Int J Clin Exp Pathol 2016;9(3):3357-3364
www.ijcep.com /ISSN:1936-2625/IJCEP0020189

Original Article

miR-144 regulates the proliferation of human vascular endothelial cells through targeting SRF

Honglei Yin[1], Lihua Zhao[1], Junbiao Zhang[2], Yan Zhang[3]

[1]Department of Cardiology I, The First Affiliated Hospital of Xinxiang Medical University, Weihui 453100, Henan, China; [2]Department of Cardiology II, The First Affiliated Hospital of Xinxiang Medical University, Weihui 453100, Henan, China; [3]CCU (Coronary Heart Disease), The First Affiliated Hospital of Xinxiang Medical University, Weihui 453100, Henan, China

Received November 20, 2015; Accepted January 23, 2016; Epub March 1, 2016; Published March 15, 2016

Abstract: Atherosclerosis, which constitutes the single most important contributor to coronary artery disease (CAD), is the leading cause of death and disability worldwide. MiR-144 has been well investigated in many human diseases, while its role in atherosclerosis remains obscure. Here, we found that miR-144 is significantly up-regulated in the plasma of CAD patients by qRT-PCR. In addition, we identified serum response factor (SRF) as a potential target of miR-144 in Human umbilical vein endothelial cells (HUVECs) by luciferase reporter assay and western blot. Results from MTT assays and flow cytometry showed that overexpression of miR-144 significantly inhibited cell proliferation, increased apoptosis and induced more cells arrest in G1/S phase in HUVEC cells. While downregulation of miR-144 in HUVEC cells showed an adverse effect. Moreover, we found that restoration of SRF partially attenuated the inhibitory effect of miR-144 on HUVEC cells proliferation. In conclusion, our study demonstrated that miR-144 regulates the proliferation of human vascular endothelial cells through targeting SRF and suggested that miR-144 might be a helpful therapeutic target for atherosclerosis treatment in the future.

Keywords: miR-144, SRF, atherosclerosis, proliferation, apoptosis

Introduction

Atherosclerosis, which constitutes the single most important contributor to coronary artery disease (CAD), is a chronic disease characterized by the accumulation of lipids and fibrous elements in the large arteries [1]. As the leading cause of death and disability worldwide, atherosclerosis accounts for approximately 29% of all mortalities [2]. Vascular endothelium stand as the first barrier to inflammation and thrombosis, the proliferation of vascular endothelial cells plays a very important role in the initiation and progress of atherosclerosis [3]. Despite the continuous advances of diagnosis and treatment on atherosclerosis over the past decades, it still remains one of the biggest threats to human health. Therefore, identifying some novel molecules involved in the proliferation of vascular endothelial cells may be helpful to improve the diagnosis and treatment of patients with atherosclerosis.

MicroRNAs (miRNAs) are a large group of non-coding RNAs containing 18-25 nucleotides and post-transcriptionally regulate the expression of multiple genes by interacting with their 3'-untranslated regions [4]. MiR-144 was originally identified as an erythroid lineage-specific microRNA, which is necessary for subsequent survival and maturation of the erythroid lineage [5]. In recent years, the biological function of miR-144 has been well investigated. For example, Cao et al. demonstrated that forced overexpression of miR-144 remarkably reduced cell proliferation, increased apoptosis, and suppressed migration and invasion of HCC cells partially by targeting E2F3 [6]. Chen et al. showed that miR-144 inhibited proliferation, enhanced apoptosis, and increased autophagy in lung cancer cells by targeted TIGAR [7]. Wang et al. reported that miR-144 was markedly downregulated in osteosarcoma cell lines and clinical specimens. Functional studies indicated that miR-144 suppresses tumor cell proliferation and metastasis in vitro as well as in vivo by directly downregulating ROCK1 and ROCK2 expression [8]. Unfortunately, the biological significance of miR-144 in the development of atherosclerosis has not yet been studied.

Serum response factor (SRF) is a member of the highly conserved MADS (refers to four members: MCM1, AGAMOUS, DEFICIENS and SRF) box family of transcription factors, which is critical in cell proliferation and cell cycle regulation [9]. SRF derives its name from the ability to bind to a serum response element (SRE) and influence gene expression [10]. A previous study reported that SRF is a downstream mediator of VEGF signaling in endothelial cells and a critical requirement for VEGF-induced angiogenesis [11]. Werth et al. showed that SRF is an essential regulator of primary human vascular smooth muscle cells proliferation and SRF knockdown caused a cell-cycle arrest in G1 phase [12]. Moreover, recent studies identified the role of SRF in the development of several types of human cancers such as esophageal squamous cell carcinoma [13], prostate cancer [14] and hepatocellular carcinoma [15], suggesting the pivotal role of SRF in cell proliferation.

In this study, we first estimated the serum levels of miR-144 and SRF in CAD patients and healthy controls. Next, we demonstrated that SRF is a candidate target of miR-144 in vascular endothelial cells. In addition, we evaluated the biological functions of miR-144 on human vascular endothelial cells and its possible mechanisms. Our findings revealed the regulatory effect of miR-144 on the proliferation of human vascular endothelial cells and the potential application of miR-144 as a candidate target in the therapy of atherosclerosis.

Materials and methods

Plasma samples

28 coronary artery disease (CAD) patients and the same amount of matched (gender and age) healthy controls (HC) were recruited to our study from September 2013 to May 2014. All subjects had undergone coronary angiography for evaluating coronary artery stenosis. Inclusion criteria for CAD patients were ≥50% narrowing of the lumina of at least one major coronary arteries and individuals without any coronary stenosis were defined as healthy controls. All blood samples were collected via venous puncture. The plasma was isolated by centrifugation, then transferred to RNase-free tubes and stored at -80°C until use. Written informed consents were obtained from all CAD patients and healthy controls. This study was performed in accordance to the declaration of Helsinki and was approved by the ethics committee of our hospital.

Cell culture and transfection

Human umbilical vein endothelial cells (HUVECs) were obtained from The American Type Culture Collection (ATCC). Cells were maintained in complete RPMI 1640 media containing 10% fetal bovine serum, 100 U/mL of penicillin and 100 μg/ml of streptomycin (all from Invitrogen, USA) in a humidified incubator at 37°C with 5% CO_2. For cell transfection, miR-144 mimics (miR-144), negative controls (miR-NC), miR-144 inhibitors (anti-miR-144) and its negative controls (anti-miR-con) were synthesized by Genepharma (China). The whole cDNA sequence of human SRF gene was amplified by polymerase chain reaction (PCR) and then cloned into pcDNA3.1 (+) vector to construct a SRF expression plasmid (pcDNA3.1-SRF). The transfections were performed with Lipofectamine 2000 (Invitrogen) according to the manufacture's manual.

RNA extraction and quantitative real-time PCR

Total RNA was extracted from plasma samples and transfected cells using TRIzol reagent (Invitrogen, USA) according to the manufacturer's instruction. The concentration of the RNA samples was measured with a NanoDrop 1000 spectrophotometer (NanoDrop Technologies, Waltham, USA) and then cDNA was synthesized by reverse transcription. Quantitative real-time PCR (qRT-PCR) was performed on ABI 7500 with SYBR Green detection (Applied Biosystems) according to the standard protocol. The qRT-PCR conditions were 95°C for 3 min and 40 cycles of 95°C for 12 s, and 62°C for 1 min. Quantitative PCR of miRNAs was performed using Taq-Man assays (Applied Biosystems, Foster City, USA). The primers were as follows: 5'-GCTGGGATATCATCATATACTG-3' (forward) and 5'-CGGACTAGTACATCATCTATACTG-3' (reverse) for miR-144; 5'-CTGCCTCAACTCGCCAGAC-3' (forward) and 5'-TCAGATTCCGACACCTGGTAG-3' (reverse) for SRF. U6 and β-actin were used as endogenous control for miRNAs and mRNAs, respectively. The relative quantitation of gene expression levels were determined by $2^{-\Delta\Delta Ct}$ method. Each sample was analyzed in triplicate.

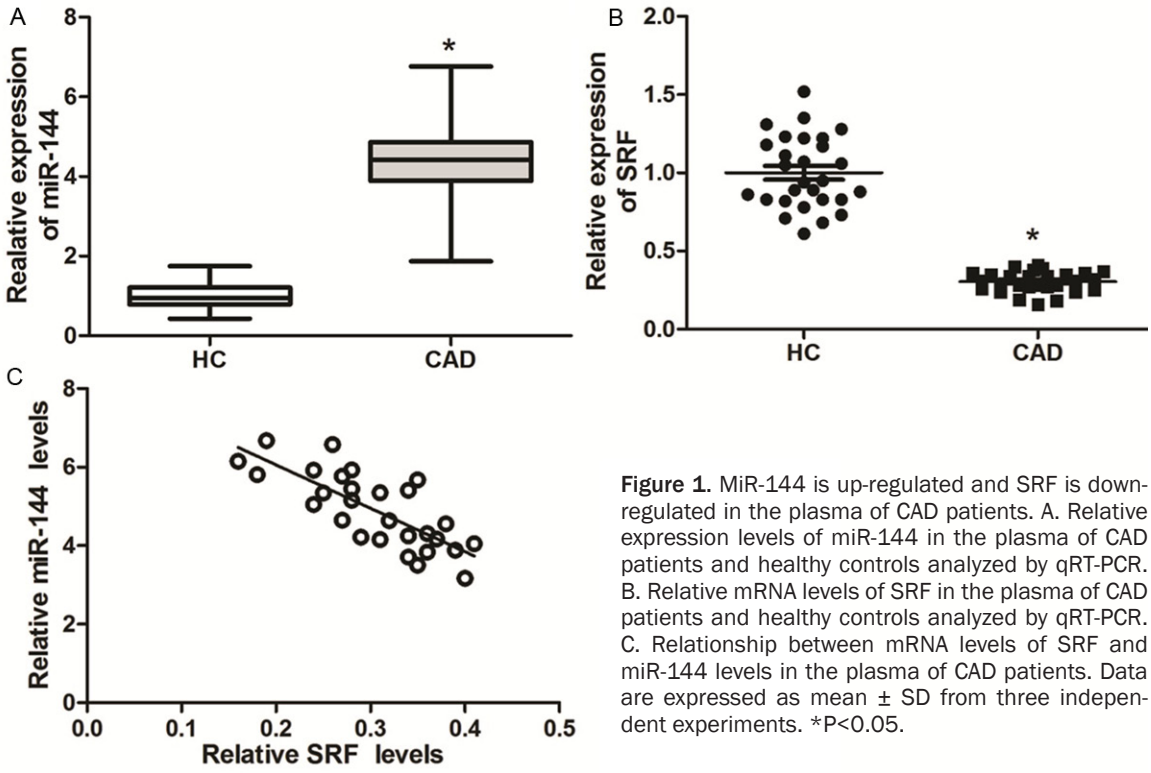

Figure 1. MiR-144 is up-regulated and SRF is down-regulated in the plasma of CAD patients. A. Relative expression levels of miR-144 in the plasma of CAD patients and healthy controls analyzed by qRT-PCR. B. Relative mRNA levels of SRF in the plasma of CAD patients and healthy controls analyzed by qRT-PCR. C. Relationship between mRNA levels of SRF and miR-144 levels in the plasma of CAD patients. Data are expressed as mean ± SD from three independent experiments. *P<0.05.

Luciferase reporter assay

For luciferase reporter assay, The 3'-UTR of SRF containing the potential binding sites of miR-144 or the mutant SRF 3'-UTR was cloned into downstream Renilla luciferase open reading frame of the pMIR miRNA Expression Reporter Vector (Ambion, ABI). HUVEC cells were plated into 24-well plate and then co-transfected with 100 nM of miR-144 mimics or negative controls and wild type (WT) or Mutant (Mut) 3'-UTR of SRF using Lipofectamine 2000 reagent (Invitrogen). HUVEC cells were harvested 48 h after transfection and the relative luciferase activity was evaluated using Dual-Luciferase Reporter System (Promega, Wisconsin, WI, USA) following the manufacture's manual. This experiment was repeated at least three times.

Western blot analysis

Total protein from HUVEC cells were extracted 48 h post-transfection by using RIPA buffer supplemented with proteinase inhibitor (YTHX Biotechnology, China) and protein concentrations were determined using the BCA method (Pierce, Rockford, USA). Then, proteins samples were electrophoresed by SDS-PAGE and transferred onto PVDF membranes (Millipore, USA). After blocking with 5% non-fat milk for 1 h at room temperature, membranes were incubated with primary antibody against SRF or β-actin (Abcam, USA) at 4°C overnight. After washing for three times, HRP conjugated secondary antibody (Santa Cruz, USA) was added and incubated for another 1 h at room temperature. Finally, the protein bands were visualized using ECL system (Pierce, Rockford, USA). Three independent experiments were performed.

MTT assay

Cell proliferation of transfected HUVEC cells was measured by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay according to the manufacturer's protocol. Briefly, the transfected cells were maintained in RPMI 1640 medium with 10% fetal bovine serum at 37°C with 5% CO_2. After incubation for 24, 48, 72 and 96 h, respectively, 20 μl MTT reagent (5 mg/ml, Sigma) was added to each well and cells were further incubated for 4 h. Then the formazan precipitate was dissolved in 150 μL of DMSO (Sigma) and the absorbance values were measured with a microplate reader (Thermo Scientific, USA) at wavelength of 450

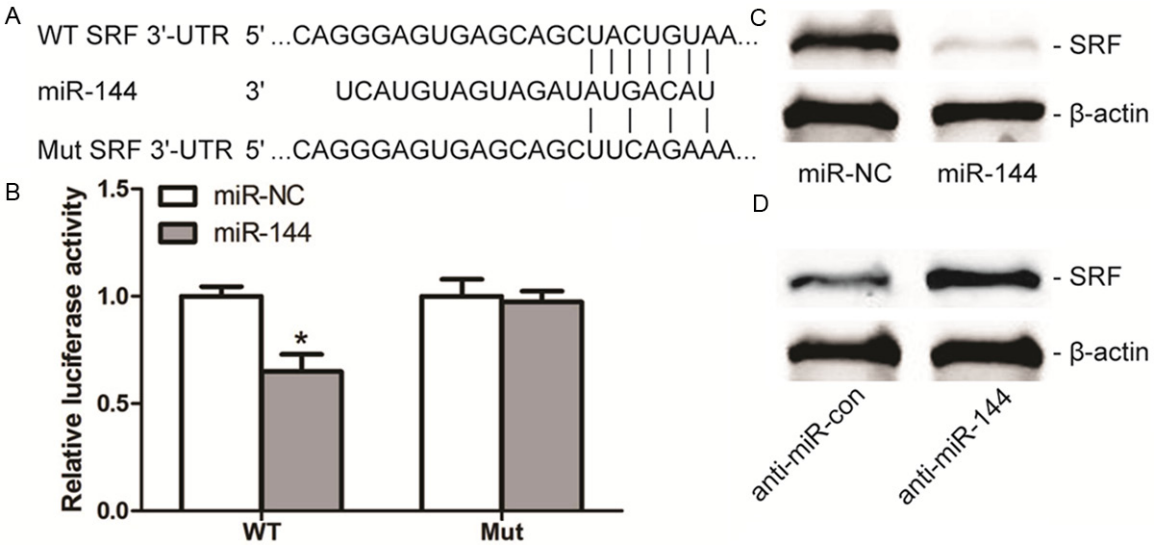

Figure 2. SRF is a direct target of miR-144 in HUVEC cells. A. The potential miR-144 targeting site in SRF 3'-UTR and the mutated sequences. B. The relative luciferase activity was measured in HUVEC cells after co-transfected with miR-144 or miR-NC with wild-type (WT) or mutated (Mut) 3'-UTR of SRF. C. The protein levels of SRF in HUVEC cells after tranfected with miR-144 mimics (miR-144) or its negative control (miR-NC). D. The protein levels of SRF in HUVEC cells after tranfected with miR-144 inhibitor (anti-miR-144) or its negative control (anti-miR-con). Data are expressed as mean ± SD from three independent experiments. *P<0.05.

nm. Each experiment was repeated in triplicate at least.

Flow cytometry

After transfection for 48 h, cells (1×105) were harvested and wished twice with cold PBS. Then, cells were incubated in PBS with 1 μg/ml Annexin V-FITC and 1 μg/ml propidium iodide (PI) at room temperature in dark for 15 minutes. Apoptotic cells were assessed by flow cytometric analysis using an Annexin V-FITC Apoptosis Detection kit (BD Biosciences) according to the manufacturer's guideline. For cell cycle analysis, the harvested cells were washed two times with PBS and fixed in cold 70% ethanol at 4°C overnight. Next, cells were re-suspended and incubated with 0.1 mg/ml RNase A and 0.05 mg/ml propidium iodide (PI) for 30 min at 4°C. After that, cell cycle was then analyzed by FACS Calibur flow cytometer (BD Bioscience). All assays were conducted in triplicate.

Statistical analysis

All data were expressed as mean ± standard deviation (SD) from at least three independent experiments. SPSS 17.0 statistical software (Chicago, IL) was used for statistical analysis. The differences between groups were analyzed using Student's t-test when comparing only two groups or one-way analysis of variance when comparing more than two groups. P<0.05 was considered statistically significant.

Results

MiR-144 is up-regulated and SRF is down-regulated in the plasma of CAD patients

28 CAD patients and the same amount of matched healthy controls were enrolled into our study to evaluate the expression levels of miR-144 and SRF in their plasma. qRT-PCR was performed on all collected plasma samples. We found that miR-144 expressions were significantly up-regulated in the plasma of CAD patients compared with healthy controls (**Figure 1A**). However, the expression levels of SRF in the plasma of CAD patients were significantly down-regulated when compared to that in healthy controls (**Figure 1B**). Besides, the expressions of SRF were inversely correlated with the levels of miR-144 in the plasma of CAD patients (**Figure 1C**), indicating a regulatory correlation between SRF and miR-144.

SRF is a direct target of miR-144 in HUVEC cells

Based on the miR target analysis results from the websites targetscan, PicTar and miRanda,

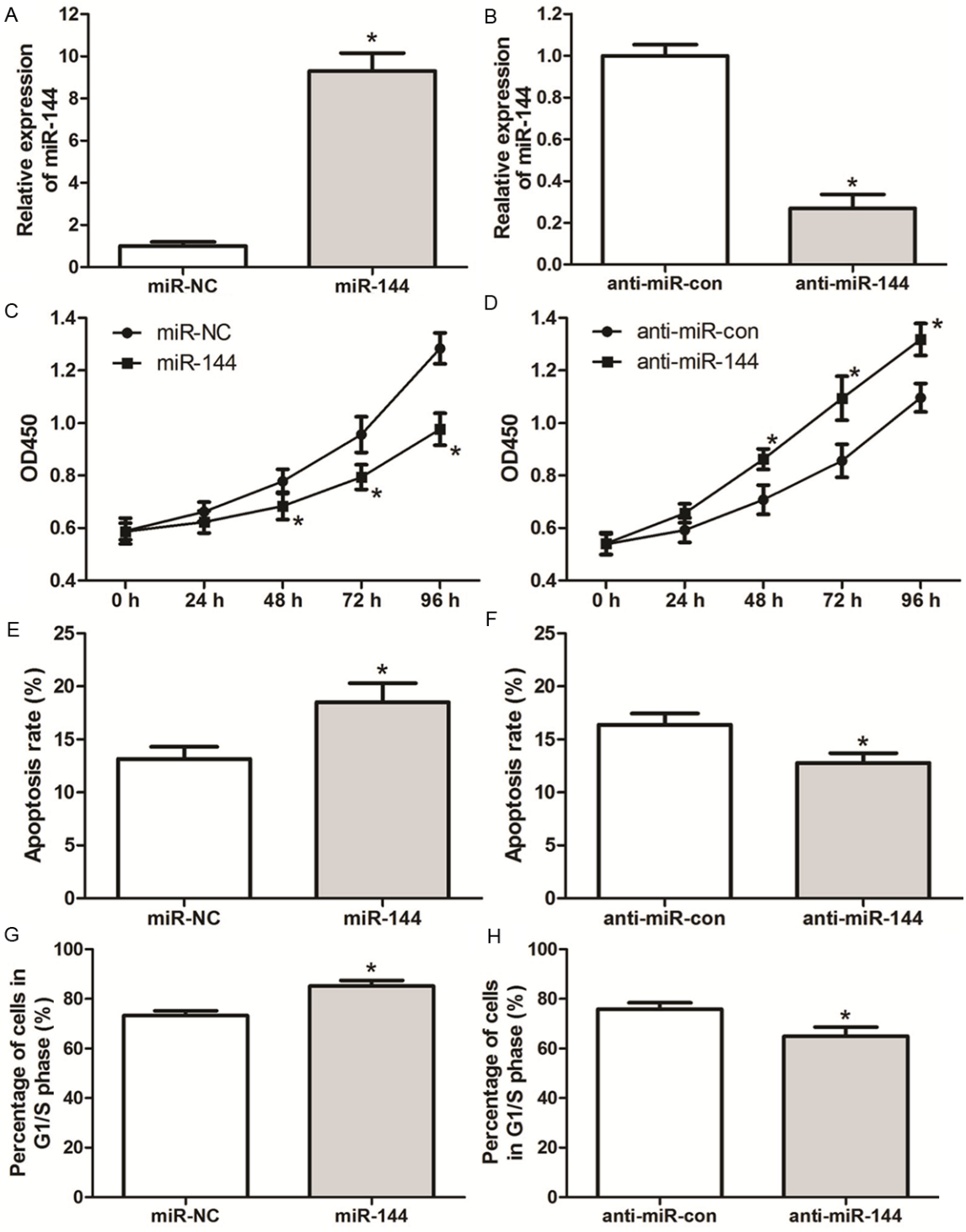

Figure 3. MiR-144 regulates the proliferation of HUVEC cells. A, B. Relative expression levels of miR-144 in HUVEC cells transfected with miR-144 mimics (miR-144) or its negative control (miR-NC). C, D. Cell proliferation of transfected HUVEC cells determined by MTT assays. E, F. Cell apoptosis rate of transfected HUVEC cells measured by flow cytometry. G, H. Percentages of transfected HUVEC cells arrest in G1/S phase measured by flow cytometry. Data are expressed as mean ± SD from three independent experiments. *P<0.05.

SRF has a putative miR-144 binding site in its 3'-UTR and was identified as a potential target of miR-144 (**Figure 2A**). To verify it, we conducted luciferase report assay and western blot. Data from luciferase report assay showed that the luciferase activity of wild type (WT) SRF

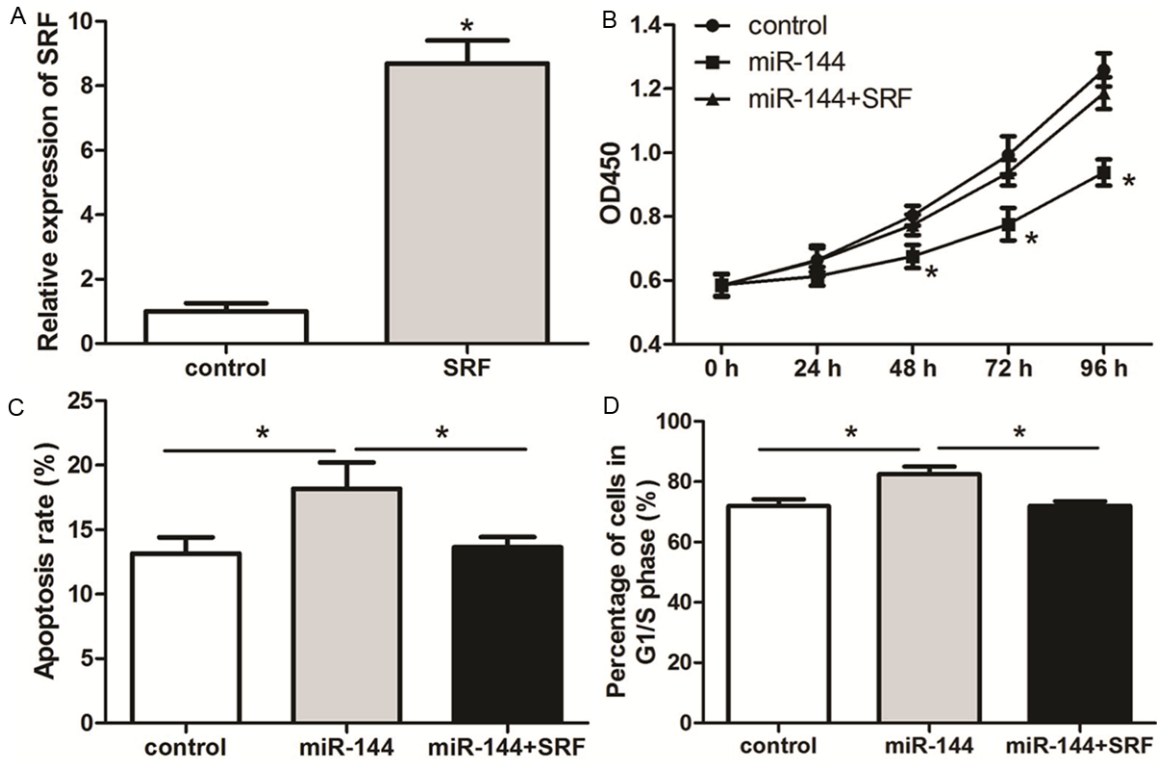

Figure 4. SRF overexpression partially attenuated the inhibitory effect of miR-144 on HUVEC cells proliferation. A. Relative mRNA levels of SRF in HUVEC cells transfected with pcDNA3.1-SRF or the control vector examined by qRT-PCR. B. Cell proliferation of transfected HUVEC cells determined by MTT assays. C. Cell apoptosis rate of transfected HUVEC cells measured by flow cytometry. D. Percentages of transfected HUVEC cells arrest in G1/S phase measured by flow cytometry. Data are expressed as mean ± SD from three independent experiments. *P<0.05.

3'-UTR was significantly inhibited by miR-144 but the mutant (Mut) SRF 3'-UTR not (**Figure 2B**). In addition, results of western blot showed that the protein levels of SRF in HUVEC cells were markedly down-regulated when transfected with miR-144 mimics while up-regulated when transfected with miR-144 inhibitors (**Figure 2C**, **2D**). These results suggest that SRF is a direct target of miR-144 in HUVEC cells.

MiR-144 regulates the proliferation of HUVEC cells

To investigate the biological effects of miR-144 on the proliferation of HUVEC cells, cells were transfected with miR-144 mimics (miR-144), miR-144 inhibitors (anti-miR-144) or their negative controls. The efficiency of transfection was confirmed by qRT-PCR (**Figure 3A**, **3B**). Then, we conducted MTT assays on treansfected cells and the results showed that the proliferation of HUVEC cells was significantly inhibited when transfected with miR-144 mimics while promoted when transfected with miR-144 inhibitors (**Figure 3C**, **3D**). We speculated that the regulatory effects of miR-144 on the proliferation of HUVEC cells may correlate with cell apoptosis or cell cycle distribution, and flow cytometry was performed to test it. Our results showed that overexpression of miR-144 in HUVEC cells resulted in increased apoptotic cells and more cells arrest in G1/S phase (**Figure 3E**, **3G**). On the contrary, downregulation of miR-144 resulted in decreased apoptotic cells and fewer cells arrest in G1/S phase (**Figure 3F**, **3H**). These data collectively indicate the regulatory effects of miR-144 on the proliferation of HUVEC cells.

SRF overexpression partially attenuated the inhibitory effect of miR-144 on HUVEC cells proliferation

After co-transfected with miR-144 mimics and pcDNA3.1-SRF (**Figure 4A**), HUVEC cells were performed with MTT assays and flow cytometry. Data from MTT assays revealed that SRF overexpression partially attenuated the inhibi-

tory effect of miR-144 on HUVEC cells proliferation (**Figure 4B**). Consistently, the effects of miR-144 mimics on HUVEC cells apoptosis and cell cycle distribution were also partially attenuated by SRF overexpression (**Figure 4C**, **4D**). These data suggest that miR-144 may play its role in HUVEC cells by partially regulate the expression of SRF.

Discussion

The endothelium is a dynamic endocrine organ which plays a critical role in vascular homeostasis by keeping a delicate balance between vasodilation and vasoconstriction [16]. In the initial stage of atherosclerosis, the dysfunctional endothelium produces proinflammatory cytokines and subsequently promoted the differentiation of circulating monocytes into macrophages [17]. Nevertheless, the balance of endothelial cells proliferation contribute to the integrity of endothelium and evidences suggest that dysfunction of endothelial cells is the earliest manifestation of atherosclerosis even without angiographic evidence [18]. Therefore, to clarify the factors regulating endothelial cells proliferation and its mechanisms in atherosclerosis is very necessary for further understanding of its initiation and progression.

In recent years, the crucial role of microRNAs in the development of atherosclerosis has been well studied. Several miRNAs have been shown to regulate the function of endothelial cells by negatively controlling the expression of their target genes, thereby involving in the initiation and progression of atherosclerosis. For example, Wu et al. found that miR-152 could inhibit HUVEC cells proliferation and migration by targeting ADAM17, indicating that miR-152 may be involved in the development of atherosclerosis [19]. Schober et al. demonstrated that miR-126-5p promotes endothelial cells proliferation and limits atherosclerosis by suppressing the expression of Dlk1, introducing a potential therapeutic approach [20]. Zhang et al. reported that miR-26a inhibits endothelial cells apoptosis by directly targeting TRPC6 and its abnormal expression is correlated with the development of atherosclerosis [21].

In our study, we firstly analyzed the plasma levels of miR-144 and SRF in CAD patients and healthy controls. We found that miR-144 is significantly up-regulated and SRF is significantly down-regulated in the plasma of CAD patients when compared with that in healthy controls. In addition, we found that the mRNA levels of SRF were inversely correlated with miR-144 expressions in the plasma of CAD patients. In this respect, we supposed that miR-144 may play its regulatory role by suppressing the expression of SRF during the development of CAD. Then, results from target prediction indicated that SRF is a potential target of miR-144. To confirmed that, luciferase report assay and western blot were performed. Data showed that the luciferase activity of SRF 3'-UTR and the protein levels of SRF were negatively regulated by miR-144. These suggest that miR-144 may play a critical role in the development and progression of CAD.

To further explore the biological effect of miR-144 on HUVEC cells, we transfected cells with miR-144 mimics, inhibitors or their negative controls, respectively. Then, MTT assays and flow cytometry were performed on transfected cells. Results showed that overexpression of miR-144 significantly inhibited the proliferation, increased apoptosis and induced more cells arrest in G1/S phase in HUVEC cells. While downregulation of miR-144 showed an adverse effect on HUVEC cells. Furthermore, we examined whether restoration of SRF could whittle the inhibitory effect of miR-144 on HUVEC cells proliferation. Data from our experiments showed that SRF overexpression partially attenuated the inhibitory effect of miR-144 on HUVEC cells proliferation. These results reveal that miR-144 regulates HUVEC cells proliferation through directly targeting SRF.

In conclusion, our study demonstrated for the first time that miR-144 is up-regulated in CAD patients and uncovered the inhibitory effect of miR-144 on HUVEC cells proliferation through directly targeting SRF. This finding provided new insights into the molecular mechanism of atherosclerosis progression and suggested that miR-144 might be a helpful therapeutic target for atherosclerosis treatment in the future.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Honglei Yin, Department of Cardiology I, The First Affiliated Hospital of Xinxiang Medical University, Weihui 453100, China. E-mail: honleiy1981@gmail.com

References

[1] Xu Z, Han Y, Liu J, Jiang F, Hu H, Wang Y, Liu Q, Gong Y and Li X. MiR-135b-5p and MiR-499a-3p Promote Cell Proliferation and Migration in Atherosclerosis by Directly Targeting MEF2C. Sci Rep 2015; 5: 12276.

[2] Li S, Fan Q, He S, Tang T, Liao Y and Xie J. MicroRNA-21 Negatively Regulates Treg Cells Through a TGF-beta1/Smad-Independent Pathway in Patients with Coronary Heart Disease. Cell Physiol Biochem 2015; 37: 866-878.

[3] Zhang W, Yan L, Li Y, Chen W, Hu N, Wang H and Ou H. Roles of miRNA-24 in regulating endothelial nitric oxide synthase expression and vascular endothelial cell proliferation. Mol Cell Biochem 2015; 405: 281-289.

[4] Yu LD, Jin RL, Gu PC, Ling ZH, Lin XJ and Du JY. Clinical significance of microRNA-130b in osteosarcoma and in cell growth and invasion. Asian Pac J Trop Med 2015; 8: 752-756.

[5] Fu YF, Du TT, Dong M, Zhu KY, Jing CB, Zhang Y, Wang L, Fan HB, Chen Y, Jin Y, Yue GP, Chen SJ, Chen Z, Huang QH, Jing Q, Deng M and Liu TX. Mir-144 selectively regulates embryonic alpha-hemoglobin synthesis during primitive erythropoiesis. Blood 2009; 113: 1340-1349.

[6] Cao T, Li H, Hu Y, Ma D and Cai X. miR-144 suppresses the proliferation and metastasis of hepatocellular carcinoma by targeting E2F3. Tumour Biol 2014; 35: 10759-10764.

[7] Chen S, Li P, Li J, Wang Y, Du Y, Chen X, Zang W, Wang H, Chu H, Zhao G and Zhang G. MiR-144 inhibits proliferation and induces apoptosis and autophagy in lung cancer cells by targeting TIGAR. Cell Physiol Biochem 2015; 35: 997-1007.

[8] Wang W, Zhou X and Wei M. MicroRNA-144 suppresses osteosarcoma growth and metastasis by targeting ROCK1 and ROCK2. Oncotarget 2015; 6: 10297-10308.

[9] Kaplan-Albuquerque N, Van Putten V, Weiser-Evans MC and Nemenoff RA. Depletion of serum response factor by RNA interference mimics the mitogenic effects of platelet derived growth factor-BB in vascular smooth muscle cells. Circ Res 2005; 97: 427-433.

[10] Kawakami-Schulz SV, Verdoni AM, Sattler SG, Jessen E, Kao WW, Ikeda A and Ikeda S. Serum response factor: positive and negative regulation of an epithelial gene expression network in the destrin mutant cornea. Physiol Genomics 2014; 46: 277-289.

[11] Chai J, Jones MK and Tarnawski AS. Serum response factor is a critical requirement for VEGF signaling in endothelial cells and VEGF-induced angiogenesis. FASEB J 2004; 18: 1264-1266.

[12] Werth D, Grassi G, Konjer N, Dapas B, Farra R, Giansante C, Kandolf R, Guarnieri G, Nordheim A and Heidenreich O. Proliferation of human primary vascular smooth muscle cells depends on serum response factor. Eur J Cell Biol 2010; 89: 216-224.

[13] He X, Xu H, Zhao M and Wang S. Serum response factor is overexpressed in esophageal squamous cell carcinoma and promotes Eca-109 cell proliferation and invasion. Oncol Lett 2013; 5: 819-824.

[14] Verone AR, Duncan K, Godoy A, Yadav N, Bakin A, Koochekpour S, Jin JP and Heemers HV. Androgen-responsive serum response factor target genes regulate prostate cancer cell migration. Carcinogenesis 2013; 34: 1737-1746.

[15] Ohrnberger S, Thavamani A, Braeuning A, Lipka DB, Kirilov M, Geffers R, Autenrieth SE, Romer M, Zell A, Bonin M, Schwarz M, Schutz G, Schirmacher P, Plass C, Longerich T and Nordheim A. Dysregulated serum response factor triggers formation of hepatocellular carcinoma. Hepatology 2015; 61: 979-989.

[16] Kasprzak JD, Klosinska M and Drozdz J. Clinical aspects of assessment of endothelial function. Pharmacol Rep 2006; 58 Suppl: 33-40.

[17] Choi BJ, Matsuo Y, Aoki T, Kwon TG, Prasad A, Gulati R, Lennon RJ, Lerman LO and Lerman A. Coronary endothelial dysfunction is associated with inflammation and vasa vasorum proliferation in patients with early atherosclerosis. Arterioscler Thromb Vasc Biol 2014; 34: 2473-2477.

[18] Egashira K. Clinical importance of endothelial function in arteriosclerosis and ischemic heart disease. Circ J 2002; 66: 529-533.

[19] Wu Y, Huang A, Li T, Su X, Ding H, Li H, Qin X, Hou L, Zhao Q, Ge X, Fang T, Wang R, Gao C, Li J and Shao N. MiR-152 reduces human umbilical vein endothelial cell proliferation and migration by targeting ADAM17. FEBS Lett 2014; 588: 2063-2069.

[20] Schober A, Nazari-Jahantigh M, Wei Y, Bidzhekov K, Gremse F, Grommes J, Megens RT, Heyll K, Noels H, Hristov M, Wang S, Kiessling F, Olson EN and Weber C. MicroRNA-126-5p promotes endothelial proliferation and limits atherosclerosis by suppressing Dlk1. Nat Med 2014; 20: 368-376.

[21] Zhang Y, Qin W, Zhang L, Wu X, Du N, Hu Y, Li X, Shen N, Xiao D, Zhang H, Li Z, Zhang Y, Yang H, Gao F, Du Z, Xu C and Yang B. MicroRNA-26a prevents endothelial cell apoptosis by directly targeting TRPC6 in the setting of atherosclerosis. Sci Rep 2015; 5: 9401.