European Review for Medical and Pharmacological Sciences 2020; 24: 3322-3330

MiR-19a suppress apoptosis of myocardial cells in rats with myocardial ischemia/reperfusion through PTEN/Akt/P-Akt signaling pathway

Z. MA[1], Y.-H. LAN[1], Z.-W. LIU[2], M.-X. YANG[3], H. ZHANG[1], J.-Y. REN[3]

[1]Department of Cardiology, Beijing Jishuitan Hospital, Beijing, China
[2]Department of Emergency, Beijing Jishuitan Hospital, Beijing, China
[3]Department of Cardiology, China-Japan Friendship Hospital, Beijing, China

Abstract. – OBJECTIVE: To detect differentially expressed micro ribonucleic acids (miRNAs) in rats with myocardial ischemia/reperfusion (MIR), and to explore the influence of miR-19a on MIR rats and its mechanism.

MATERIALS AND METHODS: Firstly, the Sprague-Dawley (SD) rats were used to prepare MIR models, RNAs were extracted, and miRNA sequencing analysis was carried out to determine differentially expressed miRNAs related to MIR. Secondly, the predicted target genes of miR-19a were collected, and WebGestalt was applied to analyze gene ontology (GO) and pathway enrichment. Thirdly, the expression of the related proteins and the apoptosis of myocardial cells in MIR rats were detected *via* Western blotting. Fourthly, the interaction between miR-19a and the target gene phosphatase and tensin homolog (PTEN) was examined through Luciferase reporter assay.

RESULTS: Compared with that in the Sham operation (Sham) group, the miR-19a expression in rat myocardial tissues in the MIR group was significantly increased ($p<0.05$). Compared with those in the miR-negative control (miR-NC) group, the messenger RNA (mRNA) and protein expressions of PTEN in the miR-19a group were notably decreased ($p<0.05$). In comparison with the miR-NC group, miR-19a group had elevated expression of phosphorylated protein kinase B (p-Akt) ($p<0.05$). The Luciferase reporter gene assay manifested the direct binding of miR-19a to PTEN mRNA.

CONCLUSIONS: MiR-19a inhibits the PTEN expression by directly binding to the 3'-UTR of PTEN mRNA, thus activating the Akt/p-Akt signaling pathway to suppress the apoptosis of myocardial cells in MIR injury.

Key Words:
Myocardial ischemia/reperfusion, PTEN, MiR-19a, Akt/p-Akt.

Introduction

Cardiovascular disease has a high mortality rate and causes lesions in the heart and blood vessels. In 2013, about 17.3 million people died of the disease worldwide, and its mortality rate increased from 25% to 40% only in China from 1990 to 2010[1,2]. Most patients with cardiovascular disease suffer from myocardial ischemia due to the reduction of cardiac blood flow caused by partial or complete occlusion of cardiovascular arteries, thus failing to obtain sufficient oxygen supply, which damages the myocardium and causes serious arrhythmia[3]. Myocardial ischemia/reperfusion (MIR) is regarded as the most effective treatment method for ischemic heart disease[4]. Paradoxically, reperfusion also leads to injury by prolonging myocardial injury during ischemia despite its improvement on the prognosis of patients with acute myocardial infarction[5]. Therefore, reducing the intervention of reperfusion injury during coronary artery recanalization is considered as a promising strategy to further reduce the infarct size and improve the prognosis of myocardial infarction, and exploring a new mechanism of MIR injury is of great significance for preventing and treating cardiovascular disease.

Micro ribonucleic acids (miRNAs) are a kind of non-coding single-stranded RNA molecules that participate in the regulation on post-transcriptional gene expression in plants and animals[6]. MiRNAs play vital roles in metabolism, proliferation, and other biological processes[7], and they are also involved in the processes of many diseases[8], such as tumor development[9] and inflammatory process[10]. MiR-19a is a member of the miR-17-92 cluster, that exerts crucial regulatory effects in the development of the heart and lung[11,12] and is also involved in aging

Corresponding Author: Jingyi Ren, MD; e-mail: renjingyi1213@163.com

and canceration[13]. So, miR-34a is able to regulate autophagy activity by targeting Atg4B, resulting in IR injury[14]. As a core member of miR-17-92 cluster, miR-19 can improve lymphangiogenesis[15] by targeting thrombospondin-1 in colorectal cancer, thus accelerating cell proliferation, migration, and invasion. Besides, miR-19a overexpression can suppress myocardial cell apoptosis resulting from ischemia *in vivo* and *in vitro* and protects myocardial cells from hypoxia/reoxygenation-induced apoptosis through the phosphatase and tensin homolog (PTEN)/phosphatidylin-ositol-3-kinase (PI3K)/phosphorylated protein kinase B (p-Akt) pathway[16].

In this study, the rat model of MIR was utilized to study the differential expression of miR-19a, thus further clarifying the effect of miR-19a on MIR, and its action mechanism was explored. The results manifested that miR-19a inhibited the apoptosis of myocardial cells by suppressing the expression of PTEN, which provides a more sufficient theoretical basis for miR-19a as a marker and target of MIR.

Materials and Methods

Reagents

MiR-19a mimics were purchased from GenePharma (Shanghai, China), Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum albumin (FBS) from Nanjing Sunshine Biotechnology Co., Ltd. (Nanjing, China), TransFast transfection reagent from Promega (Madison, WI, USA), psiCHECK-2 Luciferase plasmid from Shanghai Kelei Co., Ltd. (Shanghai, China), radioimmunoprecipitation assay (RIPA) lysate (Beyotime, Shanghai, China), polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA), enhanced chemiluminescence (ECL) Plus Western blotting detection reagent and horseradish peroxidase (HRP)-conjugated secondary antibody from ImmunoWay Biotechnology Company (Plano, TX, USA).

Rat MIR Model Preparation

A total of 20 Sprague-Dawley (SD) rats were randomly divided into two groups and anesthetized with pentobarbital. After the hearts were exposed, the left anterior descending coronary artery (LAD) was sutured with silk threads for 30 min in the operation (MIR) group. Myocardial ischemia was confirmed by electrocardiograms. Next, LAD was reperfused for 2 h, and the obtained heart tissues were further analyzed. In the sham operation (Sham) group, the heart was exposed, but the LAD was not ligated. This study was approved by the Animal Ethics Committee of Peking University Animal Center.

MiRNA Sequencing Analysis

MiRNA sequencing was implemented by Shanghai Kangchen Biotech Co., Ltd. (Shanghai, China). In short, small RNAs were separated from the total RNAs and purified firstly. Then, they were linked to the 5' end and purified, and linked to the 3' end, followed by purification. Secondly, the first-strand and second-strand complementary deoxyribonucleic acids (cDNAs) were synthesized, and Illumina Genome Analyzer IIx was employed for sequencing analysis. MiRNAs with FC >2.0 and $p<0.05$ were considered to be differentially expressed.

Target Gene Prediction and Analysis

TargetScan and miRDB were employed to predict miR-19a target genes. The first 300 target genes were taken as its target genes after the intersection of the two databases. After that, the target genes were uploaded to WebGestalt for gene ontology (GO) and pathway enrichment analyses. In addition, the first 150 target genes were taken for the visualization of the miRNA-target network using Cytoscape3.7.1[17]. Finally, the binding site between miR-19a and the target gene PTEN was predicted using TargetScan.

Cell Culture and Transfection

H9c2 cells were maintained in DMEM supplemented with 10% FBS and 1% Pen/Strep solution and cultured in an incubator with 5% CO_2 at 37°C. Thereafter, H9c2 cells were plated in a 24-well plate and transfected with 100 nM of miR-19a mimics (miR-19a) and negative control (miR-NC) using TransFast according to the instructions of miRNA transfection reagent (GenePharma, Shanghai, China). Subsequently, the complete PTEN sequence was inserted into pcDNA3.1 plasmid to construct PTEN overexpression system (pcDNA-PTEN). On the next day, H9c2 cells were transfected with the PTEN overexpression plasmid using TransFast, and at 24 h after transfection, the *in vitro* ischemia model was induced[18]. 48 h later, the cells were collected for subsequent analysis.

Detection of the Change in the Gene mRNA Level Via Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR)

The total RNAs were extracted from the cells and quantified *via* NanoDrop ND-2000 (Thermo Fisher Scientific, Waltham, MA, USA). CDNAs were synthesized with miScriptII reverse transcriptase (Qiagen, Hilden, Germany), and miR-19a was quantified using miScript SYBR Green PCR kit (Qiagen, Hilden, Germany). Then, cDNAs were synthesized using RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, K1622, Waltham, MA, USA), and PTEN was quantified with SYBR Green PCR kit (Promega, Madison, WI, USA). Finally, the cDNAs were tested on the machine based on the 7500-Fast real-time PCR system (Applied Biosystems, Foster City, CA, USA). The expression levels of miRNAs and genes were calculated by $2^{-\Delta\Delta Ct}$ method, with U6 (a small nuclear RNA) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as internal references. QRT-PCR primer sequences are shown in Table I.

Luciferase Reporter Assay

TransFast was used to co-transfect myocardial cells with psiCHECK-2 Luciferase plasmids containing wild-type PTEN (PTEN-WT) and mutant PTEN (PTEN-MUT) and miR-19a mimics or miR-NC, and the Luciferase activity was measured using a microplate reader (BioTek, Biotek Winooski, VT, USA) 48 h later.

Analysis of the Level of Related Protein Via Western Blotting

The culture solution in the plate was discarded, and an appropriate amount of lysis solution was added to each well after washing with phosphate-buffered saline (PBS). After fully shaking, the cells were scraped, and the protein samples were obtained after ultrasonication and centrifugation. The protein concentration was measured by bicinchoninic acid (BCA; Pierce, Rockford, IL, USA), and the volume of all sample proteins was set constant for isoconcentration. Following sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), the proteins in the gel were transferred onto a PVDF membrane, and incubated with anti-PTEN antibody (diluted at 1:2000) overnight at 4°C and then, with HRP-conjugated secondary antibody (diluted at 1:600) at room temperature for 1 h. Ultimately, the color was developed with ECL Plus Western blotting detection reagent.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 software (IBM Corp., Armonk, NY, USA) was adopted for statistical analysis. The data of the numerical variables were expressed as mean ± standard deviation, and independent two-sample *t*-test was employed for the comparison between the two groups. $p<0.05$ suggested that the difference was statistically significant.

Results

MiR-19a Expression Was Upregulated in MIR Rats

MiRNA sequencing analysis showed that the expression of miR-19a in the myocardial tissues of rats in MIR group was increased compared with that in the Sham group ($p<0.05$) (Figure 1), which was further verified by qRT-PCR (Figure 2).

Prediction Results of MiR-19a Target Genes

Bioinformatics tools (TargetScan and miRPathDB) were utilized to predict miR-19a target genes. After the intersection of the two databases, the first 300 target genes were taken as miR-9a target genes. Then, Cytoscape 3.7.1 was employed to visualize the interaction network between miR-19a-target for the first 150 target genes (Figure 3), and it was found that PTEN was one of miR-19a target genes.

Results of GO and Pathway Enrichment Analyses for MiR-19a Target Genes

The 300 target genes of miR-19a were uploaded to the WebGestalt online tool for enrichment analysis to obtain a series of biological processes in which the target genes participate. These biological processes covered a wide range of categories, including responses to endogenous stimuli, and regulation on cell growth, proliferation, and metabolism process (Figure 4).

MiR-19a Transfection Induced the Overexpression of MiR-19a

In order to identify whether miR-19a transfection induces the overexpression of miR-9a

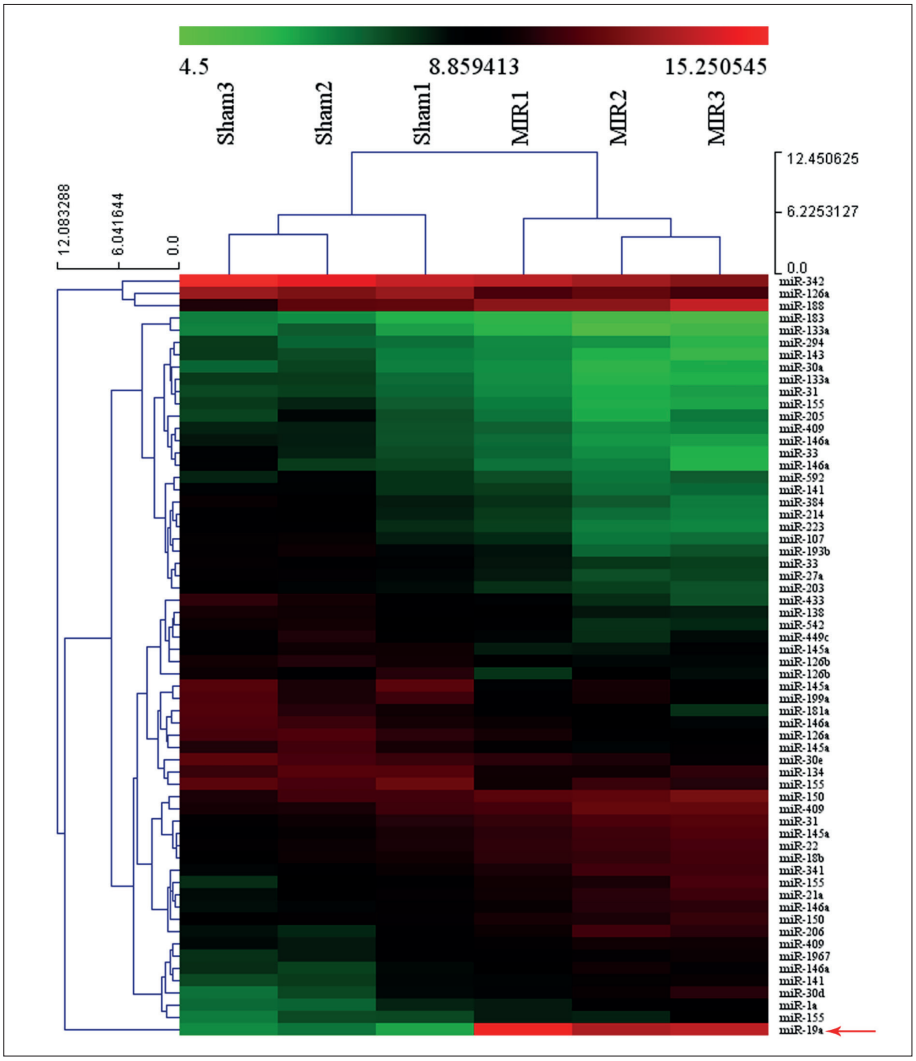

Figure 1. Heat map shows that miRNAs are differentially expressed in MIR rats. Arrow: miR-19a.

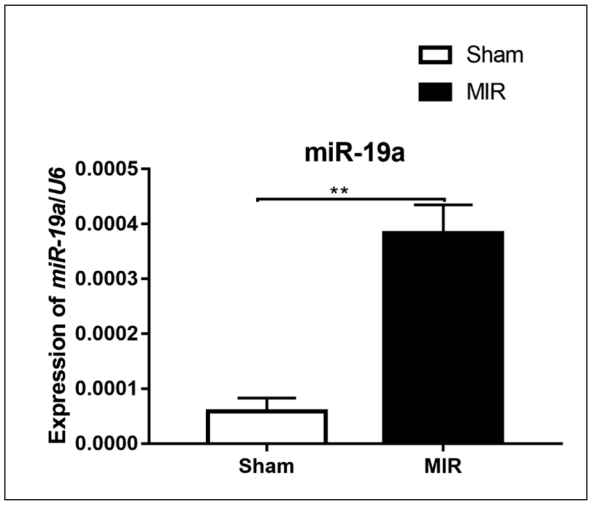

Figure 2. QRT-PCR displays that miR-19a expression in MIR rats is increased. Note: Compared with that in the Sham group, the expression of miR-19a in MIR group is increased (**p<0.01).

in myocardial cells, qRT-PCR was carried out to detect the miR-19a expression in myocardial cells after transfection. The results revealed that the expression level of miR-19a in miR-19a group was markedly raised compared with that in the miR-NC group (p<0.05) (Figure 5).

MiR-19a Inhibited the Apoptosis of Myocardial Cells

The expression of cleaved cysteinyl aspartate specific proteinase-3 (cleaved Casp-3) was detected, so as to determine whether the overexpression of miR-19a after MIR treatment affects the level of apoptosis (Figure 6A). It was found that the miR-19a overexpression reduced the expression of cleaved Casp-3 induced by MIR, and the difference was statistically significant (p<0.05) (Figure 6B).

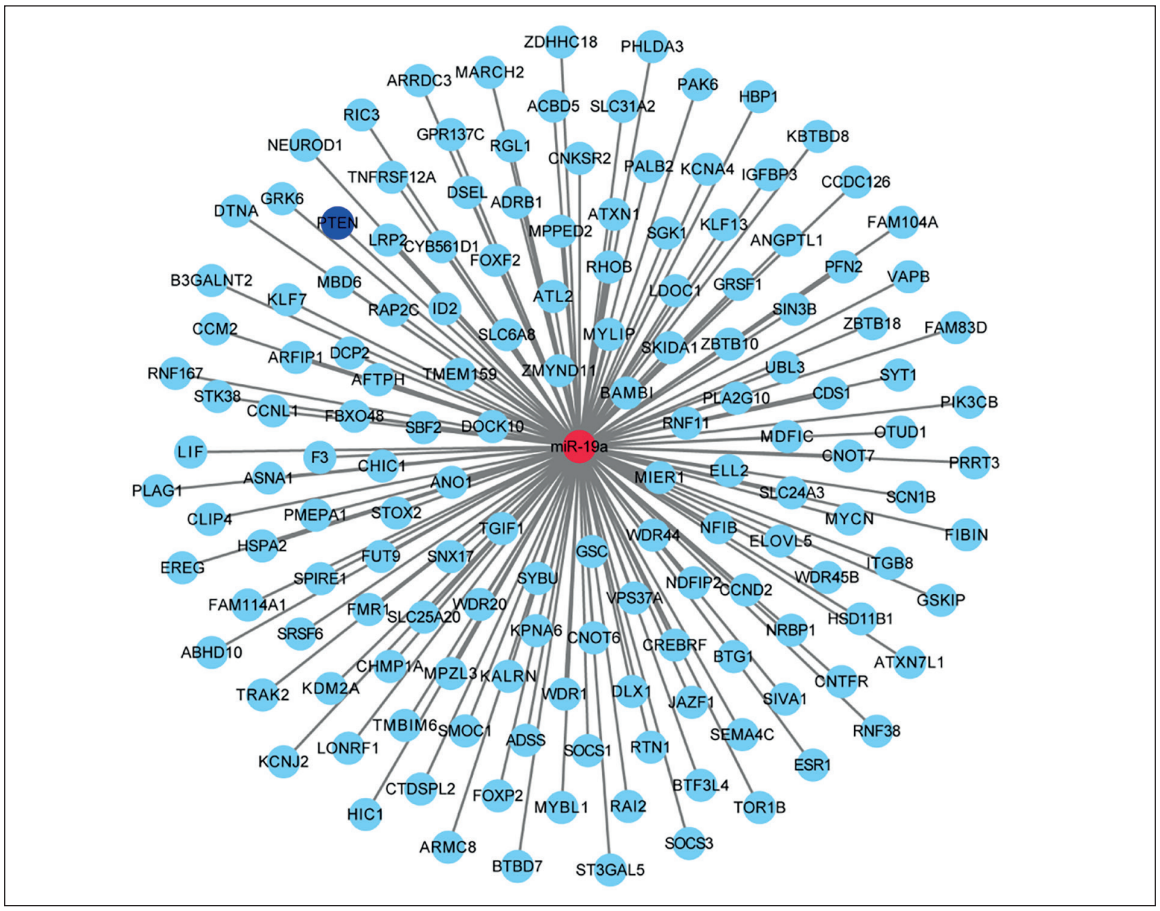

Figure 3. MiR-19a-target interaction network. Nodes: genes or miRNAs, lines: connections, red: miR-19a, light blue: target genes and dark blue: PTEN.

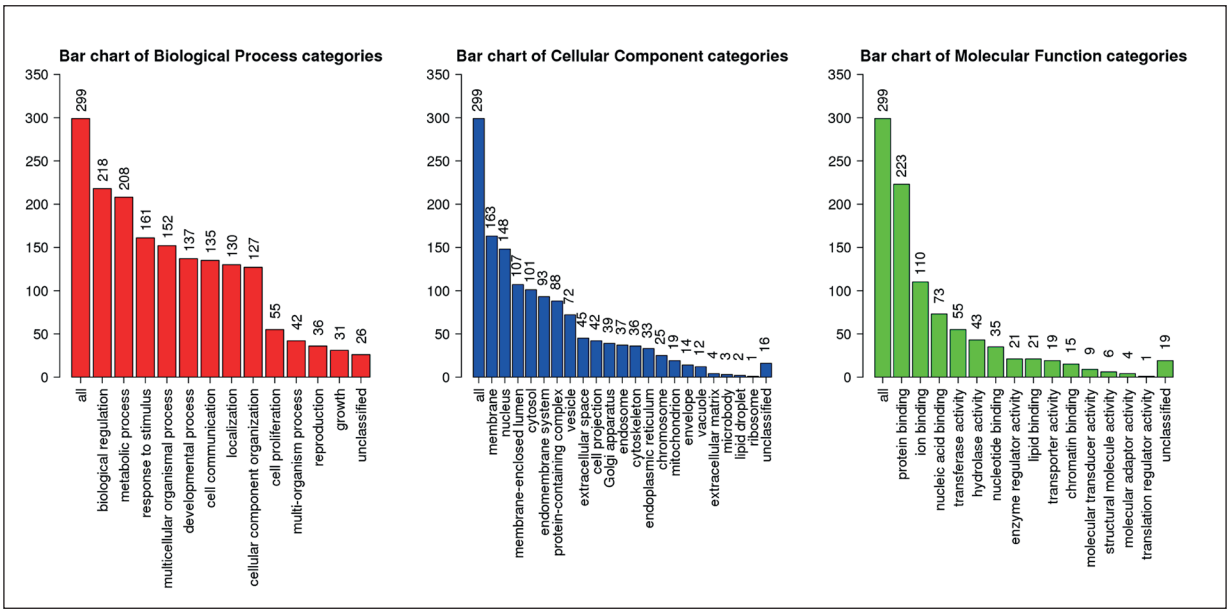

Figure 4. Statistics of GO entries enriched with miR-19a target genes. Note: BP: BP enrichment, cellular component (CC): CC enrichment, and molecular function (MF): MF enrichment.

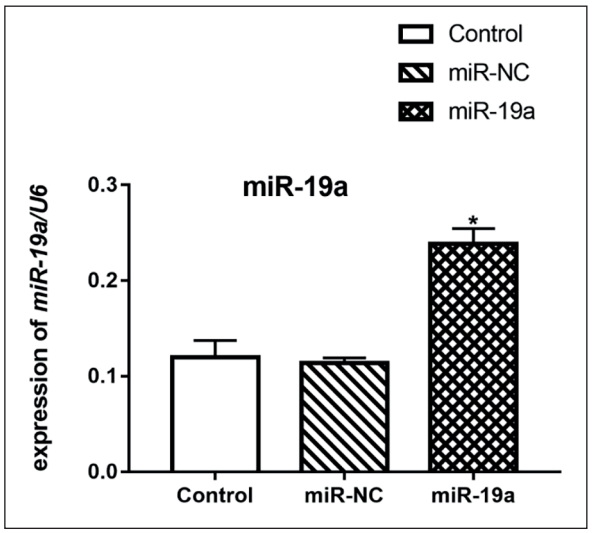

Figure 5. MiR-19a transfection induces the overexpression of miR-19a. Note: Compared with the Control group, the expression of miR-19a in miR-NC group has no evident change. In comparison with miR-NC group, the expression level of miR-19a in miR-19a group is notably raised (*p<0.05).

MiR-19a Directly Bound to PTEN to Inhibit PTEN and Activate the PI3K/Akt Signaling Pathway

To further explore the action mechanism of miR-19a in MIR, TargetScan was firstly used for prediction of the binding site between miR-19a and PTEN (Figure 7A). The results of the Luciferase reporter gene assay manifested that the Luciferase activity of the reporter plasmids with PTEN-WT in H9c2 cells was evidently decreased in the miR-19a overexpression group. However, no evident change was observed in the PTEN-MUT reporter gene system (p<0.05) (Figure 7B). QRT-PCR results demonstrated that the mRNA level of PTEN in miR-19a overexpression group declined (p<0.05, Figure 7C). According to Western blotting results, the protein level of PTEN was markedly inhibited by miR-19a, and the expressions of p-PI3K and p-Akt were raised after miR-19a was overexpressed (p<0.05, Figure 8). In conclusion, miR-19a inhibits the mRNA and protein expression levels of PTEN and activates the Akt/p-Akt signaling pathway by directly binding to PTEN.

Discussion

In this study, the enrichment analysis was firstly implemented for the biological processes and pathways involving miR-19a target genes at the gene level. The results showed that the biological processes in which miR-9a target gene participated covered a wide range of categories, including responses to endogenous stimuli, and regulation on cell growth and metabolism. The role of miR-19a in myocardial cells has been reported in previous studies. In this study, the biological processes involving miR-19a target genes were analyzed in a more comprehensive manner and it was found that that miR-19a participates in the regulation processes of cell proliferation and apoptosis. Secondly, miR-19a may influence the metabolic process of cells. Subsequent studies are expected to investigate the effect of metabolic process imbalance on MIR onset. It is known that the signal transduction pathways involving PTEN, PI3K, and p-Akt are associated with apoptosis and exert vital effects in the control of myocardial cell survival and function[19]. PTEN is a tumor suppressor often inactivated in human cancers and capable of inhibiting the PI3K pathway and Akt phosphorylation[20]. Activation or inhibition of Akt phosphorylation results

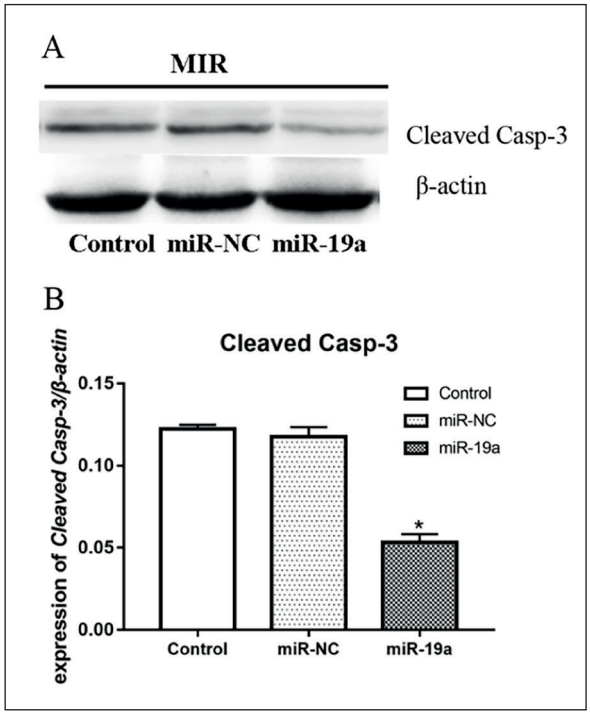

Figure 6. Expression level of cleaved Casp-3 detected by Western blotting: (**A**) protein electrophoresis results, and (**B**) protein quantification results. Note: MIR treatment induces the expression of cleaved Casp-3. Compared with that in the Control group, the expression level of cleaved Casp-3 in miR-NC group does not change significantly. Compared with that in miR-NC, the expression level of cleaved Casp-3 in miR-19a group declines remarkably (*p<0.05).

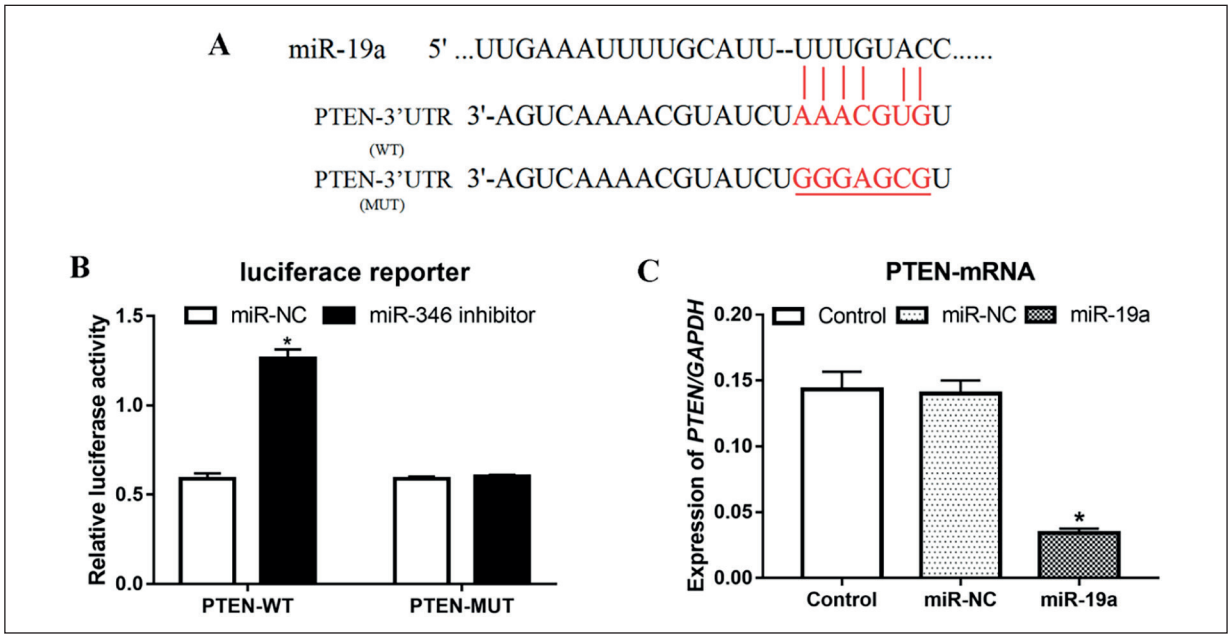

Figure 7. MiR-19a functions by directly binding to PTEN. **A,** The binding sites between normal PTEN and PTEN-MUT sequences and miR-19a. **B,** The luciferase activity of PTEN-WT and PTEN-MUT reporter genes after myocardial cells are transfected with miR-NC and miR-19a. **C,** Changes in the mRNA level of PTEN after myocardial cells are transfected with miR-19a and miR-NC. Note: $^{*}p<0.05$: miR-NC group *vs.* miR-19a group.

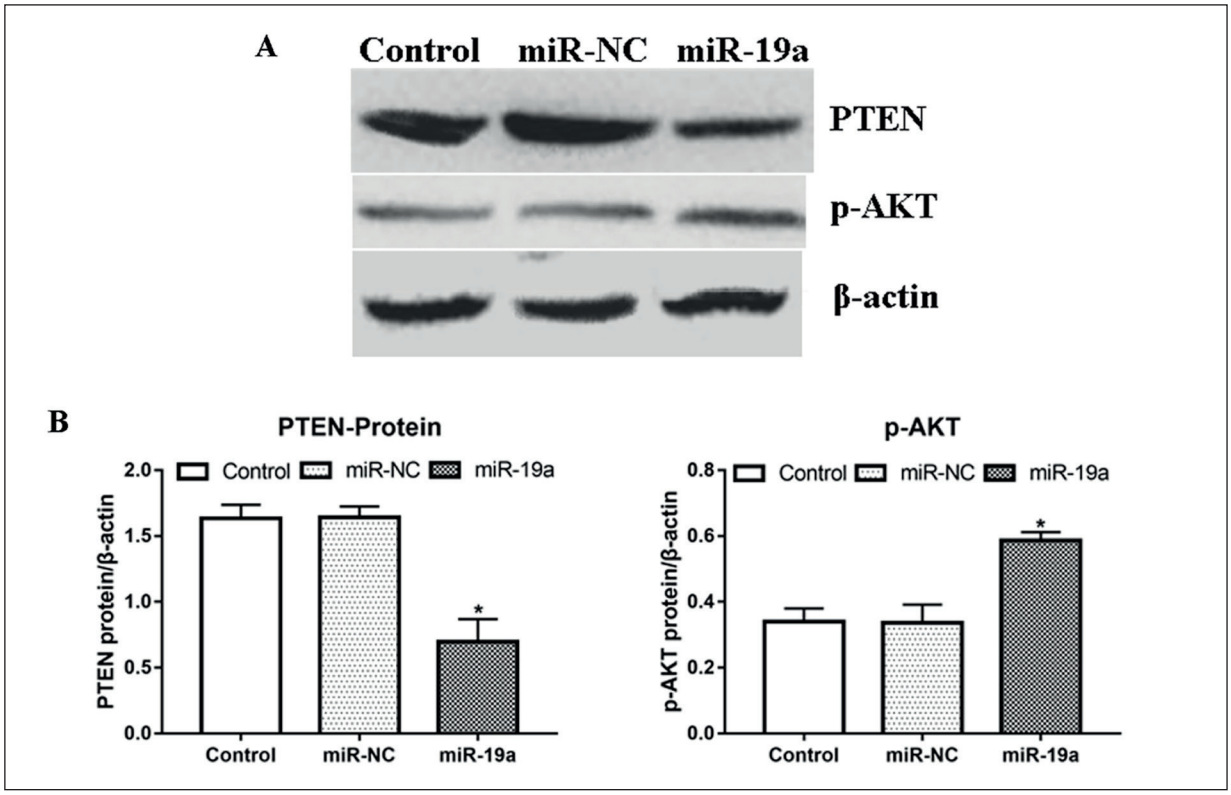

Figure 8. Verification results of related protein levels after miR-19a overexpression. **A,** Protein electrophoresis bands of PTEN, p-PI3K and p-Akt after myocardial cells are transfected with miR-NC and miR-19a. **B,** Protein quantification results of PTEN, p-PI3K and p-Akt proteins after after myocardial cells are transfected with miR-NC and miR-19a. Note: $^{*}p<0.05$: miR-NC group *vs.* miR-19a group.

in the imbalance of many biological processes, including cell apoptosis, proliferation, and metabolism[21]. In this study, the changes in the miR-19a expression were detected in the myocardial tissues of MIR rats, and the inhibitory mechanism of PTEN was explored.

It has been indicated by previous studies that miRNAs bind to mRNAs in numerous diseases and biological processes and act as mRNA inhibitors to regulate the mRNA expression at the transcriptional and post-transcriptional levels. In the meantime, increasingly more miRNA molecules have been identified to be a landmark in diseases. However, due to the complex interaction between miRNAs and their mRNA targets, the action mechanism of miRNAs is still controversial and needs to be further explored[22,23]. Li et al[24] have reported that miR-19 family is a promising biomarker and therapeutic target in the heart, blood vessels, and neurons. MiR-19a molecule also plays a crucial role in cancer transformation and often serves as an indicator for cancer prognosis[25,26]. In addition, the functions of miR-19a in myocardial ischemia processes involving the complex PTEN/PI3K/Akt signaling pathway have also been researched[16]. However, compared with these studies, the present study made related verification using myocardial cells and partial verification on the miR-9a expression *via* the rat model. However, there are still deficiencies. The *in vivo* study is not sufficient and further exploration is needed. Besides, the data reported previously are limited and the mechanism of action is still unclear. In the present study, the changes in the expression of miR-19a during MIR injury were investigated. The differential expression of miR-19a in MIR indicates its vital function in the disease progression. Moreover, the potential role of miR-19a in IR-induced myocardial cell injury was explored, and the verification of the relationship between miR-19a and target genes denotes the role of miRNA in the activation of Akt/p-Akt pathway, thus laying foundations for MIR development and the identification of miR-19a as a diagnostic marker or therapeutic target. In spite of the relatively sufficient prediction and verification in this study, there are still deficiencies. Firstly, only the effect of miR-19a on myocardial cell apoptosis through the Akt/p-Akt signaling pathway was researched, but other pathways, such as MAPK signaling pathway and NF-κB signaling pathway, were not detected. Therefore, the mechanism of miR-19a in MIR still needs further exploration. Secondly, attention should also be paid to other BPs in addition to cell apoptosis, such as GO indicating the process of metabolism. Furthermore, whether miR-19a molecule plays the same role in MIR patients still needs further clinical verification because of the differences between rats and humans and between cell tests and clinical tests.

Conclusions

To sum up, this study shows the differential expression of miR-19a in the myocardial tissues of MIR rats, laying a foundation for its role in the heart injury in the future. The mechanism exploration reveals that miR-19a inhibits the PTEN expression and activates the Akt phosphorylation, providing theoretical and scientific bases for the application of miR-19a in the treatment of MIR. The possibility of miR-19a as a potential therapeutic target for MIR is proposed, and the theoretical insights into miR-19a as a potential therapeutic target for other related diseases are also provided, which enrich and expand the role of miR-19a in diseases.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Jiang L, Krumholz HM, Li X, Li J, Hu S. Achieving best outcomes for patients with cardiovascular disease in China by enhancing the quality of medical care and establishing a learning health-care system. Lancet 2015; 386: 1493-1505.
2) Gao S, Peng J. Protective effect of Timosaponin on myocardial ischemic injury and its research mechanism. Acta Medica Mediterr 2019; 35: 529-537.
3) Shecterle LM, Terry KR, St Cyr JA. Potential clinical benefits of D-ribose in ischemic cardiovascular disease. Cureus 2018; 10: e2291.
4) Zhang ZX, Li H, He JS, Chu HJ, Zhang XT, Yin L. Remote ischemic postconditioning alleviates myocardial ischemia/reperfusion injury by up-regulating ALDH2. Eur Rev Med Pharmacol Sci 2018; 22: 6475-6484.
5) Braunwald E, Kloner RA. Myocardial reperfusion: a double-edged sword? J Clin Invest 1985; 76: 1713-1719.
6) Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 2014; 15: 509-524.

7) Calin GA, Cimmino A, Fabbri M, Ferracin M, Wojcik SE, Shimizu M, Taccioli C, Zanesi N, Garzon R, Aqeilan RI, Alder H, Volinia S, Rassenti L, Liu X, Liu CG, Kipps TJ, Negrini M, Croce CM. MiR-15a and miR-16-1 cluster functions in human leukemia. Proc Natl Acad Sci U S A 2008; 105: 5166-5171.

8) Mendell JT, Olson EN. MicroRNAs in stress signaling and human disease. Cell 2012; 148: 1172-1187.

9) Mei Q, Li F, Quan H, Liu Y, Xu H. Busulfan inhibits growth of human osteosarcoma through miR-200 family microRNAs in vitro and in vivo. Cancer Sci 2014; 105: 755-762.

10) Fabbri M, Paone A, Calore F, Galli R, Gaudio E, Santhanam R, Lovat F, Fadda P, Mao C, Nuovo GJ, Zanesi N, Crawford M, Ozer GH, Wernicke D, Alder H, Caligiuri MA, Nana-Sinkam P, Perrotti D, Croce CM. MicroRNAs bind to Toll-like receptors to induce prometastatic inflammatory response. Proc Natl Acad Sci U S A 2012; 109: E2110-E2116.

11) Zhou M, Cai J, Tang Y, Zhao Q. MiR-17-92 cluster is a novel regulatory gene of cardiac ischemic/reperfusion injury. Med Hypotheses 2013; 81: 108-110.

12) Concepcion CP, Bonetti C, Ventura A. The microRNA-17-92 family of microRNA clusters in development and disease. Cancer J 2012; 18: 262-267.

13) Borkowski R, Du L, Zhao Z, McMillan E, Kosti A, Yang CR, Suraokar M, Wistuba II, Gazdar AF, Minna JD, White MA, Pertsemlidis A. Genetic mutation of p53 and suppression of the miR-17 approximately 92 cluster are synthetic lethal in non-small cell lung cancer due to upregulation of vitamin D Signaling. Cancer Res 2015; 75: 666-675.

14) Liu XJ, Hong Q, Wang Z, Yu YY, Zou X, Xu LH. MicroRNA-34a suppresses autophagy in tubular epithelial cells in acute kidney injury. Am J Nephrol 2015; 42: 168-175.

15) Yin Q, Wang PP, Peng R, Zhou H. MiR-19a enhances cell proliferation, migration and invasion through improving lymphangiogenesis via targeting thrombospondin-1 in colorectal cancer. Biochem Cell Biol 2019. doi: 10.1139/bcb-2018-0302. [Epub ahead of print].

16) Sun G, Lu Y, Li Y, Mao J, Zhang J, Jin Y, Li Y, Sun Y, Liu L, Li L. MiR-19a protects cardiomyocytes from hypoxia/reoxygenation-induced apoptosis via PTEN/PI3K/p-Akt pathway. Biosci Rep 2017; 37. pii: BSR20170899.

17) Kohl M, Wiese S, Warscheid B. Cytoscape: software for visualization and analysis of biological networks. Methods Mol Biol 2011; 696: 291-303.

18) Ke ZP, Xu P, Shi Y, Gao AM. MicroRNA-93 inhibits ischemia-reperfusion induced cardiomyocyte apoptosis by targeting PTEN. Oncotarget 2016; 7: 28796-28805.

19) Wang ZG, Wang Y, Huang Y, Lu Q, Zheng L, Hu D, Feng WK, Liu YL, Ji KT, Zhang HY, Fu XB, Li XK, Chu MP, Xiao J. bFGF regulates autophagy and ubiquitinated protein accumulation induced by myocardial ischemia/reperfusion via the activation of the PI3K/Akt/mTOR pathway. Sci Rep 2015; 5: 9287.

20) Jiang BH, Liu LZ. PI3K/PTEN signaling in tumorigenesis and angiogenesis. Biochim Biophys Acta 2008; 1784: 150-158.

21) Seguella L, Capuano R, Pesce M, Annunziata G, Pesce M, de Conno B, Sarnelli G, Aurino L, Esposito G. S100B protein stimulates proliferation and angiogenic mediators release through RAGE/pAkt/mTOR pathway in human colon adenocarcinoma Caco-2 Cells. Int J Mol Sci 2019; 20. pii: E3240.

22) Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nat Rev Genet 2008; 9: 102-114.

23) Oliveto S, Mancino M, Manfrini N, Biffo S. Role of microRNAs in translation regulation and cancer. World J Biol Chem 2017; 8: 45-56.

24) Li X, Teng C, Ma J, Fu N, Wang L, Wen J, Wang TY. MiR-19 family: a promising biomarker and therapeutic target in heart, vessels and neurons. Life Sci 2019; 232: 116651.

25) Li Z, Li Y, Wang Y. MiR-19a promotes invasion and epithelial to mesenchymal transition of bladder cancer cells by targeting RhoB. J BUON 2019; 24: 797-804.

26) Peng Y, Huang D, Ma K, Deng X, Shao Z. MiR-19a as a prognostic indicator for cancer patients: a meta-analysis. Biosci Rep 2019; 39. pii: BSR20182370.