Life Sciences 232 (2019) 116619

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

MicroRNA-374a protects against myocardial ischemia-reperfusion injury in mice by targeting the MAPK6 pathway

Zhao-Qi Huang[a], Wei Xu[b], Jin-Lei Wu[c], Xiong Lu[a], Xi-Ming Chen[a,*]

[a] Department of Vasculocardiology, The 3rd Affiliated Hospital of Guangzhou Medical University, People's Republic of China
[b] Department of Vasculocardiology, Huadu District People's Hospital of Guangzhou, People's Republic of China
[c] Department of Vasculocardiology, Hexian Memorial Affiliated Hospital of Southern Medical University, People's Republic of China

ARTICLE INFO

Keywords:
Ischemia reperfusion injury
miR-374a-5p
Myocardial ischemia
MAPK6

ABSTRACT

Aims: Clinical treatment strategies for patients with myocardial ischemia typically include coronary artery recanalization to restore myocardial blood supply. However, myocardial reperfusion insult often induces oxidative stress and inflammation, which further leads to apoptosis and necrosis of myocardial cells. Increasing evidence suggests that microRNAs (miRNAs) participate in the pathological and physiological processes associated with myocardial ischemia reperfusion.

Main methods: In this study, we established a myocardial H/R H9C2 cell model and a mouse I/R model to detect molecules implicated in myocardial I/R regulation and to determine the underlying signal transduction pathways.

Key findings: Herein, we showed that the expression of miR-374a-5p decreased in a myocardial cell model (H9C2 cells) of hypoxia/reoxygenation (H/R) and mouse model of ischemia/reperfusion (I/R). Alternatively, overexpression of miR-374a-5p was found to ameliorate myocardial cell damage within both *in vivo* and *in vitro* models of ischemia. Further, mitogen-activated protein kinase 6 (MAPK6) was identified as a direct target of miR-374a-5p. Thus, by targeting MAPK6, miR-374a-5p was found to negatively regulate MAPK6 expression. However, up-regulation of MAPK6 functioned to inhibit the previously observed protective effect of miR-374a-5p in the H9C2 H/R model.

Significance: Taken together, our study suggests that miR-374a-5p may have protective effects against cardiac I/R injury *in vivo*, and H/R injury *in vitro*, thereby providing novel insights into the molecular mechanisms associated with ischemia/reperfusion injury and a potential novel therapeutic target.

1. Introduction

Restoring blood flow to ischemic myocardium is one of the most common treatment strategies for ischemic heart disease [1]. Restoration of blood flow minimizes damage caused by the infarct, thus reducing the rate of mortality. However, sudden return of blood flow may result in extra cardiovascular trauma, referred to as reperfusion injury [2], which can lead to serious consequences, including myocyte apoptosis and necrosis, and cardiac arrest [2]. Moreover, the efficacy of myocardial ischemia therapy is affected by the presence of ischemia/reperfusion injury (IRI) [2].

The molecular mechanisms associated with myocardial ischemia/reperfusion (I/R) have recently become a major focal point in research. MicroRNAs (miRNAs) are short, highly conserved, non-coding RNA fragments [3] that negatively regulate gene expression *via* splicing of mRNAs, effectively inhibiting protein translation. MiRNAs are widely used for gene regulation after transcription [4]. MiRNAs can regulate 30–80% of the genes in the human genome and are, therefore, potential molecular targets for many human diseases [5]. MiRNAs have also been implicated in cardiac IRI [6]. Further, they have been shown to negatively regulate post-transcriptional gene expression of cytokines, chemokines, stress proteins, and intercellular adhesion molecules, all of which are up-regulated in the tissues surrounding cardiac lesions following I/R [7,8]. Particularly, miR-374a-5p, a highly conserved sequence, was demonstrated to regulate inflammatory responses by targeting MCP-1 expression in diabetic nephropathy [9], and protect against chemical hypoxia-induced injury in PC12 cells *via* the GADD45α/JNK signaling pathway [10]. These findings suggest that miR-374a-5p may play an important role in inflammatory injury control. However, little is known about the relationship between

* Corresponding author at: Department of Vasculocardiology, The 3rd Affiliated Hospital of Guangzhou Medical University, Guangzhou, Guangdong 510150, People's Republic of China.
E-mail address: 18078805442@163.com (X.-M. Chen).

https://doi.org/10.1016/j.lfs.2019.116619
Received 3 June 2019; Received in revised form 28 June 2019; Accepted 28 June 2019
Available online 29 June 2019

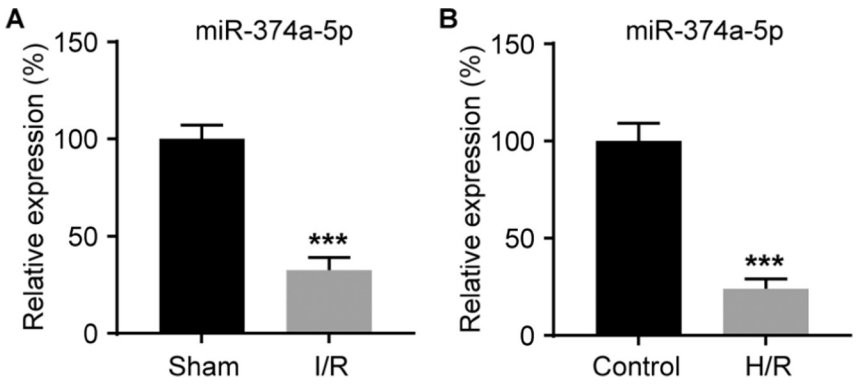

Fig. 1. MiR-374a-5p expression is down-regulated in ischemic/reperfused (I/R) myocardial tissue and hypoxic/reoxygenated (H/R) cardiomyocytes.
(A) Myocardial miR-374a-5p is down-regulated in myocardial tissue subjected to I/R injury. (B) The expression level of miR-374a-5p level in H9C2 cells decreased following exposure to H/R. Data are presented as the means ± SEM; unpaired *t*-tests were performed; $^{***}P < 0.001$; $n = 8$.

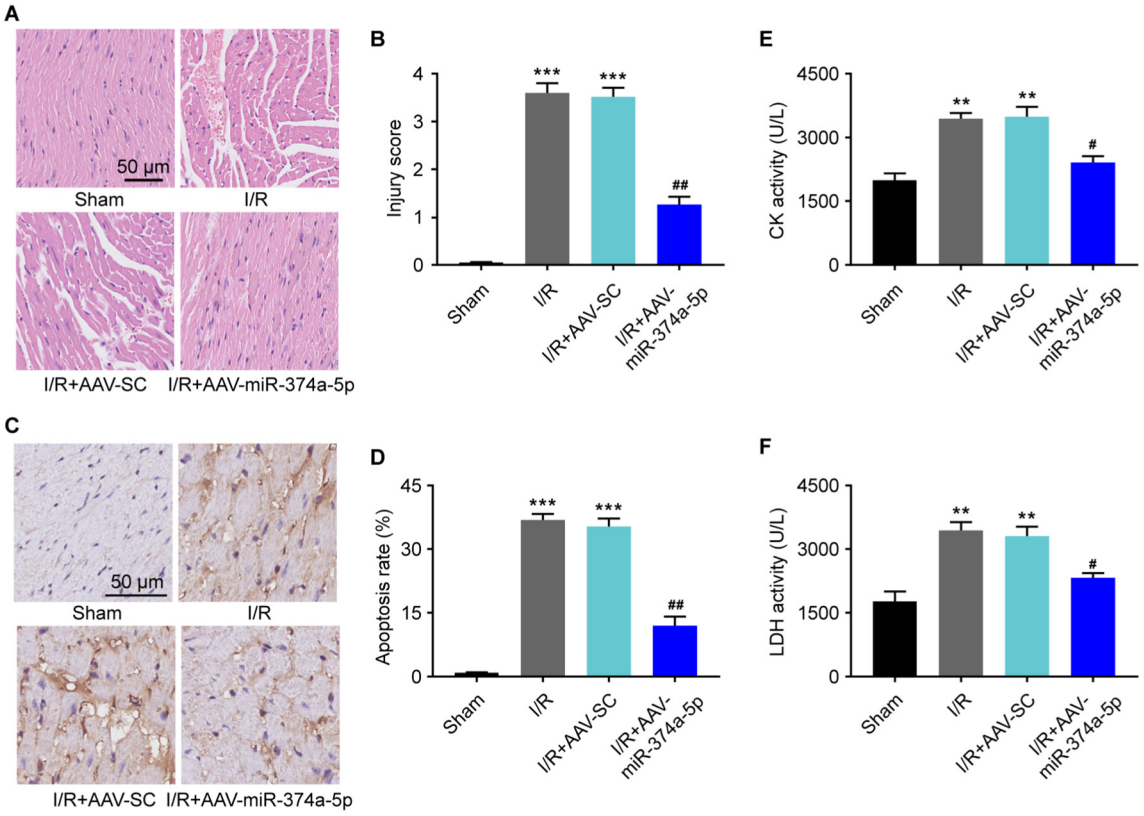

Fig. 2. Up-regulation of MiR-374a-5p protects against myocardial ischemia/reperfusion injury.
(A) Representative image of HE staining. (B) Statistical representation of the injury score. (C) Representative TUNEL staining image of the myocardial tissue slices. (D) Statistical representation of the apoptosis rate. (E) Serum CK activity level. (F) Serum LDH activity level. Compared with the Sham group, $^{**}P < 0.01$, $^{***}P < 0.001$; compared with the I/R + AAV-SC group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$; $n = 6$.

cardiomyocyte injury and miR-374a-5p under IRI conditions.

Mitogen-activated protein kinase (MAPK) signaling pathways are involved in the regulation of various cellular processes, including cell differentiation, development, apoptosis, and survival by regulating the membrane-nucleus signal transduction system [11]. Increasing evidence has shown that MAPK signaling pathways are activated in the liver [12], kidney [13], brain [14], and heart [15] during I/R injury. *In vitro* evidence shows that modulation of the MAPK signaling pathway reduces hypoxic injury [16]. As a member of MAPK signaling, overexpression of MAPK6 could promote cell apoptosis induced by hypoxia/reoxygenation (H/R) [17]. However, the effects of MAPK6 on cardiomyocytes have not been investigated, and it remains unclear whether MAPK6, a direct target of miR-374a-5p, participates in the regulation of myocardial IRI.

Thus, in this study, we established a myocardial H/R H9C2 cell model and a mouse I/R model to detect molecules implicated in myocardial I/R regulation and to determine the underlying signal transduction pathways. We sought to explore the possible molecular mechanisms employed by miR-374a-5p and MAPK6 in myocardial IRI.

2. Materials and methods

2.1. Experimental animals

Forty healthy c57BL6/J male mice weighing 18–22 g were purchased from Guangdong Medical Laboratory Animal Center. They were raised in an SPF environment, with 12 h light/dark cycles, and constant and suitable temperature (25 °C) and humidity (60%). All experiments were approved by the Administrative Panel on Laboratory Animal Care of the 3rd Affiliated Hospital at the Guangzhou Medical University. All procedures were conducted in accordance with the *Care and Use of Laboratory Animals* issued by the Chinese Association for Laboratory

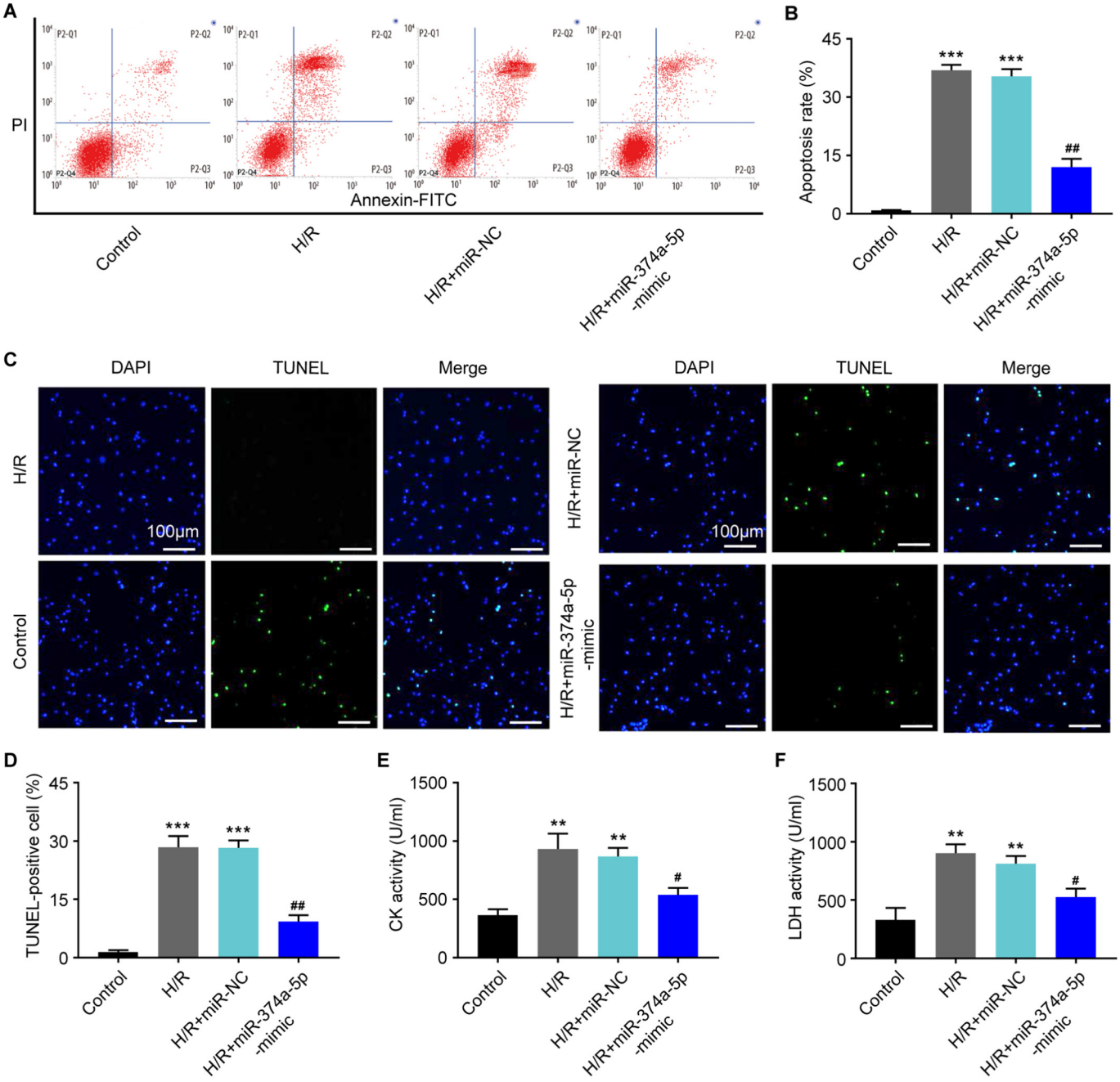

Fig. 3. Over-expression of miR–374a-5p protect H9C2 cells from hypoxia/reoxygenation induces injury.
(A) Apoptosis in H9C2 cells was analyzed using Annexin V-FITC staining assay. (B) Statistical representation of apoptosis rates. (C) TUNEL assays were employed to evaluate the level of apoptosis in H9C2 cells (D) Statistical representation of TUNEL positive cells. (E) Cellular supernatant CK activity. (F) Cellular supernatant LDH activity. Compared with the control group, $^{**}P < 0.01$, $^{***}P < 0.001$; compared with the H/R + miR-NC group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$; $n = 7$.

Animal Care.

2.2. Establishment of a myocardial IRI model

The left anterior descending coronary artery (LAD) was ligated to establish an I/R mouse model, as previously described [18]. Briefly, mice were anesthetized with 2% isoflurane (RWD, Shenzhen, oxygen flow speed 1 L/min). A 6–0 silk suture slipknot was placed around the LAD, and the slipknot was released after 30 min to allow reperfusion following myocardial ischemia, to induce cardiac IRI. Buprenorphine hydrochloride (Sigma Aldrich, USA) was subcutaneously administered as an intraoperative analgesic (0.05 mg/kg). For the sham-operation, an identical surgical procedure was performed, save for the LAD ligation. Five percent glucose (0.5–1.0 mL) was subcutaneously injected to prevent dehydration after the operation. Tissue samples were collected 24 h following induction of I/R.

2.3. Cell cultures and H/R treatment

H9C2 cells were purchased from the Chinese Academy of Sciences Shanghai Cell Bank (Catalog number GNR 5) and were maintained in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin, and incubated at 37 °C with 5% CO_2. To establish a cellular H/R model, serum/glucose-free DMEM was used. Cells were cultured in anaerobic conditions (94% N_2, 5% CO_2, and 1% O_2) and maintained at

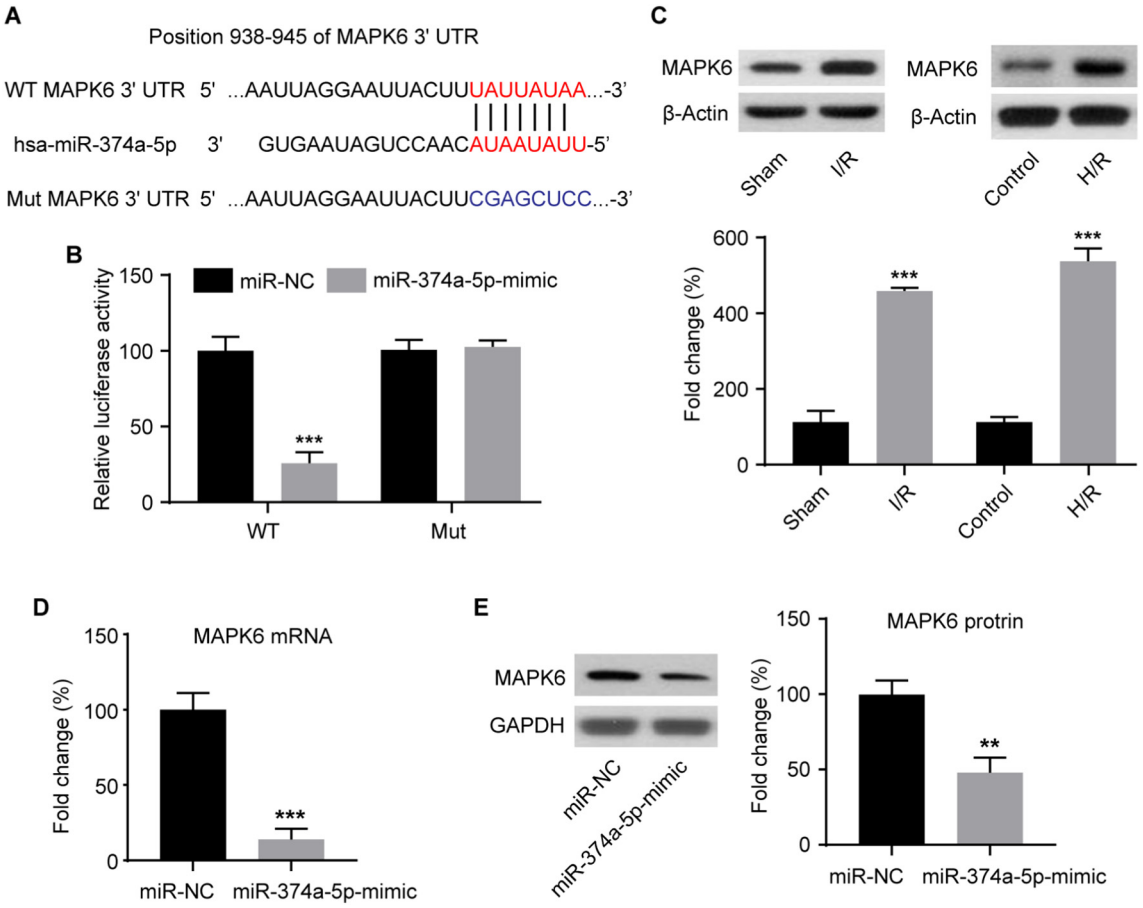

Fig. 4. MAPK6 is a target gene of miR-374a-5p.
(A) The predicted target sequences for miR-374a-5p in the MAPK6 3′ UTR. (B) Luciferase reporter assays were conducted to prove that miR-374a-5p directly binds with the wide type (WT) site of MAPK6. (C) Expression of MAPK6 following exposure of H9C2 cells to three I/R or H/R processes. (D) mRNA and (E) protein levels of MAPK6 in H9C2 cells, transfected with miR-374a-5p-mimic or a scramble control. ns, not significant; $*P < 0.05$, $**P < 0.01$; $n = 6$.

37 °C for 4 h. The cells were then transferred to the normoxic incubator for 4 h to undergo reoxygenation. Cells under normoxic conditions served as a control.

2.4. Real-time polymerase chain reaction (PCR)

TRIzol reagent (Invitrogen, USA) was used to extract total RNA from cardiac tissues or myocardial cells. The primer sequences were designed based on cDNA sequences obtained from GenBank. A cDNA Synthesis Kit (TaKaRa, Japan) was used to reverse transcribe 2 μg of RNA into cDNA. Quantitative PCR (qPCR) was performed using the MiniOpticon qPCR detection system (Bio-Rad Laboratories). The $2^{-\Delta\Delta CT}$ method was adopted to calculate the relative quantification.

2.5. In vivo and in vitro transfection

The AAV-EF1a-hsa-mir-374a-5p-eGFP virus vector was designed by Obio Technology (Shanghai). The reference sequence for miR-374a-5p was: 5′-UUAUAAUACAACCUGAUAAGUG-3′. The AAV vector packed with a scrambled control (AAV-SC) sequence served as a control. Two weeks prior to I/R modeling, 8×10^{12} AAV vector particles were intravenously injected into the tail vein of the experimental mice.

Lipofectamine™ 2000 (Invitrogen, USA) was employed to transfect miR-374a-mimic or its negative control (NC) into H9C2 cells, respectively, according to the manufacturer's instructions. Both, miR-374a-mimic and miR-NC, were purchased from GeneChem, Shanghai, China. Following incubation at 37 °C and 5% CO_2 for 6 h, the transfection efficiency was verified. Transfected cells were collected for H/R exposure.

The MAPK6 sequence was sub-cloned into a pcDNA3.1 vector (Invitrogen, USA) to construct pcDNA3.1-MAPK6-GFP (pcDNA-MAPK6), and an empty pcDNA3.1 (pcDNA-SC) was transfected as a control. Both the pcDNA-MAPK6 and pcDNA-SC vectors were synthesized by GenePharma, Shanghai, China. The transfection was performed in the same manner as that described for miR-274a.

2.6. Morphological analysis of apoptosis

Hearts were fixed with 10% buffered formalin for histopathological examination.

After dehydration and paraffin embedding, 4-μm paraffin sections were prepared and stained with hematoxylin-eosin (HE). The injuries were assessed and scored using an optical microscope (Olympus, Japan). The TUNEL apoptosis determination kit (Beyotime, Jiangsu, China) was used to detect apoptotic cells in myocardial tissue and H9C2 cells according to the manufacturer's instructions. The Image J cell counting program was used for cell quantification.

2.7. Activity of lactate dehydrogenase (LDH) and creatine kinase (CK)

Blood samples and cell supernatants were collected for quantification of LDH and CK using commercially available ELISA kits (Jianchen, Nanjing, China).

2.8. Western blot analysis

Chilled RIPA lysis buffer (Beyotime, Jiangsu, China) was added to

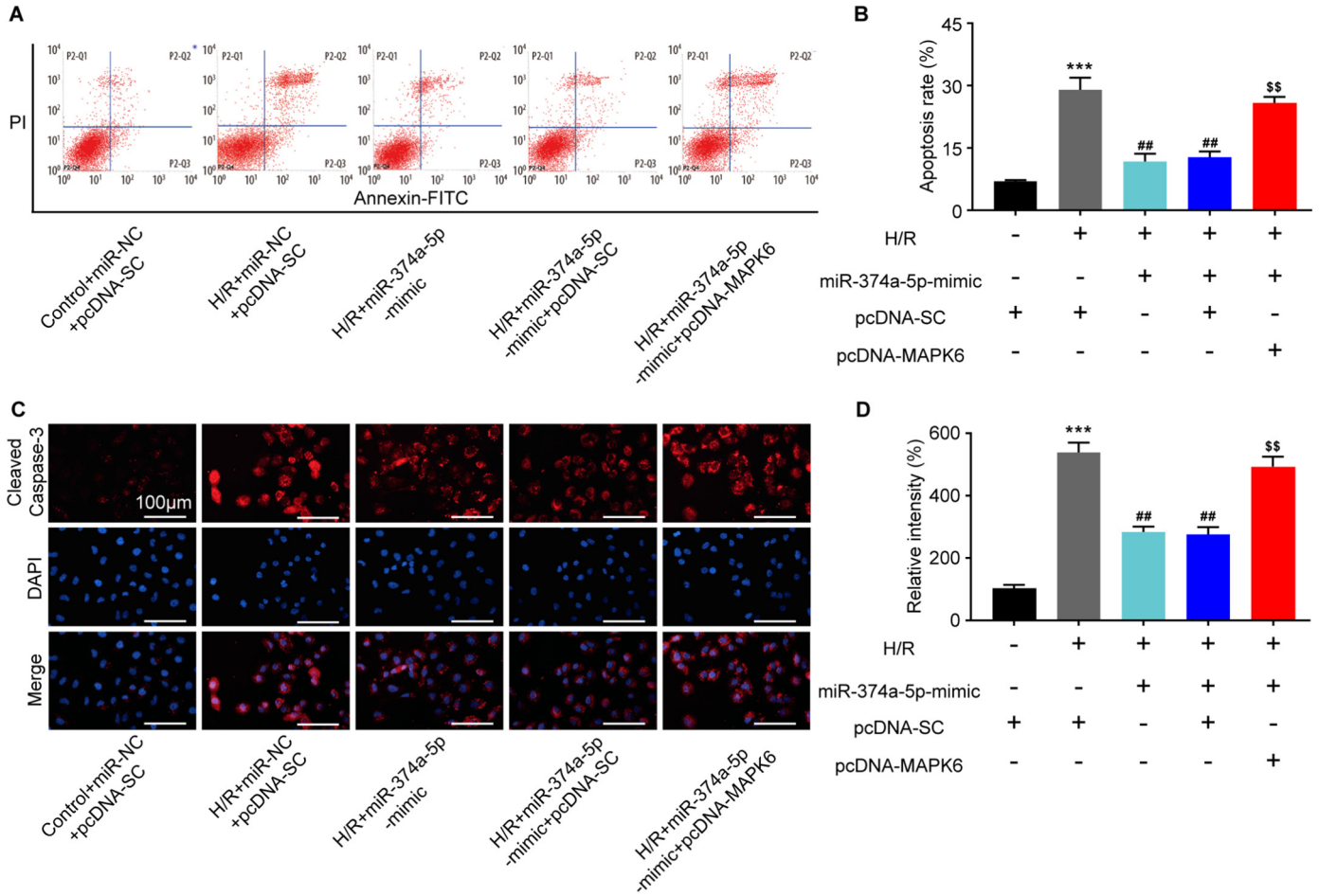

Fig. 5. miR-374a-5p confers its protective effects on H9C2 cells by targeting MAPK6.
H9C2 cells were co-transfected with miR-374a-5p-mimic or pcDNA-MAPK6, and subjected to hypoxia or not. (A) Annexin V-FITC/PI-FCM staining in H/R exposed H9C2 cells. (B) Apoptosis rate in H/R H9C2 cells. (C) Cleaved-caspase-3 immunofluorescence staining (D) Statistical representation for the relative expression of cleaved-caspase-3 in H9C2 cells. Compared with the control + miR-NC + pcDNA-SC group, $^{***}P < 0.001$; compared with the H/R + miR-NC + pcDNA-SC group, $^{\#\#}P < 0.01$; compared with the H/R + miR-374a-5p-mimic + pcDNA-SC group, $^{\$\$}P < 0.01$; $n = 8$.

cardiac tissues and H9C2 myocardial cells. Cell and tissue lysates were separated using 10% SDS-PAGE and transferred to nitrocellulose membranes and probed with primary antibodies specific for MAPK6 (1:1000, ab53277, Abcam), and GAPDH (1:500, ab181602, Abcam, Cambridge, MA, USA) overnight at 4 °C. After washing, the membranes were treated with the corresponding secondary antibodies and incubated for 1 h at 25 °C. GAPDH was used as an internal control. The western blots were visualized using Bio-Rad VersaDocTM imaging system (Bio-Rad Laboratories, Freiburg, Germany).

2.9. Flow cytometric analysis

Cells were treated with 0.25% trypsin (Boster, Wuhan, China) and collected in tubes. They were then centrifuged and washed thrice with PBS. According to the instructions in the Annexin-V-FITC cell apoptosis detection kit (BioVision, Milpitas, CA, USA), FITC and PI fluorescence was quantified *via* FACS analysis.

2.10. Cleaved-caspase-3 immunofluorescent staining

The H9C2 cells were placed on coverslips, rinsed thrice with PBS and fixed with 4% paraformaldehyde for 30 min. The cells were then permeabilized with PBS containing 0.5% Triton X-100 for 15 min. The cleaved caspase-3 antibody (AC033, Beyotime, Jiangsu, China) was applied to the coverslip and incubated in a wet box overnight at 4 °C. After washing three times with PBS, the fluorescent secondary antibody (1:500, Beyotime, China) was added and incubated for 1 h in the wet box at 25 °C. Following three additional washes with PBS, the DAPI mounting solution (Vector, USA) was added to stain the nuclear compartment. The immunoreactive cultured cells were observed using a fluorescence microscope (Olympus, Japan).

2.11. Dual-luciferase reporter gene assay

Reporter assays were performed with 293T cells, which were transfected with either wild type or mutant reporter plasmids and either miR-374a-5p mimic or the negative control. The resulting Firefly and Renilla luciferase activity were measured 48 h post-transfection using the Dual-Glo™ Luciferase Assay System, according to manufacturer's instructions (Promega, Wisconsin, USA).

2.12. Statistical analysis

Data are presented as the mean ± SEM. Unpaired two-tailed *t*-test was used for comparison between two groups. Two-way ANOVA with multiple comparisons between groups was performed to compare multiple groups, followed by Bonferroni's *post hoc* test. GraphPad Prism 7.0 was used to perform all analyses. P values < 0.05 were considered to be significant.

3. Results

3.1. MiR-374a-5p expression was down-regulated in I/R myocardial tissue and H/R cardiomyocytes

Various miRNAs were detected in the myocardial tissues, including miR- 15a/b [19], miR-21 [20], and miR-101 [21], validating the successful establishment of the I/R and H/R models (data not shown). To gain an insight into the role of MiR-374a-5p, the expression levels of miR-374a-5p in I/R myocardial tissue and H/R cardiomyocytes were determined *via* qPCR. As shown in Fig. 1, miR-374a-5p was found to be significantly down-regulated in the hearts of mice following I/R (Fig. 1A). MiR-374a-5p was similarly down-regulated in H9C2 myocardial cells under H/R conditions (Fig. 1B).

3.2. MiR-374a-5p overexpression protects against myocardial IRI

To examine whether up-regulation of miR-374a-5p protects against myocardial IRI, we packed an adeno-associated virus with miR-374a-5p plasmids (AAV-miR-374a-5p) and injected the vectors into the tail vein of mice 3 weeks prior to performing the I/R operation. The HE staining results revealed that the study group that received the sham operation had normal myocardial tissue structure (Fig. 2A). However, in the I/R group, the myocardial fiber structure was damaged, vascular walls were broken, and hemocyte infiltration was apparent. The experimental group that received pre-injection with AAV-miR-374a-5p exhibited mild tissue damage; however, the degree of myocardial tissue destruction in the IR + AAV-SC group was similar to that of the untreated I/R group (Fig. 2B). We also used TUNEL staining to detect myocardial cell apoptosis (Fig. 2C and D). Mice myocardium subjected to IRI demonstrated a significant increase in the abundance of TUNEL-positive/apoptotic cells, the level of which was seen to decrease following treatment with the miR-374a-5p vector (Fig. 2D). In addition, miR-374a-5p overexpression caused significant reduction in the serum levels of active CK (Fig. 2E) and LDH (Fig. 2F) in mice subjected to IRI.

3.3. Up-regulation of miR-374a-5p had a protective role against H/R injury

To investigate whether the up-regulation of miR-374a-5p protects H9C2 cardiomyocytes from H/R-induced apoptosis, cells were stained with Annexin V-FITC/PI (Fig. 3A). The results revealed a significant increase in apoptosis in the H/R group compared with the control, whereas the apoptotic rates were markedly reduced in H9C2 cells that were pre-transfected with the miR-374a-5p-mimic (Fig. 3B). TUNEL staining was used to quantitatively characterize the apoptosis of H9C2 cells and similar results were obtained as above (Fig. 3C, D). We also quantified the level of CK and LDH released into the cellular supernatants to determine the severity of injury experienced by the cardiomyocytes. As shown in Fig. 3E and F, the levels of CK and LDH were markedly increased in H/R-exposed cells, respectively. However, pre-transfection with the miR-374a-5p-mimic significantly reduced the activity levels of CK and LDH, indicating that miR-374a-5p may exert protective effects in H9C2 cells against H/R injury.

3.4. MAPK6 expression in myocardial cells is a direct target of miR-374a-5p

To explore the mechanism by which miR-374a-5p mitigates IRI, we predicted potential molecular targets based on TargetScan. MAPK6 were predicted as a potential target of miR-374a-5p. Further, bioinformatics predictions suggested that the 3′-UTR of MAPK6 mRNA contains putative binding sites for the miR-374a-5p seed sequence (Fig. 5C). Therefore, we constructed a dual luciferase reporter plasmid (pmirGLO- MAPK6 3′-UTR) encoding the mice MAPK6 3′-UTR that contained the miR-374a-5p binding site. This reporter plasmid (with wild type [Wt] or mutant [Mut] 374a-5p 3′-UTR) was co-transfected with miR-374a-5p-mimic into 293 T cells, and luciferase activity was assessed 48 h following transfection. Results revealed that up-regulation of miR-374a-5p significantly repressed luciferase activity in 293 T cells transfected with the wild type MAPK6 3′-UTR (Fig. 5D). This suggests that miR-374a-5p may have inhibited MAPK6 expression through direct binding to its 3′-UTR. Moreover, western blot analysis results also revealed that MAPK6 protein expression was significantly up-regulated in the I/R and H/R groups (Fig. 4A, B). However, the results from our qPCR and western blot analyses indicated that the mRNA and protein levels of MAPK6, respectively, were significantly down-regulated following transfection of H9C2 cells with miR-374a-5p-mimic (Fig. 4D and E, $P < 0.01$).

3.5. miR-374a-5p confers its protective effects on H9C2 cells by targeting MAPK6

The expression levels of miR-374a-5p and MAPK6 were simultaneously overexpressed *via* transfection, after which Annexin V-FITC/PI-FCM and cleaved-caspase-3 staining were performed and analyzed. As shown in Fig. 4A and B, when compared with the miR-374a-5p-mimic + pcDNA-SC group, the apoptosis rate in H9C2 cells transfected with the miR-374a-5p-mimic + pcDNA-MAPK6 was significantly increased ($P < 0.01$). In addition, the activation of caspase-3 was significantly promoted in the miR-374a-5p-mimic + pcDNA-MAPK6 group, as compared with the miR-374a-5p mimic + pcDNA-SC group ($P < 0.001$, Fig. 4C and D). These results suggest that miR-374a-5p was unable to protect H9C2 cells against H/R injury when MAPK6 was overexpressed.

4. Discussion

To our knowledge, this is the first study to provide evidence demonstrating that miR-374a-5p expression may be mediated by IRI, and that its expression may serve a protective role against cardiac IRI *in vivo* and *in vitro*. Moreover, our results show that miR-374a-5p confers its protective effects by targeting MAPK6.

Myocardial IRI has a complicated pathophysiological mechanism and a profound impact on the global burden due to heart disease [22]. Further, the expression of miRNAs has often been found to be altered in the presence of pathological stimulation, tissue damage, or disturbances in the surrounding tissue environment [23,24]. An increasing number of studies have found that miRNAs are involved in regulating IRI [6,25]. Recent studies have also revealed that miR-374a-5p has an important role in tumor immunology [26], tumorigenesis [27], and inflammation [28]. However, the biological role that miR-374a-5p plays in cardiac myocytes remains poorly understood. Our results revealed that the expression level of miR-374a-5p decreased in H9C2 cells following exposure to H/R conditions, and within myocardial cells in the mouse I/R model, indicating its potential protective effect in IRI. Our study shows that up-regulation of miR-374a-5p could protect cardiac cells from apoptosis both *in vivo* and *in vitro* by TUNEL staining. The level of CK and LDH is a sensitive index for evaluating the myocardial cell injury and membrane integrity [29], since the enzymes can leak out of cells upon H/R or I/R-induced cell membrane damage. Thus, the decrease in release of CK and LDH in serum and cell supernatant upon up-regulation of miR-374a-5p further validate the cardioprotective role of the miRNA *in vivo* and *in vitro*.

An emerging role for the MAPK pathway in the molecular response to cardiac damage has also been demonstrated in recent studies [30,31]. Specifically, the MAPK pathway has been shown to have an essential role in the pathogenesis of cardiovascular diseases including, atherosclerosis [32], myocardial ischemia [33] and myocardial hypertrophy [34]. Further, Hao et al. has identified MAPK6 as the functional target gene of miR-133a-5p in apoptosis induced by hepatic H/R [17]. However, since the miRNA-mediated effects on gene expression and cellular functions are cell specific, it was unclear whether MAPK6

continued to function as a target gene for miR-374a-5p in cardiac myocytes during the process of H/R. Our results, however, clearly demonstrate that MAPK6 expression was regulated by miR-374a-5p in cultured cardiac myocytes. Thus, our study verifies that MAPK6 is a functional target gene of miR-374a-5p, which may account for the observed miR-374a-5p-mediated cardio-protective effects. Rescue experiments also demonstrated that the protective effect of miR-133a-5p was lost when MAPK6 was simultaneously overexpressed. Further investigations to examine the molecular mechanism implicated in MAPK6-regulated apoptosis as well as those employed by miR-374a-5p will be the focus of our future studies.

In the present study, although our cardiac *in vivo* and *in vitro* models could controllably simulate the pathogenesis of IRI to elucidate the mechanism implicated in the research, the limitation of H9c2 cardiomyoblasts may have pathophysiologic characteristics different from myocardial cells; this needs further investigation. The emergence of cardiomyocytes derived from human induced pluripotent stem cells and engineered cell culture platforms may provide more suitable tools for establishment of cardiac IRI models [35] [36].

5. Conclusion

In conclusion, this study determined that miR-374a-5p was down-regulated during the cardiac I/R process both *in vivo* and *in vitro*. However, its up-regulation may serve to attenuate cell injury by targeting MAPK6. These results describe a novel regulatory role for miR-374a-5p following cardiac I/R, indicating a potential novel therapeutic strategy for treating myocardial IRI.

Declaration of Competing Interest

The authors report no conflicts of interest.

Acknowledgements

Funding

This work was supported by the Science and Technology Planning Project of Guangdong Province (grant number 2013B022000103).

References

[1] M. Ovize, R.A. Kloner, S.L. Hale, K. Przyklenk, Coronary cyclic flow variations "precondition" ischemic myocardium, Circulation 85 (1992) 779.
[2] E. Murphy, C. Steenbergen, Mechanisms underlying acute protection from cardiac ischemia-reperfusion injury, Physiol. Rev. 88 (2008) 581–609.
[3] S.W. Chi, J.B. Zang, A. Mele, R.B. Darnell, Ago HITS-CLIP decodes miRNA-mRNA interaction maps, Nature 460 (2009) 479.
[4] J. Hausser, A.P. Syed, N. Selevsek, E. van Nimwegen, L. Jaskiewicz, R. Aebersold, M. Zavolan, Timescales and bottlenecks in miRNA-dependent gene regulation, Mol. Syst. Biol. 9 (2013) 711.
[5] H.T. Dang, K. Satou, B.H. Tu, Finding microRNA regulatory modules in human genome using rule induction, BMC Bioinf. 9 (2008) 1–10.
[6] Y. Zhou, Q. Chen, K.S. Lew, A.M. Richards, P. Wang, Discovery of potential therapeutic miRNA targets in cardiac ischemia-reperfusion injury, J. Cardiovasc. Pharmacol. Ther. 21 (2016) 296–309.
[7] J.L. Marques-Rocha, M. Samblas, F.I. Milagro, J. Bressan, J.A. Martínez, A. Marti, Noncoding RNAs, cytokines, and inflammation-related diseases, FASEB J. 29 (2015) 3595–3611.
[8] M. Neri, I. Riezzo, C. Pascale, E. Pomara, E. Turillazzi, Ischemia/reperfusion injury following acute myocardial infarction: a critical issue for clinicians and forensic pathologists, Mediat. Inflamm. 2017 (2017) 7018393.
[9] Z. Yang, Z. Guo, J. Dong, S. Sheng, Y. Wang, L. Yu, H. Wang, L. Tang, miR-374a regulates inflammatory response in diabetic nephropathy by targeting MCP-1 expression, Front. Pharmacol. 9 (2018) 900.
[10] W. Gong, S. Qie, P. Huang, J. Xi, Protective effect of miR-374a on chemical hypoxia-induced damage of PC12 cells in vitro via the GADD45α/JNK signaling pathway, Neurochem. Res. 43 (2017) 1–10.
[11] J.M. Kyriakis, J. Avruch, Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation, Physiol. Rev. 81 (2001) 807–869.
[12] L.H. Toledopereyra, F. Lopez-Neblina, J.S. Reuben, A.H. Toledo, P.A. Ward, Selectin inhibition modulates Akt/MAPK signaling and chemokine expression after liver ischemia-reperfusion, J. Investig. Surg. 17 (2004) 303.
[13] M.J. Di, R. Davis, R.L. Safirstein, MAPK activation determines renal epithelial cell survival during oxidative injury, Am. J. Phys. 277 (1999) F195.
[14] Y. Shu, Y. Yang, P. Zhang, Neuroprotective effects of penehyclidine hydrochloride against cerebral ischemia/reperfusion injury in mice, Brain Res. Bull. 121 (2016) 115–123.
[15] D. Leshem, A. Shainberg, Y. Chepurko, B. Vidne, E. Porat, E. Hochhauser, p38 MAPK is involved in adenosine receptors' protection against ischemia–reperfusion (I/R) injury of the heart, J. Mol. Cell. Cardiol. 42 (2007) S196.
[16] J.E. Jeong, J.H. Park, C.S. Kim, S.L. Lee, H.L. Chung, W.T. Kim, E.J. Lee, Neuroprotective effects of erythropoietin against hypoxic injury via modulation of the mitogen-activated protein kinase pathway and apoptosis, Korean J. Pediatr. 60 (2017) 181–188.
[17] W. Hao, Z.H. Zhao, Q.T. Meng, M.E. Tie, S.Q. Lei, Z.Y. Xia, Propofol protects against hepatic ischemia/reperfusion injury via miR-133a-5p regulating the expression of MAPK6, Cell Biol. Int. 41 (2017) 495–504.
[18] K. Reichert, B. Colantuono, I. McCormack, F. Rodrigues, V. Pavlov, M.R. Abid, Murine left anterior descending (lad) coronary artery ligation: an improved and simplified model for myocardial infarction, J. Vis. Exp. 2017 (2017).
[19] L.F. Liu, Z. Liang, Z.R. Lv, X.H. Liu, J. Bai, J. Chen, C. Chen, Y. Wang, MicroRNA-15a/b are up-regulated in response to myocardial ischemia/reperfusion injury, J. Geriatr. Cardiol. 9 (2012) 28–32.
[20] Y. Tu, L. Wan, Y. Fan, K. Wang, L. Bu, T. Huang, Z. Cheng, B. Shen, Ischemic postconditioning-mediated miRNA-21 protects against cardiac ischemia/reperfusion injury via PTEN/Akt pathway, PLoS One 8 (2013) e75872.
[21] D. Wu, H. Jiang, S. Chen, H. Zhang, Inhibition of microRNA-101 attenuates hypoxia/reoxygenation-induced apoptosis through induction of autophagy in H9c2 cardiomyocytes, Mol. Med. Rep. 11 (2015) 3988.
[22] S. Cadenas, ROS and redox signaling in myocardial ischemia-reperfusion injury and cardioprotection, Free Radic. Biol. Med. 117 (2018) 76–89.
[23] M.V. Latronico, D. Catalucci, G. Condorelli, MicroRNA and cardiac pathologies, Physiol. Genomics 34 (2008) 239–242.
[24] A. Flemming, Heart failure: targeting miRNA pathology in heart disease, Nat. Rev. Drug Discov. 13 (2014) 336.
[25] F. Yang, T. Li, Z. Dong, R. Mi, MicroRNA-410 is involved in mitophagy after cardiac ischemia/reperfusion injury by targeting high-mobility group box 1 protein, J. Cell. Biochem. 119 (2018) 2427–2439.
[26] Y. Zhen, W. Fang, M. Zhao, R. Luo, Y. Liu, Q. Fu, Y. Chen, C. Cheng, Y. Zhang, Z. Liu, miR-374a-CCND1-pPI3K/AKT-c-JUN feedback loop modulated by PDCD4 suppresses cell growth, metastasis, and sensitizes nasopharyngeal carcinoma to cisplatin, Oncogene 36 (2017) 275–285.
[27] D. Son, Y. Kim, S. Lim, H.G. Kang, D.H. Kim, J.W. Park, W. Cheong, H.K. Kong, W. Han, W.Y. Park, K.H. Chun, J.H. Park, miR-374a-5p promotes tumor progression by targeting ARRB1 in triple negative breast cancer, Cancer Lett. 454 (2019) 224–233.
[28] Y. Xiong, J. Qiu, C. Li, Y. Qiu, L. Guo, Y. Liu, J. Wan, Y. Li, G. Wu, L. Wang, Z. Zhou, J. Dong, C. Du, D. Chen, H. Guo, Fortunellin-induced modulation of phosphatase and tensin homolog by microRNA-374a decreases inflammation and maintains intestinal barrier function in colitis, Front. Immunol. 9 (2018) 83.
[29] B. Maisch, P.M. Seferović, A.D. Ristić, R. Erbel, R. Rienmüller, Y. Adler, W.Z. Tomkowski, G. Thiene, M.H. Yacoub, Task Force on the Diagnosis and Management of Pricardial Diseases of the European Society of Cardiology, Guidelines on the diagnosis and management of pericardial diseases executive summary; the Task Force on the Diagnosis and Management of Pericardial Diseases of the European Society of Cardiology, Eur. Heart J. 25 (2004) 587–610.
[30] A.J. Muslin, MAPK signaling in cardiovascular health and disease: molecular mechanisms and therapeutic targets, Clin. Sci. 115 (2008) 203–218.
[31] J.A. Moolman, S. Hartley, J. Van Wyk, E. Marais, A. Lochner, Inhibition of myocardial apoptosis by ischaemic and beta-adrenergic preconditioning is dependent on p38 MAPK, Cardiovasc. Drugs Ther. 20 (1) (2006) 13–25.
[32] J.X. Pan, LncRNA H19 promotes atherosclerosis by regulating MAPK and NF-kB signaling pathway, Eur. Rev. Med. Pharmacol. Sci. 21 (2017) 322–328.
[33] L.Y. Dong, S. Li, Y.L. Zhen, Y.N. Wang, X. Shao, Z.G. Luo, Cardioprotection of vitexin on myocardial ischemia/reperfusion injury in rat via regulating inflammatory cytokines and MAPK pathway, Am. J. Chin. Med. 41 (2013) 1251–1266.
[34] K. Tanaka, M. Honda, T. Takabatake, Redox regulation of MAPK pathways and cardiac hypertrophy in adult rat cardiac myocyte, J. Am. Coll. Cardiol. 37 (2001) 676–685.
[35] X. Yang, T. Papoian, Moving beyond the comprehensive in vitro proarrhythmia assay: use of human-induced pluripotent stem cell-derived cardiomyocytes to assess contractile effects associated with drug-induced structural cardiotoxicity, J. Appl. Toxicol. 38 (2018) 1166–1176.
[36] H. Masumoto, T. Ikuno, M. Takeda, H. Fukushima, A. Marui, S. Katayama, T. Shimizu, T. Ikeda, T. Okano, R. Sakata, J. Yamashita, Human iPS cell-engineered cardiac tissue sheets with cardiomyocytes and vascular cells for cardiac regeneration, Sci. Rep. 4 (2014) 6716.