European Review for Medical and Pharmacological Sciences 2020; 24: 2865-2873

MiR-24 inhibits oligodendrocyte precursor cell differentiation after spinal injury by targeting adrenal medulla

C.-J. LEI[1], W. CHEN[2], M.-H. LI[3], Y. XU[4], Q.-Y. PAN[4], G. ZHENG[1], Y.-X. XU[5]

[1]Department of General Surgery, the Second Affiliated Hospital of Jianghan University, Wuhan, Hubei, China
[2]Department of Kidney Medicine, Huangshi Central Hospital, Huangshi, Hubei, China
[3]Department of Orthopedics, the Second Affiliated Hospital of Jianghan University, Wuhan, Hubei, China
[4]Department of Endocrinology, the Second Affiliated Hospital of Jianghan University, Wuhan, Hubei, China
[5]Department of Gynecology, the Second Affiliated Hospital of Jianghan University, Wuhan, Hubei, China

Changjiang Lei, Wei Chen, Minghui Li, and Ying Xu contributed equally to this work

Abstract. – OBJECTIVE: Oligodendrocyte precursor cells (OPCs) differentiate into oligodendrocytes (OLs) that provide nutrients to neurons. Adrenal medulla is (ADM) involved in nerve damage. MiR-24 participates in various diseases. However, the regulation and mechanism of miR-24 in oligodendrocyte precursor cell differentiation after spinal injury is unclear.

MATERIALS AND METHODS: Wistar rats were divided into sham operation group and model group. Real Time-PCR detects miR-24, PDGFRa and NG2 and MBP expression. OPC cells were cultured and divided into control group, miR-24 group, and si-miR-24 group followed by analysis of miR-24 expression by Real Time-PCR, expression of PDGFRa, NG2 and MBP by Western blot, as well as ADM content and secretion of IL-6 and TNF-α by enzyme-linked immunosorbent assay (ELISA).

RESULTS: Expression of miR-24, PDGFRa, and NG2 was increased in the model group and MBP and ADM expression was decreased with increased secretion of IL-6 and TNF-α. Compared with control group, the difference was statistically significant ($p<0.05$). Upregulation of miR-24 promoted the expression of PDGFRa and NG2, decreased MBP and ADM level, and increased IL-6 and TNF-α secretion. Compared with control group, the difference was statistically significant ($p<0.05$). Downregulation of miR-24 reversed the above changes, and the difference was statistically significant ($p<0.05$).

CONCLUSIONS: MiR-24 expression is increased in spinal injury. Upregulation of miR-24 expression reduces adrenal medulla expression and inhibits oligodendrocyte precursor cell differentiation.

Key Words:
Spinal injury, MiR-24, Adrenal medulla, Oligodendrocyte precursor cells, Differentiation.

Abbreviations

Adrenal medulla: ADM; basic fibroblast growth factor: bFGF; Dulbecco's Modified Eagle's Medium: DMEM; enhanced chemiluminescence: ECL; Mtableyelin basic protein: MBP; Neural/glial antigen 2: NG2; Oligodendrocyte precursor cells: OPCs; oligodendrocytes: OLs; Platelet Derived Growth Factor Receptor Alpha: PDGFRa; Polyvinylidene difluoride: PVDF; spinal cord injury: SCI.

Introduction

Spinal injury is a common trauma in spinal surgery, which can cause spinal cord injury, often leading to severe dysfunction of the limb below the injured segment[1,2]. With the continuous development of the global construction industry, industrialization and mining, and the growing development of the transportation industry, the rapid development of the construction industry has caused an increase in accidental spinal cord injuries, such as falls or car accidents. Spinal cord injuries are often accompanied by high disability and mortality rate[3,4]. A total of 100,000 cases are found each year worldwide, with predominance of young and middle-aged people[5,6]. Spinal injury is difficult to treat, and surgical treatment is mostly used at a high cost. Spinal cord injury not only brings serious physical and psychological damage to the patient, but also has a poor prognosis and causes a huge economic burden on the whole society[7]. It can damage the myelin sheath of the electrical insulating membrane outside the axon and cannot effectively provide nutrients and metabolic support

Corresponding Author: Gang Zheng, MD; e-mail: tao60180139@126.com

for neurons[8,9]. Oligodendrocytes (OLs) are myelin cells of the central nervous system, which are mainly differentiated by oligodendrocyte precursor cells (OPCs)[10]. When the myelin sheath is damaged, a large number of inflammatory mediators are released, causing pathological changes, which can lead to a large degree of damage by cascading amplification effects on the basis of primary injury, resulting in damage to oligodendrocyte formation, thereby causing myelin damage and further function loss[11,12].

As a common non-coding RNA, miRNAs are a class of non-coding single-stranded RNA molecules that are present in a variety of eukaryotic cells and viruses with approximately 22 nucleotides in length[13]. It is highly conserved in evolution and binds to specific sites at the 3'-UTR end of the target gene and is involved in the regulation of post-transcriptional levels of genes[14,15]. MiRNAs account for about 1% of the entire genome, and it is speculated that about one-third of the genes in the human genome are regulated by miRNAs[16]. MiR-24 is involved in the growth, development, apoptosis, and other biological processes of cells, and exerts its biological functions by regulating the expression of multiple target genes, regulating the development of various diseases[17,18]. However, the regulation and mechanism of miR-24 in oligodendrocyte precursor cell differentiation after spinal injury have not been elucidated.

Materials and Methods

Experimental Animals

Healthy male Wistar rats, 2 months old, SPF grade, body weight (250±20) g, were purchased from the experimental Animal Center of the unit, and fed by the SPF Animal Experiment Center. Feeding conditions included maintaining the temperature at (21 ± 1)°C and maintaining relative humidity (50-70%) under constant temperature and constant humidity conditions, ensuring a 12/day cycle every 12 hours.

Main Materials and Instruments

Sodium pentobarbital was purchased from Shanghai Zhaohui Pharmaceutical Co., Ltd (Shanghai, China). OPC cells were kept in our laboratory and stored in liquid nitrogen. Polyvinylidene difluoride (PVDF) membrane was purchased from Pall Life Sciences (Port Washington, NY, USA). Western blot related chemical reagent was purchased from Shanghai Biyuntian Biotechnology Co., Ltd. (Shanghai, China), enhanced chemiluminescence (ECL) reagent was purchased from Amersham Biosciences (Little Chalfont, Buckinghamshire, UK), rabbit anti-mouse platelet derived growth factor receptor alpha (PDGFRa) and neural/glial antigen 2 (NG2) and myelin basic protein (MBP) monoclonal antibody, horseradish peroxidase (HRP) labeled IgG secondary antibody were purchased from Cell Signaling Technology (Danvers, MA, USA). The OPC medium was supplemented with 10 ng/ml basic fibroblast growth factor (bFGF), 10 ng/ml PDGF-AA, 2% B27, 100 μg/ml streptomycin, 100 U/ml penicillin from Dulbecco's Modified Eagle's Medium (DMEM)/F12 base medium. OL differentiation medium was added to 2% B27, 100 μg/ml streptomycin, 100 U/ml penicillin from DMEM/F12 base medium. Rabbit anti-mouse TNF-α and IL-6 enzyme-linked immunosorbent assay (ELISA) kits were purchased from R&D (Minneapolis, MN, USA). MiR-24 siRNA and pcDNA miR24 plasmid were synthesized by Shanghai Shenggong Bioengineering Technology Co., Ltd (Shanghai, China). Surgical microscopy equipment was purchased from Suzhou Medical Instrument Factory (Suzhou, China). The RNA extraction kit and the reverse transcription kit were purchased from ABI (Waltham, MA, USA). Surgical microscopy equipment was purchased from Suzhou Medical Instrument Factory (Suzhou, China). The Multi-Parameter Monitor small animal physiological monitor was purchased from Shanghai Yuyan Instrument Co., Ltd (Shanghai, China). The Amp PCR System 2400 DNA Amplifier was purchased from PE Gene (Foster City, CA, USA). The iMark microplate reader was purchased from BD Corporation (San Jose, CA, USA).

Grouping and Processing of Experimental Animals

Wistar rats were randomly divided into 3 groups: control group, sham operation group (the lamina was removed without damaging the spinal cord) and model group (a rat model of spinal cord injury was established by modified ALLEN's method).

Preparation of Rat Spinal Cord Injury (SCI) Model

According to the literature, a rat SCI model was established using the modified ALLEN's weight attack method[19]. After anesthesia through intraperitoneally injection of 30 mg/kg sodium pentobarbital, the rats were fixed on the operating table. The lamina and spinous processes of the thoracic vertebra

T9-T11 were removed. The center was set in the T10 spinal cord segment and a circular area of approximately 4 mm in diameter was revealed as the lesion. According to the physiological curvature of the dorsal spinal cord of the rat, a plastic buckling pad with a length of 3 mm, a width of 2 mm and a thickness of 1 mm was prepared, and the spacer was placed in the spinal cord of the T10 spinal cord. Using a modified ALLEN's striking device, the center was set to the median blood vessel behind the spinal cord, and the striking rod moved freely from the height of 5 cm through the sleeve, directly hitting the plastic spacer. The successful model mainly included the retraction and flutter of the rat's body and the lower limbs, and the rat tail showed a swaying swing. The surgical incision was closed layer by layer, covered with a dressing to cover and fix the wound, and the conventional antibiotic was administrated for prevention of infection.

Specimen Collection

On the 20th day after operation, 5 ml peripheral blood of rats in each group were collected through the tail vein and centrifuged for 15 min. The serum was placed in an Eppendorf (EP; Hamburg, Germany) tube and stored in a -80°C refrigerator for later use. Rats in each group were sacrificed, and the spinal cord tissues of each group were taken and stored in a refrigerator at -80°C for later use.

ELISA Analysis of the Expression of TNF-α, IL-6 and ADM in Serum and Cell Supernatant of Each Group

All the samples were tested by ELISA kit for the expression of TNF-α, IL-2 and ADM in serum and cell supernatant of each group according to the ELISA kit instructions. The linear regression equation of the standard curve was calculated according to the concentration of the standard product and the corresponding OD value, and the corresponding sample concentration was calculated on the regression equation according to the OD value of the sample.

In Vitro Rat OPC Culture

Cell culture was carried out in OPC medium in the presence of 10 ng/ml bFGF, 10 ng/ml PDGF-AA, 2% B27, 100 μg/ml streptomycin, 100 U/ml penicillin for 2 days in DMEM/F12 basal medium which was then replaced by the OL differentiation medium after 2 days. Then, the cells were cultured in a 5% carbon dioxide incubator at 37°C. When these cells were in the logarithmic growth phase, the culture medium in the flask was discarded, washed twice with sterile phosphate-buffered saline (PBS), and 0.25% trypsin was added to digest the cells. The cells were resuspended in medium and seeded in 6-well plates.

Cell Transfection

MiR-24 siRNA and pcDNA miR24 plasmids were transfected into OPC cells cultured in differentiation medium according to the instructions of Lipofectamine 2000. The cells were seeded in a six-well plate, the density of the cells was 70% after 24 hours of culture, and 1.5 mL of serum-free medium was replaced. 250 μl of serum-free MEM medium was transferred to a sterile 1.5 mL EP tube, 5 μL of Lipofectamine 2000 was added, shaken evenly, and allowed to stand at room temperature for 5 min. 250 μL of serum-free MEM medium was transferred to a sterile 1.5 mL EP tube, and 5 μL of MiR-24 siRNA and pcDNA miR24 were added and shaken evenly. The solutions in the above two EP tubes were mixed together and allowed to stand at room temperature for 25 minutes. The mixed solution was transferred to the prepared six-well plate cells, and placed in a 37°C, 5% CO_2 incubator for cultivation. 48 h after transfection, cells were harvested.

Real Time-PCR Detection of the Expression of MiR-24, PDGFRa, NG2, and MBP in Spinal Cord Tissue and Cells of Each Group

The spinal cord tissue and cell mRNA of each group were extracted with TRIzol reagent, and DNA reverse transcription synthesis was performed according to the kit instructions. The primers were designed by Primer Premier 6.0 according to each gene sequence and synthesized by Shanghai Yingjun Biotechnology Co., Ltd. (Shanghai, China) (Table I). Real-time PCR detection of the gene of interest with reaction conditions: 92°C 30 s, 58°C 45 s, 72°C 35 s, a total of 35 cycles. Data was collected using the PCR reactor software and U6 was used as a reference. According to the fluorescence quantification, the starting cycle number (CT) of all samples and standards was calculated. Based on the standard CT value, a standard curve was drawn, and then the semi-quantitative analysis was carried out by the $2^{-\Delta Ct}$ method.

Western Blot

The total protein of each group was extracted: the lysate was added, the cells were lysed on ice for 15-30 min, the cells were sonicated by 5 s × 4 times, centrifuged at 4°C, 10,000 × g for 15 min, and the supernatant was transferred to a new

EP tube. The protein was quantified and stored at -20°C for Western blot experiments. The isolated protein was electrophoresed on 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (separation range 20-80 KD), and the target proteins were 52 KD and 67 KD, respectively. The gel was transferred to the PVDF membrane by semi-dry transfer method, 160 mA, 1.5 h, and blocked with 5% skim milk powder for 2 h to remove non-specific background. The membrane was incubated with different dilutions of primary antibody PDGFRa and NG2 and MBP (1:1000, 1:500, 1:1500) and β-actin (1:2000) monoclonal antibody at 4°C overnight. After washing with phosphate-buffered saline and tween (PBST), the corresponding secondary antibodies diluted in different proportions were added in the dark and incubated for 30 min, followed by washing with PBST and development after addition of enhanced chemiluminescence for 1 min. X-ray exposure imaging was performed to observe the results. X-film and strip density measurements were separately scanned using protein image processing system software and Quantity one software. The experiment was repeated four times (n = 4) and the statistical analysis too.

Statistical Analysis

Data were analyzed by SPSS 19.0 (IBM, Armonk, NY, USA) statistical software. Measurement data were expressed as mean ± standard deviation, and multiple groups of sample means were compared using one-way ANOVA with Bonferroni post-hoc analysis. $p<0.05$ indicates a statistical difference.

Results

MiR-24 Expression In Rats With Spinal Injury

Real time PCR analysis of miR-24 expression in rats with spinal injury showed that the expression of miR-24 in the model group was increased, compared with the sham operation group, the difference was statistically significant ($p<0.05$; Figure 1).

Expression of PDGFRa and NG2 In Spinal Cord Tissue of Rats With Spinal Injury

Real time PCR was used to analyze the expression of OPC markers PDGFRa and NG2 in the spinal cord of rats with spinal injury. The expression of OPC markers PDGFRa and NG2 in the spinal cord of the model group was increased. Compared with the sham operation group, the difference was statistically significant ($p<0.05$; Figure 2).

MBP Expression In Spinal Cord Tissue of Rats With Spinal Injury

Real time PCR was used to analyze the expression of OLs marker MBP in the spinal cord of rats with spinal injury. The expression of OLs marker MBP in the spinal cord of the model group was decreased. Compared with the sham operation group, the difference was statistically significant ($p<0.05$; Figure 3).

Changes In Serum ADM Content In Rats With Spinal Injury

The changes of serum ADM content in rats with spinal injury were analyzed by ELISA. The serum ADM content in rats with spinal injury was decreased, compared with the sham operation group, the difference was statistically significant ($p<0.05$; Figure 4).

Changes In Serum Inflammatory Factors IL-6 and TNF-α Expression In Rats With Spinal Injury

The expression of inflammatory factors IL-6 and TNF-α in the serum of rats with column injury was increased, compared with the sham operation group, the difference was statistically significant ($p<0.05$; Figure 5).

Table I. Primer sequences.

Gene	Forward 5′-3′	Reverse 5′-3′
U6	TGCTTCGGCAGCACATATAC	AGGGGCCATGCTAATCTTCT
miR-24	TCCACAGATCCTCCAGT	GCGTCATCGTATAGTAGCTT
PDGFRa	GACTCCAGTCATTAGCT	CTAACCTAACAACTCCAGT
NG2	CGCCTCCGGTCACTA	GTAAACTTGCTACATGGC
MBP	TAT CGCCGTCCTAACCT	TCACTAACTTAGCCATGTC

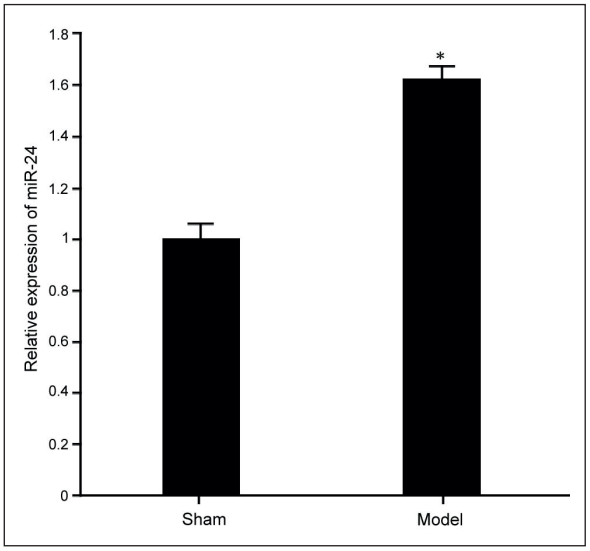

Figure 1. MiR-24 expression in spinal injured rats. Compared with the sham operation group, *p<0.05.

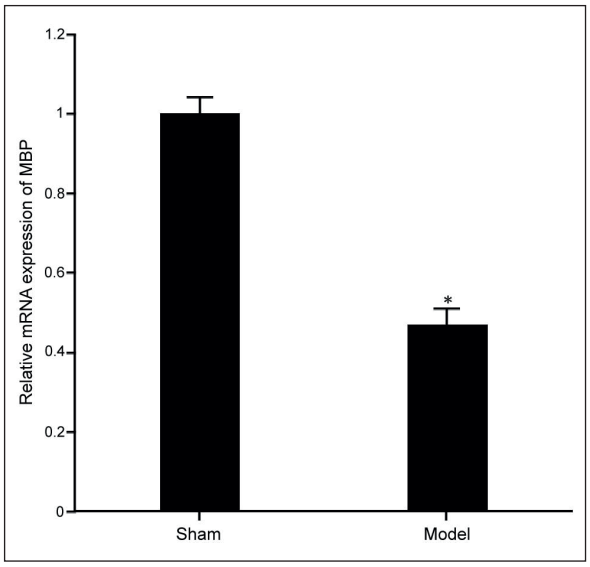

Figure 3. MBP expression in spinal cord tissue of rats with spinal injury. Compared with the sham operation group, *p<0.05.

Regulation of MiR-24 on MiR-24 Expression In OPC Cells

Transfection of pcDNA miR-24 plasmid into OPC cells up-regulated miR-24 expression, compared with the control group, the difference was statistically significant (p<0.05). MiR-24 siRNA transfection into OPC cells down-regulated miR-24 expression, compared with the control group, the difference was statistically significant (p<0.05; Figure 6).

Effect of MiR-24 on the Differentiation of OPC Cells

Western blot analysis of the effect of miR-24 on the differentiation of OPC cells showed that up-regulation of miR-24 expression could inhibit the differentiation of OPC cells, and the expression of OPC cell marker proteins PDGFRa and NG2 was increased, while the expression of OL marker protein MBP was decreased. Compared with the control group, the difference was sta-

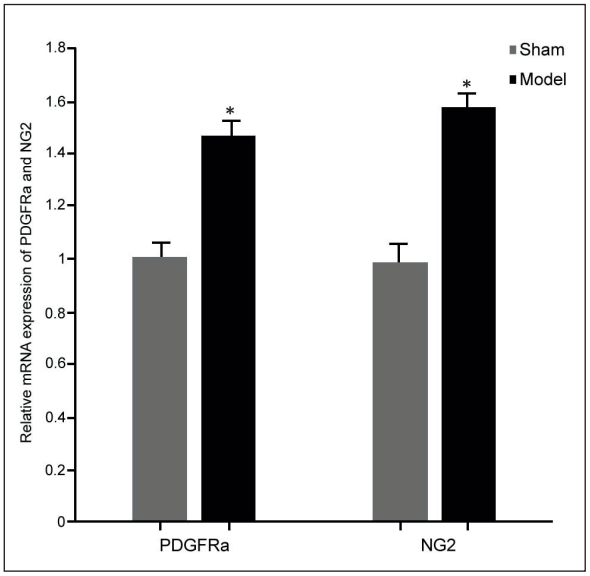

Figure 2. Expression of PDGFRa and NG2 in spinal cord tissue of rats with spinal injury. Compared with the sham operation group, *p<0.05.

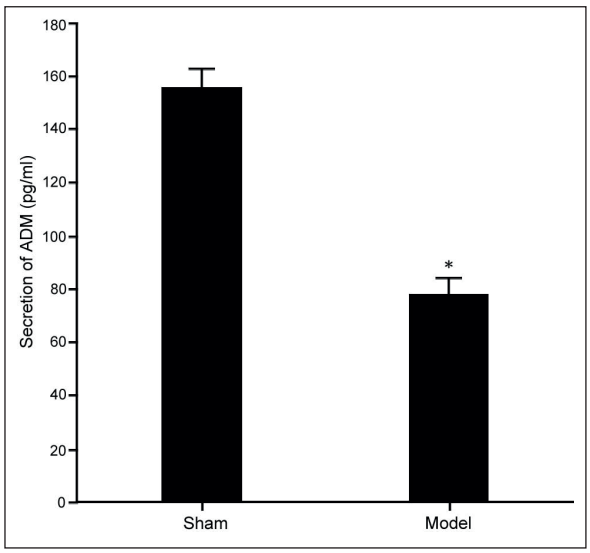

Figure 4. Changes in serum ADM content in rats with spinal injury. Compared with the sham operation group, *p<0.05.

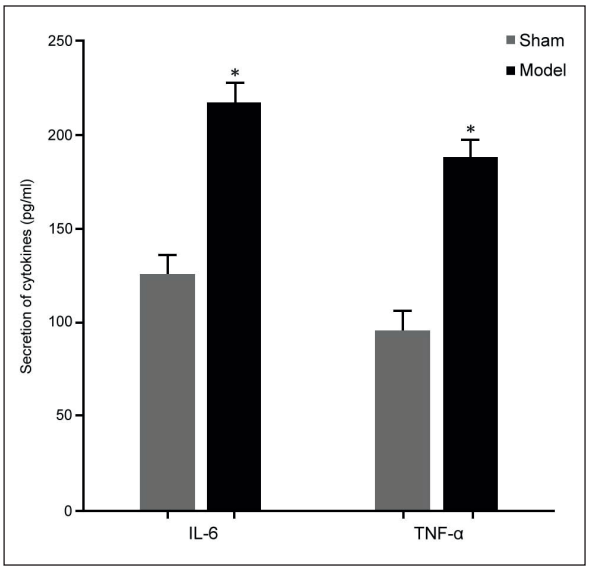

Figure 5. Changes in serum inflammatory factors IL-6 and TNF-α expression in rats with spinal injury. Compared with the sham operation group, *p<0.05.

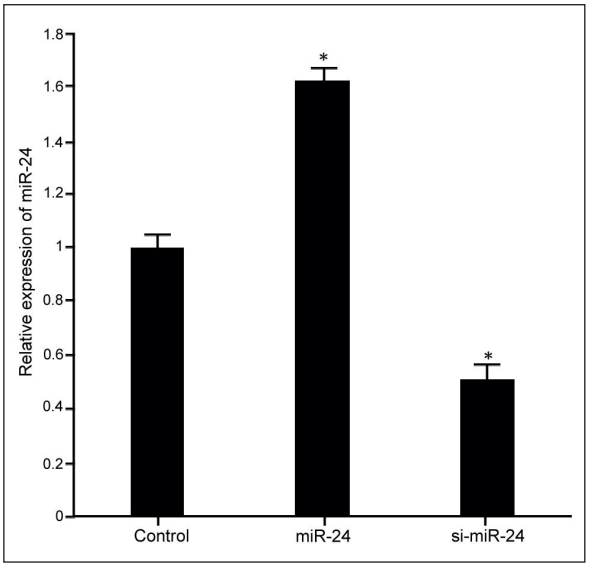

Figure 6. Regulation of miR-24 on miR-24 expression in OPC cells. Compared with the control group, *p<0.05.

tistically significant (p<0.05). Down-regulation of miR-24 expression promoted OPC cell differentiation, decreased OPC cell marker proteins PDGFRa and NG2 expression and increased OL marker protein MBP expression, compared with the control group, the difference was statistically significant (p<0.05) (Figure 7).

Effect of MiR-24 on ADM Expression In OPC Cells

Up-regulation of miR-24 expression inhibited the secretion of ADM from OPC cell supernatants, and the difference was statistically significant (p<0.05). Down-regulation of miR-24 expression promoted the secretion of ADM from OPC cell supernatants, and the difference was statistically significant (p<0.05; Figure 8).

Effect of MiR-24 on the Secretion of Inflammatory Factors In OPC Cells

Up-regulation of miR-24 expression promoted the expression of inflammatory cytokines IL-6 and TNF-α in OPC cells. The difference was statistically significant (p<0.05). Down-regulation

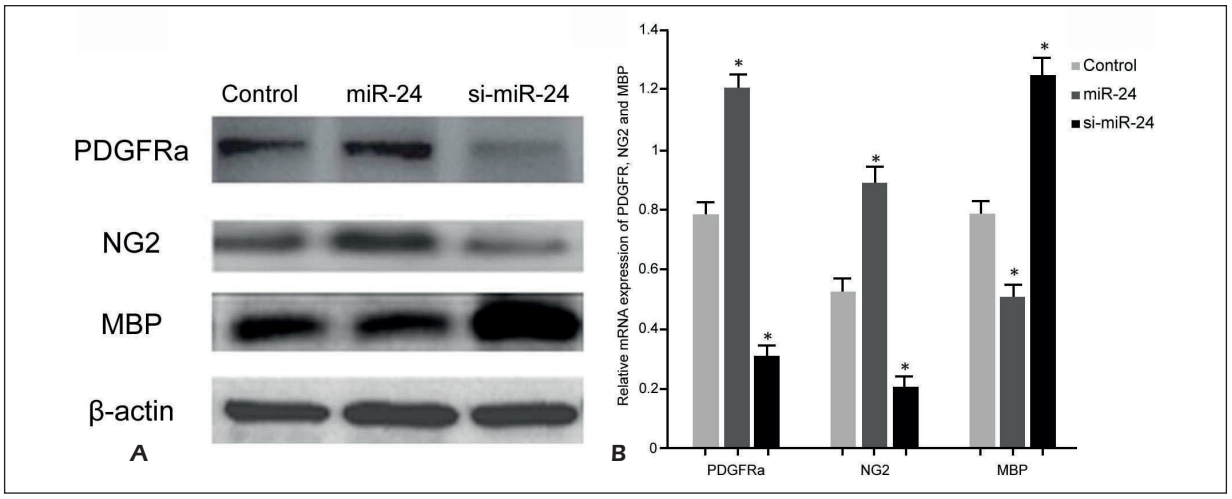

Figure 7. Effect of miR-24 on OPC cell differentiation. **A**, Western blot analysis of the regulation of miR-24 on the expression of OPC cell marker proteins PDGFRa and NG2 and the expression of OL marker protein MBP. **B**, Effect of miR-24 on the expression of PDGFRa and NG2 and MBP expression, compared with the control group, *p< 0.05.

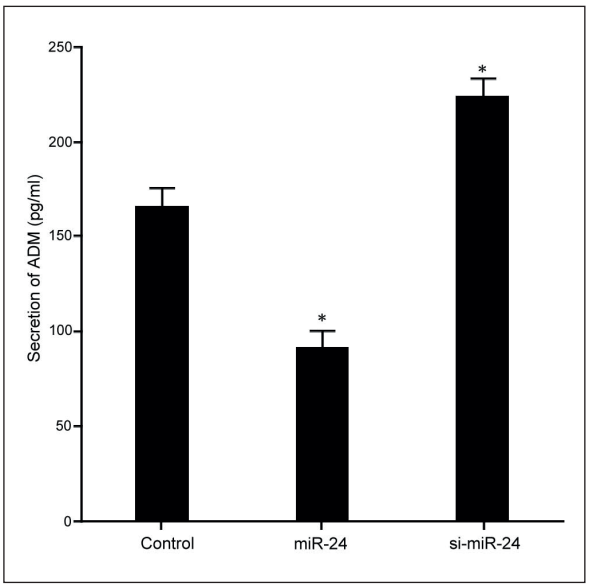

Figure 8. Effect of miR-24 on ADM expression in OPC cells. Compared with the control group, *p<0.05.

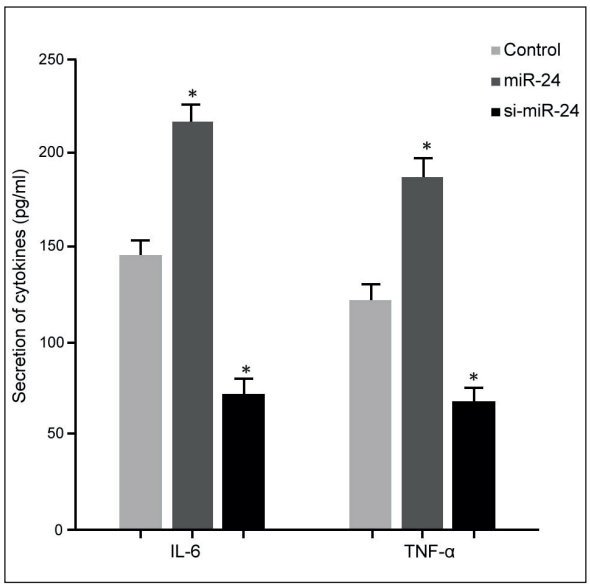

Figure 9. Effect of miR-24 on the secretion of inflammatory cytokines in OPC cells. Compared with the control group, *p<0.05.

of miR-24 expression inhibited the secretion of inflammatory cytokines IL-6 and TNF-α in OPC cells, and the difference was statistically significant (p<0.05; Figure 9).

Discussion

The pathogenesis of spinal cord injury is complex. Due to damage of nerve cells, glial scar hyperplasia, inflammation, oxidative stress and other related factors, damaged nerve tissue and axons are difficult to repair, and dysfunction is gradually worsened[20]. Therefore, elucidating the related mechanisms of spinal cord injury and analyzing its regulatory factors is conducive to the diagnosis and treatment of spinal cord injury. Under pathological and physiological conditions, oligodendrocyte precursor cells (OPCs), which act as reservoirs, can differentiate into oligodendrocytes (OLs), form myelin cells, and provide nutrients for neurons[21]. OPCs have a unique ability to differentiate into myelin-derived oligodendrocytes in cell replacement therapy for neurodegenerative diseases, and thus playing a therapeutic role[22]. During spinal cord injury, oligodendrocyte precursor cells are activated to produce myelin oligodendrocytes, which contribute to remyelination and functional recovery[23]. Therefore, how to promote the differentiation of oligodendrocyte precursor cells into myelin oligodendrocytes and promote the repair of spinal cord injury has become one of the research topics.

MiRNA variants caused by genetic mutations, epigenetic changes, or chromosomal aberrations have been shown to have an effect on the biological processes of many diseases and play an important role in cell regeneration, differentiation, proliferation, and apoptosis[24]. MiR-24 plays an important role in a variety of diseases, so the aim of this study is to analyze the role of miR-24 in spinal cord injury and oligodendrocyte precursor cell differentiation. This research revealed that the expression of miR-24 was increased in the spinal cord of the spinal cord injury model group, the expression of PDGFRa and NG2 was increased, the expression of MBP was decreased, the ADM was decreased, and the expression of IL-6 and TNF-α was increased. This result suggests that miR-24 is abnormally expressed in spinal cord injury and oligodendrocyte precursor cell differentiation is inhibited.

Further analysis was performed for the regulation of miR-24 on the differentiation of oligodendrocyte precursor cells. Adrenal medulla (ADM) isolated from human chrome cell tumor is an endogenous vasoactive peptide containing 52 amino acids, a member of the calcitonin gene-related peptide family, which has been shown to have anti-oxidative stress and can be affected by inflammation and participate in the regulation of cardiovascular diseases, kidney diseases and tumor growth and invasion diseases. High expression of ADM in central nervous system can be used

as neurotransmitters, neuromodulators and neurohormones[25,26], playing a role in the regulation of nerve damage, such as ischemic brain injury. The results showed that upregulation of miR-24 expression in OPC cells promoted the expression of PDGFRa and NG2, decreased MBP expression and ADM, and increased IL-6 and TNF-α expression. Down-regulation of miR-24 expression in OPC cells reverses these changes, inhibits the expression of PDGFRa and NG2, promotes MBP expression and ADM, and decreases IL-6 and TNF-α expression. This result suggests that miR-24 can inhibit the differentiation of OPC cells and increase the expression of inflammatory factors by regulating ADM.

Conclusions

In summary, the expression of miR-24 in spinal injury is increased. Increased expression of miR-24 reduces adrenal medulla expression and inhibits oligodendrocyte precursor cell differentiation.

Conflict of Interests

The authors declared there is no conflict of interest.

References

1) Prattley S, Oliver R, New F, Davies M, Brewin J. Ureteroscopy in patients with spinal cord injury: outcomes from a spinal injury unit and a review of literature. Transl Androl Urol 2019; 8: S352-S358.
2) Christopher A, Kraft E, Olenick H, Kiesling R, Doty A. The reliability and validity of the timed up and go as a clinical tool in individuals with and without disabilities across a lifespan: a systematic review. Disabil Rehabil 2019 Oct 26: 1-15. doi: 10.1080/09638288.2019.1682066. [Epub ahead of print]
3) Goncalves MB, Moehlin J, Clarke E, Grist J, Hobbs C, Carr AM, Jack J, Mendoza-Parra MA, Corcoran JPT. RARβ agonist drug (C286) demonstrates efficacy in a pre-clinical neuropathic pain model restoring multiple pathways via DNA repair mechanisms. iScience 2019; 20: 554-566.
4) Kigerl KA, Zane K, Adams K, Sullivan MB, Popovich PG. The spinal cord-gut-immune axis as a master regulator of health and neurological function after spinal cord injury. Exp Neurol 2020; 323: 113085.
5) Hong HN, Shim JH, Won YJ, Yoo JY, Hwang CH. Therapeutic time window for the effects of erythropoietin on astrogliosis and neurite outgrowth in an in vitro model of spinal cord injury. Medicine (Baltimore) 2018; 97: e9913.
6) Sperl A, Heller RA, Biglari B, Haubruck P, Seelig J, Schomburg L, Bock T, Moghaddam A. The role of magnesium in the secondary phase after traumatic spinal cord injury. A prospective clinical observer study. Antioxidants (Basel) 2019; 8: 509.
7) Guercio JR, Kralic JE, Marrotte EJ, James ML. Spinal cord injury pharmacotherapy: Current research & development and competitive commercial landscape as of 2015. J Spinal Cord Med 2019; 42: 102-122.
8) Cotner BA, Ottomanelli L, Keleher V, Dirk L. Scoping review of resources for integrating evidence-based supported employment into spinal cord injury rehabilitation. Disabil Rehabil 2019; 41: 1719-1726.
9) Phillips AA, Matin N, Frias B, Zheng MM, Jia M, West C, Dorrance AM, Laher I, Krassioukov AV. Rigid and remodelled: Cerebrovascular structure and function after experimental high-thoracic spinal cord transection. J Physiol 2016; 594: 1677-1688.
10) Nguyen LH, Ong W, Wang K, Wang M, Nizetic D, Chew SY. Effects of miR-219/miR-338 on microglia and astrocyte behaviors and astrocyte-oligodendrocyte precursor cell interactions. Neural Regen Res 2020; 15: 739-747.
11) Yuan T, Liu Q, Kang J, Gao H, Gui S. High-dose neural stem/progenitor cell transplantation increases engraftment and neuronal distribution and promotes functional recovery in rats after acutely severe spinal cord injury. Stem Cells Int 2019; 2019: 9807978.
12) Shi J, Tang R, Zhou Y, Xian J, Zuo C, Wang L, Wang J, Feng H, Hu S. Attenuation of white matter damage following deferoxamine treatment in rats after spinal cord injury. World Neurosurg 2019 Sep 10. pii: S1878-8750(19)32414-3. doi: 10.1016/j.wneu.2019.08.246. [Epub ahead of print]
13) Zhang Q, Li W, Liu G, Tang W. MicroRNA-24 regulates the growth and chemosensitivity of the human colorectal cancer cells by targeting RNA-binding protein DND1. J BUON 2019; 24: 1476-1481.
14) Azizi E, Ghaffari Novin M, Naji M, Amidi F, Shams Mofarahe Z. Does in vitro fertilization affect the expression of mirnas and their biogenesis pathway in preimplantation mouse embryos? Birth Defects Res 2020; 112: 62-70.
15) Zhang L, Song Y, Chen L, Li D, Feng H, Lu Z, Fan T, Chen Z, Livingston MJ, Geng Q. MiR-20a-containing exosomes from umbilical cord mesenchymal stem cells alleviates liver ischemia/reperfusion injury. J Cell Physiol 2020; 235: 3698-3710.
16) Song S, Lin F, Zhu P, Wu C, Zhao S, Han Q, Li X. Lycium barbarum polysaccharide alleviates oxygen glucose deprivation-induced PC-12 cells damage by up-regulating miR-24. Artif Cells Nanomed Biotechnol 2019; 47: 3994-4000.
17) Kokkinopoulou I, Maratou E, Mitrou P, Boutati E, Sideris DC, Fragoulis EG, Christodoulou MI. Decreased expression of micrornas targeting type-2 diabetes susceptibility genes in peripheral blood of patients and predisposed individuals. Endocrine 2019; 66: 226-239.

18) Zhang J, Cai W, Fan Z, Yang C, Wang W, Xiong M, Ma C, Yang J. MicroRNA-24 inhibits the oxidative stress induced by vascular injury by activating the Nrf2/Ho-1 signaling pathway. Atherosclerosis 2019; 290: 9-18.

19) Qin W, Li X, Peng Y, Harlow LM, Ren Y, Wu Y, Li J, Qin Y, Sun J, Zheng S, Brown T, Feng JQ, Ke HZ, Bauman WA, Cardozo CP. Sclerostin antibody preserves the morphology and structure of osteocytes and blocks the severe skeletal deterioration after motor-complete spinal cord injury in rats. J Bone Miner Res 2016; 31: 1482.

20) Burkovskiy I, Zhou J, Lehmann C. Experimental cannabinoid 2 receptor inhibition in cns injury-induced immunodeficiency syndrome. Microcirculation 2016; 23: 283-292.

21) Cheah M, Andrews MR, Chew DJ, Moloney EB, Verhaagen J, Fassler R, Fawcett JW. Expression of an activated integrin promotes long-distance sensory axon regeneration in the spinal cord. J Neurosci 2016; 36: 7283-7297.

22) Cheng L, Wang C, Yao F, Li Z, Liu W, Jing J. MicroRNA-26b inhibits oligodendrocyte precursor cell differentiation by targeting adrenomedullin in spinal cord injury. J Cell Physiol 2020; 235: 2429-2440.

23) Duncan GJ, Manesh SB, Hilton BJ, Assinck P, Plemel JR, Tetzlaff W. The fate and function of oligodendrocyte progenitor cells after traumatic spinal cord injury. Glia 2020; 68: 227-245.

24) Hutchinson JM, Isaacson LG. Elimination of microglia in mouse spinal cord alters the retrograde CNS plasticity observed following peripheral axon injury. Brain Res 2019; 1721: 146328.

25) Cui YM, Zhu ZY, Qi X, Li HH, Wu YL, Chen WL, Liu Y. Relationship between circulating concentration of Ang II, ADM and ADT and left ventricular hypertrophy in hypertension. Am J Transl Res 2019; 11: 3167-3175.

26) Yallampalli C, Chauhan M, Endsley J, Sathishkumar K. Calcitonin gene related family peptides: Importance in normal placental and fetal development. Adv Exp Med Biol 2014; 814: 229-240.