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MiR-195 enhances cardiomyocyte apoptosis induced by hypoxia/reoxygenation injury via downregulating c-myb

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Abstract. – OBJECTIVE: In this study, we explored the regulative effect of miR-195 on c-myb expression and also investigated the role of miR-195 and c-myb in cardiomyocyte apoptosis induced by hypoxia/reoxygenation (H/R) injury.

MATERIALS AND METHODS: QRT-PCR analysis was performed to measure mature miR-195 expression. H9c2 cells were transfected for miR-195 overexpression or knockdown or c-myb overexpression using Lipofectamine 2000. The cells were subjected to H/R treatment and following flow cytometric analysis of active caspase-3 or florescent study of reactive oxygen species (ROS) generation. The binding sites between miR-195 and 3'UTR of MYB mRNA were predicted using TargetScan 7.0. The binding sites were verified using dual luciferase assay and Western blot analysis.

RESULTS: MiR-195 is significantly upregulated after H/R treatment in H9c2 cells. H/R injury induced active caspase-3 expression. However, the cells with miR-195 suppression had substantially lower ratio of cells with active caspase-3. MiR-195 can decrease c-myb protein expression. Dual luciferase assay verified two binding sites between miR-195 and 3'UTR of MYB mRNA. C-myb overexpression can suppress mitochondrial superoxide generation and cardiomyocyte apoptosis after H/R.

CONCLUSIONS: MiR-195 is significantly increased due to H/R and can enhance cardiomyocyte apoptosis. MYB is a target gene of miR-195 in cardiomyocytes. The miR-195-MYB axis is involved in regulation of cardiomyocyte apoptosis induced by H/R.

Key Words:
Hypoxia/reoxygenation, Cardiomyocytes, MiR-195, c-myb.

Introduction

Timely reperfusion of occluded artery is still the major therapeutic target for ischemic heart disease (IHD), such as myocardial infarction[1,2]. However, reperfusion is also associated with myocardial ischemia-reperfusion injury, which plays a pivotal role in inducing cardiomyocyte death and left ventricular remodeling[3,4]. Therefore, ischemia-reperfusion injury is also considered as one of the most significant factors responsible for the poor cardiovascular outcomes[1,5,6]. Understanding of the molecular mechanisms of ischemia-reperfusion injury is quite fundamental for the development of effective therapeutic strategies to reduce the adverse outcomes[1].

Previous investigations[7-10] demonstrated that multiple miRNAs were significantly dysregulated during myocardial ischemia-reperfusion, such as miR-21, miR-1, miR-216, miR-126, miR-31, miR-214 and miR-29 family and might be might be used as potential biomarkers for myocardial infarction. Several studies[11,12] found that miR-195 upregulation is associated with acute myocardial infarction and the upregulation is induced by myocardial ischemia-reperfusion injury. Previously He et al[13] studies reported that miR-195 can regulate apoptosis, proliferation and cell cycle via targeting WEE1, CDK6, and Bcl-2. In cardiomyocyte, miR-195 can promote palmitate-induced apoptosis by downregulating Sirt1[14]. Inhibition of miR-195 helps to protect cardiomyocytes from hypoxia-induced death *in-vitro* and also to reduce cardiac remodeling in response to ischaemic damage *in-vivo*[15]. However, a miRNA usually involves in multiple signaling pathways by targeting multiple genes. Considering the important role of miR-195 in cardiomyocyte in responses to ischemia-reperfusion injury, the mechanism of miR-195 action need to be further studied.

Porrello et al[16] suggest that the identified target genes of miR-195 are implicated in the mitochondrial related apoptotic pathway, thereby modulating cardiomyocyte survival. MYB

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(myeloblastosis) is a family of transcription factors. Li et al[17] suggest that the c-myb protein encoded by MYB is involved in regulation of reactive oxygen species (ROS)-mediated cardiomyocyte injury. In this work, we further explored the regulative effect of miR-195 on c-myb expression and also investigated the role of miR-195 and c-myb in cardiomyocyte apoptosis induced by hypoxia/reoxygenation (H/R) injury.

Materials and Methods

Cell Culture and Treatment

Rat heart-derived H9c2 cells were routinely grown in 75 cm^2 flasks and cultured using Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37°C in a 5% CO_2 incubator.

MiR-195 mimics, miR-195 inhibitors (anti-miR-195), and the scramble negative controls were purchased from Ribobio (Guangzhou, China). C-myb expression vector (pCMV-c-myb) was purchased from Origene Company (Rockville, MD, USA). H9c2 cells were transfected with 100 nM miR-195 mimics, 50 nM anti-miR-195 or respectively or pCMV-c-myb using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA).

Hypoxia/reoxygenation (H/R) injury was induced by the method described previously[18]. Briefly, the cell culture medium was replaced with serum free low glucose DMEM and the cells were cultivated in a three gas incubator, set to 5% CO_2 and 95% N_2 at 37°C for 12 h and then by reoxygenation for 2 h. Reoxygenation was performed by replacing the serum free low glucose DMEM with normal cell medium under normoxic conditions. H9c2 cells with or without the transfection of anti-miR-195 or c-myb expression vector were subjected to induced H/R injury and then subjected to flow cytometric analysis of active caspase-3 and fluorescent analysis of ROS generation.

QRT-PCT Analysis

Total RNA in the cell samples was extracted using Trizol reagent (Invitrogen). cDNA was synthesized using miRNA-specific stem-loop primers and the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). The mature level of miR-195 was quantified by qRT-PCR using TaqMan MicroRNA Assay Kit (Applied Biosystems), with U6 snRNA used as the endogenous control. The $2^{-\Delta\Delta Ct}$ method was used to calculate relative mRNA and miRNA expression.

Flow Cytometric Analysis

The cells after H/R treatment were stained using Fluorescein Active Caspase-3 Staining Kit (ab65613, Abcam, Cambridge, UK) according to manufacturers' instructions. The ratio of cells with active caspase-3 was analyzed using a flow cytometry (FACSCalibur, BD Biosciences, Franklin Lakes, NJ, USA). Data acquisition was performed using CellQuest software (BD Bioscience).

Western Blot Analysis

H9c2 cells after transfection of miR-195 or anti-miR-195 were lyzed using the RIPA lysis buffer (Beyotime, Shanghai, China) for protein extraction. Then protein samples were denatured and 20 μg of proteins were loaded to each lane for separation in SDS-PAGE with 10% acrylamide gels. Then, the proteins were transferred to nitrocellulose membrane. The membranes were firstly incubated with anti-c-myb (1:1000, ab76009, Abcam) and then incubated with horseradish peroxidase-conjugated secondary antibodies. The protein bands were detected using an ECL chromogenic substrate (Bio-Rad, Hercules, CA, USA). Then, the signal intensity of the protein bands was quantified using densitometry (Quantity One Software, Bio-Rad).

Dual Luciferase Assay

The binding sites between miR-195 and 3'UTR of MYB were predicted using TargetScan (7.0). The results showed that there are two possible binding sites. Therefore, four short pieces of oligonucleotides of 3'UTR of MYB carrying the wild-type (WT) or mutant (MT) predicted targeting sites of miR-195 were chemically synthesized. The sequences were then cloned into the downstream of the luciferase gene of pmirGLO Dual-Luciferase miRNA Target Expression Vector (Promega Madison, WI, USA). The recombinant vectors were named as pGLO-MYB-WT1, pGLO-MYB-WT2, pGLO-MYB-MT1 and pGLO-MYB-MT2. H9c2 cells were co-transfected with 200 ng luciferase reporter vector and 100 nM miR-195 mimics or the negative controls. Luciferase activity was examined 24 hours after the transfection using the Dual-Luciferase Assay kit (Promega) according to manufacturer's instruction.

Detection of ROS Generation

To detect the generation of ROS, MitoSOX (Invitrogen), which is a live-cell permeant prober and selectively detects superoxide in mitochondria was used according to manufacturer's instruction. Briefly, H9c2 after transfection were grown on coverslips and then subjected to H/R injury. Then the coverslips were incubated with 5 μM MitoSOX reagent for 20 minutes at 37°C in dark. After the incubation, the cells were washed twice with ice cold PBS. Nuclei were stained using Prolong® Gold Antifade Reagent with DAPI (#8961, Cell Signaling, Danvers, MA, USA). The images were captured using a fluorescence microscope (Olympus IX73; Olympus, Tokyo, Japan).

Statistical Analysis

Data were presented in the form of means ± standard deviation (SD). Comparison between groups was performed using the unpaired *t*-test. A two-sided *p*-value of <0.05 was considered statistically significant.

Results

MiR-195 is Significantly Increased Due to H/R and Enhances Cardiomyocyte Apoptosis

Gao et al[12] found that the expression of miR-195 is induced due to ischemia–reperfusion injury in myocardial tissue. However, its effect on cardiomyocyte injury is not quite clear. In this study, we verified that miR-195 expression was significantly increased due to H/R injury (Figure 1). Then, we investigated how miR-195 affected H/R induced cardiomyocyte apoptosis. H9c2 cells were firstly transfected for miR-195 suppression (Figure 2A). QRT-PCR analysis confirmed that transfection of miR-195 inhibitor significantly suppressed miR-195 upregulation induced by H/R injury (Figure 2A). By performing flow cytometric analysis, we found that H/R injury induced the expression of active caspase-3 (Figure 2B-C). However, the group with miR-195 suppression had substantially lower ratio of cells with active caspase-3 (Figure 2B-C), suggesting alleviated cell injury.

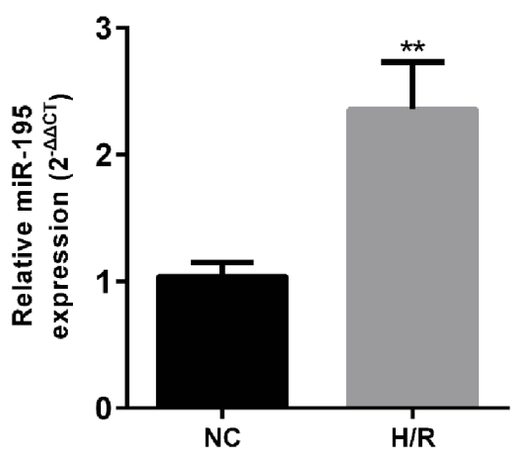

Figure 1. MiR-195 is significantly increased after H/R. QRT-PCR analysis of miR-195 expression in H9c2 cells with or without H/R treatment. $^{**}p<0.01$.

MiR-195 Directly Targets 3'UTR of MYB mRNA and Decreases c-myb Protein Expression

Then, we investigated the downstream regulation of miR-195 in cardiomyocyte. By bioinformatics analysis, we found that MYB is a highly possible target of miR-195, the 3'UTR of MYB gene has two broadly conserved binding sites with miR-195 (Figure 3A). Although H9c2 cells were rat heart-derived, the predicted binding sites between miR-195 and 3'UTR of MYB are nearly the same in rat and human gene (Figure 3A). Therefore, we used hsa-miR-195 for targeting assays. Transfection of miR-195 in H9c2 cells further reduced c-myb protein expression, while knockdown of endogenous miR-195 restored c-myb level (Figure 3B). Following dual luciferase assay showed that miR-195 mimics significantly suppressed the relative luciferase activity of the plasmids carrying WT sequences, but had no inhibitive effect on luciferase activity of the plasmids with MT sequences (Figure 3C-D). These results suggest that miR-195 directly targets MYB gene and decreases c-Myb protein expression.

C-myb is a Functional Target of miR-195 in H/R Related Cardiomyocyte Apoptosis

Since we verified that c-myb is downregulated due to miR-195 upregulation, we then investigated the function of c-myb in cardiomyocyte. ROS generation due to reoxygenation is a major cause of cardiomyocyte damage in ischemia-reperfusion injury. Therefore, we investigated whether c-myb play a role in ROS generation. By using MitoSOX, a mitochondrial superoxide indicator, we found that H/R injury leads to a large amount of mitochondrial superoxide (Figure 4A). However, H9c2 cells with c-myb overexpression had

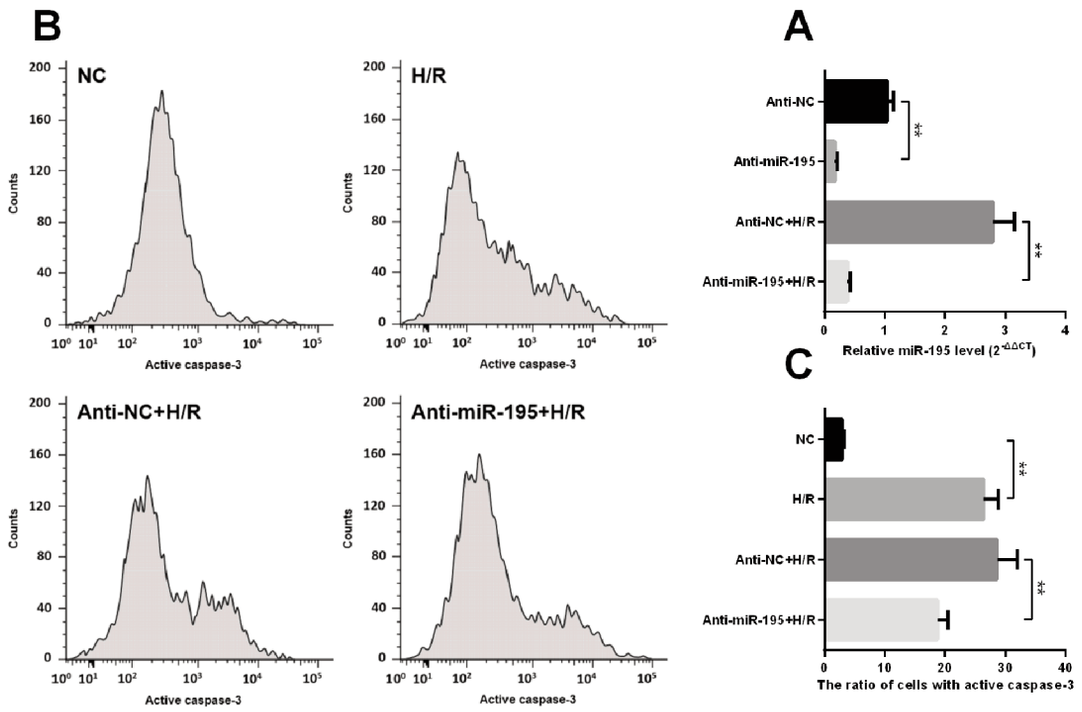

Figure 2. MiR-195 enhances cardiomyocyte apoptosis induced by H/R injury. ***A,*** QRT-PCR analysis of miR-195 levels in H9c2 cells with or without miR-195 suppression after H/R treatment. ***B,*** Representative images of flow cytometric analysis of H9c2 cells (with or without miR-195 knockdown) with active caspase-3 after H/R treatment. ***C,*** Quantification of the ratio of cells with active caspase-3 showed in Figure B. $^{**}p<0.01$.

smaller amount mitochondrial superoxide (Figure 4A). By performing flow cytometric analysis, we also observed that cells with c-myb overexpression before H/R injury had a significantly smaller amount of apoptotic cells (Figure 4B-C). These results suggest that c-myb is a functional target of miR-195 in H/R related cardiomyocyte apoptosis.

Discussion

MiR-195 is a member of the miR-15 gene family. Several studies[12,19,20] suggest that the members of this family are upregulated in overloaded hearts and in response to cardiac ischemia-reperfusion. The plasma concentration of miR-30a, miR-195 and let-7b is considered as a potential biomarker for AMI[11]. In this report, we also found that miR-195 is significantly upregulated after H/R treatment. Therefore, we decided to investigate further its regulation.

Based on the findings of previous researches[21-23], miR-195 is characterized as a regulator of cell cycle, apoptosis, cell metabolism, cell proliferation and metastasis in multiple types of cancer. Similar regulations were observed in cardiomyocytes too. Excessive ROS generation is a consequence of cardiac ischemia-reperfusion, which acts an important mechanism of following cardiac tissue damage. Zhu and Fan[24] confirmed that upregulated miR-195 expression is associated with increasing generation of ROS and subsequently enhanced cardiomyocyte apoptosis. Knockdown of miR-195 can protect cardiomyocytes from hypoxia-induced death *in-vitro* and also alleviates cardiac remodeling in response to ischemic damage *in-vivo*[15]. Therefore, miR-195 suppression is considered as a potential strategy to reduce myocardial apoptosis induced by ischemia–reperfusion injury[24,25]. In line with these findings, our flow cytometric analysis also confirmed that H9c2 cells with miR-195 suppression had substantially alleviated cell apoptosis after H/R injury.

Several investigations also explored the downstream regulations in miR-195 in multiple disease models. Generally, the identified downstream targets of miR-195 include WEE1, CDK6, Bcl-2[13], Sirt1[14], cyclin D1[22], HMGA1[26]

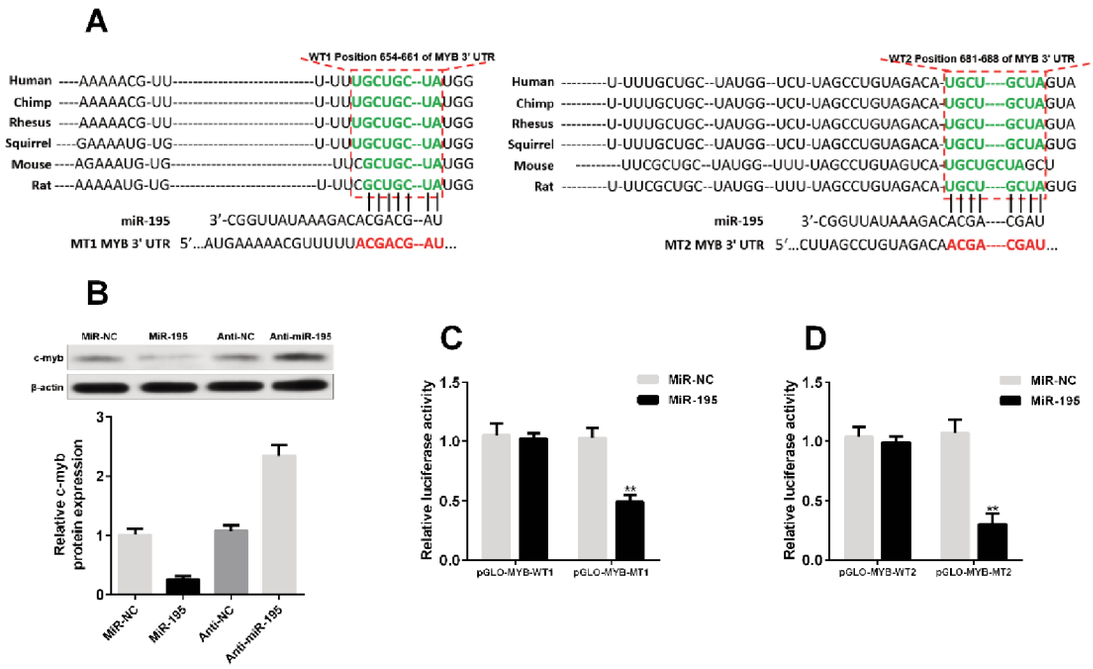

Figure 3. MiR-195 directly targets 3'UTR of MYB mRNA and decreases c-myb protein expression. ***A,*** Two predicted binding sites between miR-195 and 3'UTR of MYB. WT and MT refer to the wild type (WT) and designed sequences with mutant (MT) binding sites respectively. The 3'UTR of MYB in multiple species were compared. ***B,*** Western blot analysis and quantification relative c-myb protein in H9c2 cells after transfection of miR-195 mimics or miR-195 inhibitors (anti-miR-195). ***C-D,*** H9c2 cells were co-transfected with 100 nM miR-195 mimics and pGLO-MYB-WT1 (C) or pGLO-MYB-WT2 (D) or the corresponding control plasmid carrying mutant sequences. The relative luciferase activity was measured 24 hours after transfection. $^{**}p<0.01$.

and Wnt3a[27]. However, the exact regulative effects are tissue and microenvironment-dependent. In cardiomyocytes, miR—195 can enhance isoprenaline-induced cardiomyocyte hypertrophy by targeting HMGA1[26], promote palmitate-induced apoptosis by down-regulating Sirt1[14] and enhance ischemia-reperfusion injury induced cell death via targeting Bcl-2 and inducing mitochondrial apoptotic pathway[12]. Bcl-2 can suppress Box induced apoptosis suppression of cytochrome c (Cyt-c) releasing from mitochondria[28]. In mesenchymal stem cell, it was also observed that GATA-4 overexpression induced increased resistance to ischemia and enhanced cell survival is partially mediated by downregulating of the miR-15 family members and subsequently up-regulation of anti-apoptotic proteins in the Bcl-2 family[29]. Therefore, miR-195 are implicated in the mitochondrial-related apoptotic pathway, thereby modulating cardiomyocyte survival[16]. Based on this evidence, we decided to explore further other possible downstream effectors of miR-195 in modulating mitochondrial related apoptosis.

Zhang et al[30] found that c-myb can inhibit miR-148a by binding to the transcription factor binding site in miR-148a gene and miR-148a can post-transcriptionally downregulating Bcl-2. In cardiomyocyte, it was observed that c-myb protein is involved in regulation of ROS-mediated cardiomyocyte injury[17]. By performing bioinformatics analysis, we found that the 3'UTR of MYB had two broadly conserved binding sites with miR-195. Therefore, we hypothesized that c-myb might be a downstream effector of miR-195 in the regulation of ROS-mediated cardiomyocyte injury. By performing Western blot and dual luciferase assay, we confirmed that miR-195 directly targets 3'UTR of MYB mRNA and decreases c-myb protein expression. By using MitoSOX, a mitochondrial superoxide indicator and flow cytometric analysis, we further revealed that c-myb can suppress mitochondrial superoxide generation and cardiomyocyte apoptosis after H/R. Therefore, we infer that the miR-195-MYB axis is involved in regulation of cardiomyocyte apoptosis induced by HR.

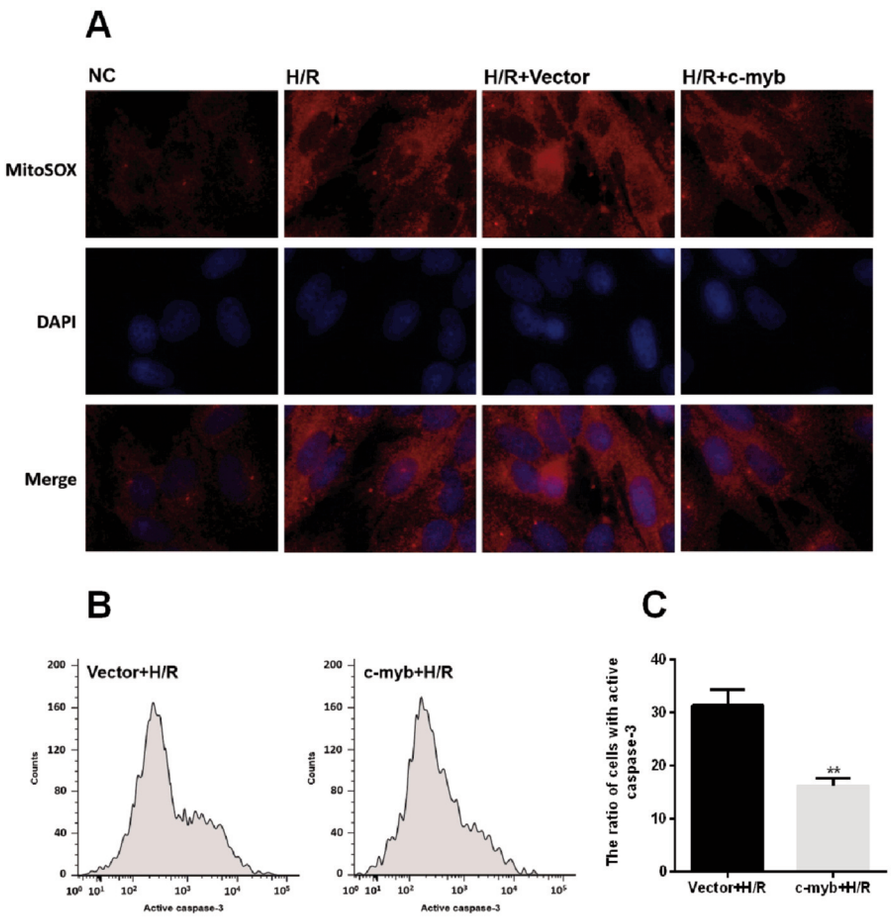

Figure 4. C-myb is a functional target of miR-195 in H/R related cardiomyocyte apoptosis. ***A,*** ROS were visualized using MitoSOX. Representative images of ROS in cells with or without c-myb overexpression after H/R treatment. Red: mitochondrial superoxide; Blue: nuclei stained with DAPI. ***B,*** Representative images of flow cytometric analysis of H9c2 cells (with or without c-myb overexpression) with active caspase-3 after H/R treatment. ***C,*** Quantification of the ratio of cells with active caspase-3 showed in Figure B. $^{**}p<0.01$.

Conclusions

MiR-195 is significantly increased due to H/R and enhances cardiomyocyte apoptosis. MYB is a target gene of miR-195 in cardiomyocytes, and the miR-195-MYB axis is involved in regulation of cardiomyocyte apoptosis induced by H/R.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Frank A, Bonney M, Bonney S, Weitzel L, Koeppen M, Eckle T. Myocardial ischemia reperfusion injury: from basic science to clinical bedside. Semin Cardiothorac Vasc Anesth 2012; 16: 123-132.

2) Yilmaz Y, Taken K, Atar M, Ergun M, Soylemez H. Protective effect of curcumin on priapism and ischemia-reperfusion injury in rats. Eur Rev Med Pharmacol Sci 2015; 19: 4664-4670.

3) Wu N, Li WN, Shu WQ, Lv Y, Jia DL. Blocking the mitochondrial permeability transition pore with cyclosporine-A can restore cardioprotection of ischemic postconditioning in hypercholesterolemic rat heart. Eur Rev Med Pharmacol Sci 2015; 19: 446-454.

4) Hamaguchi E, Tanaka K, Tsutsumi R, Sakai Y, Fukuta K, Kasai A, Tsutsumi YM. Exendin-4, glucagon-like peptide-1 receptor agonist, enhances isoflurane-induced preconditioning against myocardial infarction via caveolin-3 expression. Eur Rev Med Pharmacol Sci 2015; 19: 1285-1290.

5) Kelle I, Akkoc H, Uyar E, Erdinc M, Evliyaoglu O, Saribas S, Tunik S, Ozogul C. The combined effect of rosuvastatin and ischemic pre- or post-conditioning on myocardial ischemia-reperfusion injury

in rat heart. Eur Rev Med Pharmacol Sci 2015; 19: 2468-2476.

6) Li WN, Wu N, Shu WQ, Guan YE, Jia DL. The protective effect of fasudil pretreatment combined with ischemia postconditioning on myocardial ischemia/reperfusion injury in rats. Eur Rev Med Pharmacol Sci 2014; 18: 2748-2758.
7) Shi B, Guo Y, Wang J, Gao W. Altered expression of microRNAs in the myocardium of rats with acute myocardial infarction. BMC Cardiovasc Disord 2010; 10: 11.
8) Shan ZX, Lin QX, Fu YH, Deng CY, Zhou ZL, Zhu JN, Liu XY, Zhang YY, Li Y, Lin SG, Yu XY. Upregulated expression of miR-1/miR-206 in a rat model of myocardial infarction. Biochem Biophys Res Commun 2009; 381: 597-601.
9) Van Rooij E, Sutherland LB, Thatcher JE, Dimaio JM, Naseem RH, Marshall WS, Hill JA, Olson EN. Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. Proc Natl Acad Sci U S A 2008; 105: 13027-13032.
10) Li SP, Liu B, Song B, Wang CX, Zhou YC. miR-28 promotes cardiac ischemia by targeting mitochondrial aldehyde dehydrogenase 2 (ALDH2) in mus musculus cardiac myocytes. Eur Rev Med Pharmacol Sci 2015; 19: 752-758.
11) Long G, Wang F, Duan Q, Yang S, Chen F, Gong W, Yang X, Wang Y, Chen C, Wang DW. Circulating miR-30a, miR-195 and let-7b associated with acute myocardial infarction. PLoS One 2012; 7: e50926.
12) Gao CK, Liu H, Cui CJ, Liang ZG, Yao H, Tian Y. Roles of MicroRNA-195 in cardiomyocyte apoptosis induced by myocardial ischemia-reperfusion injury. J Genet 2016; 95: 99-108.
13) He JF, Luo YM, Wan XH, Jiang D. Biogenesis of MiRNA-195 and its role in biogenesis, the cell cycle, and apoptosis. J Biochem Mol Toxicol 2011; 25: 404-408.
14) Zhu H, Yang Y, Wang Y, Li J, Schiller PW, Peng T. MicroRNA-195 promotes palmitate-induced apoptosis in cardiomyocytes by down-regulating Sirt1. Cardiovasc Res 2011; 92: 75-84.
15) Hullinger TG, Montgomery RL, Seto AG, Dickinson BA, Semus HM, Lynch JM, Dalby CM, Robinson K, Stack C, Latimer PA, Hare JM, Olson EN, Van Rooij E. Inhibition of miR-15 protects against cardiac ischemic injury. Circ Res 2012; 110: 71-81.
16) Porrello ER, Johnson BA, Aurora AB, Simpson E, Nam YJ, Matkovich SJ, Dorn GW, 2nd, Van Rooij E, Olson EN. MiR-15 family regulates postnatal mitotic arrest of cardiomyocytes. Circ Res 2011; 109: 670-679.
17) Li X, Kong M, Jiang D, Qian J, Duan Q, Dong A. MicroRNA-150 aggravates H2O2-induced cardiac myocyte injury by down-regulating c-myb gene. Acta Biochim Biophys Sin (Shanghai) 2013; 45: 734-741.
18) Shin EJ, Schram K, Zheng XL, Sweeney G. Leptin attenuates hypoxia/reoxygenation-induced activation of the intrinsic pathway of apoptosis in rat H9c2 cells. J Cell Physiol 2009; 221: 490-497.
19) Tijsen AJ, Van Der Made I, Van Den Hoogenhof MM, Wijnen WJ, Van Deel ED, De Groot NE, Alekseev S, Fluiter K, Schroen B, Goumans MJ, Van Der Velden J, Duncker DJ, Pinto YM, Creemers EE. The microRNA-15 family inhibits the TGFbeta-pathway in the heart. Cardiovasc Res 2014; 104: 61-71.
20) Liu LF, Liang Z, Lv ZR, Liu XH, Bai J, Chen J, Chen C, Wang Y. MicroRNA-15a/b are up-regulated in response to myocardial ischemia/reperfusion injury. J Geriatr Cardiol 2012; 9: 28-32.
21) Cai C, Chen QB, Han ZD, Zhang YQ, He HC, Chen JH, Chen YR, Yang SB, Wu YD, Zeng YR, Qin GQ, Liang YX, Dai QS, Jiang FN, Wu SL, Zeng GH, Zhong WD, Wu CL. miR-195 Inhibits Tumor Progression by Targeting RPS6KB1 in Human Prostate Cancer. Clin Cancer Res 2015; 21: 4922-4934.
22) Li Z, Wang H, Wang Z, Cai H. MiR-195 inhibits the proliferation of human cervical cancer cells by directly targeting cyclin D1. Tumour Biol 2015.
23) Yongchun Z, Linwei T, Xicai W, Lianhua Y, Guangqiang Z, Ming Y, Guanjian L, Yujie L, Yunchao H. MicroRNA-195 inhibits non-small cell lung cancer cell proliferation, migration and invasion by targeting MYB. Cancer Lett 2014; 347: 65-74.
24) Zhu H, Fan GC. Role of microRNAs in the reperfused myocardium towards post-infarct remodelling. Cardiovasc Res 2012; 94: 284-292.
25) Topkara VK, Mann DL. Role of microRNAs in cardiac remodeling and heart failure. Cardiovasc Drugs Ther 2011; 25: 171-182.
26) You XY, Huang JH, Liu B, Liu SJ, Zhong Y, Liu SM. HMGA1 is a new target of miR-195 involving isoprenaline-induced cardiomyocyte hypertrophy. Biochemistry (Mosc) 2014; 79: 538-544.
27) Furuya K, Sasaki A, Tsunoda Y, Tsuji M, Udaka Y, Oyamada H, Tsuchiya H, Oguchi K. Eribulin upregulates miR-195 expression and downregulates Wnt3a expression in non-basal-like type of triple-negative breast cancer cell MDA-MB-231. Hum Cell 2016; 29: 76-82.
28) Czabotar PE, Lessene G, Strasser A, Adams JM. Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. Nat Rev Mol Cell Biol 2014; 15: 49-63.
29) Yu B, Gong M, He Z, Wang YG, Millard RW, Ashraf M, Xu M. Enhanced mesenchymal stem cell survival induced by GATA-4 overexpression is partially mediated by regulation of the miR-15 family. Int J Biochem Cell Biol 2013; 45: 2724-2735.
30) Zhang H, Li Y, Huang Q, Ren X, Hu H, Sheng H, Lai M. MiR-148a promotes apoptosis by targeting Bcl-2 in colorectal cancer. Cell Death Differ 2011; 18: 1702-1710.