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Original Article

miR-10a inhibits cardiac hypertrophy after myocardial infarction in mice by targeting HDAC4

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Abstract: Objective: Growing evidence indicates that microRNAs (miR) have been shown to regulate cardiac hypertrophy in animal models of myocardial infarction (MI). This study aimed to evaluate the role of the miR-10a in cardiac hypertrophy after myocardial infraction. Methods: The expression of miR-10a was compared in sham-operation and MI surgery. Then, the cell surface area and the cardiac hypertrophy gene expression were analyzed under miR-10a overexpression in primary cardiomyocytes. In addition, the luciferase assay and western blot were used for determine the target gene regulated by miR-10a. Lastly, the effect of miR-10a in cardiac hypertrophy was determined by in vivo overexpression of miR-10a in MI surgery group. Results: We found that miR-10a was down-regulated in cardiac hypertrophy both in vitro and in vivo. In vitro overexpressing of miR-10a inhibits cardiomyocytes hypertrophy. Then we identified a novel target gene of miR-10a, HDAC4. In contrast to miR-10a, HDAC4 was up-regulated during cardiac hypertrophy. Moreover, in vivo overexpressing miR-10a effectively attenuated myocardial infarction-induced cardiac hypertrophy through decreased the expression of HDAC4. Conclusion: Our results show that miR-10a is a key regulator or cardiac hypertrophy, thus offering a new therapeutic strategy of myocardial infarction-induced cardiac hypertrophy.

Keywords: miR-10a, myocardial infarction, HDAC4, cardiac hypertrophy

Introduction

Pathological cardiac hypertrophy is a major cause for heart failure and many heart ailments which might lead to human morbidity and mortality, especially the old [1-3]. However, the molecular mechanisms that drive the development of pathological cardiac hypertrophy remain abstruse. Myocardial infraction (MI) was happened when coronary artery occluded, which leading to myocardial hypertrophy in none-infracted zone [4, 5]. After myocardial infraction, hypertrophic growth of none-infracted tissues would results in heart failure [6].

Recent studies have proved that some microRNAs (miRNAs or miRs) are involved in cardiac hypertrophy process: some promotes cardiac hypertrophy (like miR-155, miR-221, miR-328 etc.); whereas some inhibit cardiac hypertrophy (like miR-133, miR-21-3p, miR-378 etc.) [7-12]. Lately, miR-10a was found down-regulated during cardiac hypertrophy [13]. For its high conservation and genomic localization, the miR-10a genes functions as an important regulator in several diseases [14, 15]. Yet, the roles of miR-10a genes in cardiac hypertrophy remain unclear and worth studying.

In this research, we further study the functional role of miR-10a in myocardial infarction mouse model and found that miR-10a is associated with histone deacetylase 4 (HDAC4) and modulate in cardiac hypertrophic signal pathway [16]. Our results revealed the positive regulation functions of miR-10a in cardiac hypertrophy and might provide an opportunity for development of new therapies for heart diseases.

Methods and materials

Animal experiment

All mice were in C57BL/6J background and were males aged 8-14 weeks. All mice were

maintained in a specific pathogen free facility under a 12-h dark/light cycle. Myocardial infarction (MI) surgery or sham operation was performed as previously mentioned [34]. In brief, mice were mice were anesthetized by intraperitoneal injection of chloral hydrate. Then, the left anterior descending coronary artery (LAD) was permanently ligated. Chemically modified agomirs were used for miR-10a overexpression in vivo. C57BL/6J male mice received agomir at doses of 80 mg/kg bodyweight through tail injection for every 5 days after 4 weeks recovery from MI surgery. Tissues were harvested and store at -80°C for analysis. All experimental procedures involving the care and use of mice in this study were reviewed and approved by the Ethics Committee of the Second Affiliated Hospital of Zhengzhou University.

Cell culture, transfection and cell surface area measurement

Primary neonatal mice cardiomyocytes were isolated from 1-day-old mouse as described previously. Isolated cardiomyocytes cells (ATCC) were culture in Dulbecco's modified Eagle's medium supplemented with 10% Fetal bovine serum (FBS), 1 mM glutamine, 100 units/ml penicillin, and 100 mg/ml streptomycin.

Lipofectamine 2000 was used for cell transfection, 50 μmol/L phenylephrine (Sigma-Aldrich) or an equal volume of PBS was added 48 hours after transfection. Cells were harvested 48 hours after stimulation.

Cell surface area of α-actin stained cells or unstained cells was measured as described previously [17]. 200-300 cardiomyocytes in 20-50 fields were examined in each experiment. Image J software was used to measure the area of cells.

Plasmid, RNA oligo and Luciferase assay

For construction luciferase reporter plasmids, the 3'UTR fragment of HDAC4 was amplified from mouse cDNA and inserted into pRL-TK reporter plasmids. MicroRNA double-stranded mimics and antagomir were purchased from Genepharma.

Cardiomyocytes were planted into plates then co-transfected with miR-24 mimics or negative control, pRL-TK-HDAC4-3'UTR and pGL3-basic. 48 hours after transfection, cells were lyszed and relative luciferase activities were measured on a luminometer (Promega) with the Dual Luciferase Reporter Assay System (Promega).

Real-time-PCR and Western blot

RNA was isolated using Trizol reagent from cells and tissues, 0.5 μg of RNA was reverse-transcribed into cDNA using the polyA RT system. Real Time PCR was performed on ABI Step One Real Time PCR system. The following primers were used in this study:

miR-10a: TACCCTGTAGATCCGAATTTGTG; miR-uni: CGAATTCTAGAGCTCGAGGCAGG; HDAC4-F: CTGCAAGTGGCCCCTACAG; HDAC4-R: CTGCTCATGTTGACGCTGGA; ANF-F: GCTTCCAGGCCATATTGGAG; ANF-R: GGGGGCATGACCTCATCTT; β-MHC-F: ACTGTCAACACTAAGAGGGTCA; β-MHC-R: TTGGATGATTTGATCTTCCAGGG.

Cells or tissues were lysed in RIPA lysis buffer (10 mM Tris-HCl (pH 7.5), 1% SDS, 1 mM Na3VO4, 10 mM NaF and protease inhibitor cocktail). Then protein samples (25 μg) were separated by SDS PAGE gel according to standard protocols. Anti-HDAC4 (Cell Signalling), α-actin (Sigma-Aldrich) and anti-GAPDH (Sigma-Aldrich) were used for western blotting.

Statistical analysis

All results are expressed as mean ± S.D. Statistical analysis was performed using Student's t test by GraphPad Prism 5.0. Statistical significance was considered at $P<0.05$.

Results

miR-10a is downregulated in MI-and PE-induced cardiac hypertrophy

A total of 10 male mice were used for this study. Five of these underwent myocardial infarction surgery through artificial occluded descending left coronary artery. After MI treatment for 4 weeks, the cross section of non-infarcted zone of left ventricular showed an increase in cell size and interstitial fibrosis (**Figure 1A**), suggesting the MI hypertrophic model were established successfully. We explored the expression profile of miR-10a in MI-induced ventricle samples by performing RT-PCR and found it decreased (**Figure 1B**). Similar decrease of

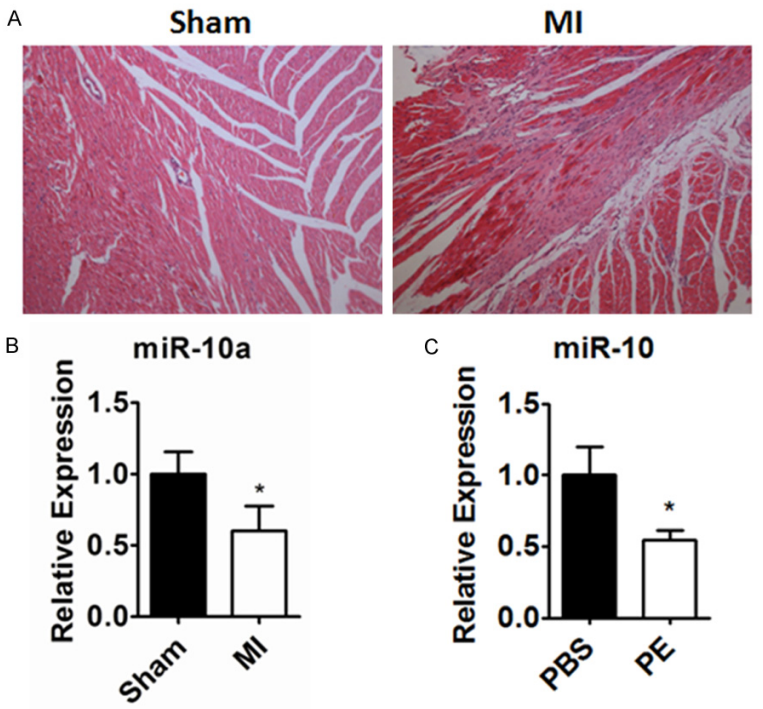

Figure 1. miR-10a is downregulated in MI- and PE-induced cardiac hypertrophy. A. Representative of H&E staining of cross section of non-infarcted zone of left ventricular samples after MI surgery (n=5). B. RT-PCR analysis of miR-10a expression in MI-induced ventricle samples. C. RT-PCR analysis of miR-10a expression in PE-induced primary cardiomyocytes. Means ± SD are shown. *P<0.05; **P<0.01.

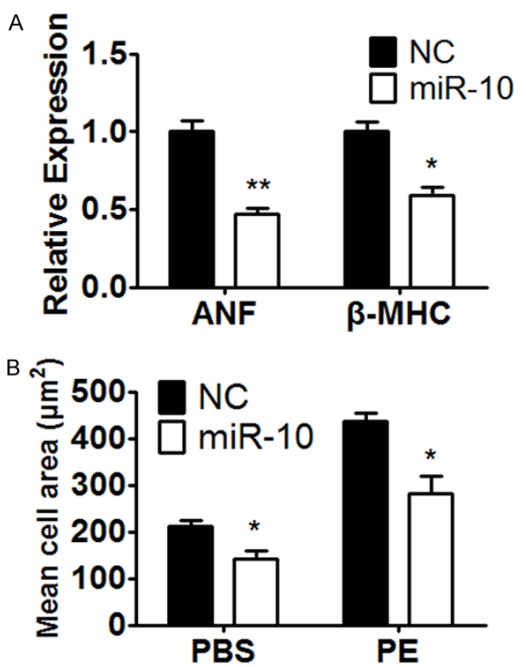

Figure 2. miR-10a inhibits cardiomyocytes hypertrophy. A. RT-PCR analysis of ANF and β-MHC mRNA levels in primary cardiomyocytes transfected with miR-10a mimics or negative control oligos (NC). B. Primary cardiomyocytes were transfected with miR-10a mimics or negative control oligos (NC), and 48 hours later, 50 μmol/L phenylephrine (PE) or equal volume of PBS was added, then cell surface area was analyzed. Means ± SD are shown. *P<0.05; **P<0.01.

miR-10a expression was found in the primary cardiomyocytes treated with phenylephrine (PE) (**Figure 1C**), which has been proved to induce cardiac hypertrophy [17, 18]. These results indicated that the expression of miR-10a was downregulated in the process of myocardial remodel cardiac hypertrophy and the miR-10a expression might likely play a critical role in cardiac hypertrophy suppression.

miR-10a inhibits cardiomyocytes hypertrophy

It has been identified that several hallmarks, including atrial natriuretic factor (ANF) and β-myosin heavy chain (β-MHC), increasing along with the development of pathological cardiac hypertrophy [18-21]. To further explore the roles of miR-10a in hypertrophic signalling pathway, we analyzed the mRNA expression levels of ANF and β-MHC in primary cardiomyocytes transfected with miR-10a mimics, taking non-sense oligos as negative control (NC). Intriguingly, we observed the expression levels of both ANF and β-MHC were significantly suppressed in cardiomyocytes with miR-10a mimics (**Figure 2A**). We then stimulated the primary cardiomyocytes with PE or PBS, and as expected, the cell surface area of cardiomyocytes with miR-10a mimics was less than that with negative control. These results, taken together, demonstrated that miR-10a inhibited cardiomyocytes hypertrophy.

HDAC4 is a direct target of miR-10a in cardiomyocytes

Based on the previous studies, we hypothesized that miR-96 could target key regulators in

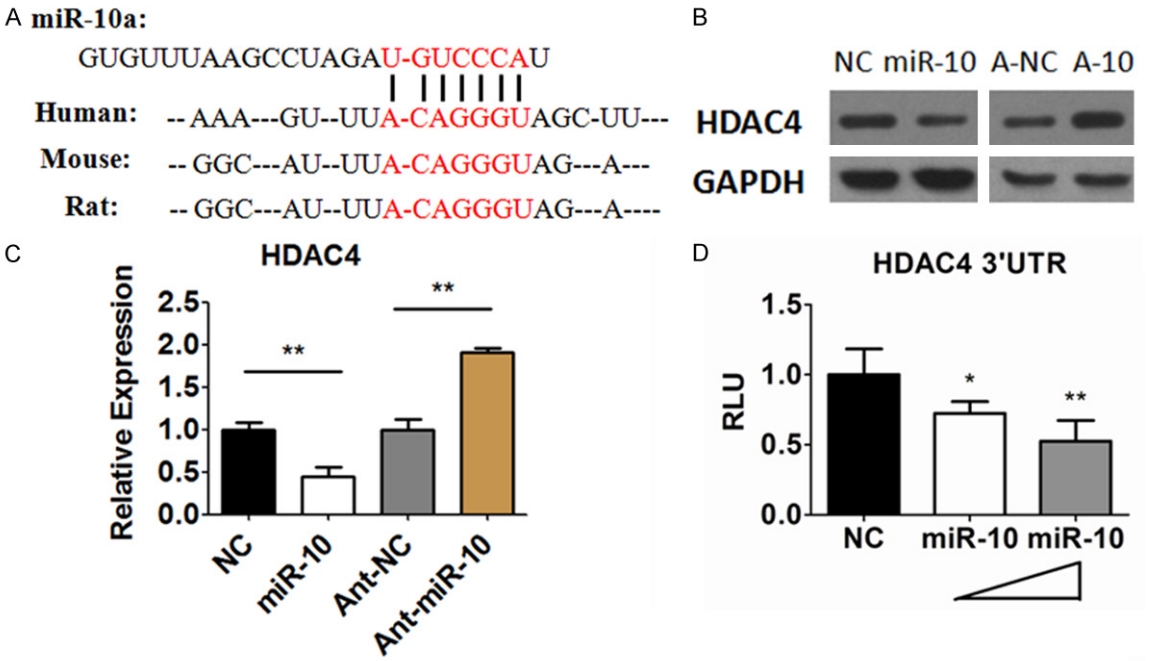

Figure 3. HDAC4 is a direct target of miR-10a in cardiomyocytes. A. Sequence alignment of miR-10a and the HDAC4 3'-UTR from various species. B. Western blot analysis of HDAC4 in primary cardiomyocytes transfected with miR-10a mimics, negative control oligos (NC), miR-10a antagomir (Ant-10) or anti-scramble (Ant-NC). GAPDH was used as a loading control. C. RT-PCR analysis of HDAC4 expression in primary cardiomyocytes transfected with miR-10a mimics, negative control oligos (NC), miR-10a antagomir (Ant-10) or anti-scramble (Ant-NC). D. The luciferase activity of reporter plasmids containing the 3'UTR of HDAC4 was determined in primary cardiomyocytes transfected different amount of miR-10a mimics. Means ± SD are shown. *P<0.05; **P<0.01.

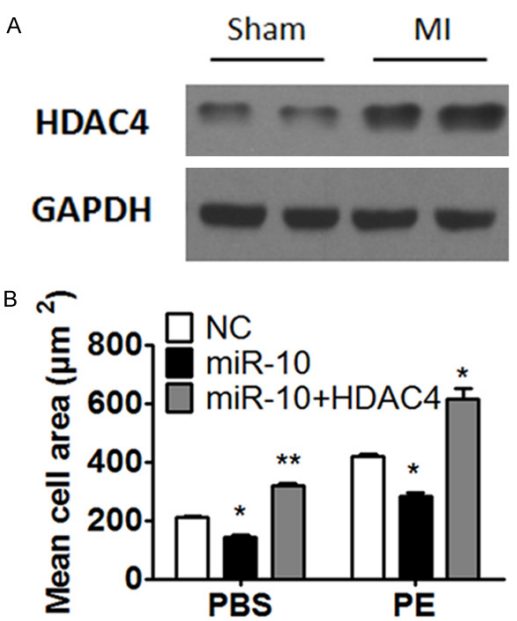

Figure 4. HDAC4 promotes cardiac hypertrophy. A. Western analysis of HDAC4 expression in Sham-operated or MI-treated mice (n=5). GAPDH was used as a loading control. B. Primary cardiomyocytes were transfected with miR-10a mimics or negative control oligos (NC), or co-transfected with HDAC4 plasmid, and 48 hours later, 50 μmol/LPEor equal volume of PBS was added, then cell surface area was analyzed. Means ± SD are shown. *P<0.05; **P<0.01.

cardiomyocytes hypertrophy. Interesting, we found that HDAC4, a key regulator of cardiomyocytes hypertrophy [22, 23], might be a potential target of miR-10a through TargetScan across species (**Figure 3A**). We analyzed HDAC4 of primary cardiomyocytes in both protein and mRNA level. As can be seen in **Figure 3B**, the HDAC4 protein expression decreased in cardiomyocytes with miR-10a mimics but increased in ardiomyocytes with miR-10a antagomir (Ant-10). The mRNA expression HDAC4 was consistent with its protein expression (**Figure 3C**). In addition, the luciferase activity of reporter plasmids containing the 3'UTR of HDAC4 was determined in primary cardiomyocytes transfected different amount of miR-10a mimics. Results showed that increasing amount of miR-10a mimics expression gradually weakened the luciferase activity of HDAC4 (**Figure 3D**). These findings raise the possibility that HDAC4 might

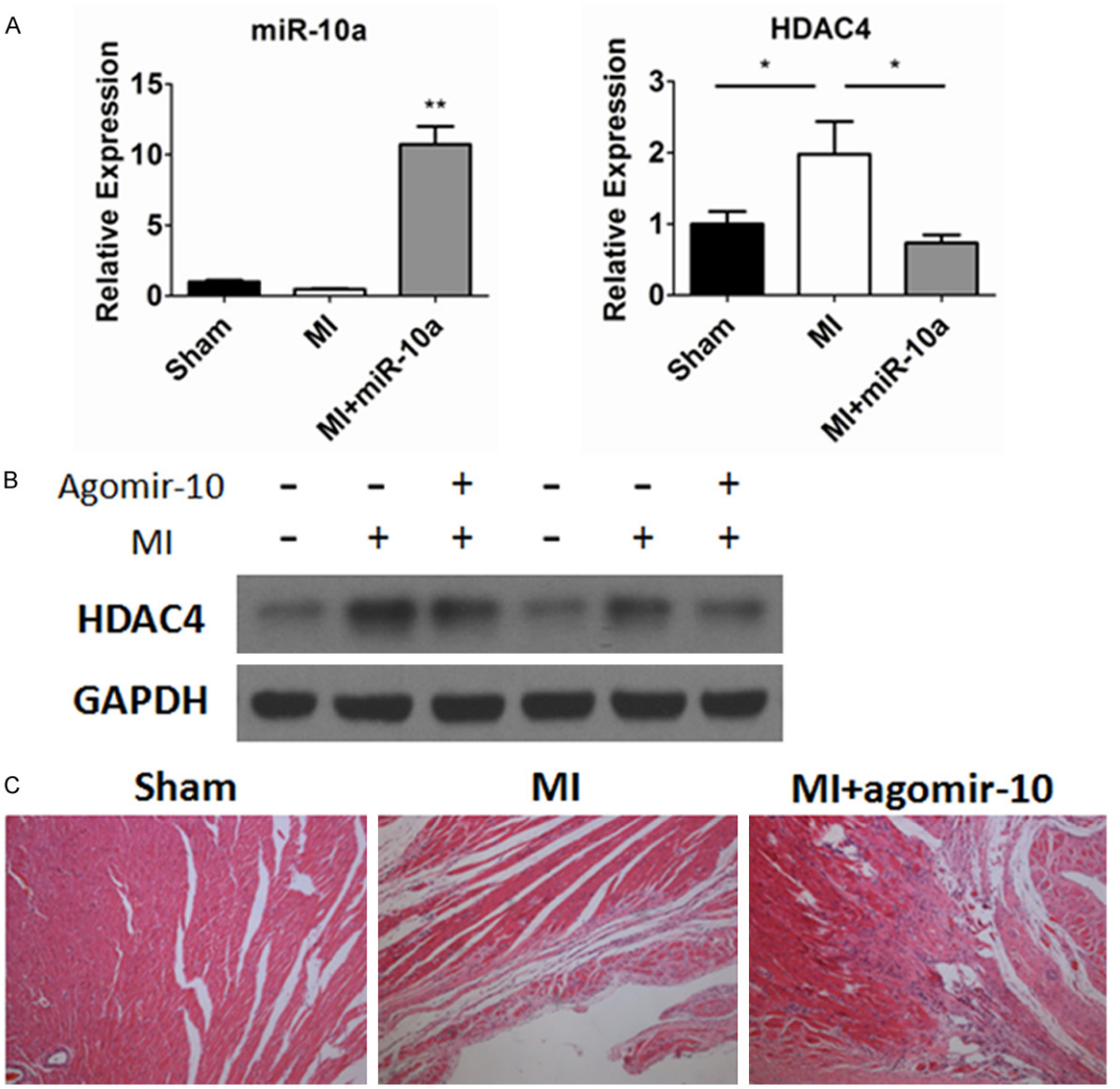

Figure 5. Restoration of miR-10a abolished the action of HDAC4 during cardiac hypertrophy. A. RT-PCR analysis of miR-10a and HDAC expression in ventricle samples of Sham-operated mice, MI-treated mice or MI-treated mice with ago miR-10a (n=3). B. Western blot analysis of miR-10a and HDAC expression in ventricle samples of Sham-operated mice, MI-treated mice or MI-treated mice with ago miR-10a (n=3). GAPDH was used as a loading control. C. Representative H&E staining images of cross section of non-infarcted zone of left ventricular samples of Sham-operated mice, MI-treated mice or MI-treated mice with ago miR-10a (n=3). Means ± SD are shown. *P<0.05; **P<0.01.

be a direct target of miR-10a in cardiomyocytes.

HDAC4 promotes cardiac hypertrophy

Western blots showed that HDAC4 expression drastically increased in MI-treated mice (**Figure 4A**), indicating that HDAC4 promotes cardiac hypertrophy. Overexpression of miR-10a inhibited cardiomyocytes hypertrophy. However, when co-expressed miR-10a and HDAC4 in cardiomyocytes, no inhibition of cardiomyocytes hypertrophy were ever observed, and mean cell area increased (**Figure 4B**). Hence, we confirmed that HDAC4 is a direct target of miR-10a in cardiomyocytes and elevate the effect of myocardial hypertrophy.

Restoration of HDAC4 abolished the action of HDAC4 during cardiac hypertrophy

To further understand the relationship between miR-10a and HDAC4 in hypertrophic signaling pathways, we next evaluated the miR-10a and

HDAC expression in ventricle samples of Sham-operated mice, MI-treated mice or MI-treated mice with ago miR-10a (**Figure 5A**). The results showed that overexpression of miR-10a significantly down-regulated the HDAC4 expression in MI-treated mice in mRNA level. Similarly, HDAC4 expression in protein level raised in MI-treated mice but suppressed in MI-treated mice with ago miR-10a (**Figure 5B**). From the representative images of cross section of ventricle samples, we observed a positive control of cardiac hypertrophy in miR-10a overexpressed sample, that the cell size as well as the percentage of interstitial fibrosis reduced (**Figure 5C**). Altogether, our data revealed that restoration of miR-10a abolished the action of HDAC4 during cardiac hypertrophy.

Discussion

miRNAs are a primordial mechanism of gene expression control that they are crucial to cellular development and nowadays are emerging as prominent players in the regulation of the biological processes in many diseases as well. The regulation functions of miRNAs have been identified in not only cardiovascular disease but also respiratory, intestinal and liver ones [24-27]. There's still a long way to go to figure out the potential functions of these biomarkers and provide clinicians with more specific guidance.

Cardiac hypertrophy is an early stage of many heart diseases. In this study, we first identified the functional role of miR-10a mediated with HDAC4 in modulating cardiac hypertrophic response. Our data showed that miR-10a expression was down-regulated in MI-treated mouse models which correspond to previous microarray study of cardiac hypertrophy [13]. Overexpression of miR-10a could suppress cardiac hypertrophic both *in vivo* and *in vitro*, and similar positive regulation functions of miR-10a has also been found in mouse models of intestinal neoplasia [28].

We additionally discovered that HDAC4 is a direct target of miR-10a in cardiomyocytes. Since it is well-known that the HDAC family, which catalyze the removal of acetyl groups from proteins, promote cardiac hypertrophic and were closely linked with heart failure, increasing attentions are paying on developing the HDAC-inhibitors that could improve cardiac functions in pathological heart conditions [29-33]. Our findings showed that the expression of HDAC4 up-regulated and lead to cardiac hypertrophic in MI mouse models, whereas restoration of miR-10a abolished the action of HDAC4 and alleviated hypertrophy of cardiomyocytes.

In conclusion, our studies reveal that miR-10a plays a protective role in cardiac hypertrophy and might serve as a novel therapeutic target for pathological cardiac hypertrophy and other heart diseases.

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Disclosure of conflict of interest

None.

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References

[1] McMullen JR, Jennings GL. Differences between pathological and physiological cardiac hypertrophy: Novel therapeutic strategies to treat heart failure. Clin Exp Pharmacol Physiol 2007; 34: 255-262.

[2] Neeland IJ, Drazner MH, Berry JD, Ayers CR, deFilippi C, Seliger SL, Nambi V, McGuire DK, Omland T, de Lemos JA. Biomarkers of chronic cardiac injury and hemodynamic stress identify a malignant phenotype of left ventricular hypertrophy in the general population. J Am Coll Cardiol 2013; 61: 187-195.

[3] Seliger SL, de Lemos J, Neeland IJ, Christenson R, Gottdiener J, Drazner MH, Berry J, Sorkin J, deFilippi C. Older adults, "malignant" left ventricular hypertrophy, and associated cardiac-specific biomarker phenotypes to identify the differential risk of new-onset reduced versus preserved ejection fraction heart failure: Chs (cardiovascular health study). JACC Heart Failure 2015; 3: 445-455.

[4] Meloni M, Caporali A, Graiani G, Lagrasta C, Katare R, Van Linthout S, Spillmann F, Campe-

si I, Madeddu P, Quaini F, Emanueli C. Nerve growth factor promotes cardiac repair following myocardial infarction. Circ Res 2010; 106: 1275-1284.
[5] Olivetti G, Quaini F, Sala R, Lagrasta C, Corradi D, Bonacina E, Gambert SR, Cigola E, Anversa P. Acute myocardial infarction in humans is associated with activation of programmed myocyte cell death in the surviving portion of the heart. J Mol Cell Cardiol 1996; 28: 2005-2016.
[6] Anversa P, Cheng W, Liu Y, Leri A, Redaelli G, Kajstura J. Apoptosis and myocardial infarction. Basic Res Cardiol 1998; 93 Suppl 3: 8-12.
[7] Heymans S, Corsten MF, Verhesen W, Carai P, van Leeuwen RE, Custers K, Peters T, Hazebroek M, Stoger L, Wijnands E, Janssen BJ, Creemers EE, Pinto YM, Grimm D, Schurmann N, Vigorito E, Thum T, Stassen F, Yin X, Mayr M, de Windt LJ, Lutgens E, Wouters K, de Winther MP, Zacchigna S, Giacca M, van Bilsen M, Papageorgiou AP, Schroen B. Macrophage microrna-155 promotes cardiac hypertrophy and failure. Circulation 2013; 128: 1420-1432.
[8] Wang C, Wang S, Zhao P, Wang X, Wang J, Wang Y, Song L, Zou Y, Hui R. Mir-221 promotes cardiac hypertrophy in vitro through the modulation of p27 expression. J Cell Biochem 2012; 113: 2040-2046.
[9] Li C, Li X, Gao X, Zhang R, Zhang Y, Liang H, Xu C, Du W, Zhang Y, Liu X, Ma N, Xu Z, Wang L, Chen X, Lu Y, Ju J, Yang B, Shan H. Microrna-328 as a regulator of cardiac hypertrophy. Int J Cardiol 2014; 173: 268-276.
[10] Diniz GP, Lino CA, Guedes EC, Moreira Ldo N, Barreto-Chaves ML. Cardiac microrna-133 is down-regulated in thyroid hormone-mediated cardiac hypertrophy partially via type 1 angiotensin ii receptor. Basic Res Cardiol 2015; 110: 49.
[11] Care A, Catalucci D, Felicetti F, Bonci D, Addario A, Gallo P, Bang ML, Segnalini P, Gu Y, Dalton ND, Elia L, Latronico MV, Hoydal M, Autore C, Russo MA, Dorn GW, Ellingsen O, Ruiz-Lozano P, Peterson KL, Croce CM, Peschle C, Condorelli G. Microrna-133 controls cardiac hypertrophy. Nat Med 2007; 13: 613-618.
[12] Nagalingam RS, Sundaresan NR, Gupta MP, Geenen DL, Solaro RJ, Gupta M. A cardiac-enriched microrna, mir-378, blocks cardiac hypertrophy by targeting ras signaling. J Biol Chem 2013; 288: 11216-11232.
[13] Feng HJ, Ouyang W, Liu JH, Sun YG, Hu R, Huang LH, Xian JL, Jing CF, Zhou MJ. Global microrna profiles and signaling pathways in the development of cardiac hypertrophy. Braz J Med Biol Res 2014; 47: 361-368.
[14] Tehler D, Hoyland-Kroghsbo NM, Lund AH. The mir-10 microrna precursor family. RNA Biol 2011; 8: 728-734.
[15] Lund AH. Mir-10 in development and cancer. Cell Death Differ 2010; 17: 209-214.
[16] Hohl M, Wagner M, Reil JC, Muller SA, Tauchnitz M, Zimmer AM, Lehmann LH, Thiel G, Bohm M, Backs J, Maack C. Hdac4 controls histone methylation in response to elevated cardiac load. J Clin Invest 2013; 123: 1359-1370.
[17] Ganesan J, Ramanujam D, Sassi Y, Ahles A, Jentzsch C, Werfel S, Leierseder S, Loyer X, Giacca M, Zentilin L, Thum T, Laggerbauer B, Engelhardt S. Mir-378 controls cardiac hypertrophy by combined repression of mitogen-activated protein kinase pathway factors. Circulation 2013; 127: 2097-2106.
[18] Jeong MH, Kim SJ, Kang H, Park KW, Park WJ, Yang SY, Yang DK. Cucurbitacin i attenuates cardiomyocyte hypertrophy via inhibition of connective tissue growth factor (ccn2) and tgf-beta/smads signalings. PLoS One 2015; 10: e0136236.
[19] Foldes G, Mioulane M, Wright JS, Liu AQ, Novak P, Merkely B, Gorelik J, Schneider MD, Ali NN, Harding SE. Modulation of human embryonic stem cell-derived cardiomyocyte growth: A testbed for studying human cardiac hypertrophy? J Mol Cell Cardiol 2011; 50: 367-376.
[20] Taegtmeyer H, Sen S, Vela D. Return to the fetal gene program: A suggested metabolic link to gene expression in the heart. Ann N Y Acad Sci 2010; 1188: 191-198.
[21] Sergeeva IA, Hooijkaas IB, Van Der Made I, Jong WM, Creemers EE, Christoffels VM. A transgenic mouse model for the simultaneous monitoring of anf and bnp gene activity during heart development and disease. Cardiovasc Res 2014; 101: 78-86.
[22] Matsushima S, Kuroda J, Ago T, Zhai P, Park JY, Xie LH, Tian B, Sadoshima J. Increased oxidative stress in the nucleus caused by nox4 mediates oxidation of hdac4 and cardiac hypertrophy. Circ Res 2013; 112: 651-663.
[23] Winbanks CE, Beyer C, Hagg A, Qian H, Sepulveda PV, Gregorevic P. Mir-206 represses hypertrophy of myogenic cells but not muscle fibers via inhibition of hdac4. PLoS One 2013; 8: e73589.
[24] Romaine SP, Tomaszewski M, Condorelli G, Samani NJ. Micrornas in cardiovascular disease: An introduction for clinicians. Heart 2015; 101: 921-928.
[25] Brown D, Rahman M, Nana-Sinkam SP. Micrornas in respiratory disease. A clinician's overview. Ann Am Thorac Soc 2014; 11: 1277-1285.
[26] Zahm AM, Hand NJ, Tsoucas DM, Le Guen CL, Baldassano RN, Friedman JR. Rectal micrornas are perturbed in pediatric inflammatory bowel disease of the colon. J Crohn's Colitis 2014; 8: 1108-1117.

[27] Povero D, Eguchi A, Li H, Johnson CD, Papouchado BG, Wree A, Messer K, Feldstein AE. Circulating extracellular vesicles with specific proteome and liver microrna are potential biomarkers for liver injury in experimental fatty liver disease. PLoS One 2014; 9: e113651.

[28] Stadthagen G, Tehler D, Hoyland-Kroghsbo NM, Wen J, Krogh A, Jensen KT, Santoni-Rugiu E, Engelholm LH, Lund AH. Loss of mir-10a activates lpo and collaborates with activated wnt signaling in inducing intestinal neoplasia in female mice. PLoS Genet 2013; 9: e1003913.

[29] Iyer A, Fenning A, Lim J, Le GT, Reid RC, Halili MA, Fairlie DP, Brown L. Antifibrotic activity of an inhibitor of histone deacetylases in doca-salt hypertensive rats. Br J Pharmacol 2010; 159: 1408-1417.

[30] Kao YH, Liou JP, Chung CC, Lien GS, Kuo CC, Chen SA, Chen YJ. Histone deacetylase inhibition improved cardiac functions with direct antifibrotic activity in heart failure. Int J Cardiol 2013; 168: 4178-4183.

[31] Williams SM, Golden-Mason L, Ferguson BS, Schuetze KB, Cavasin MA, Demos-Davies K, Yeager ME, Stenmark KR, McKinsey TA. Class i hdacs regulate angiotensin ii-dependent cardiac fibrosis via fibroblasts and circulating fibrocytes. J Mol Cell Cardiol 2014; 67: 112-125.

[32] Lee TM, Lin MS, Chang NC. Inhibition of histone deacetylase on ventricular remodeling in infarcted rats. Am J Physiol Heart Circ Physiol 2007; 293: H968-977.

[33] Nural-Guvener H, Zakharova L, Feehery L, Sljukic S, Gaballa M. Anti-fibrotic effects of class i hdac inhibitor, mocetinostat is associated with il-6/stat3 signaling in ischemic heart failure. Int J Mol Sci 2015; 16: 11482-11499.

[34] Sutton MG, Sharpe N. Left ventricular remodeling after myocardial infarction: Pathophysiology and therapy. Circulation 2000; 101: 2981-2988.