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ORIGINAL ARTICLE

Exosomal microRNA-29a mediates cardiac dysfunction and mitochondrial inactivity in obesity-related cardiomyopathy

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Abstract

Purpose Present study aims to explore the pathophysiological role of microRNA (miR)-29a in the process of obesity-related cardiomyopathy in human subjects and mice.

Methods The expression level of circulating exosomal miR-29a was measured in 37 lean and 30 obese human subjects, and correlated with cardiac parameters. The effects of miR-29a on mitochondrial activity and cardiac function were investigated by treatment of miR-29a sponge in primary mouse cardiomyocytes and diet-induced obesity-related cardiomyopathy in mice.

Results The increased circulating miR-29a level was closely associated with impaired human cardiac function, including ejection fraction ($r = -0.2663$, $p < 0.05$) and NT-proBNP levels ($r = 0.4270$, $p < 0.001$). Exosomes from obese human plasma mediated cardiomyocyte mitochondrial inactivity, but pre-treatment with miR-29a sponge attenuated the exosomal miR-29a-induced reduction of ATP production ($p < 0.001$), basal oxygen consumption ($p < 0.01$) and mitochondrial complex I activity ($p < 0.01$). In vivo mouse study, high fat diet damaged cardiac function, normal structure, and mitochondrial activity, whereas miR-29a sponge improved the cardiac status.

Conclusions Present study uncovered the correlation between circulating miR-29a and cardiac parameters in human subjects, and provided solid evidence of the therapeutic application of miR-29a sponge in combating obesity-mediated cardiac dysfunction.

Keywords MicroRNA-29a · Heart · Obesity · Exosome · Mitochondria

Introduction

Obesity is a major contributor to the elevated risks of cardiovascular diseases, hypertension, and dyslipidemia [1, 2]. The increased prevalence of obesity is closely associated to the epidemic of heart failure [1]. Studies carried out in the past decade have established obesity and the metabolic syndrome as important risk factors for subsequent heart failure development, and support the existence of obesity-related cardiomyopathy [3, 4]. Evidence showing the alterations in cardiac structure and function in obese human individuals increase, and it becomes more apparent when severe obesity exists [3]. Although obesity is closely associated to cardiac dysfunction, the mechanistic links are not well understood. Therefore, disclosing the links between obesity and cardiomyopathy is necessary for combating obesity-related cardiomyopathy.

The imbalance of energy homeostasis is a key character of obesity and obesity-related complications. The heart has a very high energy demand, which is met almost entirely by the mitochondrial oxidation of fatty acids and carbohydrates [5]. Over the past several decades, it has become widely recognized that the mitochondria serve an important role in energy production and transfer to

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myocardial cells [6]. Mitochondrial abnormalities lead severe cardiac dysfunction and structural disorders [7]. Mitochondrial complex I, as a main component of electron transfer chain (ETC), determines mitochondrial activity [8]. Downregulation of complex I activity leads decreased levels of oxygen consumption and ATP synthesis in cardiomyocytes [9]. The altered cardiac energy sources, especially lipid metabolism that result in either excessive lipid uptake or impaired lipid oxidation, which result in cardiac lipotoxicity [10]. Besides, emerging evidence has found adipose tissue as an endocrine organ secrets adipokines, including leptin, adiponectin, visfatin that act on non-adipose tissues [11–13]. Obesity affects the secretion of adipokines, which also impact the heart and vascular system. Adiponectin can accelerate fatty acid oxidation through activation of AMPK and its downstream targets in the heart [14]. These findings have demonstrated that non-cardiac factors have the abilities of affecting cardiac function and structural modification through modulating mitochondrial function.

MicroRNAs (miRs) is a group of 18–22 nt length, double-stranded non-coding RNA, which is first discovered in the nematode *Caenorhabditis elegans* [15]. Through selectively binding to complementary target sites of the 3'-UTR of mRNAs, miRs can cause translational repression and/or mRNA destabilization. Several miRs, such as miR-1a, 34a, and 155, have been closely linked to cardiac damage [16–18]. Previous studies showed that miR-29 family was significantly associated with both cardiac hypertrophy and fibrosis in cardiomyopathy [19–23]. MiR-29b was a potential therapeutic agent for angiotensin II-induced cardiac fibrosis by targeting TGF-β/Smad3 signaling [24]. In vitro study also found miR-29a could determine cardiomyocyte proliferation [25]. Meanwhile, saturated fatty acids impaired insulin signaling and glucose uptake in myocytes though induction of miR-29a [26]. The expression level of miR-29a was also increased in obese status, which exhibits adipose tissue hypoxia [27]. However, whether miR-29a participates in the process of obesity-related cardiomyopathy or the possible molecular mechanism is still unclear.

Present study aims to investigate the pathophysiological role of miR-29a in the process of obesity-related cardiomyopathy. Here, we not only disclose the clinical correlation between circulating miR-29a and cardiac parameters, but also uncover the therapeutic effects of miR-29a inhibitor in protecting against cardiac dysfunction. These findings comprehensively explain the clinical application of miR-29a as a diagnostic marker and therapeutic target for combating obesity-related cardiomyopathy.

Materials and Methods

Research subjects

From July 2015 to December 2016, we have recruited 37 lean (BMI ≤ 23 kg/m^2) and 30 obese (BMI > 28 kg/m^2) individuals undergoing cardiac ultrasound measurement at the Second affiliated hospital of Harbin Medical University. Exclusion criteria of this study included: patients younger than 18 years or older than 80 years, with known structural heart diseases, congestive heart failure, coronary heart disease, moderate to severe valvular disease, sepsis, electrolyte imbalance, chronic obstructive pulmonary disease, history of liver or renal disease, malignancy, subclinical hyperthyroidism, history of drug abuse, pregnancy, or type 2 diabetes. Written informed consent was obtained from all participants and all the procedures were approved by human ethics committee of Harbin Medical University.

Anthropometric and biological measurement

The following data of all participants were collected from medical records: age, gender, personal medical history, family medical history, clinical manifestations, physical examinations, blood biochemical tests, and echocardiograms. All blood tests were performed at the clinical laboratory of the Second affiliated hospital of Harbin Medical University. Blood biochemical tests included the levels of triglyceride, total cholesterol, high density lipoprotein-cholesterol (HDL-c), low density lipoprotein-cholesterol (HDL-c), and fasting glucose were measured on a Hitachi 747 analyzer (Roche Diagnostics, Germany). After an overnight fasting, blood samples were collected and oral glucose tolerance test (OGTT) was conducted. Plasma fasting insulin was measured by enzyme-linked immunoassays (#90095, Crystal Chem, IL). Homeostasis Model of Assessment (HOMA) index was calculated to estimate insulin resistance (IR): HOMA-IR = fasting glucose (mmol/l) × fasting insulin (mIU/l)/22.5. The circulating level of NT pro-BNP was analyzed by enzyme-linked immunoassays (#DY3604-05, R&D, MN).

Echocardiography analysis

All subjects underwent echocardiographic examinations which were obtained by using the Vevo 2100 system (Vevo). Echocardiographic parameters included heart rate, left ventricular end diastolic diameter (LVEDD), left ventricular end systolic diameter (LVESD), interventricular septum thickness (IVSd), left ventricle posterior wall thickness (LVPWd), left ventricle mass (LV mass), ejection fraction (EF).

Isolation of human circulating exosomes

Plasma was defrosted on ice and centrifuged at 3000×*g* for 10 min at 4 °C to remove particulate matter. 1–6 ml pre-cleared plasma was then diluted in equal volumes of ice cold PBS and centrifuged for 60 min at 200,000×*g*, 4 °C. Supernatants were removed and the exosomal pellet was resuspended in ice cold PBS, followed by a repeat spin. Supernatants were removed and the exosomal pellets were prepared for treatment. These isolated exosomes were immunoblotted by exosome marker CD63 and CD9.

Mouse primary cardiomyocyte experiment

Mouse primary cardiomyocytes were isolated from C57BL/6 J mice as previously described [28]. For exosome-treatment, 1×10^6 cells were pre-treated with lentivirus, and stimulated with vehicle or 2 μg exosomes.

Animal experiment

Male C57BL/6 J mice, aged 6-week, were obtained from Animal Center of Harbin Medical University (Harbin, China). All animals were housed at 24 ± 1.2 °C with a 12:12 h light-dark cycle. Mice were fed with high fat diet (HFD, Research diets), providing 60% of calories from fat or standard chow diet (STC) with 12% of calories from fat for 16 weeks, then randomly divided into 4 groups with 6 mice in each group: (1) STC-fed mice that received virus encoding control antisense (STC + Ctrl group); (2) STC-fed mice that received virus encoding miR-29a sponge (STC + miR-29a sponge group); (3) HFD-fed mice that received virus encoding control antisense (HFD + Ctrl group); (4) HFD-fed mice that received virus encoding miR-29a sponge (HFD + miR-29a sponge group). These mice were fed STC or HFD for another 4 weeks. The serum and cardiac tissues were collected and stored in −80 °C refrigerator before further measurement. Procedures involving the animal experiments were approved by the Harbin Medical University Animal Policy and Welfare Committee.

Adenosine triphosphate (ATP) measurement

Primary cardiomyocyte ATP was measured using an ATP measurement kit (Molecular Probes, Carlsbad). Briefly, the isolated primary cardiomyocytes were isolated and washed with cold PBS, and then boiled in 100 μl extraction reagent (100 mM Tris, 4 mM EDTA, adjusted to pH 7.75 with acetic acid) for 90 s. Supernatants were retrieved by centrifugation at 10,000×*g* for 60 s. ATP contents were determined by measuring the luminescence of supernatants mixed with luciferase assay buffer using a Varioskan™ Flash Multimode Reader (Thermo Scientific). ATP luminescence was normalized by protein concentration.

Oxygen consumption

Endogenous basal oxygen consumption was measured with a clark electrode in a water-jacketed chamber connected to a circulating water bath (Hansatech, Norfolk, UK).

Enzyme activity of mitochondrial complex I

The activity of the electron transfer chain (ETC) complex I (NADH: ubiquinone reductase) was determined according to the method described in previous report [29]. The complex I activity was expressed as a ratio to citrate synthase activity to account for mitochondrial enrichment.

Analysis of plasma exosomal miR-29a and cardiac gene expression

Total RNA in exosomes and cardiac tissues were extracted by using TRIzol (Invitrogen, Shanghai). Exosomal miRNA was reverse-transcribed using a PrimeScript RT reagent Kit, and subsequently quantified by Taqman quantitative kit (Invitrogen, Shanghai) in ABI real-time system (Applied Biosystems). Reverse transcription of mRNA was performed using the GoScript Reverse Transcription System (Promega), and real-time PCR analysis was performed using SYBR Green (Applied Biosystems, Alameda, CA). The sequence of primers were listed as following: IL-1β, F-TTGACGGACCCCAAAAGATG, R- AGAA- GGTGCTC ATGTCCTCA; IL-6, F- CCCAATTTCCAATGCTCTCC, R- AACGCACTAGGTTTGCCGAG; GAPDH, F- TGCA CCACCAACTGCTTAGC, R- GGATGCAGGGATGA TGTTCT. Relative gene levels were normalized to *GAPDH* level.

Histological analysis of cardiac tissues

Cardiac tissues were fixed in 4% paraformaldehyde for 24-h and embedded in paraffin. 5 μm paraffin sections were prepared and stained with hematoxylin and eosin (HE) or Sirius red (SR). To investigate the histological changes, the cardiac images were observed under a light microscope (Nikon, Tokyo).

Statistical analysis

Data were presented as mean ± SD. Student's *t*-test was used for comparing 2 groups, and one-way ANOVA was used for comparing 3 or 4 groups. GraphPad Prism 5 (GraphPad, San Diego, CA) was used to analyze the

Table 1 Anthropometric parameters and biochemical indexes between lean and obese subjects

Variables	Lean ($n = 37$)	Obese ($n = 30$)	P value
Age (years)	51.04 ± 5.13	53.01 ± 5.93	0.87
BMI (kg/m^2)	20.70 ± 0.96	31.08 ± 2.11	<0.001
Total cholesterol (mmol/l)	4.88 ± 0.62	5.97 ± 0.82	<0.01
Triglycerides (mmol/l)	0.86 ± 0.35	1.42 ± 0.63	<0.01
HDL-c (mmol/l)	1.51 ± 0.62	1.13 ± 0.29	<0.05
LDL-c (mmol/l)	3.03 ± 0.29	3.95 ± 0.42	<0.05
Fasting glucose (mmol/l)	4.72 ± 0.33	5.45 ± 0.69	<0.05
2 h glucose (mmol/l)	5.63 ± 1.11	7.93 ± 2.01	<0.01
Glucose AUC	12.94 ± 2.21	18.92 ± 4.91	<0.01
Fasting insulin (μU/ml)	4.28 ± 1.75	7.91 ± 3.84	<0.001
HOMA-IR	0.88 ± 0.54	1.69 ± 0.87	<0.001

Note: Data are means ± SEM

BMI body mass index, *AUC* area under curve, *HOMA-IR* homeostatic model assessment for insulin resistance

Table 2 Impaired cardiac function in obese subjects as compared with lean subjects

Variables	Lean ($n = 37$)	Obese ($n = 30$)	P value
Heart rate (beat rate/min)	72.31 ± 5.65	89.64 ± 7.01	<0.01
LVEDD (mm)	42.16 ± 4.44	50.43 ± 3.53	<0.01
LVESD (mm)	28.43 ± 3.87	32.13 ± 2.94	<0.05
IVSd (mm)	6.08 ± 0.53	7.43 ± 0.71	<0.01
LVPWd (mm)	5.75 ± 0.42	7.45 ± 0.69	<0.01
LV mass (g)	82.54 ± 8.11	138.22 ± 10.76	<0.001
EF (%)	65.63 ± 5.41	53.03 ± 3.01	<0.01
NT-proBNP (ng/ml)	252.06 ± 4.43	330.49 ± 7.46	<0.001
Plasma miR-29a (folds)	0.89 ± 0.09	1.73 ± 0.16	<0.001

Note: Data are means ± SEM

LVEDD left ventricular end diastolic diameter, *LVESD* left ventricular end systolic diameter, *IVSd* interventricular septum thickness, *LVPWd* left ventricle posterior wall thickness, *LV mass* left ventricle mass, *EF* ejection fraction, *NT-proBNP* N-termial pro b-type natriuretic peptide

statistical significance between sets of data. Differences were considered to be significant at $p < 0.05$.

Results

Elevated plasma miR-29a and impaired cardiac function in obese subjects

To evaluate the clinical relevance of the circulating miR-29a level and obesity-induced cardiac dysfunction, plasma from 30 obese (BMI > 28 kg/m^2) subjects and 37 age-matched lean controls (BMI ≤ 23 kg/m^2) were collected. The clinical characteristics of the study subjects according to BMI categories were summarized in Table 1. As expected, obese subjects had severe adverse metabolic profiles including abnormal lipid profiles, hyperglycemia, and impaired insulin sensitivity.

Then we measured the cardiac parameters of these subjects through ultrasound and biochemical analysis. As showed in Table 2, obese subjects exhibited the significant increasing value of the left ventricular end diastolic diameter (LVEDD) and left ventricular end systolic diameter (LVESD), as compared with lean subjects ($p < 0.05$). The cardiac wall of obese subjects was thicker than lean subjects ($p < 0.01$), as the left ventricle (LV) mass was also increased to 1.68-fold in obese subjects ($p < 0.001$), along with downregulating global LV function evaluated by ejection fraction (EF) ($p < 0.01$) compared with lean subjects. NT-proBNP as a key marker of pressure load of the heart, is considered as an accurate diagnostic parameter for cardiac failure [30]. The circulating NT-proBNP level was increased to 1.35-fold in obese subjects ($p < 0.001$), as compared with lean subjects. Meanwhile, the present data revealed that the level of miR-29a in serum was significantly increased in obese subjects ($p < 0.001$). The consistent upregulation of cardiac dysfunction and plasma miR-29a suggested that circulating miR-29a might affect cardiac function in obese human.

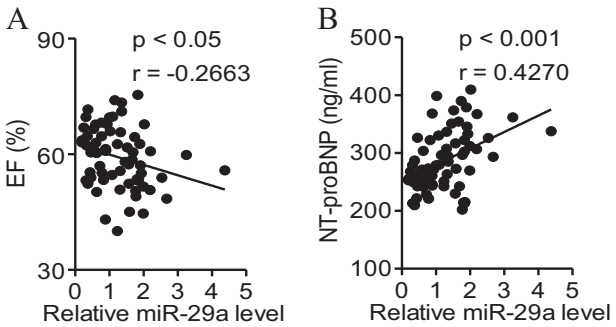

Fig. 1 Circulating miR-29a level is closely correlated with cardiac function. Plasma from 37 lean (BMI ≤ 23 kg/m^2) and 30 obese (BMI > 28 kg/m^2) individuals undergoing cardiac ultrasound measurement were collected and subjected to analysis. Correlation between plasma miR-29a level and cardiac EF% **a** and plasma NT-proBNP level **b**. Correlation was assessed by non-parametric Spearman's test

Circulating miR-29a level positively correlated cardiac dysfunction in obese subjects

To address the association between circulating miR-29a level and cardiac parameters, we further investigated their clinical correlation. As Fig. 1 showed, the plasma miR-29a level was negatively correlated with EF value (Fig. 1a; $r = -0.2663$, $p < 0.05$), but positively associated with plasma NT-proBNP level (Fig. 1b; $r = 0.4270$, $p < 0.001$).

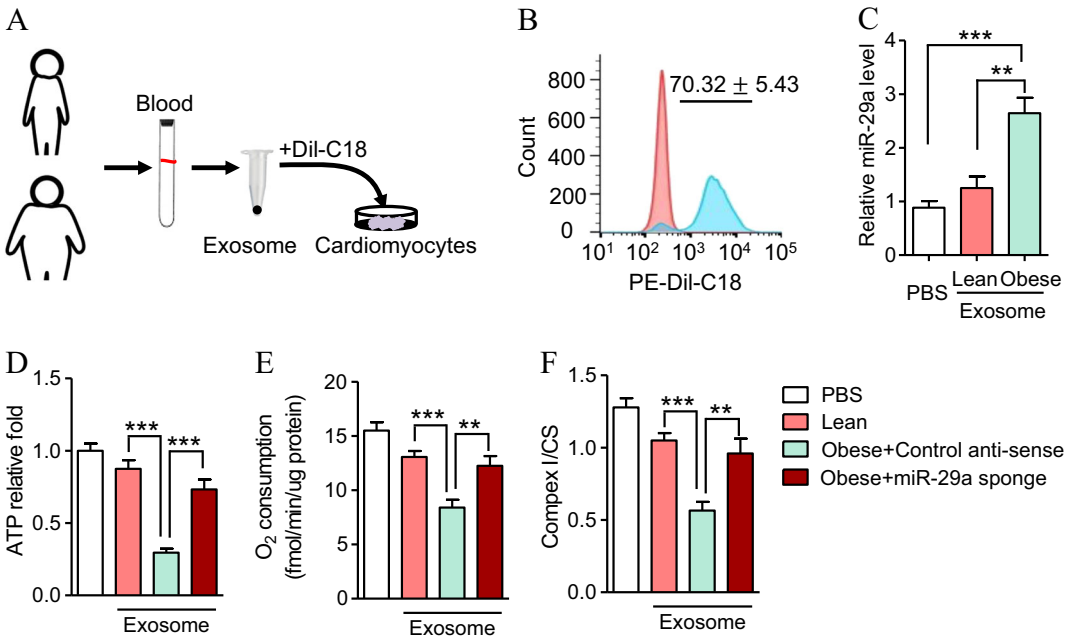

Fig. 2 Blocking human plasma-derived exosomal miR-29a maintains the mitochondrial function in primary mouse cardiomyocytes. **a–c** Human circulating exosomes were isolated and pre-stained with Dil-C18, and then applied to primary mouse cardiomyocytes for 24 h. Schematic depiction of the experiment evaluating the transportability of plasma-derived exosomal miRNAs to cardiomyocytes **a**. Flow cytometry analysis for the percentage of Dil-C18 positive cells treated with Dil-C18-stained exosomes or with blank controls **b**. Real time PCR analysis for the expression of miR-29a in cardiomyocytes stimulated with plasma-derived exosomes from obese individuals and lean subjects **c** ($n = 6$). **d–f** Cardiomyocytes were infected with 1×10^7 viral particles of lentiviruses encoding miR-29a sponge or control anti-sense followed by treatment with 2 μg exosomes for 24 h. Biochemical analysis for ATP production **d**, basal oxygen consumption **e**, and electron transport chain (ETC) complex I **f**. Data are shown as mean ± SEM (**$p < 0.01$ and ***$p < 0.001$, $n = 5$)

Obese subjects-original circulating exosomal miR-29a dampened mitochondrial function in mouse primary cardiomyocytes

Previous studies have already shown that circulating microRNAs are captured into exosomes, which can transport microRNAs to mediate intercellular communications [31]. To this end, we isolated the circulating exosomes from lean and obese individual using the ultracentrifugation methods [32]. The western blot analysis showed the extracted pallets were positively immunoblotted by exosome markers CD63 and CD9 (Fig. S1), which supported the accurate procedure of circulation exosome isolation in present study. We next stained the exosomes with the fluorescent dye Dil-C18, and treated mouse primary cardiomyocytes with the Dil-C18-labeled exosomes (Fig. 2a). 70.32 ± 5.43% of cardiomyocytes were stained with Dil-C18 after a 24-h incubation with Dil-C18-stained exosomes (Fig. 2c). Real-time quantitative PCR showed that cellular miR-29a level was significantly increased after stimulation with exosomes isolated from obese subjects (Fig. 1c) compared with lean subjects, suggesting that circulating exosomal miR-29a can be transported to cardiomyocytes.

Several studies showed that miR-29a impaired insulin signaling and energy expenditure in myocytes, and affected specific gene expression in mitochondria [26]. Mitochondrial dysfunction has been implicated in functional regressive phenotypes in various cardiac pathologies [6]. Therefore, we further investigated the inhibitory effects of circulating miR-29a on mitochondrial function in mouse primary cardiomyocytes. The primary cardiomyocytes were incubated with exosomes isolated from lean or obese subjects for 24-h. As Fig. 2d showed, mitochondrial ATP level was significantly dropped by 64.6% in obese subjects-original exosome-treated myocytes ($p < 0.001$). The obese subjects-original exosomes also decreased the basal oxygen consumption by 46.4% (Fig. 2e, $p < 0.001$). To further investigate the respiration properties, we measured the endogenous respiration activity of intact cardiomyocytes. ATP is synthesized through oxidative phosphorylation, which mainly depends on the activities of the electron transfer chain (ETC) complex I [33]. As showed in Fig. 2f, the obese subjects-original exosomes significantly decreased ETC complex I activity ($p < 0.001$). To determine the important role of exosomal miR-29a in obese subjects-original exosome-mediated mitochondrial dysfunction. We utilized miR-29a sponge, a specific inhibitor, to block the biological function of exosomal miR-29a. As showed in Fig. 2d–f, miR-29a sponge effectively reversed the mitochondrial inhibitory effect from obese subjects-original

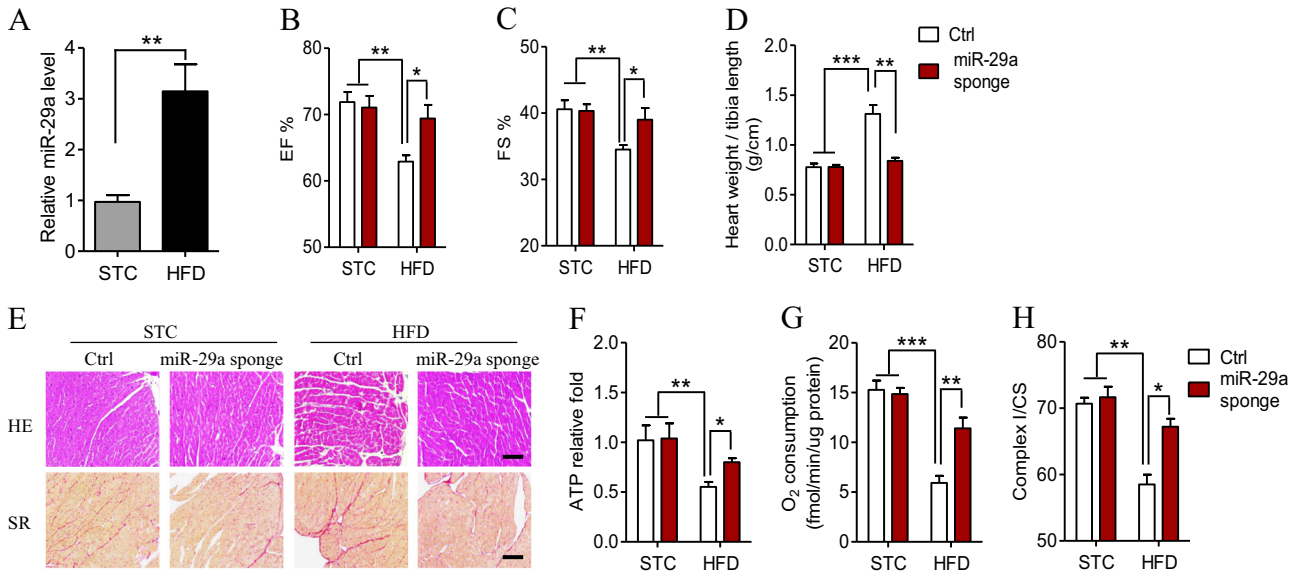

Fig. 3 MiR-29a inhibitor protects against diet-induced cardiac dysfunction in mice. Male C57BL/6 J, aged 6-week, were fed with standard chow (STC) or high fat diet (HFD) for 16 weeks, then 1×10^{10} viral particles encoding miR-29a sponge or control anti-sense (Ctrl) were administrated via tail-vein injection for 4 weeks. **a** Mouse circulating level of miR-29a. **b**, **c** Ultrasound analysis of mouse EF % **b** and FS % **c**. **d**, **e** Measurement of heart weight/ tibia length ratio **d** and histological staining of cardiac tissues (upper panel: Hematoxylin and eosin staining, lower panel: Sirius red staining; **e**). Scale bar = 100 μm. **f**–**h**. Analysis of mitochondrial function in cardiac tissues. Biochemical analysis for ATP production **f**, basal oxygen consumption **g** and electron transport chain (ETC) complex I **h**. Data are shown as mean ± SEM (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$, $n = 6$)

exosomes, including upregulation of ATP production, basal oxygen consumption, and Complex I activity. These results demonstrated circulating miR-29a contributed to obese subjects-original exosomes-mediated cardiomyocyte mitochondrial dysfunction, and closely linked the cardiac disorders.

Treatment of miR-29a sponge attenuates high fat diet-induced cardiac dysfunction in mice

To further address the role of miR-29a in the process of obesity-associated cardiomyopathy, we investigated the therapeutic benefits of miR-29a inhibitor in high fat diet-fed mice. Similarly, high fat diet (HFD) treatment significantly increased the circulating miR-29a levels (Fig. 3a, $p < 0.01$), as compared with standard chow (STC)-fed mice. Previous studies have showed that high fat diet severely damages cardiac physiology, including cardiac dysfunction, structural disorders, and energy imbalance [3, 4]. Our results also found the cardiac parameters, including the percentage of ejection fraction (EF) and fractional shortening (FS) were remarkably decreased in HFD-fed mice (Fig. 3b, c, $p < 0.01$). HFD significantly increased mouse body weight, but administration with miR-29a sponge had no significant effects in the STC-fed or HFD-fed mice (Fig. S2A). Consistent to previous studies [34, 35], the increased heart weight/tibia length ratio implied the cardiac hypertrophy in HFD-fed mice (Fig. 3d, $p < 0.001$), as compared with STC-fed mice. However, miR-29a sponge treatment effectively maintained the cardiac function and decreased heart weight/tibia length ratio in HFD-fed mice (Fig. 3b–d). Meanwhile, the hematoxylin and eosin stain (HE) and Sirius red stain (SR) showed miR-29a sponge protected against diet-induced cardiac structural damages and fibrosis (Fig. 3e), as compared with control antisense-treated mice. To determine the role of miR-29a in obesity-mediated mitochondrial dysfunction in vivo, we measured several basic mitochondrial parameters in cardiac tissues. As showed in Fig. 3f, g, HFD significantly downregulated mitochondrial function, whereas miR-29a sponge effectively reversed the mitochondrial inhibitory effects, including upregulation of ATP production (Fig. 3f, $p < 0.05$), basal oxygen consumption (Fig. 3g, $p < 0.01$) and Complex I activity (Fig. 3h, $p < 0.05$). Cardiac inflammation is a critical character in the obesity-related cardiomyopathy [2]. Therefore, we further measured the inflammatory response in heart tissues. As showed in Fig. S2, high fat diet induced the upregulation of phosphorylated IκB/IκB ratio (Fig. S2B-C, $p < 0.001$) and inflammatory cytokine levels (Fig. S2D, E, $p < 0.01$), whereas miR-29 sponge effectively inhibited these cardiac inflammation. These results demonstrated miR-29a inhibitor had the abilities of protecting high fat diet-induced cardiac dysfunction.

Discussion

Obesity and obesity-related complications, such as insulin resistance and diabetes, are positively associated with the increased miR-29a levels [36]. The imbalance of lipid metabolism and adipose tissue dysfunction is also mediated by excess miR-29a production [37, 38]. Besides, the expression of miR-29a is closely related to adipose tissue hypoxia [39], which may initiate the functional damages in heart. Present study supported the critical role of circulating miR-29a in regulating cardiac function and mitochondrial activity in obesity-related cardiomyopathy. In obese human subjects, the increased circulating miR-29a level was positively associated with cardiac dysfunction, including reduction of ejection fraction and induction of cardiac hypertrophy. The isolated exosomal miR-29a impaired mitochondrial activity in primary cardiomyocytes. However, specific miR-29a inhibitor could effectively protect against diet-induced cardiac dysfunction, structural abnormal, and mitochondrial activity in mice.

The induction of obesity-related cardiomyopathy is multifactorial, and the primary cause has yet to be identified. The abnormal alteration of left ventricle is considered as a major contributor to cardiac structural and functional changes. However, the drivers of left ventricular dysfunction is still unclear. The local endocrine and paracrine influences, including renin-angiotensin-aldosterone system (RAAS) activity, lipotoxicity, circulating factors, and oxidative stress are possible mediators of cardiac injuries under obese status [40, 41]. Among these risk factors, the energy imbalance, mainly characterized as mitochondrial dysfunction play a culprit in cardiomyopathy [6]. Several studies have revealed a reduced ability to enhance fatty acid oxidation in the face of increased lipid load, which might be attributed to mitochondrial dysfunction [7]. Increased reactive oxidative species (ROS) production coupled with increased protein and lipid peroxidation in cardiomyocyte mitochondria sufficiently stimulate the cardiac oxidant burden. The obese heart is also characterized by reduced rates of oxidative phosphorylation (OXPHOS) and diminished efficiency in ATP synthesis [9]. Therefore, targeting on upregulation of mitochondrial activity is a potential therapeutic approach to maintain cardiac function. In present study, we also found a significant reduction of mitochondrial complex I activity, oxygen consumption, and ATP production in obese cardiac tissues, accompanied with severe cardiac structural disorders and hypertrophy.

Recent functional studies using both gain-of-function and loss-of-function approaches in mice have uncovered the important roles of microRNAs in cardiac hypertrophy. Genetic deficiency of miR-34a protected ageing-induced cardiac dysfunction, but overexpression of myocyte miR-155 was linked to protection from necrotic cell death in vitro [16, 17]. Meanwhile, several studies showed that the miR-29 family was closely associated with hypertrophic cardiomyopathy [19]. Inhibition of miR-29b effectively attenuated angiotensin II-induced cardiac fibrosis [24]. Overexpression of cardiomyocyte miR-29a level could stimulate cardiomyocyte proliferation [25], whereas blocking obesity-induced miR-29a improved lipids-mediated insulin resistance [26, 27]. However, whether miR-29a participates in the process of obesity-related cardiomyopathy is unknown. Present study supported circulating miR-29a was significantly correlated with human cardiac function in obese status, and exosomal miR-29a mediated the crosstalk between heart and peripheral tissues. More interestingly, miR-29a inhibitor showed a strong therapeutic benefits on protecting against obesity-induced cardiac dysfunction, structural disorders and mitochondrial inactivity. All these findings supported miR-29a is a useful clinical diagnostic marker and therapeutic target.

The exosomes are extracellular nano-sized particles (30–100 nm in diameter) originated from endocytosis-mediated invagination of the plasma membrane. A growing body of evidence suggests that the exosomes is an important signaling mediator for intercellular communication and interorgan crosstalk, through delivery of the exosomes to the target cells, or interaction of the exosomal signaling molecules with the cell surface receptors [31, 42]. The exosomal signaling is largely determined by their composition of miRNAs, which control the gene expression in target cells by promoting mRNA degradation or blocking mRNA degradation. Researchers found blockade of exosome generation by GW4869 significantly improved cardiac function in diabetic hearts [43]. With respect to cardiovascular disease, several cardiac microRNAs, including miR-1, miR-133a, and miR-208a, have been detected in the serum [44]. Furthermore, peripheral blood mononuclear cells collected from patients with type 2 diabetes lost their angiogenic capacity after inhibition of exosomal miR-126 [45]. Similarly, our results showed that the suppression of cardiomyocyte mitochondrial activity was reversed by pre-treatment with exosomal miR-29a inhibitor in vitro, which was further confirmed in diet-induced obese mouse model.

The developed miRNA inhibitors, termed "miRNA sponges" competitively tandem binding sites to a miRNA of interest. These antisense oligonucleotides are widely used in determining the function and binding targets of specific miRNAs. Viral vectors could enforce miRNA sponge-based therapy by forcing a high prolonged expression of the sponge and by providing an efficient way for in vivo delivery [46]. Consistent with clinical correlation, our in vitro and in vivo studies supported miR-29a sponge improved cardiomyocyte mitochondrial activity and cardiac function. Our results provided evidence that miR-29a

sponge was a potential clinical therapeutic tool to alleviate obesity-related cardiomyopathy. However, the direct suppressing targets of miR-29a was still unknown, which need our continuing investigation in the further study.

In conclusions, present study uncovered the correlation between circulating miR-29a and cardiac parameters in human subjects, and provided solid evidence of the therapeutic application of miR-29a sponge in combating obesity-mediated cardiac dysfunction.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Procedures involving the clinical trial and animal experiments were approved by Human Ethics Committee and Animal Policy and Welfare of Harbin Medical University Committee.

Informed consent Informed consent was obtained from all individual participants included in the study.

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