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# LncRNA MALAT1 Promotes Neuronal Apoptosis During Spinal Cord Injury Through miR-199a-5p/PRDM5 Axis

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## ABSTRACT

**AIM:** To determine the regulation of long non-coding RNA (lncRNA) MALAT1 on neuronal apoptosis during spinal cord injury (SCI) and to explore its possible mechanisms.

**MATERIAL and METHODS:** The motor ability of SCI rat models and apoptosis in spinal cord tissue were evaluated. Primary spinal cord neurons (SCNs) were isolated and treated with H2O2 before cell transfection. The apoptosis of SCNs and expression of PRDM5 and MALAT1 were also measured. The interactions among MALAT1, miR-199a-5p, and PRDM5 were detected.

**RESULTS:** The motor ability of SCI rats decreased significantly. The proportion of apoptotic neurons increased in damaged tissue and SCN, along with an increase in the expression of apoptosis-related proteins c-caspase-3/9, autophagy-related proteins (p62 and LC3 II/I ratio), and proinflammatory factors. Moreover, overexpression of MALAT1 and PRDM5 in damaged SCN resulted in an increased apoptosis rate of neurons, elevated expression of apoptosis-related proteins, and upregulated levels of inflammatory factors. However, miR-199a-5p overexpression/PRDM5 knockdown partially counteracted the effects of MALAT1 overexpression on H2O2-induced SCNs. In addition, MALAT1 negatively regulated miR-199a-5p, which targeted PRDM5.

**CONCLUSION:** LncRNA MALAT1 promotes neuronal apoptosis during SCI by regulating the miR-199a-5p/PRDM5 axis.

**KEYWORDS:** Spinal cord injury, Long non-coding RNA MALAT1, MicroRNA-199a-5p, PRDM5, Apoptosis, Inflammatory factors

## INTRODUCTION

Spinal cord injury (SCI) causes severe neuropathic pain in 80% of patients (9). Severe cases can lead to disability and have a devastating impact on individuals and families (16). Neuronal apoptosis is responsible for the initiation of SCI, and sustained inflammatory response after SCI is a risk factor for long-term neurological dysfunction; therefore, attenuating neuronal damage and controlling inflammation are considered promising options for enhancing spinal cord repair (5,11,24). Autophagy has been documented to facilitate the recovery of neuronal function and support cell regeneration after SCI (6). Although crucial roles of apoptosis, inflammation, and autophagy in SCI have been recognised, modulation of these processes in the injured spinal cord is still a big challenge for researchers.

Long non-coding RNAs (lncRNAs) are defined as non-coding RNA molecules between 200 and 100,000 nucleotides in length (27). lncRNAs play important regulatory roles in SCI progression (29). MALAT1 is reported to be upregulated in various malignant cancers, but it can also regulate the inflammation and apoptosis of neurons (20). Knockdown of MALAT1 alleviates the SCI-induced inflammatory response, which is related to miR-199b/IKKβ/NF-κB signalling pathway (30). A previous study has proved that MALAT1 overexpression exerted neuroprotective effects in spinal cord ischemic/reperfusion injury by inhibiting neurocyte apoptosis and alleviating rat motor deficits by downregulating miR-204 (17). However, the mechanism by which MALAT1 affects SCI is largely unknown.

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PRDM5 belongs to the family of zinc finger genes that contain PR-domain proteins (21). Many members of the PRDM family are thought to have tissue-specific differences that exhibit abnormal expression under pathological conditions (14). PRDM5 has been found to regulate apoptosis in multiple cancers (31). In addition, the negative regulation of PRDM5 by microRNAs (miRNAs) can alleviate SCI (13,23). Saker et al. reported that miRNAs expression may be altered in response to SCI, which in turn may contribute to the occurrence of secondary SCI (19). Our online software predicted the binding of miR-199a-5p to both PRDM5 and MALAT1. miR-199a-5p has been shown to improve motor function and neuron proliferation, as well as inhibit apoptosis in rat spinal cord after ischemia/reperfusion insult (1). In addition, miR-199a-5p carried by exosomes suppressed apoptosis and inflammation in neural cells (22). To the best of our knowledge, the crosstalk of the MALAT1/miR-199a-5p/PRDM5 axis has not been elucidated in SCI.

This study explained the regulation of MALAT1 on neuronal apoptosis and inflammation during SCI, which might provide a reasonable basis for MALAT1 use in the clinical treatment of SCI.

## MATERIAL AND METHODS

### SCI Rat Model

Male Sprague–Dawley (SD) rats (weight: 250 ± 20 g, Hunan SJA Laboratory Animal Co., Ltd. [Changsha, Hunan, China]) were used for SCI modelling according to Allen's weight drop method (32). After the back skin of rats was sterilised, rats were injected with ketamine (60 mg/kg) and xylazine (5 mg/kg). Next, the skin and subcutaneous tissue of rats were incised at the T10 vertebral body. After the muscles were dissected, the spinous process and vertebral plate were exposed. After the T10 vertebral plate was removed by a laminar rongeur, the impactor (10 g) was dropped from a height of 5 cm on the exposed spinal cord, causing a weight-drop force of 50 gcf. Spastic twitch in the tail and paralysis of the hind limbs are characteristics of SCI modelling. The incisions were then sutured. To prevent infection, rats were intramuscularly injected with penicillin (100,000 U/d). Rats in the sham group underwent laminar removal without causing any SCI.

After SCI modelling for 6, 24, 48, and 72 h, the locomotor behaviour of the hind limbs of rats was assessed using the Basso, Beattie, and Bresnahan (BBB) locomotor rating scale (2). The rats were given BBB scores by experts familiar with the scoring criteria, and each indicator was evaluated three times. Notably, rats were assisted to urinate to avoid experimental errors.

The rats were anaesthetised after the establishment of the SCI model for 72 h, and the thorax was opened to expose the heart. Next, the syringe needle was inserted into the left ventricle into the aorta, and the right auricle was rapidly opened. The left ventricle was filled with 100 mL of normal saline to row the blood. Until the discharged fluid became clear, the rats were rapidly perfused with 200 mL of 4% formaldehyde and then slowly perfused for 60 min.

After perfusion, the skin was incised from the original incision at the back. To fully expose the spinal cord, the muscles were separated and the spinal canal was opened. Then, the spinal cord was removed with the point of impact as the centre and a 0.5 cm extension at each end for Terminal deoxynucleotidyl transferase dUTP Nick-End Labelling (TUNEL) assay and immunofluorescence (IF), quantitative reverse transcription polymerase chain reaction (RT-qPCR), and western blot. All operations were conducted in accordance with the animal ethics committee of the local hospital.

### TUNEL-IF

After the spinal cord was made into paraffin sections and dewaxed, the sections were incubated for 8 min with 50 μL of 0.1% Triton X-100. Subsequently, citric acid-sodium citrate was added for antigen retrieval, and 50 μL of TUNEL reaction solution was incubated at 37°C for 60 min. Goat serum was added and incubated for 30 min. The primary antibody of NeuN (ab177487, 1:200, Abcam, Cambridge, UK) was added and incubated overnight at 4°C. After the excess primary antibody was washed off, the secondary antibody (ab150088, 1:200) was added and incubated for 1 h. 4′,6-Diamidino-2-phenylindole (DAPI) (20 mg/mL, D9542, Sigma-Aldrich, Germany) was added for counterstaining after the secondary antibody was washed off. Finally, the cells were washed with DAPI, and an anti-fluorescence quenching agent was added. The number of apoptotic cells in the field was determined and calculated.

### Isolation and Identification of Primary Spinal Cord Neurons (SCNs)

First, spinal cord tissue was isolated from embryonic day 16 rats under an anatomical microscope, and the spinal membrane and nerve roots were removed. Trypsin was used to digest the spinal cord tissue pieces at 37°C for 15 min. After the digestion was terminated by Dulbecco's Modified Eagle Medium + 10% foetal bovine serum, the cells were flapped to prepare a cell suspension. The filtered cell suspension was centrifuged at 1,000 rpm for 5 min, and the supernatant was discarded. Then, the cells were resuspended and inoculated into a six-well plate.

After the preparation of coverslips for cell culture, primary SCNs were fixed with 4% formaldehyde before permeabilisation in 0.1% Triton X-100 and treated with bovine serum albumin. Neurons were incubated with anti-neuron-specific enolase (NSE) antibody (ab53025, 1:300) overnight at 4°C. After the excess primary antibody was washed off, the secondary antibody (ab150088, 1:200) was added and incubated at 37°C for 60 min. The neurons were washed and stained with DAPI for 3 min. The coverslip was covered on the slide and mounted with an anti-fluorescence quenching agent for observation under an inverted fluorescence microscope. After identification, the primary SCNs were passaged to the 3$^{rd}$–5$^{th}$ generation for subsequent experiments.

### $H_2O_2$-Induced Cell Injury of SCNs and Cell Transfection

The third generation of SCNs was incubated into a 96-well plate ($10^5$ cells per well) for 24 h. Then, the original culture

medium was removed, and serum-free culture medium containing 150 μM $H_2O_2$ was added for 24 h to induce neuronal injury for subsequent experiments.

The pcDNA3.1-MALAT1, sh-MALAT1, miR-199a-5p mimic, miR-199a-5p inhibitor, pcDNA3.1-PRDM5, sh-PRDM5, and corresponding negative control vectors (pcDNA3.1-NC, mimic-NC, inhibitor-NC, and sh-NC) were purchased from Shanghai Genechem Co., Ltd. The vectors (100 ng/well) were incubated with Lipofectamine 2000 reagent (11668019, Invitrogen, California, USA), Opti-MEMI (31985062, Gibco, New York, USA), and 8 ng/mL polybrene (TR-1003, Sigma-Aldrich, St. Louis, MO, USA), and SCNs were cultured in the aforementioned medium for 48 h for subsequent detection. The normal SCNs were named blank group, and the damaged SCNs were named $H_2O_2$ group.

### Detection of Cell Apoptosis Using Flow Cytometry

When the SCNs reached 80% confluence, the cells were collected and counted. SCNs ($1 \times 10^6$) were rinsed with pre-cooled phosphate-buffered saline (PBS), suspended in 1× Annexin solution, and placed in clear tubes. Annexin V-FITC (5 μL, Becton Dickinson) was added to the tubes and incubated for 15 min at 4°C without light. Propidium iodide (PI) (10 μL) was used to stain the cells away from light at 4°C for 15 min. Then, cells were treated with 400 μL of 1× Binding Buffer. Flow cytometry (Guava easyCyte HT, Millipore) was used to detect green and red fluorescence (green for Annexin V-FITC, channel FL1, and red for PI, channel FL2). The apoptosis rate of SCNs in each group was statistically analysed using a scatter diagram.

### RT-qPCR

TRIzol (1 mL, Thermo Fisher Scientific, MA, USA) was used to dissolve spinal cord tissues and SCNs, from which total RNA was extracted. Reverse transcriptase M-MLV and random primers were used to obtain the cDNA. RT-PCR was performed using the Premix Ex Taq II kit (Takara, Dalian, China) and an ABI7500 quantitative PCR instrument (Applied Biosystems, Shanghai, China). Data analysis was performed using $2^{-\Delta\Delta Ct}$ method (3). The primer sequences for each gene are listed in Table I.

### Western Blot

The lysis of spinal cord tissues and SCNs was measured with the BCA Protein Detection kit (23227, Thermo Fisher Scientific) for protein concentration. Then, the protein was diluted with sample buffer five times and treated using electrophoresis with 12% separation gel for 90 min before the non-specific response was terminated using 5% (w/v) skimmed milk powder (PM) in PBS blocking solution for 1 h. Proteins were incubated at 4°C overnight with primary antibody of c-caspase-3 (ab2302, Abcam, Cambridge, UK, 1:500), c-caspase-9 (ab2324, 1:500), PRDM5 (ab79016, 1:200), LC3B (ab192890, 1:500), and p62 (AB91526, 1:500). After the antibody was washed off, the protein was incubated with a secondary antibody (ab6721, 1:500) for 1 h. Finally, the membrane was photographed using the BioSpectrum Imaging System (UVP, USA).

**Table I:** Primer Sequences of Genes Used in This Study

| Name of primer | Sequences (5'-3') |
|---|---|
| MALAT1-F | ATGCGAGTTGTTCTCCGTCT |
| MALAT1-R | TATCTGCGGTTTCCTCAAGC |
| miR-199a-5p-F | CCCAGTGTTCAGACTAC |
| miR-199a-5p-R | TGGTGTCGTGGAGTCG |
| PRDM5-F | GATCAAGTGGGTGCTCACAA |
| PRDM5-R | CATTGATAGGGACGCTCACC |
| U6-F | CTCGCTTCGGCAGCACA |
| U6-R | AACGCTTCACGAATTTGCGT |
| GAPDH-F | GTGGCTGGCTCAGAAAAAGG |
| GAPDH-R | GGGGAGATTCAGTGTGGTGG |

***Note: F:*** *Forward;* ***R:*** *Reverse.*

### Enzyme-Linked Immunosorbent Assay (ELISA)

Expression levels of tumour necrosis factor (TNF)-α (PT516, Beyotime, Shanghai, China) and interleukin (IL)-6 (PI328, Beyotime) in rat spinal cord and SCNs were examined using an ELISA kit. Antibodies were coated on an ELISA plate, and tissue homogenates or cell lysates were added to the wells to bind the antibodies. After incubation, ELISA plates were washed. Next, the biotinylated antibody and the catalase-labelled biotinylated protein were incubated with 3,3′,5,5′-tetramethylbenzidine in the wells. Finally, the absorbance of each well in the ELISA plate was measured at 450 nm and quantified using a standard curve.

### Luciferase Reporter Assay

The starBase database predicted the binding sites of miR-199a-5p with MALAT1/PRDM5, and the wild and mutant sequences of the binding sites (WT-MALAT1, MT-MALAT1, WT-PRDM5, and MT-PRDM5) were designed, synthesised, and inserted into intopGL3-Basic for cell transfection with miR-199a-3p mimic (30 nM) or mimic NC into HEK-293T cells. The ratio of firefly luciferase activity to Renilla luciferase activity was calculated.

### Statistical Analysis

Data were analysed using GraphPad Prism 7 and expressed as the mean ± standard deviation. The data between two groups were analysed using the *t*-test, and those among multiple groups using one-way analysis of variance with Dunnett's multiple comparisons as post hoc analysis. Statistical significance was set at $p<0.05$.

## RESULTS

### MALAT1 and PRDM5 were increasingly expressed in SCI rats

The BBB scores in the model group clearly decreased at 6, 24, 48, and 72 hours, and the motor ability of model rats clearly decreased compared to that of the sham group (Figure 1A), indicating that SCI models were established.

The expression levels of p62 and LC3 II/LC3 I ratio in the spinal cord of SCI rats were determined. The p62 expression and LC3 II/LC3 I ratio increased significantly in the model group (Figure 1B), suggesting that autophagy flux was blocked in the rat spinal cord. TUNEL staining results showed that apoptotic cell numbers, c-caspase-3/9 expression, and TNF-α and IL-6 expression increased remarkably in the model group (Figure 1C–E). Therefore, SCI blocked autophagy flux in rat spinal cord tissue and promoted apoptosis and inflammatory factor secretion. In contrast to the sham group, MALAT1 and PRDM5 expression in rat spinal cord tissue remarkably increased in the model group (Figure 1F, G).

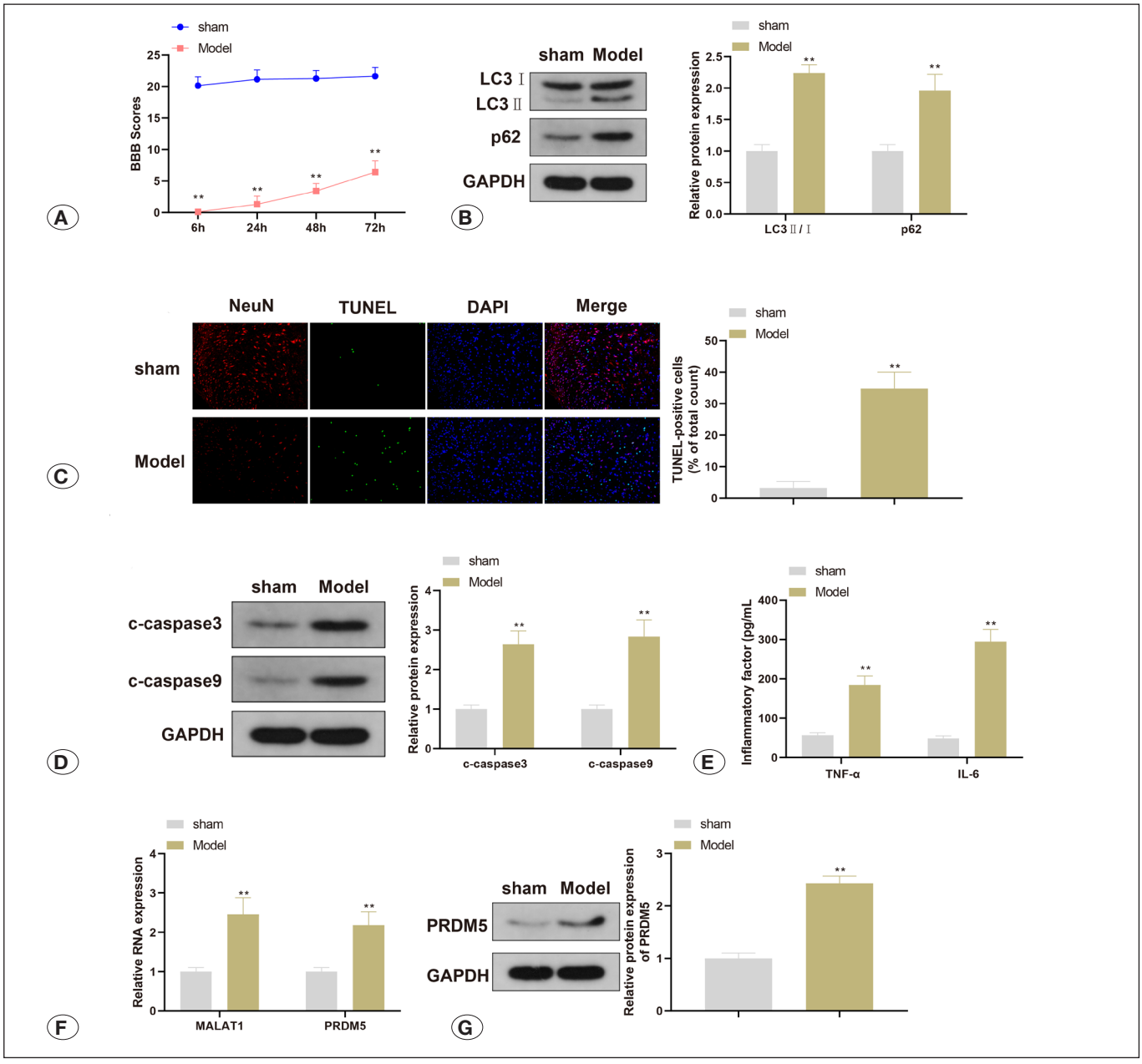


**Figure 1:** Expression patterns of MALAT1 and PRDM5 in spinal cord. Allen's weight-drop method was used for SCI modelling, and **A)** the motor ability was evaluated by Basso, Beattie, and Bresnahan score. **B)** Autophagy-related proteins p62, LC3 I, and LC3 II were detected using western blot. **C)** The neuronal apoptosis in spinal cord tissues was examined using TUNEL-IF. **D)** Apoptosis-related proteins c-caspase-3 and c-caspase-9 were assessed using western blot. **E)** The levels of inflammatory factors TNF-α and IL-6 were determined using ELISA. **F–G)** MALAT1 and PRDM5 were detected using RT-qPCR and western blot. Compared with the sham group, ¨p<0.01. SCI, spinal cord injury; TNF, tumour necrosis factor, IL, interleukin; ELISA, enzyme-linked immunosorbent assay; RT-qPCR, quantitative reverse transcription polymerase chain reaction; TUNEL-IF, Terminal deoxynucleotidyl transferase dUTP Nick-End Labelling (TUNEL) assay and immunofluorescence.

### Overexpression of MALAT1 Promoted Apoptosis of Injured SCNs

The primary SCNs were extracted from rat embryonic spinal cord tissues and identified using NSE. The expression of NSE in all cells indicated that the purity of the primary SCNs was 100% (Figure 2A).

Later, $H_2O_2$ was used to damage SCNs after SCNs were passaged to the third generation, and indicators related to autophagy and apoptosis were detected. The SCN apoptosis rate, the expression levels of autophagy-related proteins p62 and LC3 II/I ratio, apoptosis-related proteins c-caspase-3 and c-caspase-9, and the levels of inflammatory factors TNF-α and IL-6 increased in the $H_2O_2$ group compared to the blank group (Figure 2B–D). After $H_2O_2$ treatment, the apoptosis and inflammatory response were enhanced, while autophagy flow was blocked, indicating that $H_2O_2$ successfully damaged SCNs.

RT-qPCR showed that MALAT1 expression levels in the pcDNA3.1-MALAT1 group were higher than those in the control, while MALAT1 expression in the sh-MALAT1 group decreased (Figure 2E). Flow cytometry and western blot demonstrated that the apoptosis index of neurons, the expression of c-caspase-3 and c-caspase-9, and the concentration of inflammatory factors in the pcDNA3.1-MALAT1 group were higher than those in the pcDNA3.1-NC group (Figure 2F–H). Different expression was found in the sh-MALAT1 group compared to that in the sh-NC group (Figure 2F–H). Combined with the aforementioned results, MALAT1 overexpression promotes neuronal apoptosis and the release of inflammatory factors in injured SCNs.

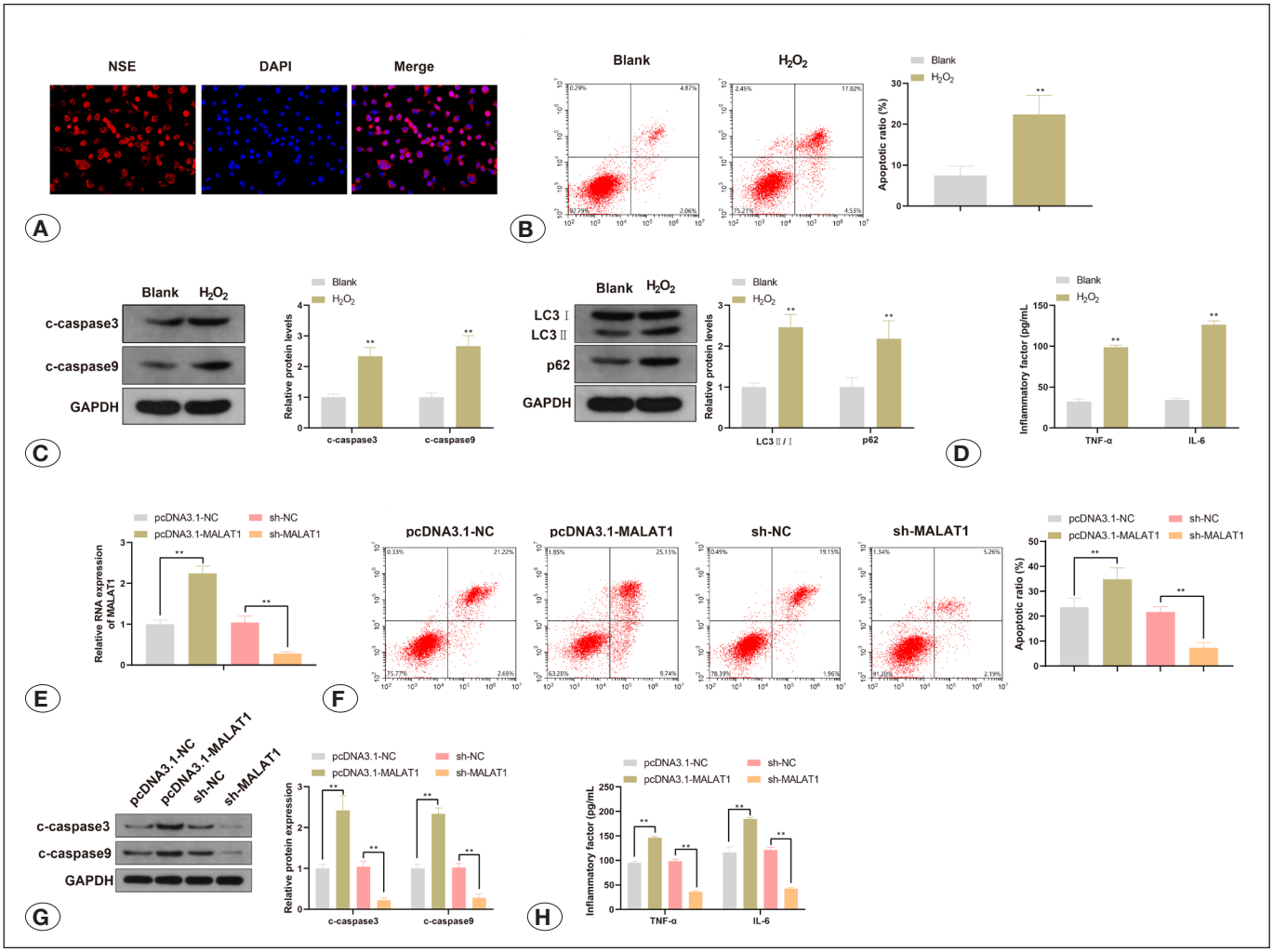


**Figure 2:** Overexpression of MALAT1 promotes apoptosis of injured SCNs. SCNs were transfected with pcDNA3.1-MALAT1, sh-MALAT1, and **(A)** immunofluorescence identified SCNs. **B)** Flow cytometry examined the apoptosis rate after injury. **C)** Autophagy-related and apoptosis-related proteins in spinal cord neurons were measured using western blot. **D)** Levels of inflammatory factors in cells were detected using ELISA. **E)** RT-qPCR evaluated the transfection efficiency. **F)** The apoptosis rate of SCNs after transfection was assessed using flow cytometry. **G)** Apoptosis-related proteins after transfection were measured using western blot. **H)** Related inflammatory factors were detected using ELISA. *p<0.05, **p<0.01. SCNs, spinal cord neurons; ELISA, enzyme-linked immunosorbent assay; RT-qPCR, quantitative reverse transcription polymerase chain reaction.

### PRDM5 overexpression enhanced apoptosis of injured SCNs

To verify whether PRDM5 enhanced cell apoptosis in injured SCNs, pcDNA3.1-PRDM5, sh-PRDM5, and negative controls were transfected into SCNs. The expression level of PRDM5 in the pcDNA3.1-PRDM5 group was elevated compared to that in the pcDNA3.1-NC group; in contrast, the expression of PRDM5 in the sh-PRDM5 group was clearly lower than that in the sh-NC group (Figure 3A, B), indicating successful transfection for subsequent experiments.

Next, the apoptosis rate of SCNs, apoptosis-related protein expression, and TNF-α and IL-6 expressions in the pcDNA3.1-PRDM5 group increased but decreased in the sh-PRDM5 group (Figure 3C–E). These results suggest that PRDM5 overexpression can promote apoptosis of injured SCNs and the release of inflammatory factors.

### MALAT1 and PRDM5 Competitively Bound miR-199a-5p

To study whether MALAT1 and PRDM5 regulated each other in damaged SCNs, the binding sites of MALAT1 and PRDM5 were predicted using starBase. There was no direct binding site between the two, but miR-199a-5p had binding sites with both MALAT1 and PRDM5; therefore, the mutation sites were designed (Figure 4A). After miR-199a-5p mimic and MT-MALAT1/MT-PRDM5 were transfected, the luciferase activity of HEK293T cells showed an insignificant difference compared to that of the mimic-NC group, while luciferase activity of cells transfected with the miR-199a-5p mimic and WT-MALAT1/WT-PRDM5 was clearly lower than that of the mimic-NC group (Figure 4B–C). These results indicate that both MALAT1 and PRDM5 can directly bind to miR-199a-5p.

RT-qPCR results revealed lower miR-199a-5p expression in the pcDNA3.1-MALAT1 group and higher miR-199a-5p

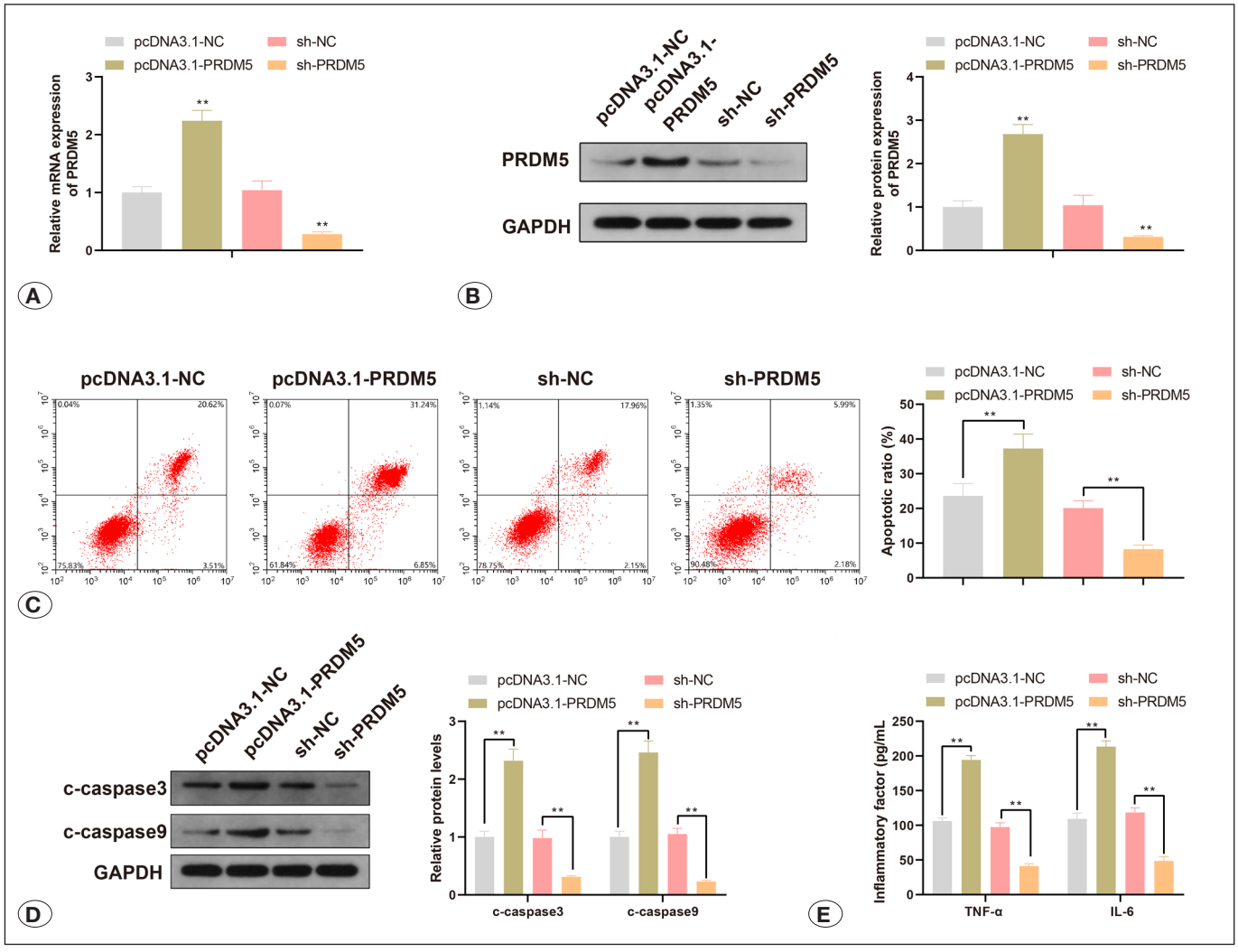


**Figure 3:** PRDM5 overexpression promotes apoptosis of injured SCNs. pcDNA3.1-PRDM5 and sh-PRDM5 were transfected into SCNs, and **(A, B)** transfection efficiency was detected. **C)** Apoptosis rate of SCNs was detected using flow cytometry. **D)** The expression levels of autophagy-related proteins and apoptosis-related proteins in SCNs were also detected. **E)** Levels of inflammatory factors TNF-α and IL-6 were measured using ELISA. *p<0.05, **p<0.01. **SCNs,** spinal cord neurons; **ELISA,** enzyme-linked immunosorbent assay; **TNF,** tumour necrosis factor; **IL,** interleukin.

expression in the sh-MALAT1 group (Figure 4D). RT-qPCR and western blot demonstrated that PRDM5 expression in the miR-199a-5p mimic group was clearly lower, and its expression clearly increased in the miR-199a-5p inhibitor group (Figure 4E, F). These results indicate that MALAT1 negatively regulates miR-199a-5p, and miR-199a-5p negatively regulates PRDM5 in SCNs.

### MALAT1 Promoted Apoptosis of Injured SCNs by miR-199a-5p/PRDM5 Axis

The $H_2O_2$-damaged SCNs were transfected or co-transfected with pcDNA3.1-NC, pcDNA3.1-MALAT1, pcDNA3.1-MALAT1 + miR-199a-5p mimic, and pcDNA3.1-MALAT1 + sh-PRDM5. In contrast to the PCDNA3.1-MALAT1 group, the apoptosis rate of neurons, expression of c-caspase-3 and c-caspase-9, and inflammatory factors in the PCDNA3.1-MALAT1 + miR-199a-5p mimic group and the pcDNA3.1-MALAT1 + sh-PRDM5 group decreased (Figure 5A–C). In contrast to the pcDNA3.1-NC group, the aforementioned indices showed significant differences in the pcDNA3.1-MALAT1 + miR-199a-5p mimic group or in the pcDNA3.1-MALAT1 + sh-PRDM5 group.

## DISCUSSION

This study aimed to determine the mechanism by which MALAT1 regulates neuronal apoptosis during SCI. MALAT1 and PRDM5 expression was upregulated and promoted neuronal apoptosis and inflammation. Overall, this study demonstrated that MALAT1 promoted apoptosis of injured SCNs by regulating the miR-199a-5p/PRDM5 axis.

SCI has an important influence on the physiological function, psychological state, and participation of patients (10). An increasing number of studies have shown that the regulation of inflammation and neuronal apoptosis plays an important role in SCI therapy (7,28). Researchers have found that unnatural expression of lncRNAs often occurs together with inflammatory responses and neuronal damage. For example, high expression of lncRNA RP11-543N12.1 is accompanied by more severe neuronal apoptosis and inflammation in AD (4). Furthermore, researchers have revealed that SNHG4 in spinal nerve ligation was upregulated, and SNHG4 knockdown attenuated the progression of neuropathic pain by suppressing neuroinflammation *in vivo* (15). According to previous studies, low expression of MALAT1 suppresses inflammation and apoptosis to mitigate hepatic ischaemia and reperfusion (I/R) injury (26). Similarly, our study showed that after induction of SCI, MALAT1 expression significantly increased, and downregulated MALAT1 decreased the expression of apoptosis-related proteins and the concentration of inflammatory factors. Thus, it can be concluded that MALAT1 promotes neuronal apoptosis and inflammation.

Additionally, we found that the expression of PRDM5 was greatly upregulated following SCI. Several studies have revealed that PRDM5 can exacerbate neuronal apoptosis. For instance, by observing the changes of PRDM5 expression and its co-localisation with active caspase-3 after SCI, PRDM5 inhibition by siRNA can reduce the apoptosis of primary neurons induced by glutamate (14). A previous study showed that downregulated PRDM5 expression can reduce active caspase-3 levels and inhibit neuronal apoptosis (13). Furthermore, PRDM5 significantly increased following the LPS-induced inflammatory response, accompanied by neuroinflammation and neuronal apoptosis (25). At the same time, our experiments demonstrated that overexpression of PRDM5 exacerbated neuronal apoptosis and inflammatory responses. In summary, the performance of MALAT1 and PRDM5 made it reasonable to speculate whether the functions of MALAT1 and PRDM5 were exerted through mutual regulation in damaged SCNs. The study also showed

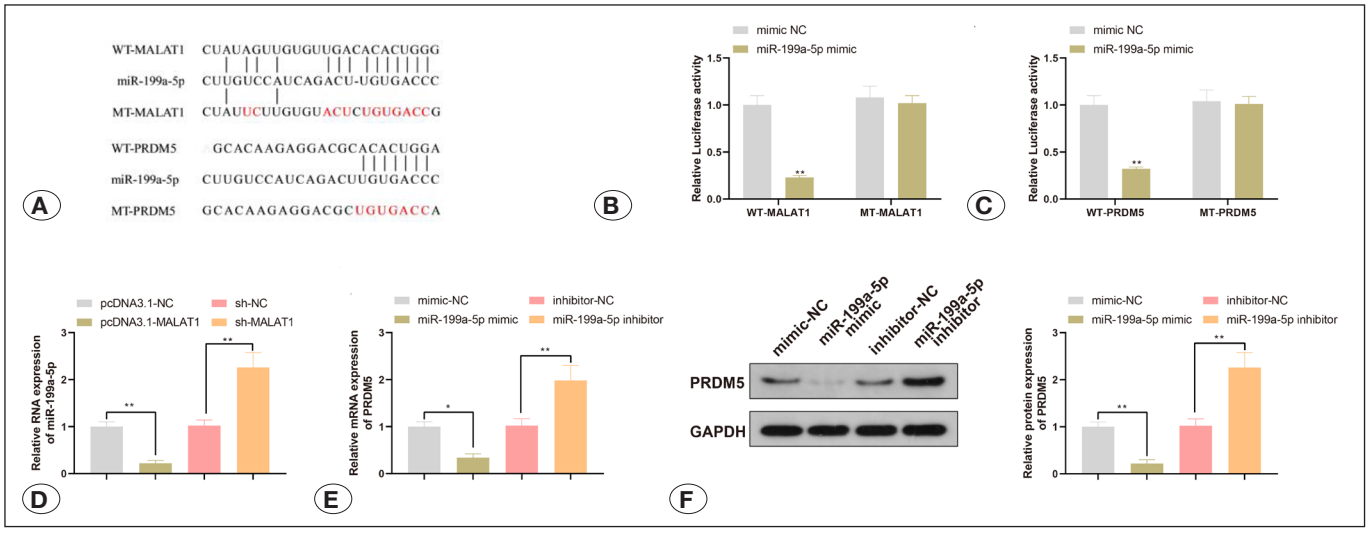


**Figure 4:** miR-199a-5p can bind both MALAT1 and PRDM5. **A)** Binding sites among MALAT1, miR-199a-5p, and PRDM5. **B, C)** The luciferase reporter vector was used to detect the interaction between MALAT1 and miR-199a-5p and between miR-199a-5p and PRDM5. **D)** RT-qPCR was used to assess miR-199a-5p expression. **E, F)** PRDM5 expression was measured using RT-qPCR and western blot. *p<0.05, **p<0.01. **RT-qPCR,** quantitative reverse transcription polymerase chain reaction.

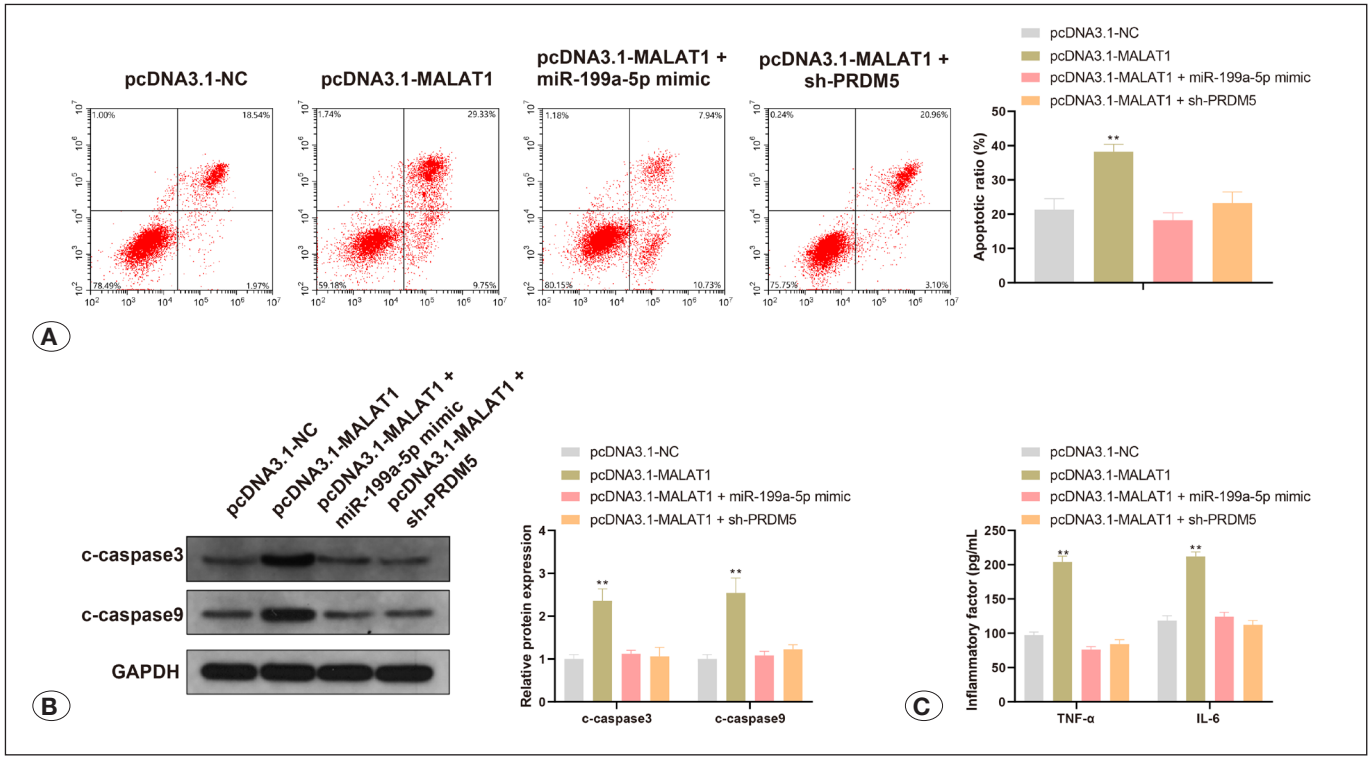


**Figure 5:** MALAT1 promotes SCN apoptosis through miR-199a-5p/PRDM5 axis. SCNs were transfected or co-transfected with pcDNA3.1-NC, pcDNA3.1-MALAT1, pcDNA3.1-MALAT1 + miR-199a-5p mimic, pcDNA3.1-MALAT1 + sh-PRDM5 mimic, and pcDNA3.1-MALAT1 + sh-PRDM5. **A)** Flow cytometry determined the apoptosis rate of SCNs. **B)** The expression levels of autophagy-related proteins and apoptosis-related proteins were examined using western blot. **C)** TNF-α and IL-6 were measured using ELISA. ¨p<0.01. SCN, spinal cord neuron; **TNF,** tumour necrosis factor; **IL,** interleukin; **ELISA,** enzyme-linked immunosorbent assay.

that there was no direct relationship between MALAT1 and PRDM5; however, in our study, miR-199a-5p could bind both MALAT1 and PRDM5. A previous study has found that targeted regulation of miR-182/miR-7a to PRDM5 could improve SCI (13). Fang et al. revealed that MALAT1 promoted apoptosis of hippocampal neurons by targeting miRNA-429 (8). Collectively, our data showed that lncRNA MALAT1 and PRDM5 competitively bound miR-199a-5p.

Although the aforementioned experiments have demonstrated the regulatory relationship between MALAT1 and miR-199a-5p and between miR-199a-5p and PRDM5, whether MALAT1 promotes SCN apoptosis through the miR-199a-5p/PRDM5 axis remains to be elucidated. Currently, studies have shown that miR-199a-5p knockdown can partially explain the suppressive effect of HUVEC-derived exosomes on cell apoptosis and inflammation, reducing the protective effect of RIPostC on the central nervous system (22). Ren et al. found that lncRNA TCTN2 promoted neuronal apoptosis by regulation of miR-216b/Beclin-1 axis (18). In addition, downregulated lncRNA ZNF667-AS1 can inhibit the inflammatory response and accelerate SCI recovery by inhibiting the JAKS-STAT signalling pathway (12). Unsurprisingly, miR-199a-5p up-regulation or PRDM5 knockdown significantly attenuated the increase in apoptosis rate and inflammatory responses induced by MALAT1 overexpression in injured SCNs. In all, MALAT1 accelerates neuronal apoptosis via the miR-199a-5p/PRDM5 axis.

In conclusion, lncRNA MALAT1 promotes neuronal apoptosis during SCI through the miR-199a-5p/PRDM5 axis. Our study showed that MALAT1 downregulation induced an increase in miR-199a-5p expression and restrained the expression of PRDM5, thereby attenuating SCI. However, it is not enough to study neuronal cells alone, and further studies are needed to determine whether MALAT1 and PRDM5 have the same effect in glial cells or other non-neuronal cells.

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## AUTHORSHIP CONTRIBUTION

The authors confirm contribution to the paper as follows:

**Study conception and design:** JY, XG

**Data collection:** XG, HC, SL

**Analysis and interpretation of results:** HC, SL, SZ, YG

**Draft manuscript preparation:** HC, SL

**Critical revision of the article:** XG , JY

**Other (study supervision, funding):** JY

All authors (XG, HC, SL, SZ, YG, JY) reviewed the results and approved the final version of the manuscript.

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