European Review for Medical and Pharmacological Sciences 2018; 22: 5416-5423

Over-expressed miR-27a-3p inhibits inflammatory response to spinal cord injury by decreasing TLR4

P. ZHANG, L.-Q. LI, D. ZHANG, Y. SHEN

Department of Spine Surgery, The Third Hospital of Hebei Medical University, Shijiazhuang, China
Peng Zhang and Liqiang Li contributed equally to this work

Abstract. – OBJECTIVE: We investigate whether microRNA-27a-3p (miR-27a-3p) can inhibit the inflammatory response of spinal cord injury by negatively regulating toll-like receptor 4 (TLR4).

PATIENTS AND METHODS: The quantitative Real-time polymerase chain reaction (qRT-PCR) assay was used to detect the expression of miR-27a-3p and TLR4 in serum samples from patients with spinal cord injury and in hydrogen peroxide-treated C8-B4 and C8-D1A cells. Dual luciferase reporter assays were used to detect targeted binding of TLR4 to miR-27a-3p. The protein expression of miR-27a-3p and TLR4 and the two inflammatory factors, tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6), were all detected by Western blot.

RESULTS: TLR4 expression was elevated and miR-27a-3p was decreased in serum samples from patients with spinal cord injury and in hydrogen peroxide-treated C8-D1A and C8-B4 cells. Dual luciferase reporter assays results demonstrated that miR-27a-3p can bind to TLR4. Up-regulation of miR-27a-3p can decrease the expression of TNF-α and IL-6 and can also reduce TLR4 expression. After overexpression of TLR4, the expression of TNF-α and IL-6 were increased.

CONCLUSIONS: miR-27a-3p can inhibit the inflammatory response of spinal cord injury by negatively regulating TLR4.

Key Words
miR-27a-3p, TLR4, Spinal cord injury, Inflammatory response

Introduction

Spinal cord injury (SCI) is one of the main causes of disability[1]. Its pathophysiological process includes primary injury and secondary injury[2]. Secondary injury initially results in the recruitment of a large number of peripheral inflammatory cells, with a strong inflammatory response and subsequent glial cell death[3,4]. After spinal cord injury, there will be not only oxidative stress reaction, cell necrosis and apoptosis, but also a large number of inflammatory mediators locally, leading to immuno-inflammatory reactions. The ability to control or suppress the inflammatory response directly affects the recovery of spinal cord injury.

MicroRNAs are small RNAs that act as a crucial regulation role at the post-transcriptional level. They are about 19 to 25 nucleotides in length and can induce the formation of gene silencing complexes to cause degradation of the target gene mRNA or interfere with its translation[5]. Recent studies have demonstrated that microRNAs are closely related to tumor diagnosis, radiotherapy and chemotherapy tolerance, and prognostic testing[6].

microRNA-27a-3p (miR-27a-3p) has been confirmed as one of the members involved in the progression of various diseases. miR-27a-3p can increase the sensitivity of acute myelogenous leukemia TRAIL therapy by targeting the interference of the expression of PLAG1[7]. miR-27a-3p can also inhibit the migration and invasion of fibroblast-like synovial cells in patients with rheumatoid arthritis by inhibiting the expression of FSTL1 gene and interfering with the transmission of toll-like receptor 4 (TLR4)/NFκB signaling pathway[8]. In the latest study have demonstrated that miR-27a-3p can alleviate mitral valve inflammation in valvular heart disease[9]. The aim of this study was to investigate whether miR-27a-3p has a similar effect on the inflammatory response to spinal cord injury.

Patients and Methods

Object and Sample Collection

Serum samples of SCI patients and non-SCI serum samples from The Third Hospital of Hebei Medical University from May 2016 to May 2017 were collected and stored in liquid nitrogen until use. All cases were diagnosed according to ASIA (2011). All patients participated in this study all signed informed consent. This study was approved by the The Third Hospital of Hebei Medical University Research Ethics Committee.

Corresponding Author: Yong Shen, MD; e-mail: sduy63@163.com

Cell Culture and Hydrogen Peroxide Treatment

Murine astrocytes C8-D1A and C8-B4 were purchased from American Type Culture Collection (ATCC) (Manassas, VA, USA) and cultured with Dulbecco's modified eagle Medium (DMEM) containing 10% (v/v) fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA, USA). All cells were cultured in a 37°C incubator containing 5% carbon dioxide. 10 μM hydrogen peroxide concentration (Sigma-Aldrich, St. Louis, MO, USA) were added to cultured C8-D1A/C8-B4 cells for 12 h and then the cells were used for detection.

Cells Transfection

The cells in log phase with better vitality were selected and transfected according to Lipofectamine 2000 instructions (Invitrogen, Carlsbad, CA, USA). miR-27a-3p NC, mimics, inhibitor and TLR4 overexpression plasmids and the negative control were transfected into cells, respectively. The culture medium was changed 6 h after transfection.

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

The total RNAs of the cells were extracted using the TRIzol kit (Invitrogen, Carlsbad, CA, USA), then reverse-transcribed into complementary DNAs (cDNAs). The cDNAs were diluted with RNase-containing deionized water and adjusted to a final concentration of 3 ng/μL. Quantitative PCR procedures were performed according to the SYBR Green PCR Kit (TaKaRa, Otsu, Shiga, Japan) instructions and the total reaction system was 10 μL. The qRT-PCR parameters were: pre-denaturation at 95°C for 15 min, denaturation at 94°C for 15 s, 55°C for 30 s, extension at 72°C for 30 s, and 40 cycles. Primer sequences were as following: TNF-α (F: 5'-CCACGTCGTAGCAGCAAACCACCAAG-3', R: 5'-CAGGTACATGGGCTCCTCATACC-3'); IL-6 (F: 5'-TGTATGAACAACGATGATGCACTT-3', R: 5'-ACTCTGGCTTTGTCTTTCTTGTTATCT-3'); U6 (F: 5'- CGCTTCGGCAGCACATATAC-3', R: 5'-TTCACGAATTTGCGTGTCAT-3'), GAPDH (F: 5'-GGAATCCACTGGCGTCTTCA-3', R: 5'-GGTTCACGCCCATCACAAAC-3'), miR-27a-3p (F: 5'-CCCAAGCTTACTGTGAAACTGTGAAACGTGAAACTGTGAAACTGTGAAACTGTGAATCTAGAGC-3', R: 5'-GCTCTAGATTTCACAGTTTCACAGTTTCACAGTTTCACAGTTTCACAGTTTCACAGTAAGCTTGGG-3'), TLR4 (F: 5'-ACCTGTCCCTGAACCCTATGAA-3' R: 5'-CTTCTAAACCAGCCAGACCTTG-3')

Serum RNA Extraction

0.25 mL of serum and 2-8 μL of Polyacryl Carrier were added to 0.75 mL of TRI Reagent BD, then 0.2 mL of chloroform was added. After shaken for 15 seconds, the serum were incubated for 2-5 min, and centrifuged at 12000 g for 15 min at 4°C. After centrifugation, the aqueous complex was transferred to another centrifuge tube. Isopropanol was added to the mixture to extract RNAs. After the gel-like or white RNA was centrifuged, a precipitate formed at the bottom of the tube. Ethanol was added and the RNA precipitate was mixed and washed. The ethanol solution was removed after centrifugation and the RNA was dried in air for 5 min. The RNA was dissolved and collected at -80°C until use.

microRNA Reverse Transcription and qRT-PCR

The reverse transcription product was diluted with RNase-containing deionized water and adjusted to a final concentration of 3 ng/μL. Quantitative PCR procedures were performed according to the SYBR Green PCR Kit instructions and the total reaction system was 10 μL. The qRT-PCR parameters were: pre-denaturation at 95°C for 15 min, denaturation at 94°C for 15 s, 55°C for 30 s, extension at 72°C for 30 s, and 40 cycles.

Western Blot

Cell lysates containing the protease inhibitor phenylmethylsulfonyl fluoride (PMSF) (Beyotime, Shanghai, China) were added to lyse cells on ice. The lysate was collected and aspirated with centrifuging at 12000 r/min for 20 min. The supernatant was taken and the concentration of total protein was measured by the bicinchoninic acid (BCA) assay (Pierce, Rockford, IL, USA). 50 μg of total protein were taken from samples for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). After finished, the membrane was blocked with skim milk for 2 hours and eluted with Tris-buffered saline and Tween 20 (TBST) 6 times for 10 min. The specific primary antibodies were added and blocked the membrane overnight. The secondary antibody was incubated the next day after the membrane was washed with TBST. Finally, the membrane was exposured.

Dual Luciferase Reporter Assay

The 3' UTR sequence of TLR4 was downloaded from the NCBI website to construct the TLR4 wild-type sequence TLR4 WT 3' UTR and the mutant sequence TLR4 MUT 3' UTR. Cells were subsequently plated and co-transfected with 50 pmol/L miR-27a-3p mimics and 80 ng TLR4 mutant plasmids. Negative controls were set. After 48 hours of transfection, the fluorescence intensity was measured.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 19.0 statistical software (IBM, Armonk, NY, USA) was used for analysis. Measurement data were analyzed by *t*-test. Data were expressed as mean±standard deviation. The difference was statistically significant at $p<0.05$.

Results

Decreased Expression of miR-27a-3p and Increased Expression of TLR4 in Patients with SCI

We first detected the expression of miR-27a-3p in peripheral serum samples of SCI patients and non-SCI patients by qRT-PCR. We found that the expression of miR-27a-3p in peripheral blood of SCI patients was strikingly lower than that of non-SCI patients (Figure 1A). And serum TLR4 expression was increased in SCI patients (Figure 1B). We also found that low expression of miR-27a-3p in hydrogen peroxide-treated C8-B4 and C8-D1A cells (Figure 1C). In order to reveal the protein expression of TLR4 in these samples, we used Western blot to detect the expression of TLR4. We found that TLR4 expression was higher in peripheral blood of SCI patients comparing to controls (Figure 1D, 1E). The expression of TLR4 mRNA and proteins were significantly increased in rat glial cells treated with hydrogen peroxide (Figure 1F-1H). Finally, we overexpressed and knocked out miR-27a-3p at the cellular level, respectively. RT-PCR detected the expected transfection effect in both C8-B4 and C8-D1A cells (Figure 1I).

Overexpression of miR-27a-3p Reduced Inflammatory Factor Expression

In order to study the role of miR-27a-3p in hydrogen peroxide-treated C8-B4 and C8-D1A cells, we overexpressed miR-27a-3p in cells to observe the expression of inflammatory cytokines IL6 and TNF-α. The results demonstrated that TNF-α and IL6 mRNA expression was strikingly downregulated (Figure 2A, 2D). Western blot experiments demonstrated that after overexpression of miR-27a-3p, the protein levels of inflammatory cytokines TNF-α and IL6 were significantly down-regulated in both cells (Figure 2B-C, 2E-F). Based on the findings, we speculate that miR-27a-3p can reduce the expression of inflammatory factors.

miR-27a-3p can Bind to TLR4 and Reduce TLR4 Expression

Further, we explored the relationship between miR-27a-3p and TLR4. The results demonstrated that TLR4 expression was strikingly down-regulated after overexpression of miR-27a-3p, while the expression of TLR4 was down-regulated when miR-27a-3p were knocked out. The protein result was same as mRNA outcome (Figure 3B, 3C). We then used online bioinformatics tools to predict the presence of the miR-27a-3p binding region in the 3'UTR region of We used online bioinformatics tools to predict the presence of the miR-27a-3p base pair binding region in the TLR4 3'UTR region (Figure 3D). We explored the relationship between miR-27a-3p and TLR4 by Dual Reporter Luciferase Assay. miR-27a-3p-mimcs bind tightly to wild-type TLR4, with a decrease in luciferase activity (Figure 3E, 3F). We conclude that miR-27a-3p and TLR4 can target binding.

TLR4 can Attenuate the Inhibitory Effect of miR-27a-3p on Inflammation

We further confirmed the relationship between TLR4 and miR-27a-3p by recovery experiments. After overexpression of miR-27a-3p, the protein expression of inflammatory cytokines TNF-α and IL6 were significantly down-regulated. Overexpression of TLR4 also partially reversed the inhibitory effect of miR-27a-3p on inflammatory responses (Figure 4A-B).

Discussion

Spinal cord injury (SCI) is a disease with high incidence, mortality, disability, and high treatment costs[10-12]. Spinal cord injuries are caused by traumatic and other violent factors, and lead to deep venous thrombosis, sexual dysfunction and other serious complications[13]. The incidence rate has been rising year by year. There are 10,000 new cases in the United States each year[14], and

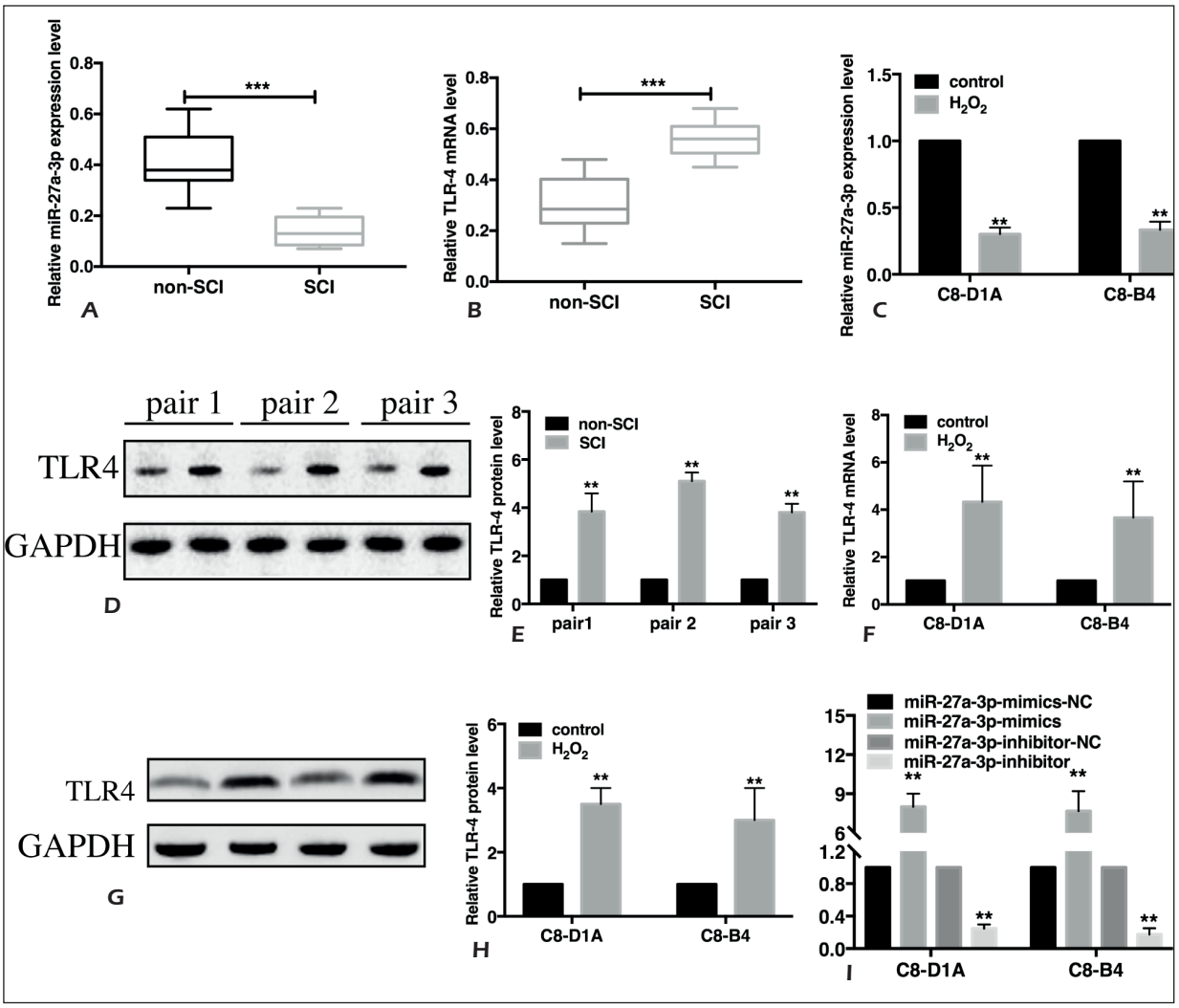

Figure 1. miR-27a-3p was lowly expressed and TLR4 was highly expressed in patients with spinal cord injury. ***A***, miR-27a-3p was lowly expressed in the peripheral blood of patients with spinal cord injury. ***B***, TLR4 was highly expressed in the peripheral blood of patients with spinal cord injury. ***C***, Hydrogen peroxide treatment significantly decreased the expression of miR-27a-3p in rat glial cells. ***D***, and ***E***, TLR4 protein was highly expressed in the peripheral blood of patients with spinal cord injury. ***F***, After treatment with hydrogen peroxide, TLR4 expression was significantly up-regulated in rat glial cells. ***G***, and ***H***, The expression of TLR4 protein was significantly up-regulated in rat glial cells after treated with hydrogen peroxide. ***I***, The corresponding cell changes after overexpression or knockout of miR-27a-3p.

the number of newly-increased cases in China is also more than 60,000 each year, which places a heavy burden on the society[15,16]. The primary injury of spinal cord injury is determined by the torsional forces, compression forces, and the degree of nerve transection that were sustained at the time of the injury. In the later stages, secondary injury will further aggravate the condition, including associated blood-spinal spinal cord barrier disorders, ischemic edema, inflammatory reactions, lipid peroxidation, free radical production, impaired ion pathways, axonal demyelination, neuronal apoptosis, and cicatrix, etc.[17,18]. The inflammatory cytokines can be expressed early after SCI, and induce the corresponding inflammatory cells to invade the damaged spinal cord to generate secondary inflammatory reactions and aggravate the damage of damaged spinal cord[19,20]. TNF-α and IL6 are the two inflammatory cytokines that act as a crucial role in this progress.

MicroRNAs are a class of 20 to 25 nucleotide non-coding single-stranded RNAs that regulate the expression of specific genes at the post-transcriptional level. MicroRNAs were first discovered in the nematode (Cenorhadbitis elegans),

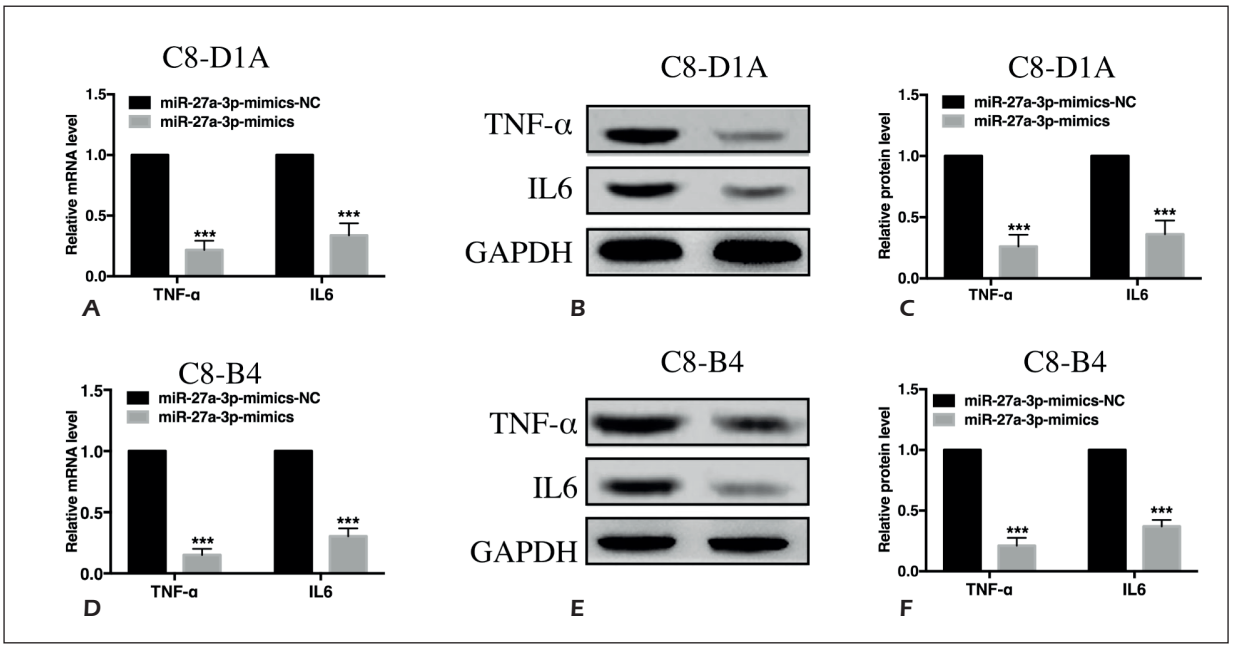

Figure 2. MicroRNA-27a can reduce the expression of inflammatory factors. **A**, After overexpression of miR-27a-3p in C8-D1A cells, the expression of inflammatory cytokines TNF-α and IL6 mRNA were significantly down-regulated. **B**, and **C**, After C8-D1A cells overexpressed miR-27a-3p, the expression of inflammatory cytokines TNF-α and IL6 were significantly down-regulated. **D**, After overexpression of miR-27a-3p in C8-B4 cells, the mRNA expression of TNF-α and IL6 were significantly down-regulated. **E**, and **F**, After overexpression of miR-27a-3p in C8-B4 cells, the protein expression of TNF-α and IL6 were significantly down-regulated.

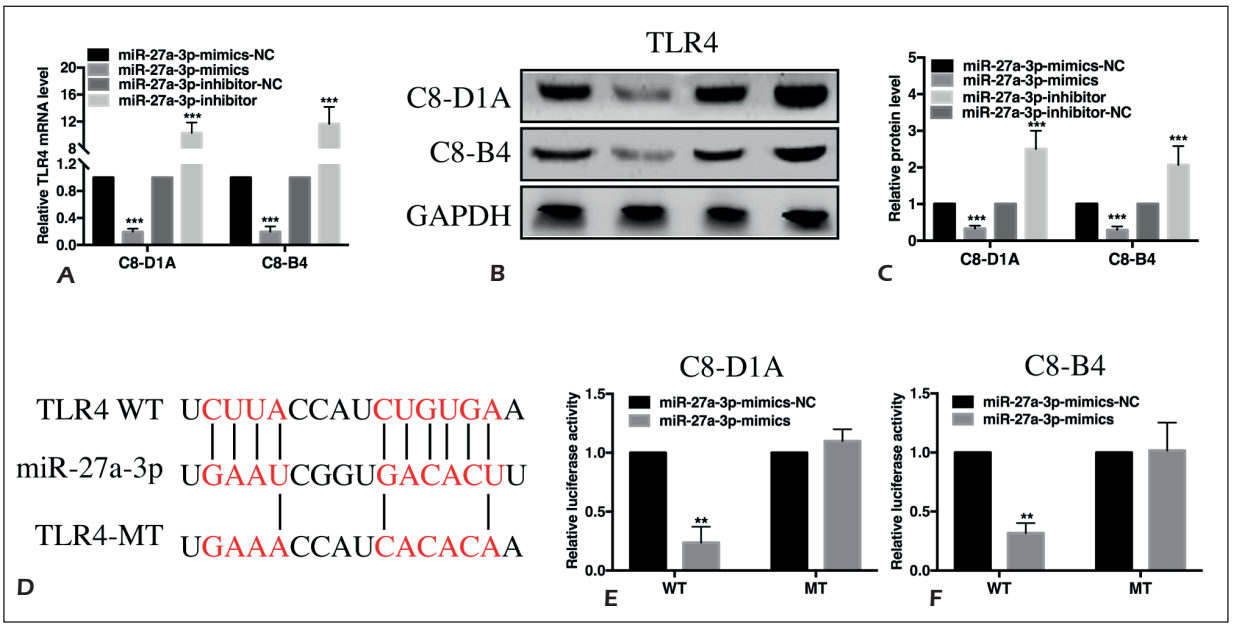

Figure 3. miR-27a-3p can reduce TLR4 expression. **A**, After overexpression of miR-27a-3p, TLR4 mRNA expression were significantly down-regulated. Interfering with miR-27a-3p, TLR4 mRNA expression were significantly up-regulated. **B**, and **C**, After overexpression of miR-27a-3p, TLR4 protein expression were significantly down-regulated, and TLR4 protein expression were significantly up-regulated after miR-27a-3p interference. **D**, miR-27a-3p can bind to TLR4 3'-UTR region. **E**, and **F**, The luciferase activity of miR-27a-3p-mimcs+TLR4 WT groups was significantly reduced.

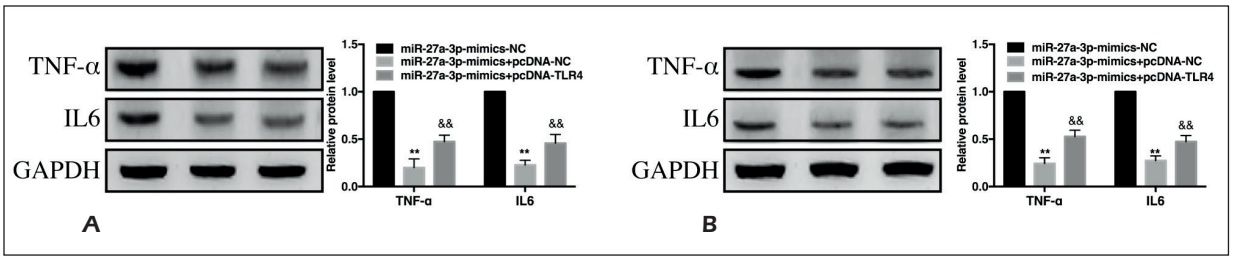

Figure 4. TLR4 can reverse the effects of miR-27a-3p in mitigating the inflammatory response. **A**, and **B**, After overexpression of miR-27a-3p, the expression of inflammatory cytokines was significantly down-regulated. Overexpression of TLR4 significantly increased the expression of inflammatory factors.

an endogenous small RNA that regulates the translation of specific proteins through RNA-RNA interactions[21]. Pathophysiological changes caused by spinal cord injury, such as apoptosis, inflammation, astrogliosis, etc., are also closely regulated by the expression of specific genes. MicroRNA is one of the important regulatory molecules and also a new target for therapeutic interventions that promote repair and regeneration. The changes in microRNA expression after SCI and the role of specific microRNA molecules are not yet reported. Abnormal expression of microRNAs may play a role in spinal cord injury and may be a potential target for therapeutic intervention after spinal cord injury[22]. Bioinformatics analysis revealed that these altered microRNAs influence key processes in the pathophysiology of spinal cord injury, including inflammation and apoptosis[23]. Up to now, studies have demonstrated that microRNAs up-regulated after spinal cord injury, including microRNA-124, microRNA-129, and microRNA-1, etc.[24]. In hepatoma, miR-27a-3p can down-regulate the expression of VE-cadherin, inhibit the occurrence of EMT, and thus inhibit the migration and invasion of hepatoma cells[25]. miR-27a-3p can also inhibit the metastasis of tumor cells by targeting the YAP1 in oral squamous cell carcinoma and inhibiting the occurrence of EMT[26]. In the valvular disease, miR-27a-3p can relieve mitral valve inflammation[9].

Toll-like receptor 4 (TLR4) is the earliest found member of the Toll-like receptors (TLRs) family, and is closely related to the inflammatory response. Toll-like receptors are a type of pattern recognition receptors that regulate immune responses and inflammatory responses *in vivo*. They can recognize multiple pathogenic molecules and influence the differentiation, maturation of immune cells and the activation of inflammatory cells through regulating downstream signal transduction. TLR4 is the most closely related member of the TLRs family with inflammatory responses. It can activate the transcription factor NF-κB through a downstream MyD88-dependent pathway or a non-MyD88-dependent pathway. Activated NF-κB can translocate into the nucleus and then regulate the expression of a variety of inflammatory mediators[27]. Recent studies have demonstrated that in addition to the classical pathway, TLR4 can also form intracellular signal transduction with microRNA that form the TLR4/microRNA signaling axis. The axis is widely involved in various pathophysiological processes[28-30]. The inflammatory reaction that occurs after spinal cord injury is a pathological link that causes secondary damage. TLR4 is an significant recognition receptor that regulates the inflammatory response *in vivo*[31].

In this study, we found that compared with normal people, the expression of miR-27a-3p in peripheral blood of SCI patients was decreased, and the expression of TLR4 was up-regulated. The same results were obtained in cells treated with hydrogen peroxide. After overexpression of miR-27a-3p, we observed that TLR4 expression was remarkedly down-regulated, and the expression of TNF-α and IL6 were also reduced. Comparing to the group that overexpressed miR-27a-3p only, over-expression of miR-27a-3p and TLR4 in cells increased the expression of inflammatory factors, indicating that overexpression of TLR4 can reverse the inhibitory effect of miR-27a-3p on inflammatory responses. By dual luciferase report assay, we found that miR-27a-3p can bind to TLR4. In conclusion, we found that overexpression of miR-27a-3p inhibited inflammatory responses after spinal cord injury by reducing TLR4 expression

Conclusions

The expression of miR-27a-3p decreased after spinal cord injury. Overexpression of miR-27a-3p can reduce the expression of TLR4, thus inhibiting the inflammatory response after spinal cord injury.

Conflict of Interests:

The authors declared no conflict of interest.

References

1) Aomar MM, Cortinas SM, Delgado TJ, Geronimo PM, Calatayud PV, Peyro GR. [Assessment of neurologic function and complications in a retrospective cohort of patients with acute spinal cord injury due to trauma treated with large-dose methylprednisolone]. Rev Esp Anestesiol Reanim 2011; 58: 583-588.
2) Ates O, Cayli SR, Gurses I, Turkoz Y, Tarim O, Cakir CO, Kocak A. Comparative neuroprotective effect of sodium channel blockers after experimental spinal cord injury. J Clin Neurosci 2007; 14: 658-665.
3) Genovese T, Cuzzocrea S. Role of free radicals and poly(ADP-ribose)polymerase-1 in the development of spinal cord injury: new potential therapeutic targets. Curr Med Chem 2008; 15: 477-487.
4) 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.
5) Gerlach CV, Vaidya VS. MicroRNAs in injury and repair. Arch Toxicol 2017; 91: 2781-2797.
6) Homami A, Ghazi F. MicroRNAs as biomarkers associated with bladder cancer. Med J Islam Repub Iran 2016; 30: 475.
7) Sun YP, Lu F, Han XY, Ji M, Zhou Y, Zhang AM, Wang HC, Ma DX, Ji CY. MiR-424 and miR-27a increase TRAIL sensitivity of acute myeloid leukemia by targeting PLAG1. Oncotarget 2016; 7: 25276-25290.
8) Shi DL, Shi GR, Xie J, Du XZ, Yang H. MicroRNA-27a inhibits cell migration and invasion of Fibroblast-Like synoviocytes by targeting Follistatin-Like protein 1 in rheumatoid arthritis. Mol Cells 2016; 39: 611-618.
9) Chen H, Zhang Z, Zhang L, Wang J, Zhang M, Zhu B. MiR-27a protects human mitral valve interstitial cell from TNF-alpha-induced inflammatory injury via up-regulation of NELL-1. Braz J Med Biol Res 2018; 51: e6997.
10) Wang H, Liu X, Zhao Y, Ou L, Zhou Y, Li C, Liu J, Chen Y, Yu H, Wang Q, Han J, Xiang L. Incidence and pattern of traumatic spinal fractures and associated spinal cord injury resulting from motor vehicle collisions in China over 11 years: an observational study. Medicine (Baltimore) 2016; 95: e5220.
11) Yang R, Guo L, Huang L, Wang P, Tang Y, Ye J, Chen K, Hu X, Cai Z, Lu C, Wu Y, Shen H. Epidemiological characteristics of traumatic spinal cord injury in Guangdong, China. Spine (Phila Pa 1976) 2017; 42: E555-E561.
12) Dai J, Xu LJ, Han GD, Sun HL, Zhu GT, Jiang HT, Yu GY, Tang XM. MiR-137 attenuates spinal cord injury by modulating NEUROD4 through reducing inflammation and oxidative stress. Eur Rev Med Pharmacol Sci 2018; 22: 1884-1890.
13) Garcia-Perdomo HA, Echeverria-Garcia F, Tobias A. Effectiveness of phosphodiesterase 5 inhibitors in the treatment of erectile dysfunction in patients with spinal cord trauma: systematic review and meta-analysis. Urol Int 2017; 98: 198-204.
14) Zhang Y, Zhang L, Shen J, Chen C, Mao Z, Li W, Gan WB, Tang P. Two-photon-excited fluorescence microscopy as a tool to investigate the efficacy of methylprednisolone in a mouse spinal cord injury model. Spine (Phila Pa 1976) 2014; 39: E493-E499.
15) Bhalala OG, Srikanth M, Kessler JA. The emerging roles of microRNAs in CNS injuries. Nat Rev Neurol 2013; 9: 328-339.
16) Spinal cord injury (SCI) 2016 facts and figures at a glance. J Spinal Cord Med 2016; 39: 493-494.
17) Dasari VR, Veeravalli KK, Dinh DH. Mesenchymal stem cells in the treatment of spinal cord injuries: a review. World J Stem Cells 2014; 6: 120-133.
18) Zhu T, Tang Q, Gao H, Shen Y, Chen L, Zhu J. Current status of cell-mediated regenerative therapies for human spinal cord injury. Neurosci Bull 2014; 30: 671-682.
19) Xu M, Wang HF, Zhang YY, Zhuang HW. Protection of rats spinal cord ischemia-reperfusion injury by inhibition of MiR-497 on inflammation and apoptosis: possible role in pediatrics. Biomed Pharmacother 2016; 81: 337-344.
20) Poniatowski LA, Wojdasiewicz P, Krawczyk M, Szukiewicz D, Gasik R, Kubaszewski L, Kurkowska-Jastrzebska I. Analysis of the role of CX3CL1 (Fractalkine) and its receptor CX3CR1 in traumatic brain and spinal cord injury: Insight into recent advances in actions of neurochemokine agents. Mol Neurobiol 2017; 54: 2167-2188.
21) Lee RC, Feinbaum RL, Ambros V. The C. Elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 1993; 75: 843-854.
22) Liu NK, Wang XF, Lu QB, Xu XM. Altered microRNA expression following traumatic spinal cord injury. Exp Neurol 2009; 219: 424-429.
23) Yunta M, Nieto-Diaz M, Esteban FJ, Caballero-Lopez M, Navarro-Ruiz R, Reigada D, Pita-Thomas DW, Del AA, Munoz-Galdeano T, Maza RM. MicroRNA dysregulation in the spinal cord following traumatic injury. PLoS One 2012; 7: e34534.
24) Strickland ER, Hook MA, Balaraman S, Huie JR, Grau JW, Miranda RC. MicroRNA dysregulation following spinal cord contusion: implications for neural plasticity and repair. Neuroscience 2011; 186: 146-160.
25) Zhao N, Sun H, Sun B, Zhu D, Zhao X, Wang Y, Gu Q, Dong X, Liu F, Zhang Y, Li X. MiR-27a-3p suppresses tumor metastasis and VM by down-regulating VE-cadherin expression and inhibiting EMT: an essential role for Twist-1 in HCC. Sci Rep 2016; 6: 23091.

26) Zeng G, Xun W, Wei K, Yang Y, Shen H. MicroRNA-27a-3p regulates epithelial to mesenchymal transition via targeting YAP1 in oral squamous cell carcinoma cells. Oncol Rep 2016; 36: 1475-1482.
27) Hally KE, La Flamme AC, Larsen PD, Harding SA. Platelet toll-like receptor (TLR) expression and TLR-mediated platelet activation in acute myocardial infarction. Thromb Res 2017; 158: 8-15.
28) O'Neill LA, Sheedy FJ, McCoy CE. MicroRNAs: the fine-tuners of toll-like receptor signalling. Nat Rev Immunol 2011; 11: 163-175.
29) Quinn SR, O'Neill LA. A trio of microRNAs that control toll-like receptor signalling. Int Immunol 2011; 23: 421-425.
30) Virtue A, Wang H, Yang XF. MicroRNAs and toll-like receptor/interleukin-1 receptor signaling. J Hematol Oncol 2012; 5: 66.
31) Ellis A, Grace PM, Wieseler J, Favret J, Springer K, Skarda B, Ayala M, Hutchinson MR, Falci S, Rice KC, Maier SF, Watkins LR. Morphine amplifies mechanical allodynia via TLR4 in a rat model of spinal cord injury. Brain Behav Immun 2016; 58: 348-356.