EXPERIMENTAL STUDY

MiR-448-5p/VEGFA Axis Protects Cardiomyocytes from Hypoxia ThroughRegulating the FAS/FAS-L Signaling Pathway

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Summary

Bioinformatics analysis showed that miR-448-5p expression in the myocardial tissue of rats with myocardial infarction significantly increased, suggesting that it may participate in myocardial cell apoptosis in myocardial infarction. This study aimed to explore the protective effects of miR-448-5p on hypoxic myocardial cells.

H9C2 cells were cultured and subjected to anoxia for 2, 4, and 8 hours to establish a hypoxia model. MiR-448-5p mimic and inhibitor were transfected into the cells; then, a dual-luciferase experiment was conducted to verify the targeting relationship between miR-448-5p and VEGFA. Cell viability and apoptosis was detected by cell counting kit-8 and flow cytometry, respectively. The expressions of apoptosis-related proteins, miR-448-5p, FAS, and FAS-L were measured using western blotting and quantitative reverse transcription-polymerase chain reaction (qRT-PCR).

Hypoxia-reduced H9C2 cell viability and promoted apoptosis. MiR-448-5p expression was increased after H9C2 cell hypoxia. MiR-448-5p mimic significantly inhibited the viability and promoted the apoptosis of hypoxia-induced model cells. Hypoxia promoted the expression of apoptosis-related protein B-cell lymphoma-2 (Bcl-2) and inhibited the expressions of Bcl-2-associated x protein (Bax), cleaved caspase-3, and caspase-3, whereas the effect of inhibitor on hypoxia-reduced H9C2 cell and apoptotic protein expression were opposite to miR-448-5p mimic. MiR-448-5p targeted VEGFA and regulated its expression. Silenced VEGFA expression significantly inhibited inhibitor effect on increasing cell viability and promoted apoptosis. In addition, miR-448-5p mimic inhibited the effect of hypoxia on promoting the expressions of FAS and FAS-L of H9C2 cells. Inhibitors had the opposite effect on cell hypoxia model.

The miR-448-5p/VEGFA axis could protect cardiomyocytes from hypoxia through inhibiting the FAS/FAS-L signaling pathway.

Key words: Cell viability, Hypoaxia injury

Cardiovascular disease is one of the major diseases that threaten human life and health. As the incidence of cardiovascular disease is increasing annually, a better understanding of its pathogenesis is of great significance to its prevention and treatment.1-3) Studies indicated that myocardial cell damage is a main factor in inducing myocardial infarction, heart failure, and other cardiovascular diseases.4,5) Apoptosis of a large number of myocardial cells can be often observed in myocardial lesion area, and as some of the lesions are irreversible, they will directly aggravate myocardial injury.6,7) The initiation and development of apoptosis is a complex process regulated by a series of genes.8) Therefore, further exploration of apoptosis-related regulatory genes of cardiac myocytes will improve the prevention and treatment of cardiovascular diseases.

MicroRNAs (miRNAs) are a type of small-molecule noncoding RNAs that mainly promote the degradation of target gene mRNAs or inhibit the translation of target genes through binding to the mRNA 3’-UTR of target gene to negatively regulate the expression of target genes at the posttranscriptional level. Moreover, miRNAs also actively participate in a series of life activities, including apoptosis, proliferation, and differentiation.9-10) Studies increasingly demonstrated that miRNA is abnormally expressed in the pathophysiological process of cardiovascular diseases such as acute myocardial infarction, and they play important roles in myocardial cell injury.11,12) Bioinformatics analysis showed that miR-448-5p expression in the myocardial tissue of rats with myocardial infarction significantly increased, suggesting that it may participate in myocardial cell apoptosis in myocardial infarction. However, the effect of miR-448-5p on hypoxia-damaged cardiac myocytes remains unclear. In recent years, FAS...
and its ligand FAS-L are the widely studied membrane-surface molecules related to cell apoptosis, and the occurrence and development of cell apoptosis are closely related to the FAS/FAS-L signaling pathway. \cite{11,14}

Therefore, in this study, we explored the effects of miR-448-5p on hypoxia-damaged myocardial cells and the relationship between miR-448-5p and the FAS/FAS-L signaling pathway. The current findings provide new understanding on the apoptotic mechanism of myocardial cells damaged by hypoxia, hoping to improve the treatment of cardiovascular diseases.

**Methods**

**Cell culture:** Rat myocardial cell line H9C2 was purchased from the Central Laboratory of China-Japan Union Hospital of Jilin University. The H9C2 cells were subcultured by subjecting them to a water bath at 37°C and centrifuged at 1000 × g at 4°C for 5 minutes. Next, the supernatant was discarded, and 1 m Dulbecco’s Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12, 11320033, Gibco, USA) was added to the cells. Afterwards, the cells were transferred to 10-cm-diameter medium, and 10 mL Mixture F-12 with 1% streptomycin-penicillin double antibody (15070063, Gibco, USA) was added at 37°C in a 5% CO₂ atmosphere. When the cell confluence reached 60%-80%, the medium was discarded, the cells were washed with 2 mL PBS three times, and 1 mL 0.25% trypsin (25200114, Thermo Fisher Scientific, USA) was added to the culture dish to digest the cells at room temperature for 2 minutes. Under an inverted phase contrast microscope, we observed that the cells gradually transformed from spindle shape to round shape. The trypsin was discarded, and the digestion was terminated by adding 4 mL DMEM/F-12 containing 10% fetal bovine serum (FBS, Gibco, USA) and 1% streptomycin-penicillin double antibody. Next, 2 mL cell suspension was added into a new culture dish, and 10 mL culture medium was further added at 37°C in a 5% CO₂ atmosphere.

**Establishment of cell hypoxia model:** H9C2 cells were divided into four groups: control group, 2-hour anoxia group, 4-hour anoxia group, and 8-hour anoxia group. The control group was treated by 20% oxygen at 37°C, whereas the other three anoxia groups were maintained in hypoxic conditions with 1% oxygen and incubated at 37°C for 2, 4, or 8 hours, respectively.

**Cell viability detection:** Cells in logarithmic growth phase were used in the experiments, and the density was adjusted to 1 × 10⁶/mL by DMEM medium with 10% FBS. The cells were inoculated into the 96-well plate; then, 10 μL of cell counting kit-8 (96992, Sigma-Aldrich, USA) solution was added; it was then incubated for 4 hours. The absorbance at 450 nm was determined through enzyme microscopy (Multiskan GO, Shanghai Bajiu Industrial Co., Ltd., http://www.89-china.com/, Shanghai, China). The experiment was conducted in triplicate to calculate the average value.

**Luciferase activity assay:** For luciferase activity assay, the sequence of VEGFA containing the binding sites of miR-448-5 p was as follows: 5’-ACTTGAGTTGGGAGGAGGATGTC-3’; Furthermore, the 3’-UTR sequence of VEGFA WT was inserted into the pmirGLO Vector (Promega, Madison, Wisconsin, USA) with XhoI and Sacl double digestion, and they were used to construct the recombinant dual-luciferase reporter vector. Meanwhile, the plasmid containing the MUT VEGFA sequence was generated through DNA synthesis (Sangon Biotech Co. Ltd., Shanghai, China), and the sequence of VEGFA that bind to miR-448-5p was as follows: 5’-ACTTGAGTTGGGAGGAGGATGTC-3’. Afterwards, the pmirGLO vector containing WT or MUT VEGFA sequence was co-transfected with miR-448-5p mimic into cells using Lipofectamine™ 2000 (Invitrogen, USA). After incubation for 48 hours, Luciferase Reporter Gene Detection Kit (RG088S, Beyotime Biotechnology, China) was used to measure the relative luciferase activity of cells.

**Cell apoptosis:** The cells were washed four times using PBS and digested by trypsin for 2 minutes. Next, trypsin was discarded, 1 mL RPMI-1640 was added to the cells, and the cells were repeatedly blown into a single cell fluid. Cell suspensions were transferred to 15-mL centrifuge tube and centrifuged at 1000 × g at 4°C for 5 minutes at 4°C. Subsequently, the supernatant was discarded, and 1 mL RPMI-1640 was added to centrifuge tube. The cells were resuspended in the solution consisted of 1 × annexin binding buffer, 5 μL fluorescein isothiocyanate annexin V, and 1 μL of 100 μg/mL propidium iodide solution. Then, 300 μL 1 × annexin binding buffer was then added to cell suspension at room temperature for 15 minutes. Finally, the stained cells were analyzed using flow cytometry (version 10.0, FlowJo, FACS Calibur™, BD, Franklin Lakes, USA).

**Transfection:** Since the most obvious changes in cell viability were found in cells are under the 8-hour hypoxia, 8-hour hypoxia was, therefore, used for the subsequent research on the conditions of hypoxia. For the cells in the 8-hour hypoxia group, the cell culture medium was placed in a 6-pore cell dish, and 2 mL of the cell solution (containing 1 × 10⁶ cells) was added at 37°C in a 5% CO₂ atmosphere to reach a cell density of 40%-60%. To prepare A transdye, 20 pmol miR-448-5p mimic (Applied Biosystems, USA), miR-448-5p inhibitor, mimic-negative control (NC), inhibitor-NC, sh-VEGFA, and sh-NC (Shanghai GenePharma Co., Ltd., China) were dissolved in 50 μL DMEM (Hyclone, USA) and thoroughly mixed. For B transdye preparation, 1 μL Lipofectamine™ 2000 was dissolved in 50 μL DMEM, set aside for 15 minutes at room temperature and then mixed with A transdye. Afterwards, the mixture of A and B transdyes were added into the corresponding hole of the cell dish, which was placed in a cell culture box at 37°C with 5% CO₂ for further culture. The medium was changed 24 hours after the transfection, and then, the cells were collected after culture for 72 hours.

**Quantitative reverse transcription-polymerase chain reaction (qRT-PCR):** The total RNAs of cells were extracted using TRIzol reagent (15596018, Thermo Fisher Scientific, USA). The concentrations of RNAs were detected using Nanodrop (FSC-6539918, eGeneralMedical, USA) and then diluted to 500 ng/mL. Total RNA (1 μg) was reverse-transcribed into cDNA using a Super-
script II First-Strand cDNA Synthesis System (Invitrogen, USA). The mRNA expression levels were determined using SYBR Green PCR Master Mix (Thermo Fisher Scientific, USA) in the 7500 Real-Time PCR system (Thermo Fisher Scientific, USA). Then, 10 μL reaction solutions consisted of 1 μL Primer, 4 μL cDNA, and 5 μL SYBR (code: DRR041A, Takara, Japan). The PCR cycle was as follows: pretreatment at 95°C for 1 minute, at 95°C for 30 seconds, at 58°C for 20 seconds, at 90°C for 30 seconds (40 cycles), at 95°C for 15 seconds, at 60°C for 15 seconds, at 95°C for 15 seconds, and final chain extension of 72°C for 7 minutes and kept at 4°C. The 2^{-ΔΔCt} method was used to determine the expression levels of RT-PCR products. All primer sequences were listed in the Table. The total proteins of cells were lysed using a RIPA Lysis and Extraction Buffer (89901, Thermo Fisher Scientific, USA). Next, the proteins were boiled for 5 minutes at 100°C for denaturation. The proteins were separated by 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the concentration of proteins was measured using DC protein assay (Biorad, USA). Then, 10 μL reaction solutions were used for statistical analysis. The data were shown as mean ± standard deviation (SD) of at least three independent experiments. The differences between multiple groups were analyzed using one-way analysis of variance. P < 0.05 was considered as statistically significant.

**Results**

Effects of hypoxia on H9C2 cell viability and apoptosis: Under hypoxia conditions, the cell viability was slightly reduced after hypoxia culture for 2 hours compared with normal culture (P < 0.05, Figure 1A), whereas the cell viability was significantly reduced after 4 and 8 hours of hypoxic culture (P < 0.001, Figure 1A). In addition, compared with the control group, cell apoptosis was slightly promoted after 2-hour hypoxic culture (P < 0.05, Figure 1B, C), and cell apoptosis was significantly increased after 4- and 8-hour hypoxic culture (P < 0.001, Figure 1B, C).

**MiR-448-5p expression and its effects on the viability and apoptosis of H9C2 cells exposed to hypoxia:** qRT-PCR results showed that the miR-448-5p expression of H9C2 cells was increased after 2-, 4-, and 8-hour hypoxic culture compared with control group (P < 0.05, Figure 1D). The miR-448-5p expression of the cells transfected with mimic was increased after hypoxic culture for 8 hours (P < 0.05, Figure 2A); however, the expression of the cells transfected with the inhibitor was inhibited after the 8-hour hypoxic culture (P < 0.05, Figure 2B). MiR-448-5p mimic and inhibitor were transfected into H9C2 cells; the results showed that, after 6-hour hypoxic culture, the viability of the cells transfected with mimic was significantly inhibited (P < 0.05, Figure 2C), whereas the viability of the cells transfected with inhibitor was greatly increased (P < 0.05, Figure 2D). Meanwhile, mimic significantly increased the cell apoptosis of H9C2 cells after hypoxia culture (P < 0.05, Figure 2E, G). Inhibitor greatly reduced the cell apoptosis of hypoxia-induced H9C2 cells (P < 0.05, Figure 2F, H).

Effects of miR-448-5p on the apoptosis-related factor of hypoxic H9C2 cells: Hypoxia inhibited apoptosis-related Bcl-2 protein expression and promoted the expressions of Bax, cleaved caspase-3, and caspase-3. MiR-448-5p mimic inhibited the expression of Bcl-2 and promoted the expressions of cleaved caspase-3 and Bax; it also promoted the expression of cleaved caspase-3/caspase-3 in H9C2 cells treated by 8-hour hypoxic culture (P < 0.05, P < 0.001, Figure 3A-C), whereas, under the same condi-

| Gene          | Primer sequence | Species  |
|---------------|-----------------|----------|
| MiR-448-5p    | Forward: 5'-TGCATATGTGAGGTTCATCCATGT-3'  |
|               | Reverse: 5'-CTCGTGGGATGGCGGGAATTTG-3'   | Human    |
| VEGFA         | Forward: 5'-TCTCCACACATGCCAGTTGTC-3'    | Human    |
| FAS           | Forward: 5'-TTTACGTGTTATATCTCCT-3'      | Human    |
| FAS-L         | Forward: 5'-TGCACTTGCTGTTAATGGG-3'      | Human    |
| β-Actin       | Reverse: 5'-CTAAGTCATAGTCCGCTAGAAGCA-3' | Human    |
Effects of hypoxia on H9C2 cell viability and apoptosis and miR-448-5p expression. 

A: CCK-8 was used to detect cell viability.
B, C: Cell apoptosis was detected using flow cytometry.
D: qRT-PCR was used to detect the expression of miR-448-5p in the hypoxia of cells. 

The above protein expressions were the opposite in H9C2 cells transfected with the inhibitor ($P < 0.001$, Figure 3D-F).

**MiR-448-5p regulated the FAS/FAS-L pathway in H9C2 cells:** Hypoxia increased the expressions of FAS and FAS-L in H9C2 cells, which were significantly increased by miR-448-5p mimic ($P < 0.001$, Figure 3A-C). The inhibitor inhibited the expressions of FAS and FAS-L in H9C2 cells ($P < 0.001$, Figure 3D-F).

**MiR-448-5p targeted VEGFA and regulates the expression level of VEGFA:** TargetScan 7.1 was used to predict the targeted gene of miR-448-5p, and the result found that VEGFA had a targeted binding relationship with VEGFA (Figure 4A). Furthermore, we found that the overexpression of miR-448-5p significantly inhibited the luciferin activity of VEGFA through dual-luciferase experiments, thus confirming that VEGFA is the target gene of miR-448-5p (Figure 4B, $P < 0.001$). Next, we detected the expression of VEGFA in the model group and found that hypoxia significantly inhibited VEGFA expression, whereas transfection with mimic to hypoxia-H9C2 cell VEGFA expression was slightly inhibited compared with model group. On the contrary, VEGFA expression was significantly increased in the hypoxia-H9C2 cell transfected with the inhibitor ($P < 0.05$, $P < 0.001$, Figure 5D, E).
miR-448-5p expression and its effects on the viability and apoptosis of hypoxic H9C2 cells. 

**A:** After the mimic was transfected to hypoxic H9C2 cells, qRT-PCR was used to detect the expression of miR-448-5p in the hypoxia of cells. 

**B:** After the inhibitor was transfected to hypoxic H9C2 cell, qRT-PCR was used to detect the expression of miR-448-5p in the hypoxia of cells.

**C:** CCK-8 was used to detect the viability of cells treated by miR-448-5p mimic or hypoxia. 

**D:** CCK-8 was used to detect the viability of cells treated with miR-448-5p inhibitor or hypoxia.

**E, G:** Apoptosis of cells treated by miR-448-5p mimic or hypoxia was detected using flow cytometry. 

**F, H:** Apoptosis of cells treated by miR-448-5p inhibitor or hypoxia was detected using flow cytometry.

qRT-PCR indicates quantitative real-time polymerase chain reaction; CCK-8, cell counting kit-8; and NC, negative control.
Figure 3. Effects of miR-448-5p on the apoptosis-related factor of hypoxic H9C2 cells. A-C: Western blotting was used to detect the expressions of apoptosis-related proteins (cleaved caspase-3, Bcl-2, caspase-3) of cells treated with miR-448-5p mimic or hypoxia. n = 3, P*** < 0.001 versus control; P<0.05, P<0.01 versus Model; P^<0.05, P<0.01 versus Model + mimic (B). P**<0.01 versus control; P<0.001 versus Model; P<0.01 versus Model + mimic (C). D-F: Western blotting was used to detect the expressions of apoptosis-related proteins of cells treated with miR-448-5p inhibitor or hypoxia. n = 3, P*** < 0.001 versus control; P<0.05, P<0.01, P<0.001 versus Model; P<0.05, P<0.01 versus Model + inhibitor (E). P**<0.01 versus control; P<0.05 versus Model; P***<0.01 versus Model + mimic (F). qRT-PCR indicates quantitative real-time polymerase chain reaction; CCK-8, cell counting kit-8; NC, negative control; cleaved caspase-3, cleaved cysteiny1 aspartate-specific proteinase-3; Bcl-2, B-cell lymphoma-2; and Bax, Bcl-2-associated x protein.

Discussion

Myocardial damage can lead to the accumulation of myocardial cell metabolites, interstitial edema, and dysfunction of endothelial cells, further causing reduced cardiac function and inducing myocardial infarction, atherosclerosis, and other cardiovascular diseases. Hypoxia injury is one of the main factors damaging myocardial cells, and studies found that insufficient oxygen supply to the myocardium can lead to myocardial metabolic disorder, insufficient energy supply, and reduced myocardial systolic function and ultimately affects the function of the whole body. Several studies have shown that hypoxia can inhibit the viability of myocardial cells and promote their apoptosis. In this study, we established a hypoxia model of H9C2 cells and found that hypoxia inhibited myocardial cell viability and increased apoptosis in a time-dependent manner, which was consistent with the study of Zhao, et al.

MiRNA plays an important role in the regulation of the growth and development of myocardial cells and their functions; for example, miR-448-5p can effectively improve spinal cord injury caused by hypoxia reperfusion. However, the effects of miR-448-5p on the pathophysiology of myocardial cells have not been fully understood. Based on this, we further explored the role of miR-448-5p in hypoxia-injured myocardial cells and observed that the expression of miR-448-5p of H9C2 cells was increased as the hypoxia time prolonged. Thus, we speculated that...
miR-448-5p had a protective effect on myocardial cells damaged by hypoxia.

Studies have shown that miR-7a/b overexpression protects H9C2 cells induced by hypoxia injury.23) Feng, et al. found that overexpressed miR-210 alleviates the hypoxia-induced injury to H9C2 cells by overexpressing miR-210 or silencing miR-210 in H9C2 cells, whereas silencing miR-210 produces the opposite results.24) To further investigate the protective effect of miR-448-5p on myocardial cells induced by hypoxia injury, miR-448-5p mimic and inhibitor were transfected into the hypoxia model, and the results showed that the cell viability of hypoxia model was inhibited; moreover, its apoptosis was significantly promoted when miR-448-5p was overexpressed, and inhibiting miR-448-5p promoted the viability and reduced the apoptosis of the hypoxia model.

Bcl-2, Bax, cleaved caspase-3, and caspase-3 proteins are apoptosis-related proteins.25) Zhao, et al. demonstrated that miR-101a significantly reverses the hypoxia-induced upregulation of the expressions of Bax and caspase-3 and the downregulation of Bcl-2 in cardiac fibroblasts.25) In this study, we also detected the above proteins.
miR-448-5p targeted VEGFA and regulates the expression level of VEGFA. A: TargetScan v7.1 was used to detect the target relationship between miR-448-5p and VEGFA. B: A dual-luciferase experiment was conducted to verify the targeting relationship between miR-448-5p and VEGFA (n = 3, P*** < 0.001 versus mimic control). C: The VEGFA expression in H9C2 cells treated with miR-448-5p mimic or inhibitor or hypoxia was detected by qRT-PCR (n = 3, P*** < 0.001 versus control; P(< 0.001 versus model + mimic-NC; P### < 0.001 versus model + inhibitor-NC). D, E: The VEGFA expression in H9C2 cells treated with miR-448-5p mimic or inhibitor or hypoxia was detected using Western blotting. (n = 3, P*** < 0.001 versus control; P(< 0.001 versus model + mimic-NC; P### < 0.001 versus model + inhibitor-NC).

The FAS/FAS-L system is the most important apoptotic receptor pathway. FAS, an important death receptor on the cell surface, is activated and transmits apoptotic signals after binding with its ligand, thereby inducing apoptosis of target cells. Studies confirmed that FAS/FAS-L induces apoptosis of myocardial cells during myocardial ischemia reperfusion. The relationship between apoptotic proteins to further explore the effect of miR-448-5p on the apoptosis of hypoxia-damaged myocardial cells. The results demonstrated that hypoxia inhibited the apoptotic expression of Bcl-2 protein and promoted the expressions of Bax, cleaved caspase-3, and caspase-3, whereas the overexpression of miR-448-5p reversed the above protein expressions in model group, and the inhibition of miR-448-5p reversed the effects of overexpressed miR-448-5p.
Figure 6. miR-448-5p regulated the apoptosis-related factor of hypoxic H9C2 cells by targeting VEGFA. A, B: Western blotting was used to detect the VEGFA expression in hypoxia-induced H9C2 cells treated with miR-448-5p inhibitor or sh-VEGFA. C: qRT-PCR was used to detect the VEGFA expression in hypoxia-induced H9C2 cells treated with miR-448-5p inhibitor or sh-VEGFA. D: CCK-8 was used to detect the viability of hypoxia-induced H9C2 cells treated with miR-448-5p inhibitor or sh-VEGFA. E-G: Western blotting was used to detect the expressions of apoptosis-related proteins (cleaved caspase-3, Bcl-2, Bax, caspase-3) of hypoxia-induced H9C2 cells treated with miR-448-5p inhibitor or sh-VEGFA. (n = 3, \( P^{***} < 0.001 \), \( P^{**} < 0.01 \), \( P^{*} < 0.05 \), versus model; \( P^{***} < 0.001 \), \( P^{**} < 0.01 \), \( P^{*} < 0.05 \), versus model + inhibitor + sh-NC).
miRNA and the FAS/FAS-L signaling pathway has been increasingly revealed; for example, the overexpression of miR-199b inhibits the expression of FAS protein and thereby inhibits the expression of FAS-mediated apoptosis of colorectal cancer cells, and miR-25 inhibits the apoptosis induced by ischemia/reperfusion injury by downregulating the FAS/FAS-L signaling pathway. In this study, the relationship between miR-448-5p and the FAS/FAS-L signaling pathway was further detected to reveal the apoptotic mechanism of myocardial cells damaged by hypoxia. The results of this study showed that overexpressed miR-448-5p promoted the effect of hypoxia on promoting FAS and FAS-L expressions in H9C2 cells, suggesting that miR-448-5p might regulate hypoxia-induced myocardial apoptosis through regulating the FAS/FAS-L signaling pathway.

It is well known that miRNAs can interpret or inhibit the translation of mRNA through complementary binding to the 3′ noncoding region of the target gene mRNA, thereby regulating gene expression at the transcription level. Studies have found that downregulating miR-448 can reduce spinal cord ischemia/reperfusion injury by targeting SIRT1 and upregulating its expression. Therefore, we further explored the genes that may be targeted in the process of miR-448-5p regulating hypoxic cell damage. The target gene prediction website showed that miR-448-5p and VEGFA have binding sites, and their targeting relationship was confirmed by luciferase experiments. VEGFA is a major driver of angiogenesis and vasculogenesis, and it is well known that miRNAs can interpret or inhibit the translation of mRNA through complementary binding to the 3′ noncoding region of the target gene mRNA, thereby regulating gene expression at the transcription level. Therefore, the relationship between miR-448-5p and VEGFA was further detected to reveal the relationship between miR-448-5p and the FAS/FAS-L signaling pathway. VEGFA promotes the therapeutic efficacy of mesenchymal stem cells and cardiac stem cells as a therapy for cardiovascular disease. However, the current study is a preliminary study. In the future, we will start animal experiments to continue to explore the functional effects of miR-448-5p on myocardial infarction mice.

Conclusion

In conclusion, this work demonstrates that miR-448-5p targeting VEGFA might protect cardiomyocytes damaged by hypoxia by inhibiting the FAS/FAS-L signaling pathway. However, the current study is a preliminary study. In the future, we will start animal experiments to continue to explore the functional effects of miR-448-5p on myocardial infarction mice.

Disclosure

Conflicts of interest: The authors report no conflict of interest.

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