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ORIGINAL ARTICLE

MiR-132 Inhibits Expression of SIRT1 and Induces Pro-inflammatory Processes of Vascular Endothelial Inflammation through Blockade of the SREBP-1c Metabolic Pathway

Liwei Zhang · Dangsheng Huang · Qiushuang Wang · Dong Shen · Yumei Wang · Bingyang Chen · Jinqian Zhang · Luyue Gai

Abstract
Purpose Inflammation participates centrally in all stages of atherosclerosis (AS), which begins with pro-inflammatory processes and inflammatory changes in the endothelium, related to lipid metabolism. MicroRNA (miRNA) inhibition of inflammation related to SIRT1 has been shown to be a promising therapeutic approach for AS. However, the mechanism of action is unknown.
Methods We investigated whether miRNAs regulate the SIRT1 and its downstream SREBP-lipogenesis-cholesterogenesis metabolic pathway in human umbilical vein endothelial cells (HUVECs). HUVECs were transfected with miR-132 mimics and inhibitors, and then treated with or without tumor necrosis factor α (TNFα). The effects of miR-132 on pro-inflammatory processes, proliferation and apoptosis were assessed.
Results We identified that the relative 3' UTR luciferase activities of SIRT1 were significantly decreased in miR-132 transfected HUVECs (0.338±0.036) compared to control (P=0.000). miR-132 inhibited SIRT1 expression of mRNA level in HUVECs (0.53±0.06) (P<0.01) as well as proteins of SIRT1. mRNA expression and protein levels of SREBP (0.45±0.07), fatty acid synthase (FASN) (0.55±0.09) and 3-hydroxy-3-methylglutaryl CoA reductase (HMGCR) (0.62±0.08) (P<0.01), which are downstream regulated genes, were reduced in HUVECs by miR-132. MiR-132 promoted pro-inflammatory processes and apoptosis of HUVECs induced by TNF-α, and inhibited its proliferation, viability and migration.
Conclusions SIRT1 mRNAs are direct targets of miR-132. miR-132 controls lipogenesis and cholesterogenesis in HUVECs by inhibiting SIRT1 and SREBP-1c expression and their downstream regulated genes, including FASN and HMGCR. Inhibition of SIRT1 by miR-132 was associated with lipid metabolism-dependent pro-inflammatory processes in HUVECs. The newly identified miRNA, miR-132 represents a novel targeting mechanism for AS therapy.

Keywords MicroRNA · SIRT1 · Inflammation · SREBP-1c · Lipid metabolism · Atherosclerosis

Introduction

Atherosclerosis is the major cause of myocardial infarction and stroke and thus constitutes the leading cause of mortality in the world. Atherosclerosis is a specific form of arteriosclerosis in which an artery wall thickens as a result of the accumulation of calcium and fatty materials such as cholesterol and triglyceride. It is a syndrome affecting arterial blood vessels, a chronic inflammatory response in the walls of arteries. It is caused largely by the accumulation of macrophages and white blood cells and promoted by low-density lipoproteins (LDL, plasma proteins that carry cholesterol and triglycerides) without adequate removal of fats and cholesterol

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from the macrophages by functional high-density lipoproteins (HDL). It is characterized by the formation of plaques within the arteries [1].

Recent evidence has implicated involvement of microRNAs (miRNA) in regulating angiogenesis and endothelial function [2–5].

miRNA-132 (miR132) is a highly conserved miRNA that is induced by the neurotrophin BDNF in a CREB-dependent manner [6, 7]. MiR-132 has also been shown to regulate cellular excitability in cultured cells, possibly via regulation of ion channels [8, 9]. Using primary human adipose-derived stem cells as a source of preadipocytes and in vitro differentiated adipocytes, Strum JC et al. found that IL-8 and monocyte chemoattractant protein-1 (MCP-1) are constitutively secreted by both cell types and induced in response to serum deprivation. MicroRNA profiling revealed the rapid induction of miR-132 in these cells when switched to serum-free medium. Furthermore, miR-132 overexpression was sufficient to induce nuclear factor-kappaB translocation, acetylation of p65, and production of IL-8 and MCP-1. Inhibitors of miR-132 decreased acetylated p65 and partially inhibited the production of IL-8 and MCP-1 induced by serum deprivation. MiR-132 was shown to inhibit silent information regulator 1 (SIRT1) expression through a miR-132 binding site in the 3'-untranslated region of SIRT1. Thus, in response to nutritional availability, induction of miR-132 decreases SIRT1-mediated deacetylation of p65 leading to activation of nuclear factor-kappaB and transcription of IL-8 and MCP-1 in primary human preadipocytes and in vitro differentiated adipocytes [10].

SIRT1 is an NAD-dependent deacetylase that regulates apoptosis in response to oxidative and genotoxic stress [11, 12]. Resveratrol attenuates endothelial inflammation by inducing autophagy, which was in part mediated through the activation of the cAMP PRKA-AMPK-SIRT1 signaling pathway [13, 14]. Recent in vivo studies have suggested that Sterol regulatory element binding protein (SREBP)-1c deacetylation by SIRT1 on Lys-289 and Lys-309 inhibits SREBP-1c activity by decreasing its stability and its association with lipogenic target genes [15]. SREBP-1c is a transcription factor that controls the expression of genes related to fatty acid and triglyceride synthesis in tissues with high lipid synthesis rates.

Targeting the aberrant SREBP-lipogenesis-cholesterogenesis pathway may lead to new approaches to the treatment of artherosclerosis. SIRT1 is believed to regulate vascular endothelial pro-inflammatory processes by deacetylating molecular targets that include SREBP-1c.

MiR-132 might regulate cellular pro-inflammatory processes, proliferation, viability, migration and apoptosis through the SIRT1-SREBP-1c pathway implicating energy metabolism. miR-132 is progressively expressed in endothelial cells. The prediction from miRBase indicates that SIRT1 is a potential target of miR-132 in endothelial cells. Thus, in the present study, we aimed to investigate whether vascular endothelial pro-inflammatory processes and atherosclerosis progression is determined by a miR-132-dependent SIRT1 loss of function.

Materials and Methods

Cell Culture and Treatment

Human umbilical vein endothelial cells (HUVECs) were isolated from umbilical cord veins as reported previously [16] and cultured on gelatin-coated plastic dishes (Dibco Biocult, Uxbridge, 1–50350) with M199 medium supplemented with 10 % fetal bovine serum at 37 °C in a humidified atmosphere containing 5 % CO_2. All experiments were performed following 3–6 passages when the cells reached 80-90 % confluence. During the logarithmic growth phase, cells were exposed to TNF (10 ng/mL) for 4 h. The ethics review board at the first Affiliated Hospital of Chinese PLA General Hospital approved this study, and informed consent was obtained from all patients before participation.

Reagents

Human miRNA precursors, miRNA inhibitors, TaqMan miRNA assay, mirVanaTM miRNA isolation kit and Lipofectamine 2,000 were purchased from Life Technologies. The 3'UTR luciferase reporter DNA constructs of SIRT1 (HmiT017704-MT05) were purchased from GeneCopoeia (Rockville, MD). CellTiter 96H AQueous One Solution Cell Proliferation Assay (mitochondrial MTS assay) and Caspase-GloH 3/7 Assay Systems were obtained from Promega (Madison, WI).

MiRNA Transfection

Transient transfection of miRNA precursors or inhibitors was carried out using Lipofectamine 2,000 according to the manufacturer's protocol. Human miR-132 (PM12827) precursors, anti-miR-132 (MH12827) and negative control (miR-NC; AM17110) were used for assays.

Quantitative Real-time Reverse Transcription-polymerase Chain Reaction (qRT-PCR)

Total RNA was prepared from cells using the RNeasy Mini kit (Qiagen, Valencia, CA) and subjected to reverse transcription by SuperScriptH III reverse transcriptase (Life Technologies) according to the manufacturer's instructions. A hot start at 95 °C for 5 min was followed by 40 cycles at denaturation at 95 °C for 15 s, annealing of the primers at 58 °C for 30 s and elongation at 72 °C for 30 s using ABI 7,500 Fast Real-Time

PCR System (Life Technologies). Data were normalized to 18S rRNA or GAPDH and represented as the average fold of three independent duplicates. To determine intrinsic miR-132 expression, miRNA was prepared from cells using the mirVanaTM miRNA isolation kit (Life Technologies). Mature miRNA was quantified by the TaqMan miRNA assay (Life Technologies) in accordance with the manufacturer's instructions. The data were normalized by RNU6B.

ELISA for sICAM1

The sICAM1 levels in the endothelial cell-conditioned medium were measured using an ELISA kit according to the manufacturer's instructions.

Western Blot Analysis

Cell lysates were prepared from cells using a lysis buffer [50 mM Tris (pH 8), 150 mM NaCl, 0.02 % NaN3, 0.1 % SDS, 1 % NP-40 and 0.5 % sodium deoxycholate] containing 1 mM phenylmethylsulfonyl fluoride and protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN). Protein concentration was determined by Bradford assay using Coomassie Plus Protein Reagent (Thermo Scientific, Rockford, IL). Western blot was performed using the Novex system (Life Technologies).

Primary antibodies anti-SREBP-1c, FASN, HMGCR, AR, ICAM1, MMP9 andβ-actin, (Santa Cruz Biotechnology, Santa Cruz, CA), and secondary antibodies which were conjugated with horseradish peroxidase (GE Healthcare, Piscataway, NJ) were used. Detection of protein bands was done using Enhanced Chemiluminescence Western Blotting Detection Reagents (GE Healthcare).

Fatty Acid and Cholesterol Quantification

The amounts of long chain fatty acids and cholesterols were determined using the Free Fatty Acid Quantification Kit and Cholesterol/Cholesteryl Ester Detection Kit (Abcam, Cambridge, MA) in cells. The amounts of fatty acids and cholesterols were normalized by total cell numbers. The relative fatty acid or cholesterol (%) was assigned as 100 % in control.

Cell Proliferation, Viability and Migration Assays

Cells (6,000 cells/well) were plated on 96-well plates and transfected with miR-132, inhibitor of miR-132 or NC. Cell proliferation was measured 3 d post-transfection by MTS assay (Promega) according to the manufacturer's instructions. Cell Counting Kit-8 (CCK-8) kit purchased from DOJINDO (Japan), which was used to determine cell viability. Cells were pretreated with above methods and then incubated in 96-well plate for 16 h. In each well, 10 μL CCK-8 solutions was added. Cells were incubated at 37 °C for 4 h, absorbance values were measured with plate reader at 450 nm. In vitro cell migration was determined in Boyden chambers precoated with collagen I (Sigma-Aldrich, St. Louis, MO; for migration assay) or growth factor-depleted Matrigel matrix (BD Bioscience, San Jose, CA; for invasion assay). Cells (1.5×10^5 cells/well) were seeded into the inside of the precoated upper chambers. After incubation at 37°Cwith 5 % CO_2 for 48 h, the numbers of migrating cells were measured by a crystal violet staining method.

Apoptosis Assays

For Annexin V staining, a fluorescence-activated prostate cancer cell sorting analysis was done 72 h post-transfection of miRNAs using the Annexin V-FITC Apoptosis Detection Kit I (BD Bioscience) and a FACScan flow cytometer with CellQuest software (Becton Dickinson Labware, Lincoln Park, NJ). For the caspase activity assay, cells were measured for caspase 3/7 enzymatic activities by Caspase-GloH 3/7 Assay Systems (Promega). Data were normalized by total proteins.

Statistics

Continuous normally distributed variables were represented graphically as mean±standard deviation (SD). For statistical comparison of quantitative data between groups, analysis of variance (ANOVA) or *t*-test was performed. To determine differences between groups not normally distributed, medians were compared using Kruskal-Wallis ANOVA. χ^2 test was used when necessary for qualitative data. The degree of association between variables was assessed using Spearman's nonparametric correlation. All statistical analyses were carried out using SPSS software version 13.0 (SPSS Inc., Chicago, IL, USA). Probabilities of 0.05 or less were considered to be statistically significant.

Results

MiR-132 Inhibits Expression of SIRT1, SREBP1-c and their Downstream Genes in HUVECs

To investigate whether miRNAs regulate the SIRT1 and its downstream SREBP-lipogenesis-cholesterogenesis metabolic pathway in HUVECs, we first used DIANA microT v4.0 online software (http://diana.cslab.ece.ntua.gr/) to predict if one or more miRNAs target SIRT1, the key factor that regulates fatty acid, lipid and cholesterol biosynthesis and homeostasis. The miRNAs, miR-132, were retrieved which

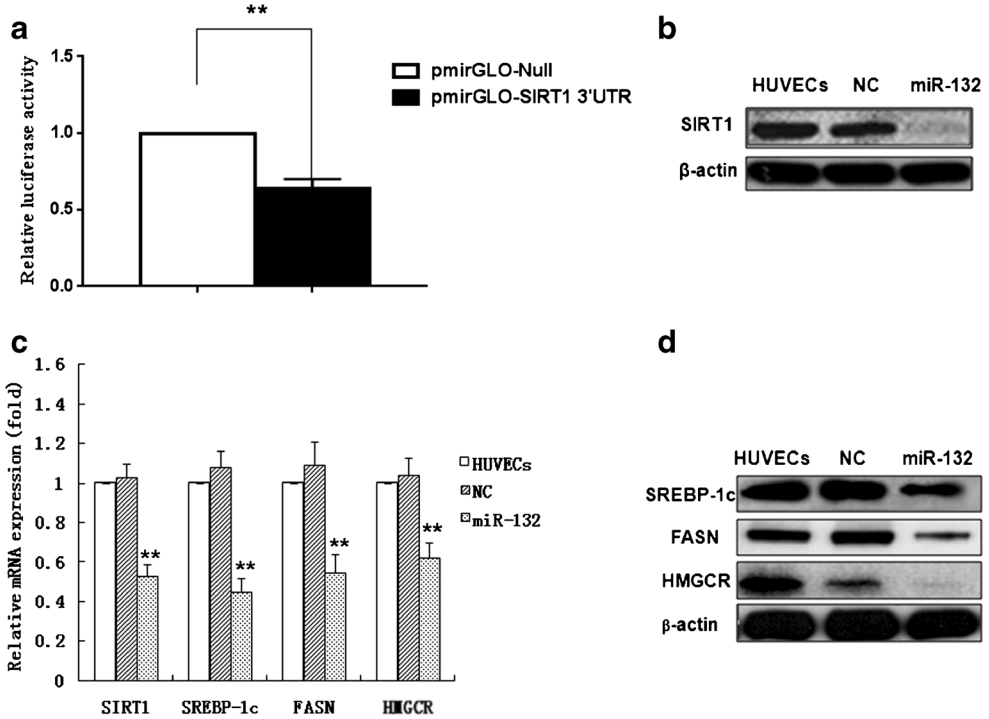

Fig. 1 MiR-132 inhibits expression of SIRT1, SREBP1-c and their downstream genes in HUVECs. To investigate whether miRNAs regulate SIRT1 and its downstream SREBP-lipogenesis-cholesterogenesis metabolic pathway in HUVECs, we first used DIANA microT v4.0 online software (http://diana.cslab.ece.ntua.gr/) to predict if one or more miRNAs target SIRT1, the key factor that regulates fatty acid, lipid and cholesterol biosynthesis and homeostasis. miR-132 was retrieved that potentially co-targeted 3' UTRs of SIRT1 mRNAs. To further verify if miR-132 directly binds with 3' UTRs of SIRT1, we performed 3' UTR luciferase reporter assays of SIRT1 in miR-132 transfected HUVECs (**a**). To validate whether miRNAs control the SREBP-lipogenesis-cholesterogenesis pathway in HUVECs, we performed Western blot (**b**) and qRT-PCR (**c**) quantification analyses in HUVECs. To validate whether the miRNAs control the SREBP-lipogenesis-cholesterogenesis pathway in HUVECs through SIRT1, we performed Western blot analyses in HUVECs (**d**). The data are presented as mean±SD from three independent experiments. *P<0.05, **P<0.01

potentially co-target 3' UTRs of SIRT1 mRNAs. To further verify if miR-132 directly binds with 3' UTRs of SIRT1, we performed 3' UTR luciferase reporter assay and found that the relative 3' UTR luciferase activities of SIRT1 were significantly decreased in miR-132 transfected HUVECs (0.338±0.036) compared to control (Fig. 1a) (P=0.000). The results confirm that SIRT1 mRNAs are direct targets of miR-132. To validate whether the miRNAs control the SREBP-lipogenesis-cholesterogenesis pathway in HUVECs, we performed Western blot (Fig. 1b) and qRT-PCR quantification analyses. miR-132

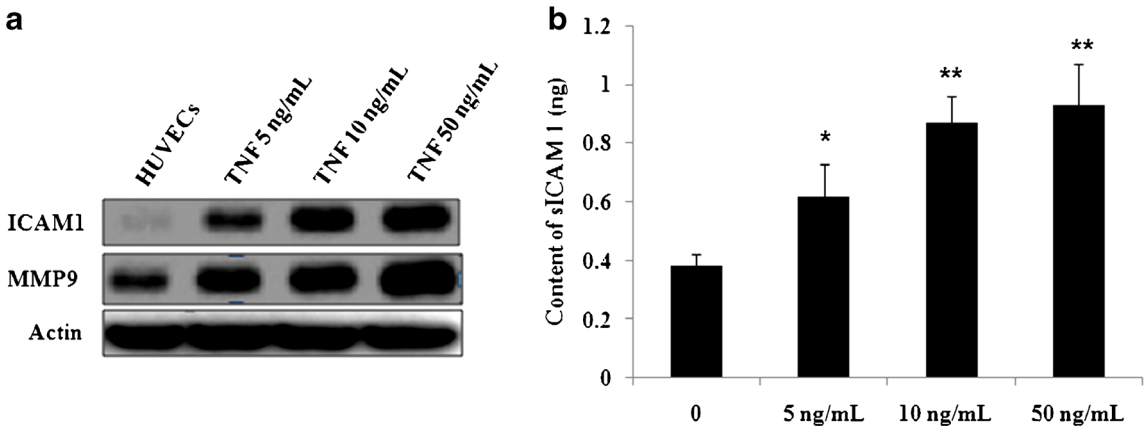

Fig. 2 TNFα induced pro-inflammatory processes of endothelial (**a**) Cells were incubated with different concentrations of TNFα (5, 10 and 50 ng/mL) for 4 h. The expression of ICAM1 and MMP9 was detected by western blot. (**b**) Cells were incubated with different concentrations of TNFα (5, 10 and 50 ng/mL) for 4 h. The levels of sICAM1 in the medium were detected via ELISA. The data are presented as mean±SD from three independent experiments. *P<0.05, **P<0.01

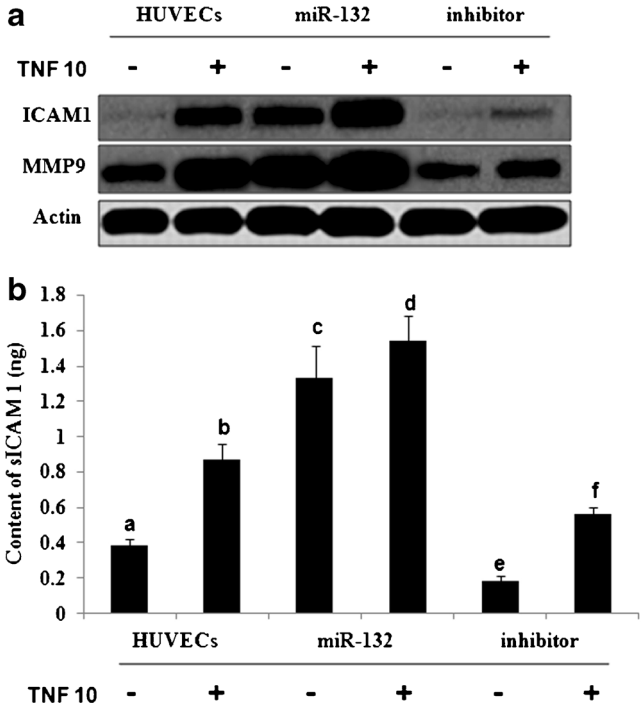

Fig. 3 Mir-132 promoted TNFα -induced pro-inflammatory processes of endothelial (**a**) Cells were pretreated with miR-132 or inhibitor of it for 48 h, and then followed by treatment with or without TNFα (10 ng/mL) for an additional 24 h. The expression of ICAM1 and MMP9 was detected by western blot. (**b**) The levels of sICAM1 in the medium were detected via ELISA. The control group was treated with 0.2 % DMSO. The data are presented as mean±SD from three independent experiments. (b vs. a, $P<0.01$; c and d vs. a, $P<0.01$; d vs. b, $P<0.01$; e vs. a, $P<0.01$; f vs. a, $P<0.05$; f vs. b, $P<0.01$; c vs. e, $P<0.01$; d vs. f, $P<0.01$)

inhibited the expression of mRNAs in HUVECs (0.53±0.06) (Fig. 1c) ($P<0.01$) as well as proteins (Fig. 1b) of SIRT1, respectively.

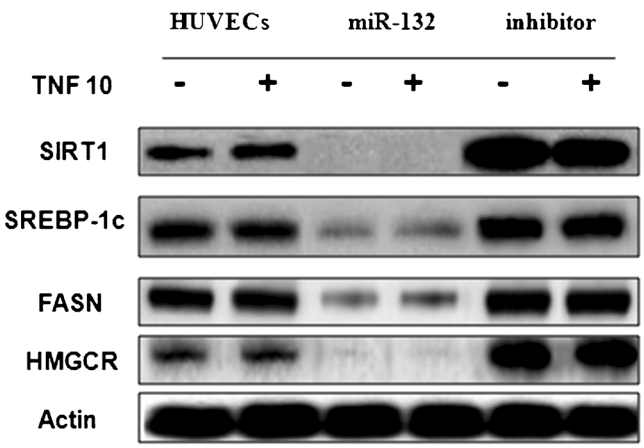

Fig. 4 Inhibit of miR-132 increased expression levels of SIRT1, SREBP1-c and their downstream genes in HUVECs. To further test the specificity of miR-132 for SREBP-lipogenesis-cholesterogenesis, antisense oligonucleotides against miR-132 were used as miR-132 inhibitors. By blocking endogenous miR-132 in HUVECs, mRNA of SIRT1, SREBP-1c and their downstream gene expression was detected. The data are presented as mean±SD from three independent experiments

mRNA Expression of SREBP (0.45±0.07), FASN (0.55±0.09) and HMGCR (0.62±0.08) [17, 18], which are downstream regulated genes, were reduced in HUVECs by miR-132 (Fig. 1c). Their protein levels were inhibited too (Fig. 1d). Because SREBP1c, FASN and HMGCR are important enzymes for de novo synthesis of fatty acid and cholesterol respectively, we subsequently examined the levels of fatty acid and cholesterol in cells.

Pro-Inflammatory Processes-Promoting Activity of miR-132 on HUVECs

In accordance with previous observations, treatment with different concentrations (5, 10 and 50 ng/mL) of

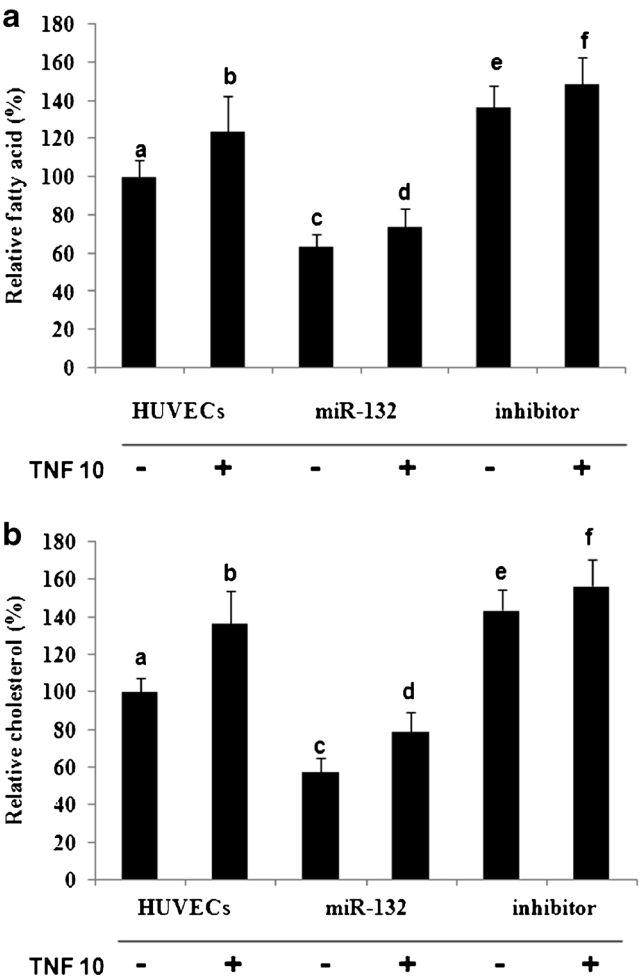

Fig. 5 MiR-132 decreased the amounts of fatty acid and cholesterol in HUVECs. To further test the specificity of miR-132 for SREBP-lipogenesis-cholesterogenesis, antisense oligonucleotides against miR-132 were used as miR-132 inhibitors. By blocking endogenous miR-132 in HUVECs, the amounts of intracellular fatty acid (**a**) and cholesterol (**b**) were detected. The data are presented as mean±SD from three independent experiments. (**a**) b vs. a, $P<0.05$; c and d vs. a, $P<0.05$; d vs. b, $P<0.01$; e and f vs. a, $P<0.05$; f vs. b, $P<0.05$; c vs. e, $P<0.01$; d vs. f, $P<0.01$. (**b**) b vs. a, $P<0.01$; c and d vs. a, $P<0.01$; d vs. b, $P<0.01$; e and f vs. a, $P<0.01$; c vs. e, $P<0.01$; d vs. f, $P<0.01$

TNFα for 4 h significantly increased the expression of ICAM1 (intercellular adhesion molecule 1) and MMP9 [matrix metallopeptidase 9 (gelatinase B, 92 kDa gelatinase, 92 kDa type IV collagenase)] (Fig. 2a) as well as the soluble ICAM (sICAM1) level (Fig. 2b) in endothelial cells (Fig. 2b: control: 0.38±0.04; 5 ng/mL 0.62±0.11; 10 ng/mL 0.87±0.09; 50 ng/mL 0.93±0.14). As expected, TNFα induced sICAM1 levels as well as the expression of ICAM1 and MMP9 in HUVECs.

To determine whether inflammation could be promoted by miR-132 in HUVECs, we investigated cells pretreated with miR-132 or its inhibitor for 48 h, and then followed by treatment with or without TNFα 10 (10 ng/mL) for an additional 24 h. The results showed that miR-132 induced sICAM1 level as well as the expression of ICAM1 and MMP9 in HUVECs (Fig. 3a and b). Moreover, the sICAM1 level as well as the expression of ICAM1 induced by miR-132 and/or TNFα could be prohibited significantly by inhibitors of miR-132 (Fig. 3b: c vs. e, $P<0.01$; d vs. f, $P<0.01$) (Fig. 3b: a, 0.38±0.04; b, 0.87±0.09; c, 1.33±0.18; d, 1.54±0.14; e, 0.18±0.03; f, 0.56±0.04).

Pro-Inflammatory Processes-Promoting Activity of miR-132 in HUVECs Related to SIRT1 and SREBP-Lipogenesis-Cholesterogenesis

To further test the specificity of miR-132 for SREBP-lipogenesis-cholesterogenesis, antisense oligonucleotides against miR-132 were used as miR-132 inhibitors. By blocking endogenous miR-132 in HUVECs with or without pro-inflammatory processes, miR-132 inhibitors increased SIRT1, SREBP-1c and their downstream gene expression (Fig. 4). As shown in Fig. 5a and b, the amounts of intracellular fatty acid and cholesterol were significantly decreased in miR-132-transfected HUVECs compared with the control groups. These results suggest that miR-132 inhibited pro-inflammatory processes related to SIRT1-SREBP signaling through reduction of SIRT1 expression and decreased the levels of fatty acid and cholesterol in HUVECs. Conversely, by blocking miR-132 in HUVECs, miR-132 inhibitors increased the amounts of intracellular fatty acid (Fig. 5a) and cholesterol (Fig. 5b).

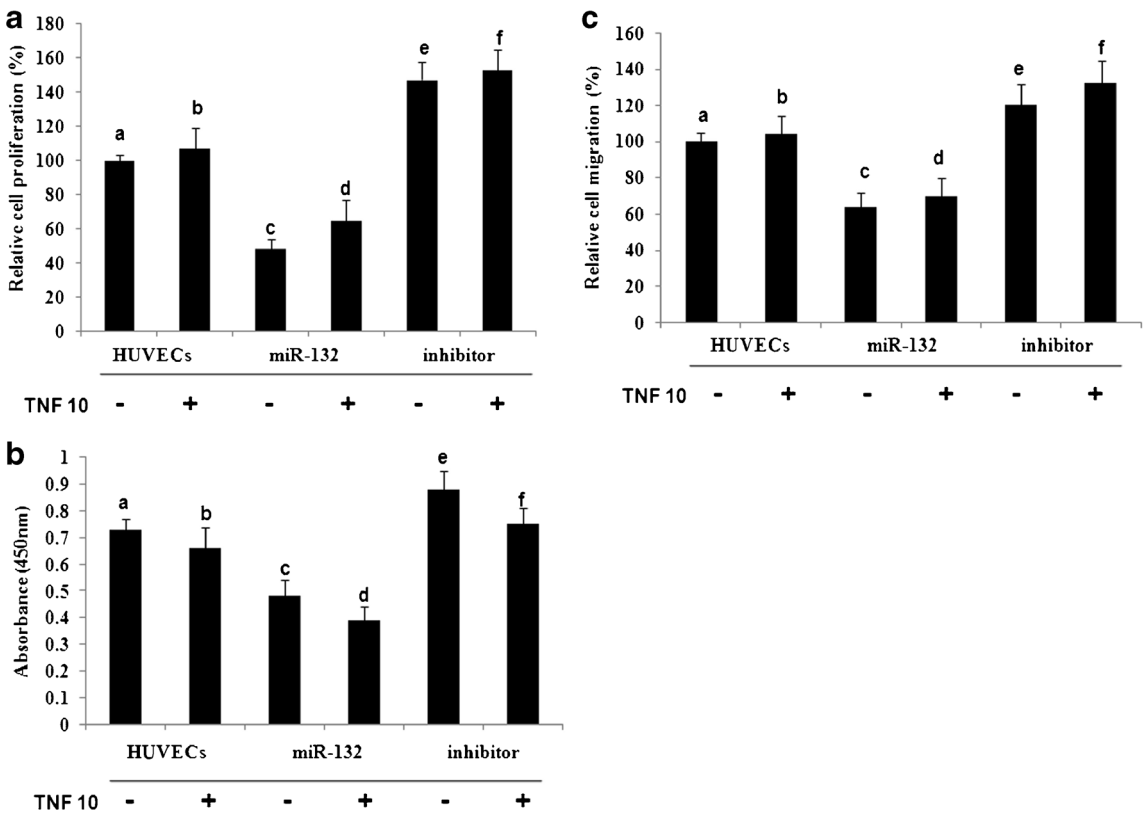

Fig. 6 MiR-132 suppress HUVECs proliferation, viability and migration. MiR-132 inhibited cell proliferation in HUVECs compared with control (**a**). The relative cell proliferation (%) was assigned as 100 % in control cells. Cell viability (**b**) and migration (**c**) were significantly decreased by miR-132 compared with the control groups. The data are presented as mean±SD from three independent experiments. (**a**) c and d vs. a, $P<0.05$; e and f vs. a, $P<0.05$. (**b**) c and d vs. a, $P<0.01$; e and f vs. a, $P<0.01$. (**c**) c and d vs. a, $P<0.05$; e and f vs. a, $P<0.05$

MiR-132 Suppress Cell Proliferation, Viability and Migration in HUVECs

To assess the potential for biological consequences elicited by miR-132, we re-expressed and downloaded miR-132 in HUVECs and performed a series of functional assays relevant to cell function. When HUVECs were transfected with miR-132, proliferation of both cell types was inhibited in comparison with control cells (Fig. 6a). MiR-132 also significantly inhibited in vitro viability (Fig. 6b) and migration (Fig. 6c) in HUVECs. Conversely, by over-expressing miR-132 in HUVECs, miR-132 inhibitors increased cell proliferation, viability and migration (Fig. 6). These data suggest that miR-132 suppress pathways relevant to cell viability and migration.

Apoptotic Death in HUVECs

To determine if miR-132 induce apoptosis in HUVECs, Annexin V-FITC/7-AAD staining measurements and caspase activity assays were carried out. The results of Annexin VmiR-FITC/7-AAD staining and flow cytometric analysis revealed that miR-132 increased the apoptotic cell fractions (both early and late apoptotic cell fractions, $P<0.05$) in HUVECs compared with the control groups, especially in groups treated with TNFα (Fig. 7a, $P<0.05$). Caspase 3/7 activities were also significantly induced by miR-132 in HUVECs (Fig. 7b, $P<0.01$) These results suggest that miR-132 induces HUVECs death through activation of a caspase-dependent apoptotic mechanism.

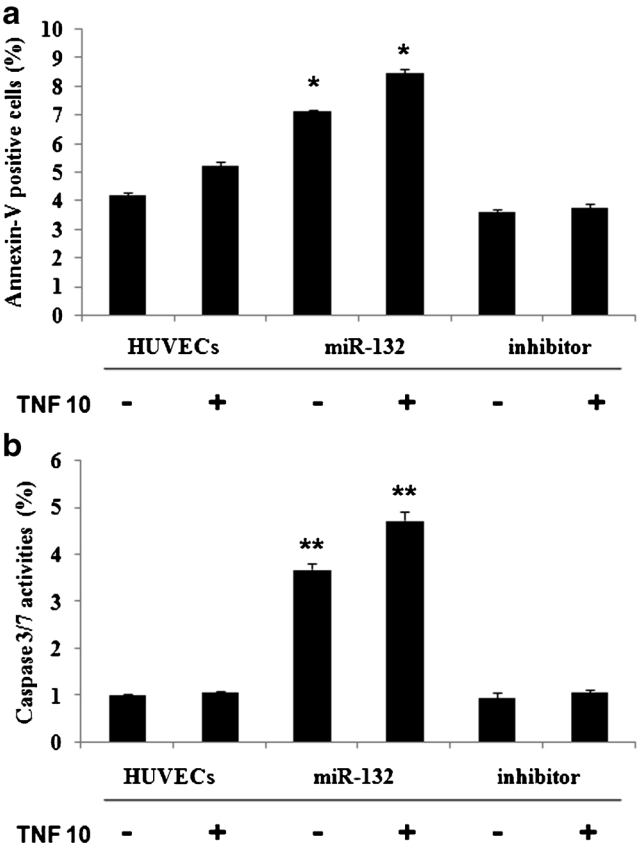

Fig. 7 MiR-132 induces apoptotic death of HUVECs. An Annexin V-FITC/7-AAD staining apoptotic assay and flow cytometry were performed in HUVECs. MiR-132 increased the apoptotic cell fractions (both early and late apoptotic cell fractions; $P<0.01$) compared with the control groups (**a**). Caspase 3/7 activities were significantly increased by miR-132 in HUVECs (**b**). Caspase 3/7 activities (fold) were assigned as 1.0 in non-treated cells. The data are presented as mean±SD from three independent experiments. *$P<0.05$, **$P<0.01$

Discussion

In this study, we identified one miRNA that plays an important role in the regulation of lipogenesis and cholesterogenesis in HUVECs. MiRNAs have been reported to regulate multiple important biological processes including metabolism [19] and are potentially of use in AS therapy [20]. However, how miRNAs mediate aberrant fat metabolism and homeostasis in HUVECs remains unclear. We found that miR-132 inhibited fatty acid and cholesterol biosynthesis through down-regulation of SIRT1 and key lipogenic or cholesterogenic transcription factors, SREBP-1c, and their downstream regulated genes including FASN and HMGCR. The inhibitor of miR-132 could induce a reversed phenomenon.

SREBP-1 controls genes involved in fatty acid, lipid and cholesterol biosynthesis [21–23], dysregulation of SREBPs and their downstream targeted genes associated with lipogenesis and cholesterogenesis has been implicated in cancer. Examples include FASN, a metabolic oncogene [24], and HMGCR, the rate-limiting step in cholesterol biosynthesis; both proteins have been reported to be involved in the development and progression of cancer [25, 26]. Since SREBP transcription factors have been shown to be disregulated both in endothelial dysfunction and metabolic disorders [27], targeting the aberrant SREBP-lipogenesis-cholesterogenesis pathway may lead to new approaches to the treatment of AS. Our results showed that SIRT1 mRNAs are direct targets of miR-132. MiR-132 controls lipogenesis and cholesterogenesis in HUVECs by inhibiting SIRT1 and SREBP-1c expression and downregulating their targeted genes, including FASN and HMGCR. Inhibition of SIRT1 by miR-132 was associated with lipid metabolism-dependent pro-inflammatory processes in HUVECs. The newly identified miRNA, miR-132 represents a novel targeting mechanism for AS therapy.

Accumulating evidence indicates that AS is the result of a prolonged and excessive inflammatory process in the vascular wall, which often begins with inflammatory changes in the

endothelium, characterized by the expression of SIRT1, SREBP-1c and their downstream molecules [28]. Pharmacological and dietary strategies have targeted these disorders to control AS, and some natural food products may be candidates for the control or prevention of AS [29].

TNFα may induce sICAM1 levels as well as the expression of ICAM1 and MMP9 in HUVECs. To determine whether pro-inflammatory processes could be promoted by miR-132 in HUVECs, we investigated cells which were pretreated with miR-132 or its inhibitor for 48 h, and then followed by treatment with or without TNFα. Our results showed that miR-132 induced sICAM1 level as well as the expression of ICAM1 and MMP9 in HUVECs . Moreover, the induction of sICAM1 as well as the expression of ICAM1 induced by miR-132 and/or TNFα could be prohibited significantly by the inhibitor of miR-132 .

To further test the specificity of miR-132 for SREBP-lipogenesis-cholesterogenesis, antisense oligonucleotides against miR-132 were used as miR-132 inhibitors. By blocking endogenous miR-132 in HUVECs with or without pro-inflammatory processes, miR-132 inhibitors increased SIRT1, SREBP-1c and their downstream gene expression. As shown in Fig. 5, the amounts of intracellular fatty acid and cholesterol were significantly decreased in miR-132-transfected HUVECs compared with the control groups. These results suggest that miR-132 inhibited pro-inflammatory processes related to SIRT1-SREBP signaling through reduction of SIRT1 expression and decreased the levels of fatty acid and cholesterol in HUVECs. Conversely, by blocking miR-132 in HUVECs, miR-132 inhibitors increased the amounts of intracellular fatty acid and cholesterol.

Zhang et al. have shown that endothelial SIRT1 is an anti-atherosclerosis factor [30.] The possible underlying mechanism may be related to inhibition of oxLDL-induced apoptosis, upregulation of eNOS expression, and improvement of endothelial relaxation. Together with recent studies revealing that SIRT1 mediates the life span effect of calorie restriction and that calorie restriction may decrease atherosclerosis [31–34], this suggest that SIRT1 may, although as yet speculative, be an underlying mediator of calorie restriction's atheroprotective effect and that SIRT1 may be a useful target for atherosclerosis prevention and treatment [30]. In our study, we found that miR-132 controls lipogenesis and cholesterogenesis in HUVECs by inhibiting SIRT1 and SREBP-1c expression and downregulating their targeted genes, including FASN and HMGCR. Inhibition of SIRT1 by miR-132 was associated with lipid metabolism-dependent pro-inflammatory processes in HUVECs.

Because of its documented ability to regulate cellular characteristics in an activity dependent manner [35], a role for miR132 in energy metabolism in HUVECs was investigated. MiR-132 reduced cell proliferation, viability and migration, and induced caspase-dependent apoptosis in HUVECs. These data suggest that miR-132 plays a role in promoting pro-inflammatory processes by inhibiting SIRT1 and SREBP-1c expression, and thereby reprogramming lipogenesis and cholesterogenesis.

In summary, our study demonstrates that miR-132 inhibits the expression of SIRT1 and SREBP-1c as well as their downstream regulated genes, and reprograms lipogenesis and cholesterogenesis in HUVECs. Additional studies are warranted to define the regulatory mechanisms involving miR-132 in HUVECs. Down-expression of miR-132 suppresses proliferation of HUVECs and induces apoptotic cell death in vitro. It suggests that miR-132 may promote atherosclerosis. Taken together, miR-132 directly or indirectly regulates a cohort of genes with significant biological roles in lipid and cholesterol anabolism and homeostasis, cell proliferation and apoptosis progression in HUVECs. This small, non-coding RNA therefore provides a potential therapeutic target for treatment of atherosclerosis.

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Conflict of Interest Statement All authors declare that there is no conflict of interest.

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