miRNA-21 improve myocardial damage induced by Ischemia-reperfusion by regulation PTEN

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Abstract: miRNAs are rich in myocardial cells, involved in myocardial apoptosis, arrhythmia, myocardial hypertrophy and heart failure. In this study, we found that miRNA-21 over-expression had effects to improve myocardial pathology and myocardial apoptosis which induced by Ischemia-reperfusion in vitro. We found that PTEN was a direct target of miRNA-21 in myocardial damage induced by Ischemia-reperfusion. Meanwhile, miRNA-21 regulated the PTEN/AKT/ERK/P21 pathway. Our results suggested that miRNA-21 over-expression has effects to improve myocardial damage induced by Ischemia-reperfusion by targeting PTEN.

Key words: myocardial damage, Ischemia-reperfusion, miRNA-21, PTEN

Introduction

The recovery of blood reperfusion after myocardial ischemia can lead to myocardial damage, which can lead to the decrease of myocardial function and even endanger the life. This phenomenon is called myocardial ischemia reperfusion injury (1), It has become one of the important causes of coronary heart disease especially acute myocardial infarction treatment effect of blood supply reconstruction, mainly for reperfusion induced arrhythmias, myocardial stunning, microvascular obstruction, lethal myocardial reperfusion injury.

microRNAs (miRNAs) are a kind of endogenous consists of about 22 nucleotides encoding non small single stranded RNA, and the target mRNA 3 'non encoding region of complete or incomplete pairing, degradation of target mRNA or inhibiting the translation, down-regulation of protein level in the negative regulation of the expression of target gene transcription (2). miRNAs were involved in many physiological and pathological processes, such as apoptosis, cell differentiation, angiogenesis, lipid metabolism, energy metabolism, and so on. About 30% of human genes are regulated by miRNAs (3, 4). The relative studies found that miRNA-21 was an important role in cell apoptosis (5-7). Nevertheless, It has been unclear that the effects and mechanisms of miRNA-21 in development of myocardial apoptosis induced by Ischemia-reperfusion.

Materials and methods

Animals and treatments

Rats were purchased from Nanjing Medicinal University, male, body weight 250~300g, the rats were random divided into 3 groups: NC group which treated with normal; BL group which injected the empty vector from the caudal vein; miRNA groups were injected miRNA-21 from the caudal vein. The rats of BL and miRNA groups were injected mixed liquor which contained ketamine (80 mg/kg), Phenothiazine (5mg/kg) and Atropine (0.6mg/kg). After anaesthesia, Tracheal intubation, using small animal ventilator to control breathing. Under aseptic condition,

thoracotomy was performed on the left fourth intercostal space, exposing the heart, clamping the left anterior descending coronary artery 5 min, and then restoring the perfusion 5 min, so repeated 4 times. After the completion of the ischemic treatment, the recovery of blood flow was 3 h, which induced ischemia reperfusion injury.

H&E staining

At 24 h after reperfusion, the myocardial tissues of difference groups were collected in mice, the myocardial tissues were paraffin embedded sections, Hematoxylin eosin (H & E) staining, after that, the pathological changes were observation under optical microscope (×200).

TUNEL assay

The myocardial tissues of difference groups in mice were fixed in the 10% formaldehyde, conventional paraffin embedding, slicing every 1mm, Cell apoptosis was detected by in situ port end labeling according to the TUNEL kit; the slice were observation under optical microscope (×200). The apoptotic cells were brown. The apoptosis index and total cell number were analyzed by image analysis software.

IHC assay

The TLR4, JAK2 and STAT3 proteins expressions were measured in the sections by S-P method, the detail steps were following by instructions. The PTEN, AKT, Erk and P21 anti-bodies were purchased from Abcam Company (USA) which the concentrations were 1:50; the use of ultra sensitive ELISA Kit (mouse / rabbit), liquid DAB chromogenic enzyme substrate kit were purchased from Maixin Biotechnology Co., ltd. (China).

WB assay

The myocardial tissue was cut in the homogenizer and homogenized, adding 400 μl single detergent cracking liquid contained PMSF and placed on ice, after cleavage for 30min, the lysate was moved into 1.5ml centrifuge tube, centrifugal at 4 ℃ as 12000r/min for 5min, the supernatant were taken out, and measured the protein concentration by BCA method, taking the same amount of protein by SDS-PAGE, The protein was transferred to PVDF membrane, and defatted milk powder (TBST buffer)

was used to seal 1h at room temperature, adding primary antibodies including PTEN, AKT, Er, P21 and GAPDH (Abcam, USA) as 1:1000, cultured at 4℃ over-night, washing by TBST for 3 times, adding second antibody as 1:100, cultured at room temperature for 2 h, washing by TBST for 3 times, development of chemiluminescence (ECL) method.

Statistical analysis

The relative data were analyzed by SPSS 19.0 software (Chicago, USA). The data were express as mean±SD (standard deviation). Group data were compared by one-way ANOVA, and LSD was used for multiple comparisons between groups. $P < 0.05$, there were significantly difference.

Results

miRNA-21 and myocardial pathology

Some myocardial cells showed coagulation or banded necrosis in the NC and BL group; Compared with NC group, Mild myocardial interstitial edema, but the myocardial cell line is clear, no other pathological changes in the miRNA group. The detailed data were shown in Figure 1.

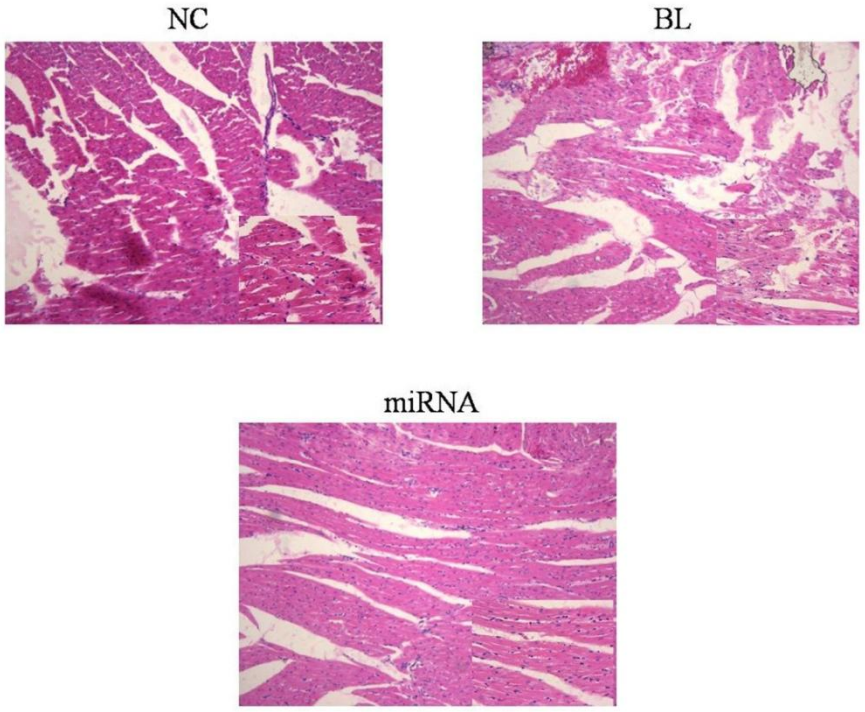

Figure 1. The pathology of difference groups by H＆E staining (200×)

miRNA-21 has effects to myocardial apoptosis in vitro

Compared with NC group, the positive apoptosis myocardial cell of miRNA group was significantly suppressed ($P<0.05$). The relative data were shown in Figure 2.

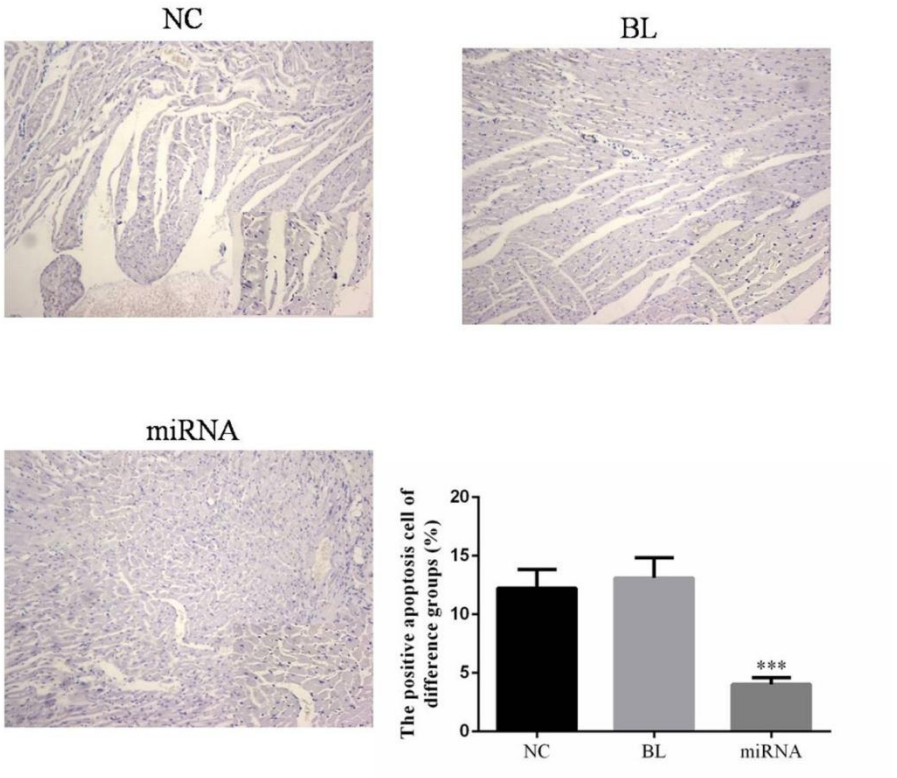

Figure 2. The cell apoptosis of difference groups by TUNEL assay (200×)

***: Compared with NC group

miRNA-21 has effects to relative proteins expressions by IHC

Compared with NC group, the PTEN and P21 proteins expression of miRNA group were significantly suppressed (P＜0.05, respectively, Figure 3 and Figure 6); The ERK and AKT protein expression of miRNA groups were significantly increased (P＜0.05, respectively, Figure 4 and Figure 5).

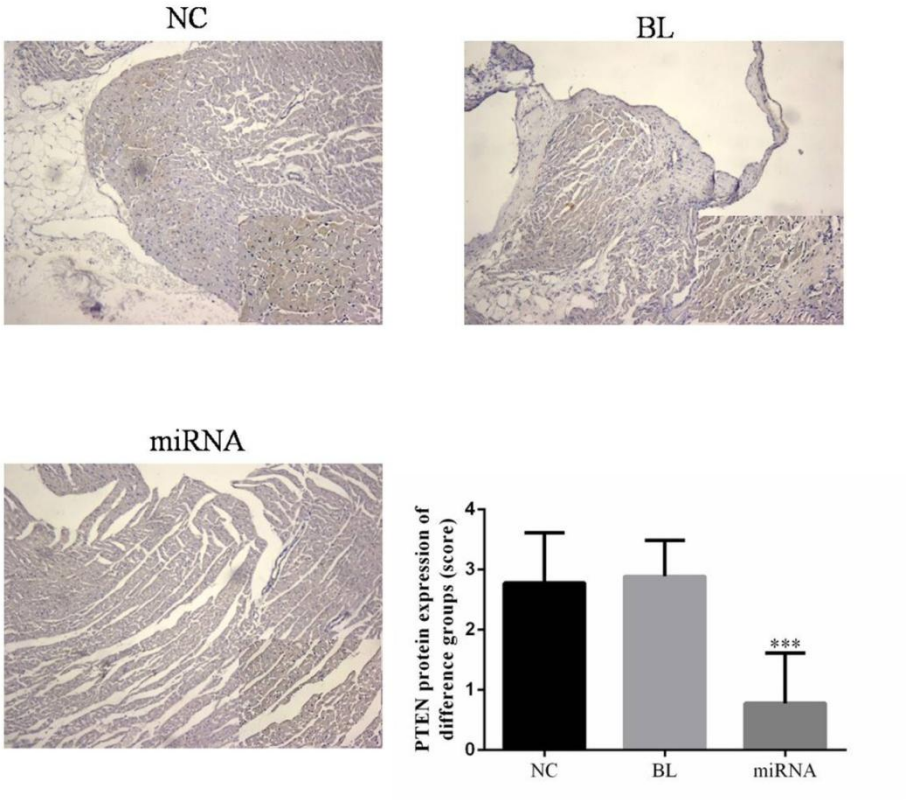

Figure 3. The PTEN protein expression of difference groups by IHC (200×)

***: Compared with NC group

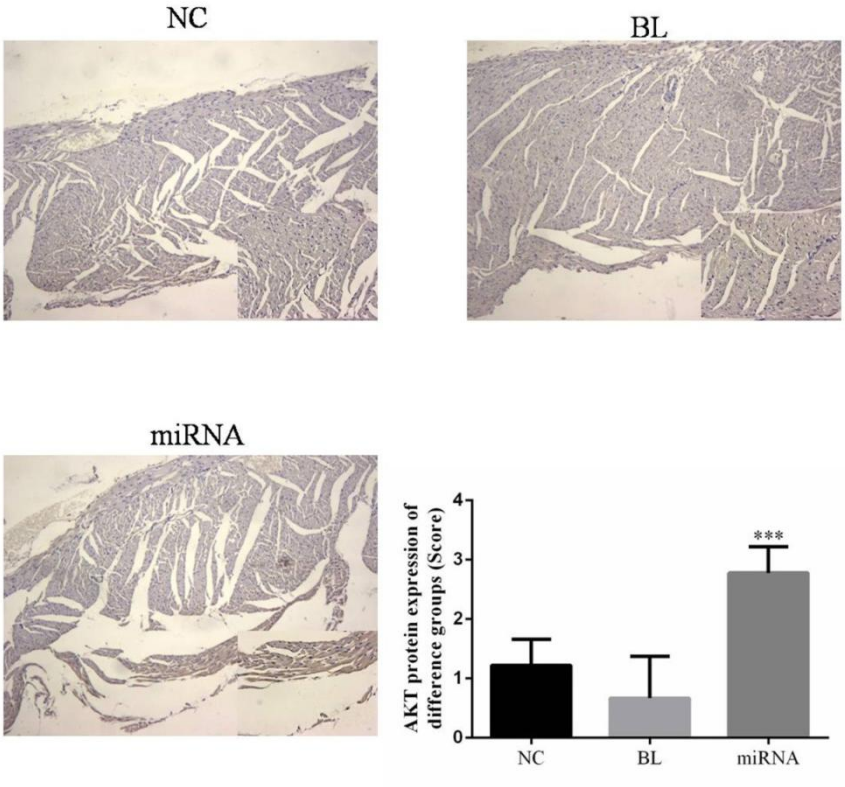

Figure 4. Then AKT protein expression of difference groups by IHC (200×)

***: Compared with NC group

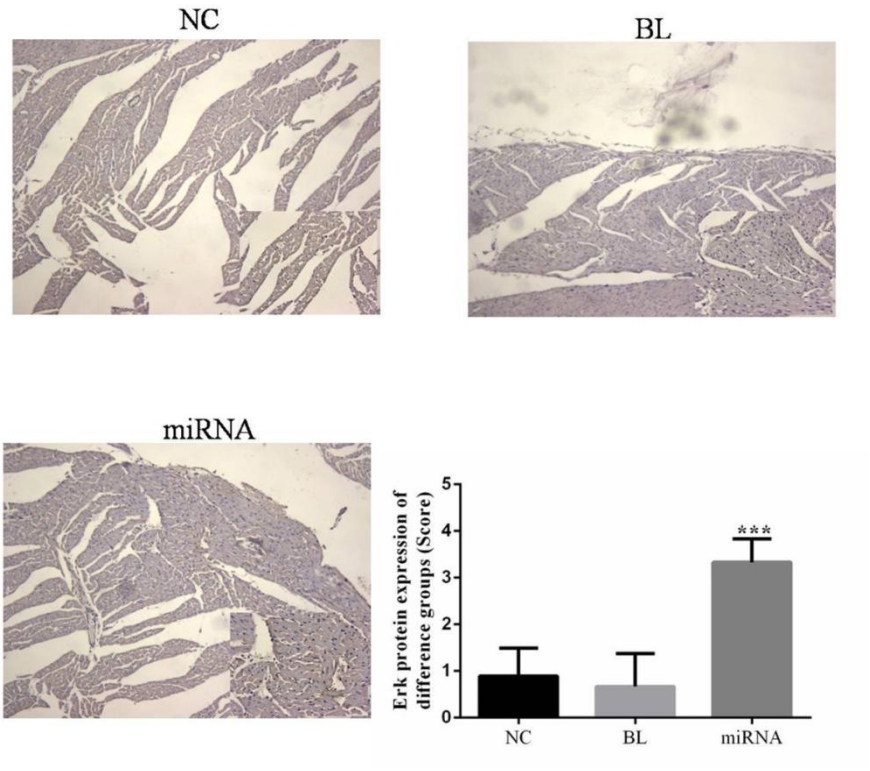

Figure 5. The ERK protein expression of difference groups by IHC (200×)

***: Compared with NC group

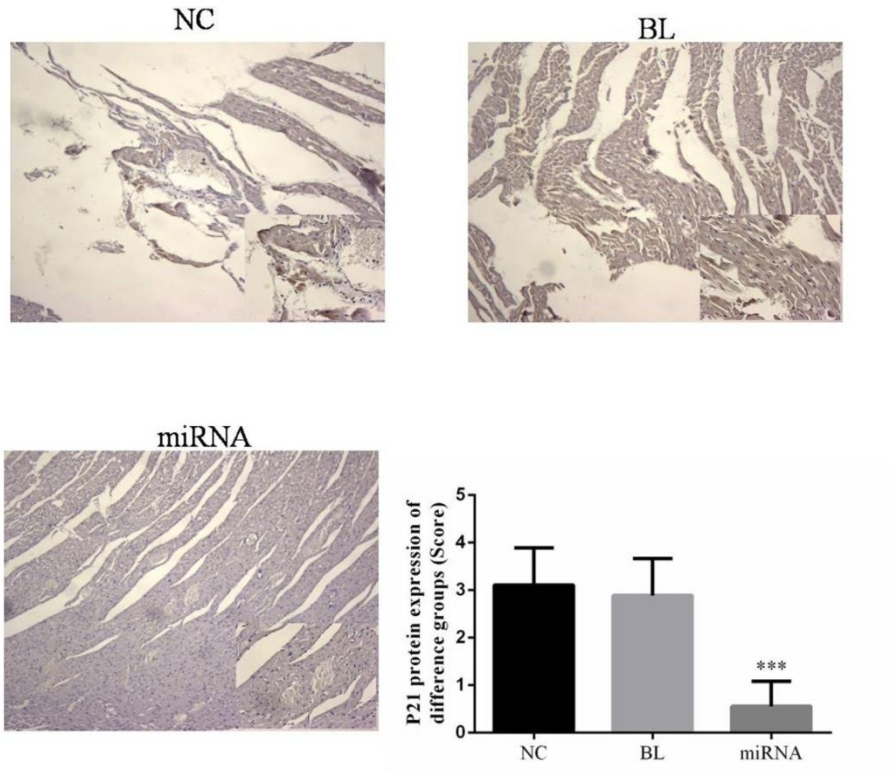

Figure 6. The P21 protein expression of difference groups by IHC (200×)

***: Compared with NC group

miRNA-21 has effects to relative proteins expression by WB assay

Compared with NC group, the PTEN and P21 proteins expression of miRNA group were significantly suppressed ($P<0.05$, respectively); The ERK and AKT protein expression of miRNA groups were significantly increased ($P<0.05$, respectively). The relative data were shown in Figure 7.

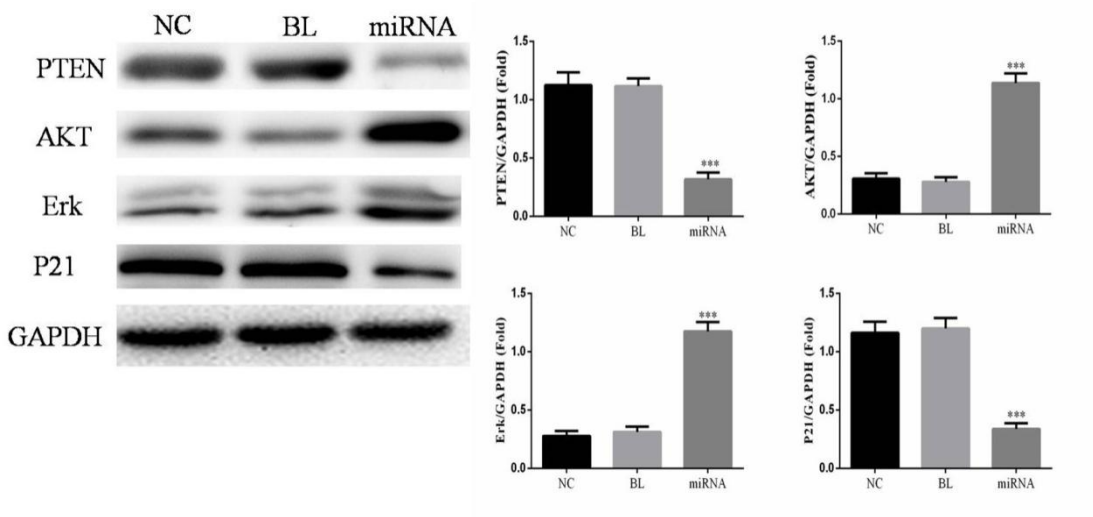

Figure 7. The relative proteins expressions of difference groups by WB assay

***: Compared with NC group

Discussion

Cardiomyocyte apoptosis is closely related to cardiomyopathy, myocardial hypertrophy and the myocardial toxicity of antibiotics, and cardiomyocyte apoptosis may also be involved in the occurrence and development of cardiovascular diseases. Recent studies have demonstrated that miRNAs is involved in the regulation of apoptotic signaling pathways (8-12). The previous study confirmed miRNA-21 in normal vascular smooth muscle cells has anti-apoptotic effect (13). H_2O_2 induced myocardial injury also confirmed that overexpression of miR-21 can inhibit cardiomyocyte apoptosis by regulating the transcription of its target gene PDCD4 (14).

In our present study, we found that miRNA-21 over-expression had effects to suppress cardiomyocyte apoptosis and improve myocardial pathology induced by myocardial ischemia reperfusion. Furthermore, we evaluated the relative protein expression which relative with cell apoptosis and miRNA-21 to explain the mechanism of miRNA-21 in myocardial damage induced by Ischemia-reperfusion.

PTEN/AKT/Erk pathway was an important signaling pathway in development of cell apoptosis (15-17). Meanwhile, miRNA-21 was considered as regulator to target PTEN in some previous studies (18, 19). P21 is a member of the CIP / KIP family of cyclin dependent kinase inhibitors (20, 21). P21 was a downstream gene of PTEN/AKT/Erk, P21 up-regulation improved cell apoptosis by regulation cell cycling in G1 phase (22). In our present study, we found that the PTEN and P21 proteins expressions were stimulated and AKT and Erk proteins expressions were suppressed in myocardial damage induced by Ischemia-reperfusion, however, the PTEN and P21 proteins expressions were suppressed and AKT and Erk proteins expressions were up-regulation in miRNA-21 over-expression group.

In conclusion, miRNA-21 over-expression could improve myocardial damage induced by Ischemia-reperfusion by targeting PTEN in vivo study.

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