Research Article

Clinical significance of miR-142-5p in spinal cord injury caused by spinal trauma and its functional role in the regulation of inflammation

Xiaoqian Zhang[1], Qing Dong[2], Wei Geng[2], Linlin Yang[2]

[1]Department of Laboratory, PKUCare Luzhong Hospital, Zibo, People's Republic of China, [2]Cyrus Tang Hematology Centre of Soochow University, Suzhou, People's Republic of China

Objective: Spinal cord injury (SCI) is a severe traumatic disease in the central nervous system, and can result in neuronal injury. Altered miRNA expression is identified to be involved in the pathogenesis of SCI.
Design: This study investigated the clinical value of miR-142-5p in SCI patients, and explored its functional role in the regulation of inflammatory.
Setting: The First Affiliated Hospital of Soochow University.
Participants: Ninety-eight patients with acute spinal trauma.
Interventions: All patients were recruited, and divided into complete SCI group, incomplete SCI group and normal nerve function group.
Outcome Measures: Real-time quantitative PCR (qRT-PCR) was used to detect the expression levels of miR-142-5p. CCK-8 and flow cytometry assay were performed to evaluate the cell viability and apoptosis. ELISA assay was applied to estimate the levels of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α).
Results: Serum miR-142-5p level was significantly increased in SCI patients, especially the complete SCI cases. ROC curve analysis suggested miR-142-5p could distinguish SCI patients from normal nerve function patients and was associated with the severity of SCI. A positive association was detected between miR-142-5p and serum levels of IL-6, TNF-α in SCI patients. Downregulation of miR-142-5p significantly reduced the protein levels of both IL-6 and TNF-α in LPS treated PC12 cells, and weakened LPS induced cell apoptosis.
Conclusion: MiR-142-5p is a potential biomarker for the occurrence of SCI in acute spinal trauma patients. Downregulation of miR-142-5p plays an anti-inflammatory effect for SCI patients.

Keywords: MiR-142-5p, Diagnosis, Spinal cord injury, Inflammatory

Introduction

Spinal cord injury (SCI), a severe traumatic disease in the central nervous system, is predominantly caused by mechanical and ischemic trauma to the spinal cord. SCI consists of two injuries, including the primary injuries that occur at initial impact and the secondary injuries that develop soon after the injury.[1] SCI can lead to the inflammatory response in the spine, further result in neuronal injury.[2–4] It is believed that anti-inflammatory treatments are beneficial for the recovery of SCI. Patients with SCI always have a relatively poor prognosis and may suffer from a limb movement disorder, loss of cognitive function, even paralysis, giving the family and society a heavy burden.[5] It is suggested that early diagnosis and reasonable treatment measures for patients with incomplete SCI may improve their prognosis to some extent.[6]

MicroRNAs (miRNAs) are endogenous, non-coding RNAs, with a length of approximately 20–24 nucleotides. As a result of the crucial role of miRNAs in post-transcriptional regulation of protein-coding genes, their regulatory roles in various diseases have been widely concerned by researchers.[7,8] Many miRNAs have been identified to be aberrantly expressed in a variety of central nervous system diseases, including SCI.[9,10] The dysregulation of miRNAs is

Correspondence to: Linlin Yang, Cyrus Tang Hematology Centre of Soochow University, No. 199, Ren'ai Road, Suzhou, Jiangsu 215000, People's Republic of China; Ph: +86-18051292806. Email: el60sptf@163.com yangllsu@163.com

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reported to be involved in the progression of SCI via regulating inflammatory, cell apoptosis, and regeneration, such as miR-100, miR-129-5p and so on.[11–13] MiR-142-5p has been reported to be involved in the progression of neurological diseases. For example, Sierksma *et al.* determined the upregulation of miR-142-5p in patients with Alzheimer's disease.[14] Another study by Wu *et al.* reported that, miR-142-5p is overexpressed in intact sciatic nerves of mice underwent peripheral nerve crush injury, suggesting the close association of miR-142-5p with nerve injury.[15] Additionally, a study in multiple sclerosis (MS) indicated that miR-142-5p is highly expressed in the frontal white matter from MS patients, and the dysregulation of miR-142-5p is involved in the pathogenesis of autoimmune neuroinflammation.[16] However, the role of miR-142-5p in SCI has not been reported.

Therefore, the present study investigated the clinical significance of miR-142-5p in SCI patients caused by spinal trauma. Furthermore, considering the important role of miR-142-5p in the inflammatory response, its influence on inflammatory responses was also explored in PC-12 cells.

Materials and methods

Study population

This study protocol was approved by the Ethics Committee of The First Affiliated Hospital of Soochow University, and written informed consent was collected from each participant.

A total of 98 patients with acute spinal trauma were recruited, who were admitted to The First Affiliated Hospital of Soochow University from August 2015 to December 2018. All patients were older than 18 years old and were admitted to the hospital within 24 h after trauma. All patients were divided into three groups according to the SCI level evaluated by American Spinal Injury Association (ASIA) Impairment Scale in International Standards for Neurological Classification of Spinal Cord Injury[17]: complete SCI group, incomplete SCI group and normal nerve function group. 27 patients were diagnosed with complete SCI, including 17 males and 10 females, in which 18 had cervical spine injuries and 9 had lumbar and thoracic injuries. 47 cases were diagnosed with incomplete SCI, including 28 males and 19 females, in which 32 had cervical spine injuries and 15 had lumbar and thoracic injuries. 24 cases were with normal nerve function, including 14 males and 10 females, 16 of them underwent cervical spine injuries and 8 underwent lumbar and thoracic injuries. Patients with normal nerve function were identified as the control group. The exclusion criteria were as follows: (1) have a history of SCI or traumatic brain injury; (2) have the severe cardiopulmonary disease, such as myocardial infarction or acute coronary syndrome, severe arrhythmia, acute respiratory distress syndrome; (3) suffer from acute severe craniocerebral injury; (4) with Malignant tumor, acute and chronic infection, severe liver and kidney insufficiency, cerebrovascular disease, autoimmune disease, and blood system disease.

Sample collection

5 ml peripheral blood samples were collected from each participant after admission (within 24 h after trauma), and the serum samples were stored at −80°C before usage.

Cell culture and transfection

The PC12 cells were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China), and cultured in DMEM medium (Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS). Cells were seeded into a 96-well plate with a density of 5×10^3 cells per well, and cultured for 24 h. Then cells were treated with 5μg/ml LPS for 12 h to mimic SCI induced inflammatory injury condition *in vitro*. The miR-142-5p mimic, miR-142-5p inhibitor and the negative control (miR-NC) were provided by Gene-Pharma (Shanghai, China). Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) was used for cell transfection.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Trizol Reagent (Invitrogen, Carlsbad, CA, USA) was used to extract total RNA according to the manufacture's protocol. Then reverse transcription reactions were performed using the miScript Reverse Transcription Kit (QIAGEN, Hilden, Germany). The qRT-PCR was performed to evaluate the expression level of miR-142-5p by using a SYBR Green I Real-Time PCR Kit (GenePharma, Shanghai, China) on an Applied Biosystems 7900 Real-Time PCR system (Applied Biosystems, Foster City, CA). The relative expression levels of miR-142-5p were calculated using the comparative delta CT ($2^{-\Delta\Delta Ct}$) method and normalized to U6.

Cell viability assay

Cell Counting Kit-8 assay (CCK-8, Dojindo Molecular Technologies, Gaithersburg, MD) was performed to evaluate the cell viability. 10μl CCK-8 solution was mixed into the culture medium, then the cells were

cultured at 37 °C with 5% CO_2 for 1 h. The optical density of each well was determined at 450 nm using a microplate reader (Bio-Tek Instruments, Winooski, VT, USA). Cell viability was evaluated as the percentage of the control group.

Flow cytometry assay

Cell apoptosis was detected by using a FITC Annexin V Apoptosis Detection kit (BD Biosciences, Franklin Lakes, NJ). After treatment, cells in different groups were collected and washed, then stained by binding buffer. Subsequently, the cells were incubated with annexin V-FITC and propidium iodide (PI) in darkness for 10 min at 37°C. The cell apoptosis was detected in a FACS Calibur flow cytometer (BD, Biosciences), and the apoptotic rate (%) was expressed as the number of apoptotic cells over the total number of cells.

Measurement of cytokines

The protein levels of IL-6, TNF-α were measured using ELISA kits according to the manufacturer's protocols (R&D Systems China Co., Ltd.).

Statistical analysis

All experiments were repeated three times. SPSS version 18.0 software (SPSS Inc.) and GraphPad Prism 5.0 software (GraphPad Software, Inc.) were used for data analysis. Demographic and clinical data were analyzed using an independent-samples t-test or a chi-squared test. Differences between two groups were analyzed using Student's t-test, and a comparison of multiple groups was calculated using a one-way analysis of variance, followed by Tukey's multiple-comparisons test. The associations between serum levels of miR-142-5p and inflammatory cytokines were assessed by Pearson's correlation coefficient. Receiver operating characteristic (ROC) curve analysis was performed to assess the diagnostic value of miR-142-5p in the patients. $P < 0.05$ was identified to be statistically significant.

Results

Demographics of the study population

All patients were divided in to three groups: complete SCI group (n = 27), incomplete SCI group (n = 47) and normal nerve function group (n = 24). As shown in Table 1, there was no significant difference in age and sex among the three groups (all $P > 0.05$). But the body temperature was significantly different among the three groups ($P < 0.05$).

Expression level of miR-142-5p in SCI patients

The expression levels of miR-142-5p were compared among different groups using qRT-PCR. As shown in Fig. 1(A), compared with the normal group, miR-142-5p expression level increased significantly in SCI group ($P < 0.001$). According to the SCI severity, all SCI patients were divided into incomplete SCI group and complete SCI group. The qRT-PCR results indicated that compared with incomplete SCI group, miR-142-5p level was upregulated significantly in complete SCI group (Fig. 1(B), $P < 0.001$). These data suggested that miR-142-5p was overexpressed in SCI patients, and the expression level of miR-142-5p might be associated with the severity of SCI.

Clinical value of miR-142-5p for SCI patients

A ROC curve was made to assess the usefulness of miR-142-5p for identifying SCI patients from normal nerve function patients. As shown in Fig. 2(A), the diagnostic sensitivity and specificity were 89.2% and 75.0%, respectively at the cutoff value of 1.089, and the area under the curve (AUC) was 0.877. Additionally, the diagnostic value of miR-142-5p in complete SCI patients compared with incomplete patients was further detected. It was found that the AUC value was 0.879 yield the sensitivity of 77.8% and specificity of 89.4% at the cutoff value of 1.345 (Fig. 2(B)). We concluded that serum miR-142-5p could be used to distinguish SCI patients from normal nerve function patients, and the level of miR142-5p was associated with the severity of SCI.

Correlation between serum miR-142-5p level and inflammatory cytokines

Considering the important role of miR-142-5p in the inflammatory response, we further analyzed the association between serum levels of miR-142-5p and inflammatory cytokines in SCI patients. As shown in Fig. 3 (A), a remarkable positive association was detected between serum miR-142-5p and IL-6 level in SCI patients ($r = 0.701$, $P < 0.001$). Additionally, similar

Table 1 General data of the study population.

Parameters	Normal (n = 24)	Incomplete SCI (n = 47)	Complete SCI (n = 27)	*P* value
Age (years)	39.71 ± 6.30	39.64 ± 6.52	39.04 ± 5.32	0.903
sex (male/female)	14/10	28/19	17/10	0.938
Body temperature (°C)	37.23 ± 0.33	37.47 ± 0.47	37.58 ± 0.60	0.033*

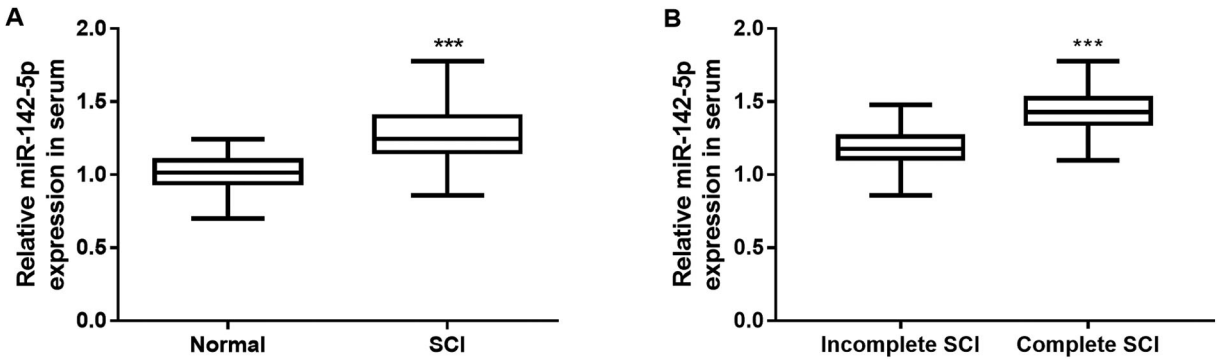

Figure 1 The expression levels of miR-142-5p among different groups. (A) Compared with the normal group, miR-142-5p expression level increased significantly in SCI group. (B) MiR-142-5p level was also upregulated significantly in complete SCI group compared with incomplete SCI group. *** $P < 0.001$.

correlation was also detected between serum miR-142-5p and TNF-α level (r = 0.820, $P < 0.001$).

Influence of miR-142-5p on LPS triggered inflammatory damage in PC12 cells

PC12 cell was subjected to LPS to mimic SCI induced inflammatory injury condition *in vitro*. The results suggested that LPS treatment significantly inhibited the PC12 cell viability and promoted the cell apoptosis compared with control group (Fig. 4(B,C), $P < 0.001$). Additionally, we further explored the effect of LPS on inflammatory cytokines expression in the cell model. It was observed that the protein levels of IL-6 and TNF-α were all significantly increased by LPS treatment in PC12 cells (Fig. 4(D,E)). These results indicated that LPS triggered inflammatory responses of neuron cells.

To further investigate the influence of miR-142-5p on inflammatory injury induced by LPS in PC-12 cells, the level of miR-142-5p was regulated by cell transfection. As shown in Fig. 4(A), miR-142-5p mimic transfection aggravated the increased level of miR-142-5p induced by LPS, whereas miR-142-5p inhibitor transfection significantly reversed the increase of miR-142-5p level. The CCK-8 results suggested that miR-142-5p upregulation aggravated the inhibitory effect of LPS on cell viability, but miR-142-5p downregulation significantly weakened LPS induced cell viability inhibition in PC12 cells (Fig. 4(B)). Consistently, the flow cytometry assay also indicated that overexpression of miR-142-5p

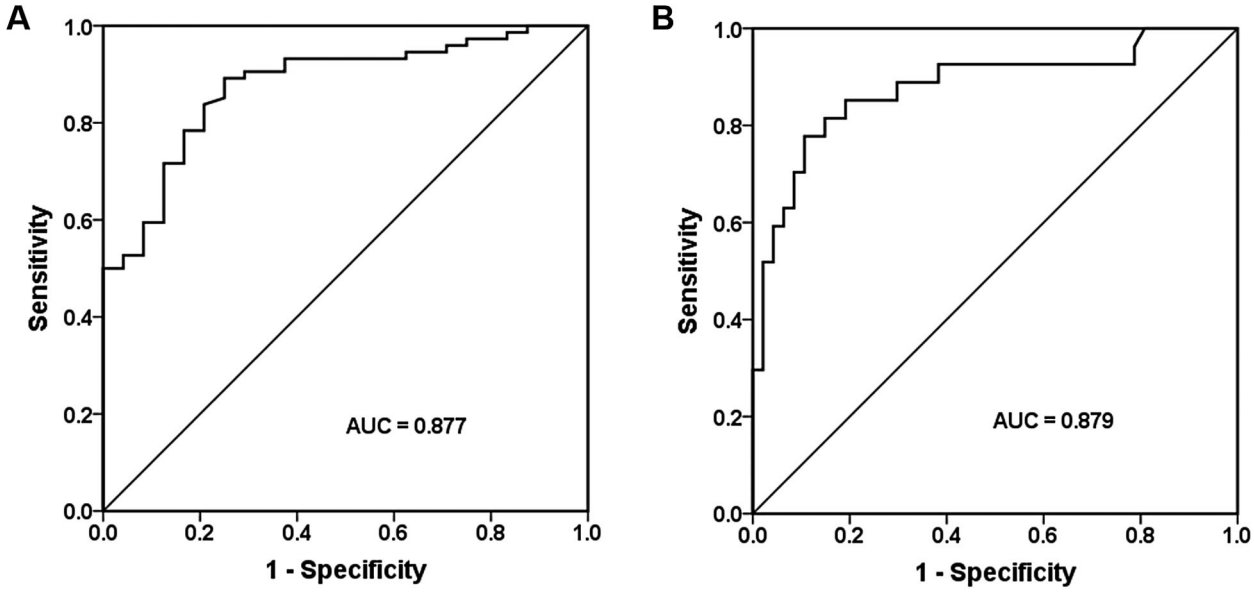

Figure 2 Diagnostic value of miR-142-5p in SCI patients. (A) MiR-142-5p could identify SCI patients from normal nerve function patients, the diagnostic sensitivity and specificity were 89.2% and 75.0% respectively, and the area under the curve (AUC) was 0.877. (B) MiR-142-5p could distinguish incomplete patients from complete SCI patients. The AUC value was 0.879 yield the sensitivity of 77.8% and specificity of 89.4% at the cutoff value of 1.345.

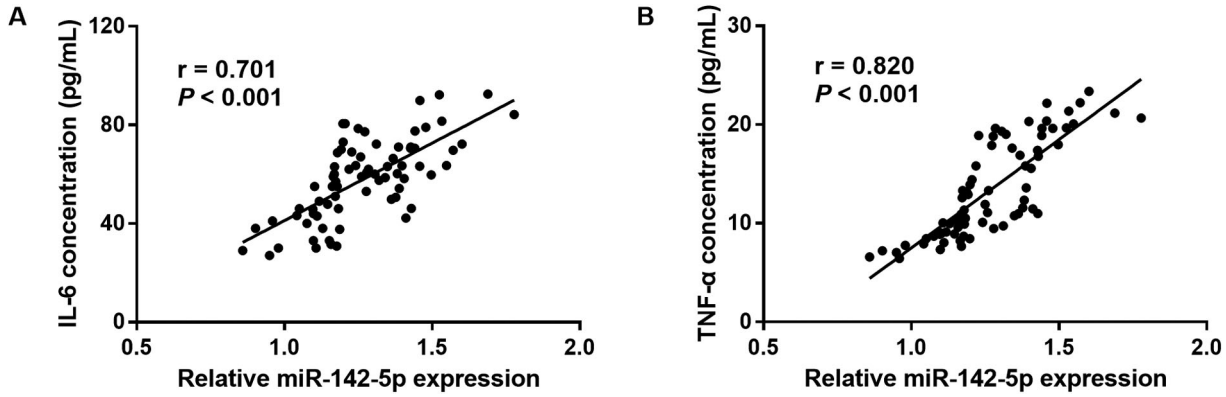

Figure 3 Correlation between serum miR-142-5p level and inflammatory cytokines in SCI patients. Serum miR-142-5p level was positively associated with IL-6 (A, r = 0.701, $P < 0.001$) and TNF-α (B, r = 0.820, $P < 0.001$) level in SCI patients.

aggravated LPS induced neuron cell apoptosis, which was reversed by the downregulation of miR-142-5p (Fig. 4(C)). Additionally, the ELISA results indicated that miR-142-5p overexpression intensified the inductive effect of LPS on IL-6 and TNF-α, while downregulation of miR-142-5p significantly reduced the protein levels of both IL-6 and TNF-α in LPS treated PC12 cells (Fig. 4 (D,E)). It was concluded that the downregulation of miR-142-5p attenuated LPS triggered neuron cell apoptosis and neuroinflammation.

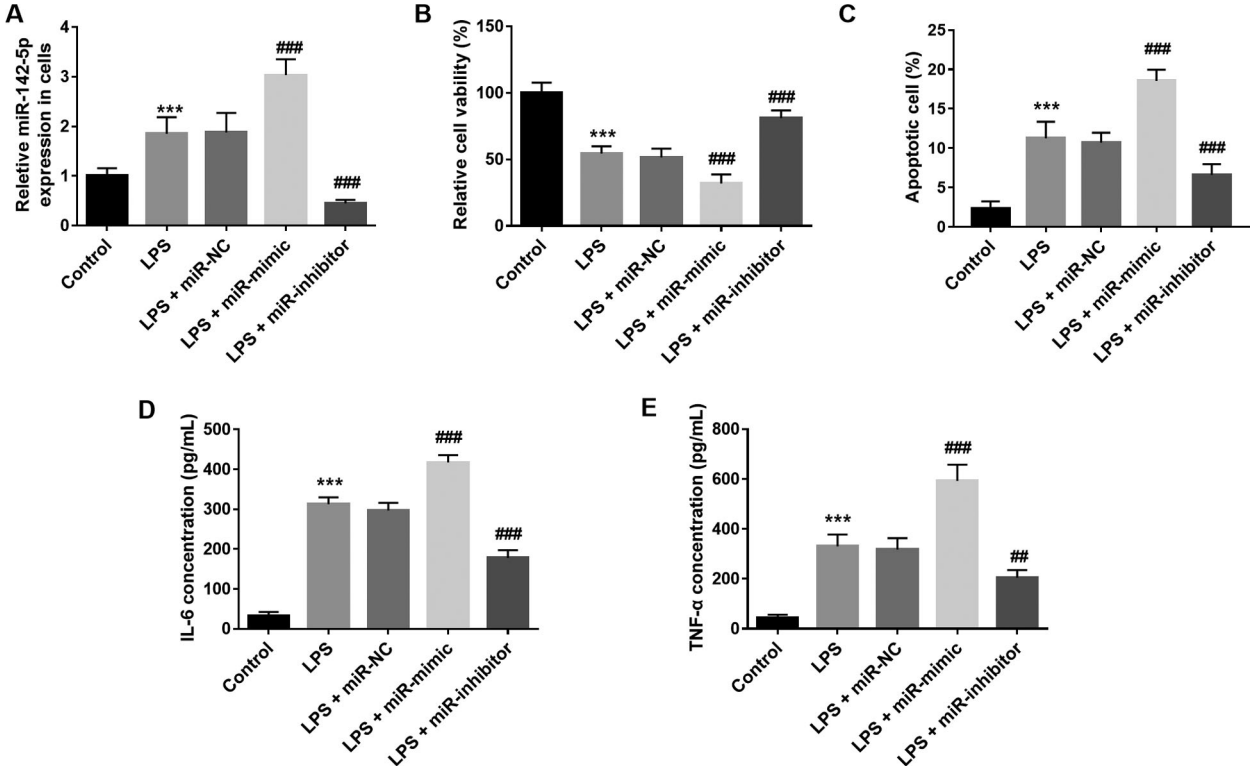

Figure 4 Influence of miR-142-5p on LPS triggered inflammatory damage in PC12 cells. (A) miR-142-5p mimic transfection aggravated the increased level of miR-142-5p induced by LPS, whereas miR-142-5p inhibitor transfection significantly reversed the increase of miR-142-5p level. (B) miR-142-5p upregulation aggravated the inhibitory effect of LPS on cell viability, but miR-142-5p downregulation significantly weakened LPS induced cell viability inhibition in PC12 cells. (C) Overexpression of miR-142-5p aggravated LPS induced neuron cell apoptosis, which was reversed by the downregulation of miR-142-5p. (D–E) MiR-142-5p overexpression intensified the inductive effect of LPS on IL-6 and TNF-α, while downregulation of miR-142-5p significantly reduced the protein levels of both IL-6 and TNF-α in LPS treated PC12 cells. *** $P < 0.001$, compared with control group; ## $P < 0.01$, ### $P < 0.001$, compared with LPS group.

Discussion

SCI is a complicated pathophysiologic process that involves a cascade of cellular and biochemical events, such as oxidative stress, hypoxia, inflammation, vascular endothelial damage, platelet aggregation.[18,19] The main reason for the high disability rate and poor functional recovery associated with SCI is irreversible structural and functional changes in neurons induced by progressive secondary damage after the primary injury.[20] It is known that early interventions are a focus of treatment for enhancing neurologic potential after SCI.[21] Therefore, early diagnosis for patients with SCI is of great significance for the timely treatment and improvement of prognosis.

MiRNAs are implicated in various biological processes by regulating their target mRNA expression.[22] The dysregulation of miRNAs has been widely reported in a variety of human diseases, and the clinical value of miRNAs in human disease is widely concerned.[23] In recent years, many kinds of research have focus on the close correlation between miRNA and SCI, and several miRNAs are identified to be involved in the pathogenesis of different forms of SCI.[24,25] A study by Ma *et al.* determined that miR-130a is low expressed in the peripheral blood mononuclear cells (PBMCs) of SCI patients, the altered expression of miR-130a is involved in the pathogenic mechanism of SCI by enhanced TNF-1α expression.[24] Another study by Jiang *et al.* proved that miR-489-3p participates in the progression of SCI via regulating neurite growth with the involvement of PI3 K/AKT pathway.[26] Wang *et al.* reported that the expression level of miR-142-5p is increased in the hippocampal mitochondria and cytoplasm of rats following traumatic brain injury.[27] In the present study, a cohort of patients with acute spinal trauma were recruited to explore the expression level changes of miR-142-5p in the serum. It was observed that the miR-142-5p level was overexpressed in the serum of SCI patients, and it might be associated with the severity of SCI. But in the current study, the serum miR-142-5p levels were just detected in patients within 24 h after trauma, it is interesting to explore its expression changes with the passage of time, which should be investigated in future study.

Previous study has suggested that miR-142-5p is a potential biomarker for many human diseases. For example, as Iwasaki *et al.* suggested, in the patients with De novo donor-specific HLA antibody (DSA), miR-142-5p may serve as a potential biomarker for early detection of chronic antibody-mediated rejection in kidney transplantation.[28] Another study by Wiczling *et al.* suggested that miR-142-5p was aberrantly expressed in ovarian cancer patients compared with the healthy controls using a Bayesian multilevel model, demonstrating its potential diagnostic value for ovarian cancer.[29] In the current study, a ROC curve was made to assess the diagnostic value of miR-142-5p in SCI, it was found that serum miR-142-5p could distinguish SCI patients from normal nerve function patients, and the level of miR142-5p was associated with severity of SCI.

Recent studies have shown that miR-142-5p is a key regulator of immune cell functions, and is implicated in the regulation of inflammatory diseases.[30,31] Altered miRNA expression has been identified in the T cells and SCI patients, and further regulates the expression of downstream target molecules, contributing to the pathogenesis of different forms of SCI.[24] Furthermore, secondary SCI has been reported to be closely associated with cellular inflammatory response.[32] Considering the crucial role of inflammatory in regulating the pathogenesis of SCI, we further analyzed the association between serum levels of miR-142-5p and inflammatory cytokines in SCI patients. As expected, a positive association was detected between serum miR-142-5p level and inflammatory cytokines, including IL-6 and TNF-α.

Considering the crucial role of miR-142-5p in the inflammatory response, a cell-based model of SCI inflammatory injury in PC12 cells was constructed to further investigate the influence of miR-142-5p on SCI inflammatory injury. The results indicated that the downregulation of miR-142-5p significantly reduced the protein levels of both IL-6 and TNF-α in LPS treated PC12 cells. Additionally, inhibition of miR-142-5p also significantly weakened LPS induced cell apoptosis in PC12 cells. It was concluded that inhibition of miR-142-5p attenuated LPS triggered inflammatory response in PC12 cells. With the increasing knowledge of the pathogenesis of SCI, the dysfunction and over secretion of multiple pro-inflammatory factors have been concerned, especially TNF-α.[33,34] A recent study reported that TNF-α regulated synaptic plasticity in the hippocampus and spinal cord in mice after peripheral nerve injury.[33] Another study in a SCI rat model supported that TNF-α inhibition promoted neurological function recovery through regulating the Wnt signaling pathway.[34] Our present results indicated that the downregulation of miR-142-5p significantly reduced the protein levels of TNF-α, which was in accordance with the previous evidence. We concluded that miR-142-5p might be a novel regulator of the inflammatory response

of SCI patients. Consistently, in a study about post-traumatic stress disorder (PTSD), downregulation of miR-142-5p was suggested to attenuate neuroinflammation in the hippocampus of PTSD rats.[35] In addition, miR-142-5p was also reported to play a neuroprotective role in protecting against isoflurane-induced neurological impairment through regulating neuron cell viability and apoptosis.[36] All evidence supported our present results. However, in the present study, only *in vitro* studies were performed, and the role of miR-142-5p in SCI should be further verified *in vivo*. In addition, the clinical information in this is incomplete, which may results in result bias, such as the muscle or bone injury information of the patients. Maybe another indepent patients group who have acute limb trauma should be recruited to verify the expression level of miR-142-5p in these cases.

In conclusion, the current study results indicated that miR-142-5p was upregulated in SCI patients, and might be a promising biomarker for the occurrence of SCI in acute spinal trauma patients. Downregulation of miR-142-5p played an anti-inflammatory effect for SCI inflammatory injury. It provides a novel concept for the diagnosis and therapeutic interventions of SCI.

Disclaimer statements

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Conflicts of interest The authors have declared no conflict of interest.

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