



Mechanisms of myocardial ischemic injury repair by bone marrow mesenchymal stem cell-derived miR-183-5P targeting FOXO1

Quancheng Lin¹, Jian Liu², Qingyang Chen¹, Yan Li³, Feiyue Dai¹, Xinyu Chen^{4*}

¹ Central Intensive Care Unit, The First Hospital of Hunan University of Chinese Medicine, Changsha, Hunan, China

² Medical Innovation Experiment Center, The First Hospital of Hunan University of Chinese Medicine, Changsha, Hunan, China

³ Vascular Tumor Interventional Department, The First Hospital of Hunan University of Chinese Medicine, Changsha, Hunan, China

⁴ Internal Medicine-Cardiovascular Department, The First Hospital of Hunan University of Chinese Medicine, Changsha, Hunan, China

ARTICLE INFO

Original paper

Article history:

Received: February 13, 2023

Accepted: April 28, 2023

Published: April 30, 2023

Keywords:

Bone marrow mesenchymal stem cells, acute myocardial infarction, miR-183-5P; FOXO1, myocardial ischemia

ABSTRACT

The homing rate of transplanted mesenchymal stem cells (BMSCs) after acute myocardial infarction (AMI) is generally low, with only 0%-6% of the number of transplanted stem cells distributed to the heart; therefore, this study will investigate the therapeutic effects and mechanisms of miR-183-5p-modified BMSCs cells on myocardial ischemia and hypoxia caused by AMI. In this experiment, After first establishing the BMSCs ischemic-hypoxic injury model, the rats were divided into healthy group, model group, BMSCs group and BMSCs+ miR-183-5P group, where the healthy group was taken to normal culture, the model group caused myocardial ischemic-hypoxic damage, the BMSCs group underwent BMSCs stem cell transplantation on the basis of the model group, and the BMSCs+ miR-183-5P group was group was cultured with BMSCs-derived miR-183-5P on the basis of the model group. Myocardial tissue sections of rats in each group were taken for HE staining and histopathological changes were observed by light microscopy. The proliferation, apoptosis and migration ability of the cells were detected by CCK-8 method, flow cytometry and Transwell transfer method. The target gene of miR-183-5P was predicted using bioinformatics software, and the binding of miR-183-5P to FOXO1 was investigated. The expression of FOXO1 was analysed using qRT-PCR and protein blotting techniques. The qRT-PCR results showed that the expression of miR-183-5P was higher in BMSCs of the BMSCs group and BMSCs+ miR-183-5P group compared with the model group, and the expression was highest in the BMSCs+ miR-183-5P group ($P<0.05$). The value-added ability and the migration capacity of BMSCs in the BMSCs group and BMSCs+ miR-183-5P group were increased compared with the model group, and the BMSCs+ miR-183-5P group BMSCs had the highest proliferation capacity and the migration capacity ($P<0.05$). In contrast, the apoptotic capacity of BMSCs was significantly reduced in the BMSCs group and BMSCs+ miR-183-5P group compared with the model group, and the apoptotic capacity of BMSCs was lowest in the BMSCs+ miR-183-5P group ($P<0.05$). The bioinformatics software RegRNA 2.0 was used to predict that the specific target gene that may be regulated by miR-183-5P is FOXO1 and confirmed that miR-183-5P does indeed have a targeting relationship with the FOXO1 pathway. After upregulation of miR-183-5P expression, the expression of FOXO1 mRNA was higher in BMSCs of the BMSCs group and BMSCs+ miR-183-5P group compared with the model group, and the expression was highest in the BMSCs+ miR-183-5P group ($P<0.05$). The Western blotting showed that the expression of FOXO1 mRNA was higher in BMSCs of the BMSCs group and BMSCs+ miR-183-5P group compared with the model group, especially the expression was highest in the BMSCs+ miR-183-5P group ($P<0.05$). In conclusion, BMSCs-derived miR-183-5P can target and regulate FOXO1 to increase the proliferation and migration of BMSCs and reduce their apoptosis, and can also reduce myocardial tissue edema and inflammatory response by increasing the expression of FOXO1 mRNA, which can increase the survival rate of BMSCs and provide a clinical basis for BMSCs transplantation.

Doi: <http://dx.doi.org/10.14715/cmb/2023.69.4.14>

Copyright: © 2023 by the C.M.B. Association. All rights reserved.

Introduction

Acute myocardial infarction (AMI) is one of the most dangerous diseases in cardiovascular disease (CVD) due to its sudden onset, high disability rate and high lethality. Prolonged ischemia occurs after myocardial infarction, resulting in irreversible damage to cardiomyocytes (i.e., coagulative necrosis), as well as edema and neutrophil infiltration in the intercellular space (1-2). The current common treatment is to rapidly restore myocardial ground

blood flow, but myocardial remodeling may still occur in up to 60% of AMI patients (3), and currently, conventional treatments, including drugs, interventions, or surgical procedures, are unable to rescue the already necrotic myocardial cells, which will eventually progress to changes in the size, shape, structure, and function of the entire heart (4). Stem cells are a type of cell with the ability to self-renew and differentiate, and the rapid emergence and development of the stem cell field in the last decade or so have

* Corresponding author. Email: chenxinyucxy@126.com

brought new hope for the treatment of AMI (5). Mesenchymal stem cells (BMSCs) are totipotent adult stem cells with multi-directional differentiation potential, immune regulation, hematopoietic support and self-replication, and can be transformed into adipose, muscle, endothelial, liver, cardiac muscle and other tissue cells under certain conditions. They have the advantages of being less likely to cause immune rejection, having low immunogenicity, and not involving ethics and morality (6-7). Therefore, BMSCs are the star cells for stem cell transplantation therapy after myocardial ischemic-hypoxic injury to improve recent cardiac function and inhibit ventricular remodeling in the ischemic heart (8). However, related studies have found that the homing rate of BMSCs transplantation after AMI is generally low, with only 0%-6% of the number of transplanted stem cells actually distributed to the heart; therefore, improving the homing rate is the key to exploiting the role of BMSCs transplantation in AMI therapy (9).

MicroRNA (miRNA) regulates gene expression through the targeted regulation of messenger RNA (mRNA), thereby regulating the biological behavior of cells (10). An increasing number of studies claim (11-12) that a variety of miRNAs can be involved in cellular autophagy, development, etc. The FOXO1 transcription factor can participate in organism growth and development, metabolism, etc. by regulating various physiological processes such as cellular oxidative stress, proliferation, and apoptosis, and is a member of the FOXO subfamily of the FOX family (13). FOXO1 genes are widely expressed in adults including the heart, brain, lung, liver, kidney, skeletal muscle, pancreas and other tissues and organs, and FOXO1 is expressed mainly in muscle tissue during the embryonic period and in adipose tissue in adults (14-15). Studies have shown that FOXO1 currently plays an important role as an oncogenic molecule in the development of breast cancer. miR-183-5p.1 can target and regulate FOXO1 to perform the function of promoting cell proliferation or apoptosis. A study by Ling-Li et al (16) showed that overexpression of RP4 could bind to miR-183-5p.1. endogenously and competitively to upregulate FOXO1 expression, resulting in the inhibition of breast cancer cell proliferation. It was shown that regulation of miR-183-5p gene expression targeting FOXO1 could regulate the progression of breast cancer, but whether BMSCs-derived miR-183-5p could regulate FOXO1 to repair myocardial damage caused by myocardial ischemia and hypoxia is not yet known. It has been found that the survival rate of BMSCs after transplantation is extremely low, and the vast majority of BMSCs can be apoptotic for various reasons within 1 week after transplantation (17). Therefore, there is a need to find effective interventions to effectively increase the number of BMSCs homing to the damaged parts of the myocardium, improve the survival rate, and enhance the therapeutic value of BMSCs transplantation in AMI patients (18).

Therefore, this study will investigate the therapeutic effects of miR-183-5p-modified BMSCs cells on myocardial ischemia and hypoxia caused by AMI and its mechanism of action for further investigation.

Materials and Methods

Experimental materials

Lipofectamine 3000, cell lysate, protease inhibitor, fe-

tal bovine serum, RT-PCR reverse transcription kit, TRIzol reagent and fluorescence quantification kit (Thermo Fisher, USA); Transwell chamber (Unicon Biotechnology, Beijing); CCK-8 kit, cDNA synthesis kit (Tiangen Biochemistry, Beijing); EdU kit (Shanghai Enzyme Linkage Biology); dual-luciferase reporter gene detection kit (Beijing Yuanpinghao Biotechnology); fluorescence quantitative PCR instrument (Roche, USA); primers miR-183-5P, FOXO1, GAPDH (Biometra, Germany); DMEM/F12 medium, RPMI-1640 medium (Shanghai Zhongqiao Xinzhou Biotechnology); anti-miR-183-5P, FOXO1 antibodies (Abeam, USA); Negative control (NC) mimics (Shanghai Jima Pharmaceuticals); GAPDH (Shanghai Weihao Biotechnology); EL340 ELISA (BioTek Winooski, USA).

Experimental methods

Sample collection

Bone marrow cells were collected from SD bone marrow donor rats, and tibia bone was separated after dislocation and execution, and bone marrow cells were extracted, and stem cells were cultured and passaged, and the 3rd generation cells were taken for establishing the model of ischemic and hypoxic injury of BMSCs, after taking healthy male clean grade SD rats weighing 200±45g, and they were divided into the healthy group, model group, BMSCs group, BMSCs+ miR-183-5P group, where the healthy group was taken in normal culture, the model group caused myocardial ischemic and hypoxic damage, the BMSCs group underwent BMCs stem cell transplantation on the basis of the model group, and the BMSCs+ miR-183-5P group was cultured with BMSCs-derived miR-183-5P on the basis of the model group.

Establishment of a hypoxic injury model

The 3rd generation cells were collected and the cell concentration was adjusted to 1×10^4 /mL to prepare for transplantation. 24 h before implantation, the BMSCs were placed in a hypoxic constant temperature cell culture incubator and nitrogen gas was introduced into them to reduce their oxygen volume fraction to 0.03, CO₂ volume fraction to 0.05, and saturation temperature to 37°C. After 24 h, the cells were moved to a normal oxygen-content incubator for 2 h. The 3rd generation BMSCs were co-incubated with Hoechst 33342 at a final mass concentration of 10 µg/mL for 30 min for Hoechst labeling.

Histopathological observation of myocardium

The myocardial tissues of SD rats in each group were taken, fixed, dehydrated, paraffin-embedded, sectioned and processed, then HE stained, and the myocardial histopathological changes were observed under the light microscope.

Quantitative real-time fluorescence polymerase chain reaction (qRT-PCR)

RNA was extracted from BMSCs by the TRIzol method and reverse transcribed to cDNA using TaqMan™ advanced miRNA cDNA synthesis tool. RT-qPCR was performed by SYBR fluorescent PCR technique. GAPDH is an intrinsic control between FOXO1 and miRNA. The results of qRT-PCR were analyzed by Step One Plus real-time PCR system. Relative expression levels were estimated using the $2^{-\Delta\Delta Ct}$ method. The primers and primer

Table 1. Real-time PCR primers as well as primer sequences.

	Primer	Primer sequences
FOXO1	Forward	5'-GGATGTCCATTCTATGGTGTACC-3'
	Reverse	5'-TTTCGGGATTGCTTATCTCAGAC-3'
miR-183-5P	Forward	5'-CGCGTATGGCACTGGTAGAA-3'
	Reverse	5'-AGTGCAGGGTCCGAGGTATT-3'
GAPDH	Forward	5'-ATTCCATGGCACCGTCAAGGCTGA-3'
	Reverse	5'-TTCTCCATGGTGGTGAAGACGCCA-3'

order are listed in Table 1.

Bioinformatics software prediction and dual luciferase reporter gene detection of targeting relationship between miR-183-5P and target genes

The bioinformatics analysis software of RegRNA2.0 was applied to predict the regulation of specific targets by miR-183-5P. BMSCs were cotransfected with FOXO1-WT plasmid, FOXO1-MUT plasmid and negative control sequences or miR-183-5P mimics, and compared by the method of dual luciferase reporter gene kit.

Cell Counting Kit 8 (CCK-8) assay

Transfected cells were inoculated in 60-well plates at 1×10^3 (200 μ l), and after 4 h, the cell culture medium solution was aspirated and DMED medium containing 10% CCK-8 was added for incubation for 1 h. The growth of cells on days 1, 2, 3, 4 and 5 was measured with the CCK-8 kit, and the growth curves were determined. It was strictly implemented according to the operational standards of CCK-8.

Flow cytometry analysis

The cells of each group were digested, made into 1×10^6 ml of cell suspension, centrifuged, washed with PBS, re-suspended with 150 μ l of buffer, added into 10 μ l Annexin V-FITC and 5 μ l PI staining solution, incubated for 15 min with protection from light and detected by flow cytometry.

Transwell migration assay

The cells of each group were made into 1×10^5 cells/ml, inoculated into the upper chamber of Transwell, and the lower chamber was put into serum-free culture medium containing SDF-1 α , incubated for 12 h, removed and washed, fixed with paraformaldehyde, stained with crystal violet, aspirated with floating color, observed and detected the absorbance at 565 nm.

The cell migration after 0, 24 and 48 h incubation was observed using a conventional microscope, and image analysis was performed with the corresponding calculations.

Western blot assay

The above BMSCs were obtained and lysed to obtain supernatant, BCA method for protein concentration determination, protein sample: loading buffer = 1:4, boiling for preparation of protein samples, gel preparation and loading, electrophoresis and membrane transfer, protein closure, primary antibody (miR-183-5P, FOXO1, GAPDH, 1:1000), secondary antibody incubation (mouse anti-rabbit 1:200), and TBST wash, development. The experiment was repeated 5 times.

Statistical analysis

Statistical analysis was performed using SPSS21.0 and GraphPad Prism, and the measurement data were expressed as ($\bar{x} \pm SD$), conforming to the normal distribution and satisfying chi-square; the measurement data were expressed as (%), and all data in this study were compared between groups using the F-test and the LSD method. Results were considered significantly different at $P < 0.05$.

Results

Histopathological observation of myocardium in each group of rats

The HE staining results showed that in the healthy group (Figure 1A): myocardial fibers were neatly arranged, the cytoplasm was eosin, and there were no edema and inflammatory cells in the interstitium; in the model group (Figure 1B): myocardial fibroblasts were proliferating, myocardial fibers were edematous, and some fibers were replaced by loose connective tissue, and a large number of inflammatory cells were infiltrated; in the BMSCs group (Figure 1C): myocardial tissue was partially edematous and inflammatory, and myocardial cells were The BMSCs group (Figure 1C): myocardial tissue had some edema and inflammation, and myocardial cells were arranged neatly compared with the model group; BMSCs+miR-183-5P group (Figure 1D): myocardial cells were arranged more neatly, and tissue edema and inflammation were less severe.

Increasing the expression of miR-183-5P in BMSCs by Bone marrow mesenchymal stem cell-derived miR-183-5P

The qRT-PCR results showed that the expression of miR-183-5P was significantly lower in BMSCs of the

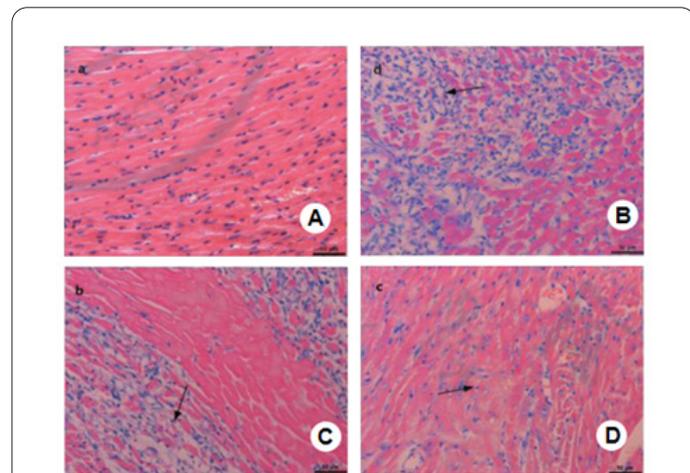


Figure 1. Staining of myocardial tissue in each group of mice ($\times 200$).

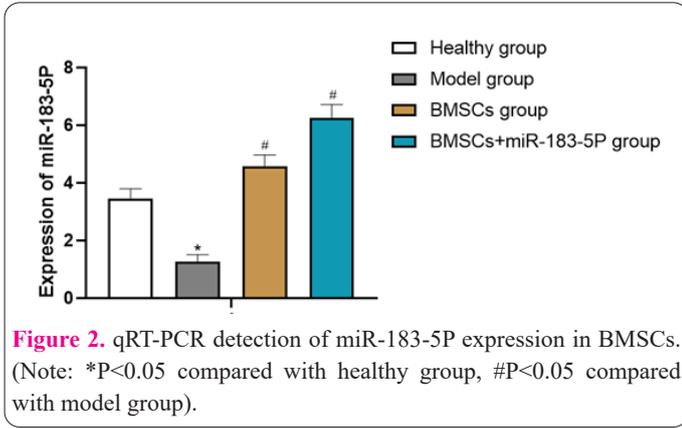


Figure 2. qRT-PCR detection of miR-183-5P expression in BMSCs. (Note: *P<0.05 compared with healthy group, #P<0.05 compared with model group).

model group compared with the healthy group, and the expression of miR-183-5P was significantly higher in BMSCs of the BMSCs group and BMSCs+ miR-183-5P group compared with the model group, and the expression was highest in the BMSCs+ miR-183-5P group (P< 0.05). See Figure 2.

Increasing the proliferation and migration ability of BMSCs and decreasing the apoptosis of BMSCs by bone marrow mesenchymal stem cell-derived miR-183-5P

There was no significant difference in the proliferation ability of several groups of cells on days 1-2 of transfection; from day 3, the proliferation ability of BMSCs in the model group was significantly reduced compared with the healthy group (P<0.05), and the value-added ability of BMSCs in the BMSCs group and BMSCs+ miR-183-5P group was significantly increased compared with the model group, and BMSCs in the BMSCs+ miR-183-5P group had the highest proliferative capacity (P<0.05). See Figure 3.

After transfection, the migration ability of BMSCs in the model group was significantly decreased compared with the healthy group (P<0.05), and the migration ability of BMSCs in the BMSCs group and BMSCs+ miR-183-5P group was significantly increased compared with the model group, and the migration ability of BMSCs in the BMSCs+ miR-183-5P group was the highest (P<0.05). See Figure 4.

The apoptotic capacity of BMSCs was significantly increased in the model group compared with the healthy group (P<0.05), and the apoptotic capacity of BMSCs was significantly decreased in the BMSCs group and BMSCs+ miR-183-5P group compared with the model group, and the apoptotic capacity of BMSCs was the lowest in the BMSCs+ miR-183-5P group (P<0.05). See Figure 5.

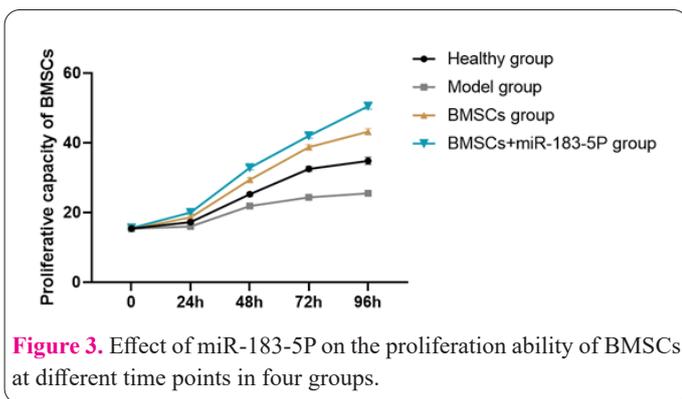


Figure 3. Effect of miR-183-5P on the proliferation ability of BMSCs at different time points in four groups.

Prediction of miR-183-5P target genes using bioinformatics software

The specific target gene that may be regulated by miR-183-5P was predicted to be FOXO1 using the bioinformatics software RegRNA 2. 0. The binding site of miR-183-5P to FOXO1 is shown in Figure 6.

Base sequence complementary interaction between miR-183-5P and FOXO1 gene mRNA

The relative luciferase activity of the miR-183-5P+ FOXO1-WT cotransfected group was significantly lower than that of the miR-183-5P+ FOXO1-MUT cotransfected group (P<0.05). See Figure 7.

miR-183-5P can target and promote the expression of FOXO1 mRNA

After upregulating miR-183-5P expression, the expression of FOXO1 mRNA was significantly higher in BMSCs of the BMSCs group and BMSCs+ miR-183-5P group compared with the model group, and the expression

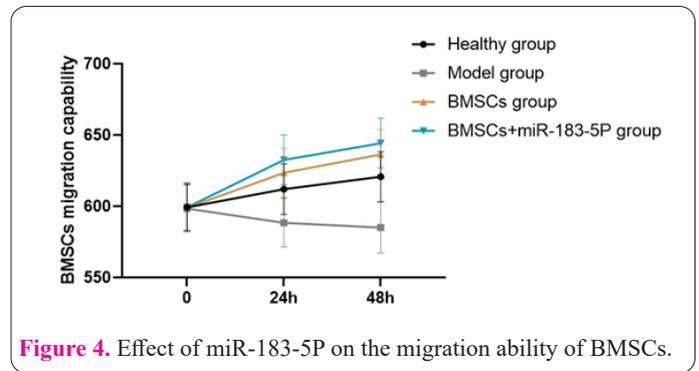


Figure 4. Effect of miR-183-5P on the migration ability of BMSCs.

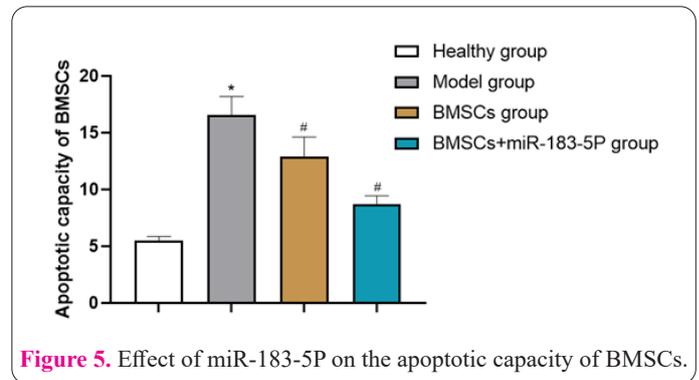


Figure 5. Effect of miR-183-5P on the apoptotic capacity of BMSCs.

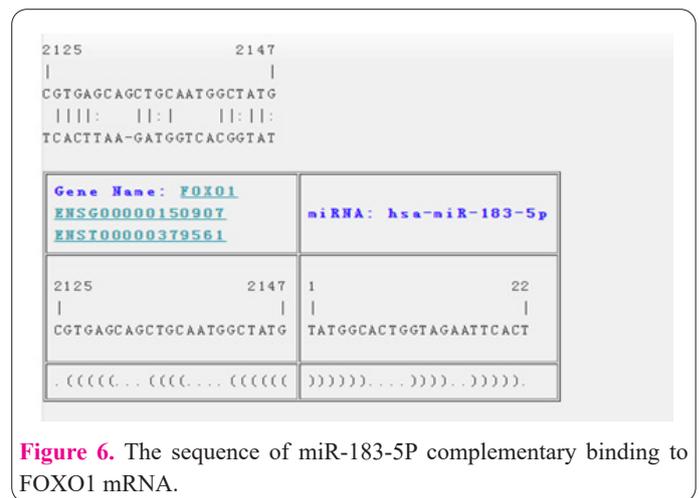


Figure 6. The sequence of miR-183-5P complementary binding to FOXO1 mRNA.

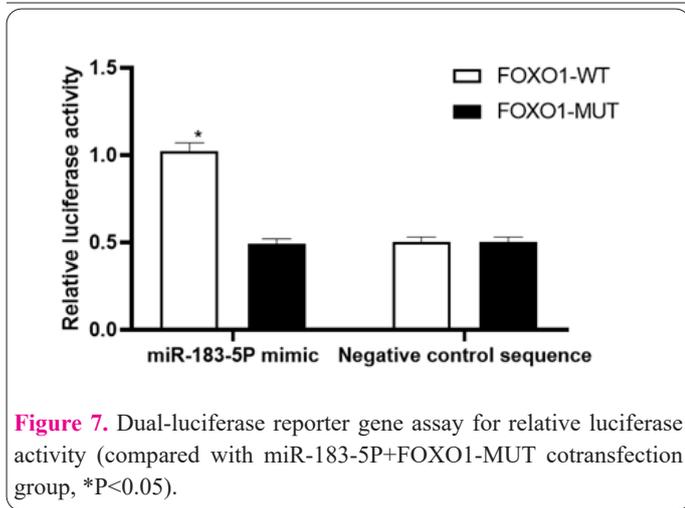


Figure 7. Dual-luciferase reporter gene assay for relative luciferase activity (compared with miR-183-5P+FOXO1-MUT cotransfection group, *P<0.05).

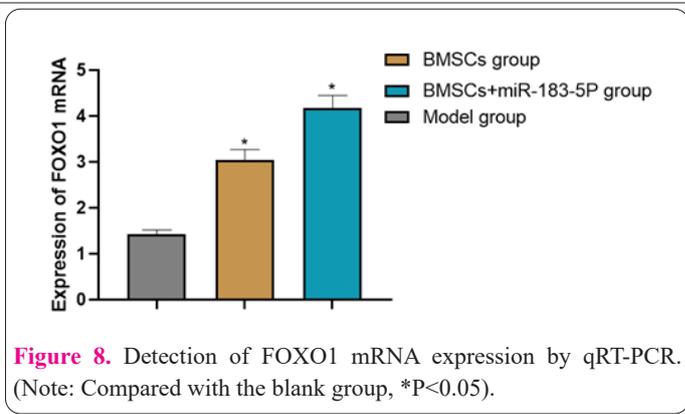


Figure 8. Detection of FOXO1 mRNA expression by qRT-PCR. (Note: Compared with the blank group, *P<0.05).

was highest in BMSCs+ miR-183-5P group (P<0.05). See Figure 8.

Promotion of the expression of FOXO1 mRNA and BMSCs by bone marrow mesenchymal stem cell-derived miR-183-5P

The results of the Western blotting assay showed that the expression of FOXO1 mRNA was lower in BMSCs of the model group compared with the healthy group (P<0.05), and the expression of FOXO1 mRNA was significantly higher in BMSCs of the BMSCs group and BMSCs+ miR-183-5P group compared with the model group, especially the expression of FOXO1 mRNA in BMSCs+ miR-183-5P group showed the highest expression (P<0.05). See Figure 9.

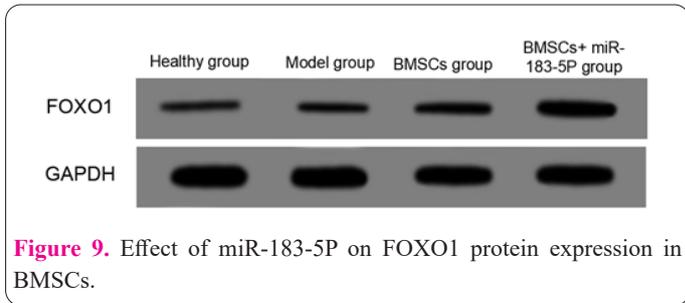


Figure 9. Effect of miR-183-5P on FOXO1 protein expression in BMSCs.

Discussion

Currently, in the study of BMSCs transplantation for ischemic heart disease, it has been found that BMSCs transplanted into ischemic myocardium can differentiate into cardiomyocyte-like cells with myocardial fiber contractility, increasing myocardial compliance and elasticity, significantly reducing infarct size and improving left ventricular function. It also effectively promotes the repair of infarcted myocardium and inhibits ventricular remodeling through paracrine mechanisms (19-20). Studies have confirmed that transplantation of MSCs after myocardial infarction can promote the anti-apoptotic effect of cardiomyocytes under hypoxic conditions through paracrine effects and can significantly reduce myocyte apoptosis (21). BMSCs can differentiate into vascular endothelial cells or increase the capillary formation and improve collateral circulation by secreting vascular endothelial growth factor, thus improving myocardial perfusion, vascular regeneration facilitates the establishment of collateral circulation and long-term survival of the transplanted cells, limiting the expansion of the infarcted area (22). As a special microenvironment, the stem cell "nest" is the basis for the existence of stem cells, and it regulates the behavior of stem cells through different signaling pathways so that the growth, renewal and differentiation of stem cells are in a stable balance under physiological conditions (23). Through literature analysis and previous studies, in stem cell transplantation for the treatment of ischemic heart disease, it can be found that although BMSCs transplantation has a good research prospect in the treatment of AMI, the

low level of homing and differentiation limits the potential functions of stem cells (24). Therefore, this study investigated the therapeutic effect of BMSCs cells modified by miR-183-5p on myocardial ischemia and hypoxia caused by AMI and its mechanism of action, aiming to provide a new clinical basis for BMSCs transplantation.

The results of the present study showed that qRT-PCR results showed that the expression of miR-183-5P in BMSCs in the model group was significantly lower compared with the healthy group, and the expression of miR-183-5P in BMSCs in the BMSCs group and BMSCs+ miR-183-5P group was significantly higher compared with the model group, and the BMSCs+ miR-183-5P group had the highest expression (P<0.05). Compared with the healthy group, the proliferation ability of BMSCs in the model group was significantly reduced (P<0.05), and the value-added ability of BMSCs in the BMSCs group and BMSCs+ miR-183-5P group was significantly increased compared with the model group, and the proliferation ability of BMSCs in the BMSCs+ miR-183-5P group was the highest (P<0.05); after transfection, compared with the healthy group, the The migration ability of BMSCs was significantly reduced (P<0.05) in the model group compared with the healthy group, and the migration ability of BMSCs was significantly increased in the BMSCs group and BMSCs+ miR-183-5P group compared with the model group, and the migration ability of BMSCs was highest in the BMSCs+ miR-183-5P group (P<0.05); the apoptotic ability of BMSCs was significantly increased in the model group compared with the healthy group (P<0.05), and the apoptotic capacity of BMSCs was significantly lower in the BMSCs group and BMSCs+ miR-183-5P group compared with the model group, and the apoptotic capacity of BMSCs in the BMSCs+ miR-183-5P group was the lowest (P<0.05). The bioinformatics software RegRNA 2.0 was used to predict that the specific target gene that might be regulated by miR-183-5P was FOXO1. Dual luciferase reporter gene assay confirmed that miR-183-5P could complementarily bind to the 3' untranslated region of FOXO1 mRNA, which further confirmed that FOXO1 was the target gene of miR-183-5P. miR-183-5P+FOXO1-WT

cotransfection group showed significantly lower relative luciferase activity than miR-183-5P+ FOXO1-MUT cotransfection group ($P<0.05$). After upregulation of miR-183-5P expression, the expression of FOXO1 mRNA was significantly higher in BMSCs of the BMSCs group and BMSCs+ miR-183-5P group compared with the model group, and the expression was highest in the BMSCs+ miR-183-5P group ($P<0.05$). The Western blotting assay showed that, compared with the healthy group The expression of FOXO1 mRNA in BMSCs was lower in the model group compared with the healthy group ($P<0.05$), and significantly higher in the BMSCs and BMSCs+ miR-183-5P groups compared with the model group, with the highest expression in the BMSCs+ miR-183-5P group ($P<0.05$) (25, 26). The BMSCs+ miR-183-5P group showed the least inflammatory manifestation, which also demonstrated the repairing effect of BMSCs-derived miR-183-5P targeting FOXO1 on myocardial tissue ischemia and hypoxia, similar to the findings of Zhang Changjiang et al (25).

In summary, miR-183-5P of BMSCs origin can target and regulate FOXO1 to increase the proliferation and migration of BMSCs and reduce their apoptosis, and can also increase the survival rate of BMSCs by increasing the expression of FOXO1 mRNA to reduce myocardial tissue edema and decrease the inflammatory response, which provides a clinical basis for the transplantation of BMSCs. However, there are some shortcomings in this study, such as the expression of miR-183-5P in clinical specimens of AMI myocardial tissues has not been validated, so clinical specimens of AMI myocardial tissues will be collected later to further explore the correlation between miR-183-5P expression levels and clinical characteristics of AMI myocardial tissues.

Acknowledgments

Construction Project of Chen Xinyu Inheritance Studio-National Famous Traditional Chinese Medicine Expert (No.2022-75 Letter from Department of Chinese Medicine Education in China)
National Natural Science Foundation of China; (81704061,81173213);
Hunan province innovative province construction special fight against new
Coronavirus pneumonia project (2020SK3009);
R&D plan for key areas of the Hunan Provincial Department of Science and Technology (2019SK2321);
Hunanscienceandtechnologytalenthostingproject(2020TJ-N01);
Hunan Development and Reform Commission Innovation Guidance Project (Hunan
Development and Reform Investment 2019-412);
Special Project for the Construction of Key Laboratory of 'Four Seasons Adjusting
Yang' for Heart Disease (Xiang TCM Letter51);
Hunan Engineering Research Center for Preventive Treatment of Diseases in 'Four
Seasons Adjusting Yang' (No. 1006 of Hunan Development to High Technology);
Hunan University of Traditional Chinese Medicine first-class discipline open fund
(2022ZYX01,2022ZYX11,2021ZYX41)

References

- Boyanpally A, Cutting S, Furie K. Acute Ischemic Stroke Associated with Myocardial Infarction: Challenges and Management. *Semin Neurol.* 2021;41(4):331-339. doi:10.1055/s-0041-1726333
- DeFilippis AP, Chapman AR, Mills NL, et al. Assessment and Treatment of Patients With Type 2 Myocardial Infarction and Acute Nonischemic Myocardial Injury. *Circulation.* 2019;140(20):1661-1678. doi:10.1161/CIRCULATIONAHA.119.040631
- Luo Q, Li JY. Effect of adjuvant therapy with ginseng rhizome injection on ventricular remodeling and serum bFGF and VEGF levels in patients with acute myocardial infarction. *Journal of Integrative Cardiovascular and Cerebrovascular Diseases.* 2020,18(10):1573-1575. doi:10.12102/j.issn.1672-1349.2020.10.016.
- Zhang J, Liang MJ. Research progress on the mechanism of Chinese medicine intervention in ventricular remodeling after acute myocardial infarction. *Journal of Traditional Chinese Medicine.* 2021,27(8):128-132,137.
- Min S. F., Zou Y., Cui G. F., et al. Ultrasound assessment of the therapeutic value of mesenchymal stem cells in acute heart infarction in rats. *Chinese Journal of Ultrasound Medicine.* 2021,37(6):702-705. doi:10.3969/j.issn.1002-0101.2021.06.032.
- Chu DT, Phuong TNT, Tien NLB, et al. An Update on the Progress of Isolation, Culture, Storage, and Clinical Application of Human Bone Marrow Mesenchymal Stem/Stromal Cells. *Int J Mol Sci.* 2020;21(3):708. Published 2020 Jan 21. doi:10.3390/ijms21030708
- Yang W, Ma B. A Mini-Review: The Therapeutic Potential of Bone Marrow Mesenchymal Stem Cells and Relevant Signaling Cascades. *Curr Stem Cell Res Ther.* 2019;14(3):214-218. doi:10.2174/1574888X13666180912141228
- Guo X, Bai Y, Zhang L, et al. Cardiomyocyte differentiation of mesenchymal stem cells from bone marrow: new regulators and its implications. *Stem Cell Res Ther.* 2018;9(1):44. Published 2018 Feb 26. doi:10.1186/s13287-018-0773-9
- Hosseinpour A, Kheshti F, Kazemi A, Attar A. Comparing the effect of bone marrow mono-nuclear cells with mesenchymal stem cells after acute myocardial infarction on improvement of left ventricular function: a meta-analysis of clinical trials. *Stem Cell Res Ther.* 2022;13(1):203. Published 2022 May 16. doi:10.1186/s13287-022-02883-3
- Lu TX, Rothenberg ME. MicroRNA. *J Allergy Clin Immunol.* 2018;141(4):1202-1207. doi:10.1016/j.jaci.2017.08.034
- Liu Q, Kong Y, Guo X, et al. GSK-3 β inhibitor TDZD-8 prevents reduction of aquaporin-1 expression via activating autophagy under renal ischemia reperfusion injury. *FASEB J.* 2021;35(8):e21809. doi:10.1096/fj.202100549R
- Xiong Q, Song N, Li P, et al. RNA and quantitative proteomic analysis of Dictyostelium knock-out cells lacking the core autophagy proteins ATG9 and/or ATG16 [published correction appears in *BMC Genomics.* 2021 Jul 19;22(1):555]. *BMC Genomics.* 2021;22(1):444. Published 2021 Jun 15. doi:10.1186/s12864-021-07756-2
- Zeng T, Guo YY, Guo JY, et al. Construction of astrocyte U251 stable trans-FOXO1 gene cell line and its protein-interacting biotin validation. *Chinese Journal of Pharmacology.* 2022,38(5):799-800. DOI:10.12360/CPB202111130.
- Hu Aini, Wang Yuping, Yang Mei, et al. Expression of FOXO1 gene in gestational diabetic blood and correlation with insulin. *Guizhou Medicine.* 2020,44(4):514-516. doi:10.3969/j.issn.1000-744X.2020.04.003.
- Gao HN, Bai RX, Zhao PW, et al. Effect of miR-581-regulated

- FOXO1 on autophagy in ovarian cancer SKOV3 cells. *Cancer Control Research*,2022,49(5):403-407. doi:10.3971/j.issn.1000-8578.2022.21.1202.
16. Ling L, Lu MY, Jia S, et al. RP4 upregulates FOXO1 through miR-183-5p.1 to inhibit breast cancer cell proliferation. *Chinese Journal of Biochemistry and Molecular Biology*,2019,35(8):894-900. doi:10.13865/j.cnki.cjbmb.2019.08.16.
17. Ning K, Yang B, Chen M, et al. Functional Heterogeneity of Bone Marrow Mesenchymal Stem Cell Subpopulations in Physiology and Pathology. *Int J Mol Sci*. 2022;23(19):11928. Published 2022 Oct 7. doi:10.3390/ijms231911928
18. Song Zhongyang, WANG Gongchen, Zhang Zhiming, et al. Protective effects of tonic yang reversible soup combined with BMSCs on myocardium in a model of myocardial ischemia-reperfusion injury in rats. *Journal of Beijing University of Traditional Chinese Medicine*,2020,43(2):115-124. doi:10.3969/j.issn.1006-2157.2020.02.005.
19. Liu DY, Wang XY, Wang L, et al. Mesenchymal stem cell exosome transplantation for ischemic heart disease. *Chinese Tissue Engineering Research*,2023,27(15):2435-2442.
20. Litwinowicz R, Kapelak B, Sadowski J, Kędziora A, Bartus K. The use of stem cells in ischemic heart disease treatment. *Kardiolog Torakochirurgia Pol*. 2018;15(3):196-199. doi:10.5114/kitp.2018.78446
21. Wen Z, Mai Z, Zhu X, et al. Mesenchymal stem cell-derived exosomes ameliorate cardiomyocyte apoptosis in hypoxic conditions through microRNA144 by targeting the PTEN/AKT pathway. *Stem Cell Res Ther*. 2020;11(1):36. Published 2020 Jan 23. doi:10.1186/s13287-020-1563-8
22. Ju C, Li Y, Shen Y, et al. Transplantation of Cardiac Mesenchymal Stem Cell-Derived Exosomes for Angiogenesis. *J Cardiovasc Transl Res*. 2018;11(5):429-437. doi:10.1007/s12265-018-9824-y
23. Li WANGYANG, Xiong F. Effect of early intervention of Tao Hong Si Wu Tang on the homing of mesenchymal stem cells in fracture healing in rats. *China Bone Injury*,2022,35(4):367-374. doi:10.12200/j.issn.1003-0034.2022.04.014.
24. Sun WX, Zhao YC, Zhao RZ. Mesenchymal stem cell transplantation for the treatment of myocardial infarction: problems, cruxes and new breakthroughs. *Chinese Tissue Engineering Research*,2021,25(19):3103-3109.
25. Zhang CJ, Liang Guiyou. Bone marrow mesenchymal stem cells for myocardial ischemia-reperfusion injury: mechanism of action of exosomes and factors. *Chinese Tissue Engineering Research*,2019,23(9):1455-1460. doi:10.3969/j.issn.2095-4344.1599.
26. Singh L, Priya K, Chaudhary K, Comparison assessment regarding the awareness, behaviors and attitude towards HIV/AIDS of dental students in comparison to other allied sciences, *SPR*, 2021, Volume 1, issue 1, Page No.: 27 - 31.