European Review for Medical and Pharmacological Sciences 2020; 24: 491-498

MiRNA-411 attenuates inflammatory damage and apoptosis following spinal cord injury

F. SUN[1], S.-G. LI[2], H.-W. ZHANG[1], F.-W. HUA[1], G.-Z. SUN[1], Z. HUANG[3]

[1]General Hospital of Heilongjiang General Administration of Agriculture and Reclamation, Haerbin, China
[2]The Seventh Medical Center of General Hospital PLA Beijing Municipality, Beijing, China
[3]Department of Orthopedics, Guanghua Integrative Medicine Hospital, Shanghai, China

Abstract. – OBJECTIVE: To investigate the role and regulate the target of miRNA-411 on spinal cord injury.

MATERIALS AND METHODS: The microglia cultured *in vitro* was activated by lipopolysaccharide (LPS) to express the inflammatory phenotype. The inflammatory response through miRNA-411 transfection in microglia was measured to certain whether increased miRNA-411 suppressed interleukin-18 (IL-18) level to attenuate the inflammation amplification *via* downregulating JNK pathway. Furthermore, we established spinal cord injury (SCI) model in SD rats and further explored the glial inflammatory degree and neurological recovery following miRNA-411 treatment. Lastly, we estimated the hindlimbs function of SCI rats with miRNA-411 administration or not within four weeks at post-SCI.

RESULTS: *In vitro*, miRNA-411 inhibited IL-18 expression and downregulated JNK pathway, along with that inflammatory microglia were declined. In SCI rats, we detected the decreased amounts of inflammatory microglia and reduction of the inflammatory factors after miRNA-411 treatment. IL-18 and JNK pathway was also restrained resulted from increased miRNA-411. In addition, apoptosis degree in injury site reduced and survived axons were relatively multiple in the miRNA-411 group compared with the SCI group. The Basso-Beattie-Bresnahan (BBB) locomotor scores of miRNA-411 treated rats were superior to those in rats with no treatment.

CONCLUSIONS: MiRNA-411 increase ameliorates the inflammatory microglia-induced neurological lesion and promotes neural recovery by JNK pathway inhibition *via* negative targeting IL-18 in SCI.

Key Words:
MiRNA-411, Spinal cord injury, JNK pathway, IL-18.

Introduction

Spinal cord injury (SCI) is one of the most serious neurological diseases in the central nervous system (CNS)[1,2], causing the destructive impact of organicism[3]. Following injury, the blood-spinal cord barrier (BSCB) is disrupted so that hemocytes and leukocytes reach to the lesion[4]. Accumulating leukocytes and cell debris alter vasopermeability leading to infiltration[5]. Besides, resident microglia stimulate the response to primary damage, which will recruit increased microglia and astrocytes to the surroundings of the damaged tissue epicenter[6]. As a result of the disorder of neuroimmunology and neuropathology, the aggravating neuroinflammatory action will provoke neurotoxicity and neuroinhibition, eventually, the remained neural tissue will be unable to compensate for normal neurophysiological activities[7]. Therefore, how to restrain inflammation following SCI widely influences architectural repair and functional remodeling of neuronetwork. MiRNAs are a group of non-coding RNA at length of 22-24 nucleotides and play a necessary role in the regulation of the biological procedures[8]. Evidently, there are various MiRNAs that occur exceptional variation following SCI, which signifies that miRNAs crucially participate in SCI pathogenesis[9]. MiRNA-411 has been reported to an effective tumor target miRNA[10]; in fact, decreased miRNA-411 enhanced bladder cancer growth *via* targeting MLLT11[11]. Autoregulatory loop between miRNA-411 and MAPK pathway promoted proliferation and differentiation of rhabdomyosarcoma[12]. Oncogenic microRNA-411 boosted lung carcinogenesis by negatively targeting SPRY4 and TXNIP[13]. However, Most et al[14] have demonstrated that miRNA-411 is a potential factor of neuromodulation. How to regulate miRNA-411 content following SCI maybe guide the neurologic recovery to an advantageous aspect. Interleukin-18 (IL-18) is one of the IL-1 family as an inflammatory magnify-

Corresponding Author: Zheng Huang, MD; e-mail: 13501772762@126.com

ing agent[15,16]. Because of the pleiotropic nature of IL-18, IL-18 receptor is expressed in various types of malignant cells and normal cells including natural killer cells (NK), macrophages, T lymphocytes, and B lymphocytes, microglia, astrocytes, and even neurons[17,18]. The activity of IL-18 is mainly involved in the organismal defense processes, ranging from inflammatory enhancement and immune regulation against body offenses to the tissue damage whose expression is unrestricted, leading to the development of the chronic inflammation[19]. Therefore, following SCI, exploring a target that effectively regulates IL-18 is crucial to the control of the neuroinflammatory levels. In the current study, we have verified that as a promising target, miRNA-411 increase suppressed IL-18 expression and downregulated IL-18-induced JNK pathway. Of note, SCI-induced neuroinflammation and apoptosis were alleviated through miRNA-411 treatment. Hence, hindlimbs functional recovery was improved with miRNA-411 treatment.

Materials and Methods

Primary Microglia Extraction and Culture

We sacrificed 3 days-old neonatal rats in 75% alcohol and removed the cerebral cortex in trypsin in order to dissolve the organization. Then, the obtained cells compound immigrated into medium containing glutamine. Two weeks later, the cells were put on a shaking bed to extract microglia at 37° C When purified microglia reached to 95%, we collected and incubated them with Dulbecco's Modified Eagle's Medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA) containing 10% defined fetal bovine serum (FBS; Gibco, Rockville, MD, USA) and 1% double antibiotics. When the confluence reached 90%, we transfected miRNA-411 mimic into microglia, and 100 ng/ml lipopolysaccharide (LPS) was used to stimulate them.

Animals and Grouping

The Male Sprague Dawley (SD) rats aged at six-weeks-old and weighted from 200g to 220g were selected to establish the SCI model. The Experimental Ethics have been approved by the General Hospital of Heilongjiang General Administration of Agriculture and Reclamation. All animals were kept in a room with a constant temperature at 22-25°C, humidity at 50%-60% and 12/12h circadian rhythms. Available food and water were given to rats until they were sacrificed. Three groups were needed in this study. In the Sham group (n=8) was performed a laminectomy. In the SCI group (n=8) was established the SCI model, and we injected normal saline (NS). In the SCI+miRNA-411 mimic group (n=8) was injected miRNA-411 mimic following SCI.

Surgical Procedure of SCI

Briefly, the rats were injected with 10% chloral hydrate for preoperative anesthesia. Following skin antisepsis, the rats were cut an incision at T10, and their muscles were bluntly dissected until lamina was visible. Following laminectomy, we impacted T10 spinal cord of rats with an Allen impactor. The hindlimbs extension and the tail-flick reflex signified a successful modeling. Artificial urination was performed twice a day until the urination function returns to normal.

Real Time-Polymerase Chain Reaction (qRT-PCR) and Western Blot

The total RNA was isolated from spinal cord tissue or microglia with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. After that, RNA was reverse transcribed to cDNA with PrimeScript™ RT Master Mix (Applied Biosystems, Foster City, CA, USA). RT-PCR was performed to quantify miRNA-411, JNK, and Glyceraldehyde phosphate dehydrogenase (GAPDH) mRNA expression levels. RT-PCR was conducted using SYBR Green Master Mix (Applied Biosystems, Foster City, CA, USA). GAPDH was used for normalization. The primers used for RT-PCR are shown in Table I. The relative mRNA expression levels were calculated by the $2^{-\Delta\Delta Ct}$ methods.

The tissues and microglia cells were harvested in radioimmunoprecipitation assay (RIPA; Beyotime, Shanghai, China) lysis buffer. The proteins were isolated using a Nuclear/Cytosol Fractionation Kit (Beyotime, Shanghai, China) according to the manufacturer's instructions. The protein concentrations were measured with the enhanced bicinchoninic acid (BCA) Protein Assay Kit (Beyotime, Shanghai, China). After blocking with 5% skim milk for 1 hour at room temperature and probed overnight with anti-CD11b (Abcam, Cambridge, CA, USA, 1:1000), anti-p-JNK (Abcam, Cambridge, CA, USA, 1:1000), anti-JNK (Abcam, Cambridge, CA, USA, 1:1000), anti-NF-κB (Abcam, Cambridge, CA, USA, 1:1000), anti-caspase-8 (Abcam, Cambridge, CA, USA,

1:1000), anti-bcl-2 (Abcam, Cambridge, CA, USA, 1:1000), and anti-GAPDH (Cell Signaling Technology, Danvers, MA, USA, 1:1000). After being washed with PBST, the membrane was incubated with secondary antibody (Abcam, Cambridge, CA, USA, 1:2000) for 2 h at room temperature. The protein bands were visualized and detected using the enhanced chemiluminescence system. GAPDH was used as the controls.

Immunofluorescence

The spinal cord samples in each group were washed three times with Phosphate-Buffered Saline (PBS), fixed with 4% formaldehyde for 24 h at room temperature, and then dehydrated in sucrose solution. The dehydrated samples were embedded in paraffin and cut to sections (6 μm). The sections underwent rehydration, deparaffinage, antigen retrieval, and blocked with 5% bovine serum albumin (BSA) for 1 h. Subsequently, the sections were incubated overnight at 4°C with a primary antibody against CD11b (Abcam, Cambridge, CA, USA, 1:500), IBA-1 (Abcam, Cambridge, CA, USA, 1:500) or caspase-3 (Abcam, Cambridge, CA, USA, 1:500), followed by Cy3-conjugated goat anti-rabbit IgG antibody (Abcam, Cambridge, CA, USA, 1:200) for 2 h at room temperature. The nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI; Beyotime, Shanghai, China, 1:500), and the sections were visualized using a fluorescence microscope (Zeiss, Oberkochen, Germany).

Basso-Beattie-Bresnahan (BBB) Locomotor Rating Method

The locomotor function of rats hindlimbs was estimated *via* BBB locomotor rating scale. The rats were allowed to free the activities in an open field within 5 minutes and two blinded researchers scored hindlimbs motor ability of every rat. We recorded the scores at 1, 7,14, 21, 28 days following SCI.

Statistical Analysis

All data are normality displayed by the means ± standard deviations. The data compared between the two groups were analyzed using the Student's *t*-test. The comparison between multiple groups was done using One-way ANOVA test followed by the post-hoc test (Least Significant Difference). *p*-values <0.05 were considered statistically significant.

Results

Inflammatory Inhibition in Microglia Results From Increased MiRNA-411 Downregulating IL-18-Induced JNK Pathway

To assure whether miRNA-411 increase in microglia influences LPS-induced alteration of inflammatory phenotype, we initially got through miRNA-411 mimic transfection in microglia and stimulated microglia using LPS. MiRNA-411 in RNA level was visualized using RT-PCR technique, displaying that in LPS activated microglia there was miRNA-411 a significant decrease, but miRNA-411 mimic transfection remarkably increased miRNA-411 expression (Figure 1A). Then, the microglia inflammatory phenotype was measured by Western blot, it was found that miRNA-411 increase suppressed microglia stimulation to the phenotype of inflammation (Figures 1B, 1C). Besides, IL-18 level was detected with ELISA assay, and we discovered that IL-18 expression in LPS activated microglia was apparently elevated, however, following treatment of miRNA-411 IL-18 content was prominently reduced in microglia (Figure 1D). Notably, we measured in Western blot result that JNK pathway induced by IL-18, which was considered as a pathway of the inflammatory development, was inhibited following miRNA-411 treatment (Figures 1E, 1F). Therefore, through *in vitro* cultured microglia, we verified that the microglial inflammatory level was inversely regulated by a miRNA-411 increase by descending IL-18 expression to inhibit the JNK pathway.

MiRNA-411 Increase Alleviates Glial Inflammatory Reaction by Suppressing IL-18 Level in SCI

Further, we established SCI model in rats to certain whether a variety of miRNA-411 level influences the extent of glial inflammation. Firstly, we detected miRNA-411 levels using RT-PCR within one week following SCI, finding that the spinal cord trauma led to a miRNA-411 reduction, but miRNA-411 mimic treatment rescued miRNA-411 decrease trend during the first week after injury (Figure 2A). Moreover, to verify whether miRNA-411 descending in SCI is associated with glial inflammation, we used immunofluorescence technique to reflect a range of microglial activation. Inflammatory microglia expressed high-level CD11b factor; hence, microglia underwent IBA-1 and CD11b co-staining

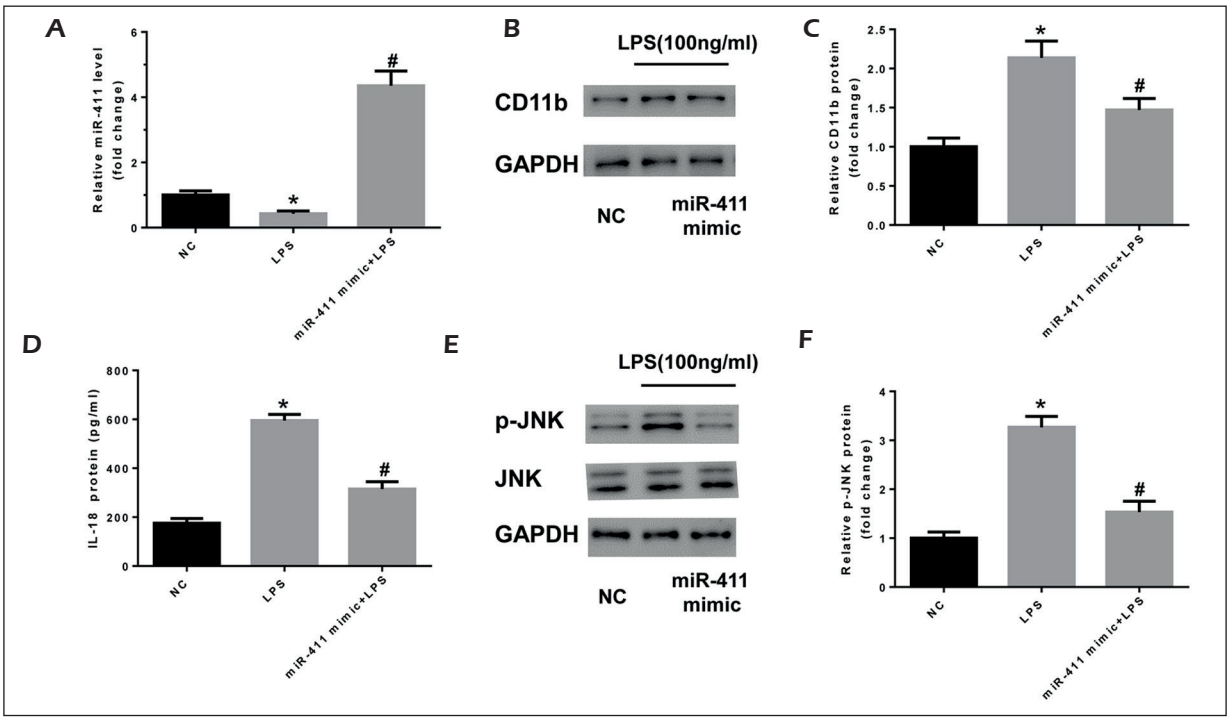

Figure 1. Inflammatory inhibition in microglia results from increased miRNA-411 downregulating IL-18-induced JNK pathway. **A,** The RNA level of miRNA-411 in NC, LPS, and miR-411 mimic+LPS group. **B,** The protein bands of CD11b and GAPDH in NC, LPS, and miR-411 mimic+LPS group. **C,** Grey value analysis of CD11b in the three groups is considered to be the statistical difference. **D,** IL-18 protein level in NC, LPS, and miR-411 mimic+LPS group. **E,** p-JNK, JNK, and GAPDH protein bands in NC, LPS, and miR-411 mimic+LPS group. **F,** p-JNK levels in the three group exist statistic difference.

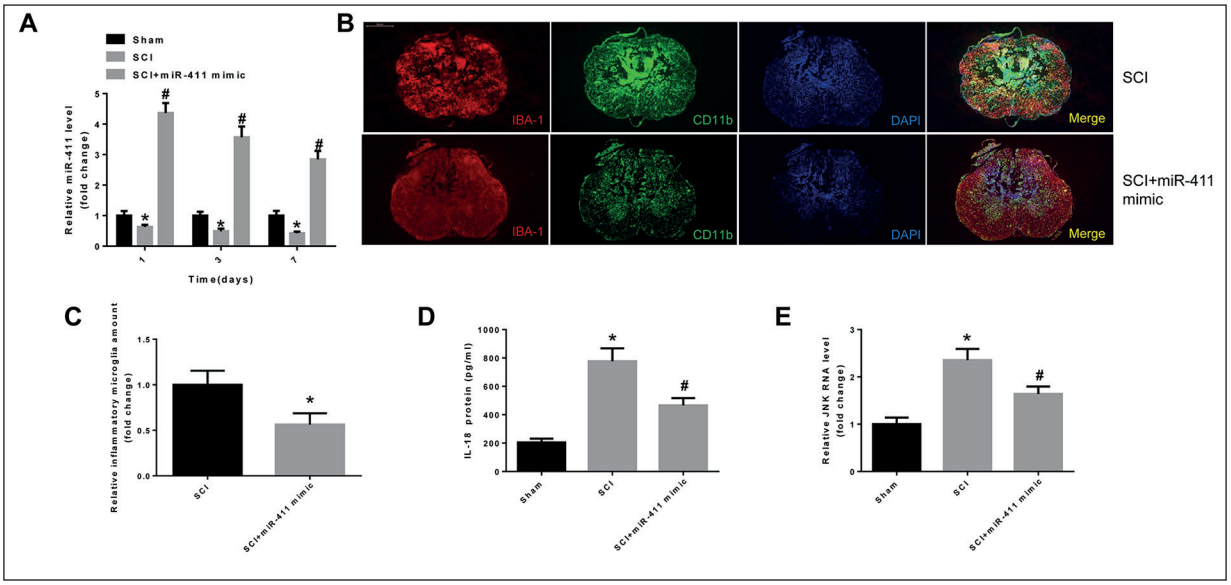

Figure 2. MiRNA-411 increase alleviates glial inflammatory reaction by suppressing IL-18 level in SCI. **A,** MiRNA-411 changes within a week post modeling in Sham, SCI, and SCI+miR-411 mimic group. **B,** CD11b and IBA-1 co-staining of immunofluorescence at 3 days post-injury in SCI and SCI+miR-411 mimic group (magnification: 10×). **C,** Relative CD11b-positive area in injury core is statistical significance between two groups. **D,** IL-18 protein difference in the three groups at 3 days following SCI. **E,** JNK RNA level in the three groups at 3 days after SCI.

in the injured spinal cord to materialize image (Figures 2B, 2C). According to the results, we demonstrated that the area of the glial inflammation was remarkably reduced *via* miRNA-411 mimic treatment. Meanwhile, we speculated that miRNA-411 elevation similarly induced IL-18 restraining to conclude the glial inflammatory inhibition *in vivo*. Therefore, we continued to measure IL-18 (Figure 2D) and its downstream JNK pathway levels (Figure 2E) using ELISA and RT-PCR. It was displayed that, following miRNA-411 mimic treatment, IL-18 and JNK RNA declined their expression in the injured spinal cord. The all above consequences led us to draw a conclusion that increased miRNA-411 attenuated the glial inflammation reaction *via* inducing IL-18 and JNK pathway suppression *in vivo* SCI model.

Accumulated MiRNA-411 Reduces Apoptosis in Lesion by Inhibiting Caspase System

Apoptosis induces the primary death of neural cells following SCI, and it also intimately relates to the inflammation development. Hence, we explored that increased miRNA-411 rescued cells simultaneously by weakening neural apoptosis along with inflammatory restraining. Through immunofluorescence staining, we visualized apoptosis key enzyme caspase-3 level in injured epicenter at 7 days following SCI, finding that miRNA-411 mimic administration receded caspase-3 expression (Figures 3A, 3B). Moreover, as the associated apoptosis activated the target and enzyme system, NF-κB and caspase-8 were detected with Western blot, and it was discovered that caspase-8 and NF-κB were decreased with miRNA-411 mimic treatment, yet the bcl-2 level, an antagonist of apoptosis, was inversely increased due to miRNA-411 increase (Figures 3C, 3D). Therefore, we drew a conclusion that miRNA-411 accumulation declined apoptosis development by inhibiting caspase and NF-κB.

MiRNA-411 Increase Improves Hindlimbs Locomotor Recovery Following SCI in Rats

Locomotor functional recovery was evaluated *via* BBB motor rating scale. The rats in each group were accepted to hindlimbs motor evaluation at 1, 7, 14, 21, 28 days, respectively. According to the scores, we made an analysis and exhibited the results in Figure 4. The Sham group took almost normal scores for four weeks, while the SCI group and miRNA-411 mimic group stayed

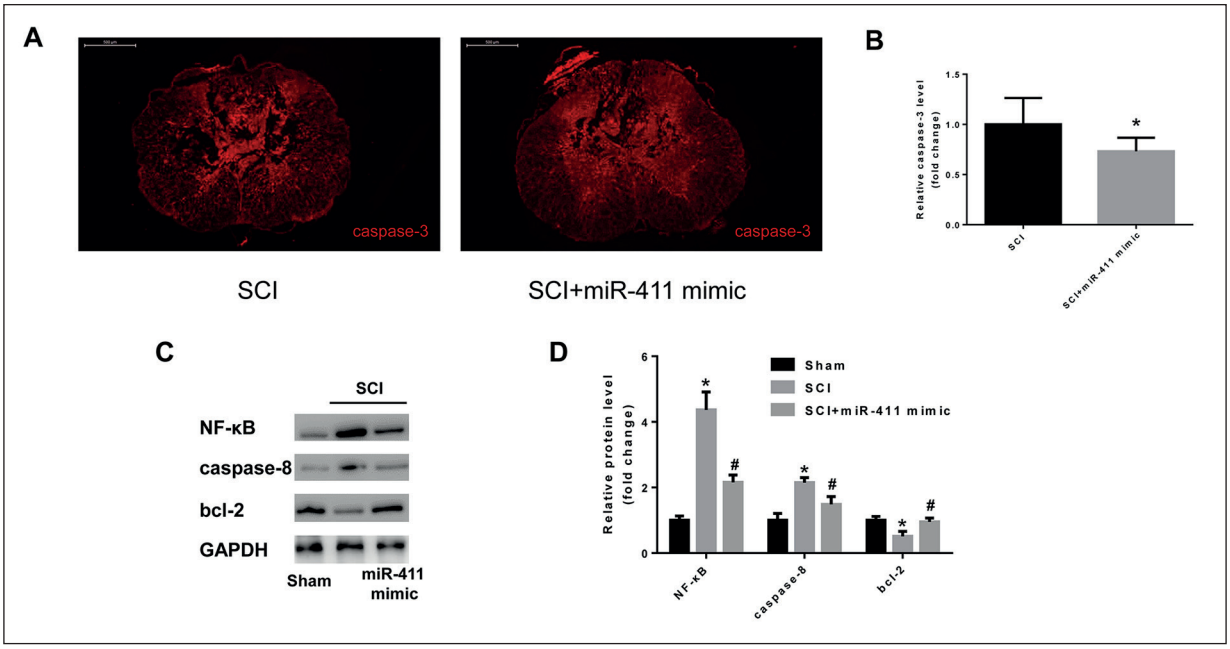

Figure 3. Accumulated miRNA-411 reduces the apoptosis in the lesion by inhibiting the caspase system. **A,** Caspase-3 images of immunofluorescence in SCI and SCI+miR-411 mimic group at 7 days post-SCI (magnification: 10×). **B,** The relative caspase-3-positive area in injury site is statistical significance between two groups. **C,** NF-κB, caspase-8, and bcl-2 protein bands in the three group at 7 days post-injury. **D,** The protein difference among groups is statistical significance.

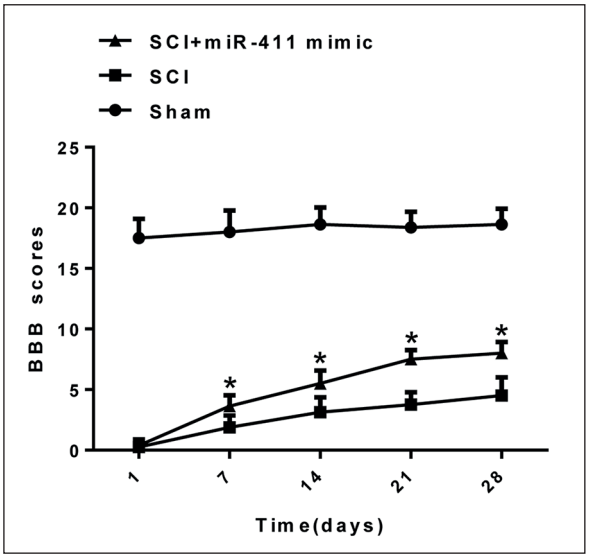

Figure 4. MiRNA-411 increase improves the hindlimbs locomotor recovery following SCI in rats. BBB scores among groups at 1, 7, 14, 21, 28 days following SCI.

originally at a low level and the motor recovery remarkably improved at 7 days at post-SCI. Importantly, miRNA-411 mimic treatment significantly promoted recovery compared with that in the SCI group from 7 days to 28 days. Therefore, miRNA-411 increase following SCI could effectively accelerate the locomotor recovery in rats.

Discussion

SCI-induced destructive inflammatory damage is considered to dominate the secondary injury in the pathological development, in which microglia undergo inflammatory activation and mediate the apoptosis procedure[20,21]. Resident microglia activation seen as a critical process for response to trauma induces neural tissue and structure disruption by releasing nitric oxide and secreting pro-inflammatory cytokines[22,23]. The secondary damage following SCI is associated with the alteration of the microglial inflammatory type, including the synthesis of the neurotoxic substances and a release of the inflammatory mediators[24]. Besides, a crop of pathological alterations was subject to the modulation of microglial inflammation, such as cytomembrane peroxidation, neurons loss, and demyelination change[25]. Due to the complicated mechanism of SCI, inflammation and apoptosis are widely thought as specific features during its process.

MiRNAs aberrant variations following SCI have been demonstrated to be involved in a series of biological events including astrocytic glial scar[26], anti-apoptosis[27], and neutrophils expression[28]. Gong et al[29] have suggested that miRNA-411 elevation is in favor of functional recovery *via* down-regulating FasL following SCI in rats and miRNA-411 can regulate IL-18 expression in tumor[30]. Hence, we made a hypothesis, the suppression of IL-18 in SCI-induced glial inflammation attributed to miRNA-411 increase maybe minimizes the inflammatory signaling pathway and state. Besides, we speculated whether miRNA-411 treatment could have a significant effect on apoptosis. Until now, miRNA-411 has been reported to a significative regulatory target in various tumor diseases; however, its potential therapeutic value in neurology has been seldom probed yet. Therefore, we explored how does miRNA-411 work, following SCI.

In the current study, between SCI+miRNA-411 mimic group and SCI group, it is evident to notice that an increased miRNA-411 is accompanied with improved neural recovery. We discovered that miRNA-411 mimic treatment both *in vitro* and *in vivo* inhibits microglia-induced inflammatory reaction *via* inversely regulating IL-18 and its downstream JNK pathway. Moreover, apoptosis is another factor leading to neural loss following SCI. Hence, the suppression of the apoptosis in injured spinal cord tissue may also promote neurologic recovery and attenuate secondary damage. We firstly verified miRNA-411 effect in the inflammatory inhibition. Considering the connection between inflammation and apoptosis, we continued to probe whether increased miRNA-411 both exerts an anti-apoptosis role in SCI. Scholars have suggested that NF-κB is involved in the modulation of apoptosis because of excessive inflammation. Likewise, our researches make favorable support to the perspective that NF-κB is restrained by increased miRNA-411 expression. Thus, bcl-2 level is enhanced, but the caspase system is crippled. Prospectively, miRNA-411 is exactly a promising target that induces glial inflammatory inhibition and anti-apoptosis reaction by influencing IL-18 and JNK pathway in SCI.

Conclusions

It has been demonstrated in this work that miRNA-411 can be an effective factor that works by inhibiting glial inflammation and cell apoptosis *via* downregulation of IL-18 and JNK pathway to alleviate SCI.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

Project of Scientific and Technical Committee of Changning District (CNKW2018Y13).

References

1) Dubreuil CI, Winton MJ, McKerracher L. Rho activation patterns after spinal cord injury and the role of activated Rho in apoptosis in the central nervous system. J Cell Biol 2003; 162: 233-243.
2) Zhang K, Zheng J, Bian G, Liu L, Xue Q, Liu F, Yu C, Zhang H, Song B, Chung SK, Ju G, Wang J. Polarized macrophages have distinct roles in the differentiation and migration of embryonic spinal-cord-derived neural stem cells after grafting to injured sites of spinal cord. Mol Ther 2015; 23: 1077-1091.
3) Pruss H, Tedeschi A, Thiriot A, Lynch L, Loughhead SM, Stutte S, Mazo IB, Kopp MA, Brommer B, Blex C, Geurtz LC, Liebscher T, Niedeggen A, Dirnagl U, Bradke F, Volz MS, DeVivo MJ, Chen Y, von Andrian UH, Schwab JM. Spinal cord injury-induced immunodeficiency is mediated by a sympathetic-neuroendocrine adrenal reflex. Nat Neurosci 2017; 20: 1549-1559.
4) Yu Q, Huang J, Hu J, Zhu H. Advance in spinal cord ischemia reperfusion injury: blood-spinal cord barrier and remote ischemic preconditioning. Life Sci 2016; 154: 34-38.
5) Tilley SL, Wagoner VA, Salvatore CA, Jacobson MA, Koller BH. Adenosine and inosine increase cutaneous vasopermeability by activating A(3) receptors on mast cells. J Clin Invest 2000; 105: 361-367.
6) Caito S, Yu Y, Aschner M. Differential response to acrylonitrile toxicity in rat primary astrocytes and microglia. Neurotoxicology 2013; 37: 93-99.
7) Conductier G, Blondeau N, Guyon A, Nahon JL, Rovere C. The role of monocyte chemoattractant protein MCP1/CCL2 in neuroinflammatory diseases. J Neuroimmunol 2010; 224: 93-100.
8) Acunzo M, Croce CM. MicroRNA in cancer and cachexia--a mini-review. J Infect Dis 2015; 212 Suppl 1: S74-S77.
9) Liu NK, Wang XF, Lu QB, Xu XM. Altered microRNA expression following traumatic spinal cord injury. Exp Neurol 2009; 219: 424-429.
10) Guo L, Yuan J, Xie N, Wu H, Chen W, Song S, Wang X. MiRNA-411 acts as a potential tumor suppressor miRNA via the downregulation of specificity protein 1 in breast cancer. Mol Med Rep 2016; 14: 2975-2982.
11) Jin H, Sun W, Zhang Y, Yan H, Liufu H, Wang S, Chen C, Gu J, Hua X, Zhou L, Jiang G, Rao D, Xie Q, Huang H, Huang C. MicroRNA-411 downregulation enhances tumor growth by upregulating MLLT11 expression in human bladder cancer. Mol Ther Nucleic Acids 2018; 11: 312-322.
12) Sun M, Huang F, Yu D, Zhang Y, Xu H, Zhang L, Li L, Dong L, Guo L, Wang S. Autoregulatory loop between TGF-beta1/miR-411-5p/SPRY4 and MAPK pathway in rhabdomyosarcoma modulates proliferation and differentiation. Cell Death Dis 2015; 6: e1859.
13) Wang SY, Li Y, Jiang YS, Li RZ. Investigation of serum miR-411 as a diagnosis and prognosis biomarker for non-small cell lung cancer. Eur Rev Med Pharmacol Sci 2017; 21: 4092-4097.
14) Most D, Salem NA, Tiwari GR, Blednov YA, Mayfield RD, Harris RA. Silencing synaptic MicroRNA-411 reduces voluntary alcohol consumption in mice. Addict Biol 2019; 24: 604-616.
15) Dinarello CA. Membrane interleukin-18 revisits membrane IL-1alpha in T-helper type 1 responses. Eur J Immunol 2012; 42: 1385-1387.
16) Netea MG, Kullberg BJ, Verschueren I, Van Der Meer JW. Interleukin-18 induces production of proinflammatory cytokines in mice: no intermediate role for the cytokines of the tumor necrosis factor family and interleukin-1beta. Eur J Immunol 2000; 30: 3057-3060.
17) Gerdes N, Sukhova GK, Libby P, Reynolds RS, Young JL, Schönbeck U. Expression of interleukin (IL)-18 and functional IL-18 receptor on human vascular endothelial cells, smooth muscle cells, and macrophages: implications for atherogenesis. J Exp Med 2002; 195: 245-257.
18) Hoshino K, Tsutsui H, Kawai T, Takeda K, Nakanishi K, Takeda Y, Akira S. Cutting edge: generation of IL-18 receptor-deficient mice: evidence for IL-1 receptor-related protein as an essential IL-18 binding receptor. J Immunol 1999; 162: 5041-5044.
19) Yellayi S, Braun S, Domingues HG, Kityatana N, Murali R, Johnson RP, Mansfield K, Westmoreland SV, O'Neil SP. Cloning and characterization of rhesus IL-18 binding protein, a natural antagonist to IL-18. Cytokine 2010; 51: 232-239.
20) Weaver LC, Marsh DR, Gris D, Meakin SO, Dekaban GA. Central mechanisms for autonomic dysreflexia after spinal cord injury. Prog Brain Res 2002; 137: 83-95.
21) Fleming JC, Norenberg MD, Ramsay DA, Dekaban GA, Marcillo AE, Saenz AD, Pasquale-Styles M, Dietrich WD, Weaver LC. The cellular inflammatory response in human spinal cords after injury. Brain 2006; 129 (Pt 12): 3249-3269.
22) Choi SH, Aid S, Kim HW, Jackson SH, Bosetti F. Inhibition of NADPH oxidase promotes alternative and anti-inflammatory microglial activation during neuroinflammation. J Neurochem 2012; 120: 292-301.
23) Ng SY, Semple BD, Morganti-Kossmann MC, Bye N. Attenuation of microglial activation with minocycline is not associated with changes in neurogenesis after focal traumatic brain injury in adult mice. J Neurotrauma 2012; 29: 1410-1425.

24) Qu WS, Tian DS, Guo ZB, Fang J, Zhang Q, Yu ZY, Xie MJ, Zhang HQ, Lü JG, Wang W. Inhibition of EGFR/MAPK signaling reduces microglial inflammatory response and the associated secondary damage in rats after spinal cord injury. J Neuroinflammation 2012; 9: 178.

25) Kang JQ, Chong ZZ, Maiese K. Akt1 protects against inflammatory microglial activation through maintenance of membrane asymmetry and modulation of cysteine protease activity. J Neurosci Res 2003; 74: 37-51.

26) 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.

27) 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.

28) 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.

29) Gong ZM, Tang ZY, Sun XL. MiR-411 suppresses acute spinal cord injury via downregulation of Fas ligand in rats. Biochem Biophys Res Commun 2018; 501: 501-506.

30) Yamamoto K, Seike M, Takeuchi S, Soeno C, Miyanaga A, Noro R, Minegishi Y, Kubota K, Gemma A. MiR-379/411 cluster regulates IL-18 and contributes to drug resistance in malignant pleural mesothelioma. Oncol Rep 2014; 32: 2365-2372.