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miR-9 knockdown inhibits hypoxia-induced cardiomyocyte apoptosis by targeting Yap1

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ABSTRACT

Aims: Aberrantly expressed miRNAs are demonstrated to be involved in the development of congenital heart disease (CHD). miR-9 was proposed to be upregulated in cardiac tissues from CHD cases. However, the role of miR-9 in hypoxia-induced cardiomyocytes and the potential mechanism are far from being addressed.

Main methods: qRT-PCR and western blot analysis were performed to detect miR-9 and Yes-associated protein 1 (Yap1) expressions in hypoxic H9c2 cells. CCK-8, flow cytometry analysis, caspase-3/7 activity assay were applied to evaluate cell proliferation, apoptosis, and caspase-3/7 activity, respectively. The interaction between miR-9 and Yap1 was explored by luciferase reporter assay, qRT-PCR and western blot.

Key findings: miR-9 was upregulated and Yap1 was downregulated in H9c2 cells in response to hypoxia in a time-dependent manner. Knockdown of miR-9 promoted cell proliferation, and inhibited apoptosis and caspase-3/7 activity in hypoxic H9c2 cells, while miR-9 overexpression exerted the opposite effects on hypoxic H9c2 cells. In addition, Yap1 was a direct target of miR-9 in H9c2 cells. Yap1 knockdown suppressed cell proliferation and promoted apoptosis in hypoxia-exposed H9c2 cells. Yap1 knockdown attenuated the effect of anti-miR-9 on cell proliferation and apoptosis in hypoxia-exposed H9c2 cells.

Significance: miR-9 knockdown inhibited hypoxia-induced cardiomyocyte apoptosis by targeting Yap1. Our study provided a novel insight into the mechanism of the adaptation of cardiomyocytes to chronic hypoxia.

1. Introduction

Congenital heart disease (CHD) is the most frequent type of human congenital malformation in the fetal age and is the leading cause of infant mortality worldwide with an incidence rate of 6–8 per 1000 live births, which poses a heavy health burden on families and society [1]. Researches have demonstrated that the etiology of CHD is multifactorial, and multiple genetic and environmental causes are responsible for the occurrence of CHD [2,3]. Chronic hypoxia is one of the basic pathophysiological processes, which can affect mitochondrial oxidative metabolism, resulting in heart remodeling [4]. Although patients with CHD are chronically perfused with low-oxygen blood, most of them are able to adapt chronic hypoxia and rarely progress to cardiac failure [5]. Thus, exploring the pathophysiological changes of CHD and the underlying mechanisms of protective adaptation of cardiomyocytes to chronic hypoxia is helpful to provide novel therapeutic strategies for attenuating myocardial damage during cardiac surgery.

microRNAs (miRNAs) are a group of small endogenous non-coding RNAs with approximately 18–24 nucleotides in length and negatively regulate gene expression post-transcriptionally by binding to the 3′untranslated region (3′UTR) of target mRNA, leading to inhibition of translation or mRNA degradation [6]. miRNAs have been demonstrated to play a crucial regulatory role in many aspects of cellular function, such as cell proliferation, apoptosis, differentiation and development [7]. Notably, compelling evidence has suggested that aberrantly expressed miRNAs are involved in the development of CHD [8]. For example, miR-23b expression was increased in the patients with cyanotic CHD and hypoxia-exposed H9c2 cells, which promoted cardiomyocyte apoptosis and reduced cell growth under hypoxic conditions [9]. miR-182 expression was downregulated in the patients with cyanotic CHD and hypoxia-induced cardiomyocytes, and exerted protection effects through suppressing HES1 in hypoxia-induced cardiomyocytes [10]. miR-9, a brain-enriched miRNA, was proposed to be upregulated in cardiac tissues from CHD cases [11]. However, the role of miR-9 in hypoxia-induced cardiomyocytes and the potential mechanism are far from being addressed.

As a key downstream effector of the hippo signaling pathway, Yes-associated protein 1 (Yap1) is a transcriptional coactivator interacting with TEA domain family members (TEAD) and regulates organ size and regeneration, as well as normal tissue homeostasis by modulating cell

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proliferation, growth and apoptosis [12]. Yap1 has recently attracted increasing interest due to its critical role in the regulation of heart development and cardiomyocyte proliferation [13,14]. Activation of Yap1 has been well-documented to promote cardiomyocyte proliferation, differentiation and regeneration [15]. Yap1 was previously reported to be downregulated in pediatric hearts with ventricular septal defects (VSDs), the most common type of CHD [16]. However, the detailed role of Yap1 in hypoxic cardiomyocytes remains to be further elucidated.

In the present study, we investigated the expression and role of miR-9 in hypoxia-treated cardiomyocytes, as well as the interaction between miR-9 and Yap1. We found that miR-9 was upregulated in hypoxia-cultured H9c2 cells and miR-9 knockdown restored hypoxia-induced proliferation reduction and inhibited hypoxia-induced apoptosis of cardiomyocytes by targeting Yap1.

2. Materials and methods

2.1. Cell culture and treatment

The embryonic rat ventricular myocardial H9c2 cells were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained in Dulbecco's modified Eagle's medium (DMEM, Thermo Fisher scientific, Waltham, MA, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco, Grand Island, NY, USA), and 1% penicillin/streptomycin (Gibco) at 37 °C in an incubator containing 95% air and 5% CO_2. After incubation in serum-free medium overnight, cells in hypoxia group were cultured in an *Invivo*200 cultivator (Ruskin Technology Ltd., Bridgend, UK) and exposed to a gaseous mixture of 1% O_2, 5% CO_2 and 94% N_2 for durations of 24, 48, and 72 h at 37 °C. Cells in normal group were cultivated under the same conditions except for 21% O_2 concentrations.

2.2. Cell transfection

miR-9 mimics, mimic negative control (miR-NC), miR-9 inhibitor (anti-miR-9), inhibitor negative control (anti-miR-NC), siRNA targeting Yap1 (si-Yap1), and siRNA negative control (si-NC) were synthesized from GenePharma Co., Ltd. (Shanghai, China). When grown to 70–80% confluence, H9c2 cells were transfected with miR-9 mimics, anti-miR-9, si-Yap1, or respective controls, or cotransfected with anti-miR-9 or anti-miR-NC and si-Yap1 or si-NC using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). H9c2 cells were then incubated in the hypoxic condition for 48 h.

2.3. Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from treated H9c2 cells using Trizol Reagent (Invitrogen) and the concentration of extracted total RNA was detected using a spectrophotometer (Thermo Fisher Scientific). Reverse transcription into cDNA was then performed using a PrimeScript RT reagent kit (Takara, Otsu, Japan) and specific stem-loop RT primers. To quantify miR-9 and Yap1 mRNA expressions, qPCR was conducted using a mirVana™ qRT-PCR microRNA detection kit (Thermo Fisher Scientific) and SYBR Premix Ex Taq GC kit (Takara), respectively. All PCR reactions were performed on a Step-One plus real-time PCR System (Applied Biosystems, Carlsbad, CA, USA). U6 small nuclear RNA (snRNA) and GAPDH were used as the normalization for miRNA and mRNA, respectively. The primer sequences used were as follows: miR-9, forward 5′-GCCCGCTCTTTGGTTATCTAG-3′, reverse 5′-CCAGTGCAGG GTCCGAGGT-3′; U6, forward 5′-CTCGCTTCGGCAGCACA-3′, reverse 5′-AACGCTTCACGAATTTGCGT-3′; Yap1, forward 5′-AGAAC AATGAC GACCAATAGCTC-3′, reverse 5′-GCT GCTCATGCTTAGTCCAC-3′; GAPDH, forward 5′-TATGACTCTACCCACGGCAAGT-3′, reverse 5′-ATACTCAGCACCAGCATCACC-3′.

2.4. Western blot analysis

Total protein was extracted from treated H9c2 cells using ice-cold RIPA lysis buffer (Beyotime, Shanghai, China) supplemented with 1 mM phenylmethylsulfonyl fluoride (PMSF; Sigma-Aldrich, St. Louis, MO, USA). The protein samples (50 μg/lane) were loaded on 10% SDS-PAGE and then blotted onto a polyvinylidene difluoride membrane (Millipore, Billerica, MA, USA). Following being blocked with 5% non-fat dry milk for 2 h, the membranes were probed at 4 °C overnight with primary antibodies against Yap1 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and β-actin (Santa Cruz Biotechnology, Inc.) and then incubated with secondary antibodies conjugated to horseradish peroxidase (Santa Cruz Biotechnology, Inc.) for 1 h at room temperature. Finally, immunoreactive protein bands were visualized using the Enhanced Chemiluminescence (ECL) system (Amersham Pharmacia, Piscataway, NJ, USA) and band intensities were quantified using an LAS-3000 image analyzer (Fuji Photo Film Co., Tokyo, Japan).

2.5. Cell proliferation assay

Cell Counting Kit-8 (CCK-8) assay was performed to assess cell proliferation. The treated H9c2 cells were cultured in 96-well plates at a density of 2000 cells/well and cultured for 24 h, 48 h, and 72 h. At the indicated time point, 10 μL of CCK-8 reagent (Dojindo, Tokyo, Japan) was added to each well and incubated for 2 h at 37 °C. The absorbance at 450 nm was determined with a microplate reader (Bio-Tek Instruments, Winooski, VT, USA).

2.6. Flow cytometry analysis

Cell apoptosis was evaluated by flow cytometry with Annexin V-fluorescein isothiocyanate (FITC) Apoptosis Detection Kit (BD Biosciences, San Diego, CA, USA). Briefly, the treated H9c2 cells were collected and washed with PBS, followed by resuspension in 100 μL 1 × binding buffer at a concentration of 1×10^5 cells/mL. Subsequently, the cells were incubated with 5 μL of Annexin V-FITC and 5 μL propidium iodide (PI) for 15 min in the dark. Cell apoptotic rate was analyzed by FACScan Flow Cytometer (BD Biosciences).

2.7. Caspase-3/7 activity assay

The activity of caspase-3/7 was measured using a commercially available Caspase-Glo 3/7 assay kit (Promega, Madison, WI, USA). Briefly, the treated H9c2 cells were seeded into 96-well plates at a density of 1×10^4 cells/well and incubated with the culture medium containing 100 μL of Caspase-Glo 3/7 reagent for 2 h. The activity of caspse-3/7 was then measured using VICTOR-X3 Multilabel Plate Reader (Perkin Elmer, Santa Clara, CA, USA).

2.8. Luciferase reporter assay

The wild-type (WT) 3′UTR of Yap1 carrying the binding sites of miR-9 was synthesized and cloned into the pMIR-REPORT luciferase reporter plasmids (Promega), namely WT-Yap1. The mutant Yap1 3′UTR reporter construct (MUT-Yap1) was generated using Quickchange XL site-directed mutagenesis kit (Agilent Technologies, Santa Clara, CA, USA). For luciferase reporter assay, H9c2 cells were seeded into 96-well plates and cotransfected with miR-9 mimics or miR-NC and WT-Yap1 or MUT-Yap1 using Lipofectamine 2000 (Invitrogen). After 48 h of transfection, luciferase activities were measured using a Dual-Luciferase Reporter Assay System (Promega) following the manufacturer's instructions.

2.9. Statistical analysis

All results are shown as mean ± standard deviation (SD) and

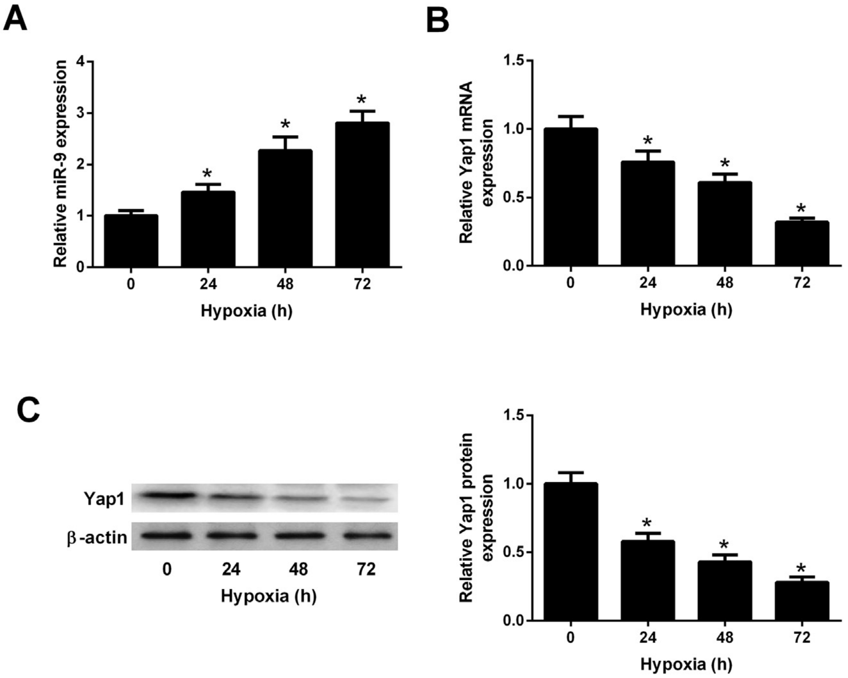

Fig. 1. Expressions of miR-9 and Yap1 in hypoxia-cultured H9c2 cells. (A) The expression of miR-9 was detected by qRT-PCR in H9c2 cells exposed to hypoxia for 24, 48, and 72 h. (B and C) The mRNA and protein levels of Yap1 in H9c2 cells under hypoxic conditions for 24, 48, and 72 h were examined by qRT-PCR and western blot, respectively. $^*P < 0.05$ vs 0 h.

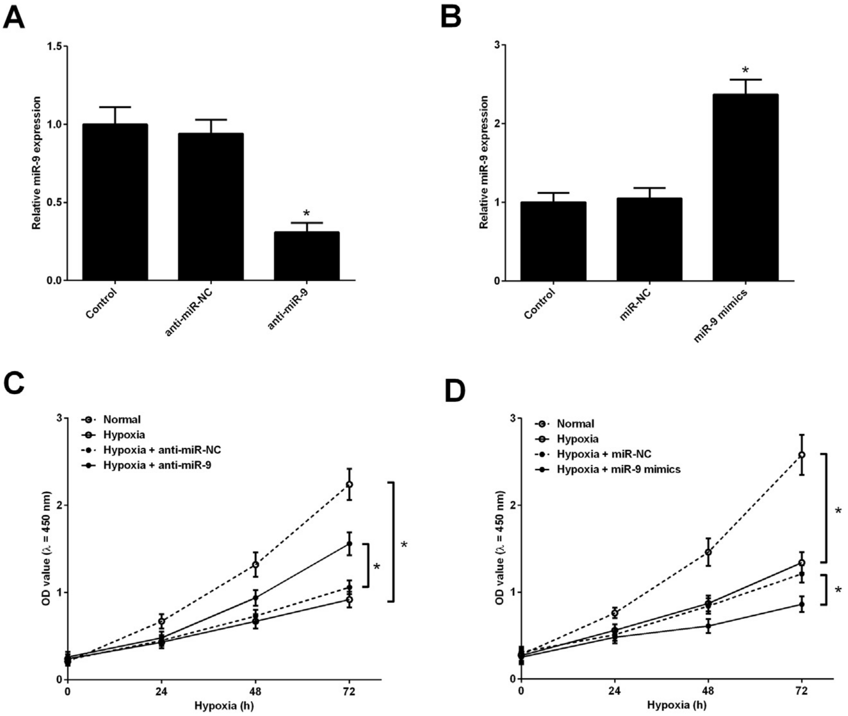

Fig. 2. Effects of miR-9 on cell proliferation in hypoxia-induced H9c2 cells. (A) The expression of miR-9 was measured by qRT-PCR in H9c2 cells transfected with anti-miR-9 or anti-miR-NC for 48 h. $^*P < 0.05$ vs anti-miR-NC. (B) The expression of miR-9 was detected by qRT-PCR in H9c2 cells 48 h after transfection with miR-9 mimics or miR-NC. $^*P < 0.05$ vs miR-NC. (C) CCK-8 assay was conducted to assess cell proliferation in H9c2 cells after transfection with anti-miR-9 or anti-miR-NC, followed by treatment with hypoxia for appointed time. $^*P < 0.05$. (D) CCK-8 assay was applied to detect cell proliferation in H9c2 cells after transfection with anti-miR-9 or anti-miR-NC, followed by induction of hypoxia for appointed time. $^*P < 0.05$.

statistical analysis was carried out using Graphpad Prism version 5.0 (GraphPad Software, San Diego, CA, USA). The statistical differences were assessed using Student's *t*-test or one-way analysis of variance (ANOVA). *P* values of < 0.05 were considered as statistically significant.

3. Results

3.1. miR-9 was upregulated and Yap1 was downregulated in hypoxia-cultured H9c2 cells

H9c2 cells were exposed to hypoxia for 24, 48, and 72 h and the expressions of miR-9 and Yap1 were detected by qRT-PCR and western blot. The results showed that miR-9 expression was continuously increased (Fig. 1A) while Yap1 expression at mRNA (Fig. 1B) and protein (Fig. 1C) levels were gradually decreased in hypoxia-exposed H9c2 cells in a time-dependent manner.

3.2. Knockdown of miR-9 restored hypoxia-induced proliferation reduction in H9c2 cells

To clarify the potential roles of miR-9 in hypoxic cardiomyocytes, gain-of-function and loss-of-function approaches were performed in H9c2 cells by transfecting with miR-9 mimics, anti-miR-9, or matched

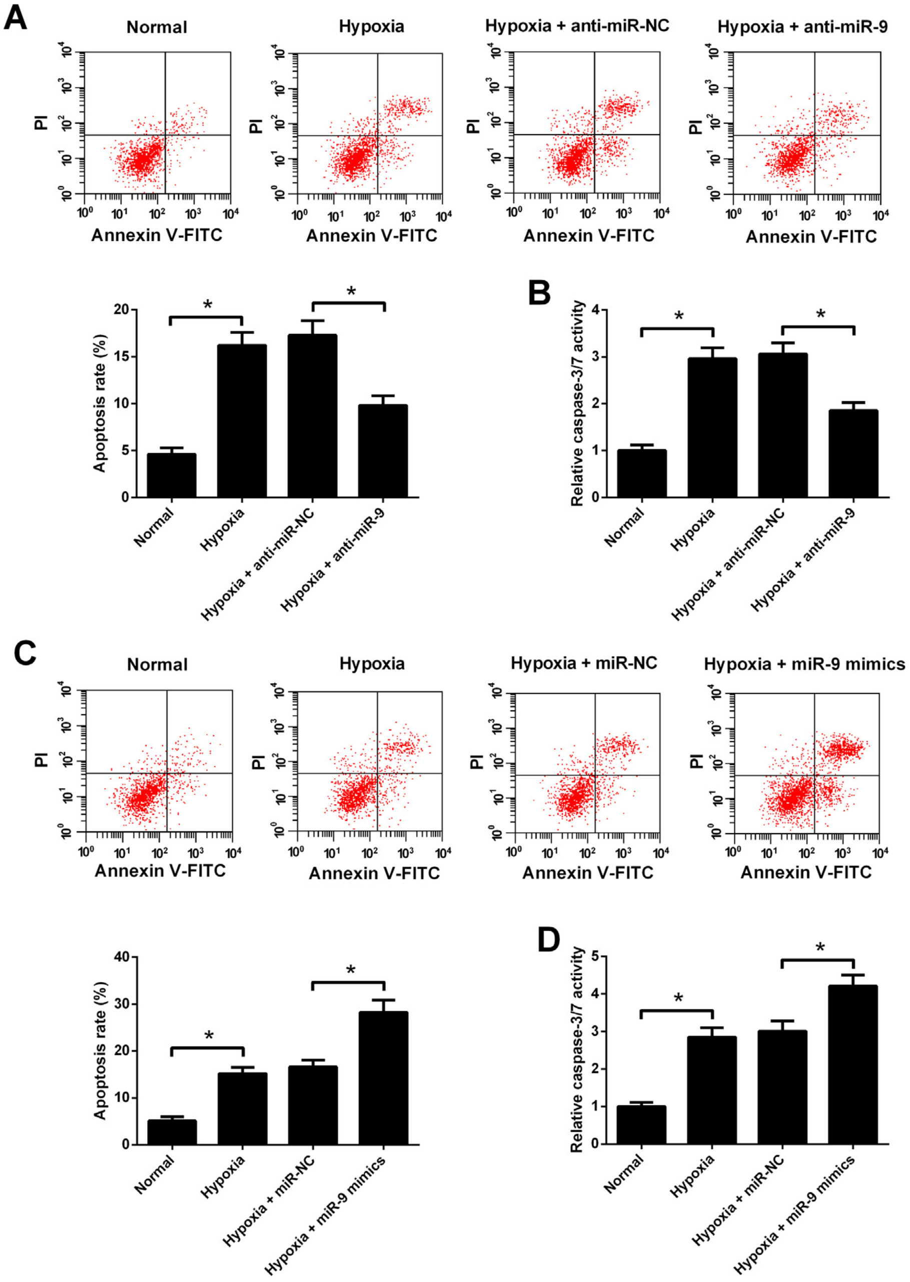

Fig. 3. Effects of miR-9 on apoptosis of hypoxia-induced H9c2 cells. (A and C) Apoptosis of H9c2 cells was evaluated by flow cytometry analysis after transfection with anti-miR-NC, anti-miR-9, miR-9 mimics, or miR-NC, followed by induction of hypoxia for 48 h. (B and D) Caspase-3/7 activity was measured after H9c2 cells were introduced with anti-miR-NC, anti-miR-9, miR-9 mimics, or miR-NC, and then exposed to hypoxia for 48 h. $^*P < 0.05$.

controls, followed by hypoxia treatment. As demonstrated by qRT-PCR, miR-9 expression was notably decreased following transfection with anti-miR-9 (Fig. 2A) and overexpressed by miR-9 mimics (Fig. 2B) in H9c2 cells. The effect of miR-9 on cell proliferation was evaluated by CCK-8 assay and the subsequent results revealed that exposure to hypoxia effectively suppressed cell proliferation in H9c2 cells. However, decrement of miR-9 distinctly restored hypoxia-induced proliferation inhibition (Fig. 2C) while overexpression of miR-9 intensified hypoxia-induced proliferation reduction (Fig. 2D) in H9c2 cells. These results suggested that knockdown of miR-9 restored hypoxia-induced proliferation reduction in H9c2 cells.

3.3. Knockdown of miR-9 inhibited hypoxia-induced apoptosis in H9c2 cells

The effect of miR-9 on apoptosis was explored by flow cytometry analysis. As shown in Fig. 3A and C, cell apoptosis was induced after the induction of hypoxia in H9c2 cells. However, transfection with anti-miR-9 weakened hypoxia-induced apoptosis while introduction with miR-9 mimics enhanced hypoxia-induced apoptosis in H9c2 cells. Meanwhile, caspase-3/7 activity assay revealed that caspase-3/7 activity was elevated following treatment with hypoxia in H9c2 cells. However, miR-9 knockdown by anti-miR-9 hindered the caspase-3/7 activity in H9c2 cells under hypoxic condition (Fig. 3B). Forced expression of miR-9 promoted the caspase-3/7 activity in hypoxia-induced H9c2 cells (Fig. 3D). Therefore, we concluded that knockdown of miR-9 inhibited hypoxia-induced apoptosis in H9c2 cells.

3.4. Yap1 was a direct target of miR-9

To elucidate the molecular mechanism underlying the biological

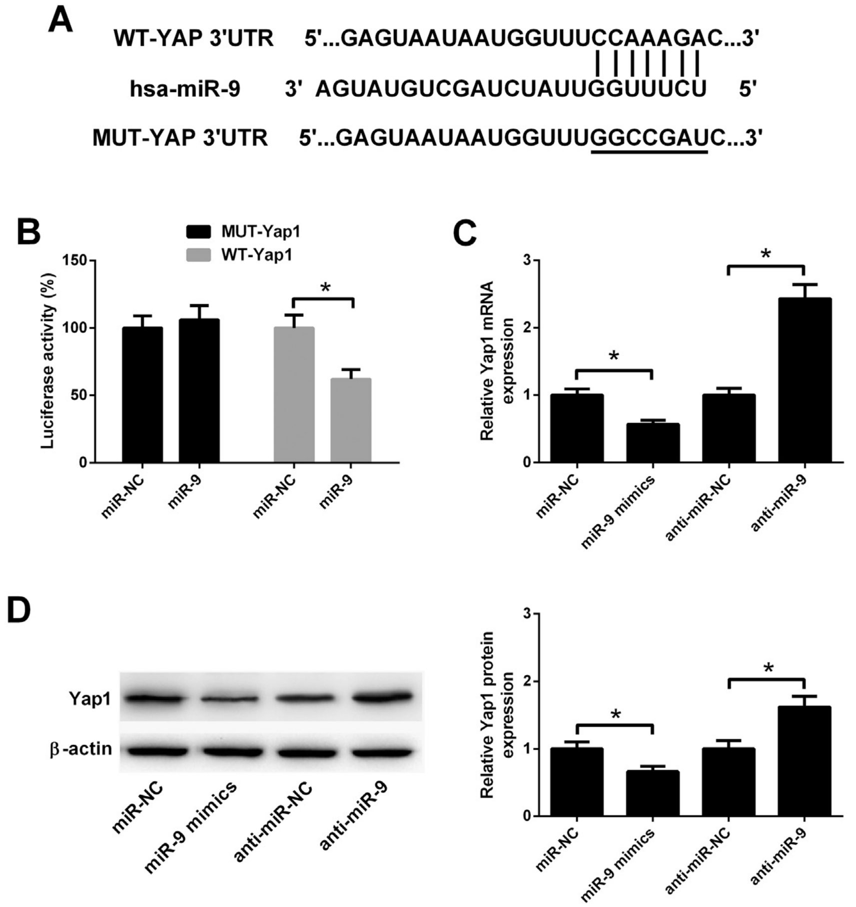

Fig. 4. Yap1 was a direct target of miR-9. (A) TargetScan algorithm predicted that the 3′UTR of Yap1 mRNA contained the potential binding sites of miR-9. (B) Luciferase reporter assay was conducted to measure the luciferase activity after H9c2 cells were cotransfected with miR-9 mimics or miR-NC and WT-Yap1 or MUT-Yap1. (C) qRT-PCR and western blot analyses were performed to determine the mRNA and protein levels of Yap1 in H9c2 cells 48 h after introduction with miR-9 mimics, anti-miR-9, or matched controls. $^*P < 0.05$.

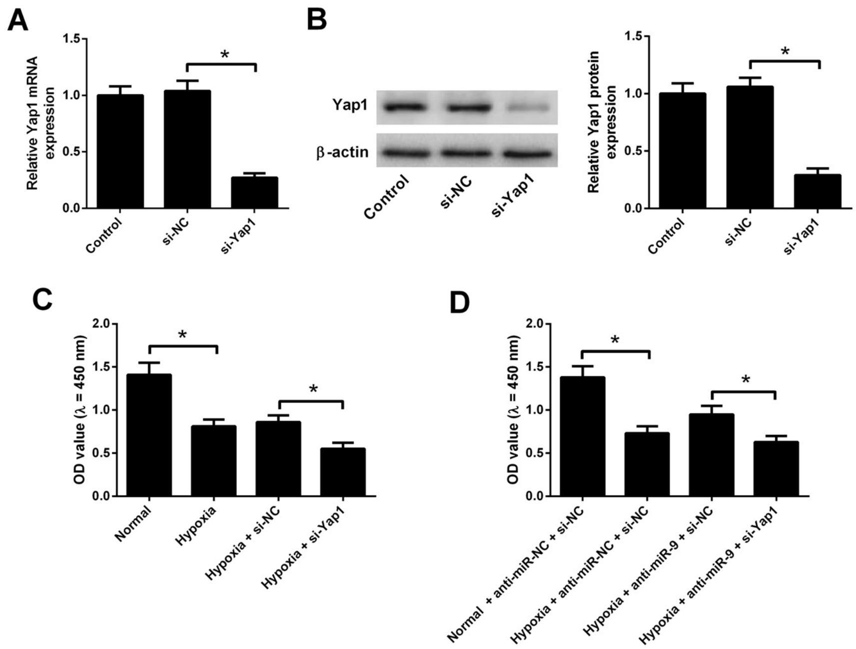

Fig. 5. Yap1 knockdown attenuated the effects of anti-miR-9 on the proliferation in hypoxia-cultured H9c2 cells. (A and B) qRT-PCR and western blot analysis were performed to detect the mRNA and protein levels of Yap1 in H9c2 cells with si-Yap1 or si-NC transfection, respectively. (C) Cell viability was determined by CCK-8 assay in H9c2 cells after transfection with si-Yap1, followed by induction of hypoxia for 48 h. (D) Cell viability was evaluated by CCK-8 assay after H9c2 cells were cotransfected with anti-miR-9 or anti-miR-NC and si-Yap1 or si-NC, and then treated with hypoxia for 48 h. $^*P < 0.05$.

function of miR-9 in hypoxic cardiomyocytes, a bioinformatics analysis using TargetScan algorithm predicted the potential targets of miR-9 and found that the 3′UTR of Yap1 contained the complementary binding sites of miR-9, as shown in Fig. 4A. The subsequent luciferase reporter assay validated that miR-9 overexpression reduced the luciferase activity of WT-Yap1 in H9c2 cells, but failed to decrease the luciferase activity of MUT-Yap1 (Fig. 4B). To further explore the regulatory effect of miR-9 on Yap1 expression, H9c2 cells were transfected with miR-9 mimics, anti-miR-9, or matched controls. qRT-PCR and western blot analysis revealed that the mRNA and protein levels of Yap1 were suppressed by transfection of miR-9 mimics but promoted following introduction with anti-miR-9 in H9c2 cells (Fig. 4C and D). These results

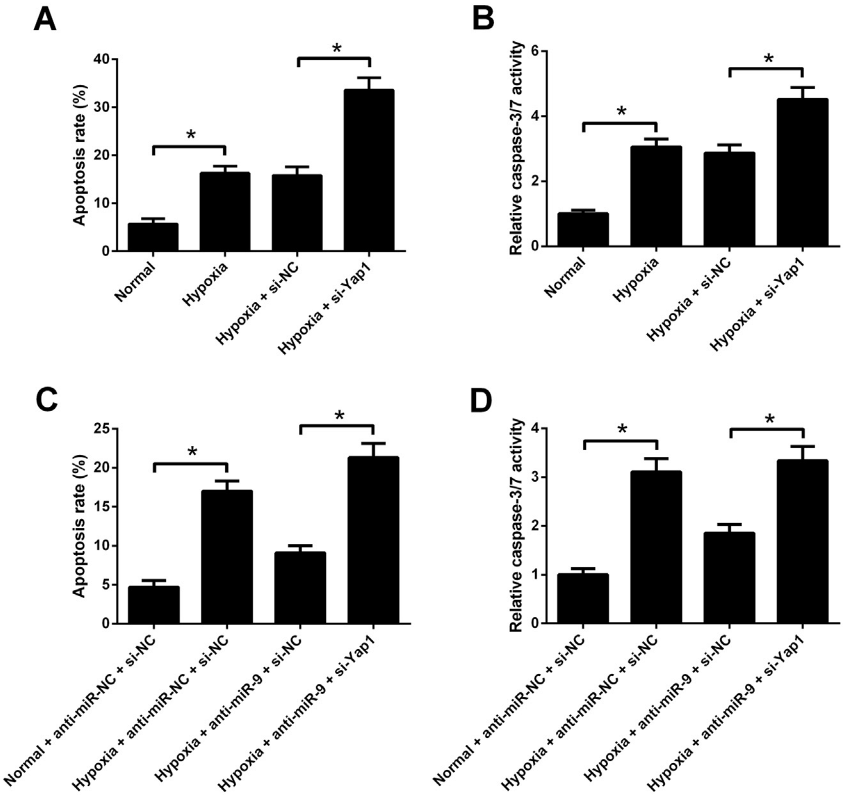

Fig. 6. Yap1 knockdown resisted the effect of anti-miR-9 on apoptosis in hypoxia-cultured H9c2 cells. (A) Cell apoptosis was examined by flow cytometry analysis after H9c2 cells were introduced with si-Yap1 or si-NC, and then treated with hypoxia for 48 h. (B) Caspase-3/7 activity was measured by caspase-3/7 activity assay after H9c2 cells were treated with si-Yap1 or si-NC, and then exposed to hypoxia for 48 h. (C) Cell apoptosis was examined by flow cytometry analysis after H9c2 cells were cotransfected with anti-miR-9 or anti-miR-NC and si-Yap1 or si-NC, followed by treatment with hypoxia for 48 h. (D) Caspase-3/7 activity was measured by caspase-3/7 activity assay after H9c2 cells were cotransfected with anti-miR-9 or anti-miR-NC and si-Yap1 or si-NC, and then exposed to hypoxia for 48 h. $^{*}P < 0.05$.

demonstrated that Yap1 was a direct target of miR-9.

3.5. Yap1 knockdown attenuated the effects of anti-miR-9 on proliferation in hypoxia-cultured H9c2 cells

To determine the effects of Yap1 in hypoxic cardiomyocytes, H9c2 cells were transfected with si-Yap1 or si-NC prior to treatment with hypoxia. The transfection efficiency was confirmed by qRT-PCR and western blot analysis and the results showed that the mRNA and protein levels of Yap1 were substantially declined in si-Yap1-treated H9c2 cells (Fig. 5A and B). CCK-8 assay manifested that depletion of Yap1 evidently exacerbated hypoxia-induced proliferation reduction in H9c2 cells with respect to si-NC group (Fig. 5C). Moreover, Yap1 silencing significantly antagonized the promotion of proliferation by anti-miR-9 in hypoxia-induced H9c2 cells (Fig. 5D). Collectively, these data suggested that Yap1 knockdown attenuated the effects of anti-miR-9 on the proliferation in hypoxia-cultured H9c2 cells.

3.6. Yap1 knockdown resisted the effect of anti-miR-9 on apoptosis in hypoxia-cultured H9c2 cells

Flow cytometry analysis suggested that suppressed expression of Yap1 potentiated hypoxia-induced apoptosis in H9c2 cells as compared with si-NC group (Fig. 6A). Moreover, caspase-3/7 activity assay revealed that Yap1 knockdown greatly increased caspase-3/7 activity in hypoxia-treated H9c2 cells when compared with si-NC group (Fig. 6B). Rescue experiments further demonstrated that the inhibitory effect of anti-miR-9 on hypoxia-induced apoptosis in H9c2 cells was abolished following Yap1 silencing (Fig. 6C). We also discovered that Yap1 depletion ameliorated anti-miR-9-induced repression on caspase-3/7 activity in H9c2 cells under hypoxic conditions (Fig. 6D). Therefore, we concluded that Yap1 knockdown resisted the effect of anti-miR-9 on apoptosis in hypoxia-cultured H9c2 cells.

4. Discussion

CHD is the most common type of birth defect, accounting for the high infant morbidity and mortality [17]. Chronic hypoxia is widely recognized as a common feature in patients with CHD and contributes to their pathophysiological process [18]. It has been suggested that hypoxia contributes to the pathogenesis of apoptosis, and the degree of hypoxic-induced cardiomyocyte injury is positively related to the number of apoptotic cells [19]. Herein, our study demonstrated that miR-9 expression was continually increased while Yap1 expression was gradually decreased in H9c2 cells in response to hypoxia in a time-dependent manner. Moreover, we firstly revealed that inhibition of miR-9 restored hypoxia-induced proliferation reduction and inhibited hypoxia-induced apoptosis in H9c2 cells, suggesting that inhibition of miR-9 was probably a potential cardioprotective mechanism during chronic hypoxia.

miR-9, a miRNA of recognized neural functions, has been reported to function as either an oncogenic or tumor suppressive gene in tumor progression, suggesting the context-dependent roles in different types of tumors [20,21]. Accumulating evidence has indicated that miRNAs play critical regulatory roles in the embryonic development of the heart, cardiac pathophysiology and cardiovascular function [22,23]. Up to now, the role of miR-9 in heart diseases has been investigated by several studies. For example, miR-9-5p mimics aggravated hypoxia-induced injury to H9c2 cells while inhibition of miR-9-5p attenuated cardiac remodeling following myocardial infarction (MI) and maintained heart function [24]. Upregulation of miR-9 ameliorated high glucose-induced proliferation, differentiation and collagen accumulation of human cardiac fibroblasts [25]. miR-9 expression was downregulated upon hypertrophic stimulation with isoproterenol and aldosterone and enforced expression of miR-9 attenuated cardiac hypertrophy by suppressing myocardin expression [26]. In the present study, we found that miR-9 expression was continually increased in H9c2 cells in response to hypoxia in a time-dependent manner, consistently with the previous study [11]. Functional analyses demonstrated that inhibition of miR-9 promoted cell proliferation and suppressed apoptosis in H9c2 cells under hypoxic conditions while miR-9 mimics exerted the opposite effects on hypoxic H9c2 cells. These results suggested that upregulated miR-9 might be a mechanism contributing to the pathogenesis of CHD.

To further explore the molecular mechanism of miR-9, bioinformatics analysis by TargetScan predicted the potential targets of miR-9 and found that the 3′UTR of Yap1 contained the potential binding sites complementary to miR-9. Recent studies have suggested that Yap1, the nuclear target of Hippo signaling, plays an important role in the regulation of cardiomyocyte proliferation, regeneration, and embryonic heart development [13]. Activation of Yap1 stimulated heart growth primarily by regulating cardiomyocytes proliferation, but not physiological cardiomyocyte hypertrophy [13]. On the other hand, deletion of Yap in the embryonic mouse heart impeded cardiomyocyte proliferation, causing myocardial hypoplasia and lethality [27]. Forced overexpression of Yap1 in adult mice promoted cardiac regeneration and improved contractility after MI [28]. In addition, Yap1 deficiency attenuated phenylephrine-induced cardiac hypertrophy and exacerbated injury in response to chronic MI [29]. Using luciferase reporter assay, qRT-PCR and western blot analysis, our study validated that Yap was a direct target of miR-9 in H9c2 cells. We also manifested that hypoxia induced the decrease of Yap1 expression in time-dependent manner in H9c2 cells, which was in line with the previous studies [16]. Moreover, we found that Yap1 knockdown significantly suppressed cell proliferation and promoted apoptosis in hypoxia-exposed H9c2 cells. Rescue experiments revealed that Yap1 knockdown attenuated the effects of anti-miR-9 on cell proliferation and apoptosis in hypoxia-induced H9c2 cells, suggesting that miR-9 knockdown restored hypoxia-induced proliferation reduction and inhibited hypoxia-induced cardiomyocyte apoptosis by targeting Yap1.

5. Conclusion

In summary, hypoxia induced upregulation of miR-9 and down-regulation of Yap1 in cardiomyocytes. miR-9 knockdown inhibited hypoxia-induced cardiomyocyte apoptosis by targeting Yap1. Our study provided a novel insight into the mechanism of the adaptation of cardiomyocytes to chronic hypoxia and miR-9 might be a potential significant target for the clinical treatment of the CHD patients.

Conflict of interest

The authors declare that they have no conflict of interest.

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