European Review for Medical and Pharmacological Sciences 2019; 23: 4089-4096

MicroRNA-204-5p targets SOX11 to regulate the inflammatory response in spinal cord injury

J.-S. FENG[1], J.-D. SUN[2], X.-D. WANG[1], C.-H. FU[1], L.-L. GAN[1], R. MA[1]

[1]Intensive Care Unit, Gansu Provincial Hospital, Lanzhou, China
[2]Department of Spinal Cord Injury Rehabilitation, Gansu Province Hospital Rehabilitation Center, Lanzhou, China

Junshuai Feng and Jundi Sun contributed equally to this work

Abstract. – OBJECTIVE: The purpose of this study was to investigate whether microRNA-204-5p can regulate the inflammatory response of spinal cord injury (SCI) by targeting SOX11.

PATIENTS AND METHODS: Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was used to detect the expression of microRNA-204-5p in patients with SCI. The mouse SCI model was established to detect the recovery of the grip strength of the upper and lower limbs. Then, the expression of microRNA-204-5p in these mice with SCI was detected by qRT-PCR, and the levels of the inflammatory factors Toll-like receptor 4 (TLR4) and iNOS were examined by Western blot. Subsequently, microRNA- 204-5p was overexpressed in the mouse SCI model using lentivirus, and the changes in mouse grip strength and the inflammatory factor levels were observed. SOX11 was then searched as the target gene of microRNA-204-5p through bioinformatics analysis, and its expression in patients or mice with SCI was examined using qRT-PCR. SOX11 expression was again detected after the overexpression or knockdown of microRNA-204-5p in cells. The binding of microRNA-204-5p to SOX11 was verified by dual-luciferase reporting assay. After microRNA-204-5p and SOX11 were co-overexpressed in cells, the levels of TLR4 and iNOS were analyzed. Furthermore, the changes in the grip strength were observed in mice with SCI after simultaneous up-regulation of microRNA-204-5p and SOX11.

RESULTS: Micro-204-5p level was conspicuously decreased in the population with SCI. And the SCI mouse model showed that the upper and lower limb strength conspicuously decreased and began to recover after 7 days. During the seven days, microRNA-204-5p level in the SCI mice decreased with time, while the levels of the inflammatory cytokines TLR4 and iNOS conspicuously increased. After microRNA-204-5p was overexpressed in SCI mice, their upper and lower limb strength was conspicuously restored, while the levels of TLR4 and iNOS were also remarkably decreased. The bioinformatics analysis revealed that there exist some binding sites between microRNA-204-5p and SOX11, and we found that SOX11 expression was conspicuously enhanced in the plasma of the SCI patients. Meanwhile, the SOX11 level in SCI mice was also conspicuously increased, and it was time-dependent. The expression of SOX11 was decreased after the upregulation of microRNA-204-5p, while the opposite result was observed after the downregulation of microRNA-204-5p. In addition, the result of the dual-luciferase reporter gene assay revealed that microRNA-204-5p could bind to SOX11 in a targeted manner. Meanwhile, the up-regulation of SOX11 was partially relieved by the inhibitory effect of microRNA-204-5p on TLR4 and iNOS. Moreover, the simultaneous overexpression of SOX11 and microRNA-204-5p partially reversed the impact of the up-regulated microRNA-204-5p alone on the recovery of the upper and lower limb strength in SCI mice.

CONCLUSIONS: The low expression of microRNA-204-5p in patients with SCI can affect the levels of the inflammatory cytokines TLR4 and iNOS and improve SCI by targeting SOX11.

Key Words:
MicroRNA-204-5p, Spinal cord injury, Inflammatory response, SOX11.

Introduction

Spinal cord injury (SCI) is one of the most common and devastating injuries observed in the spine and neurosurgery, usually caused by motor vehicle accidents, sports injuries, and diving accidents[1]. Recently, it has been shown that the spinal cord microglia, a major source of inflammatory mediators that experience a deep activation in response to injury, can be activated and lead to pain[2]. Moreover, SCI can induce the activation of

Corresponding Author: Ru Ma, MM; email: fjsh1982jd@163.com

microglia related to pain phenomena, which can promote excitotoxicity by releasing neurotoxins after SCI[3].

When SCI happens, a series of inflammatory responses are activated. The destruction of the blood-spinal cord barrier (BSCB) results in the accumulation of various inflammatory cytokines[4]. The inflammatory cytokines, a large class of small soluble proteins, are mediators and regulators for the complex functional interactions and reactions of the immune system[5]. During spinal cord injury, the infiltration and activation of the leukocyte glial cells can aggravate the tissue damage by releasing proteases, reactive oxygen intermediates, lysosomal enzymes, and proinflammatory cytokines[6]. Although the role of inflammation in this phase is complex, there are also some beneficial aspects, such as the removal of cell debris. However, many studies have shown that the inflammatory response spreads damage to surrounding tissues, induces apoptotic cell death, and damages spontaneous regeneration and functional recovery[7]. In order to protect the damaged spinal cord from these secondary pathological processes, several methods for manipulating the inflammatory response have been evaluated and found to be effective. These methods include the use of monoclonal antibodies to block or neutralize the specific cytokine signaling, the delivery of anti-inflammatory drugs, and the use of genetically modified animals[8,9]. However, more in-depth studies on the specific mechanisms and the regulatory modes of the inflammatory reactions in the nervous system are needed, so as to further understand the mechanism of the immune inflammatory reactions after nervous system injury and provide a scientific theoretical basis for clinical treatment.

A miRNA is a single-stranded, non-coding, small-molecule RNA of about 18 to 22 nucleotides in length, which binds to the target gene mRNA in a fully complementary or incompletely complementary form at the transcriptional level, thereby regulating gene expression and exerting its biological effects[10,11]. Currently, studies[12-14] have shown that the micro ribonucleic acids (miRNAs) are able to participate in the pathophysiological process of SCI, including inflammation, cell apoptosis, and other aspects. It has been reported that microRNA-219 plays a pivotal role in the differentiation of the oligodendrocytes, and microRNA-381 promotes cell proliferation of neurons by inhibiting Hes1 expression[15,16]. In addition to their role in the normal spinal cord, some evidence suggests that miRNA dysregulation is closely associated with SCI[17].

In this study, we established a SCI mouse model and performed *in vitro* cell experiments to investigate whether microRNA-204-5p can regulate the inflammatory response of SCI by targeting SOX11.

Patients and Methods

Clinical Sample Collection

30 people were collected from the blood samples of patients with SCI. The samples collected 3-7 days after SCI were all marked as 3-7 days after SCI. The control sample used blood samples from the physical examination center health checkup. All blood samples involved in the experiment were taken after informing the patient/student. They also signed the written informed consent form. This investigation was approved by the Ethics Committee of Gansu Provincial Hospital, China.

SCI Mouse Model

30 female C57bl/6J mice aged 8-10 weeks were fed with normal diet under a normal light-dark cycle. This study was approved by the Animal Ethics Committee of Gansu Provincial Hospital Animal Center. In the injury group (SCI group) a longitudinal incision was made from the neck to expose the c4-6 spinous process, and the C5 lamina was removed by ophthalmic shearing to expose the dural sac. The hammer position of the spinal cord percussion instrument was adjusted to C5 spinal cord and then, the hemostasis and suture were performed after a successful stroke. In the Sham-operated group (Sham group), similarly, a longitudinal incision was made from the neck to expose the c4-6 spinous process, and the C5 lamina was removed by ophthalmic shearing to expose the dural sac. In this process, the dural sac and spinal cord should not be injured. The mice used in the experiment were purchased from Shanghai Slack (Shanghai, China). On the 1st, 3rd, and 5th day after SCI modeling, 80 mg/kg of microRNA-204-5p-mimics and SOX11 overexpression plasmid were injected into SCI mice through the tail vein.

Forefoot Grip Strength Measurement

The grip strength measurements were recorded 4 times for each successful measurement and averaged. If the mouse was unable to grasp the

crossbar due to a severe forelimb movement disorder, the strength was recorded as 0.

Cell Culture and Processing

The human renal epithelial cell line (293T) was purchased from the Kunming Cell Bank of the Typical Culture Collection of the Chinese Academy of Sciences (Kunming, China). The 293T cells were cultured in The Roswell Park Memorial Institute (RPMI–1640, Hyclone, South Logan, UT, USA) medium containing 10% fetal bovine serum (FBS, Hyclone, South Logan, UT, USA) and 1% streptomycin along with penicillin at 37°C with 5% CO_2 in an incubator. NC, microRNA-204-5p-mimics, and SOX11 overexpression plasmid were transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) transfection reagent when 293T cell density reached 60%.

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

QIAzoI Lysis Reagent was added into 200 μL of serum and let stand for 5 min. Then, the microRNANeasy serum/plasma spike-in control was added and mixed well. Subsequently, the RNA was extracted using chloroform and RNeasy Minelute spin columns adsorption column. Total RNA of 293T cells was extracted by TRIzol (Invitrogen, Carlsbad, CA, USA) method. The purity was measured by an ultraviolet spectrophotometer and then, the extracted RNA was stored at -80°C until use. The complementary deoxyribonucleic acid (cDNA) was obtained by reverse transcription, and the SYBR Green method was used for PCR detection. The primer sequences were as follows: microRNA-204-5p: (F: 5'-GACGCTTTCCCTTTGTCATCCT-3'; R: 5'-GTGCAGGGTCCGAGGTATTC-3'); U6 (F: 5'-CTCGCTTCGGCAGCACA-3'; R: 5'-AACGCTTCACGAATTTGCGT-3'); SOX11 (F: 5'-GGTGGATAAGGATTTGGATTCG-3'; R: 5'-GCTCCGGCGTGCAGTAGT-3'); TLR4 (F: 5'-CCTGTCCCTGAACCCTATGA-3'; R: 5'-CCAGAACCAAACGATGGACT-3'); iNOS (F: 5'-GGCAGCCTGTGAGACCTTTG-3'; R: 5'-GCATTGGAAGTGAAGCGTTTC-3'); GAPDH (F: 5'-ACCCACTCCTCCACCTTTGA-3'; R: 5'-CTGTTGCTGTAGCCAAATTCGT-3').

Western Blot

The total protein was collected from each group, subjected to dodecyl sulfate and sodium salt-polyacrylamide gel electrophoresis (SDS-PAGE) electrophoresis, transferred into a polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA), blocked for 2 h at room temperature, and incubated with a specific primary antibody (Abcam, Cambridge, MA, USA) overnight. In the next day, the protein was incubated with horseradish peroxidase-labeled secondary antibody at room temperature for 2 h and the results of the analysis were observed after enhanced chemiluminescence (ECL) detection.

Enzyme-Linked Immunosorbent (ELISA) Assay

The double antibody sandwich Avidin Biotin Complex-enzyme-linked immunosorbent (ABC-ELISA; Novus Biologicals, Littleton, CO, USA) was used. The monoclonal antibody was coated with the monoclonal antibody on the ELISA plate and the target protein in the standard sample then, the cell samples were combined with the monoclonal antibody, and the biotinylated target protein was added to form an immune complex attached to the plate. After a mixture of horseradish peroxidase-labeled Streptavidin and biotin, the substrate working solution was added in, and finally the stop solution sulfuric acid was added to stop the reaction. The optical density (OD) value was measured at 450 nm. The protein concentration to be measured could be directly proportional to the OD value, and the protein concentration could be obtained according to the standard curve.

Luciferase Reporter Gene Assay

293T cells (3105 cells per well) were plated into a 24-well plate, and then the wild-type or mutant psiCHECK-2 SOX11 vector (Generay, Shanghai, China) and microRNA- 204-5p mimic were co-transfected into the cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). After 24 h of transfection, the cells were collected and their luciferase activities were measured using a dual luciferase reporter assay system (Promega, Madison, WI, USA).

Statistical Analysis

All data were statistically analyzed by Statistical Product and Service Solutions (SPSS) 17.0 (SPSS Inc., Chicago, IL, USA) statistical software. The data of each group were expressed as mean ± standard deviation (x ± s). The *t*-test was used to analyze the data between the two groups. $p<0.05$ was considered statistically significant ($^*p<0.05$).

Results

MicroRNA- 204-5p was Under-Expressed in SCI Patients and Models

Our study found that the level of microRNA-204-5p in the plasma of the SCI patients was conspicuously lower than that in healthy people (Figure 1A). Then, the mice SCI model was constructed to detect the grip strength of their dual forelimbs, left forelimbs, and right forelimbs after blunt spinal cord contusion. It was found that the SCI injured mice were severely impaired in motor function during one week of injury and could not perform any action. After 7 days from the operation, the grip strength of both of the two claws or the left and right forepaws alone gradually and slightly improved with time, but there was still a serious motor dysfunction. In the Sham-operated group, the strength decreased after surgery, but as the wound gradually recovered, it gradually returned to the preoperative level (Figure 1B-1D). At the same time, qRT-PCR results revealed that the level of microRNA-204-5p gradually decreased on the third day after SCI and showed the lowest on the seventh day (Figure 1E). Western blot results indicated that the SCI mice had a time-dependent effect on the expression of the inflammatory factors including TLR4 and iNOS after spinal cord blunt trauma surgery (Figure 1F). These results suggested that microRNA-204-5p might be involved in the development of SCI with a sustained inflammatory response.

MicroRNA-204-5p Played a Protective Role in SCI

To further investigate the role of microRNA-204-5p in the development of SCI, we up-regulated microRNA-204-5p in SCI mice and found that these mice showed decreased grip strength after spinal contusion. However, their grip force values were still conspicuously higher than those of the control mice which began to recover on

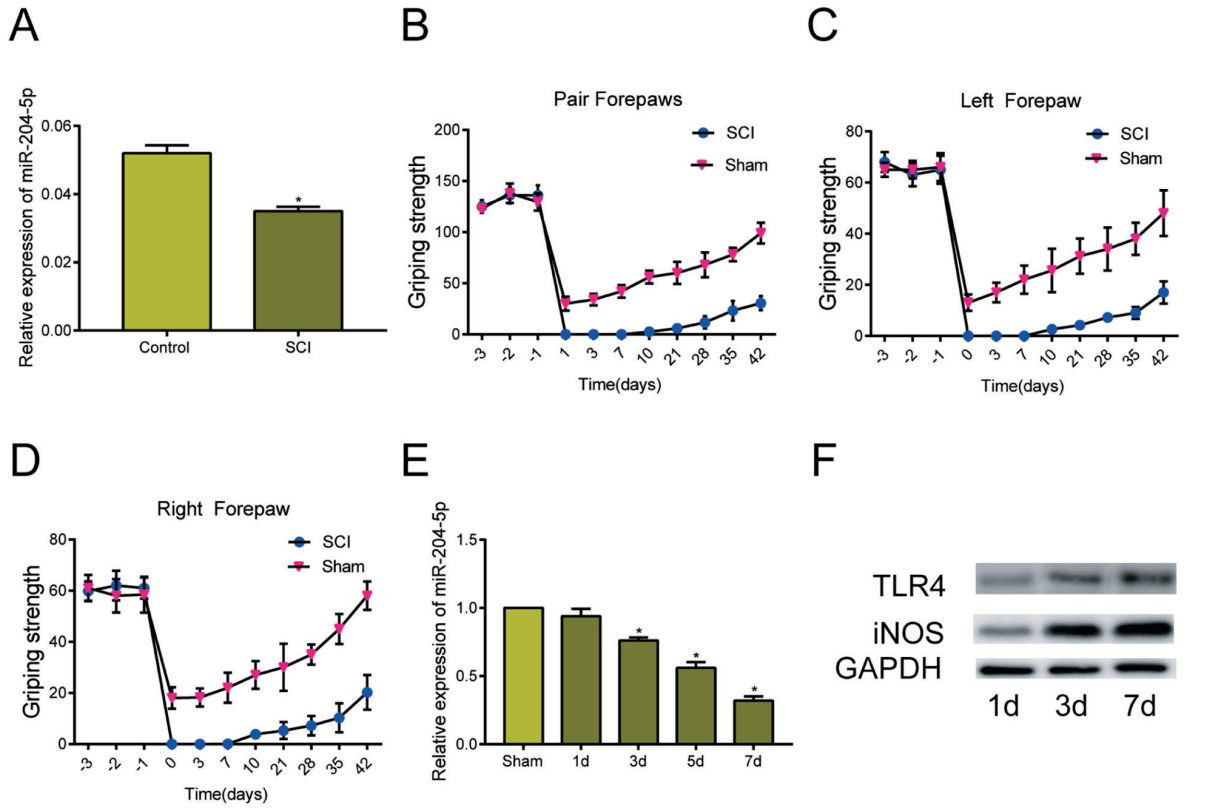

Figure 1. Low expression of microRNA-204-5p in SCI patients and models. ***A,*** In the plasma of SCI patients, the expression of microRNA-204-5p was significantly lower than that of the normal group. ***B, C, D,*** Handgrip strength of mice in each group was measured. The Sham operation group was Sham group in the figure, and spinal cord injury group was SCI group, indicating that the model was successfully constructed. ***E,*** The expression of microRNA-204-5p was significantly decreased in SCI mice, which was time-dependent. ***F,*** The expression of inflammatory cytokines TLR4 and iNOS was significantly increased in the SCI model, with a certain time effect.

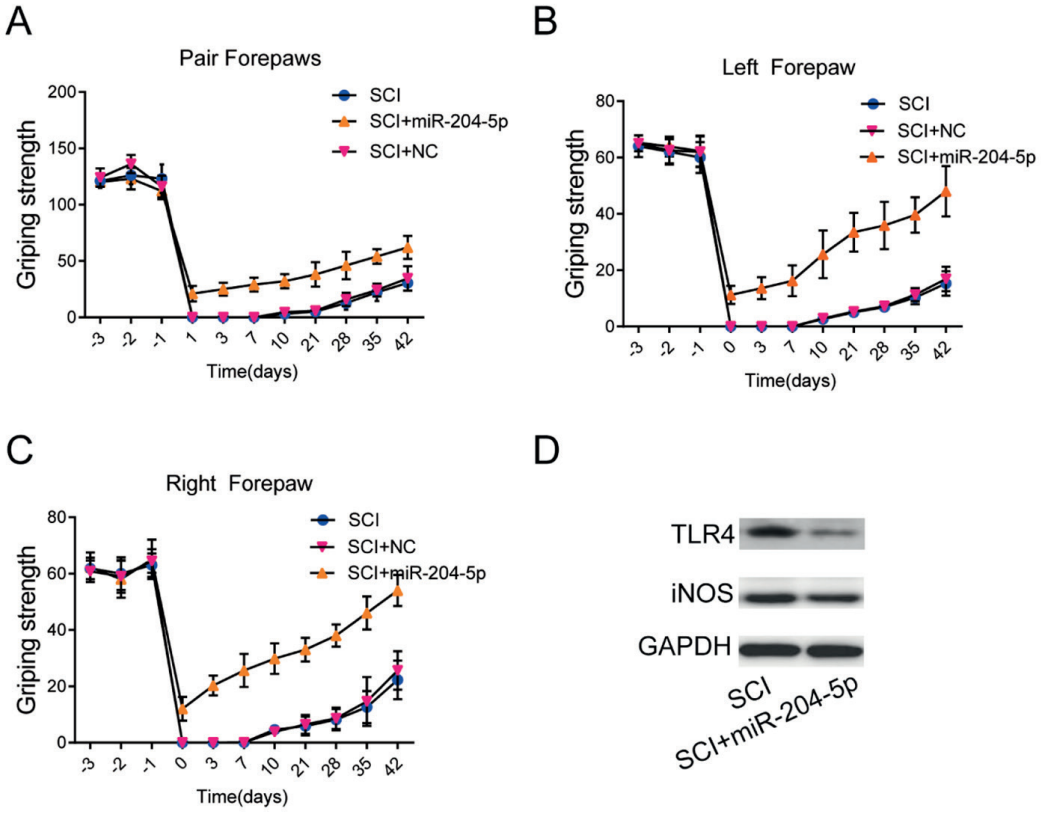

Figure 2. MicroRNA-204-5p plays a protective role in SCI. ***A-C,*** Compared with the control group, after up-regulating the expression of microRNA-204-5p in the SCI model, the grip strength of mice in each group increased significantly. ***D,*** In the SCI model, the protein expressions of TLR4 and iNOS were significantly decreased after the up-regulation of microRNA-204-5p.

the third day after surgery. MicroRNA-204-5p enhanced the recovery rate of the postoperative grip strength in mice, both the recovery rate and recovery degree were conspicuously better than in the control SCI mice (Figure 2A-2C). Furthermore, after up-regulating the microRNA- 204-5p expression in SCI mice, we found that the expression of the inflammatory factors such as TLR4 and iNOS was conspicuously reduced (Figure 2D). The above results suggested that microRNA-204-5p can alleviate the inflammation in SCI mice and thus exert a protective role in the development of SCI.

SOX11 was Identified as the Target Gene of MicroRNA-204-5p

To further explore the protective role of microRNA-204-5p in the development of SCI, we found a potential binding site for microRNA-204-5p and SOX11 by bioinformatics analysis (Figure 3A). Furthermore, further ELISA assays revealed that SOX11 expression was conspicuously higher in the plasma of SCI patients than in the normal group (Figure 3B). At the same time, we found that SOX11 mRNA levels were increased with time (Figure 3C). To observe the relationship between microRNA- 204-5p and SOX11, we overexpressed or knocked down microRNA-204-5p in 293T cells and found that the former treatment enhanced the SOX11 mRNA expression, while the latter inhibited that (Figure 3D). In addition, the dual-luciferase reporter gene results indicated that upregulation of miR-204-5p in 293T cells transfected with psi-CHECK-2 SOX11 vector has reduced the fluorescence activity (Figure 3E). The above results suggested that microRNA-204-5p can target SOX11 to regulate its expression.

MicroRNA- 204-5p Played a Protective Role in SCI by Regulating SOX11

To figure out whether microRNA-204-5p plays a protective role in SCI by regulating SOX11, we simultaneously overexpressed microRNA-204-5p and SOX11 in SCI mice. Western blot results showed that the protein levels of TLR4 and iNOS in SCI mice with up-regulated-microRNA-204-5p were significantly decreased, while the simulta-

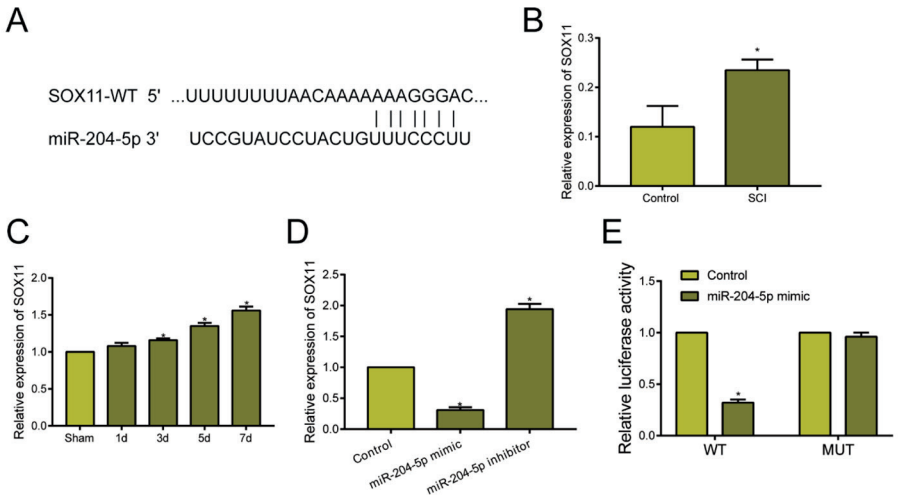

Figure 3. SOX11 is the target gene of microRNA-204-5p. ***A,*** There are potential binding sites between microRNA-204-5p and SOX11. ***B,*** In the plasma of SCI patients, the expression of SOX11 was significantly higher than that of the normal group. ***C,*** The expression of SOX11 was significantly increased in SCI mice, and it was time dependent. ***D,*** In 293T tool cells, the expression of SOX11 was significantly decreased after the up-regulation of microRNA-204-5p, while the expression of SOX11 was significantly increased after the downregulation of microRNA-204-5p. ***E,*** The results of the dual luciferase reporter gene showed that there was a binding relationship between the two groups.

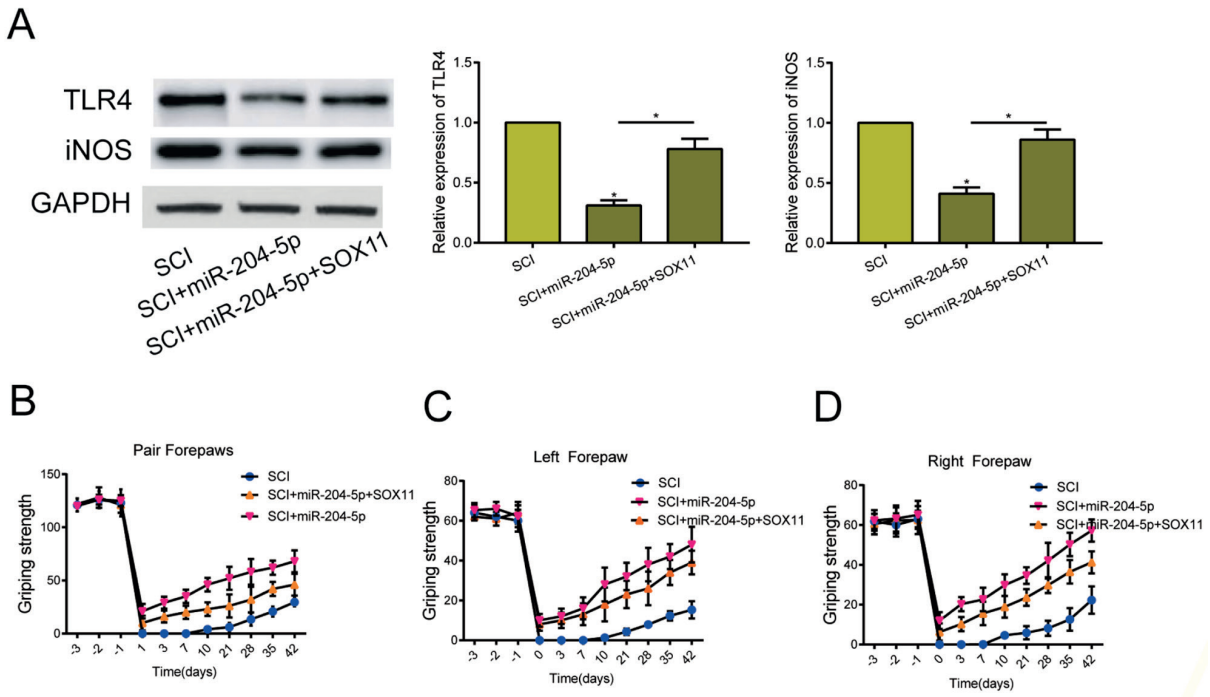

Figure 4. MicroRNA-204-5p functions by regulating SOX11. ***A,*** In SCI mice, the simultaneous up-regulation of SOX11 expression can partially relieve the inhibitory effect of microRNA-204-5p on TLR4 and iNOS. ***B-D,*** In SCI models, the up-regulation of SOX11 also partially reversed the protective effect of microRNA-204-5p on SCI

neous upregulation of SOX11 partially reversed the inhibitory effect of microRNA-204-5p on the content of inflammatory cytokines (Figure 4A). Subsequently, it was found in the grip strength measurement that the simultaneous up-regulation of microRNA-204-5p and SOX11 partially eliminated the therapeutic effect of microRNA-204-5p on the recovery of the postoperative handgrip strength (Figures 4B-4D). The above experimental results indicated that microRNA-204-5p could

reduce the inflammation in SCI mice by regulating SOX11 and played a protective role in the development of SCI.

Discussion

Spinal cord injury remains a major public health problem in developed countries and worldwide, and the pathophysiological features of SCI are secondary to primary lesions[18]. The oxidative stress is a series of adaptive responses caused by the imbalance between active oxygen and antioxidant system in the body[19]. Studies[20,21] have shown that the alleviation of the early inflammatory responses can improve neurobehavioral recovery after SCI. Our experimental results indicated that the reduction of the level of the inflammatory factors in SCI mice contributed to the recovery of mouse grip strength.

MiRNAs can play a regulatory role in the progression of SCI, as miRNAs regulate gene expression at the post-transcriptional level[22-24]. Hachisuka et al[25] have found that microRNAs widely affect the pathological response and signaling networks after SCI. It has been reported that microRNA-204-5p is down-regulated in the tubulointerstitial inflammation, and that the high expression of microRNA-204-5p has an inhibitory effect on inflammation[26]. However, the role of microRNA-204-5p in the development of SCI is still unclear. Our results showed that microRNA- 204-5p was lowly expressed in SCI and was time-dependent. The up-regulation of microRNA-204-5p attenuated the inflammatory response caused by SCI and promoted recovery after SCI.

SOX11, a transcription factor, plays a pivotal role in the embryonic development of the central nervous system, neuronal growth and survival, and in the recovery of adult neurons in damaged tissues[27,28]. It has been reported that SOX11 is essential in the pulmonary inflammatory response[29]. Our results showed that SOX11 expression was conspicuously increased in the plasma of SCI patients. Similarly, in the SCI mouse injury model, the SOX11 expression showed a time-dependent increase after spinal cord blunt trauma, accompanied by an increase of the inflammatory cytokines. At the same time, the simultaneous up-regulation of SOX11 and microRNA-204-5p can eliminate the influence of the up-regulated microRNA-204-5p on the recovery of SCI injury and the inhibition of inflammation.

Conclusions

We found that microRNA-204-5p was under-expressed in SCI patients and could inhibit the inflammatory response of the SCI mice model as well as promote their recovery of the grip strength by targeting SOX11. Therefore, our study demonstrated that microRNA-204-5p can play a pivotal regulatory role in the repair process of SCI, and may be an important target to promote the repair of SCI.

Conflict of interest

The authors declare no conflicts of interest.

References

1) Hyun JK, Kim HW. Clinical and experimental advances in regeneration of spinal cord injury. J Tissue Eng 2010; 2010: 650857.
2) Zhao P, Waxman SG, Hains BC. Modulation of thalamic nociceptive processing after spinal cord injury through remote activation of thalamic microglia by cysteine cysteine chemokine ligand 21. J Neurosci 2007; 27: 8893-8902.
3) Gomes-Leal W, Corkill DJ, Freire MA, Picanço-Diniz CW, Perry VH. Astrocytosis, microglia activation, oligodendrocyte degeneration, and pyknosis following acute spinal cord injury. Exp Neurol 2004; 190: 456-467.
4) Gris D, Marsh DR, Oatway MA, Chen Y, Hamilton EF, Dekaban GA, Weaver LC. Transient blockade of the CD11d/CD18 integrin reduces secondary damage after spinal cord injury, improving sensory, autonomic, and motor function. J Neurosci 2004; 24: 4043-4051.
5) DiSabato DJ, Quan N, Godbout JP. Neuroinflammation: the devil is in the details. J Neurochem 2016; 139 Suppl 2: 136-153.
6) Okada S. The pathophysiological role of acute inflammation after spinal cord injury. Inflamm Regen 2016; 36: 20.
7) Sun X, Jones ZB, Chen XM, Zhou L, So KF, Ren Y. Multiple organ dysfunction and systemic inflammation after spinal cord injury: a complex relationship. J Neuroinflammation 2016; 13: 260.
8) Garcia E, Aguilar-Cevallos J, Silva-Garcia R, Ibarra A. Cytokine and growth factor activation in vivo and in vitro after spinal cord injury. Mediators Inflamm 2016; 2016: 9476020.
9) Anwar MA, Al Shehabi TS, Eid AH. Inflammogenesis of secondary spinal cord injury. Front Cell Neurosci 2016; 10: 98.
10) Yu CH, Xing FY, Zhang JY, Xu JQ, Li YC. A combination of mRNA expression profile and miRNA expression profile identifies detection biomarkers

in different tumor stages of laryngeal squamous cell carcinoma. Eur Rev Med Pharmacol Sci 2018; 22: 7296-7304.

11) Thomas KT, Gross C, Bassell GJ. MicroRNAs sculpt neuronal communication in a tight balance that is lost in neurological disease. Front Mol Neurosci 2018; 11: 455.

12) Zhu H, Xie R, Liu X, Shou J, Gu W, Gu S, Che X. MicroRNA-494 improves functional recovery and inhibits apoptosis by modulating PTEN/AKT/mTOR pathway in rats after spinal cord injury. Biomed Pharmacother 2017; 92: 879-887.

13) Wang X, Su X, Gong F, Yin J, Sun Q, Lv Z, Liu B. MicroRNA-30c abrogation protects against spinal cord ischemia reperfusion injury through modulating SIRT1. Eur J Pharmacol 2019; 851: 80-87.

14) Alizadeh A, Karimi-Abdolrezaee S. Microenvironmental regulation of oligodendrocyte replacement and remyelination in spinal cord injury. J Physiol 2016; 594: 3539-3552.

15) Wang H, Moyano AL, Ma Z, Deng Y, Lin Y, Zhao C, Zhang L, Jiang M, He X, Ma Z, Lu F, Xin M, Zhou W, Yoon SO, Bongarzone ER, Lu QR. MiR-219 cooperates with miR-338 in myelination and promotes myelin repair in the CNS. Dev Cell 2017; 40: 566-582.

16) Ruan W, Ning G, Feng S, Gao S, Hao Y. MicroRNA381/Hes1 is a potential therapeutic target for spinal cord injury. Int J Mol Med 2018; 42: 1008-1017.

17) Martirosyan NL, Carotenuto A, Patel AA, Kalani MY, Yagmurlu K, Lemole GJ, Preul MC, Theodore N. The role of microRNA markers in the diagnosis, treatment, and outcome prediction of spinal cord injury. Front Surg 2016; 3: 56.

18) Qiao F, Atkinson C, Kindy MS, Shunmugavel A, Morgan BP, Song H, Tomlinson S. The alternative and terminal pathways of complement mediate post-traumatic spinal cord inflammation and injury. Am J Pathol 2010; 177: 3061-3070.

19) Han S, Arnold SA, Sithu SD, Mahoney ET, Geralds JT, Tran P, Benton RL, Maddie MA, D'Souza SE, Whittemore SR, Hagg T. Rescuing vasculature with intravenous angiopoietin-1 and alpha v beta 3 integrin peptide is protective after spinal cord injury. Brain 2010; 133: 1026-1042.

20) Genovese T, Esposito E, Mazzon E, Di Paola R, Caminiti R, Bramanti P, Cappelani A, Cuzzocrea S. Absence of endogenous interleukin-10 enhances secondary inflammatory process after spinal cord compression injury in mice. J Neurochem 2009; 108: 1360-1372.

21) Zhang W, Cheng L, Hou Y, Si M, Zhao YP, Nie L. Plumbagin protects against spinal cord injury-induced oxidative stress and inflammation in wistar rats through Nrf-2 upregulation. Drug Res (Stuttg) 2015; 65: 495-499.

22) Wang W, Shen H, Xie JJ, Ling J, Lu H. Neuroprotective effect of ginseng against spinal cord injury induced oxidative stress and inflammatory responses. Int J Clin Exp Med 2015; 8: 3514-3521.

23) Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 2014; 15: 509-524.

24) Hu JZ, Huang JH, Zeng L, Wang G, Cao M, Lu HB. Anti-apoptotic effect of microRNA-21 after contusion spinal cord injury in rats. J Neurotrauma 2013; 30: 1349-1360.

25) Hachisuka S, Kamei N, Ujigo S, Miyaki S, Yasunaga Y, Ochi M. Circulating microRNAs as biomarkers for evaluating the severity of acute spinal cord injury. Spinal Cord 2014; 52: 596-600.

26) Li H, Wang J, Liu X, Cheng Q. MicroRNA-204-5p suppresses IL6-mediated inflammatory response and chemokine generation in HK-2 renal tubular epithelial cells by targeting IL6R. Biochem Cell Biol 2019; 97: 109-117.

27) Jankowski MP, Cornuet PK, McIlwrath S, Koerber HR, Albers KM. SRY-box containing gene 11 (Sox11) transcription factor is required for neuron survival and neurite growth. Neuroscience 2006; 143: 501-514.

28) Haslinger A, Schwarz TJ, Covic M, Lie DC. Expression of Sox11 in adult neurogenic niches suggests a stage-specific role in adult neurogenesis. Eur J Neurosci 2009; 29: 2103-2114.

29) Mitamura Y, Nunomura S, Nanri Y, Arima K, Yoshihara T, Komiya K, Fukuda S, Takatori H, Nakajima H, Furue M, Izuhara K. Hierarchical control of interleukin 13 (IL-13) signals in lung fibroblasts by STAT6 and SOX11. J Biol Chem 2018; 293: 14646-14658.