Effects of miR-26a-5p and PTEN on Myocardium of Myocardial Ischemia-reperfusion Mice

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This study aimed to investigate the effects of miR-26a-5p and PTEN on myocardium of myocardial ischemia-reperfusion mice. Twelve C57/B6 male mice were randomly selected and divided into control group and mouse model group with 6 mice in each group, in which no surgical modeling was normally performed. Mice were killed 2 hours after operation to collect myocardial tissue. H9C2 was transfected with miR-26a-5p-mimic and blank vector to overexpress miR-26a-5p, and then normal group, hypoxia/reoxygenation group (cell model group), blank group and overexpression group were established respectively. Protein expressions of PTEN, Caspase-3, Caspase-9, P13K and p-Akt in tissues and cells of mice were detected. Expressions of miR-26a-5p and PTEN in myocardial tissue and cells of mice were detected. Apoptosis was detected. The relationship between miR-26a-5p and PTEN was determined. Expressions of miR-26a-5p in tissues of mouse model group were lower than those of normal group, while PTEN expressions were opposite ($p<0.05$). Expressions of miR-126 in overexpression group were increased compared with those of cell model group and blank group, and decreased compared with those of control group ($P<0.05$). Expressions of PTEN in overexpression group were lower than those in cell model group and blank group, and higher than those in control group ($p<0.05$). Compared with control group, miR-26a-5p was decreased and PTEN was increased in cell model group ($P<0.05$). Compared with blank group and cell model group, expressions of PTEN, Caspase-3 and Caspase-9 proteins in overexpression group were significantly decreased, while expressions of P13K and p-Akt proteins were significantly increased ($p<0.05$). Apoptosis rate in overexpression group was significantly lower than that in cell model group and blank group ($p<0.05$). Dual-luciferase reporter proved that there was a targeted regulatory relationship between miR-26a-5p and PTEN. In conclusion, up-regulation of miR-26a-5p regulates PTEN/PI3K/p-Akt signaling pathway and reduces myocardial cell apoptosis, thus improving ischemia-reperfusion injury.

Key words: miR-26a-5p, PTEN, myocardial ischemia-reperfusion
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INTRODUCTION

Due to continuous changes in people's living standards and diet structure in recent years, the incidence rate of basic diseases is also increasing. Some surveys showed that [1] in 2013, more than 20% of adults in China had cardiovascular diseases, and about 3.5 million people died of cardiovascular diseases every year. The mortality rate is increasing, which is mainly closely related to ischemic heart disease death. Ischemic heart disease (IHD), also known as coronary atherosclerotic heart disease (CHD), is a common clinical cardiovascular disease [2]. As atherosclerosis occurs in the coronary artery wall, the vascular lumen is gradually narrowed or blocked, resulting in myocardial hypoxia, ischemia and necrosis [3]. At present, clinical treatment of CHD is mainly through timely thrombolysis and percutaneous coronary intervention, but patients will suffer from cardiac ischemia-reperfusion injury during the treatment [4].

Ischemia-reperfusion is a common phenomenon in clinical surgical treatment, which is more common in liver, heart and brain operations [5]. Cardiac ischemia-reperfusion can cause cardiac contraction disorder, arrhythmia, microangiopathy

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and myocardial cell apoptosis, thus causing irreversible damage [6]. Therefore, how to reduce ischemia-reperfusion injury is a hot topic in cardiovascular field in recent years. As a tumor suppressor gene, deletion of phosphate gene and tension homologue gene on chromosome ten (PTEN) plays a regulatory role in the process of cell growth and apoptosis [7]. Studies have shown that [8] PTEN can regulate the activity of Akt by inhibiting PI3K and play a role in regulating cell apoptosis. Therefore, whether PTEN expressions can be regulated to improve apoptosis inhibited by ischemia-reperfusion is crucial.

microRNA (miR) has been a hot research topic in various fields in recent years. miR is a 22nt long non-coding short-chain RNA. Studies have shown that miR can be combined by regulating mRNA 3'-endnon-translated region of downstream target gene, thus playing a role in regulating downstream target gene [9, 10]. miR-26a-5p, as one of the important members of miR family, studies show that it has certain regulatory effects in lung cancer, osteosarcoma and hepatoblastoma [11-13]. However, the role of miR-26a-5p in ischemia-reperfusion is currently less studied. In this study, we detected and found that miR-26a-5p can target and regulate PTEN expressions through dual-luciferase reporter.

Therefore, this study provides references for clinical treatment by exploring effects of miR-26a-5p and PTEN on myocardium of mice with myocardial ischemia-reperfusion.

DATA AND METHODS

Cell and Animal Sources

H9C2 myocardial cells were purchased from American ATCC Corporation (ATCC®CRL-1446). Twelve C57/B6 male mice, aged 8 weeks, with a mass of 23~25 g, were purchased from Beijing Weitonglihua Experimental Animal Technology Co., Ltd., China. The animal experiment certificate number was SCXK (Beijing) 2015-0001. After bought back, mice were sent to the standard feeding room of the animal research center of Guangxi Medical Universityfor one week. The room temperature was 22~26 ℃, the relative humidity was 50%~65%, and the circulation system alternated between day and night for 12 hours.

Main Materials and Instruments

Isoflurane for animals (R510-22, Rayward Life Science and Technology, Shenzhen City, China); Fetal Bovine Serum (FBS), PBS (US Gibco Company, 10437028, 10010010049); trypsin, RIPA, BCA Protein Kits (US Thermo Scientific, A40007, 89901, 23229); TRIzol™ Reagent, ECL Chemiluminescence Detection Kit; Lipofect AMINE 2000 Kit (US Invitrogen Company, 15596018, 35050, 11668500); PTEN, P13K, p-Akt, Caspase-3, Caspase-9,β-Actin, HRP-labeled goat anti-rabbit IgG secondary antibody (Abcam company of the United States, ab32199, ab32360, ab214430, ab52298, ab38449, ab6721); Trans Script Green miRNA Two-Step qRT-PCR Super Mix, Trans Script II Green Two-Step qRT-PCR Super Mix (Beijing, China, Trans Gen Biotech, AQ202-01, AQ301-01); Annexin V/PI Apoptosis Detection Kit (Shanghai Yisheng Biotechnology Co., Ltd., 40302ES20); dual-luciferase reporter gene detection kit (China Beijing Solarbio Co., Ltd., D0010); miR-26a-5p, PTEN and miR-26a-5p overexpressions (miR-26a-5p-mimic) and blank sequence (miR-26a-5p-NC) were designed and synthesized by Shanghai Bioengineering Co., Ltd.; PCR instrument (ABI Company, 7500, USA); R500 general-purpose small animal anesthesia machine (Rayward Life Science and Technology, Shenzhen, China, R500).

Establishment of Animal Model

Six mice were taken as mouse model group. Mice in model group were anesthetized with 2% isoflurane and maintained at 1.5-2%. Skin was cut off in the left 3-4 ribs of mice, muscles were bluntly separated, and myocardium was quickly squeezed out after finding out the heart. Ligation was performed at 2 mm below the junction of the left aurcle and arterial cone. Then, the heart was put back into chambers of the heart, the muscle was compounded and suturing was performed finally. Observing mice with electrocardiogram, ST segment elevation indicated successful model making. After 30 min of ligation, the ligature was pulled out to resume perfusion. The remaining 6 mice were not treated as normal. Abdominal anesthesia was performed with 1% pentobarbital 2 hours after operation. Myocardial tissue of mice was collected and then neck was severed and executed. Collected myocardial tissue was taken for subsequent experiments.

Establishment of Hypoxia/Reoxygenation Cells and Plasmid Transfection

Repurchased H9C2 cells were cultured in 10% DMED medium (10% FBS, penicillin-streptomycin double-antibody) and transferred to 37℃, 5% CO_2 for 48 h. After 48 hours, cell proliferation was observed, DMED medium (no 10% FBS, penicillin-streptomycin double-antibody) was replaced, and cultured in 37℃ anoxic incubator for 12 hours. After anoxia was completed, the culture medium was replaced with 10% DMED culture medium, which was transferred to 37℃, 5% CO_2 for 24 hours to complete anoxia/reoxygenation models of myocardial cells. After the modeling of H9C2 cells was completed, 50 nmol/L miR-26a-5p was transfected into myocardial cells by Lipofect AMINE 2000 kit. Cells were divided into control

group (H9C2 was not subjected to hypoxia/reoxygenation modeling), cell model group, blank group (miR-26a-5p-NC) and overexpression group (miR-26a-5p-mimic).

Detection of Expressions of miR-26a-5p, PTEN and mRNA

Collected cells and myocardial tissue were extracted with TRIzol kit for total RNA, and extracted total RNA was detected for purity, concentration and integrity by ultraviolet spectrophotometer and agarose gel electrophoresis. The detection method of miR-26a-5p was as follows: Trans Script® miRNA RT Enzyme Mix and 2×TS miRNA Reaction Mix were used to reverse transcription with total RNA, and operation steps were strictly in accordance with manufacturer's kit. Then PCR amplification experiment was carried out. PCR reaction system was as follows: 1 μL of cDNA, 0.4 μL of upstream and downstream primers each, 10 μl of 2×Trans Taq®Tip Green qPRC Super Mix, 0.4 μL of Passive Reference Dye(50X), finally ddH2O was added to make up to 20 μL. PCR reaction conditions were as follows: Predegeneration at 94℃ for 30 s, degeneration at 94℃ for 5 s, annealing at 60℃ for 30 s, with a total of 40 cycles. Each sample was provided with 3 repeated holes, and experiment was carried out for 3 times. U6 was used as internal reference and $2^{-\Delta\Delta ct}$ was used to analyze the data of this study. Detection schemes of PTEN and mRNA were as follows: Reverse transcription was performed by 5X Trans Script® II All-in-One Super Mix for qPCR and gDNA Remover kits, and procedures were strictly in accordance with manufacturer's kits. Then PCR amplification experiment was carried out. PCR reaction system was as follows: 1 μL of cDNA, 0.4 μL of upstream and downstream primers each, 10 μL of 2X Trans Script® Tip Green qPCR Super Mix, Passive Reference Dye (50X). Finally, Nuclease-free Water was added to make up to 20 μL. PCR reaction conditions were as follows: Predegeneration at 94℃ for 30 s, degeneration at 94℃ for 5 s, annealing at 60℃ for 30 s, with a total of 40 cycles. Each sample was provided with 3 repeated holes, and experiment was carried out for 3 times. GAPDH was used as internal reference and $2^{-\Delta\Delta ct}$ was used to analyze the data of this study. MiR-26a-5p upstream sequence 5'-ATGGTTCGTGGGTTCAAGTAATCGATGAGGC-'3, downstream sequence 5'-GCAGGGTCCGAGTATTCG-'3; PTEN upstream sequence 5'-CCGCTGTGTGTGGTGatatc-'3, downstream sequence 5'-GAATGTATTTACCCAAAAGTGAAATT-'3; U6 upstream sequence 5'-CTCGCTTCGGCACA-'3, downstream sequence 5'-AACCTTCACGAATTTGCGT-'3; GAPDH upstream sequence 5'-GGAAGGTGAGGTCGGAG-'3, downstream sequence 5'-CGTTCTCACCTGCGG-'3.

Protein Detection

Cultured myocardial cells were extracted with RIPA lysis method to extract total protein, protein concentration was detected with BCA method, protein concentration was adjusted to 4 μg/μL, 12% SDS-PAGE electrophoresis separation was carried out, the membrane was transferred to PVDF membrane after ionization, staining was carried out with ponceau working solution, PBST was soaked for 5 min, washing was carried out, sealing was carried out with 5% skim milk powder for 2 h, PTEN, P13K, p-Akt, Caspase-3, Caspase-9, β-Actin primary antibody (1: 1000) were added to seal overnight at 4℃. The membrane was washed to remove primary antibody, horse radish peroxidase labeled goat anti-rabbit secondary antibody (1: 5000) was added, incubated at 37℃ for 1 h, rinsed with PBS 3 times for 5 min each time. Developing in darkroom, absorbing excess liquid on membrane with filter paper, ECL emitting light and developing. Protein bands were scanned and gray value was analyzed in Quantity One software, where the relative expression level of protein was equal to target protein band gray value / β-Actin protein band gray value.

Detection of Apoptosis

Transfected cells were digested with 0.25% trypsin, washed twice with PBS after digestion, added with 100 μL of binding buffer, prepared into 1*106 /mL suspension, sequentially added with Annexin V-FITC and PI, incubated in dark at room temperature for 5 min, and detected with FC500MCL flow cytometer system. The experiment was repeated for 3 times and averaged.

Prediction of Target Genes

Targetscan7.2 and miRwalk were used to predict downstream target gene of miR-26a-5p. Reporter gene plasmids containing wild-type and mutant PTEN 3'UTR, psiCHECK-2 plasmids (Promega, C8021, USA), miR-26a-5p-mimc and their controls were transfected into H9C2 cells for 48 hours and then used. Dual-luciferase reporter gene detection kits were used for operation. Finally, collected cells were detected by chemiluminiscence ELIASA. Three repetitions were designed for each group of experiments, and each experiment was repeated for three times.

Statistical Methods

In this study, SPSS 20.0 software was used to analyze the collected data, Graph Pad Prism 7 software was used to draw relevant pictures, and K-S was used to analyze the data distribution; measurement data were expressed as mean ± standard deviation (SD ± meas) and t test was used;

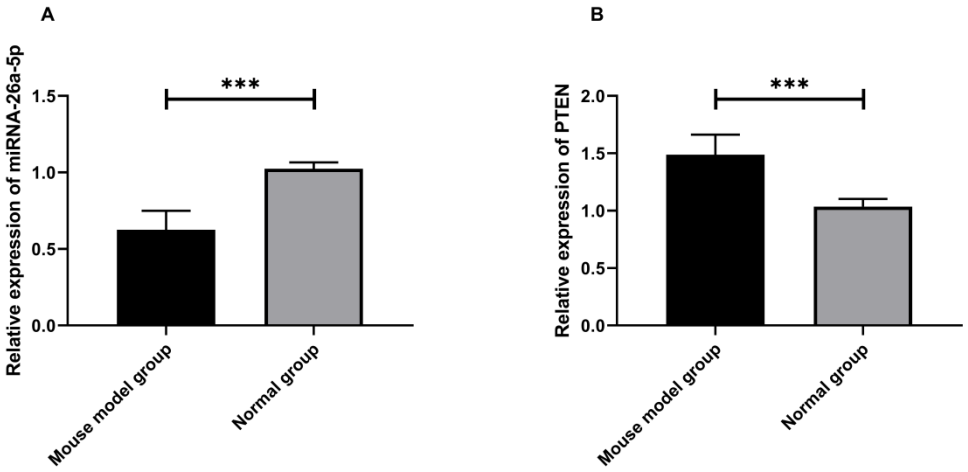
independent-samples T test was used for inter-group comparison, denoted by t; one-way ANOVA was used for multi-group comparison, denoted by f; LSD-t test was used for post-event comparison, and when $p<0.05$, there were statistical differences.

RESULTS

Expressions of miR-26a-5p and PTEN in Tissues of Mice in Model Group

QRT-PCR was used to detect expressions of miR-26a-5p and PTEN in myocardial tissue of mice after modeling. It was found that expressions of miR-26a-5p in myocardial tissue of mice in model group were significantly lower than those of mice in normal group, and there were significant differences in expressions of PTEN ($p<0.05$). See Figure 1 for details.

Figure 1
Relative Expressions of miR-26a-5p and PTEN in Myocardial Tissue of Animal Model



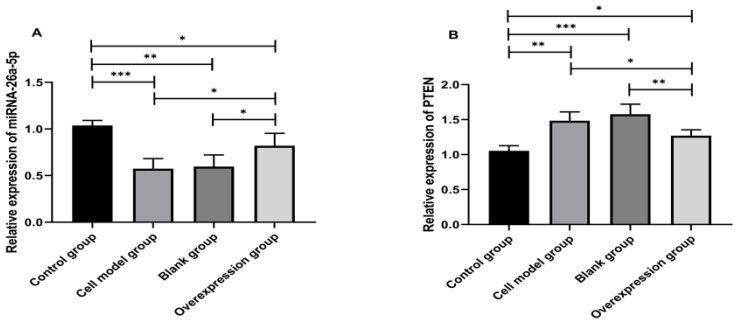
A. Expressions of miR-26a-5p in myocardial tissue of model group (0.625±0.124) were lower than those of control group (1.025±0.041), with differences (t=7.502, p<0.001).

B. PTEN expressions in myocardial tissue of model group (1.488±0.174) were lower than those of control group (1.036±0.066), with differences (t=5.949, p<0.001)

Expressions of miR-26a-5p and PTEN in Myocardial Cells after Transfection

QRT-PCR detection found that expressions of miR-26a-5p and PTEN in transfected cells of each group were significantly different ($F_{miR\text{-}26a\text{-}5p}=11.938$, $P_{MIR\text{-}26a\text{-}5p}=0.003$; $F_{PTEN}=13.344$, $P_{PTEN}=0.002$), in which expressions of miR-126 after overexpression transfection of myocardial cells were significantly higher than those of cell model group and blank group, but significantly lower than those of control group ($p<0.05$). Observation of PTEN expressions in myocardial cells of each group showed that PTEN expressions in overexpressed group were significantly lower than those in cell model group and blank group, but significantly higher than those in control group ($p<0.05$). There was no significant difference in miR-26a-5p and PTEN expressions between cell model group and blank group ($p>0.05$); compared with control group, miR-26a-5p was significantly decreased, PTEN was significantly increased, and there were differences ($p<0.05$).See Figure 2 for details.

Figure 2
Expressions of miR-26a-5p and PTEN in Myocardial Cells after Transfection

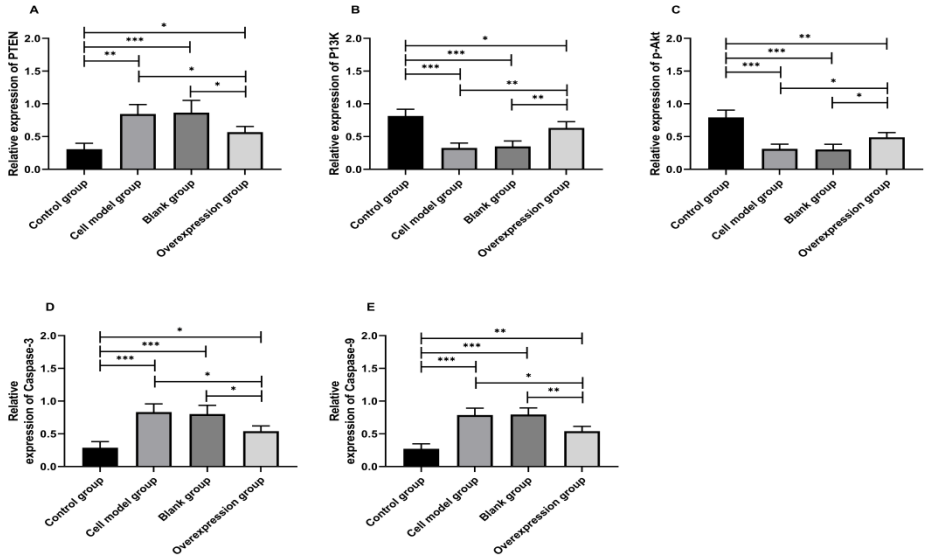


A. Relative expressions of miR-26a-5p in myocardial cells after transfection
B. Relative expressions of PTEN in myocardial cells after transfection
* means p<0.05, * * means p<0.01, and * * * means p<0.001.

Expressions of Proteins in Cells

Expressions of PTEN, P13K, p-Akt, Caspase-3, Caspase-9 proteins in transfected cells were detected by WB, which found that compared with control group, expressions of PTEN, Caspase-3, Caspase-9 proteins in cell model group, blank group and overexpression group increased significantly, while expressions of P13K and p-Akt proteins decreased significantly (p<0.05). Compared with blank group and cell model group, expressions of PTEN, Caspase-3 and Caspase-9 proteins in overexpression group significantly decreased, while expressions of P13K and p-Akt proteins significantly increased (p<0.05). Expressions of PTEN, P13K, p-Akt, Caspase-3, Caspase-9 proteins in cell model group and blank group had no significant difference (p>0.05), as shown in Figure 3.

Figure 3
Expressions of PTEN, P13K, p-Akt, Caspase-3, Caspase-9 Proteins in Cells



A. Expressions of PTEN protein in cells of each group after transfection
B. Expressions of P13K protein in cells of each group after transfection
C. Expressions of p-Akt protein in cells of each group after transfection
D. Expressions of Caspase-3 protein in cells of each group after transfection
E. Expressions of Caspase-9 protein in cells of each group after transfection
WB bar graph
* means p<0.05, * * means p<0.01, and * * * means p<0.001.

Apoptosis after Transfection

Detection of apoptosis in each group after transfection showed that compared with control group, the apoptosis rate in cell model group, blank group and overexpression group was significantly increased ($p<0.05$), while the apoptosis rate in overexpression group was significantly lower than that of cell model group and blank group ($p<0.05$), and there was no difference in the apoptosis rate between cell model group and blank group ($p>0.05$), as shown in Figure 4.

Figure 4
Apoptosis after Transfection
Apoptosis Rate in Each Group

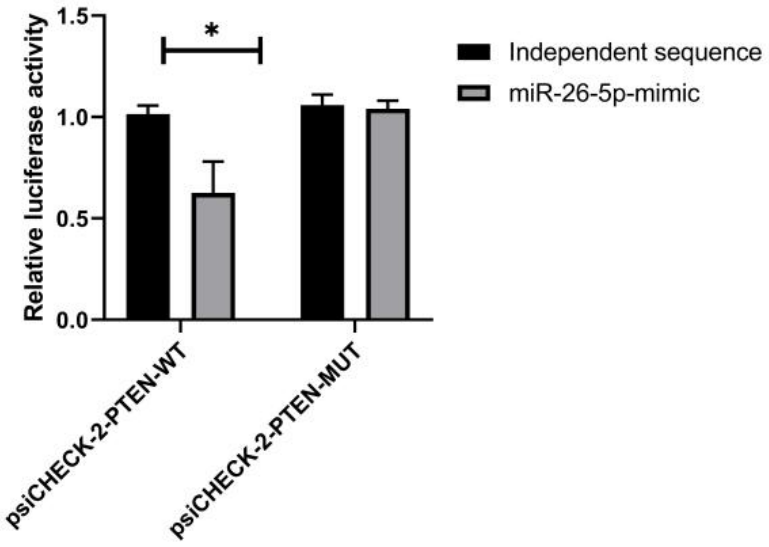
A

Mirna	Refseqid	Genesymbol	Score	Position	Binding Site	Au	Me	N Pairings	Targetscan	Mirdb	Mirtarbase
hsa-miR-26a-5p	NM_000314	PTEN	0.92	3UTR	8461,8484	0.71	-6.107	18	LINK	Link	MIRT001095
hsa-miR-26a-1-3p	NM_000314	PTEN	0.85	3UTR	2679,2699	0.57	-7.705	18	--	--	MIRT622568
hsa-miR-26b-5p	NM_000314	PTEN	0.85	3UTR	4848,4870	0.59	-8.574	18	LINK	Link	MIRT438485
hsa-miR-26b-3p	NM_000314	PTEN	0.92	3UTR	2934,2969	0.57	-9.185	19	--	--	--
hsa-miR-27a-5p	NM_000314	PTEN	1.00	5UTR	545,565	0.23	-4.65	18	--	--	--
hsa-miR-27a-5p	NM_000314	PTEN	0.92	5UTR	820,841	0.31	-9.687	17	--	--	--
hsa-miR-27a-5p	NM_000314	PTEN	1.00	3UTR	4887,4905	0.53	-12.255	17	--	--	--
hsa-miR-27a-5p	NM_000314	PTEN	0.82	5UTR	972,992	0.44	-9.137	15	--	--	--
hsa-miR-27a-3p	NM_000314	PTEN	0.88	5UTR	199,247	0.38	-4.673	19	--	--	--
hsa-miR-28-5p	NM_000314	PTEN	1.00	3UTR	3398,3421	0.63	-6.87	18	--	--	--

B

Position 2619-2626 of PTEN 3' UTR	5'	...UUACAUGUCUGAAGUUACUUGAA...
hsa-miR-26a-5p	3'	UGGAUAGGACCUAAUGAACUU

C



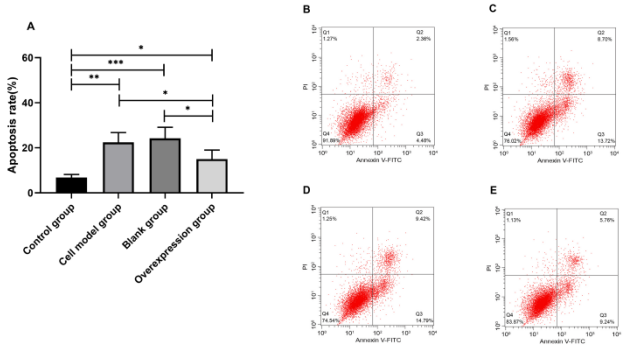
X

* means $p<0.05$, * * means $p<0.01$, and * * * means $p<0.001$.

Identification of miR-26a-5p Target Gene

Prediction of miR-26a-5p downstream target genes by Targetscan7.2 and miRwalk found that PTEN and miR-26a-5p had targeted binding sites, so we verified it by dual-luciferase reporter. Activity of luciferase in miR-26a-5p-mimc group in psiCHECK-2-PTEN-WT was significantly lower than that of independent sequence group ($p<0.05$), while activity of luciferase in miR-26a-5p-mimc group in psiCHECK-2-PTEN-MUT group was not significantly different from that of independent sequence group ($p>0.05$), as shown in Figure 5.

Figure 5
Dual-luciferase Reporter of miR-26a-5p and PTEN



A. PTEN binding targets were predicted by mirwalk3.0 online software miR-26a-5p.
B. Binding targets of miR-26a-5p and PTEN5'-3'-UTRT were predicted by Targetscan7.2 online software.
C. Activity detection of dual-luciferase reporter gene
* means that comparison between the two groups is $p<0.05$.

DISCUSSION

At present, the most effective treatment for ischemic heart disease is reperfusion therapy, but it may cause ischemia-reperfusion injury to patients while recovering the blood supply in the ischemic region of patients' heart [14]. After myocardial ischemia-reperfusion injury occurs, myocardial tissue will have large necrosis area, inflammatory reaction, etc. In the long run, the occurrence of inflammatory cascade reactions will not only aggravate case of patients, but may endanger the life safety of patients to some extent [15]. Therefore, how to improve case of patients is one of the urgent problems for clinical scholars to solve.

PTEN is a newly discovered tumor suppressor gene in recent years. Protein products are mainly distributed in cytoplasm, karyotheca and nucleus [16]. Studies have shown that [17] PTEN has lipid and protein phospholipase activities, and its phospholipase can antagonize PI3K inhibition of p-Akt and thus play a role in regulating apoptosis. Previous studies have shown that [18] PTEN can antagonize PI3K inhibition of p-Akt and promote tumor apoptosis through overexpression. Other studies have shown that expressions of PTEN in [19] myocardial cells will obviously increase. Therefore, we propose whether PTEN expressions can be negatively regulated to reduce myocardial cell apoptosis and improve the injury of ischemia-reperfusion to patients, thus achieving preventive or therapeutic effects.

In recent years, many studies have found that miRs can participate in cardiac remodeling and occurrence of cardiovascular diseases. In studies of Wang et al. [20], activated macrophages can secrete exosomes containing miR-155 into myocardial fibroblasts, which can inhibit the proliferation of fibroblasts and accelerate inflammatory response, leading to exacerbation of myocardial infarction in patients. As an important member of miR family, miR-26a-5p is closely related to the occurrence and development of tumors and cardiovascular diseases [21]. Previous reports by Zheng et al [22] showed that miR-26a-5p can induce collagen expressions of myocardial fibroblasts by targeted regulation of ULK1. However, this study found that miR-26a-5p and PTEN have binding targets and regulatory relationships. Therefore, in this study, we explore expressions and influences of miR-26a-5p and PTEN in ischemia-reperfusion myocardial cells to provide new references for clinical treatment.

In this study, we first established a mouse heart ischemia-reperfusion model. By detecting relative expressions of miR-26a-5p and PTEN in the myocardial tissue of the model mouse, we found that expressions of miR-26a-5p in the myocardial tissue of model mouse group were significantly lower than those of normal group, while expressions of PTEN were significantly higher, which indicated that expressions of miR-26-5p would decrease after ischemia-reperfusion and might be a potential CHD diagnostic indicator. Subsequently, we established an in vitro cell line hypoxia/reoxygenation cell model, and transferred the overexpressed miR-26a-5p plasmid into cells for culture, further detected relative expressions of miR-26a-5p and PTEN in cells, and found that expressions of cell model group and control group were consistent with those of mouse model. In the study of Keyes et al. [23], it was found that myocardial injury in myocardial ischemia-reperfusion model was significantly improved by inhibiting PTEN expressions. However, in this study, we found that miR-26a-5p expressions were significantly increased and PTEN expressions were inhibited after transferring the plasmid that overexpressed miR-26a-5p into anoxic/reoxygenation cells. Further detection of apoptosis showed that the apoptosis rate of overexpressed group was significantly lower than that of cell model group and blank group.

Moreover, dual-luciferase reporter proved that miR-26a-5p could target PTEN, which suggested that overexpressed miR-26a-5p could target PTEN expressions and reduce apoptosis.

However, it was not clear how miR-26-5p regulated apoptosis. Therefore, we further detected expressions of PI3K and p-Akt in cells. PI3K and PTEN could interact with each other and had feedback regulation effects, which was of great significance in cell proliferation, apoptosis and metabolism [24]. Akt, as a closely related downstream kinase in PI3K pathway, plays a role by phosphorylating it [25]. Research by Ravingerová et al. [26] showed that PI3K/p-Akt signaling pathway could regulate survival and size of myocardial cells after activation. This study found that PTEN protein expressions in overexpression group were significantly inhibited, and PI3K and p-Akt protein expressions were significantly increased, which indicated that up-regulation of miR-26a-5p could inhibit PTEN expressions, thus activating PI3K and p-Akt signaling pathways and inhibiting myocardial cell apoptosis. Finally, we also detected expressions of apoptosis-related proteins Caspase-3 and Caspase-9. Caspase participates in the regulation of cell growth, differentiation and apoptosis, among which Caspase-3 and Caspase-9 are important regulatory factors in Caspase family [27]. We found that expressions of Caspase-3 and Caspase-9 protein were significantly inhibited compared with model group after overexpression of miR-26a-5p. This suggested that up-regulation of miR-26a-5p could reduce myocardial cell apoptosis by inhibiting PTEN protein activity and activating PI3K/p-Akt signals by reducing expressions of Caspase-3 and Caspase-9 proteins.

However, this study still has certain limitations. Firstly, we have not established a miR-26a-5p inhibitor vector, and it is unclear whether the inhibition of miR-26a-5p expressions specifically affects myocardial cell apoptosis. Secondly, this study does reduce cell apoptosis by up-regulating miR-26a-5p expressions, but there is still a certain difference compared with normal group, which may be related to measurement. Finally, as a basic experiment, whether this study can play a role in clinic is still unknown. Therefore, we hope to add more groups in future studies, adjust the measurement, and try to carry out clinical research to supplement our research results.

To sum up, up-regulation of miR-26a-5p regulates PTEN/PI3K/p-Akt signaling pathway and reduces myocardial cell apoptosis, thus playing a role in improving ischemia-reperfusion injury.

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