MicroRNA-129-5p targets keap-1 to protect against cardiomyocyte hypertrophy via Nrf2 pathway

Huiming Ye (✉ huimingyedoc@aliyun.com)

Beijing Shijitan Hospital, Capital Medical University <https://orcid.org/0000-0002-1077-8006>

Guiyu Xu

Capital Medical University Affiliated Beijing Shijitan Hospital

Dexian Zhang

Capital Medical University Affiliated Beijing Shijitan Hospital

Rupeng Wang

Capital Medical University Affiliated Beijing Shijitan Hospital

Research article

Keywords: Cardio hypertrophy, Oxidative stress, MiR-129a-5p, keap-1, Nrf2 pathway

Posted Date: January 2nd, 2020

DOI: <https://doi.org/10.21203/rs.2.19838/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Cardiac hypertrophy is a common pathological process of many cardiac diseases and persistent cardiac hypertrophy is the main cause of heart failure and sudden cardiogenic death. It is of great value to elucidate the mechanism of cardiac hypertrophy for better prevention and treatment.

Methods: The protein levels were measured by western blotting or RT-qPCR. cardiomyocytes hypertrophy was evaluated by [3H]-leucine incorporation assay. oxidative stress was measured by corresponding detection kits. The target relationship was measured by Luciferase reporter gene assay. Morphological change of cardiomyocyte was measured by immunofluorescence staining.

Results: In our study, we for the first time revealed the effects and regulatory mechanism of miR-296-5p in cardiac hypertrophy in vitro . We found suppressed expression of miR-129-5p and elevated expression of keap-1 in Ang II-induced cardiomyocyte hypertrophy model. MiR-129-5p mimic effectively suppressed Ang II-induced hypertrophic responses and oxidative stress. Further experiments showed that keap-1 is a target of miR-129-5p, and miR-129-5p inhibitor promoted cardiomyocyte hypertrophy and oxidative stress by elevating keap-1. Besides, si-keap-1 mediated the activation of Nrf2 pathway, while miR-129-5p inhibitor inactivated the Nrf2 pathway by further elevating keap-1.

Conclusions: MiR-129-5p mimic protects against Ang II induced cardiomyocyte hypertrophy via activating Nrf2 pathway by targeting keap-1.

Background

Cardiac hypertrophy is a common pathological process of many cardiac diseases, such as long-term hypertension, hypertrophic cardiomyopathy and so on. Early cardiac hypertrophy is a compensatory increase in volume and quality of the heart in order to maintain cardiac function under the stimulation of various physiological and pathological factors, characterized by increased cardiomyocyte size, and thickened ventricular walls. However, persistent cardiac hypertrophy is the main cause of heart failure and sudden cardiogenic death [1, 2]. Therefore, it is of great importance to elucidate the mechanism of cardiac hypertrophy to prevent and reverse cardiac hypertrophy for the treatment of various heart systematic diseases.

Pressure load and stimulation of various neurohumoral factors are the main inducing factors of cardiac hypertrophy. Among which, angiotensinII (AngII) is the most important active hormone in Renin-Angiotensin-Aldosterone System (RAAS) and the strongest factor of cardiac hypertrophy at present [3]. By binding to AT1 receptors, Ang II activates many signaling molecules involved in cardiac hypertrophy [4]. Continuous activation of RAAS can induce cardiac hypertrophy, fibrosis and necrosis, induce oxidative stress response, and lead to cardiac function gradually from compensation to de-compensation [5]. Therefore, Ang II sustained-release stimulation-induced cardiac hypertrophy is an ideal research model.

With the development of molecular biology, molecular genetics, immunology and other related disciplines, gene therapy in a variety of diseases, including cardiovascular diseases, has also been rapidly developed [6–8]. Gene therapy is an effective and safe treatment method, which can compensate defective genes or correct abnormal genes by introducing foreign normal genes into target cells to achieve the purpose of treatment [9]. MicroRNA (miRNA) has been demonstrated to play important regulation function in kinds of diseases, thus more and more miRNAs have been regarded as targets of gene therapy in cancer and other diseases [10, 11]. It has been confirmed that ectopic expressed miRNAs are commonly found in cardiac hypertrophy model and defective human heart samples [12]. For example, decreased expression of both miR-133 and miR-1 was found in mouse and human models of cardiac hypertrophy [13]. Besides, Ramachandran S et al. demonstrated that down-regulated miR-129-5p was a sensitive and specific biomarker for heart failure in univentricular heart disease and the levels of miR-129-5p were inversely related to the degree of clinical heart failure [14]. However, the mechanisms by which miR-129-5p affects the progression of cardiac hypertrophy and how we can utilize the mechanisms for the treatment of cardiac hypertrophy have not been thoroughly studied.

NF-E2-related factor 2 (Nrf2) is an important transcription factor that regulates antioxidant stress and plays cardiac protection role [15]. Chen D et al. found that Nrf2 deficiency aggravated AngII-induced cardiac injury by increasing hypertrophy and enhancing inflammation [16]. Kelch-like ECH-associated protein 1 (Keap1), an intracellular sensor for oxidative stress, is a specific receptor for Nrf2 and it mediated the degradation of Nrf2 through ubiquitination. The dissociating Keap1/Nrf2 complex promoted the nuclear transfer of Nrf2, inducing the activation of Nrf2 pathway [17]. Thus, the Keap1-Nrf2 signaling pathway may also play an important role in cardiac hypertrophy by regulating oxidative stress and we conducted a related study.

In our present study, we found that overexpression of miR-129-5p alleviated AngII-induced cardiomyocyte hypertrophy and oxidative stress in in vitro cardiomyocyte hypertrophy model. Further study revealed that miR-129-5p mimic exerted protective effect by targeting Keap1 and activating the Nrf2 pathway. Our study for the first time revealed the mechanisms of miR-129-5p in cardiac hypertrophy, providing novel targets for further research and treatment of cardiac hypertrophy.

Material And Methods

Cell culture

Human cardiac myocytes (HCM) were purchased from ScienCell, USA (Cat. #6200) and were maintained in a humidified incubator at 37°C/5% CO₂.

Ang II-induced cardiomyocyte hypertrophy in vitro

In our experiments, the HCM were incubated with 100 nmol/L Ang II (Sigma) for 48 h to induce the in vitro cardiomyocyte hypertrophy model. The serum-free medium containing Ang II was changed every 24 h.

Cell transfection

MiR-129-5p mimic and miR-129-5p NC were purchased from GenePharma (Shanghai, China). SiRNA-miR-129-5p, siRNA-keap-1 and corresponding siRNA-NC were designed and synthesized by Invitrogen. Human cardiac myocytes were transfected with miR-129-5p mimics or miR-129-5p mimics NC for 72 h for the following experiments according to the instructions of Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA in HCM was isolated using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Reverse transcription was conducted reverse transcriptase amplification kit (Fermentas, New York, USA). Specific reverse transcription primers and quantitative PCR primers were obtained from RiboBio Co. Ltd. (Guangzhou, China). qRT-PCR was performed by the SYBR Premix DimerEraser on a 7900HT system. U6 and GAPDH were regarded as the internal references for miRNA and mRNA, respectively. The qRT-PCR results were analyzed by the $2^{-\Delta\Delta C_t}$ method. The following primers were used: MiR-129-5p (forward, 5'-ACACTCCTTTTTGCGTCTGGGCTTGC-3' and reverse, 5'-TGGTGTCTGTGGAGTCG-3'), Keap-1 (forward, 5'-AGTTACTTGTCCCGGTCCTG-3' and reverse, 5'-TTTCCAACCTCCGCACAAAGG-3'), Nppa (forward, 5'-ATGGGCTCCTTCTCCATCAC-3' and reverse, 5'-TCTTCGGTACCGGAAGCTG-3') and Myh7 (forward, 5'-GCAGCTTATCAGGAAGGAATAC-3' and reverse, 5'-CTTGCGTACTCTGTCACTC-3').

Western blot

Protein in HCM was extracted using ice-cold RIPA buffer (Beyotime, Shanghai, China). Protein concentration was valued using the BCA protein assay kit (cwbiotech, China). The protein sample mixed with loading buffer was incubated for 8 min in a boiling water bath and then average protein (40 µg) was split by 10% of SDS polyacrylamide mini-gel and was then transferred onto Polyvinylidene Fluoride (PVDF) membranes (Millipore, Massachusetts, USA) and blocked with 5% skim milk for 1 h at room temperature. Then the membranes were incubated with the primary antibodies and the corresponding HRP-conjugated secondary antibodies. At last, the membranes were treated with enhanced chemiluminescence detection kit (Super Signal West Pico; Pierce) and the proteins were analyzed using the image processing program ImageJ. GAPDH was used as an endogenous reference.

[3H]-leucine incorporation assay

[3H]-leucine incorporation assay was used to evaluate cardiomyocytes hypertrophy as previously described [18]. Briefly, HCM were seeded in 96-well plates at the density of 5×10^4 cells/well and were transfected with miR-129-5p mimics or miR-129-5p NC as indicated. Then, 0.1 µM Ang II was used to stimulate cardiomyocyte hypertrophy and 1 µCi [3H]-leucine was added to each well. After stimulation with Ang II for 60 h, the cells were harvested by precipitation with 10% trichloroacetic acid on ice for 30 min and then were solubilized with 1 mol/L NaOH overnight at 4°C. The samples were neutralized with 1 mol/l HCl and the level of [3H] was determined by using a β counter to assess [3H]-leucine incorporation.

Detection of SOD, CAT, MDA, NO levels

The activities of oxidative stress related enzymes superoxide dismutase (SOD), catalase (CAT), and malonyldialdehyde (MDA) and nitric oxide (NO) was detected using corresponding detection kits following the manufacturer's instructions. The kits of SOD, CAT, and MDA were purchased from Nanjing Jiancheng Bioengineering Institute. The kit of NO was purchased from Beyotime Biotechnology.

Bioinformatics analysis

Potential targets of miR-129-5p were predicted by performing a search in the following online database: miRanda (<http://www.microrna.org/microrna/home.do>), TargetScan (<http://www.targetscan.org/>) and miRmap (<http://mirmap.ezlab.org/app/>).

Luciferase reporter gene assay

HCM were firstly inoculated into 12-well plates at a density of 1×10^5 cells per well. 3'-UTR of the Keap-1 gene containing putative miR-129-5p targeting site, was amplified by chemical synthesis and was inserted into the psiCHECK2 vector (Promega, Madison, WI, USA). When the confluence was up to 70%, HCM were transfected with related mixtures including 50 ng keap-1 wild-type or keap-1 mutant-type 3'-UTR reporter plasmids, miR-129-5p mimics or miR-129-5p NC in a final concentration of 20 nM, and Lipofectamine 2000 for 48 h. Luciferase activity was detected using the dual-luciferase reporter gene kit (Beyotime, Shanghai, China).

Immunofluorescence

For immunofluorescence staining, the HCM were incubated with the primary antibody against α -actinin (1:200, Sigma, St. Louis, Missouri, U.S.A.) and the fluorescent secondary antibody (1:200, Alexa Fluor 488, Invitrogen) according to the instructions. Nuclear staining was conducted with Hoechst (Sigma, St. Louis, Missouri, U.S.A.). The cells were observed by a fluorescence microscope (Zeiss, Heidenheim, Baden-Wuerttemberg, Germany).

Statistical analysis

Statistical analysis was carried out using SPSS 21.0 (SPSS Inc., IL, USA). The Student's t-test was used for the comparisons between two independent groups. Differences among multiple groups were carried out by the one-way analysis of variance followed by a Student–Newman–Keuls post hoc test. P-values < 0.05 were considered as being statistically significant.

Results

Overexpression of miR-129-5p inhibits AngII-induced cardiomyocyte hypertrophy

AngII-treated HCM were transfected with siRNA-miR-129-5p mimic or siRNA-NC, respectively. Relative expression of miR-129-5p and keap-1 mRNA was detected through qRT-PCR. The results in Fig. 1A showed that AngII stimulation largely inhibited the expression of miR-129-5p and suppressed miR-129-5p

was then elevated in the presence of miR-129-5p mimic. On the contrary, the expression of keap-1 was induced by AngII stimulation and was suppressed by miR-129-5p overexpression detected through qRT-PCR (Fig. 1B) and western blot (Fig. 1C), respectively. Besides, immunofluorescence staining showed that cell surface area was obviously increased by AngII stimulation and was decreased by miR-129-5p mimic (Fig. 1D). In addition, the expression change of myocardial hypertrophy marker genes Nppa and Myh7 also demonstrated that AngII-induced cardiomyocyte hypertrophy was obviously relieved by the addition of miR-129-5p mimic (Fig. 1E). What is more, Ang II-induced [3H]-leucine incorporation was significantly decreased after miR-129-5p mimic treatment, also indicating that miR-129-5p overexpression inhibited cardiomyocyte hypertrophy (Fig. 1F). The above results demonstrate that overexpression of miR-129-5p inhibits AngII-induced cardiomyocyte hypertrophy.

Overexpression of miR-129-5p suppresses AngII-induced oxidative stress

AngII-induced oxidative stress is another important inducer of cardiomyocyte hypertrophy. The results in Fig. 2A-2B showed that the activities of antioxidant enzymes SOD and CAT were strongly destroyed by AngII stimulation and then were partly recovered by miR-129-5p mimic. At the same time, the accumulation of MDA was increased by AngII and was cleared by miR-129-5p mimic (Fig. 2C). Just in the opposite, the generation of NO was suppressed by AngII and was increased by miR-129-5p mimic (Fig. 2D). The above results all point out that overexpression of miR-129-5p effectively suppresses AngII-induced oxidative stress.

Keap-1 is a target of miR-129-5p

We have introduced that miRNA exerts regulating effect by specifically binding to the 3'UTR of mRNA, so the potential targets of miR-129-5p were predicted through bioinformatics analysis. The predictive complementary sequences were shown as in Fig. 3A. The luciferase reporter gene assay in Fig. 3B further showed that the combination of keap-1-WT and miR-129-5p mimic largely decreased fluorescence intensity compared with the combination of keap-1-WT and miR-129-5p NC. However, the combination of keap-1-MUT and miR-129-5p mimic or miR-129-5p NC both have no effect on fluorescence intensity, indicating that there exists targeting relationship between keap-1 and miR-129-5p. The result of qRT-PCR in Fig. 3C and the result of the western blot in Fig. 3D showed that the expression of keap-1 could be elevated by siRNA-miR-129-5p and be suppressed by miR-129-5p mimic, also demonstrating that keap-1 acts as a target of miR-129-5p.

MiR-129-5p inhibitor promotes cardiomyocyte hypertrophy and oxidative stress by elevating keap-1

We further explored the effects of miR-129-5p/keap-1 axis in cardio hypertrophy. The result in Fig. 4A showed that elevated keap-1 expression in AngII group was decreased by siRNA-keap-1, but was then

largely elevated in the presence of siRNA-miR-129-5p. Then, the results of immunofluorescence staining in Fig. 4B showed that siRNA-keap-1 decreased cell surface area of cardiomyocyte which was increased by AngII before. Then, co-transfection of siRNA-miR-129-5p again enlarged cell surface area of cardiomyocyte compared with the AngII + siRNA-keap-1 group, indicating that miR-129-5p inhibitor promotes cardiomyocyte hypertrophy by elevating keap-1. Besides, cardiomyocyte hypertrophy in different groups was further demonstrated in Fig. 4C by detecting the expression of Nppa and Myh7 through qRT-PCR and in Fig. 4D through the [3H]-leucine incorporation assay. The above results demonstrate that miR-129-5p inhibitor promotes cardiomyocyte hypertrophy by elevating keap-1.

Similarly, the activities of oxidative stress related enzymes SOD (Fig. 4E) and CAT (Fig. 4F) were destroyed in AngII + siRNA-keap-1 + siRNA-miR-129-5p group compared with the AngII + siRNA-keap-1 group. At the same time, the expression of MDA (G) was elevated and the production of NO (H) was decreased by siRNA-miR-129-5p group compared with the AngII + siRNA-keap-1 group, indicating that miR-129-5p inhibitor promotes oxidative stress by elevating keap-1.

Keap-1 mediated the inactivation of Nrf2 pathway by miR-129-5p inhibitor

We have known that keap-1 is a specific receptor for Nrf2 and the Nrf2 pathway is closely associated with oxidative stress, thus we further explored whether miR-129-5p inhibitor affects cardiomyocyte hypertrophy through the Nrf2 pathway. The results of the western blot in Fig. 5 showed that expression of Nrf2 was elevated in cytoplasm and was suppressed in nucleus in AngII induced group, indicating that the Nrf2 pathway was inactivated in case of cardiomyocyte hypertrophy. Then we found addition of siRNA-keap-1 activated the Nrf2 pathway by promoting nuclear transport of Nrf2, so as to induce further antioxidant stress response to protect against cardiomyocyte hypertrophy. However, the further transfection of siRNA-miR-129-5p abolished the activating effect of siRNA-keap-1 on Nrf2 pathway by strongly suppressing nuclear transport of Nrf2. Thus, we concluded that the knockdown of miR-129-5p inactivated the Nrf2 pathway by targeting keap-1 in cardiomyocyte hypertrophy, thus suggesting that overexpression of miR-129-5p may alleviate AngII-induced cardiomyocyte hypertrophy by activating the Nrf2 pathway.

Discussion

Recently years, unreasonable daily routines, dietary habits and some other factors give rise to increased incidence of cardiovascular diseases, which seriously threatens human health. Cardiac hypertrophy is a compensatory response of the heart to increase the wall tension by increasing the wall thickness under the stimulation of various pathological factors [19]. Early cardiac hypertrophy is conducive to maintaining the normal function of the heart. However, sustained cardiac hypertrophy will lead to heart failure and sudden death [20]. Therefore, the study on the mechanism of cardiac hypertrophy is of great significance to find the target of prevention and treatment of cardiac hypertrophy [21].

It is found that there are many kinds of miRNAs in the endothelial cells and smooth muscle cells of vascular walls and myocardial cells, which are involved in many cell biology processes, such as cell proliferation, migration, and differentiation, playing an important role in normal development of the heart and blood vessels [22]. Previous studies also have demonstrated that many miRNAs, such as miR-1 [23], miR-101b [24] are both down-regulated in cardiac hypertrophy, and overexpression of miR-1 and miR-101b both play an anti-hypertrophic role by suppressing hypertrophic responses. At the same time, there are also some other miRNAs, such as miR-297 [25], miR-22 [26] are up-regulated in cardiac hypertrophy and the deletion of these related miRNAs also has the effect to attenuate hypertrophic responses. Xiao N et al. found that miR-129-5p was down-regulated in the serum of chronic heart failure (CHF) patients and transfection of miR-129-5p improved heart function and hemodynamic parameters, as well as attenuated oxidative stress and inflammation factors in CHF rats [27]. However, the direct effect and regulation mechanism of miR-129-5p in cardiac hypertrophy has not been explored yet. In our present study, we found that miR-129-5p was down-regulated in Ang-II induced cardiac hypertrophic cells and the overexpression of miR-129-5p successfully suppressed hypertrophic responses, which were exhibited through the immunofluorescence staining, western blot, and the [3H]-leucine incorporation assay.

Oxidative stress is closely related to cardiac hypertrophy and oxidative stress is considered to be a major inducer for the signal transduction in cardiac cells pathological conditions [28]. Thus, a large number of studies have shown that inhibition of oxidative stress can significantly prevent and improve cardiac hypertrophy [29–31]. In our study, we found that overexpression of miR-129-5p largely rescued the activities of oxidative stress related enzymes SOD and CAT and increased the generation of NO in AngII induced cardiac hypertrophic cells. At the same time, the accumulation of MDA was cleared by miR-129-5p mimic, indicating that overexpression of miR-129-5p effectively suppresses AngII-induced oxidative stress. Thus, miR-129-5p mimic may act as an antioxidant to prevent cardiac hypertrophy.

We have learned from previous studies that Keap1-Nrf2 signaling pathway is very important in oxidative stress defense mechanism and the Keap1-Nrf2 signaling pathway has become a prospective target for the prevention and treatment of oxidative stress-related diseases, including cancer, cardiovascular, inflammatory diseases and so on [32, 33]. Keap1, the negative regulator of Nrf2, acts as an important role in the activation of Keap1-Nrf2 signaling pathway [34]. For example, Liao W et al. reported that miR-140-5p attenuated oxidative stress in Cisplatin induced acute kidney injury by activating Keap1-mediated Nrf2/ARE pathway [35]. In our study, we found that the expression of Keap-1 was elevated by AngII stimulation and was then suppressed by miR-129-5p mimic. Bioinformatics analysis predicted complementary sequences between miR-129-5p and Keap-1 and the luciferase reporter gene further demonstrated the targeting relationship between them. In addition, we found that inhibiting effect of siRNA-Keap-1 on AngII-induced hypertrophic responses and oxidative stress was largely neutralized in the presence of siRNA-miR-129-5p. From another point of view, it demonstrated that miR-129-5p mimic suppressed cardiomyocyte hypertrophy and oxidative stress by down-regulating Keap-1.

In addition, we found that co-transfection of siRNA-miR-129-5p abolished the activating effect of siRNA-Keap-1 on Nrf2 pathway by strongly suppressing nuclear transport of Nrf2, also indicating that miR-129-

5p mimic exerts anti-hypertrophic role through activating the Keap1-Nrf2 pathway. Ying Y et al. revealed that Phloretin prevented diabetic cardiomyopathy by dissociating Keap1/Nrf2 complex and inhibiting oxidative stress [36]. Li R et al. also pointed out that Baicalin protected against diabetic cardiomyopathy through Keap1/Nrf2/AMPK-mediated antioxidative and lipid-lowering effects [37]. However, the specific association between Keap1/Nrf2 and cardiac hypertrophy has never been clearly stated. Thus, we for the first time find that the activation of Keap1/Nrf2 pathway may have therapeutic effect on cardiac hypertrophy, providing a theoretical basis for the application of Nrf2 pathway activator in cardiac hypertrophy.

Taken together, we constructed an in vitro cardiomyocyte hypertrophy model by stimulating the HCM with Ang II. We for the first time revealed that miR-129-5p mimic has the potential as a therapeutic target on cardiac hypertrophy and it may exert anti-hypertrophic role through activating the Keap1-Nrf2 pathway, proving novel research interest preventive and curative procedures of cardiac hypertrophy. However, there are also limitations to our work. For example, whether miR-129-5p overexpression could attenuate cardiac hypertrophy in vivo model still need further exploration. Thus, we will do more related research in our following experiments.

Abbreviations

MiRNA, microRNA; HCM, Human cardiac myocytes; qRT-PCR, Quantitative real-time polymerase chain reaction; SOD, superoxide dismutase; CAT, catalase; MDA, malonyldialdehyde; NO, nitric oxide; AngII, angiotensinII; RAAS, Renin-Angiotensin-Aldosterone System; mRNA, message RNA; 3'UTR, 3'-untranslated region; Nrf2, NF-E2-related factor 2; Keap1, Kelch-like ECH-associated protein 1.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests

Funding

Not applicable

Authors' contributions

HY did all the experiments, analyzed all data and was a major contributor in writing the manuscript. GX, DZ and RW did some experiment work. All authors read and approved the final manuscript.

Acknowledgements

Not applicable

References

1. Ferrario CM. Cardiac remodelling and RAS inhibition. *Ther Adv Cardiovasc Dis*. 2016;10:162-71.
2. Shimizu I, Minamino T. Physiological and pathological cardiac hypertrophy. *Ital Heart J*. 2000; 2:42-9.
3. Lifton RP, Gharavi AG, Geller DS. Molecular mechanisms of human hypertension. *Cell*. 2001;104:545-56.
4. Piratello AC, Moraes-Silva I, Paulini J, Souza PR, Sirvente R, Salemi V, et al. Renin angiotensin system and cardiac hypertrophy after sinoaortic denervation in rats. *Clinics (Sao Paulo, Brazil)*. 2010;65:1345-50.
5. Liu CT, Liu MY. Daily sesame oil supplementation attenuates local renin-angiotensin system via inhibiting MAPK activation and oxidative stress in cardiac hypertrophy. *J Nutr Biochem*. 2017;42:108-116.
6. Smith E, Blomberg P. Gene therapy - from idea to reality. *Lakartidningen*. 2017;114.
7. Yla-Herttuala S, Baker AH. Cardiovascular Gene Therapy: Past, Present, and Future. *Mol Ther*. 2017;25:1095-106.
8. Gabisonia K, Recchia FA. Gene Therapy for Heart Failure: New Perspectives. *Curr Heart Fail Rep*. 2018;15:340-9.
9. Philippidis A. Gene Therapy Briefs. *Hum Gene Ther Clin Dev*. 2018;29:172-5.

10. Rupaimoole R, Slack FJ. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. *Nat Rev Drug Discov.* 2017;16:203-22.
11. Woosook K, Barron DA, Rebeca SM, Chan KS, Tran LL, Yang F, et al. RUNX1 is essential for mesenchymal stem cell proliferation and myofibroblast differentiation. *Proc Natl Acad Sci U S A.* 2014;111:16389-94.
12. Tatsuguchi M, Seok HY, Callis TE, Thomson JM, Chen JF, Newman M, et al. Expression of microRNAs is dynamically regulated during cardiomyocyte hypertrophy. *J Mol Cell Cardiol.* 2007;42:1137-41.
13. Care A, Catalucci D, Felicetti F, Bonci D, Addario A, Gallo P, et al. MicroRNA-133 controls cardiac hypertrophy. *Nat Med.* 2007;13:613-8.
14. Ramachandran S, Lowenthal A, Ritner C, Lowenthal S, Bernstein HS. Plasma microvesicle analysis identifies microRNA 129-5p as a biomarker of heart failure in univentricular heart disease. 2017;12:e0183624.
15. Chen QM, Maltagliati AJ. Nrf2 at the heart of oxidative stress and cardiac protection. *Physiol Genomics.* 2018;50:77-97.
16. Ye Y, Zhang F, Chen Q, Huang Z, Li M. LncRNA MALAT1 modified progression of clear cell kidney carcinoma (KIRC) by regulation of miR-194-5p/ACVR2B signaling. *Mol Carcinog.* 2019;58:279-92.
17. Fukutomi T, Takagi K, Mizushima T, Ohuchi N, Yamamoto M. Kinetic, thermodynamic, and structural characterizations of the association between Nrf2-DLGex degron and Keap1. *Mol Cell Biol.* 2014;34:832-46.
18. Yu L, She T, Li M, Han L, Cheng M. Tetramethylpyrazine inhibits angiotensin II-induced cardiomyocyte hypertrophy and tumor necrosis factor-alpha secretion through an NF-kappaB-dependent mechanism. *Int J Mol Med.* 2013;32:717-22.
19. Rosca MG, Tandler B, Hoppel CL. Mitochondria in cardiac hypertrophy and heart failure. *J Mol Cell Cardiol.* 2013;55:31-41.
20. Nakamura M, Sadoshima J. Mechanisms of physiological and pathological cardiac hypertrophy. *Nat Rev Cardiol.* 2018;15:387-407.
21. Mosterd A, Hoes AW. Clinical epidemiology of heart failure. *Heart.* 2007;93:1137-46.
22. Wojciechowska A, Braniewska A, Kozar-Kaminska K. MicroRNA in cardiovascular biology and disease. *Adv Clin Exp Med.* 2017;26:865-74.
23. Diniz GP, Lino CA, Moreno CR, Senger N, Barreto-Chaves MLM. MicroRNA-1 overexpression blunts cardiomyocyte hypertrophy elicited by thyroid hormone. *J Cell Physiol.* 2017;232:3360-8.
24. Lee JS, Yang DK, Park JH, Kim JO, Park WJ, Cho C, et al. MicroRNA-101b attenuates cardiomyocyte hypertrophy by inhibiting protein kinase C epsilon signaling. *FEBS letters.* 2017;591:16-27.
25. Bao Q, Zhao M, Chen L, Wang Y, Wu S, Wu W, et al. MicroRNA-297 promotes cardiomyocyte hypertrophy via targeting sigma-1 receptor. *Life Sci.* 2017;175:1-10.
26. Huang ZP, Chen J, Seok HY, Zhang Z, Kataoka M, Hu X, et al. MicroRNA-22 regulates cardiac hypertrophy and remodeling in response to stress. *Circ Res.* 2013;112:1234-43.

27. Xiao N, Zhang J, Chen C, Wan Y, Wang N, Yang J. miR-129-5p improves cardiac function in rats with chronic heart failure through targeting HMGB1. *Mamm Genome*. 2019. 30:276-88.
28. Rababa'h AM, Guillory AN, Mustafa R, Hijawi T. Oxidative Stress and Cardiac Remodeling: An Updated Edge. *Curr Cardiol Rev*. 2018;14:53-59.
29. Seddon M, Looi YH, Shah AM. Oxidative stress and redox signalling in cardiac hypertrophy and heart failure. *Heart*. 2007;93:903-7.
30. Feng H, Cao J, Zhang G, Wang Y. Kaempferol Attenuates Cardiac Hypertrophy via Regulation of ASK1/MAPK Signaling Pathway and Oxidative Stress. *Planta medica*. 2017;83:837-845.
31. Adebisi AO, Adebisi OO, Owira PM. Naringin Mitigates Cardiac Hypertrophy by Reducing Oxidative Stress and Inactivating c-Jun Nuclear Kinase-1 Protein in Type I Diabetes. *J Cardiovasc Pharmacol*. 2016;67:136-44.
32. Magesh S, Chen Y, Hu L. Small molecule modulators of Keap1-Nrf2-ARE pathway as potential preventive and therapeutic agents. *Med Res Rev*. 2012;32:687-726.
33. Deshmukh P, Unni S, Krishnappa G, Padmanabhan B. The Keap1-Nrf2 pathway: promising therapeutic target to counteract ROS-mediated damage in cancers and neurodegenerative diseases. *Biophys Rev*. 2017;9:41-56.
34. Suzuki T, Yamamoto M. Molecular basis of the Keap1-Nrf2 system. *Free Radic Biol Med*. 2015;88:93-100.
35. Liao W, Fu Z, Zou Y, Wen D, Ma H, Zhou F, et al. MicroRNA-140-5p attenuated oxidative stress in Cisplatin induced acute kidney injury by activating Nrf2/ARE pathway through a Keap1-independent mechanism. *Exp Cell Res*. 2017;360:292-302.
36. Ying Y, Jin J, Ye L, Sun P, Wang H, Wang X. Phloretin Prevents Diabetic Cardiomyopathy by Dissociating Keap1/Nrf2 Complex and Inhibiting Oxidative Stress. *Front Endocrinol (Lausanne)*. 2018;9:774.
37. Li R, Liu Y. Bailcalin Protects against Diabetic Cardiomyopathy through Keap1/Nrf2/AMPK-Mediated Antioxidative and Lipid-Lowering Effects. *Oxid Med Cell Longev*. 2019;2019:3206542.

Figures

Figure 1

Overexpression of miR-129-5p inhibits AngII-induced cardiomyocyte hypertrophy. The expression of miR-129-5p (A) was detected through qRT-PCR. The expression of keap-1 mRNA was detected through qRT-PCR (B) and western blot (C), respectively. Immunofluorescence staining showed that the morphological change of cardiomyocyte in different groups (D). The expression of myocardial hypertrophy marker genes Nppa and Myh7 was detected through qRT-PCR (E). [3 H]-leucine incorporation assay was

conducted to value cardiomyocyte hypertrophy (F). **P < 0.01. The bars showed means ± SD of three independent experiments.

Figure 2

Overexpression of miR-129-5p suppresses AngII-induced oxidative stress. The activities of oxidative stress related enzymes SOD (E), CAT (F), and MDA (G) and NO (H) was detected using corresponding detection kits following the manufacturer's instructions. **P < 0.01. The bars showed means ± SD of three independent experiments.

Figure 3

Keap-1 is a target of miR-129-5p. Bioinformatics analysis showed predictive complementary sequences between keap-1 and miR-129-5p (A). Luciferase reporter gene assay was conducted to further demonstrate the targeting relationship between keap-1 and miR-129-5p (B). qRT-PCR (C) and western blot (D) were employed to detect the expression of keap-1. GAPDH was used as an internal reference. **P < 0.01. The bars showed means $\pm$ SD of three independent experiments.

Figure 4

MiR-129-5p inhibitor promotes cardiomyocyte hypertrophy and oxidative stress by elevating keap-1. The expression of keap-1 was detected through western blot (A). The morphological change of cardiomyocyte was observed through immunofluorescence staining (B). The expression of Nppa and Myh7 was detected through qRT-PCR (C). The [³H]-leucine incorporation assay was conducted here to value cardiomyocyte hypertrophy. The activities of oxidative stress related enzymes SOD (E), CAT (F), and MDA (G) and NO (H)

was detected using corresponding detection kits following the manufacturer's instructions. $**P < 0.01$. The bars showed means $\pm$ SD of three independent experiments.

Figure 5

Keap-1 mediated the inactivation of Nrf2 pathway by miR-129-5p inhibitor. The expression of Nrf2 in cytoplasm (A) and nucleus (B) was detected through western blot. GAPDH and Histone H3 were used as internal references, respectively. $**P < 0.01$. The bars showed means $\pm$ SD of three independent experiments.