmacy and Medical Sciences, University of South

Australia, Adelaide, Australia; ^cDepartment of Orthopedics, The People's Hospital of Yuxi City, The 6th

Affiliated Hospital of Kunming Medical University, Yuxi; ^dDepartment of Orthopedics, the Fifth Hospital of Harbin, Harbin, China

Key Words

Glial cell line derived neurotrophic factor (GDNF) • Mesenchymal stem cells (MSCs) • miR-383 • Spinal cord injury (SCI)

Abstract

Background/Aims: Transplantation of bone-marrow-derived mesenchymal stem cells (MSCs) has been used to treat spinal cord injury (SCI) to enhance tissue repair and neural cell regeneration. Glial cell line derived neurotrophic factor (GDNF) is an identified neural growth and survival factor. Here, we examined whether modification of GDNF levels in MSCs may further increase the potential of MSCs in promoting neural cell regeneration and subsequently the therapeutic outcome. **Methods:** We examined the mRNA and protein levels of GDNF in human MSCs by RT-qPCR and Western blot, respectively. Bioinformatics analyses were done to predict microRNAs (miRNAs) that target GDNF in MSCs. The functional binding of miRNAs to GDNF mRNA was examined by a dual luciferase reporter assay. MSCs were transduced with adeno-associated virus (AAV) carrying null or antisense for miR-383 (as-miR-383), which were transplanted into nude rats that underwent SCI. The intact tissue, cavity volume, and recovery of locomotor activity were assessed. **Results:** MSCs expressed very low GDNF protein, but surprisingly high levels of GDNF mRNA. Bioinformatics analyses showed that miR-383 inhibited protein translation of GDNF, through binding to the 3'-UTR of the GDNF mRNA. MSCs transduced with AAV-as-miR-383 further increased the intact tissue percentage, decreased cavity volume, and enhanced the recovery of locomotor activity in nude rats that underwent SCI, compared to MSCs. **Conclusions:** Suppression of miR-383 may increase the therapeutic potential of human bone-marrow-derived MSCs in treating SCI via augmentation of GDNF protein levels.

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

G.-J. Wei and G An contributed equally to this work.

Da-Ming Dong

Department of Orthopaedics, The 1st Affiliated Hospital of Harbin Medical University, 23 Youzheng Street, Harbin 150001 (China)
Tel. +8645185555233, E-Mail dongdaming1965@hotmail.com

Introduction

Bone-marrow-derived mesenchymal stem cells (MSCs) have the potential of differentiating into different mesenchymal lineages [1-5]. Recently, MSCs have been shown to have significant therapeutic effects on a variety of diseases, since mesenchymal cells play a critical role in tissue repair and organ remodeling. For example, Li et al showed that transplantation of MSCs mitigates the severity of cirrhosis by secreting BMP7 to contradict the fibrogenic effects of transforming growth factor beta 1 (TGF-beta1) in the injured liver [5]. In another elegant study, transplantation of mesenchymal stem cells (MSCs) alleviated dextran sulfate sodium -induced colitis through their crosstalk with macrophages via TGF-beta receptor signaling [4]. Moreover, transplantation of MSCs has been shown to promote post-injury cardiac muscle repair through augmentation of neovascularization [3].

Spinal cord injury (SCI) is a common disease in humans. Bone-marrow-derived MSCs have been used to transplant into spinal cord after injury to enhance tissue repair and neural cell regeneration [6-8]. Glial cell line derived neurotrophic factor (GDNF) was initially found to be a neural growth and survival factor [9], but, subsequently, found to be a critical trophic factor for development of enteric, sympathetic and parasympathetic neurons. Hence, overexpression of GDNF in MSCs appears to be an attractive strategy to further increase the potential of MSCs in promoting neural cell regeneration and subsequently the therapeutic outcome.

MicroRNAs (miRNAs) are non-coding small RNAs that mediate post-transcriptional gene regression, mainly through Watson-Crick pairing to the 3'-untranslated region (3'-UTR) of the mRNA of a specific gene. During many physiological and pathological events, miRNAs appear to play a critical role [10-13]. Among all miRNAs, the function of miR-383 has been found to have different functions in different conditions [14-19]. However, a role of miR-383 in regulation of the therapeutic potential of MSCs in SCI has not reported. Here we addressed this question.

In the current study, we examined the mRNA and protein levels of GDNF in human MSCs by RT-qPCR and Western blot, respectively. Bioinformatics analyses were done to predict microRNAs (miRNAs) that target GDNF in MSCs. The functional binding of miRNAs to GDNF mRNA was examined by a dual luciferase reporter assay. MSCs were transduced with adeno-associated virus (AAV) carrying null or antisense for miR-383 (as-miR-383), which were transplanted into nude rats that underwent SCI. The intact tissue, cavity volume and recovery of locomotor activity were assessed. We found that MSCs expressed very low GDNF protein, but a surprisingly high level of GDNF mRNA. Bioinformatics analyses showed that miR-383 inhibited protein translation of GDNF, through binding to the 3'-UTR of the GDNF mRNA. MSCs transduced with AAV-as-miR-383 further increased the intact tissue percentage, decreased cavity volume, and enhanced the recovery of locomotor activity in nude rats that underwent SCI, compared to those receiving MSCs.

Materials and Methods

Protocol approval

All the experimental methods in the current study have been approved by the research committee at Harbin Medical University. All the experiments have been carried out in accordance with the guidelines from the research committee at Harbin Medical University. All animal experiments were approved by the Institutional Animal Care and Use Committee at Harbin Medical University (Animal Welfare Assurance). Surgeries were performed in accordance with the Principles of Laboratory Care, supervised by a qualified veterinarian.

AAV production and transduction of MSCs

The Human Embryonic Kidney 293 cell line (HEK293) was used for virus production. We used a pAAV-CMVp-GFP plasmid (Clontech, Mountain View, CA, USA), a packaging plasmid carrying the serotype 2 rep

(which is the best among all serotypes for transduction of the MSCs) and cap genes, and a helper plasmid carrying the adenovirus helper functions (Applied Viromics, LLC, Fremont, CA, USA) for generating AAVs in this study. The constructs with a 2A sequence were cloned into a pAAV-CMVp-GFP backbone at the site between CMVp and GFP. AAVs were produced by co-transfecting HEK293 cells with the prepared pAAV-CMVp-miR-383/pAAV-CMVp-as-miR-383/pAAV-CMVp-Null plasmids, R2C8 (containing AAV2 Rep and AAV2 capsid genes) and pAd5 (containing adenovirus helper genes) by Lipofectamine 2000 (Invitrogen, St. Louis, MO, USA). The viruses were purified using CsCl density centrifugation and then titrated by a quantitative densitometric dot-blot assay. These viruses were used to transduce MSCs using a MOI of 50. The transduced cells expressed GFP, which allowed them to be purified by flow cytometry.

Cell isolation and culture

Human bone-marrow derived MSCs were isolated from a 40-year-old male healthy donor, who had received proper informed consent form after IRB approval was obtained. MSCs were collected into DMEM culture medium (Dulbecco's Modified Eagle's Medium, Gibco, San Diego, CA, USA). The cells were centrifuged and re-suspended in DMEM containing inactivated 10% fetal bovine serum (FBS, Gibco), 3.7g/l HEPES (N-2-hydroxyethylpiperazine-N'-2-ethane-sulphonic acid, Sigma-Aldrich, St. Louis, MO, USA), 1% 200 mmol/l L-glutamine 100x (Gibco) and 1% PSA (Gibco). The cell number and viability were determined by trypan blue staining (Gibco). The cells were incubated in a humidified chamber with 5% CO₂ at 37°C for 72 h. The adherent cells were considered MSCs and were maintained in culture until reaching 80% confluence. The passage 6 was used for the current study. A human GDNF-expressing glial cell line, B49, was obtained from American Type Culture Collection (ATCC, Rockville, MD, USA). B49 cells were cultured in RPMI1640 medium (Invitrogen, Carlsbad, CA, USA) supplemented with 15% FBS in a humidified chamber with 5% CO₂ at 37 °C.

Differentiation of MSCs

A MSC clone was selected and subjected to chondrogenetic, osteogenic, and adipogenic differentiation assays. For chondrogenetic induction, 2.5×10⁵ MSCs were induced with 5ml chondrogenetic induction medium containing 10μg transforming growth factor β1 (TGFβ1, R&D System, Los Angeles, CA, USA), 50μg insulin growth factor 1 (IGF-1, R&D System). The cells were maintained in the chondrogenetic induction medium for 14 days and then subjected to Alcian blue staining. For osteogenic induction, cells were digested and seeded onto a 24-well plate at a density of 10⁴ cells/well, and then maintained in osteogenic induction medium containing 10nmol/l Vitamin D3 (Sigma-Aldrich) and 10mmol/l β-phosphoglycerol and 0.1μmol/l DMSO for 14 days before subjected to Von kossa staining. For adipogenic induction, cells were digested and seeded onto a 24-well plate at a density of 10⁴ cells/well, and then maintained in the adipogenic induction medium containing 0.5mmol/l 3-isobutyl-1-methylxanthine (IBMX), 200μmol/l indomethacin, 10μmol/l insulin and 1μmol/l DMSO for 14 days before subjected to Oil red O staining.

SCI model, GDNF injection and MSC transplantation

SCI was induced in male nude rats (SLAC Laboratory Animal, Shanghai, China) of 200g body weight. The nude rats are immune-deficient, and therefore have limited immune responses to the transplanted human cells. Rats were deeply anesthetized by intraperitoneal injection of chloral hydrate (70 mg/ml, 0.4 ml per 100 g, Sigma-Aldrich). After skin incision, laminectomy at the Th8 vertebral level was performed. The impact rod (diameter 2 mm, weight 10 g) of an impactor was centered above Th8 and dropped from a height of 25 mm to induce SCI. Immediately after laminectomy or SCI, MSCs were injected at 10⁶/5μl and/or GDNF was injected at 100μg/5μl in saline intraspinally into the rats, using a 25G needle. The local injection of MSCs reduced great scale of cell migration out of the injected area. After surgery, rats received daily doses of gentamicin (25 mg/kg, Sigma-Aldrich) intramuscularly for 7 consecutive days. Bladders of injured rats were manually emptied twice daily until spontaneous voiding occurred.

Quantitative real-time PCR (RT-qPCR)

Total RNA were extracted from cultured cells with miRNeasy mini kit (Qiagen, Hilden, Germany) for cDNA synthesis. Quantitative real-time PCR (RT-qPCR) was performed in duplicates with QuantiTect SYBR Green PCR Kit (Qiagen). All primers were purchased from Qiagen. Data were collected and analyzed using 2-ΔΔCt method. Values of genes were first normalized against α-tubulin, and then compared to experimental controls.

Table 1. Prediction of human GDNF-binding miRNAs by Bioinformatics. Predicted targets of each miRNA family are sorted by total context++ score. The representative miRNA is the miRNA in its family with the most favorable (lowest) total context++ score. From all the candidates with the highest score (low values), has-miR-383 was detected to be highly expressed in human MSCs

miRNA	Position in the UTR	context++ score
hsa-miR-133a	53-60	-0.56
hsa-miR-1271-5p	1038-1045	-0.42
hsa-miR-96-5p	1038-1045	-0.36
hsa-miR-9-5p	2897-2903	-0.36
hsa-miR-182-5p	1039-1045	-0.24
hsa-miR-125b-5p	277-283	-0.2
hsa-miR-4319	277-283	-0.18
hsa-miR-142-5p	2914-2920	-0.16
hsa-miR-326	892-898	-0.15
hsa-miR-411-3p	853-860	-0.12
hsa-miR-379-3p	853-860	-0.12
hsa-miR-383	2866-2872	-0.12
hsa-miR-212-5p	100-106	-0.08
hsa-miR-493-5p	2924-2930	-0.08
hsa-miR-145-5p	210-216	-0.06

Western blot

Proteins were isolated from cultured cells. Primary antibodies were rabbit anti-GDNF and anti- α -tubulin (Cell Signaling, Carpinteria, CA, USA). Secondary antibody is HRP-conjugated anti-rabbit (Dako, Carpinteria, CA, USA). Figure images were representative from 5 repeats. α -tubulin was used as a protein loading control.

Luciferase-reporter activity assay

The target gene of miR-365 was predicted by TargetScan, using the context++ score system, as described [20]. The candidate miRNAs are shown in Table 1. The dual-luciferase reporter plasmids, p3'-UTR-GDNF (containing the wild-type GDNF 3'-UTR binding site in luciferase reporter plasmid (RiboBio Co. Ltd., China)) and p3'-UTR-GDNF-mut (containing the mutant GDNF 3'-UTR; mut) were constructed. For the luciferase assay, the constructed plasmid (500ng) and miR-383 (mir-383) (100 nmol/l) were co-transfected into MSCs using Lipofectamine™ 3000 Reagent (Invitrogen, USA). Then the luciferase activity was detected with the dual-luciferase reporter assay system (Promega, USA) after co-transfection cells for 48 hours, following the manufacturer's protocol.

Histological assessment

For histological assessment, 28 days after transplantation rats were anesthetized with chloral hydrate, prior to intracardiac perfusion with 4% paraformaldehyde (PFA, Sigma-Aldrich). The tissue was resected and fixed in PFA for 6 hours. After overnight incubation in 30% sucrose, samples were frozen in liquid nitrogen and embedded in tissue freezing medium. Non-fixed tissue was used for RT-PCR and Western blot. Twenty micron longitudinal tissue sections were then stained with Azur-eosin for measurement of tissue and cavity volume. The combined area of any cysts was calculated and subtracted from the total tissue area of intact four section to estimate total tissue remaining as described previously [21].

Behavioral assessment

Locomotor recovery was evaluated using the open-field BBB locomotor ratio scale, as described before [21]. Briefly, the motor functions of the lower limbs of the rats were evaluated in a blind way. There are 5 groups of rats of 10 each. Group 1, sham, no SCI and no MSCs; group 2, SCI, no MSCs; group 3, SCI, null-transduced MSCs; group 4, SCI, null-transduced MSCs and single injection of GDNF; group 5, SCI, as-miR-383-transduced MSCs. For the scoring system, the motor functions of the lower limbs are divided into 22 grades. While 0 points indicate hind leg paralysis, 21 indicates completely normal function. The BBB score observation period lasted for 4 min.

Statistical analysis

All values represent the mean $\pm$ standard deviation (SD). Statistical analysis of group differences was carried out using a one-way analysis of variance (ANOVA) test (SPSS 12.0, Chicago, IL, USA) followed by the Fisher's Exact Test to compare two groups. Ten rats were used in each experimental group. A value of $p < 0.05$ was considered statistically significant after Bonferroni correction.

Fig. 1. Human MSCs express low GDNF protein but high GDNF mRNA. GDNF levels in human MSCs were compared to a GDNF-expressing glial cell line, B49. (A) GDNF protein levels by Western blot (B) GDNF mRNA levels by RT-qPCR. * $p < 0.05$. NS: non-significant. N=5.

Fig. 2. MiR-383 targets 3'-UTR of GDNF mRNA to inhibit its translation in MSCs. (A) The expression levels of 15 GDNF-binding miRNAs in MSCs by RT-qPCR. (B) Bioinformatics analyses show the binding site of miR-383 on the 3'-UTR of GDNF mRNA. (C) MSCs cells were transfected with either miR-383 or antisense for miR-383 (as-miR-383). The MSCs were also transfected with a null sequence as a control (null). Modulation of miR-383 levels in these cells was confirmed by RT-qPCR. (D) Then, the intact 3'-UTR of GDNF mRNA (GDNF 3'-UTR), together with a 3'-UTR with mutant at miR-383-binding site of GDNF mRNA (GDNF 3'-UTR mut), was then cloned into luciferase reporter plasmids. MSCs cells were co-transfected with 1 μ g as-miR-383/null plasmids and 1 μ g GDNF 3'-UTR or GDNF 3'-UTR mut plasmids or co-transfected with 1 μ g miR-383/null plasmids and 1 μ g GDNF 3'-UTR or GDNF 3'-UTR mut plasmids. The luciferase activity was determined. * $p < 0.05$. N=5.

Results

Human MSCs express low GDNF protein but high GDNF mRNA

We isolated human MSCs, and examined the GDNF levels in MSCs, compared to a GDNF-expressing glial cell line, B49. To our surprise, MSCs expressed very low GDNF protein (Fig. 1A), but comparable level of GDNF mRNA to B49 cells (Figure 1B). These data suggest a possibility of presence of post-transcriptional control of GDNF in MSCs.

MiR-383 targets 3'-UTR of GDNF mRNA to inhibit its translation in MSCs

Next, we tried to find out whether GDNF protein translation may be regulated by miRNAs. Thus, we performed bioinformatics analyses to identify the GDNF target sequence for miRNAs that binds to the 3'-UTR of GDNF mRNA. Based on a scoring system named "context++ score" [20], we found 15 candidate miRNAs that effectively target GDNF (Table

Fig. 3. Preparation of miR-383-depleted MSCs. MSCs were transduced with AAV carrying as-miR-383 or control null. (A-B) The construct also contained a GFP reporter to allow visualization of the transduced cells in culture (A), and to allow purification of transduced cells by flow cytometry (B). (C) Western blot for GDNF in as-miR-383-transduced MSCs. * $p < 0.05$. N=5.

1). Then we measured the expression levels of these miRNAs in MSCs, and specifically found that miR-383 expression is extremely high in MSCs, compared to others (Fig. 2A). Bioinformatics analyses showed that miR-383 bound to 3'-UTR of GDNF mRNA at 2866th-2872nd base site (Fig. 2B). To determine whether the binding of miR-383 to GDNF mRNA may functionally inhibit protein translation of GDNF, we transfected MSCs cells with either miR-383 or antisense for miR-383 (as-miR-383). The MSCs were also transfected with a null sequence as a control (null). Modulation of miR-383 levels in these cells was confirmed by RT-qPCR (Fig. 2C). Then, the intact 3'-UTR of GDNF mRNA (GDNF 3'-UTR), together with a 3'-UTR with mutant at miR-383-binding site of GDNF mRNA (GDNF 3'-UTR mut), was cloned into luciferase reporter plasmids. MSCs cells were co-transfected with 1μg as-miR-383/null plasmids and 1μg GDNF 3'-UTR or GDNF 3'-UTR mut plasmids or co-transfected with 1μg miR-383/null plasmids and 1μg GDNF 3'-UTR or GDNF 3'-UTR mut plasmids. The results suggest that miR-383 may specifically target 3'-UTR of GDNF mRNA to inhibit its translation in MSCs (Fig. 2D).

Preparation of miR-383-depleted MSCs

Next, we knocked down miR-383 in MSCs to see whether it may increase the protein levels of GDNF. If true, we also aimed to test the therapeutic potential of using these miR-383-depleted MSCs in animal models for SCI. Hence, we used AAV carrying as-miR-383 or control null to transduce MSCs. The construct also contained a GFP reporter to allow visualization of the transduced cells in culture (Fig. 3A), and to allow purification of transduced cells by flow cytometry (Fig. 3B). We found that overexpression of as-miR-383 in MSCs increased GDNF levels by about 16 fold (Fig. 3C).

MSC property of miR-383-depleted MSCs

Before we transplanted these miR-383-depleted MSCs into nude rats, we tried to confirm that alteration of miR-383 levels did not change the MSC property of MSCs. We selected an as-miR-383-transduced MSCs and analyzed for cell surface markers CD73, CD90, CD105, CD31, CD45 and HLA-DR, which confirmed the MSC phenotype of this clone (Fig. 4A-B). Afterwards, we confirmed the MSC properties of this clone by 3 differentiation assay (Fig. 4C-E).

Fig. 4. MSC property of miR-383-depleted MSCs. (A) A selected clone of as-miR-383-transduced MSCs was analyzed for cell surface markers CD73, CD90, CD105, CD31, CD45 and HLA-DR for confirmation of MSC phenotype. (B) A representative image of this clone in culture. (C-E) The MSC clone was examined for differentiation properties by Von kossa staining to evaluate osteogenic induction (C), Oil red O staining to evaluate adipogenic induction (D) and Alcian blue staining to evaluate chondrogenetic induction (E). Scale bars are 50µm.

Depletion of miR-383 in MSCs increases their therapeutic potentials in treating SCI in rats

Finally, we examined the effects of miR-383 depletion in MSCs *in vivo* in rat model for SCI. SCI was induced in a nude rat. Immediately after laminectomy or SCI, MSCs of null transduced or as-miR-383 transduced were injected intraspinally. Another control was MSCs-null with single injection of GDNF. The rats were then followed up for 28 days (Fig. 5A). We found that miR-383-depletion in MSCs further increased the intact tissue percentage (Fig. 5B), decreased cavity volume (Fig. 5C), and enhanced the recovery of locomotor activity in nude rats that underwent SCI (Fig. 5D), compared to null-transduced MSCs. MiR-383-depletion in MSCs on SCI recovery was at least as effective as additional injection of GDNF (Fig. 5B-D). Thus, depletion of miR-383 in MSCs increases their therapeutic potentials in treating SCI in rats.

Discussion

Bone-marrow-derived MSCs have been used to treat SCI. However, enhancement of tissue repair and neural cell regeneration are needed to improve therapeutic outcomes [6-8]. Thus, very recent approaches have been applied to modify gene expression of MSCs, using successful experiences from studies of other fields. For example, Liu et al. showed that during treating mouse model of Alzheimer's disease with MSCs, depletion of miR-937 in MSCs significantly reduced the deposition of Abeta, increased the levels of BDNF, and significantly improved the therapeutic outcome in mice [22]. In another study, Yan et al. reported that transplantation of AAV carrying as-miR-548e partially mimicked the effects of MSC transplantation on collagen-induced arthritis in mice, possibly through suppressing miR-548e-mediated IkappaB inhibition [23].

Fig. 5. Depletion of miR-383 in MSCs increases their therapeutic potentials in treating SCI in rats. (A) Schematic of *in vivo* experiment. SCI was induced in nude rats. Immediately after laminectomy or SCI, MSCs and/or GDNF were injected intraspinally. The rats were then followed up for 28 days. There are 5 groups of rats of 10 each. Group 1, sham, no SCI and no MSCs; group 2, SCI, no MSCs; group 3, SCI, null-transduced MSCs; group 4, SCI, null-transduced MSCs and single injection of GDNF; group 5, SCI, as-miR-383-transduced MSCs. (B) Intact tissue percentage in 4 groups (C) Cavity volume in 4 groups (D) The assessment of locomotor activity by BBB method. * $p < 0.05$. $N = 10$.

GDNF, neural growth and survival factor, was recently used to modify MSCs to improve their effects on SCI [24]. In this report, the authors overexpressed GDNF mRNA in MSCs by thousands of times to reach improvement of therapeutic effects comparable to our study. However, we think that since here we found that GDNF in MSCs is post-transcriptionally controlled, the most effective approach should be removal or dismissal of this control, rather than simply overwhelmingly expressing high mRNA for GDNF. First, extreme overexpression of one mRNA will occupy a lot of cellular machinery, which may affect other biological processes. Second, the extreme overexpression of one mRNA to contradict an antagonizing miRNA may induce upregulation of that miRNA and cause protein expression and function disorder, leading to occurrence of ER stress.

Here, we knocked down a highly expressed GDNF-antagonizing miRNA in MSCs, which effectively increased the protein levels of GDNF. To the best of our knowledge, this is the first study to show that the therapeutic effects of MSCs on SCI model are augmented through modification of a miRNA in MSCs. Further elucidation of the underlying mechanisms may involve determination of the miR-383 target genes other than GDNF, e.g. ZEB2, GATA6, VEGF-A, which are important regulators for neovascularization and extracellular matrix degradation. The miR-383 depletion may enhance the expression of these genes in MSCs, which also contribute to the tissue repair during SCI recovery.

Compared to trophic factor infusion (here, GDNF injection), a cell-based therapy has several advantages. First, the dose is regulated in a biological manner and thus results in less side effects. Second, the release of the factors is consistent rather the pulse release by trophic factor infusion, and thus may result in a better outcome. Here, we found that compared to MSCs plus GDNF injection, knock-down of as-miR-383 in MSCs has a comparable effect on the increases in the intact tissue percentage and the recovery of locomotor activity in nude rats that underwent SCI, but has an improved effects on the decreases in cavity volume.

To summarize, the results from this study highly suggest that suppression of miR-383 may increase the therapeutic potential of human bone-marrow-derived MSCs in treating SCI via augmentation of GDNF protein levels.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (Grant No. 81371368).

Disclosure Statement

The authors declare no competing financial interests.

Reference

- 1 Wang M, Zhang G, Wang Y, Liu T, Zhang Y, An Y, Li Y: Crosstalk of mesenchymal stem cells and macrophages promotes cardiac muscle repair. *Int J Biochem Cell Biol* 2015;58:53-61.
- 2 Cao X, Han ZB, Zhao H, Liu Q: Transplantation of mesenchymal stem cells recruits trophic macrophages to induce pancreatic beta cell regeneration in diabetic mice. *Int J Biochem Cell Biol* 2014;53:372-379.
- 3 Zhang J, Wu Y, Chen A, Zhao Q: Mesenchymal stem cells promote cardiac muscle repair via enhanced neovascularization. *Cell Physiol Biochem* 2015;35:1219-1229.
- 4 Liu W, Zhang S, Gu S, Sang L, Dai C: Mesenchymal Stem Cells Recruit Macrophages to Alleviate Experimental Colitis Through TGFbeta1. *Cell Physiol Biochem* 2015;35:858-865.
- 5 Li B, Shao Q, Ji D, Li F, Chen G: Mesenchymal Stem Cells Mitigate Cirrhosis through BMP7. *Cell Physiol Biochem* 2015;35:433-440.
- 6 Ribeiro TB, Duarte AS, Longhini AL, Pradella F, Farias AS, Luzo AC, Oliveira AL, Olalla Saad ST: Neuroprotection and immunomodulation by xenografted human mesenchymal stem cells following spinal cord ventral root avulsion. *Scientific reports* 2015;5:16167.
- 7 Ding Y, Zhang RY, He B, Liu Z, Zhang K, Ruan JW, Ling EA, Wu JL, Zeng YS: Combination of electroacupuncture and grafted mesenchymal stem cells overexpressing TrkC improves remyelination and function in demyelinated spinal cord of rats. *Scientific reports* 2015;5:9133.
- 8 Chen D, Zeng W, Fu Y, Gao M, Lv G: Bone marrow mesenchymal stem cells combined with minocycline improve spinal cord injury in a rat model. *Int J Clin Exp Pathol* 2015;8:11957-11969.
- 9 Lin LF, Doherty DH, Lile JD, Bektesh S, Collins F: GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. *Science* 1993;260:1130-1132.
- 10 Zhu P, Zhang J, Zhu J, Shi J, Zhu Q, Gao Y: MiR-429 Induces Gastric Carcinoma Cell Apoptosis Through Bcl-2. *Cell Physiol Biochem* 2015;37:1572-1580.
- 11 Zhang T, Tian F, Wang J, Jing J, Zhou SS, Chen YD: Atherosclerosis-Associated Endothelial Cell Apoptosis by MiR-429-Mediated Down Regulation of Bcl-2. *Cell Physiol Biochem* 2015;37:1421-1430.
- 12 Sun DK, Wang JM, Zhang P, Wang YQ: MicroRNA-138 Regulates Metastatic Potential of Bladder Cancer Through ZEB2. *Cell Physiol Biochem* 2015;37:2366-2374.
- 13 Song W, Li Q, Wang L, Wang L: Modulation of FoxO1 Expression by miR-21 to Promote Growth of Pancreatic Ductal Adenocarcinoma. *Cell Physiol Biochem* 2015;35:184-190.
- 14 Lian J, Tian H, Liu L, Zhang XS, Li WQ, Deng YM, Yao GD, Yin MM, Sun F: Downregulation of microRNA-383 is associated with male infertility and promotes testicular embryonal carcinoma cell proliferation by targeting IRF1. *Cell Death Dis* 2010;1:e94.
- 15 Huang H, Tian H, Duan Z, Cao Y, Zhang XS, Sun F: microRNA-383 impairs phosphorylation of H2AX by targeting PNUITS and inducing cell cycle arrest in testicular embryonal carcinoma cells. *Cell Signal* 2014;26:903-911.

- 16 Yin M, Wang X, Yao G, Lu M, Liang M, Sun Y, Sun F: Transactivation of microRNA-320 by microRNA-383 regulates granulosa cell functions by targeting E2F1 and SF-1 proteins. *J Biol Chem* 2014;289:18239-18257.
- 17 Zhao L, Gu H, Chang J, Wu J, Wang D, Chen S, Yang X, Qian B: MicroRNA-383 regulates the apoptosis of tumor cells through targeting Gadd45g. *PLoS One* 2014;9:e110472.
- 18 Liao XH, Zheng L, He HP, Zheng DL, Wei ZQ, Wang N, Dong J, Ma WJ, Zhang TC: STAT3 regulated ATR via microRNA-383 to control DNA damage to affect apoptosis in A431 cells. *Cell Signal* 2015;27:2285-2295.
- 19 Pei L, Meng S, Yu W, Wang Q, Song F, Ma L: Inhibition of MicroRNA-383 Ameliorates Injury After Focal Cerebral Ischemia via Targeting PPARgamma. *Cell Physiol Biochem* 2016;39:1339-1346.
- 20 Agarwal V, Bell GW, Nam JW, Bartel DP: Predicting effective microRNA target sites in mammalian mRNAs. *ELife* 2015;4.
- 21 Basso DM, Beattie MS, Bresnahan JC: A sensitive and reliable locomotor rating scale for open field testing in rats. *J Neurotrauma* 1995;12:1-21.
- 22 Liu Z, Wang C, Wang X, Xu S: Therapeutic Effects of Transplantation of As-MiR-937-Expressing Mesenchymal Stem Cells in Murine Model of Alzheimer's Disease. *Cell Physiol Biochem* 2015;37:321-330.
- 23 Yan X, Cen Y, Wang Q: Mesenchymal stem cells alleviate experimental rheumatoid arthritis through microRNA-regulated IkappaB expression. *Sci Rep* 2016;6:28915.
- 24 Mukhamedshina YO, Garanina EE, Masgutova GA, Galieva LR, Sanatova ER, Chelyshev YA, Rizvanov AA: Assessment of Glial Scar, Tissue Sparing, Behavioral Recovery and Axonal Regeneration following Acute Transplantation of Genetically Modified Human Umbilical Cord Blood Cells in a Rat Model of Spinal Cord Contusion. *PLoS One* 2016;11:e0151745.