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MiR-223-5p inhibitor suppresses microglia inflammation and promotes Nrg-1 in rats of spinal cord injury

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Abstract. – OBJECTIVE: The aim of this study was to investigate the role of microRNA-233-5p (miR-233-5p) in spinal cord injury (SCI), and to explore the possible underlying mechanism.

MATERIALS AND METHODS: Microglia were first isolated from neonate rats and cultured in a suitable environment *in vitro*. Lipopolysaccharide (LPS) and interleukin-4 (IL-4) were used to activate microglia. The expressions of miR-223-5p, inducible nitric oxide synthase (iNOS) and arginase 1 (Arg-1) were measured by qRT-PCR, respectively. After transfection of miR-233-5p inhibitor, the expression levels of miR-223-5p, iNOS and Arg-1 in cells were detected as well. A moderate SCI model was successfully established in rats (10 g fallen on T10 spinal cord at the height of 5 cm). Subsequently, inflammation indexes at miR-223-5p peak moment were observed. Meanwhile, its neuro-protective effect at 28 days after SCI was estimated. Finally, Basso, Beattie, and Bresnahan (BBB) rating scale was applied to evaluate the hindlimb locomotor function of rats at 1, 3, 7, 14, 21, 28 days after SCI.

RESULTS: MiR-223-5p inhibitor significantly promoted M2 microglia expression and degenerated M1 microglia expression *in vitro*. SCI obviously elevated the level of miR-223-5p in injured spinal cord tissues within one week, which reached a peak at 5 days after injury. Meanwhile, miR-223-5p inhibitor remarkably reduced the expressions of inflammatory factors, including interleukin-1 beta (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) at 3 days after SCI, as well as increased neuregulin1 (NRG-1) expression. However, miR-223-5p inhibitor significantly declined the levels of apoptosis key enzyme-caspase-3 and glia reaction marker-glial fibrillary acidic protein (GFAP) at 7 and 28 days after SCI, respectively. As a result, BBB rating scale demonstrated that hindlimb locomotor function was significantly recovered in miR-223-5p injection group.

CONCLUSIONS: MiR-223-5p was up-regulated in M1 microglia, whereas down-regulated in M2 microglia. MiR-223-5p inhibitor could significantly increase M2 microglia expression, while decrease M1 microglia expression *in vitro*. *In vivo*, miR-223-5p inhibitor suppressed the inflammatory response and reinforced NRG-1 level to reduce glia reaction and neuron apoptosis. Thereby, its treatment promoted the hindlimb locomotor function of rats.

Key Words:
MiR-223-5p, Glia inflammation, Microglia, Locomotor function, SCI, Neuregulin1, Nrg-1.

Introduction

Spinal cord injury (SCI) is a common nervous system disease occurring after a vertebral column trauma. Some of the main symptoms of SCI are transient ruins of nervous function and stress disorder of sensorimotor function[1,2]. In severe injuries, SCI appears permanent limb paralysis below the level of lesions[3]. Previous studies have indicated that complicated reasons are involved in the germination of SCI. With the emergence of primary injury sites, a series of biological feedbacks and pathological alterations will occur in the spinal cord[4]. An injury of the blood-spinal cord barrier (BSCB) and activation of spontaneous immune lead to ischemia and inflammation of the injured marginal spinal cord and extend to distal[5,6]. Meanwhile, the activation of inflammatory microglia and the release of inflammatory cytokines in the early stage of SCI result in an intense local inflammatory microenvironment. This may eventually promote the apoptosis of neurons and the proliferation of glial cells[7-9]. Due to large-

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scale glial environment in the middle and late stages of SCI, these pathological variables prevent neuronal repair and axon regeneration as well[10,11]. The recovery of SCI is closely related to the early inflammatory activation of microglia. Meanwhile, the degree of nerve regeneration depends on secondary inflammatory glial proliferation[12,13]. Traditionally, microglia is divided into two types, including: M1 inflammatory microglia and M2 anti-inflammatory microglia. This suggests that the increase of M1 type microglia contributes to the increase of inflammatory level, while M2 cells release anti-inflammatory factors to inhibit inflammatory progression[14,15]. MicroRNA (miRNA) is a class of non-coding single-stranded RNA molecules with about 22 nucleotides in length. MiRNAs are encoded by endogenous genes, which play an important role in regulating gene expression in cells[16]. Disorders in miRNA expressions are often associated with multiple pathological processes in the development of diseases[17,18]. Various alterations have been found in the expressions of miRNAs after SCI. Meanwhile, some miRNAs have been reported related to specific pathological factors[19]. Therefore, we speculated that the changes in miRNA expression after SCI in microglial polarization might lead to the regulation of inflammatory glial activation and aggravate the apoptosis level of neurons. Izumi et al[20] have displayed that inhibition of miR-223 reduces the activation of neutrophils after SCI. However, our present studies have shown that inhibition of miR-223-5p significantly declines the expression of M1 microglia, whereas enhanced the anti-inflammatory effect of M2 microglia. Activation of inflammatory cytokines is alleviated in rats by polarizing microglia expression and increasing the level of Nrg-1 factor. Moreover, neuronal apoptosis and glial scar extension are greatly reduced as well. Besides, we investigated the exact role of miR-223-5p in polarizing glial inflammation after SCI. Our results indicated that its inhibitor significantly increased the expression of anti-inflammatory microglia, thereby reducing secondary nerve disruption. The above findings suggested that miR-223-5p inhibitor could promote locomotor function.

Materials and Methods

Microglia Isolation and Treatment

This study was approved by the Animal Ethics Committee of Fudan University Animal Center. Primary microglia were first isolated from the cerebrum of 3-day-old neonate Sprague Dawley (SD) rats. Specific steps were as follows: Young rats were sacrificed, and their heads were immersed in 75% alcohol for 5 minutes. Brain tissues were then collected and placed in cold phosphate-buffered saline (PBS) containing 5% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA). Pial meninges and blood vessels on the surface of the cerebral cortex were removed with micro-dissection tweezers, followed by washing with PBS containing 5% FBS. Trypsin was added to the cerebral cortex for intercellular substance lysis, which was terminated by an equal volume of culture medium. The lysate was centrifuged at 1000 rpm for 5 min to remove the remaining PBS. Culture medium containing 10% FBS was added into a centrifuge tube, and the cells were inoculated into a culture flask covered with poly-D-lysine. After 10 to 14 days, the microglia were shaken down in a shaking bed (200 rpm, 3-4 h). On the next day, another batch of microglia (200 rpm, 2 h) was shaken down again. The density of inoculation was adjusted to 5-10^5 /mL. After microglia grew to a suitable density, they were treated with starvation for 24 hours. Subsequently, lipopolysaccharide (LPS) (100 ng/mL) and IL-4 (200 ng/mL) were used to activate the resting glial cells for 24 hours (pretreated with miR-223-5p inhibitor in inhibitor group). Finally, the cells were chosen for total RNA and protein extraction.

Animals and Grouping

A total of 70 female SD rats were sacrificed for the present study. All rats were aged from 6 to 8 weeks and weighted at 180-200 g. They were divided into three groups, including: sham group, SCI group and miR-223-5p inhibition group. In the sham group, a laminectomy was performed at T10 vertebra. In the SCI group, moderate spinal cord contusion was produced. In miR-223-5p inhibition group, miR-223-5p inhibitor was injected into the epidural space. Rats were fed at 25°*C* and 55% humidity, with free access to food and water. The circadian rhythm was 12/12 h light/dark cycle every day.

Surgery Procedure of SCI

0.8 mL 10% chloral hydrate was intraperitoneally injected for anesthesia. Back and skin disinfection preparation was performed in rats. An incision was first implemented at T10 spinous. Separation of fascia and deep muscles was continuously performed to reach the bony laminar layer. Then laminectomy was made at T10 to expose the spinal cord, and a collision needle was used to impact the spinal cord (10 g, 5 cm). When hematomyelia was visualized at impact location

and delayed hindlimb swing and tail-flick reflex occurred, the model was successfully established in rats. Subsequently, miR-223-5p inhibitor was injected into the endorachis at injury location in the corresponding group. Then, we gradually sutured the incision and disinfected the skin. All modeled rats were placed on a heat booster to maintain body temperature and await anesthesia resuscitation. Artificial urination was provided in modeled rats until they recovered urination reflex.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

1 mL TRIzol (Invitrogen, Carlsbad, CA, USA) was added to the spinal cord tissue and homogenized after shearing. Nucleic acid protein complex was completely separated after 5 minutes of incubation at room temperature. For microglia, 0.5 mL TRIzol was added to each of the six orifice plates, and ice shaker was used for 10 minutes. Subsequently, 0.2 mL chloroform was added to 1 mL TRIzol, and the tubes were violently shaken for 15 seconds, followed by incubation at room temperature for 3 minutes. After centrifugation for 15 minutes at 10000 rpm and 4°*C*), the upper water phase was collected and isopropyl alcohol was added. Next, the mixture was vibrated and placed at room temperature for 10 minutes. After centrifugation for 10 minutes at 10000 rpm and 4°*C*), RNA precipitation was obtained and the supernatant was discarded. After washing RNA precipitation with 75% ethanol, the mixture was centrifuged at 10000 rpm and 4°*C* for 5 minutes. After discarding the supernatant, 30 μL RNase free water was added to dissolve. The concentrations of extracted RNAs were measured on Nano Drop by determining absorbance at 260 nm, 230 nm and 280 nm, respectively. A260/A280 between 1.8 and 2.0 indicated standard quality of extracted RNA, which could be used in subsequent experiments. Quantitative analysis of mRNA was achieved using Prism 7300 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). 25 μL reaction system was used, including: 12.5 μL of SYBR Green, 10 Mm of primers (0.5 mL each from the stock), 10.5 μL of water and 0.5 μL of template. Specific PCR conditions were as follows: denaturation at 95°C for 10 min, 40 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 30 s and extension at 72°C for 30 s. Experimental data was analyzed by SDS software and EXCEL. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal reference. Comparative threshold cycle (Ct) method, namely, the $2^{-\Delta\Delta Ct}$ method was used to calculate fold amplification. Primer sequences used in this study were as follows: miR-233-5p, F: 5'-CCTGAATGATAGTGAGGAAC-3', R: 5'-GCTGAACGATTTGCCACACCA-3'; IL-6, F: 5'-CTTGTCCTATAGAAGCACATG-3', R: 5'-ATCATTTCCACAGCCCTGTG-3'; TNF-α: F: CCACCACGCTCTTCTGTCTACTG, R: GGGCTACGGGCTTGTCACT; IL-1β: F: CCCTGCAGCTGGAGAGTGTGG, R: TGTGCTCTGCTTGAGAGGTGCT; U6: F: GCTTCGGCAGCACATATACTAAAAT, R: CGCTTCAGAATTTGCGTGTCAT; GAPDH: F: CGCTCTCTGCTCCTCCTGTTC, R: ATCCGTTGACTCCGACCTTCAC.

Western Blotting Analysis

Spinal cord tissues or differently treated microglia were transformed into proteins on ice using a total protein extraction kit containing protease inhibitors and phosphatase inhibitors. Protein-containing compounds were centrifuged at 13000 rpm and 4°C for 15 minutes. The concentration of extracted protein was determined by the double bicinchoninic acid (BCA) method (Pierce, Rockford, IL, USA). After separated by 10% sodium dodecyl sulfate-polyacrylamide gel, protein samples were transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA) at 4°C for 2 h. 5% non-fatty milk was prepared with Tris-buffered saline containing Tween-20 (TBST), and the membranes were blocked with the prepared solution for 1 h. After washing 3 times with TBST, the membranes were incubated with primary antibodies (iNOS, Abcam, Cambridge, MA, USA, Rabbit, 1:100; Arg-1, Abcam, Cambridge, MA, USA, Rabbit, 1:500; Nrg-1, Abcam, Cambridge, MA, USA, Rabbit, 1:1000; caspase-3, Cell Signaling Technology, Danvers, MA, USA, Rabbit, 1:1000; GFAP, Cell Signaling Technology, Danvers, MA, USA, Rabbit, 1:1000;) at 4°C overnight. After washing 3 times with TBST, the membranes were incubated with corresponding secondary antibodies (Goat Anti-Rabbit IgG, YiFeiXue Biotechnology, Nanjing, China, 1:3000) at room temperature for 2 h. After that, the membranes were washed again with TBST for 3 times. Finally, immune-reactive bands were visualized by the enhanced chemical luminescence (ECL) method.

Behavioral Analysis

Locomotor function was estimated *via* the open-field BBB method in rats. Behavior of rats was observed, and their hindlimb function was estimated on 1, 3, 7, 14, 21 and 28 days after injury and miR-

233-5p treatment, respectively. Hindlimb BBB score was obtained by two investigators' evaluation who were blinded to the treatment group.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 18.0 statistical software (SPSS Inc., Chicago, IL, USA) was used for every statistical analysis. Measurement data were expressed as $\bar{x}\pm s$. The *t*-test was used to compare the difference between two groups. Single-factor analysis of variance (ANOVA) was used to compare the differences among different groups. Least Significant Difference (LSD) test or Student-Newman-Keuls (SNK) test was used for pairwise comparison under normal homogeneity of variance. $p < 0.05$ was considered statistically significant. All experiments were repeated for at least 3 times.

Results

MiR-233-5p Inhibitor Reduced M1 Microglia Expression and Increased M2 Microglia Expression

LPS and IL-4 were first used to activate resting microglia to M1 type and M2 type, respectively. Western blot was applied to detect the protein levels of M1 microglia maker iNOS and M2 microglia marker Arg-1 (Figure 1A and 1C). Results displayed that miR-223-5p inhibitor could significantly reduce iNOS expression, while increase Arg-1 expression (Figure 1B and 1D). All the

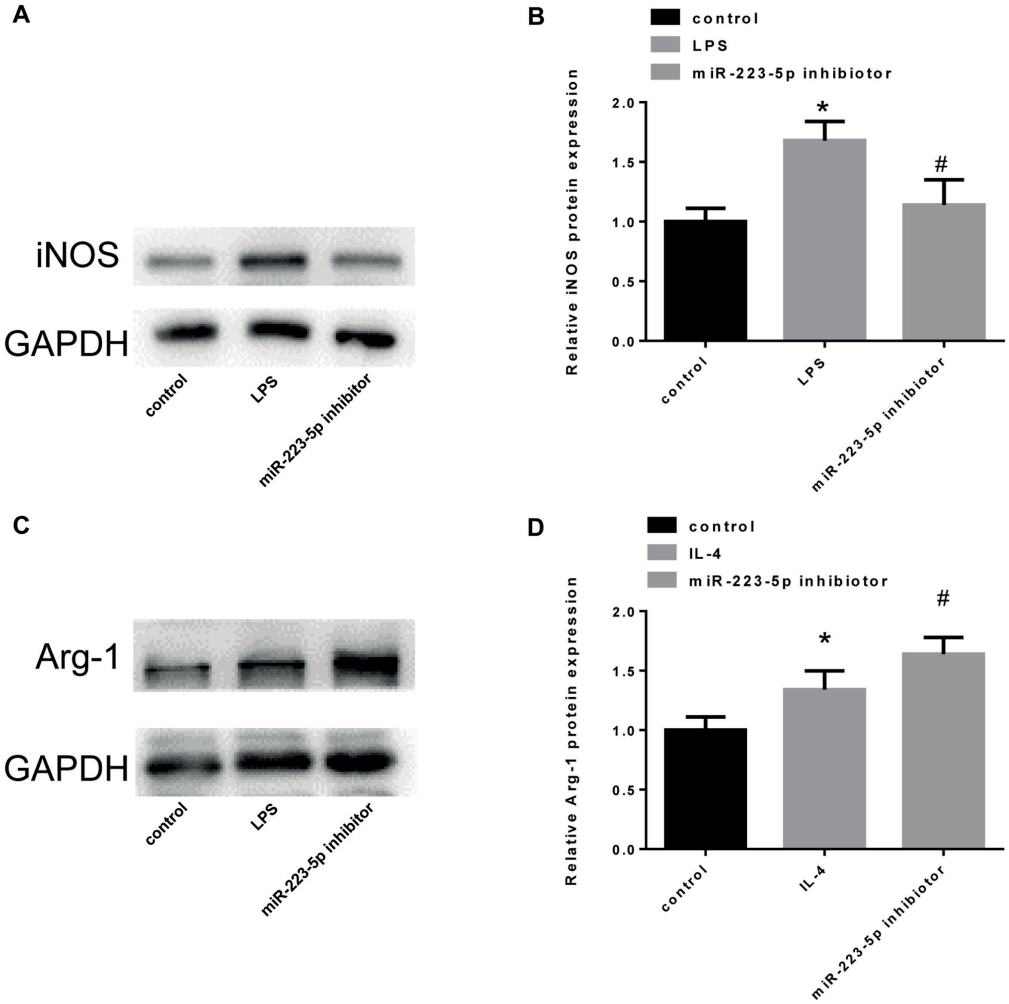

Figure 1. MiR-233-5p inhibitor reduced M1 microglia expression and increased M2 microglia expression. **A,** Protein levels of iNOS and GAPDH in microglia treated with LPS and miR-233-5p inhibitor, respectively. **B,** Protein expression levels of iNOS in each group, showing statistically significant difference. **C,** Protein expression levels of Arg-1 and GAPDH in microglia treated with IL-4 and miR-233-5p inhibitor. **D,** According to the analysis of gray value, the expression of Arg-1 in each group was statistically significant.

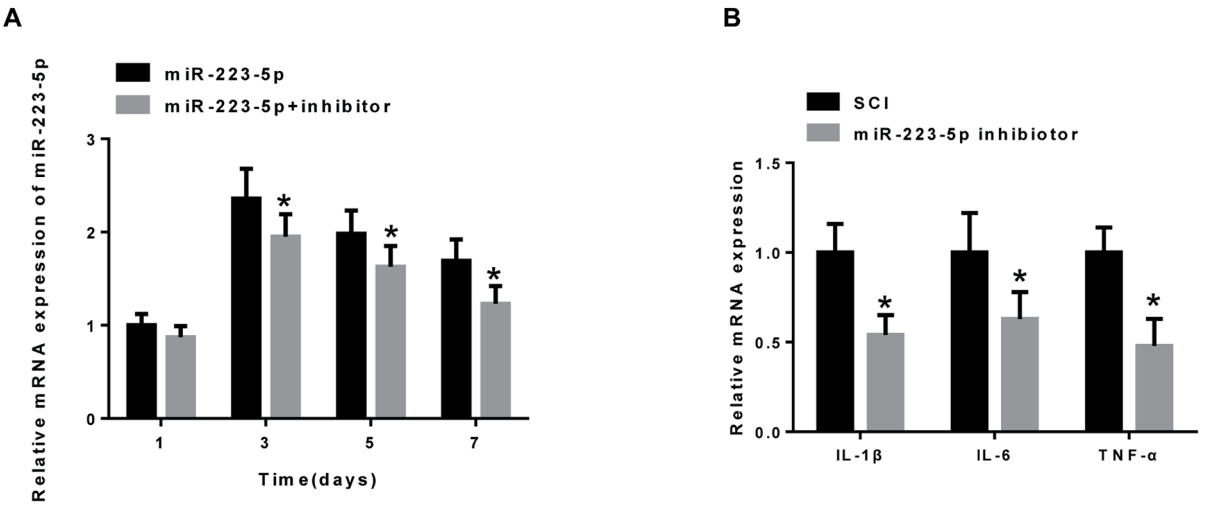

Figure 2. MiR-223-5p increased, while its inhibitor decreased inflammatory factors after SCI. **A,** QRT-PCR analysis of miR-233-5p expression in sham group, SCI group and SCI treated with miR-233-5p inhibitor group. **B,** The expressions of inflammatory factors, including IL-1 IL-1β, IL-6 and TNF-α, at 3 days after injury.

results suggested that miR-223-5p inhibitor suppressed M1 microglia formation and promoted M2 microglia formation.

MiR-223-5p Increased, while its Inhibitor Decreased Inflammatory Factors after SCI

The RNA changes of miR-223-5p in rat SCI model within one week after injury was measured by qRT-PCR, the differences among groups were calculated. Our results showed that miR-223-5p expression increased significantly within one week after injury, which reached peak on the third day (Figure 2A). However, its corresponding inhibitor could obviously reduce the mRNA expression of miR-223-5p. Based on the above results, we measured the transcription levels of inflammatory factors among different groups on the third day after SCI. QRT-PCR results showed that inhibition of miR-223-5p remarkably reduced the mRNA expressions of IL-1β, IL-6 and TNF-α (Figure 2B). Our findings suggested that miR-223-5p increased significantly after SCI. Moreover, inhibition of miR-223-5p could alleviate inflammatory response after SCI.

Restrain of miR-223-5p increased Nrg-1, Contributing to Remission of Apoptosis and Glial Reaction

Nrg-1 is a member of the growth factor family involved in neuronal survival and functional repair[21]. It is also an intercellular signal transduction protein produced by glial cells and neurons of the nervous system. Nrg-1 can bind to ErbB receptor of the tyrosine kinase family[22]. Neuro-protective effects can be induced by affecting the development and differentiation of nerve cells as well as myelin sheath and synapse formation, inhibiting nerve injury, neuronal apoptosis, glial reaction, inflammatory reaction, and inducing cytokine expression[23]. Subsequently, we detected the protein levels of Nrg-1 and caspase-3 at 7days after SCI. The protein expression of GFAP at 28 days after injury was measured by Western blot as well (Figure 3A and 3C). Results indicated that inhibition of miR-223-5p significantly elevated the protein level of Nrg-1, resulting in caspase-3 suppression and GFAP decrease (Figure 3B and 3D). Based on these results, we believed that inhibition of miR-223-5p increased the expression of Nrg-1, and reduced apoptosis and glial scar formation.

MiR-223-5p Inhibition Promoted Hindlimb Locomotor Function in Rats of SCI Model

Behavioral tests were performed on untreated rats and rats treated with miR-223-5p inhibitor at 1, 3, 7, 14, 21, and 28 days after SCI, respectively. Throughout the whole testing period, the performance of rats in the sham group remained unchanged. Functional recovery was also assessed in open-field testing using a 21-point BBB locomotor test. After SCI, corresponding to the low BBB score, the miR-223-5p group showed moderate recovery as early as 7 days after injury. Motor function improved significantly faster at 1-2 weeks after injury. Meanwhile, the recovery of

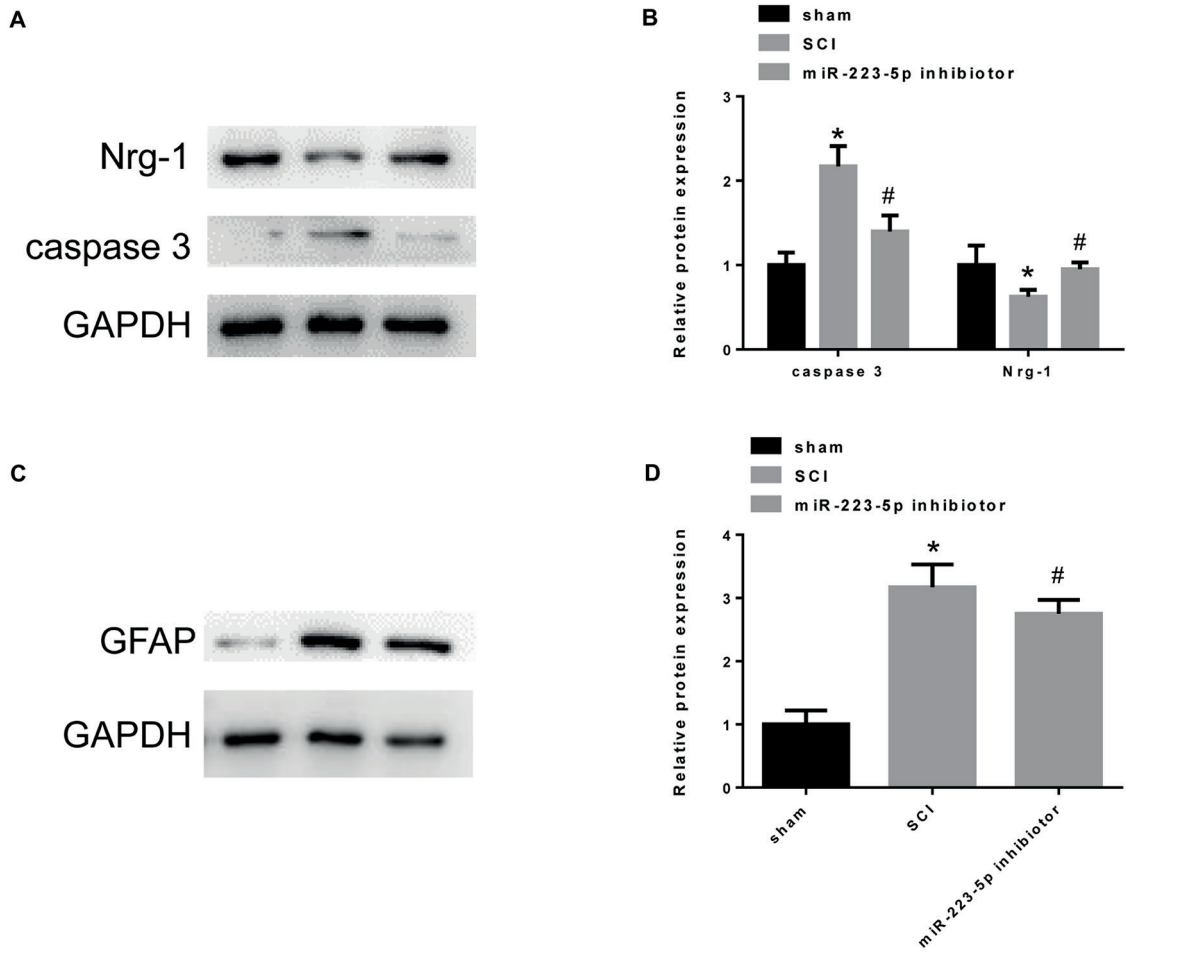

Figure 3. Restrain of miR-223-5p increased Nrg-1, contributing to remission of apoptosis and glial reaction. **A,** Protein expressions of Nrg-1, caspase-3 and GAPDH in different groups at 7 days after injury. **B,** The expressions of Nrg-1and caspase-3 in each group showed statistically significant difference based on gray value analysis. **C,** Protein bands of GFAP and GAPDH in different group at 28 days after SCI. **D,** The expressions of GFAP in each group showed statistically significant difference based on gray value analysis.

motor function in the group treated with inhibitor was significantly faster than that of the SCI group (Figure 4). Therefore, we suggested that MiR-223-5p inhibition promoted hindlimb locomotor function after SCI.

Discussion

Immune response and glial inflammation caused by the primary lesion of spinal cord are important factors leading to the expansion and severity of secondary injury area[24]. The production of a large number of inflammatory factors and the activation of inflammatory cells can eventually result in the deterioration of neuronal living environment as well as the initiation of apoptotic processes[25]. Meanwhile, inflammation activates inflammation of microglia and scarring astrocytes to form the glial scar structure. This inhibits the release of neurotrophic factor and impedes neuronal repair[26]. In the long term, it is not conductive to axon regeneration and nerve fiber re-junction[27]. Therefore, how to effectively control inflammation of SCI in the early stage has positive significance for the reconstruction of nerve structure and improvement of neural function. MiRNA has been observed to regulate the pathophysiological responses in many aspects after SCI[28]. Up-regulation of miR-223-5p after SCI is involved in the immune inflammatory response. In this study, we demonstrated that miR-223-5p was correlated with glial inflammation medi-

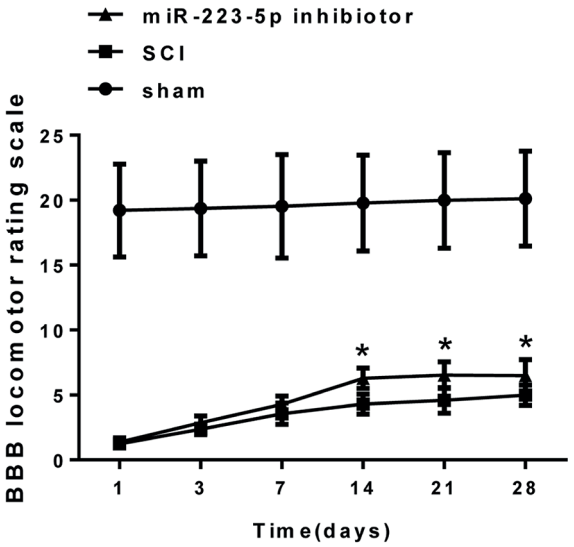

Figure 4. MiR-223-5p inhibition promoted hindlimb locomotor function in rats of SCI model. Locomotor function in miR-223-5p inhibitor group was significantly different from that of SCI group on the 7th day after SCI.

ated by glial cell polarization. The expression of neuro-trophic factor Nrg-1 was significantly up-regulated, thereby inhibiting neuronal apoptosis and glial scar formation. Therefore, inhibition of miR-223-5p significantly improved the repair of neurons and the improvement of neural function. In the present study, we identified new regulatory targets of miR-223-5p in SCI. Meanwhile, we also achieved the goal of restoring motor function by inhibiting its effects *in vitro*. However, the role of other pathological changes in SCI was not clarified, which remained to be further explored.

In summary, our report provided a new coordinate for the treatment strategy and research direction of central nervous injury. We also made new achievements in the regulation of inflammatory response after SCI, which laid a solid foundation for neuro-therapy.

Conclusions

This study provides evidence that inhibition of miR-223-5p promoted M2 microglia polarization and suppressed inflammation reactions. Moreover, the increased Nrg-1 expression reduced nerve apoptosis and glia scar formation, eventually improving hindlimb motor function recovery.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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References

1) Sharma HS. Early microvascular reactions and blood-spinal cord barrier disruption are instrumental in pathophysiology of spinal cord injury and repair: novel therapeutic strategies including nanowired drug delivery to enhance neuroprotection. J Neural Transm (Vienna) 2011; 118: 155-176.
2) Phillips AA, Krassioukov AV, Ainslie PN, Cote AT, Warburton DE. Increased central arterial stiffness explains baroreflex dysfunction in spinal cord injury. J Neurotrauma 2014; 31: 1122-1128.
3) Bareyre FM, Kerschensteiner M, Raineteau O, Mettenleiter TC, Weinmann O, Schwab ME. The injured spinal cord spontaneously forms a new intraspinal circuit in adult rats. Nat Neurosci 2004; 7: 269-277.
4) Hovden H, Frederiksen JL, Pedersen SW. Immune system alterations in amyotrophic lateral sclerosis. Acta Neurol Scand 2013; 128: 287-296.
5) Romero MI, Smith GM. Adenoviral gene transfer into the normal and injured spinal cord: enhanced transgene stability by combined administration of temperature-sensitive virus and transient immune blockade. Gene Ther 1998; 5: 1612-1621.
6) Fan L, Wang K, Shi Z, Die J, Wang C, Dang X. Tetramethylpyrazine protects spinal cord and reduces inflammation in a rat model of spinal cord ischemia-reperfusion injury. J Vasc Surg 2011; 54: 192-200.
7) Detloff MR, Fisher LC, McGaughy V, Longbrake EE, Popovich PG, Basso DM. Remote activation of microglia and pro-inflammatory cytokines predict the onset and severity of below-level neuropathic pain after spinal cord injury in rats. Exp Neurol 2008; 212: 337-347.
8) Shan S, Qi-Liang MY, Hong C, Tingting L, Mei H, Haili P, Yan-Qing W, Zhi-Qi Z, Yu-Qiu Z. Is functional state of spinal microglia involved in the anti-allodynic and anti-hyperalgesic effects of electroacupuncture in rat model of monoarthritis? Neurobiol Dis 2007; 26: 558-568.
9) Norimatsu Y, Ohmori T, Kimura A, Madoiwa S, Mimuro J, Seichi A, Yatomi Y, Hoshino Y, Sakata Y.

FTY720 improves functional recovery after spinal cord injury by primarily nonimmunomodulatory mechanisms. Am J Pathol 2012; 180: 1625-1635.

10) Hu J, Zhang G, Rodemer W, Jin LQ, Shifman M, Selzer ME. The role of RhoA in retrograde neuronal death and axon regeneration after spinal cord injury. Neurobiol Dis 2017; 98: 25-35.

11) Reisert I, Wildemann G, Grab D, Pilgrim C. The glial reaction in the course of axon regeneration: a stereological study of the rat hypoglossal nucleus. J Comp Neurol 1984; 229: 121-128.

12) Detloff MR, Fisher LC, McGaughy V, Longbrake EE, Popovich PG, Basso DM. Remote activation of microglia and pro-inflammatory cytokines predict the onset and severity of below-level neuropathic pain after spinal cord injury in rats. Exp Neurol 2008; 212: 337-347.

13) Shan S, Qi-Liang MY, Hong C, Tingting L, Mei H, Haili P, Yan-Qing W, Zhi-Qi Z, Yu-Qiu Z. Is functional state of spinal microglia involved in the anti-allodynic and anti-hyperalgesic effects of electroacupuncture in rat model of monoarthritis? Neurobiol Dis 2007; 26: 558-568.

14) Yu A, Zhang T, Duan H, Pan Y, Zhang X, Yang G, Wang J, Deng Y, Yang Z. MiR-124 contributes to M2 polarization of microglia and confers brain inflammatory protection via the C/EBP-alpha pathway in intracerebral hemorrhage. Immunol Lett 2017; 182: 1-11.

15) Habib P, Slowik A, Zendedel A, Johann S, Dang J, Beyer C. Regulation of hypoxia-induced inflammatory responses and M1-M2 phenotype switch of primary rat microglia by sex steroids. J Mol Neurosci 2014; 52: 277-285.

16) Chang W, Wang J, Tao D, Zhang Y, He J, Shi C. Identification of a novel miRNA from the ovine ovary by a combinatorial approach of bioinformatics and experiments. J Vet Med Sci 2016; 77: 1617-1624.

17) Dvorankova B, Motlik J, Holikova Z, Vacik J, Smetana KJ. Dolichos biflorus agglutinin-binding site expression in basal keratinocytes is associated with cell differentiation. Biol Cell 2002; 94: 365-373.

18) Walker RM, Rybka J, Anderson SM, Torrance HS, Boxall R, Sussmann JE, Porteous DJ, McIntosh AM, Evans KL. Preliminary investigation of miRNA expression in individuals at high familial risk of bipolar disorder. J Psychiatr Res 2015; 62: 48-55.

19) Liu NK, Wang XF, Lu QB, Xu XM. Altered microRNA expression following traumatic spinal cord injury. Exp Neurol 2009; 219: 424-429.

20) Izumi B, Nakasa T, Tanaka N, Nakanishi K, Kamei N, Yamamoto R, Nakamae T, Ohta R, Fujioka Y, Yamasaki K, Ochi M. MicroRNA-223 expression in neutrophils in the early phase of secondary damage after spinal cord injury. Neurosci Lett 2011; 492: 114-118.

21) Li Y, Li H, Liu G, Liu Z. Effects of neuregulin-1beta on growth-associated protein 43 expression in dorsal root ganglion neurons with excitotoxicity induced by glutamate in vitro. Neurosci Res 2013; 76: 22-30.

22) Raabe TD, Deadwyler G, Varga JW, Devries GH. Localization of neuregulin isoforms and erbB receptors in myelinating glial cells. Glia 2004; 45: 197-207.

23) Velanac V, Unterbarnscheidt T, Hinrichs W, Gummert MN, Fischer TM, Rossner MJ, Trimarco A, Brivio V, Taveggia C, Willem M, Haass C, Mobius W, Nave KA, Schwab MH. Bace1 processing of NRG1 type III produces a myelin-inducing signal but is not essential for the stimulation of myelination. Glia 2012; 60: 203-217.

24) Zhang ZT, Zhang XG, Yu HY, Xu ZT. MicroRNA-490-3p inhibits inflammation and apoptosis induced by spinal cord injury via targeting MK2. Eur Rev Med Pharmacol Sci 2018; 22: 8849-8856.

25) Bellucci A, Westwood AJ, Ingram E, Casamenti F, Goedert M, Spillantini MG. Induction of inflammatory mediators and microglial activation in mice transgenic for mutant human P301S tau protein. Am J Pathol 2004; 165: 1643-1652.

26) Yuan J, Liu W, Zhu H, Chen Y, Zhang X, Li L, Chu W, Wen Z, Feng H, Lin J. Curcumin inhibits glial scar formation by suppressing astrocyte-induced inflammation and fibrosis in vitro and in vivo. Brain Res 2017; 1655: 90-103.

27) Jeno CB, Coggeshall RE. Long-term patterns of axon regeneration in the sciatic nerve and its tributaries. Brain Res 1985; 345: 34-44.

28) Dong J, Lu M, He X, Xu J, Qin J, Cheng Z, Liang B, Wang D, Li H. Identifying the role of microRNAs in spinal cord injury. Neurol Sci 2014; 35: 1663-1671.