miR-126-5p regulates H9c2 cell proliferation and apoptosis under hypoxic conditions by targeting IL-17A

YIN REN, RUANZHONG BAO, ZHUJUN GUO, JIN KAI, CHEN-GE CAI and ZHU LI

Department of Cardiology, Taizhou People's Hospital of Jiangsu Province, Taizhou, Jiangsu 225300, P.R. China

Received May 29, 2019; Accepted June 19, 2020

DOI: 10.3892/etm.2020.9499

Abstract. Accumulating evidence has indicated that microRNAs (miRNAs/miRs) regulate the occurrence and development of various diseases, including diabetes, osteoporosis and cardiovascular conditions. However, the role of miRNAs in acute myocardial infarction (AMI) is not completely understood. The present study aimed to evaluate the therapeutic efficacy and mechanisms underlying the effects of miR-126-5p on H9c2 cell proliferation and apoptosis by targeting interleukin (IL)-17A. A total of 40 patients with AMI and 40 healthy volunteers were recruited in the present study and the expression levels of serum miR-126-5p and IL-17A were determined. Following confirmation that IL-17A was a target of miR-126-5p via a dual-luciferase reporter assay, H9c2 cells were exposed to hypoxic conditions. H9c2 cell viability and apoptosis were subsequently assessed. Additionally, the protein expression levels of apoptosis-associated proteins were detected following transfection. Compared with healthy individuals, miR-126-5p expression was significantly decreased in the serum samples of patients with AMI, whereas IL-17A, the target of miR-126-5p, was significantly increased. Following hypoxic treatment, miR-126-5p overexpression enhanced H9c2 cell viability compared with the NC group, which was subsequently reversed following co-transfection with pcDNA3.1-IL-17A. Additionally, the results indicated that hypoxia-induced H9c2 cell apoptosis was significantly reduced following transfection with miR-126-5p mimics via the PI3K/AKT signaling pathway compared with the NC group. The present study indicated that miR-126-5p may serve as a novel miRNA that regulates H9c2 cell viability and apoptosis by targeting IL-17A under hypoxic conditions. Therefore, miR-126-5p may serve as a crucial biomarker for the diagnosis of AMI.

Correspondence to: Dr Zhu Li, Department of Cardiology, Taizhou People's Hospital of Jiangsu Province, 336 Taihu Road, Taizhou, Jiangsu 225300, P.R. China
E-mail: zhu_li419@outlook.com

Key words: microRNA-126-5p, interleukin-17A, H9c2 cells, proliferation, apoptosis, acute myocardial infarction

Introduction

Myocardial infarction occurs when blood flow decreases, causing damage to heart muscle (1). Acute myocardial infarction (AMI) frequently induces cardiac dysfunction, eventually resulting in heart failure, which is characterized by cardiac dysfunction and cell death (2,3). During AMI, the myocardium undergoes a series of structural alterations, including decreased proliferation or abnormal apoptosis of myocardial cells (4,5). Therefore, studying H9c2 cell viability and apoptosis following hypoxic treatment was considered to be a suitable model of AMI for the present study.

MicroRNAs (miRNAs/miRs), a class of non-coding small endogenous RNAs 18-22 nucleotides in length, regulate the expression of target genes by post-transcriptionally binding to the 3' untranslated region (3'UTR) of their target genes (6,7). Numerous miRNAs have been reported to participate in the development of various disorders, including diseases of the cardiovascular system (8-10). The miR-126 locus gives rise to two mature miRNAs: miR-126-3p and miR-126-5p (11). Additionally, deletion of miR-126 affects vascular integrity and angiogenesis (12,13). A previous study demonstrated that miR-126 expression was downregulated in patients with AMI following the onset of symptoms (14), indicating that miR-126 may function as a potential biomarker for the diagnosis of AMI. However, the possible roles and mechanisms underlying miR-126-5p in AMI are not completely understood.

Interleukin (IL)-17A is one of 30 known types of ILs and one of the 6 known members of the IL-17 cytokine family (15). IL-17A is an early promoter of T-cell-induced inflammatory response, which can be amplified by promoting the release of pre-inflammatory cytokines (16). IL-17A serves a crucial role in the inflammatory reaction, tissue damage and immunological defense (17-19). A previous study revealed that IL-17A may be involved in H9c2 cell development (20). However, the association between miR-126-5p and IL-17A and their functions in AMI are not completely understood.

Materials and methods

Serum samples. A total of 40 serum samples from patients with AMI (male, 27; female, 13; age, 43-62 years; mean age, 55.3±3.8 years) and 40 serum samples from healthy volunteers (male, 24; female, 16; age, 40-67 years; mean age, 56.2±4.3 years) were collected from the Taizhou People's

Hospital of Jiangsu Province (Taizhou, China) between December 2015 and January 2017. The inclusion criteria were as follows: i) Electrocardiogram with characteristic alterations including the emergence of Q, the spread of ST segment elevation and the dynamic evolution of ST-T; ii) elevated serum biomarkers for myocardial necrosis; iii) myocardial necrosis detected by serum biomarkers; and iv) an intracoronary thrombus identified by angiography. The exclusion criteria were as follows: i) Severe renal dysfunction; ii) myocarditis; iii) rhabdomyolysis or myositis; iv) cardiomyopathy; v) acute pulmonary embolism; vi) cardiothoracic surgery, cardiac procedure or chest-wall trauma; and/or vii) central nervous system pathology.

The present study was approved by the Ethics Committee of Taizhou People's Hospital of Jiangsu Province. Written informed consent was obtained from each patient or volunteer prior to sample collection. All samples were stored at -80˚C until further experimentation.

Cell culture. The rat myocardial H9c2 cell line (Shanghai Yanji Biological Technology Co., Ltd.) was cultured in DMEM (Hyclone; Cytiva) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) and 100 μg/ml streptomycin (Gibco; Thermo Fisher Scientific, Inc.) in a humidified incubator with 5% CO_2 at 37˚C. H9c2 cells were seeded (seeding density, $1x10^6$ cells/well) into six-well plates and transfected with 0.4 nM miR-126-5p mimics or negative controls (NC) using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) for 24 h at 37˚C until use. miR-126-5p mimics (5'-CGCGTACCA AAAGTAATAATG-3') and NC (5'-GTGTAACACGTCTATACG CCCA-3') were designed and synthesized by Shanghai GenePharma Co., Ltd.

Plasmid construction and transfection. To produce IL-17A overexpression pcDNA3.1-IL-17A plasmids, IL-17A coding sequence lacking the 3'UTR was cloned into the pcDNA3.1 vector (Guangzhou RiboBio Co., Ltd.). Subsequently, H9c2 cells (seeding density, $1x10^5$ cells/well) were transfected with 40 nM pcDNA3.1 and 40 nM pcDNA3.1-IL-17A plasmids using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) for 2 h at 37˚C. Following this, H9c2 cells were cultured at 37˚C for a further 24 h until subsequent analysis.

Dual-luciferase reporter assay. TargetScan (version no. 7.2; targetscan.org/vert_72) predicted that IL-17A was a target of miR-126-5p. Subsequently, $2x10^4$ cells/well H9c2 cells were co-transfected with 40 nM wild-type or mutant IL-17A 3'UTR plasmids (Guangzhou RiboBio Co., Ltd.) and miR-126-5p mimics or NCs using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). Following incubation for 24 h at room temperature, luciferase activities were measured using a Dual-Luciferase Reporter Assay system (Promega Corporation). Firefly luciferase activities were normalized to *Renilla* luciferase activities.

Hypoxic treatment. Control cells were cultured in DMEM (Hyclone; Cytiva) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) and 100 μg/ml streptomycin (Gibco; Thermo Fisher Scientific, Inc.) in a humidified incubator with 5% CO_2 at 37˚C until use. For hypoxic treatment, H9c2 cells were cultured in a tri-gas incubator (Thermo Fisher Scientific, Inc.) containing 94% N_2, 5% CO_2 and 1% O_2. Following incubation for 48 h at room temperature, H9c2 cells were harvested for cell viability and apoptosis analysis.

Cell viability assay. Cell viability was determined by performing Cell Counting Kit-8 (CCK-8) assays (Dojindo Molecular Technologies, Inc.), according to the manufacturer's protocol. Following hypoxic treatment, transfected cells were seeded (seeding density, $1x10^5$ cells/well) into 96-well plates and cultured with 5% CO_2 at room temperature. Following incubation for 12, 24 or 48 h, 10 μl CCK-8 solution was added to each well for 2 h and incubated at room temperature until analysis. Subsequently, the absorbance of each well was measured at a wavelength of 450 nm using a multi-mode microplate reader (Biotek Instruments, Inc.).

Flow cytometry assay. Cell apoptosis was detected via flow cytometry using a Annexin V-FITC/propidium iodide (PI) reagent kit (Sigma-Aldrich; Merck KGaA), according to the manufacturer's protocol. Early and late apoptosis was assessed accordingly. Following hypoxic treatment, transfected cells were seeded (seeding density, $3x10^4$ cells/well) into 12-well plates and cultured at 5% CO_2 for 48 h at 37˚C. Subsequently, cells were labeled with Annexin V-FITC and PI for 10 min in the dark at room temperature. Cell apoptosis was assessed using a FACSCalibur flow cytometer (BD Biosciences) and FlowJo software (version no. 10; BD Biosciences).

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from serum samples and cells using TRIzol® (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Total RNA (2 μl) was reverse transcribed into cDNA using a First-strand cDNA synthesis kit (Invitrogen; Thermo Fisher Scientific, Inc.) for 2 h at 37˚C. Subsequently, qPCR was performed using a SYBR®-Green I kit (cat. no. S9430; Sigma-Aldrich; Merck KGaA) using the ABI Prism 7700 sequence detection system (Applied Biosystems; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. The following thermocycling conditions were used for qPCR: Initial denaturation or 3 min at 94˚C; 30 cycles of annealing at 62˚C for 10 sec and extension at 72˚C for 10 sec; and final extension at 72˚C for 10 min. Primers were purchased from Sigma-Aldrich, Merck KGaA and the sequences used were as follows: miR-126-5p forward, 5'-GGAATGTAAGGAAGT GTG-3' and reverse, 5'-GAGCAGGCTGGAGAA-3'; IL-17A forward, 5'-TCCCACGAAATCCAGGATGC-3' and reverse, 5'-GGATGTTCAGGTTGACCATCAC-3'; U6 forward, 5'-CTT CGGCAGCACATATAC-3' and reverse, 5'-GAACGCTTCACG AATTTGC-3'; and GAPDH forward, 5'-ACATGTTCCAATAT GATTCC-3' and reverse, 5'-TGGACTCCACGACGTACTCAG-3'. miRNA and mRNA expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method (21) and normalized to the internal reference genes U6 and GAPDH, respectively.

Western blotting. Total protein was extracted from serum samples and cells using RIPA buffer (Sigma-Aldrich; Merck KGaA). Total protein was quantified using a bicinchoninic acid protein assay kit (Beyotime Institute of Biotechnology). Proteins (10 μg/lane) were separated via 10% SDS-PAGE (cat. no. P1200-25T; Beijing Solarbio Science & Technology Co., Ltd.) and transferred to PVDF membranes (Beijing Solarbio Science & Technology Co.,

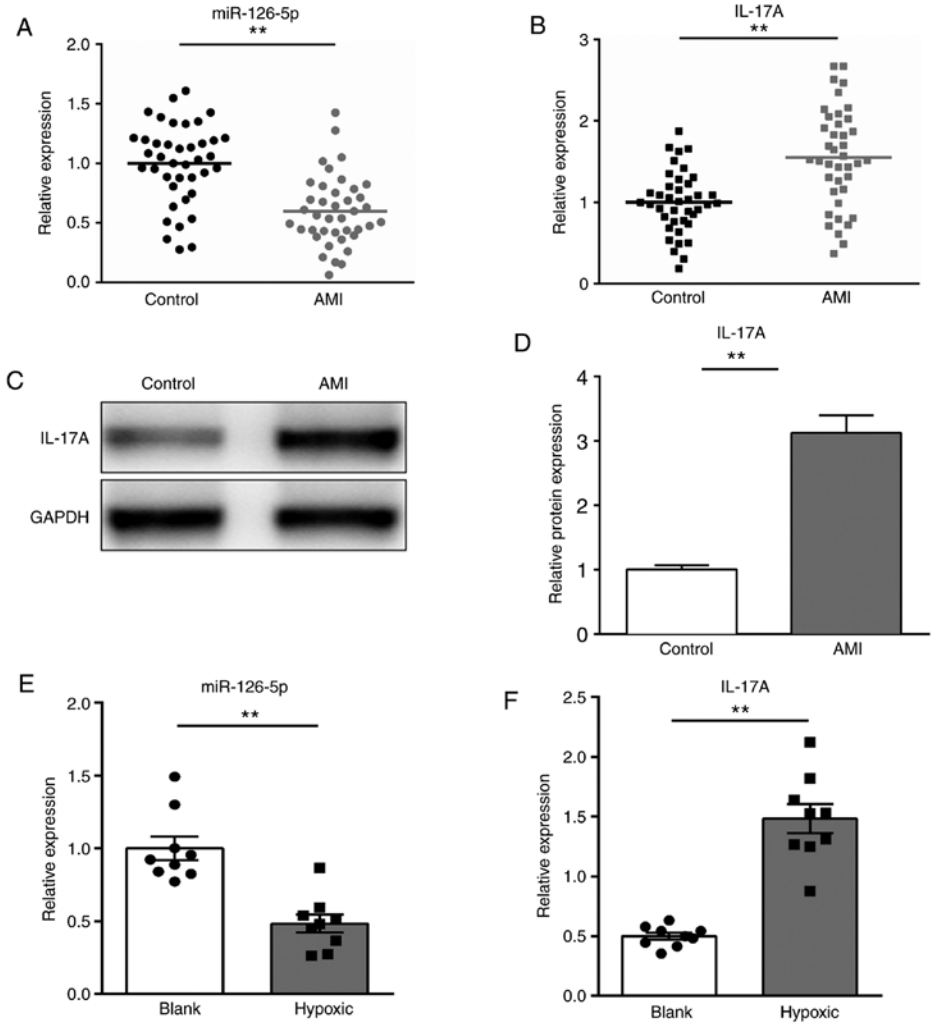

Figure 1. miR-126-5p expression is downregulated and IL-17A is upregulated in serum samples obtained from patients with AMI and H9c2 cells following hypoxic treatment. (A) miR-126-5p and (B) IL-17A expression in patients with AMI and healthy individuals. IL-17A protein expression levels in the serum of patients with AMI and healthy individuals were (C) determined by western blotting and (D) quantified. (E) miR-126-5p and (F) IL-17A expression levels in H9c2 cells following hypoxic treatment. **P<0.01. miR, microRNA; IL, interleukin; control, healthy volunteers; AMI, acute myocardial infarction; hypoxic, H9c2 cells following hypoxic treatment; control, H9c2 cells.

Ltd.). The membranes were blocked with 5% non-fat milk in TBST (containing 0.1% Tween-20; cat. no. T1081-500; Beijing Solarbio Science & Technology Co., Ltd.) for 50 min at room temperature. Subsequently, the membranes were incubated at 4˚C overnight with the following primary antibodies: Rabbit anti-IL-17A (1:1,000; cat. no. ab136668; Abcam), rabbit anti-B-cell lymphoma 2 (Bcl-2; 1:1,000; cat. no. ab32124; Abcam), rabbit anti-AKT (1:500; cat. no. ab8805; Abcam), rabbit anti-phosphorylated (p)-AKT (1:1,000; cat. no. 4060; Cell Signaling Technology, Inc.), rabbit anti-PI3K (1:1,000; cat. no. ab32089; Abcam), rabbit anti-p-PI3K (1:1,000; cat. no. ab182651; Abcam), rabbit anti-Bcl-2 associated X protein (Bax; 1:1,000; cat. no. ab32503; Abcam), rabbit anti-cleaved caspase-3 (1:500; cat. no. ab13847; Abcam) and rabbit anti-GAPDH (1:2,500; cat. no. ab9485; Abcam). After washing with 0.1% TBST, the membranes were incubated with a goat anti-rabbit IgG H&L secondary antibodies (1:1,000; cat. no. ab6940; Abcam) for 50 min at room temperature. Protein bands were visualized using an enhanced chemiluminescence kit (Thermo Fisher Scientific, Inc.). Protein expression levels were quantified using ImageJ software (version no. 1.16; National Institutes of Health) with GAPDH as the loading control.

Statistical analysis. Statistical analyses were performed using SPSS software (version no. 21.0; IBM Corp.). Data are presented as the mean ± standard deviation. Comparisons between two groups were analyzed using unpaired Student's t-test. Comparisons among multiple groups were analyzed using one-way ANOVA followed by Tukey's post-hoc test. $P<0.05$ was considered to indicate a statistically significant difference. All experiments were performed in triplicate.

Results

miR-126-5p expression is downregulated and IL-17A expression is upregulated in the serum of patients with AMI and in H9c2 cells following hypoxic treatment. To verify whether miR-126-5p participated in the pathogenesis of AMI, the expression of miR-126-5p and its predicted target, IL-17A, in the serum of patients with AMI and healthy individuals was determined via RT-qPCR. The expression of miR-126-5p was significantly downregulated, whereas IL-17A mRNA and protein expression levels were significantly upregulated in patients with AMI compared with healthy individuals ($P<0.01$; Fig. 1A-D).

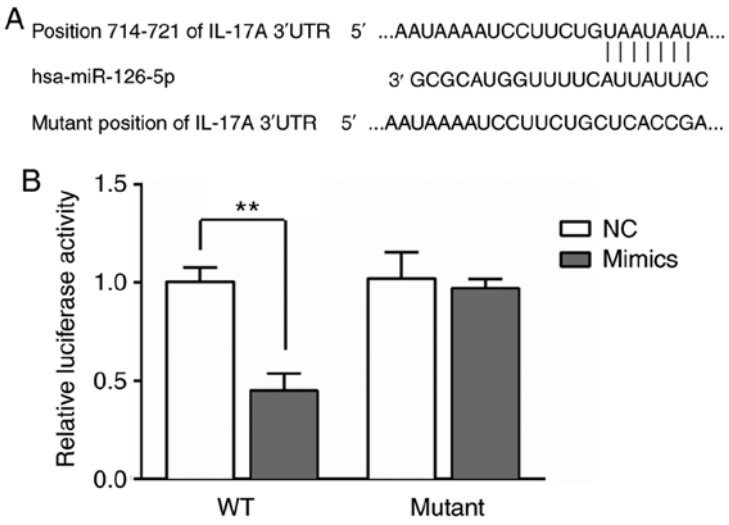

Figure 2. IL-17A is a target of miR-126-5p. (A) The binding site between miR-126-5p and IL-17A was predicted using TargetScan. (B) Dual-luciferase reporter assay results. **P<0.01. IL, interleukin; miR, microRNA; NC, negative control; WT, wild-type; UTR, untranslated region.

Furthermore, the expression levels of miR-126-5p and IL-17A in H9c2 cells following hypoxic treatment were also determined. The expression of miR-126-5p was significantly decreased, whereas IL-17A expression levels were significantly increased after hypoxic treatment compared with the control group (P<0.01; Fig. 1E and F). The results indicated that miR-126-5p may participate in AMI progression.

IL-17A is a target of miR-126-5p. The IL-17A 3'UTR contained predicted miR-126-5p binding sites (Fig. 2A). miR-126-5p mimics significantly decreased the luciferase activity of wild-type IL-17A 3'UTR compared with the NC group (P<0.01). However, the luciferase activity of mutant IL-17A 3'UTR was not significantly altered by miR-126-5p mimics compared with NC (P>0.05; Fig. 2B).

Transfection efficiency of miR-126-5p and IL-17A. The expression of miR-126-5p was significantly increased following transfection with miR-126-5p mimics compared with the NC group (P<0.01). However, the expression of miR-126-5p was not significantly altered in the NC group compared with the control group (P>0.05; Fig. 3A). Additionally, the mRNA and protein expression levels of IL-17A were significantly increased following transfection with pcDNA3.1-IL-17A vector (P<0.01; Fig. 3B and C) compared with the pcDNA3.1 group. Following co-transfection with miR-126-5p mimics and pcDNA3.1-IL-17A, the expression levels of miR-126-5p and IL-17A were measured. miR-126-5p expression levels were significantly reduced in the IL-17A group compared with the control group, whereas co-transfection with miR-126-5p mimics partially reversed the effects of IL-17A overexpression (P<0.01; Fig. 3D) compared with the IL-17 group. Meanwhile, IL-17A expression was significantly reduced in the mimics group compared with the control group, co-transfection with pcDNA3.1-IL-17 vector partially reversed the effects of miR-126-5p overexpression (P<0.01; Fig. 3E) compared with the mimics group.

miR-126-5p overexpression promotes H9c2 cell survival under hypoxic conditions. Under hypoxic conditions, H9c2 cell viability was significantly increased in the miR-126-5p mimics group compared with the NC group (P<0.01). The results also indicated that co-transfection with pcDNA3.1-IL-17A reversed the effects of miR-126-5p mimics on cell viability compared with the mimics group (P<0.01; Fig. 4).

miR-126-5p overexpression suppresses H9c2 cell apoptosis under hypoxic conditions. Under hypoxic conditions, H9c2 cell apoptosis was significantly decreased in the miR-126-5p mimics group compared with the NC group (P<0.01), which was reversed by co-transfection with pcDNA3.1-IL-17A (P<0.01; Fig. 5).

miR-126-5p regulates H9c2 cell apoptosis via the PI3K/AKT signaling pathway. Under hypoxic conditions, the expression levels of IL-17A, Bax and cleaved caspase-3 were significantly decreased, whereas p-PI3K, p-AKT and Bcl-2 were significantly increased in the miR-126-5p mimics group compared with the NC group (P<0.05). However, co-transfection with pcDNA3.1-IL-17A reversed miR-126-5p mimic-mediated effects on protein expression (Fig. 6).

Discussion

The present study indicated that miR-126-5p expression was significantly reduced in the serum of patients with AMI compared with healthy volunteers. Previous studies have demonstrated that miR-126-5p was downregulated in H9c2 cells and may inhibit H9c2 cell apoptosis, inflammation and angiogenesis following an antioxidant stimulus (22,23). A recent study reported that the expression of miR-126-5p was significantly decreased in patients with AMI (24), which was consistent with the results of the present study.

IL-17A is a proinflammatory cytokine that is primarily produced by Th17 cells (25). Accumulating evidence has confirmed that IL-17A may serve as a potential diagnostic marker for the development of various acute or chronic diseases (26,27). Furthermore, the IL-17A gene is highly expressed in patients with AMI (28). Bcl-2 and Bax are members of the Bcl-2 family that regulate cell apoptosis by inhibiting or inducing apoptosis

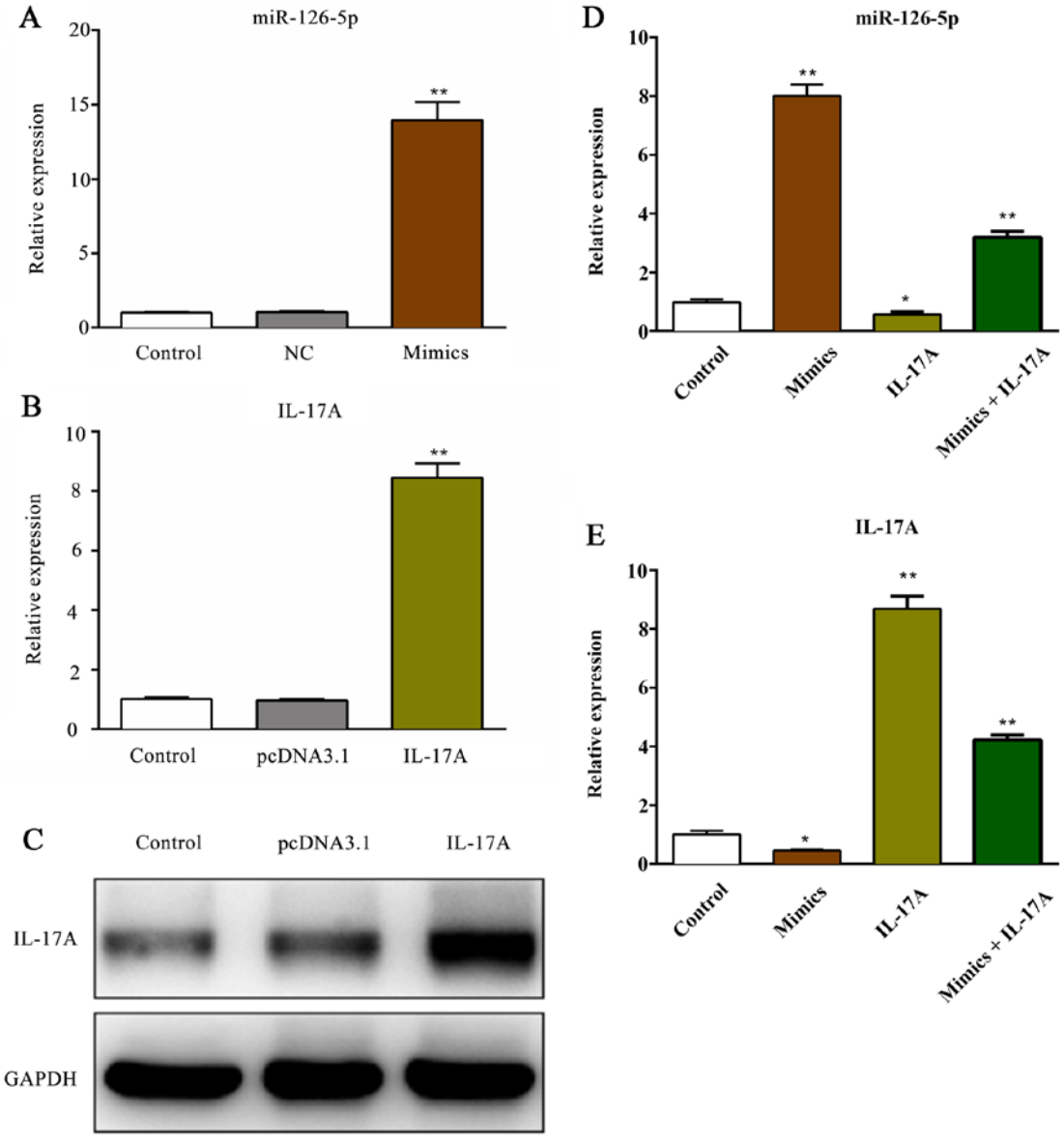

Figure 3. Transfection efficiency of miR-126-5p and IL-17A. (A) miR-126-5p expression following transfection with NC or miR-126-5p mimics. IL-17A (B) mRNA and (C) protein expression levels following transfection with pcDNA3.1 or pcDNA3.1-IL-17A. (D) miR-126-5p and (E) IL-17A expression following co-transfection with miR-126-5p mimics and pcDNA3.1-IL-17A. *P<0.05 and **P<0.01. miR, microRNA; IL, interleukin; NC, negative control; IL-17A, cells transfected with pcDNA3.1-IL-17A vector.

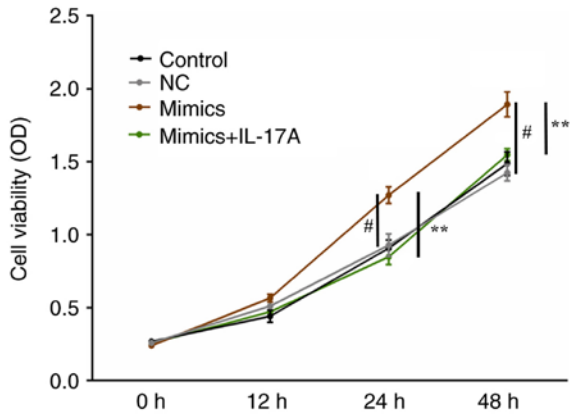

Figure 4. miR-126-5p overexpression enhances H9c2 cell survival under hypoxic conditions. $^{\#}$P<0.01, mimics vs. NC group; **P<0.01, mimic + IL-17A vs. mimics group. miR, microRNA; NC, negative control; mimics + IL-17A, cells transfected with miR-126-5p mimics and pcDNA3.1-IL-17A vector; IL, interleukin.

in a wide variety of cellular activities (29). Previous studies have reported that Bcl-2 overexpression may suppress hypoxia-induced H9c2 cell apoptosis (30,31), whereas Bax knockdown may protect H9c2 cells against hypoxia-induced apoptosis (32). Further studies reported that IL-17A inhibition increased the expression of Bcl-2 and decreased the expression of Bax (33-35). Caspase-3 is a member of the caspase family and serves a crucial role in the execution phase of cell apoptosis (36). Caspase-3 knockdown may protect against hypoxia-induced H9c2 cell apoptosis (37). Several studies have revealed that cleaved caspase-3 expression could be suppressed by IL-17A inhibition (38,39). Hence, miR-126-5p may promote H9c2 cell viability and protect against hypoxia-induced H9c2 apoptosis by downregulating Bax and cleaved caspase-3 expression, and upregulating Bcl-2 expression, which was in accordance with present study. PI3K/AKT is an intracellular signaling pathway that is important for the modulation of the cell cycle and is directly associated with cell viability

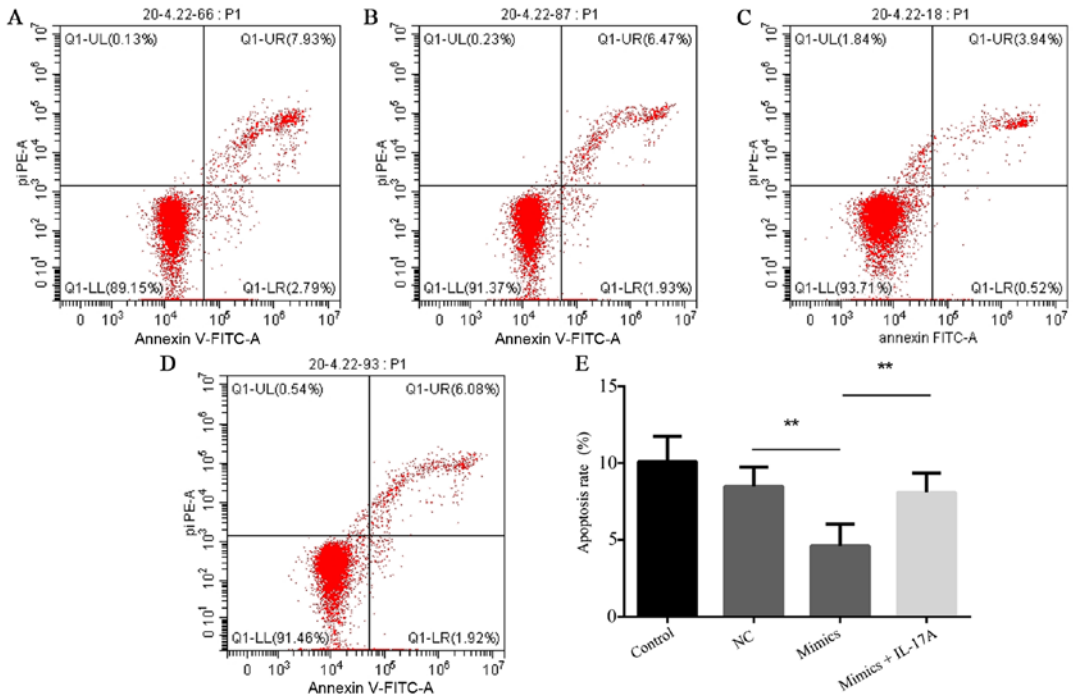

Figure 5. miR-126-5p overexpression suppresses H9c2 cell apoptosis under hypoxic conditions. Representative scatter plots for the (A) control, (B) NC, (C) mimics and (D) mimics + IL-17A groups. (E) Quantification of H9c2 cell apoptosis. **P<0.01. miR, microRNA; NC, negative control; mimics + IL-17A, cells transfected with miR-126-5p mimics and pcDNA3.1-IL-17A vector; IL, interleukin.

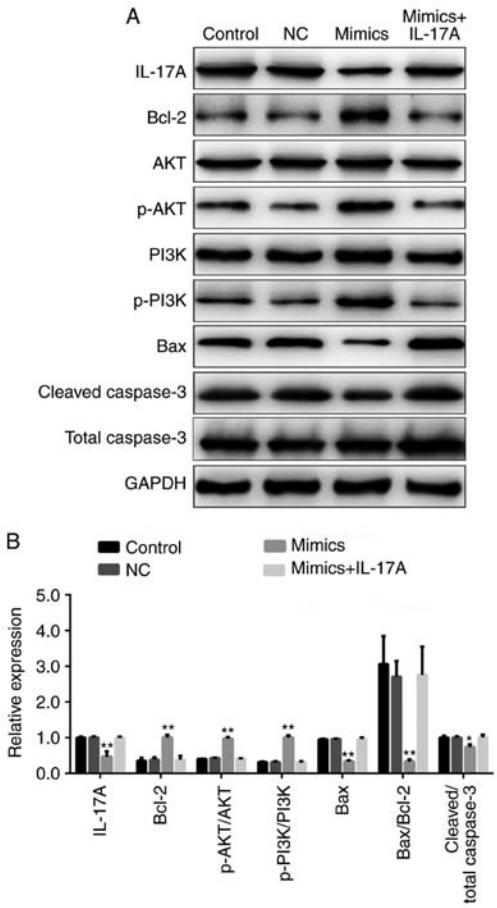

Figure 6. miR-126-5p regulates H9c2 cell apoptosis via the PI3K/AKT signaling pathway. Protein expression levels were (A) determined by western blotting and (B) semi-quantified. *P<0.01 and **P<0.05. miR, microRNA; NC, negative control; mimics + IL-17A, cells transfected with miR-126-5p mimics and pcDNA3.1-IL-17A vector; p, phosphorylated; IL, interleukin.

and apoptosis (40,41). Several studies have demonstrated that activation of the PI3K/AKT signaling pathway attenuates hypoxia-induced H9c2 cell apoptosis (42-44). The present study indicated that compared with the NC group, miR-126-5p overexpression promoted H9c2 cell viability and protected against hypoxia-induced H9c2 apoptosis by targeting IL-17A via the PI3K/AKT signaling pathway. To verify the interaction between miR-126-5p and IL-17A, miR-126-5p and IL-17A were overexpressed in the same cells in the present study. Nonetheless, future studies should perform IL-17A knockdown experiments to further verify the mechanism identified in the present study.

In conclusion, the present study identified a potential mechanism underlying AMI development associated with miR-126-5p. However, further studies are required to confirm the results of the present study.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YR, RB, ZG and JK drafted the manuscript. YR, RB and ZG designed and conceptualized the current study. YR, CGC and

ZL contributed to data acquisition and supervision. JK, CGC and ZL performed data analysis, interpretation and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent for participation

The present study was approved by the Ethics Committee of Taizhou People's Hospital of Jiangsu Province, Taizhou, China. Written informed consent was obtained from all patients and volunteers prior to sample collection.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM, *et al*: Bone marrow cells regenerate infarcted myocardium. Nature 410: 701-705, 2001.
2. Bissessor N and White H: Valsartan in the treatment of heart failure or left ventricular dysfunction after myocardial infarction. Vasc Health Risk Manag 3: 425-430, 2007.
3. DeBusk R, Drory Y, Goldstein I, Jackson G, Kaul S, Kimmel SE, Kostis JB, Kloner RA, Lakin M, Meston CM, *et al*: Management of sexual dysfunction in patients with cardiovascular disease: Recommendations of The Princeton Consensus Panel. Am J Cardiol 86: 175-181, 2000.
4. Schaper J and Schaper W: Reperfusion of ischemic myocardium: Ultrastructural and histochemical aspects. J Am Coll Cardiol 1: 1037-1046, 1983.
5. Fein FS, Kornstein LB, Strobeck JE, Capasso JM and Sonnenblick EH: Altered myocardial mechanics in diabetic rats. Circ Res 47: 922-933, 1980.
6. Montgomery RL and van Rooij E: Therapeutic advances in MicroRNA targeting. J Cardiovasc Pharmacol 57: 1-7, 2011.
7. Calin GA and Croce CM: MicroRNA signatures in human cancers. Nat Rev Cancer 6: 857-866, 2006.
8. Wu W: MicroRNA: Potential targets for the development of novel drugs? Drugs RD 10: 1-8, 2010.
9. Ai J, Zhang R, Li Y, Pu J, Lu Y, Jiao J, Li K, Yu B, Li Z, Wang R, *et al*: Circulating microRNA-1 as a potential novel biomarker for acute myocardial infarction. Biochem Biophys Res Commun 391: 73-77, 2010.
10. Pan ZW, Lu YJ and Yang BF: MicroRNAs: A novel class of potential therapeutic targets for cardiovascular diseases. Acta Pharmacol Sin 31: 1-9, 2010.
11. Villain G, Poissonnier L, Noueihed B, Bonfils G, Rivera JC, Chemtob S, Soncin F and Mattot Y: miR-126-5p promotes retinal endothelial cell survival through SetD5 regulation in neurons. Development 145: dev156232, 2018.
12. Fish JE, Santoro MM, Morton SU, Yu S, Yeh RF, Wythe JD, Ivey KN, Bruneau BG, Stainier DY and Srivastava D: miR-126 regulates angiogenic signaling and vascular integrity. Dev Cell 15: 272-284, 2008.
13. Wang S, Aurora AB, Johnson BA, Qi X, McAnally J, Hill JA, Richardson JA, Bassel-Duby R and Olson EN: The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. Dev Cell 15: 261-271, 2008.
14. Long G, Wang F, Duan Q, Chen F, Yang S, Gong W, Wang Y, Chen C and Wang DW: Human circulating microRNA-1 and microRNA-126 as potential novel indicators for acute myocardial infarction. Int J Biol Sci 8: 811-818, 2012.
15. von Stebut E, Boehncke WH, Ghoreschi K, Gori T, Kaya Z, Thaci D and Schäffler A: IL-18A in psoriasis and beyond: Cardiovascular and metabolic implications. Front Immunol 10: 3096, 2020.
16. Bai H, Gao X, Zhao L, Peng Y, Yang J, Qiao S, Zhao H, Wang S, Fan Y, Joyee AG, *et al*: Respective IL-17A production by γδ T and Th17 cells and its implication in host defense against chlamydial lung infection. Cell Mol Immunol 14: 850-861, 2017.
17. Kuwabara T, Ishikawa F, Kondo M and Kakiuchi T: The role of IL-17 and related cytokines in inflammatory autoimmune diseases. Mediators Inflamm 2017: 3908061, 2017.
18. Shaikh SB, Bhat SG and Bhandary YP: Curcumin attenuates IL-17A mediated pulmonary SMAD dependent and non-dependent mechanism during acute lung injury in vivo. Mol Biol Rep 47: 5643-5649, 2020.
19. Yang P, Qian FY, Zhang MF, Xu AL, Wang X, Jiang BP and Zhou LL: Th17 cell pathogenicity and plasticity in rheumatoid arthritis. J Leukoc Biol 106: 1233-1240, 2019.
20. Huang KD, Shen Y, Wei X, Zhang FQ, Liu YY and Ma L: Inhibitory effect of microRNA-27b on interleukin 17 (IL-17)-induced monocyte chemoattractant protein-1 (MCP1) expression. Genet Mol Res 15: 2, 2016.
21. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
22. Luo Q, Guo D, Liu G, Chen G, Hang M and Jin M: Exosomes from miR-126-overexpressing adscs are therapeutic in relieving acute myocardial ischaemic injury. Cell Physiol Biochem 44: 2105-2116, 2017.
23. Li B, Tao Y and Huang Q: Effect and mechanism of miR-126 in myocardial ischemia reperfusion. Genet Mol Res 14: 18990-18998, 2015.
24. Xue S, Liu D, Zhu W, Su Z, Zhang L, Zhou C and Li P: Circulating miR-17-5p, miR-126-5p and miR-145-3p are novel biomarkers for diagnosis of acute myocardial infarction. Front Physiol 10: 123, 2019.
25. Butcher MJ, Wu CI, Waseem T and Galkina EV: CXCR6 regulates the recruitment of pro-inflammatory IL-17A-producing T cells into atherosclerotic aortas. Int Immunol 28: 255-261, 2016.
26. Erbel C, Chen L, Bea F, Wangler S, Celik S, Lasitschka F, Wang Y, Böckler D, Katus HA and Dengler TJ: Inhibition of IL-17A attenuates atherosclerotic lesion development in apoE-deficient mice. J Immunol 183: 8167-8175, 2009.
27. de Boer OJ, van der Meer JJ, Teeling P, van der Loos CM, Idu MM, van Maldegem F, Aten J and van der Wal AC: Differential expression of interleukin-17 family cytokines in intact and complicated human atherosclerotic plaques. J Pathol 220: 499-508, 2010.
28. Chen XM, Zhang T, Qiu D, Feng JY, Jin ZY, Luo Q, Wang XY and Wu XL: Gene expression pattern of TCR repertoire and alteration expression of IL-17A gene of γδ T cells in patients with acute myocardial infarction. J Transl Med 16: 189, 2018.
29. Edlich F: BCL-2 proteins and apoptosis: Recent insights and unknowns. Biochem Biophys Res Commun 500: 26-34, 2018.
30. Liu Y, Yang L, Yin J, Su D, Pan Z, Li P and Wang X: MicroRNA-15b deteriorates hypoxia/reoxygenation-induced cardiomyocyte apoptosis by downregulating Bcl-2 and MAPK3. J Investig Med 66: 39-45, 2018.
31. Liu N, Shi YF, Diao HY, Li YX, Cui Y, Song XJ, Tian X, Li TY and Liu B: MicroRNA-135a regulates apoptosis induced by hydrogen peroxide in rat cardiomyoblast cells. Int J Biol Sci 13: 13-21, 2017.
32. Zhou YL, Sun Q, Zhang L and Li R: miR-208b targets Bax to protect H9c2 cells against hypoxia-induced apoptosis. Biomed Pharmacother 106: 1751-1759, 2018.
33. Olsson Åkefeldt S, Maisse C, Belot A, Mazzorana M, Salvatore G, Bissay N, Jurdic P, Aricò M, Rabourdin-Combe C, Henter JI, *et al*: Chemoresistance of human monocyte-derived dendritic cells is regulated by IL-17A. PLoS One 8: e56865, 2013.
34. Sui G, Qiu Y, Yu H, Kong Q and Zhen B: Interleukin-17 promotes the development of cisplatin resistance in colorectal cancer. Oncol Lett 17: 944-950, 2019.
35. Chen X, Yu X, Li X, Li L, Li F, Guo T, Guan C, Miao L and Cao G: miR-126 targets IL-17A to enhance proliferation and inhibit apoptosis in high-glucose-induced human retinal endothelial cells. Biochem Cell Biol 98: 13, 2019.
36. Fan TJ, Han LH, Cong RS and Liang J: Caspase family proteases and apoptosis. Acta Biochim Biophys Sin (Shanghai) 37: 719-727, 2005.
37. Jiang YQ, Chang GL, Wang Y, Zhang DY, Cao L and Liu J: Geniposide prevents hypoxia/Reoxygenation-induced apoptosis in H9c2 cells: Improvement of mitochondrial dysfunction and activation of GLP1R and the PI3K/AKT signaling pathway. Cell Physiol Biochem 39: 407-421, 2016.

38. Cruz A, Ludovico P, Torrado E, Gama JB, Sousa J, Gaifem J, Appelberg R, Rodrigues F, Cooper AM, Pedrosa J, *et al*: IL-17A promotes intracellular growth of mycobacterium by inhibiting apoptosis of infected macrophages. Front Immunol 6: 498, 2015.
39. Li N, Gao S, Wang J, Zhu Y and Shen X: Anti-apoptotic effect of interleukin-17 in a mouse model of oxygen-induced retinopathy. Exp Eye Res 187: 107743, 2019.
40. Mao Y, Xi L, Li Q, Cai Z, Lai Y, Zhang X and Yu C: Regulation of cell apoptosis and proliferation in pancreatic cancer through PI3K/Akt pathway via Polo-like kinase 1. Oncol Rep 36: 49-56, 2016.
41. Maurya AK and Vinayak M: PI-103 attenuates PI3K-AKT signaling and induces apoptosis in murineT-cell lymphoma. Leuk Lymphoma 58: 1153-1161, 2017.
42. Liu MH, Li GH, Peng LJ, Qu SL, Zhang Y, Peng J, Luo XY, Hu HJ, Ren Z, Liu Y, *et al*: PI3K/Akt/FoxO3a signaling mediates cardioprotection of FGF-2 against hydrogen peroxide-induced apoptosis in H9c2 cells. Mol Cell Biochem 414: 57-66, 2016.
43. Li L, Zhou Y, Li Y, Wang L, Sun L, Zhou L, Arai H, Qi Y and Xu Y: Aqueous extract of Cortex Dictamni protects H9c2 cardiomyocytes from hypoxia/reoxygenation-induced oxidative stress and apoptosis by PI3K/Akt signaling pathway. Biomed Pharmacother 89: 233-244, 2017.
44. Zhang Z, Li H, Chen S, Li Y, Cui Z and Ma J: Knockdown of microRNA-122 protects H9c2 cardiomyocytes from hypoxia-induced apoptosis and promotes autophagy. Med Sci Monit 23: 4284-4290, 2017.