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MiR-21 inhibitor improves locomotor function recovery by inhibiting IL-6R/JAK-STAT pathway-mediated inflammation after spinal cord injury in model of rat

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Abstract. – OBJECTIVE: To investigate the function of miRNA-21 and interleukin-6 receptor/Janus Kinase-Signal transducer and activator of transcription (IL-6R/JAK-STAT) pathway in microglia on inflammatory responses after spinal cord injury (SCI).

MATERIALS AND METHODS: This study first detected respectively the protein level of inflammatory factor inducible nitric oxide synthase (iNOS) and tumor necrosis factor alpha (TNF-α) by Western blotting after transfection of miR-21 or administration of miR-21 inhibitor in activated microglia cells of rat *in vitro*. The quantitative Real-time polymerase chain reaction (qRT-PCR) was utilized to detect the expression of IL-6R under two different interventions. Next, we established a model of spinal cord injury in rat and inspected miR-21 and IL-6R in SCI rat by qRT-PCR. In addition, the protein levels of iNOS and TNF-α in SCI rat were detected by Western blotting. MiR-21 inhibitor was injected into the injured area of SCI rat to delve into the function of miR-21 down-expression on iNOS and TNF-α expression by Western blot as well as the RNA levels of IL-6R, JAK and STAT3 by qRT-PCR. Furthermore, the SCI rat with movement and coordination of hindlimbs was observed by Basso-Beattie-Bresnahan locomotor rating scale (BBB scale) after miR-21 down-expression.

RESULTS: Compared with the microglia transfected with miR-21, the execution of inhibitor in microglia effectively relieved the expression of IL-6R and the breakout of iNOS and TNF-α. Meanwhile, the increase of miR-21 was significantly observed in SCI rat along with significant improvement of inflammatory response-related factors including iNOS and TNF-α. After that, we injected SCI rat with miR-21 inhibitor into the spinal cord injury area and found the inhibition of miR-21 decreased the protein levels of iNOS and TNF-α. Simultaneously, down-expression of miR-21 evidently declined the RNA levels of IL-6R, JAK, and STAT3 in SCI rat. Compared with the sham-operated rat, the movement and coordination of hindlimbs of the SCI group displayed dramatic dysfunction. However, miR-21 down-expression elevated the movement and coordination of hindlimbs of the SCI rat than those of the only injury group.

CONCLUSIONS: Inhibition of miR-21 can promote the recovery of spinal cord injury by down-regulating IL-6R/JAK-STAT signaling pathway and inhibiting inflammation.

Key Words:
miR-21 inhibitor, IL-6R/JAK-STAT signaling pathway, Inflammatory response, Spinal cord injury.

Introduction

Spinal cord injury (SCI) is a devastating nervous system disease. After SCI, there are varying degrees of sensory and motor dysfunction[1,2]. The unsatisfactory prognosis of SCI can cause the incomplete or complete disability, which seriously affects the self-care ability and quality of life of patients[3]. There are two mechanisms of spinal cord injury: one is primary spinal cord injury caused by mechanical force, the other is secondary spinal cord injury subjected to immune inflammation[4]. However, the latter is considered to play a controllable factor in the recovery process of spinal cord injury and how to effectively control the inflammatory response after injury is very influential to the recovery of impairment[5]. Interleukin-6 (IL-6) plays a significant role in immune regulation, inflammatory response and

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hematopoietic regulation[6]. The interleukin-6 receptor system consists of two peptide chains: α chain, also known as specific binding chain, or IL-6 binding receptor protein, is ligand specific receptor (IL-6R), molecular weight is 80 kda. β chain is also called signal transduction chain and has a molecular weight of 130 kda (gp130)[7, 8]. IL-6R and gpl30 are both in the form of membranous soluble receptor. The biological function of IL-6 can only be transduced by forming IL-6/IL-6R/gp130 complex with its receptor[9]. Janus Kinase2 binds to gp130 or leukemia inhibitory factor receptor (ILFR) and is activated by gp130: gp130 or gp130:ILFR dimer and phosphorylation of six tyrosine residues on gp130 and ILFR for signal transduction[10]. The Src homologous domain 2 (SH-2) on transcription activator 3 (STAT3) and transcriptional activator 1 (STAT1) interacts with phosphorylated tyrosine to form homodimer or heterodimer. The dimer is separated from its binding sites and transferred to nucleus after tyrosine phosphorylation to regulate target gene expression[11, 12]. It causes inflammatory reaction in the injured area, induces cascade effect and participates in secondary spinal cord injury.

MicroRNA is a series of RNA molecule that is length around 21-23 nucleotides in eukaryotes and MiRNA comes from some transcription from DNA but can not be further translated into protein (belong to non-coding RNA)[13]. They are involved in the regulation of post-transcriptional gene expression in many physiological and pathological processes of SCI. MiR-210 boosted angiogenesis and astrogliosis, and enhanced locomotor effect recovery following SCI[14]. MiR-142-3p modulates sensory function recovery of spinal cord injury[15]. Moreover, miR-21 can regulate astrocytic proliferation and compensatory hypertrophy and process of glial scar formation after SCI and inhibition of miR-21 function contributes to the formation of a looser glial scar[16]. However, the association of miR-21 with inflammation-mediated secondary spinal cord injury has not been reported, nor has the specific effect of miR-21 in inflammatory response to spinal cord injury been demonstrated. This study demonstrated that the expression of miR-21 improved IL-6R expression in microglia after spinal cord injury, and promoted the occurrence of immune inflammation. The inhibitor of miR-21 could effectively reduce the expression of IL-6R, thereby it inhibited the inflammatory effect, protected spinal cord tissue and improved the recovery of locomotor function.

Materials and Methods

Animals

Twenty-four 8-10 weeks male Sprague-Dawley (SD) rats, weight of 280-320 g, were obtained from Tianjin University (Tianjin, China). All rats were resided under half light/half dark conditions per day and fed with standard food and water. We randomly divided the experimental rats into three groups (n=8 per group): Sham operation group (performed laminectomy merely), SCI group (model of moderate spinal cord contusion in rats, then the injury site was injected with the same dose of saline (same dose as inhibitor groups) immediately after injury), and miR-21 inhibitor group (injected with exogenous miR-21 inhibitor in the injury area after SCI). This study was approved by the Animal Ethics Committee of Tianjin Hospital Animal Center (Tianjin, China).

Rat Primary Microglia Culture

We extracted the primary microglia from the spinal cord of naive male Sprague Dawley (SD) rats (two-days old, obtained from Tianjin University). Rat spinal cord was ground into single cells and plated on 75 cm^2 poly-L-lysine-overspread flask containing the Dulbecco's modified eagle medium (DMEM) (Gibco, Rockville, MD, USA) with bovine 10% fetal serum and 1% penicillin/streptomycin, extending over a period of 2 weeks. Microglia were separated by slight vibration and applied to nylon screen to dislodge lumps. Microglia were then indicated on poly-L-lysine-overspread 6-Well plates (5×10^5 cells/well). Extractive microglia were launched by lipopolysaccharide (LPS, 1 μg/mL) and further cultured for the experimental preparation after 24 h.

Model of SCI In Rat

The model of SCI in rat was performed with moderate contusion of spinal cord utilizing Infinite Horizon Impactor device (Precision Systems and Instrumentation, Lexington, KY, USA). After intraperitoneal injection of chloral hydrate into the each rat (280 mg/kg), iodophor disinfection was performed on the operative site. Afterwards, we cut through the disinfected skin and disconnected the muscle layer to completely reveal the lamina. Laminectomy was performed to expose adequately the spinal cord at T9-T11. The impact striker fell at the height of 20 mm on the exposed spinal cord. Satisfactory spinal cord injury models are identified as follows: observation of spinal cord ischemia, tail

flick reflex, leg swing and hysteresis hind limb paralysis. The impact striker was removed soon and the incision was sutured following the hit of spinal cord hit. SCI rats were resided singly with available food procurement and the temperature was sternly controlled at 20-25°C.

Behavioral Analysis

The locomotor function was evaluated with BBB scale at 1, 3, 7, 14 day following SCI, respectively. The score was between 0 (exhaustive paralysis) and 21 (normal movement). The evaluation criteria included hind limb movement, weight bearing, and coordinated movement of forelimbs and hind limbs. Two different researchers observed the movement of rat within 4 minutes of open field for a double-blind score.

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

The rat was euthanized and the spinal cord injury area was removed as the central upper and lower 5 mm tissue (the corresponding segments of spinal cord tissue were taken from Sham group.), which was preserved in the 4 mL centrifuge tube. At the beginning of the experiment, the spinal cord tissue and cultured rat microglia were released into a pre-cooled mortar to grind with TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Then chloroform, isopropanol and ethanol were added to extract RNA, and the concentration of RNA was determined by nano-drop method. Reverse transcription kit was utilized to reverse transcription of total RNA into complementary deoxyribonucleic acid (cDNA). qPCR was performed with the SYBR mix (TaKaRa, Otsu, Shiga, Japan). The relative expression levels of miR-21, IL-6R JAK2 and STAT3 were calculated by $2^{-\Delta\Delta CT}$ method. The primer sequences are as follows: miR-21 (forward): 5′-ACACTCCAGCTGGGTAGCTTATCAGACTGA-3′; miR-21 (reverse): 5′-TGGTGTCGTGGAGTCG-3′; IL-6R (forward): 5′-CATGTGCGTCGCCAGTAGT-3′; IL-6R (reverse): 5′-AGCTCAAACCGTAGTCTGTAG-3′; JAK2 (forward): 5′-TGGACAGGATAGCAGTGAGATTC-3′; JAK2 (reverse): 5′-TTCTTCCTCCATTCCCAGTTCTT-3′; STAT3 (forward): 5′-ATCACGCCTTCTACAGACTGC-3′; STAT3 (reverse): 5′-CATCCTGGAGATTCTCTACCACT-3′; GAPDH (forward): 5′-CGGAGTCAACGGATTTGGTCGTAT-3′; GAPDH (reverse): 5’-AGCCTTCTCCATGGTGGTGAAGAC-3’;

Western Blotting

The rat spinal cord tissue and cultured rat microglia were lysed with whole cell lysis assay (Keygen Biotech, Nanjing, China) cell lysate containing protease inhibitor, and the total protein was extracted on ice. Afterwards protein concentration was determined by double chondroic acid (BCA) method (Pierce, Rockford, IL, USA). The protein was transferred to polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA) after electrophoresis in 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel. Next, the membrane was sealed by 5% skim milk and washed 3 times with Tris-buffered saline containing 0.2% Tween 20 (TBST) and immersed into anti-iNOS antibody of rabbit (1:250), anti-TNF-α antibody of rabbit (1:500) and rabbit glyceraldehyde 3-phosphate dehydrogenase (GAPDH) overnight at 4°C. The membrane was rinsed with TBST and immersed into the corresponding secondary antibody (goat anti-rabbit antibody 1: 10000) at room temperature for 1 hours. After it was washed 3 times, the membrane was cleaned and visualized with electrochemiluminescence (ECL) Western blotting substrate (Thermo Fisher Scientific, Waltham, MA, USA).

Statistical Analysis

Statistical product and service solutions (SPSS 18.0) software (SPSS Inc., Chicago, IL, USA) was used to carry out statistical analysis. The data were expressed as mean ±standard deviation ($\bar{x}$ ±s). The difference between the two groups was tested by *t*-test. When $p<0.05$, the difference was considered to be statistically significant.

Results

The Release of Inflammatory Factors in Microglia was Markedly Elevated After miR-21 Transfection in vitro

Inflammatory cytokine are regarded as major impactive factors in secondary SCI, including iNOS and TNF-α. MiR-21 is considered to be a potential regulatory factor for the level of inflammatory cytokines following SCI. The inflammatory cytokines at the protein level was detected by Western blotting. All of these cytokines are reported to be up-regulated in the activated microglia of rat following miR-21 transfection (Figure 1A and 1B). These data suggest that miR-21 transfection of microglia can regulate the expression of pro-inflammatory cytokines at the transcriptional level after activation with LPS.

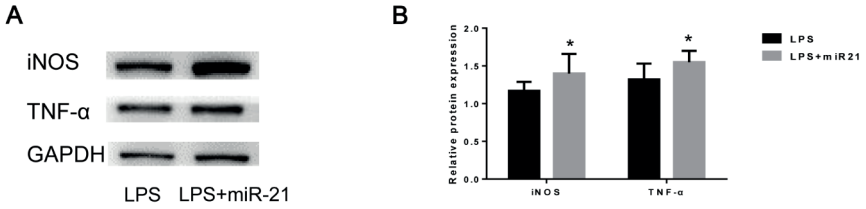

Figure 1. The release of inflammatory factors in microglia was markedly elevated after miR-21 transfection *in vitro*. ***A,*** The protein levels of iNOS and TNF-α increased significantly after miR-21 transfection compared with those of microglia only activated by LPS. ***B,*** It was found that the increase of iNOS and TNF-α after transfection of miR-21 through the grey level analysis was statistically significant.

MiR-21 Inhibitor Depressed the Accumulation of Inflammatory Cytokines in Microglia in vitro

We continued to explore the function of miR-21 inhibitors in the changes of inflammatory factors. After the miR-21 inhibitor was administered, the protein expression of iNOS and TNF-α in microglia decreased significantly compared with the miR-21 transfection group (Figure 2A and 2B). The outcomes indicated that miR-21 inhibitor could effectively inhibit the accumulation of inflammatory factors in microglia and reduce the degree of inflammation. In depth, the phenomena suggest that inhibiting the expression of miR-21 may result in neuro-protective effect by reducing the inflammatory reaction of microglia after spinal cord injury *in vivo.*

The Activation of miR-21 in Spinal Cord After SCI was Significantly Increased in vivo

After modeling spinal cord injury, the expression of miR-21 in Sham group was maintained at a stable and low level within the first seven days. However, the expression of miR-21 in SCI group was apparently more active than that in Sham group, and reach the peak 3 day after SCI (Figure 3A). Meanwhile, the level of IL-6R mRNA in SCI group increased and reached its peak on the 3rd day after the injury, which was distinctly incremental than that in Sham group (Figure 3B). In addition, the expression of iNOS and TNF-α protein was detected by Western blot method. The result reflected that the expression of iNOS and TNF-α protein increased with the prolongation of injury time and reached its peak on the 3rd day after the injury compared with the Sham group (Figure 3C). It was proved that the high expression of miR-21 after spinal cord injury might be involved in the secondary inflammatory reaction.

MiR-21 Inhibitor Relieved Inflammatory Response After SCI by Depressing IL-6R/JAK-STAT Pathway in vivo

To further probe the relationship of miR-21 and IL-6R in inflammatory response after SCI, we injected miR-21 inhibitor intrathecally into spinal cord of rats with SCI to achieve the down-expression of miR-21 (Figure 4A). After that, the results revealed that the expression of IL-6R in miR-21 inhibitor group decreased remarkably compared with SCI Group (Figure 4B). The inhibition of

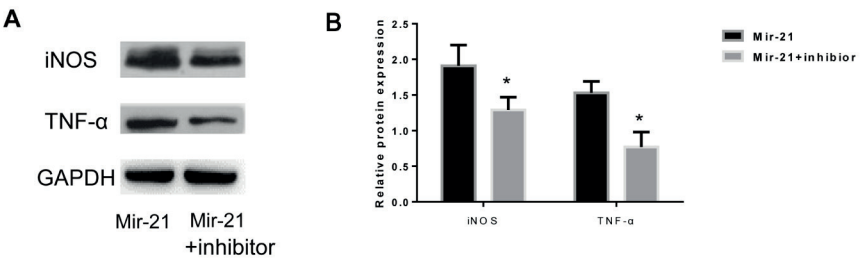

Figure 2. MiR-21 inhibitor depressed the accumulation of inflammatory cytokines in microglia *in vitro*. ***A,*** The protein expression of iNOS and TNF-α following the administration of miR-21 inhibitor was remarkably lower than the miR-21 transfection group. ***B,*** Through the grey level analysis, the reduction of protein expression of iNOS and TNF-α following the administration of miR-21 inhibitor was considered to be statistically significant.

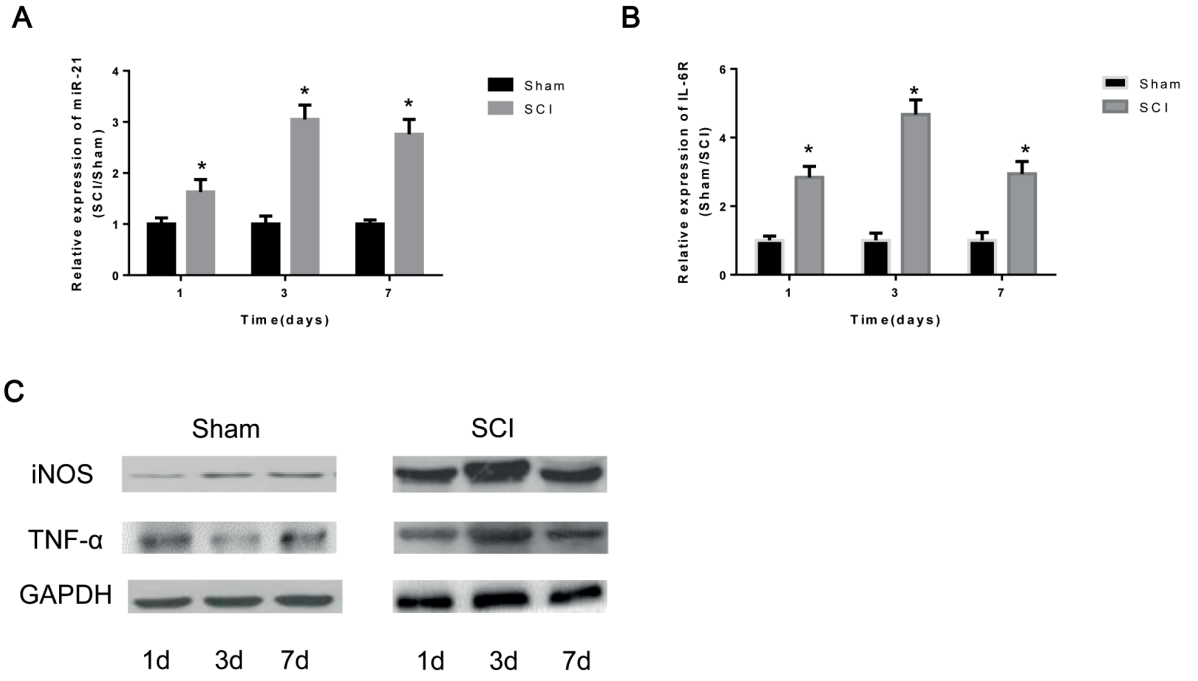

Figure 3. The activation of miR-21 in spinal cord after SCI was significantly increased *in vivo*. ***A,*** Compared with the Sham group, the expression of miR-21 increased dramatically after SCI within a week and had statistical significance. ***B,*** After one week after SCI, the increased expression of IL-6R was in accord with the increase of miR-21.***C,*** Compared with the Sham Group, the protein levels of iNOS and TNF-α increased significantly and were at the peak on the third day after SCI.

miR-21 also resulted in a significant decline in the level of iNOS and TNF-α compared with that in the SCI group (Figure 4C). At the same time, the RNA levels of JAK2 and STAT3 also declined significantly (Figure 4D and 4E). On the whole, the results testified that the inhibition of miR-21 down-regulated the effect of IL-6R/JAK-STAT signaling pathway and thus alleviated the inflammatory response after SCI.

MiR-21 Inhibitor Effectively Improved the Recovery of Locomotor Function After Spinal Cord Injury

We evaluated the BBB locomotor score at 1, 3, 7, 14 days following SCI respectively (Figure 5). The BBB score of Sham-operation group was 17.97±0.85, 18.68±0.77; 19.49±0.69 and 19.88 ±0.63, respectively. However, the SCI group was as follows: BBB score decreased dramatically (1.16±***0.32,*** 1.65 ±0.82, 2.85±0.58 and 4.41±***0.63*** respectively (p<0.01)). In addition, MiR-21 inhibitor group (1.85±0.64, 4.41±0.52, 6.36±0.44, and 8.35±0.91) at 1, 3, 7, and 14 days (p<0.01). The observation identified with the neuro-protective function of MiR-21 inhibitor and proved that the administration of MiR-21 inhibitor effectively improved the recovery of locomotor function after spinal cord injury.

Discussion

MicroRNA is a series of RNA molecule that is length of 21-23 nucleotides in eukaryotes and MiRNA comes from some transcription from DNA but belongs to non-coding RNA[17, 18]. MiR-21 is low expressed in uninjured spinal cord, while is significantly increased after SCI. These evidence suggested that miR-21 is involved in the procedure of spinal cord injury[19]. These results on the diversification after spinal cord injury have proved that the expression of miR-21 takes important effect in the nerve response after injury.

Researches[16] have shown that miR-21 can improve astrocytic hyperplasia and glial scar formation after SCI but inhibition of miR-21 effect results in the formation of a looser glial scar, which creates an enabling environment for axonal regeneration. However, in this work, we found that miR-21 increased the level of inflammatory factor

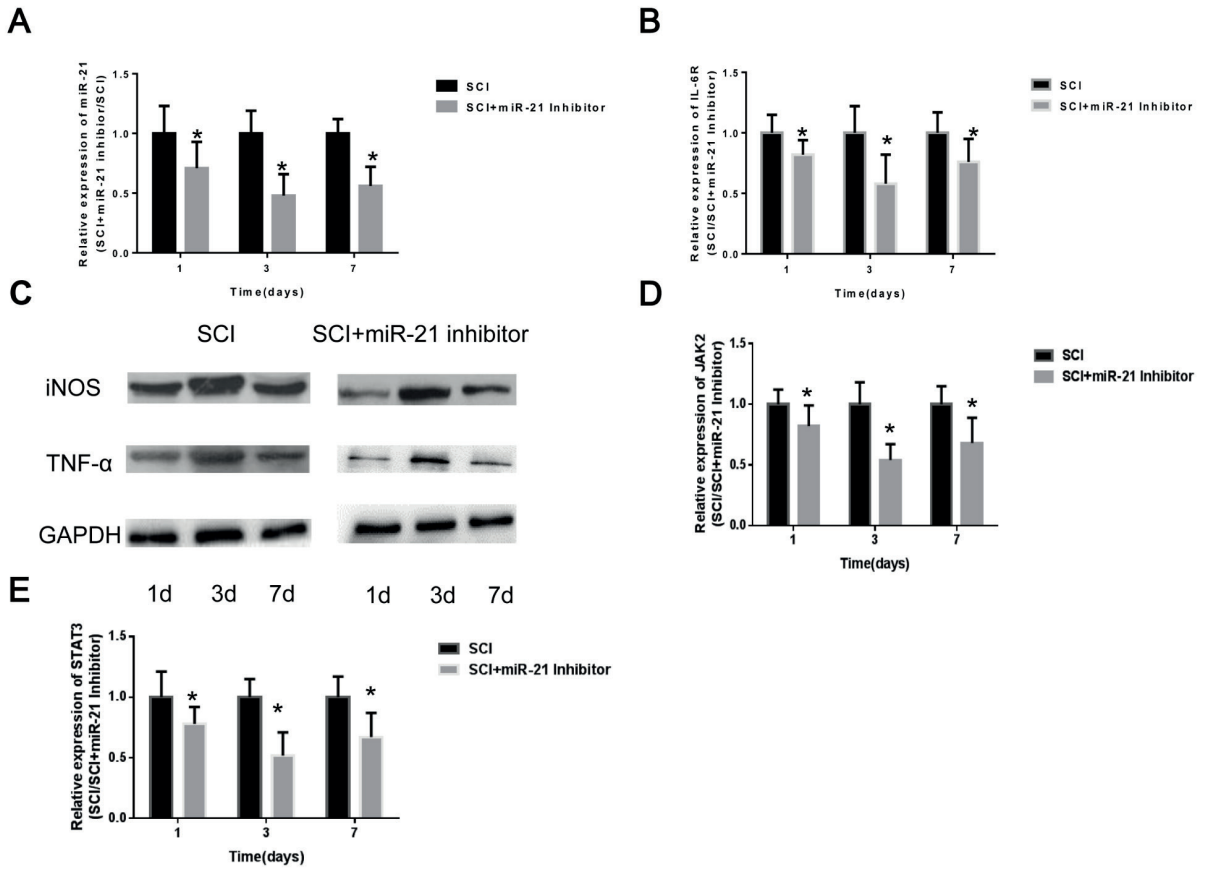

Figure 4. MiR-21 inhibitor relieved inflammatory response after SCI by depressing IL-6R/JAK-STAT pathway *in vivo*. ***A,*** The expression of miR-21 was significantly decreased in seven days after injury with miR-21 inhibitor. ***B,*** The level of RNA of IL-6R after injection of miR-21 inhibitor was significantly lower than SCI group in one week after SCI. ***C,*** The protein levels of iNOS and TNF-α were significantly down-regulated within a week following injection of miR-21 inhibitor. ***D,*** The RNA level of JAK2 was also significantly suppressed within a week following SCI after the intervention of miR-21 inhibitor. ***E,*** The RNA level of STAT3 was significantly down-regulated within a week after miR-21 inhibitor intervention following SCI.

iNOS and TNF-α in microglia, suggesting that the high expression of miR-21 might aggravate the inflammatory response after spinal cord injury. IL-6R is two transmembrane glycoprotein chains including a subunit and B subunit. The former, with a molecular weight of 80 kda, binds to IL-6 and then couples with B subunit, while the latter, with a molecular weight of 130 kda (gp130), is involved mainly in signal transduction[20, 21]. Both of them can form high affinity IL-6R and exert cellular effect. Currently, it is believed that IL-6 is involved in the regulation of immune and inflammatory response by activation of IL-6R, which can promote the occurrence of inflammation[22-24]. The activation of IL-6R increased significantly after spinal cord injury. Inhibition of the activity of IL-6R can effectively reduce the production of inflammatory factors and is beneficial to the recovery of spinal cord nerve. The high expression of miR-21 can activate the function of IL-6R, while the specific inhibition of miR-21 expression had the opposite effect. Thus, the achievement of suppression for IL-6R/JAK-STAT pathway caused the pro-inflammatory effect of IL-6 was greatly weakened after spinal cord injury.

In this study, we found that *in vitro* miR21 transinfected microglia cells could induce a more destructive inflammatory response, while inhibition of miR-21 could weaken the release of pro-inflammatory factors. Moreover, the expression of miR-21 and IL-6R/JAK-STAT pathway in spinal cord after SCI in rats was observably higher than that in Sham group. However, the level of IL-6R, JAK2 and STAT3 in the miR-21 inhibitor group

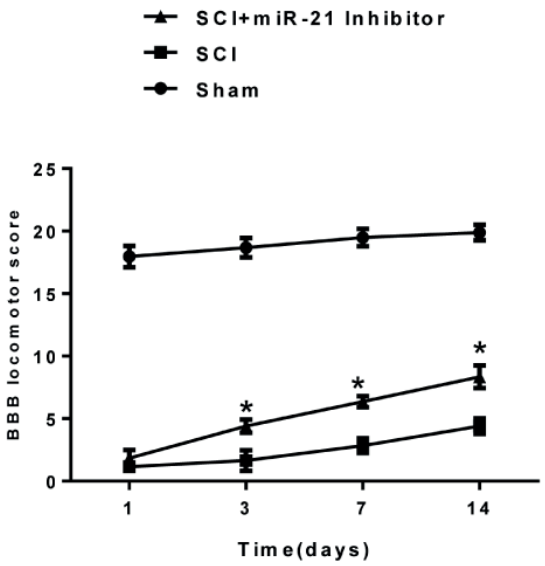

Figure 5. MiR-21 inhibitor effectively improved the recovery of locomotor function after SCI.

after the application of the miR-21 inhibitor was markedly lower than that in the SCI group. Meanwhile, the expression of iNOS and TNF-α in the miR-21 inhibitor group also revealed a significant downward trend compared with that in the SCI group. After SCI, rats with spinal cord injury had severe locomotor dysfunction in hindlimbs compared with those in Sham group. Nevertheless, the locomotor function of rats treated with intrathecal injection of miR-21 inhibitor after spinal cord injury was remarkably improved compared with that of simple injury group.

Briefly, the results demonstrated that the administration of miR-21 inhibitor could down-regulate the intensity of IL-6R/JAK-STAT pathway-mediated inflammatory response, improve the peripheral environment of the nerve and promote the recovery of motor function after spinal cord injury. It also can provide a reference target for the treatment of SCI.

Conclusions

The abatement of miR-21 expression with miR-21 inhibitor can improve motor function recovery by blocking IL-6R/JAK-STAT pathway to depress the pro-inflammatory impact of IL-6 and reduce inflammatory response, which is beneficial to the remodeling of spinal cord structure after SCI.

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Baek A, Cho SR, Kim SH. Elucidation of gene expression patterns in the brain after spinal cord injury. Cell Transplant 2017; 26: 1286-1300.
2) Dai J, Xu LJ, Han GD, Sun HL, Zhu GT, Jiang HT, Yu GY, Tang XM. MicroRNA-125b promotes the regeneration and repair of spinal cord injury through regulation of JAK/STAT pathway. Eur Rev Med Pharmacol Sci 2018; 22: 582-589.
3) Song Z, Wang Z, Shen J, Xu S, Hu Z. Nerve growth factor delivery by ultrasound-mediated nanobubble destruction as a treatment for acute spinal cord injury in rats. Int J Nanomedicine 2017; 12: 1717-1729.
4) Smith RR, Brown EH, Shum-Siu A, Whelan A, Burke DA, Benton RL, Magnuson DS. Swim training initiated acutely after spinal cord injury is ineffective and induces extravasation in and around the epicenter. J Neurotrauma 2009; 26: 1017-1027.
5) Bao F, Omana V, Brown A, Weaver LC. The systemic inflammatory response after spinal cord injury in the rat is decreased by alpha4beta1 integrin blockade. J Neurotrauma 2012; 29: 1626-1637.
6) Gadient RA, Otten UH. Interleukin-6 (IL-6)--a molecule with both beneficial and destructive potentials. Prog Neurobiol 1997; 52: 379-390.
7) Zaccaro L, Bucci E, Vitale RM, Perretta G, Fattorusso R, Benedetti E, Saviano M, Pedone C. Synthetic peptides mimicking the interleukin-6/gp130 interaction: a two-helix bundle system. Design and conformational studies. J Pept Sci 2003; 9: 90-105.
8) Ishihara K, Sawa S, Ikushima H, Hirota S, Atsumi T, Kamimura D, Park SJ, Murakami M, Kitamura Y, Iwakura Y, Hirano T. The point mutation of tyrosine 759 of the IL-6 family cytokine receptor gp130 synergizes with HTLV-1 pX in promoting rheumatoid arthritis-like arthritis. Int Immunol 2004; 16: 455-465.
9) Naruishi K, Takashiba S, Nishimura F, Chou HH, Arai H, Yamada H, Murayama Y. Impairment of gingival fibroblast adherence by IL-6/sIL-6R. J Dent Res 2001; 80: 1421-1424.
10) Hutchins NA, Chung CS, Borgerding JN, Ayala CA, Ayala A. Kupffer cells protect liver sinusoidal endothelial cells from Fas-dependent apoptosis in sepsis by down-regulating gp130. Am J Pathol 2013; 182: 742-754.
11) Park IH, Li C. Characterization of molecular recognition of STAT3 SH2 domain inhibitors through molecular simulation. J Mol Recognit 2011; 24: 254-265.
12) Zhao W, Jaganathan S, Turkson J. A cell-permeable Stat3 SH2 domain mimetic inhibits Stat3 activation and induces antitumor cell effects in vitro. J Biol Chem 2010; 285: 35855-35865.
13) Krol J, Sobczak K, Wilczynska U, Drath M, Jasinska A, Kaczynska D, Krzyzosiak WJ. Structural features

of microRNA (miRNA) precursors and their relevance to miRNA biogenesis and small interfering RNA/short hairpin RNA design. J Biol Chem 2004; 279: 42230-42239.

14) Ujigo S, Kamei N, Hadoush H, Fujioka Y, Miyaki S, Nakasa T, Tanaka N, Nakanishi K, Eguchi A, Sunagawa T, Ochi M. Administration of microRNA-210 promotes spinal cord regeneration in mice. Spine (Phila Pa 1976) 2014; 39: 1099-1107.

15) Wang T, Yuan W, Liu Y, Zhang Y, Wang Z, Chen X, Feng S, Xiu Y, Li W. miR-142-3p is a potential therapeutic target for sensory function recovery of spinal cord injury. Med Sci Monit 2015; 21: 2553-2556.

16) Bhalala OG, Pan L, Sahni V, McGuire TL, Gruner K, Tourtellotte WG, Kessler JA. microRNA-21 regulates astrocytic response following spinal cord injury. J Neurosci 2012; 32: 17935-17947.

17) 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.

18) Guo X, Lu R. Characterization of virus-encoded RNA interference suppressors in Caenorhabditis elegans. J Virol 2013; 87: 5414-5423.

19) Ning B, Gao L, Liu RH, Liu Y, Zhang NS, Chen ZY. microRNAs in spinal cord injury: potential roles and therapeutic implications. Int J Biol Sci 2014; 10: 997-1006.

20) Yamasaki K, Taga T, Hirata Y, Yawata H, Kawanishi Y, Seed B, Taniguchi T, Hirano T, Kishimoto T. Cloning and expression of the human interleukin-6 (BSF-2/IFN beta 2) receptor. Science 1988; 241: 825-828.

21) Abeywardena MY, Leifert WR, Warnes KE, Varghese JN, Head RJ. Cardiovascular biology of interleukin-6. Curr Pharm Des 2009; 15: 1809-1821.

22) Ustundag B, Yilmaz E, Dogan Y, Akarsu S, Canatan H, Halifeoglu I, Cikim G, Aygun AD. Levels of cytokines (IL-1beta, IL-2, IL-6, IL-8, TNF-alpha) and trace elements (Zn, Cu) in breast milk from mothers of preterm and term infants. Mediators Inflamm 2005; 2005: 331-336.

23) Suzuki M, Hashizume M, Yoshida H, Mihara M. Anti-inflammatory mechanism of tocilizumab, a humanized anti-IL-6R antibody: effect on the expression of chemokine and adhesion molecule. Rheumatol Int 2010; 30: 309-315.

24) Song SN, Tomosugi N, Kawabata H, Ishikawa T, Nishikawa T, Yoshizaki K. Down-regulation of hepcidin resulting from long-term treatment with an anti-IL-6 receptor antibody (tocilizumab) improves anemia of inflammation in multicentric Castleman disease. Blood 2010; 116: 3627-3634.