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The mechanism by which miR-let-7b-modified BMSCs mediating Nogo/NgR signaling pathway in the repair of spinal cord injury

Beiren Yang[1] and Aihua Yu[2]*

[1]Department of Orthopedics, Yichang Traditional Chinese Medicine Hospital, Yichang, Hubei, 434000, China
[2]Department of Orthopedics, Zhijiang People´s Hospital, Zhijiang, Hubei, China

Abstract: **Background**: Spinal cord injury (SCI) leads to severe and often permanent neurological deficits. The inhibitory Nogo/NgR signaling pathway constitutes a major barrier to axonal regeneration. Transplantation of bone marrow mesenchymal stem cells (BMSCs) and modulation of microRNAs, such as miR-let-7b, represent promising therapeutic strategies for neural repair. **Objectives**: This study aimed to investigate the role and mechanism of miR-let-7b-modified BMSCs in repairing SCI, focusing on their interaction with the Nogo/NgR signaling pathway. **Methods**: *In vitro*, an oxygen-glucose deprivation (OGD) neuronal model (VSC4.1 cells) was co-cultured with BMSCs or miR-let-7b-modified BMSCs. Neuronal marker expression (NSE, MAP-2, Tau) and targeting relationships were assessed via immunocytochemistry, RT-PCR, and dual-luciferase assays. In vivo, 40 SD rats with spinal cord hemisection were divided into control, BMSCs, miR-let-7b+BMSCs, and NgR antagonist groups. Hindlimb functional recovery was evaluated using the Tarlov score and Rivlin inclined plane test. **Results**: miR-let-7b-modified BMSCs significantly increased neuronal marker expression in vitro ($P<0.05$). In vivo, this group showed sustained and optimal functional recovery from day 7 post-injury. The modified BMSCs directly targeted the Nogo gene, significantly reducing its protein expression, whereas the NgR antagonist did not affect Nogo levels. **Conclusion**: miR-let-7b-modified BMSCs promote functional recovery after SCI by targeting the Nogo gene and inhibiting the Nogo/NgR signaling pathway, likely via an exosome-mediated mechanism, thereby improving the inhibitory microenvironment for neural repair.

Keywords: Bone marrow mesenchymal cells; MiR-let-7b; Nogo/NgR signaling pathway; Spinal cord injury

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INTRODUCTION

Spinal cord injury (SCI) refers to damage caused by external factors, resulting in spinal vertebral fracture or spinal cord compression or injury (Kirshblum *et al.*, 2021). SCI has a high disability rate and mortality (Hu *et al.*, 2021). It is estimated that the annual incidence of SCI worldwide is 22/1 million (Lin *et al.*, 2020) and the case number is about 2.5 million. The most frequent group is young people between 20 and 30 years old. Despite a near-normal life expectancy, individuals often experience severe disabilities that substantially diminish their quality of life, imposing a heavy burden on both the individuals and society. According to conventional understanding, this type of damage is considered permanent and irreversible (Wu *et al.*, 2020). Adult neurons lack the inherent regenerative ability and the damage often leads to permanent neurological deficits (Kirshblum *et al*., 2021). However, the transected peripheral nerve fibers have strong regenerative ability, suggesting that the spinal cord axon itself lacks the regenerative ability or the microenvironment of the spinal cord restricts its growth (Wang *et al.*, 2020). The spinal cord microenvironment of patients with SCI is very complex and the factors that inhibit axon regeneration (Zhang *et al.*, 2024) include microglia immune-mediated inflammatory response, scar tissue formed at the injury site and the associated inhibitory microcirculation formed by the central nervous system myelin-associated inhibitory factors (MAIFs) exposed after injury (Chambel and Cruz, 2023). The Nogo/NgR signaling pathway is the most studied reticular signaling pathway among MAIFs (Ji *et al.*, 2023). The Nogo protein, a myelin-associated inhibitor, strongly suppresses axonal growth. They exert potent axon growth inhibitory effects through cell contact, thereby limiting neural plasticity and repair. Inhibition of their activity can significantly enhance axonal regeneration capacity at the lesion site (Chambel and Cruz, 2023).There are three configurations of Nogo protein and the three have the same C-terminal Nogo-66. Research has found that the C-terminal (Nogo-66) has axon growth inhibitory effect. NgR was identified as the receptor for Nogo-66. It was initially isolated using a fusion protein of Nogo-66 and human placental alkaline phosphatase (AP)(Howard and Strittmatter, 2023). NgR is widely distributed throughout the central nervous system (CNS), localizing to the cell bodies, axons, cytoplasm and cell membranes of various neural cells. Within the growth cone, NgR extends into both the central (C) and peripheral (P) domains, with the highest density observed in the P-domain. This region serves as a critical site where multiple myelin-derived axon inhibitory proteins converge to exert their effects (Jiang *et al.*, 2021). With the development of cell therapy, exosomes from multipotent mesenchymal stem cells have shown therapeutic potential in a variety of diseases (Zhou *et al.*, 2020). In the CNS injury model, MSCs-exosomes

**Corresponding author:* e-mail: 15997615173@163.com
All author contributed equally to this work

promoted cortical neuron axons growth. Three-dimensionally cultured human bone marrow-derived MSCs show more exosomes secretion and can promote the functional restoration of traumatic brain injury in rats (Zhang *et al.*, 2020). The results indicate that BMSCs can be a good treatment tool for SCI. The Let-7 family is a type of human miRNAs discovered earlier. It can regulate the biological behavior of stem cells and is closely related to the occurrence of many human diseases. It is divided into 10 subtypes. Let-7b is one of them. The differentiation efficiency of BMSCs infected with miR-let-7b into neurons is improved and the levels of NSE, MAP-2 and Tau nerve cell marker proteins are also increased. Therefore, this study is based on the potential of BMSCs infected with miR-let-7b to repair SCI. From the perspective of animal experiments and cell experiments, this study explores the mechanism of miR-let-7b-modified BMSCs repairing SCI and focuses on analyzing its relationship with Nogo/NgR signaling pathway and provides new ideas for treating SCI.

MATERIALS AND METHODS

Laboratory animals and reagents

Experimental animals

Forty healthy female Sprague-Dawley (SD) rats were obtained from the Zhejiang Laboratory Animal Center. The animals were housed under a 12/12-hour light/dark cycle with ad libitum access to food and water. Before the establishment of the injury model, the rats were trained in behavior for one week and the rats with large differences in behavior were eliminated.

Main experimental reagents

Vector pHBAd-MCMV-GFP was purchased from Hanbio (Shanghai, China). miR-let-7 virus, restriction endonuclease, T4 ligase, plasmid DNA, fetal bovine serum and pancreatin were from GenePharma (Shanghai, China). Membrane filter, DMEM, RPMI 1640, Trypsin, DMSO, double antibody, β-actin monoclonal antibody, NSE antibody, MAP-2 antibody and Tau antibody were from Santa Cruz Biotechnology, Inc (USA). Goat anti-mouse fluorescent secondary antibody (Cell Signaling Technology, USA.) and goat anti-rabbit fluorescent secondary antibody were from Abbkine Scientific Co., Ltd (Wuhan, China).

Culture and identification of BMSC cells

BMSCs were thawed from liquid nitrogen storage in a 37°C water bath, centrifuged, and resuspended in complete culture medium (α-MEM supplemented with 10% FBS and 1% penicillin/streptomycin). Observe when the proliferation rate reaches 90%, then the cell was washed with PBS for 3 times. 20 ml of α-MEM basal medium was added and the cells were collected 3 days later.

Characterization of BMSC

Observe and count the cell suspension under a microscope, then transfer the suspension to a centrifuge tube, add specific antibodies CD90, CD45, CD44 (fluorescein labeled, 1:200), incubate in the dark for half an hour and use PBS to rinse it well.

miR-let-7b infection of BMSCs

The third generation rat BMSCs were inoculated in a 10cm culture dish at a density of 1x106cells/well, a 6-well plate was seeded at a density of 5x10cells/well and a 24-well plate was seeded at a density of 2x10cells/well and cultured normally. 24h after seeding, they are ready for transfection. Dilute the miR-let-7b virus solution with 3 mL of high-sugar serum-free medium, calculate the amount of virus solution required for MOI of 50 and add an appropriate amount of polybrene to a concentration of 2 mg/mL, shake and mix well, add it to a petri dish with medium removed and incubate for 6 hours. After the incubation, remove the virus liquid and serum-free medium and add 10 mL of complete cell culture medium and culture for 48 hours to obtain miR-let-7b gene-modified bone marrow mesenchymal stem cells.

Cultivation of spinal anterior horn motor neurons (VSC4.1) and establishment of oxygen glucose deprivation (OGD) model

VSC4.1, a spinal cord anterior horn motor neuron cell line, was cultured in 90% RPM11640 containing double antibodies and 10% fetal bovine serum. The medium was changed every other day and passaged regularly. VSC4.1 was seeded into a 6-well plate at a density of 5x10 cells/well (2x10 cells/well for 24-well plate) and cultured for 24h. After washing, the cells were incubated with 2 mL of D-Hank's solution and placed in a sealed hypoxia chamber (Model 43.441). An oxygen consumption catalyst and a hypoxia indicator strip were also introduced into the chamber to establish and monitor the hypoxic conditions. After 7 hours, the oxygen and sugar deprivation was relieved, the D-Hank's solution was removed and complete medium was used for cell culture.

Co-culture model

The co-culture of BMSCs and VSC4.1 was achieved through a Trans-well plate (pore size: 0.4um), which can prevent the two cells from contacting each other, but cell secretions such as cytokines can pass freely. BMSCs were seeded in the upper chambers of Trans-well plates at densities of 5×10^4 cells/well (6-well plate) or 2×10^4 cells/well (24-well plate). Subsequently, VSC4.1 cells were plated in the corresponding lower compartments at the same densities and subjected to OGD injury. After 7 hours of injury, the cells containing different BMSCs were inoculated for co-cultivation. After 24 hours, VSC4.1 cells were collected for protein extraction or immunofluorescence staining.

Spinal cord injury model

The rats used in the experiment were all female SD rats weighing 220g-250g, purchased from Zhejiang Experimental Animal Center and kept at a temperature of

21±2°C, humidity of 50%-55% and light/darkness for 12h /12h (22:00-10:00 dark) environment. A contusive SCI model was established at the T10 vertebral level using a modified Allen's weight-drop apparatus. After shaving, rats were fixed in the middle of the operating table in a prone position and the spine was pressed from the head with the index finger. The T10 vertebra was identified as the most prominent point of the dorsal spinal curvature. After disinfection by Iodophor wipes, the skin was cut 8mm before and after T10 along the midline of the back, muscle tissue around T9-T11was separagted to expose the T10 vertebral plate. The rongeur was used to lift the T10 vertebral plate to expose the spinal cord intact and undamaged. Type I spinal cord percussion instrument was used to complete the spinal cord impact injury model. Adjust the impact cylinder (weight 10g) to just above the center of the T10, adjust the height of 5cm from the spinal cord plane and then activate the switch to hit the cylinder for free fall. The impact was in the center of the T10 spinal cord. The dura mater turned purple, the spinal cord was swollen, the median vein was congested and thickened, local bleeding occurred, the rat's tail swung vigorously from side to side and the hind limbs tremble are signs of the successful establishment of the SCI model

Bone marrow mesenchymal stem cell transplantation

BMSCs and BMSCs+miR-let-7b were digested, centrifuged and counted to make 1x106cells/10uL cell suspension. The rats with SCI were fixed to the brain stereotaxic instrument. The micro syringe with 10 μL of the above cell suspension was fixed to the stereotaxic instrument, adjust the position so that the needle tip was aligned with the SCI site. Then move the micro-syringe so that the needle tip enters the spinal cord 2mm, then push out 0.5mm and inject BMSCs at a speed of 1μL/min. The injection was completed in 10min, leave the needle for another 5 minutes and then slowly withdraw the micro-syringe. No fluid outflow is a sign of successful cell transplantation. The surgical incision is sutured layer by layer. The rats were given brown sugar water to replenish energy after wakeup.

Hind limb motor function score

The modified Tarlov score (Basso *et al*.,1995) and the Rivlin inclined plane test (Dong *et al*.,2015) were used to evaluate the hindlimb function. Improved Tarlov scoring criteria: 0 points, completely paralyzed, no response to lower limbs during acupuncture; 1 point, complete paralysis, lower limbs responding to acupuncture, but limbs unable to move; 2 points, limbs can move, but cannot stand or stand unstable (<5s); 3 points, can stand, but cannot walk; 4 points, can walk several steps, but is unstable; 5 points, can walk slowly, but not flexible, there are certain defects; 6 points, normal walking. Rivi-lin inclined board experiment: Put the rat head to the right on the modified Rivlin inclined board and gradually increase the angle from the horizontal position (0°) to the maximum that the rat can stay on the board for 5s without falling.

Detection of NSE, MAP-2, Tau and Nogo levels by immunocytochemistry

After infection and induced differentiation for 2 h, cells were washed, fixed and placed in a 4ºC refrigerator overnight. The paraformaldehyde was aspirated the next day, cells were rinsed with 500 μl of 0.1% TBST buffer solution 3 times. Cells were blocked with 500μl of 5% fetal bovine serum for 1 hour and probed with diluted primary antibodies includding NSE antibody (1.0 mg/L), MAP-2 antibody (1.0 mg/L), Tau antibody (1.0 mg/L) and Nogo-66 antibody (1.0 mg/L), followed by probing with Cy3-labeled secondary antibody. After washing, a fluorescence microscope was used for observation and ImagePro Plus 6.0 was used for image analysis.

RT-PCR detection of the mRNA levels of NSE, MAP-2, Tau and Nogo-66

PCR amplification of cDNA was done according to instructions of Gotaq qPCR Master Mix (Promega) and Light Cycler RT-PCR system (Roche). The PCR conditions were as follows: 94ºC for 30 s, 60ºC for 30 s, for a total of 30 cycles, followed by a final extension at 72ºC for 5 min. The relative mRNA expression levels of NSE, MAP-2 and Tau were determined using the $2^{-\Delta\Delta Ct}$ method, with β-catenin as the internal reference. The primers and their sequences are listed in table 1.

Luciferase experiment

BMSCs+miR-let-7b cells in logarithmic phase were inoculated at a concentration of 3×103 cells/well. When the confluence reached 70%, transfections were performed using Lipofectamine2000. The groups were: wild type plasmid + negative control group, wild-type plasmid + miR-let-7b mimic group, mutant plasmid + negative control group and mutant plasmid + miR-let-7b mimic group.

Statistical analysis

All experimental data mentioned above were analyzed using SPSS 27.0 and GraphPad Prism software. Data analysis was performed with one-way ANOVA. Data are presented as X ± S in the charts. A P-value < 0.05 was considered statistically significant.

RESULTS

Morphological observation of BMSCs

(1) On the 5th day after culture, the observed morphology of BMSCs was polygonal, with colony-like growth, as shown in fig. 1-A.The third generation of BMSCs were arranged in a vortex and radial pattern, as shown in fig. 1-B.

(2) Identification of BMSC surface markers: The expression of CD90 (expression rate of 95.10%) and CD44 (77.04%) of adherent cells were both positive; and further observation revealed that the expression rate of CD45 on the negative table was 2.03% (Fig. 2). The above results

indicated that the BMSC culture was successful and subsequent experiments can be carried out.
(3) miR-let-7b successfully modified BMSCs: The BMSCs+miR-let-7b group showed the strongest fluorescence after three days of culture. The infection rate of BMSCs in the negative control group and BMSCs+miR-let-7b was 84.6% and 85.7%, respectively. Comparison of two sets of fluorescence was shown in fig. 3.

miR-let-7b modified BMSCs promoted the repair of SCI
(1) The level of neuron markers increased significantly in the cell experiment. The levels of NSE, MAP-2 and Tau in the NgR antagonist group and the BMSCs group were sharply up-regulated than controls ($P<0.05$). The levels of the three proteins in the BMSCs group were higher than the NgR antagonist group, but the three protein expressions were not significantly different between the 2 groups. The expression of NSE, MAP-2 and Tau in the miR-let-7b+BMSCs group was sharply increased than BMSCs group ($P<0.05$) (Fig. 4).
(2) BMSCs modified with miR-let-7b promoted the repair of SCI in animal experiments.

Model Verification and General Observations Following surgery, all rats exhibited bilateral hindlimb paralysis, confirming the successful induction of SCI. Two to three weeks after the operation, the rats with SCI began to recover to varying degrees, such as voluntary activities, joint extension and extension of the hind limbs and no difference was found in autonomous urination function ($P> 0.05$).

Tarlov score and Rivlin inclined plane test score in four groups at different periods
No significant differences in the modified Tarlov scores or Rivlin inclined plane test angles were observed among the four groups at 1 or 3 days post-injury. At 7, 14, 21, 28 days after operation, there was no statistically significant difference between BMSCs group and NgR antagonist group and the differences between other groups were statistically significant ($P<0.05$). (Tables 2 and 3).

miR-let-7b modified BMSCs may mediate the Nogo/NgR signaling pathway to promote the repair of SCI
(1) Compared to controls, Nogo protein level of NgR antagonist group was not affected ($P>0.05$); Nogo level of the BMSCs group and miR-let-7b+BMSCs group was sharply decreased than the controls ($P <0.05$) (Fig. 5).
(2) The miR-let-7b had a specific binding region within Nogo gene and Nogo is the target of miR-let-7b. The fluorescence intensity of mutant plasmid was dramatically higher than wild-type plasmid ($P<0.05$) (Fig. 6).

DISCUSSION

Promoting functional regeneration within the central nervous system (CNS) following injury remains a major unmet clinical challenge. Neural stem cell transplantation and blocking the inhibitory effect of nerve regeneration inhibitors are two major strategies for treating SCI. Among various regenerative strategies, cell transplantation, including the use of neural stem cells and mesenchymal stem cells, holds considerable promise for SCI treatment.

This study mainly studied the repair effect of miR-let-7b-modified BMSCs on SCI through cell experiments and animal experiments and focused on whether there is a targeting relationship between the Nogo/NgR signaling pathway and miR-let-7b. According to the results, a co-culture model of miR-let-7b-modified BMSCs and oxygen-glucose deprived VSC4.1 were successfully established, as well as SCI model animals. The levels of neuron markers of four groups were detected by immunocytochemistry, the miR-let-7b modified BMSCs group had significantly higher levels of NSE, MAP-2 and Tau. Among the three neuron markers selected in this experiment, NSE is a glycolytic enzyme that specifically exists in neurons and neuroendocrine cells. In the study of Huang *et al*. (Huang *et al.*, 2021b), NSE served as a specific marker of neuron damage. MAP-2 is widely used in neurobiology research. It is one of the earliest adopted markers in primitive neuroepithelium.

According to Tang *et al*.'s research on neuronal cells (Tang *et al.*, 2020), MAP-2 is also involved in the composition of cytoskeletal proteins and is one of the important protein components that constitute the neuron skeleton. It plays a very important role in the formation of intracellular network structure and maintenance of cell morphology. Meng research on Tau (Meng *et al.*, 2024) pointed out that it mainly appears in the neuronal cells of the central nervous system and the content of other cells is very small, which can be used as a marker molecule of nerve axons. Hu and other teams's summary of the application of microRNAs (Hu *et al.*, 2020) mentioned that miR-let-7b can promote the differentiation of mesenchymal stem cells into neural cells, which is an important research topic for the treatment of SCI. In the present study, a dorsal hemisection SCI model was established in 40 rats and a standard model of dorsal hemisection SCI was established to observe the general condition of the rats after the operation. After bone marrow mesenchymal stem cell transplantation, no difference was found in the recovery of 4 groups of rats. According to the results of the Rivlin inclined plate experiment and the Tarlov score, the scores of the three groups of rats after 7 days were significantly improved compared to controls and miR -let-7b+BMSCs group has the highest score, which indicates that miR-let-7b modified BMSCs can improve the microenvironment of SCI through cell-to-cell communication. The Nogo protein, which is expressed on the surface of oligodendrocytes in the central nervous system, is an important inhibitor of nerve regeneration in the local microenvironment of SCI. Therefore, our study detected the expression of Nogo protein and explored its targeting relationship with miR-let-7b in cell experiments.

Table 1: PCR primers and primer sequences.

Primers		Sequences
MAP-2	Forward primer	5'-CCACCCAGAAAACCTCATGA-3'
	Reverse primer	5'-CATCCCGGTAAGTCCAATCA-3'
NSE	Forward primer	5'-TGAGCTACTAGGTTGTGTGCTT-3'
	Reverse primer	5'-AACCACACAACCTACTACCTCA-3'
Tau	Forward primer	5'-CTCAACTGGTGTCGTGGA-3'
	Reverse primer	5'-AACGCTTCACGAATTTGCGT-3'
Nogo-A	Forward primer	5'-GAT-GATTCGATTCCTGAAGTCC-3'
	Reverse primer	5'-AAAGGTCA-CACGCCAATTCTAA-3'
β-catenin	Forward primer	5'-CCAGCGTGGACAATGGCTAC-3'
	Reverse primer	5'-TGAGCTCGAGTCATTGCATAC-3'

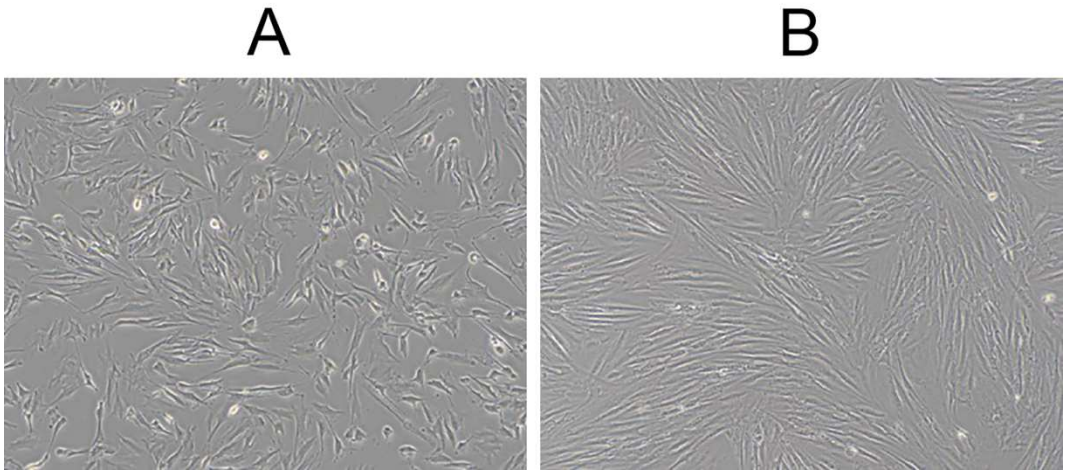

Fig. 1: Morphological identification of BMSC (×200).
(A) The morphology of BMSCs cultured for 5 days. (B). The morphology of BMSCs subcultured to the third passage.

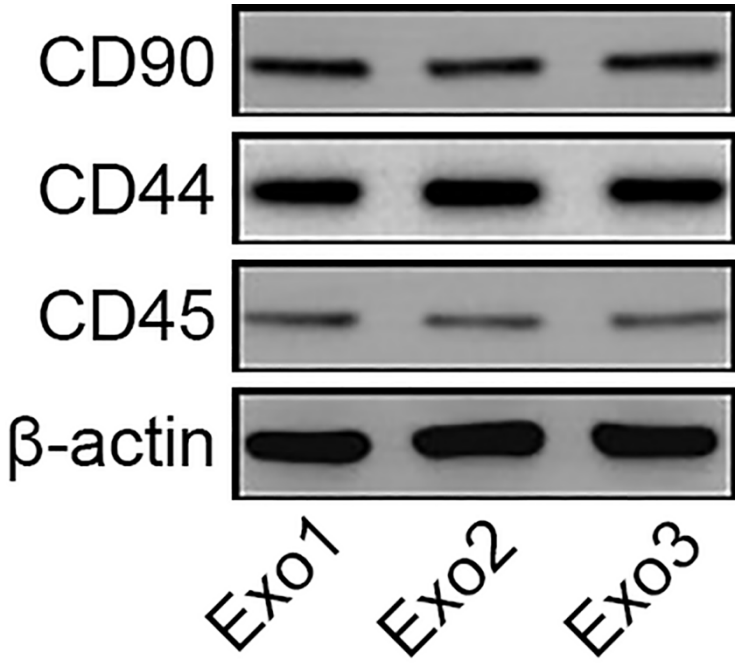

Fig. 2: Identification of BMSC surface markers.

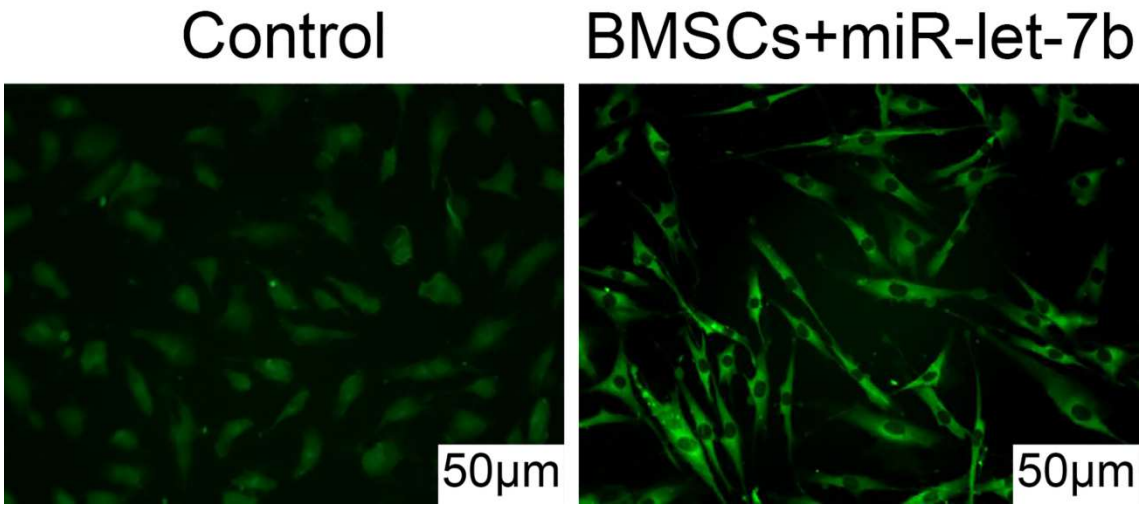

Fig. 3: Fluorescence comparison (×200).
A is the negative control group. B is the morphology of BMSCs in the BMSCs+miR-let-7b group cultured to the 5th day under a fluorescence microscope.

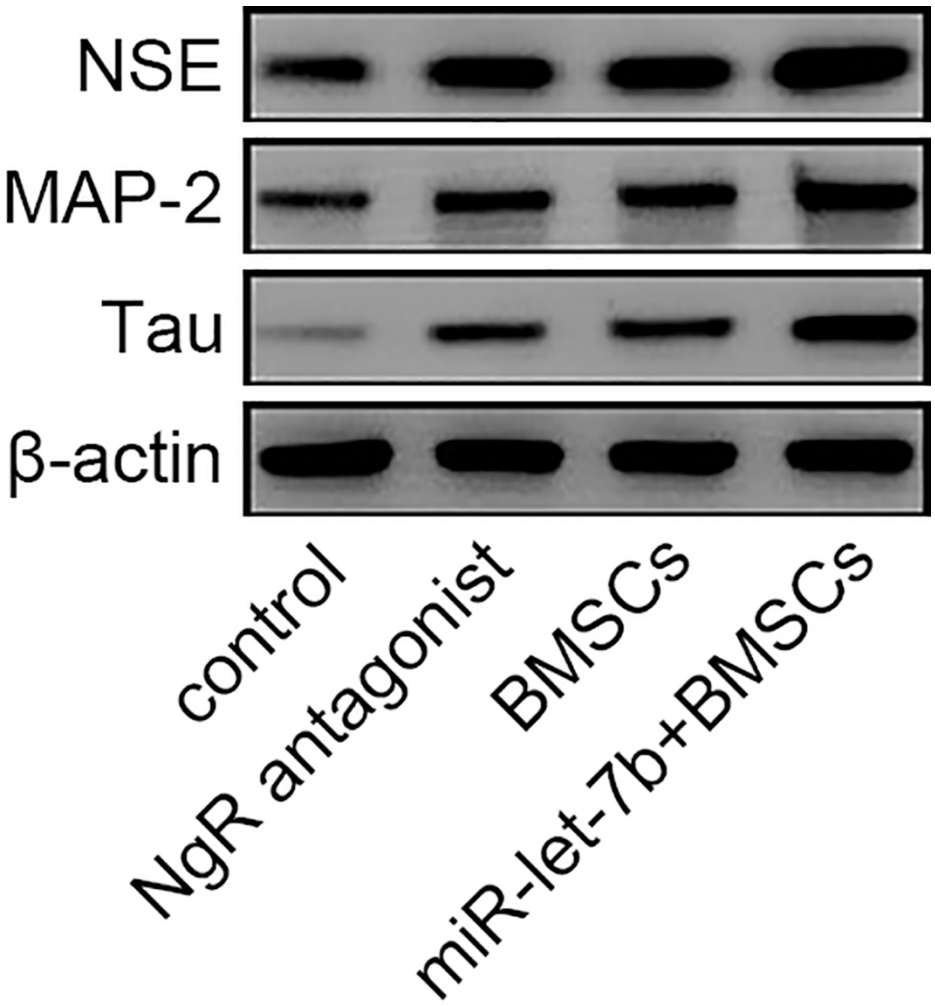

Fig. 4: Western blot comparison of the expression levels of NSE, MAP-2 and Tau in the four groups.

Table 3: Points of Rivlin incline test score at different time points after operation ($\bar{x} \pm s$).

Group	n	1d	3d	7d	14d	21d	28d
Control	10	27.71±0.81	31.08±1.07	48.94±1.22	52.54±1.44	57.14±1.24	61.74±1.76
BMSCs	10	28.22±0.74	30.91±1.13	51.28±1.36*	59.54±1.71*	61.14±1.39*	64.54±1.22*
miR-let-7b+BMSCs	10	27.84±0.94	32.08±1.05	54.24±1.13*#	64.54±1.08*#	63.14±1.14*#	67.74±1.64*#
NgR antagonist	10	29.19±0.81	31.08±1.12	51.07±1.34*	59.54±1.64*	61.14±1.41*	64.64±1.18*
F	-	6.541	7.269	6.445	7.698	6.745	7.329
P	-	0.000	0.000	0.000	0.000	0.000	0.000

Note: The sample size for each group was n = 10. Data are presented as mean ± standard deviation. One-way ANOVA was used for intergroup comparisons. *P < 0.05 indicates a statistically significant difference compared with the control group; #P < 0.05 indicates a statistically significant difference compared with the BMSCs group.

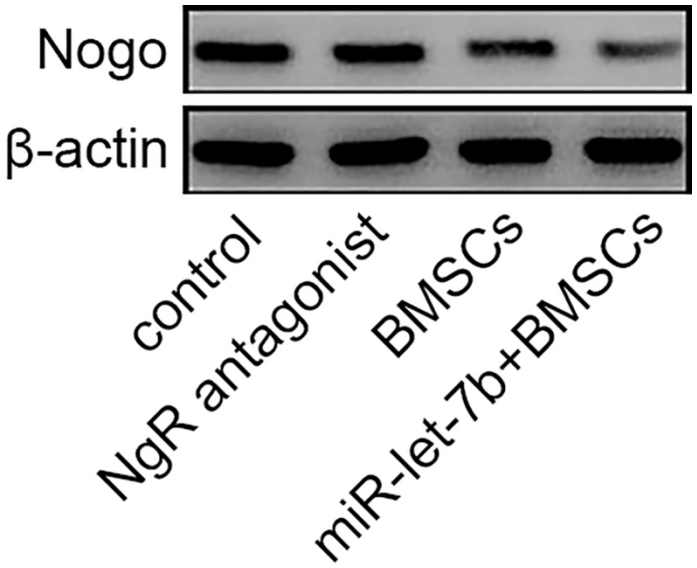

Fig. 5: Western blot analysis of Nogo protein levels in the four groups.

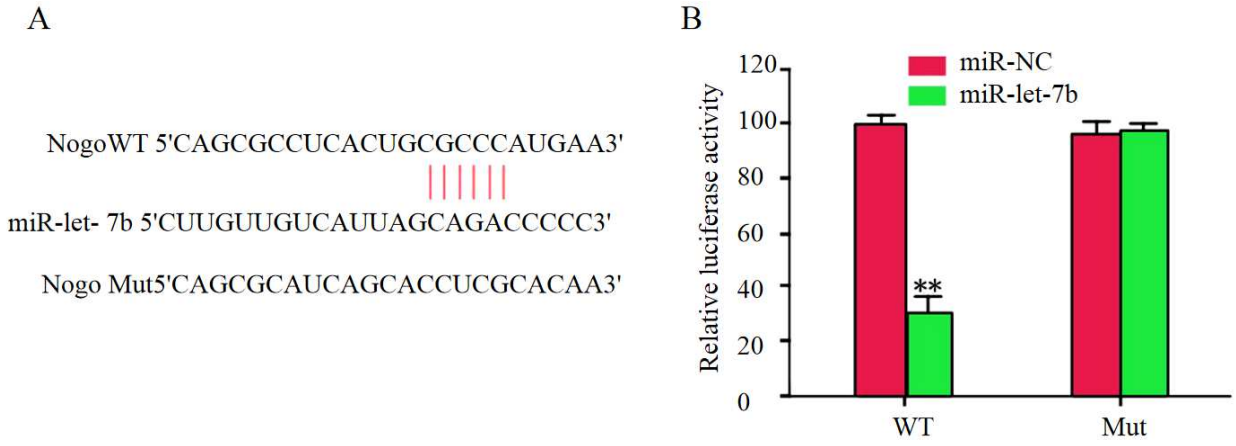

Fig. 6: Dual luciferase gene reporter assay.
(A) The binding site; (B) The luciferase activity. Note: **P<0.01

Immunoblotting showed that Nogo protein in NgR antagonist group had little change compared with controls; while Nogo protein in BMSCs group and miR-let-7b+BMSCs group was significantly lower than controls. In addition, according to the dual luciferase gene reporter assay, miR-let-7b targeted the Nogo, indicating that the miR-let-7b modified BMSCs promoted the repair of SCI via Nogo/NgR signal pathway.

In the experiment of transplantation of BMSCs into neural tissues (Fu *et al.*, 2025, Huang *et al.*, 2021a, Liu *et al.*, 2022, Matheakakis *et al.*, 2021, Yang *et al.*, 2024), it was found that they mainly differentiated into astrocytes, but rarely differentiated into neuron-like cells, indicating that the differentiation of neural stem cells and the environment factors after transplantation were closely related (Cargnoni *et al.*, 2021) and the microenvironment determines the differentiation outcome of neural stem cells and the characteristics of differentiated cell subtypes (Bazzoni *et al.*, 2020, Matheakakis *et al*., 2021). However, mesenchymal stem cell transplantation promotes functional recovery not by differentiation into damaged tissues, but by indirectly promoting axon regeneration by secreting nutritional factors and cytokines to improve the damaged microenvironment (Odegaard *et al.*, 2020, Xie *et al.*, 2020). However, autologous stem cell transplantation has several problems such as low survival rate, tumor risk and poor targeting (Ahuja *et al.*, 2020, Ernst *et al.*, 2021, Griffin and Bradke, 2020, Helissey *et al.*, 2020). Although good results have been achieved in animal experiments, there are still many problems in clinical treatment of SCI. Let-7b is one of the Let-7 family members and its role in the differentiation of BMSCs into neurons is currently unclear (Wang *et al*., 2020). miR-let-7b may play a role in the lateral differentiation of BMSCs into neurons (Sanooghi *et al.*, 2022). Increasing the level of Let-7b can promote the differentiation of MSCs into neurons (Liu *et al.*, 2025), indicating that the role of Let-7b in the process of lateral differentiation of BMSCs into neurons is not unique (Huang *et al*., 2021b), it may contribute to the control of certain signal pathways, thereby improving the induction efficiency. Studies on doctrine in the Nogo/NgR signaling pathway show that blocking the binding of Nogo-66 to NgR can effectively improve the repair of SCI. In this study, miR-let-7b modified BMSCs reduced the expression of Nogo-66, thereby increasing the repair effect of SCI. NgR is not only the receptor of Nogo-66, but also the receptor of MAG and OMGP. The silencing of NgR is likely to block the inhibitory effects of the three myelin-related nerve regeneration inhibitors at the same time.

CONCLUSION

In summary, miR-let-7b modified BMSCs may repair SCI by targeting the Nogo/NgR signaling pathway. However, this study may have some shortcomings. For example, some miRNAs other than miR-let-7b might exist in the exosomes of miR-let-7b modified BMSCs. The main components of BMSCs exosomes for the repair of SCI and their specific molecular mechanisms remain to be studied. This research is just basic experiments, how to transform it to clinical research requires a lot of further experiments. In addition, the role of endogenous BMSCs in the body also needs further analysis.

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Author's contributions
All authors participated in the completion of the manuscript. Beiren Yang was responsible for drafting the manuscript and Aihua Yu provided the overarching direction and guidance.

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Data availability statement
The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethical approval
This study was approved by the ethic committee of Huanggang Central Hospital of Yangtze University (Ethical Approval No. 2025030B). All animal experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and relevant animal ethical guidelines.

Conflict of interest
All authors declare that they have no competing interests. This study was not influenced by any commercial or financial relationships that could be construed as potential sources of bias, including but not limited to interests from funding bodies, companies, employment, consultancies, patent licensing, etc.

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