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microRNA-181a is upregulated in human atherosclerosis plaques and involves in the oxidative stress-induced endothelial cell dysfunction through direct targeting Bcl-2

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Abstract. – OBJECTIVE: Atherosclerosis is featured as artery wall thickness as a result of invasion and accumulation of white blood cells and proliferation of intimal smooth muscle cells. Endothelial dysfunction has been linked to a variety of vascular diseases, including atherosclerosis. MicroRNAs play essential roles during the atherosclerotic plaques formation. In this study, we investigate the roles of miR-181a in the oxidative stress-induced endothelial cells dysfunction.

MATERIALS AND METHODS: The expressions of miR-181a were compared between human atherosclerotic plaques and normal blood vessels. The Bcl-2 protein expression was measured by Western blot and mRNA expression was measured by qRT-PCR. HUVECs were transiently transfected with pre-miR-181a or control microRNAs by Lipofectamine 2000. The viability of HUVECs in response to H_2O_2 was measured by MTT assay.

RESULTS: We report miR-181a is upregulated in human atherosclerotic plaques compared with the normal blood vessel. The miR-181a is induced by H_2O_2 treatments. The exogenous overexpression of miR-181a accelerates the apoptosis rates of HUVECs in response to H_2O_2. We identify Bcl-2 as a direct target of miR-181a. Also, we observed H_2O_2 treatments inhibited Bcl-2 expressions at both protein and mRNA levels. Inhibition of miR-181a restores Bcl-2 expressions, leading to increased resistance to H_2O_2. Moreover, restoration of Bcl-2 in miR-181a-overexpressing HUVECs renders cells tolerate higher concentrations of H_2O_2. Finally, a reverse correlation between miR-181a and Bcl-2 expression in human atherosclerosis plaques is illustrated.

CONCLUSIONS: Our results revealed an essential role of miR-181a in the development of atherosclerosis through the regulation of the endothelial dysfunction, providing mechanisms for the development of new antioxidant drugs for the treatment of atherosclerosis.

Key Words:
micorRNA-181a, Bcl-2, Human atherosclerosis plaques, Oxidative stress.

Introduction

MicroRNAs (miRNAs) are 21-23 nucleotide small non-coding RNAs that modulate the stability of their target messenger RNAs (mRNA) through the post-transcriptional suppression[1,2]. Therefore, identification of miRNA targets is critical to elucidate the biological functions of miRNAs. In addition, it has been reported that miRNAs involve in multiple biological processes, including differentiation, proliferation, migration and apoptosis[3,4]. MiR-181 family members contain four highly conserved mature miRNAs: miR-181a, -181b, -181c and -181d, locating on three different chromosomes[5]. Evidence indicates that miR-181s are aberrantly expressed in tumor tissues and play essential roles for oncogenic processes[6]. Moreover, other miRNAs have been described that they possess important roles in the regulation of endothelial function such as miR-146a, which could prevent endothelial cells (EC) senescence[7], or miR-217 and miR-34a, which could promote EC senescence[8,9].

Endothelium lines the interior of blood vessels and exhibits complex functions in the vascular system to mediate blood vessel tone, hemostasis, neutrophil recruitment, hormone trafficking, and fluid filtration[10,11]. Consequently, endothelial dysfunction has been linked to a variety of vascular diseases, including atherosclerosis, diabetes mellitus, and hypercholesterolemia[12]. It has been known that oxidative stress caused by reactive oxygen species plays a pivotal role in controlling the endothelial function in the cardiovascular system[13], suggesting targeting the oxidative stress-induced endothelial dysfunction might contribute to the mitigation of these vascular diseases.

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Bcl-2 family, which consists about 20 pro- and anti-apoptotic proteins plays essential roles in the regulation of apoptosis via the mitochondrial pathway[14]. Bcl-2 protein is one of the family members and specifically considered an important anti-apoptotic protein[15]. Previous *in vivo* experiments[16,17] demonstrated that mice lacking macrophage Bcl-2 acquired advanced atherosclerotic lesions resulting from the increased apoptosis, indicating downregulation of Bcl-2 contributes to the formation of atherosclerotic lesions.

In this study, we explore the miRNA-modulated sensitivity of human endothelial cells to oxidative stress. By identification of the direct target of miR-181a, we investigate the functions of miR-181a in atherosclerosis.

Materials and Methods

Cell Culture and Atherosclerotic Plaque Tissue Collection

The primary HUVECs were purchased from Lonza (Shanghai, China) and were maintained in a humidified incubator containing 5% CO_2 at 37°C. HUVECs were cultured in complete endothelial cell growth medium containing 2% FBS, which was purchased from Lonza (Shanghai, China). HUVECs were used between passage 3 and 5. Human advanced atherosclerotic plaques were collected during carotid endarterectomy of patients from our hospital during 2012-2014. Total ten plaque tissues from different patients were analyzed in this study. Tissues were immediately stored at -80°C until processing for RNA isolation. Approval was obtained from the Ethical Committee at the Department of Internal Medicine, Tianjin Huanhu Hospital, Tianjin, China.

Reagents and Antibodies

Antibodies used for this project were purchased from: Rabbit monoclonal anti-Bcl-2 (human specific) (Cell Signaling, Danvers, MA, USA #4223); Mouse monoclonal anti-β-actin (Cell Signaling, Danvers, MA, USA #3700). H_2O_2 was purchased from Sigma-Aldrich Chemical Co. (Shanghai, China). Transfection agent (Lipofectamine 2000) was purchased from Invitrogen (Carlsbad, CA, USA).

Reverse Transcription-PCR and Real Time-PCR

Total RNA was harvested with the RNeasy mini kit (Qiagen, Inc., Valencia, CA, USA) according to the manufacturer's instructions. Briefly, lysates of cells were passed through a Qiashredder (Qiagen, Valencia, CA, USA) and the eluted lysates mixed 1:1 with 70% ethanol. The lysates were applied to a mini-column and after washing and DNAse I digestion, the RNAs were eluted in 30-50 μl of RNAse-free water. The quantity and quality of total RNA samples were checked by agarose-gel-electrophoresis and using the Bioanalyzer RNA 6000 Nano assay (Agilent, Waldbronn, Germany). cDNA synthesis was performed using a SuperScript First-Standard Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Quantitative PCR analyses were performed using Assay-on-Demand primers and the TaqMan Universal PCR Master Mix reagent (Applied Biosystems, Foster City, CA, USA). The primers for q-PCR were: Bcl-2: Forward: 5'-CTGCACCTGACGCCCTTCACC-3'; Reverse: 5'-CACATGACCCCACCGAACTCAAAGA-3' and β-actin: forward, 5'-CTGGCTCCTAGCACCATGAAGAT-3' and reverse 5'-GGTGGACAGTGAGGCCAGGAT-3'. The expression levels of β-actin were used to normalize the relative expression levels. All reactions were performed in triplicate. The relative amounts of mRNA were calculated by using the comparative $2^{-\Delta\Delta CT}$ method.

MicroRNA expressions were measured using the TaqMan microRNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA) and TaqMan microRNA assays kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's protocols.

miRNAs, siRNAs or Plasmid DNA Transfection

For overexpression of miRNAs, 100 nM of pre-miR-181a or negative control mock-miRNA (GenePharma, Beijing, China) was used. For knockdown of miRNAs, 100 nM of miR-181a inhibitor or negative control miRNA inhibitor (GenePharma, Beijing, China) was used. For siRNA transfection, 50 nM of siBcl-2 or control siRNA was used. Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) was used for the transfection of miRNAs or siRNA according to the manufactory's instruction. Cells were harvested 48 hours after transfection. Overexpression vector containing human Bcl-2 was obtained from Addgene (Cambridge, MA 02139, USA) and transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

Luciferase Assay

The luciferase assay was performed by transfection of the pMIR-reporter luciferase vector containing the wild-type 3'-UTR or 3'-UTR with binding site mutations of Bcl-2 and the empty vector constructed according to the methods previously described[17]. Cells at the density of $2x10^5$ per well in 24-well plates were co-transfected with pMIR-REPORT luciferase reporters (Thermofisher, Waltham, MA, USA) with 3'-UTR of wild type Bcl-2 or binding site mutant Bcl-2, pre-miR-181a using Lipofectamine 2000 reagent. Forty-eight hour later, cells were harvested and lysed with passive lysis buffer (Promega, Madison, WI, USA). Luciferase activity was measured by using a dual luciferase reporter assay (Promega, Madison, WI, USA). The pRL-TK vector (Promega, Madison, WI, USA) was used as an internal control. The results were expressed as relative luciferase activity (firefly Luc/Renilla Luc). Mutations in the miR-181a seed-matching sequences were made with the QuikChange Mutagenesis Kit (Stratagene, La Jolla, CA, USA).

Cell Viability Assay

Cell viability was measured by MTT assay. HUVECs treated with H_2O_2 were measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich Chemical Co., Shanghai, China) dye absorbance. In brief, $4x10^4$ cells/well were seeded in 96-well plates (Nunc) for MTT assays. After exposure to the indicated amounts of H_2O_2 for 24 h, 20 μl MTT (Sigma-Aldrich Chemical Co., Shanghai, China) solution (2 mg/ml in PBS) was added to each well of the 96-well plates. The plates were incubated for an additional 4 h at 37°C. Media in plates were withdrawn by pipetting, and 200 μl of DMSO was added to each well to solubilize the formazan crystals. The optical density was measured at 570 nm using a microplate reader (Synergy™ 2; BioTek Instruments Inc., Winooski, VT, USA).

Western Blot

Cells were harvested and lysed in buffer containing 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 2 mM EDTA, 1% Triton, 1 mM phenylmethylsulfonyl fluoride, and protease inhibitor mixture (Sigma-Aldrich Chemical Co., Shanghai, China) for 20 min on ice. Lysates were cleared by centrifugation at 14,000 rpm at 4°C for 10 min. Supernatants were collected, and protein concentrations were determined by the Bradford assay (Bio-Rad, Hercules, CA, USA). The proteins were then separated with a SDS/polyacrylamide gel and transferred to a nitrocellulose membrane (Bio-Rad, Hercules, CA, USA). The membranes were blocked by 5% milk for 1 hour at room temperature. Membranes were washed by TBST and incubated with primary antibody in 5% milk blocking buffer at a cold room for overnight. After the membranes have been incubated with horseradish peroxidase-linked secondary antibodies, blots were detected using an enhanced chemiluminescence (ECL) system.

Statistical Analysis

All continuous variables are expressed as means ± standard deviation (SD). Statistical significance was calculated by standard *t*-test. The correlation of miR-181a and Bcl-2 were evaluated by Pearson's product-moment correlation coefficient. A p-value of < 0.05 was considered significant. The GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA) was used for all statistical analyses.

Results

MiR-181a is Upregulated in Human Atherosclerotic plaques

MicroRNAs have been reported to participate in cardiovascular diseases and atherosclerosis[18]. In this study, we investigated the roles of miR-181a in atherosclerosis. To assess the clinical relevance of miR-181a in atherosclerosis, we analyzed the expressions of miR-181a in human atherosclerotic plaques compared with normal vessels by Taq man-based real-time quantitative reverse transcription-PCR (qRT-PCR). Using the median expression value of miR-181a as a cutoff point, we observed significantly upregulated expression of miR-181a in atherosclerotic plaques. The median expression level of miR-181a was 3-4 folds increased in atherosclerotic plaques, compared its expression in normal vessels (Figure 1), indicating the differentially expressed miR-181a might have important roles in atherosclerosis.

MiR-181a involves in the Oxidative Stress-induced endothelial Cell Dysfunction

A previous study[19] reported that H_2O_2 promoted apoptotic cell death of HUVECs. To characterize the roles of miR-181a in the H_2O_2-induced HUVECs cell death, we assessed whether non-

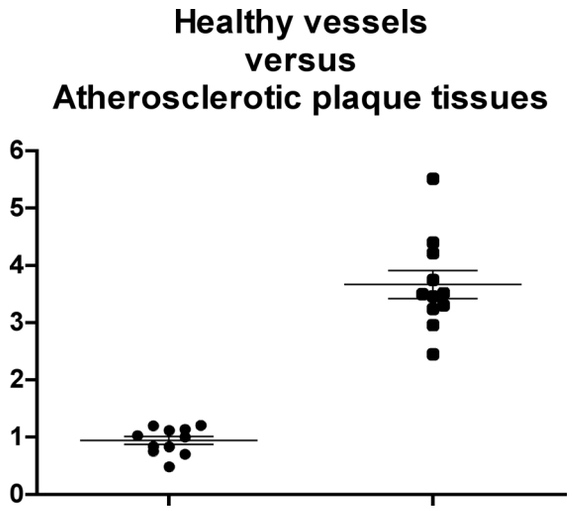

Figure 1. Differentially expressed miR-181a in healthy vessels versus atherosclerotic plaque tissues. Each group has 10 clinical samples. The expressions of miR-181a or Bcl-2 mRNA were measured by real-time quantitative.

toxic H_2O_2 treatments could regulate the expression of miR-181a. As we expected, miR-181a expressions were induced in response to H_2O_2 treatments at 40 and 80 μM for 24 hours (Figure 2A), leading us hypothesized miR-181a might regulate the oxidative stress-induced HUVEC cell death. To test this, we transfected miR-181a precursor or control microRNAs into HUVECs (Figure 2B) to compare the cell death rates under the treatments of H_2O_2. Our findings demonstrated that the exogenous overexpression of miR-181a markdly increased the sensitivity of HUVEC cells to H_2O_2 (Figure 2C, 2D). The IC50 of HUVECs in response to H_2O_2 was 328 μM. However, overexpression of miR-181a decreased the IC50 to 215 μM (Figure 2C), suggesting miR-181a possesses important functions in the endothelial dysfunction during the oxidative stress induced atherosclerosis.

BCL-2 is a Direct Target of miR-181a in Endothelial cells

To explore the putative mechanisms for the miR-181a modulated sensitivity to H_2O_2, we searched microRNAs databases for potential tar-

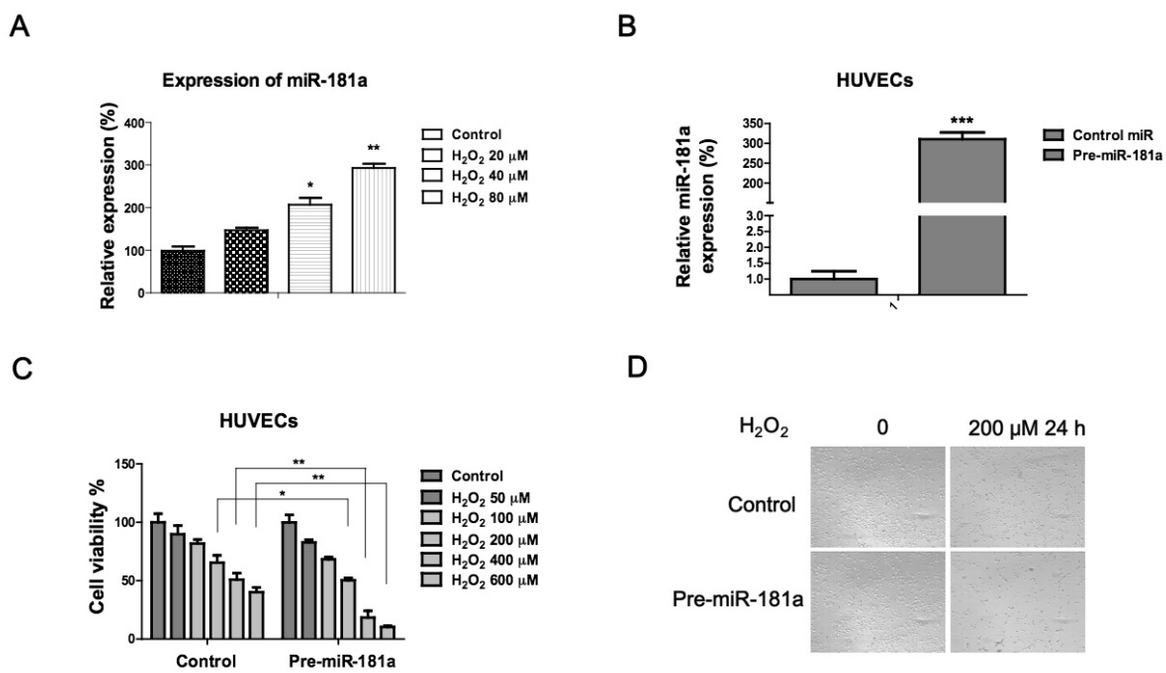

Figure 2. MiR-181a regulates the sensitivity of HUVECs to oxidative stress. ***A,*** HUVECs were treated with H_2O_2 at 0, 20, 40 or 80 μM for 24 hours, followed by the measurements of miR-181a expression by qRT-PCR. ***B,*** HUVECs were transfected with control microRNAs or pre-miR-181a for 48 hours; then, the miR-181a expression was measured by qRT-PCR. ***C,*** HUVECs were transfected with control microRNAs or pre-miR-181a for 48 hours. Cells were then treated with H_2O_2 at 0, 50, 100, 200, 400 or 600 μM for 24 hours, followed by the cell viability analysis by MTT assay. ***D,*** HUVECs were transfected with control microRNAs or pre-miR-181a for 48 hours. Cells were then treated with H_2O_2 at 0 or 200 μM for 24 hours. Then cells were visualized using a phase-contrast microscope. Columns, mean of three independent experiments; bars, SE. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

gets of miR-181a using computational miRNA target prediction algorithms, as detailed at TargetScan (http://targetscan.org, Release 5.1), we identified the Bcl-2 gene which contains a highly conserved binding site of miR-181a at the 3'-UTR site (Figure 3A). Notably, the seed sequences in 3'UTRs of multiple species targeted by miR-181 were highly evolutionarily conserved (Figure 3B), suggesting a critical role of miR-181a-Bcl-2 in pathological processes of atherosclerosis. To verify that miR-181a could inhibit Bcl-2 expression at the post-transcriptional level, protein levels of Bcl-2 was analyzed in HUVECs (Figure 3C) by Western blot with transfection of pre-miR-181a or control microRNAs. We observed significantly decreased Bcl-2 protein expressions in miR-181a overexpressing cells (Figure 3C). To validate whether miR-181a could direct target 3' UTR of BCL-2, we co-transfected 293T cells with constructs of luciferase control reporter, luciferase target reporter containing the original 3'UTR of Bcl-2 (WT BCL-2) or 3'UTR mutant of the miR-181a binding sites on Bcl-2. As we expected, transfection of miR-181a decreased the luciferase activity of the reporter with wild-type 3'-UTR of Bcl-2 (Figure 3D). However, there is only a slight difference in luciferase activity with the co-transfection of the 3'UTR mutant Bcl-2 vector with control microRNAs or pre-miR-181a. These results generally reflect non-specific effects, validating Bcl-2 is a direct target of miR-181a.

Inhibition of miR-181a Protects the H_2O_2-Induced Endothelial Cell Death

To determine whether inhibition of miR-181a could regulate the vascular endothelial cells sensitivity to oxidative stress, we first assessed the effects of H_2O_2 on the expression of Bcl-2. Results showed both of the protein (Figure 4A) and mRNA (Figure 4B) levels of Bcl-2 were suppressed by treatments of H_2O_2 at 50 μM and 100 μM. Combined with our above results in Figure 2A and Figure 3, these results indicate a correlation between the H_2O_2-induced endothelial cells apoptosis and the H_2O_2-modulated expressions of miR-181a and Bcl-2. We next knocked down the Bcl-2 expression by transfection of the specific siRNA. As we expected, knocking down of Bcl-2 significantly increased the sensitivity of HUVECs to H_2O_2 (Figure 4C), suggesting the miR-181a-mediated inhibition of Bcl-2 might directly contribute to the sensitization of HUVECs to ox-

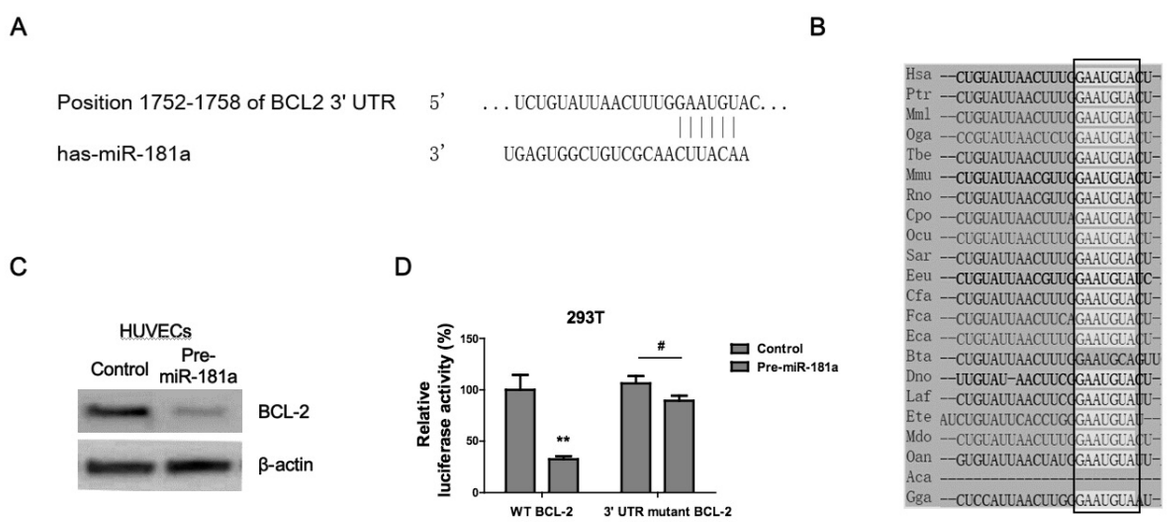

Figure 3. Bcl-2 is a direct target of miR-181a in HUVECs. ***A,*** Target prediction from Targetscan.org, the position 1752-1758 of Bcl-2 3' UTR contains putative binding sites for miR-181a. ***B***, The 3' UTR of Bcl-2 contains highly conserved sequence for binding of miR-181a in multiple species. ***C,*** HUVECs were transfected with 100 nM negative control microRNAs or pre-miR-181a for 48 hrs. Cell lysates were prepared for Western blotting analysis. β-actin was used as a loading control. ***D,*** Human 293T cells were co-transfected with luciferase reporter plasmids with wild-type 3'-UTR of Bcl-2 or mutant 3'-UTR of Bcl-2 and negative control microRNAs or pre-miR-181a using Lipofectamine 2000 reagent. Forty-eight hours post transfection, cells were harvested and lysed with passive lysis buffer. Luciferase activities were measured by a dual luciferase reporter assay. The results were expressed as relative luciferase activity (firefly LUC/Renilla LUC). Columns, mean of three independent experiments; bars, SE. **: $p < 0.01$; $^{\#}$: $p > 0.05$ (no significance).

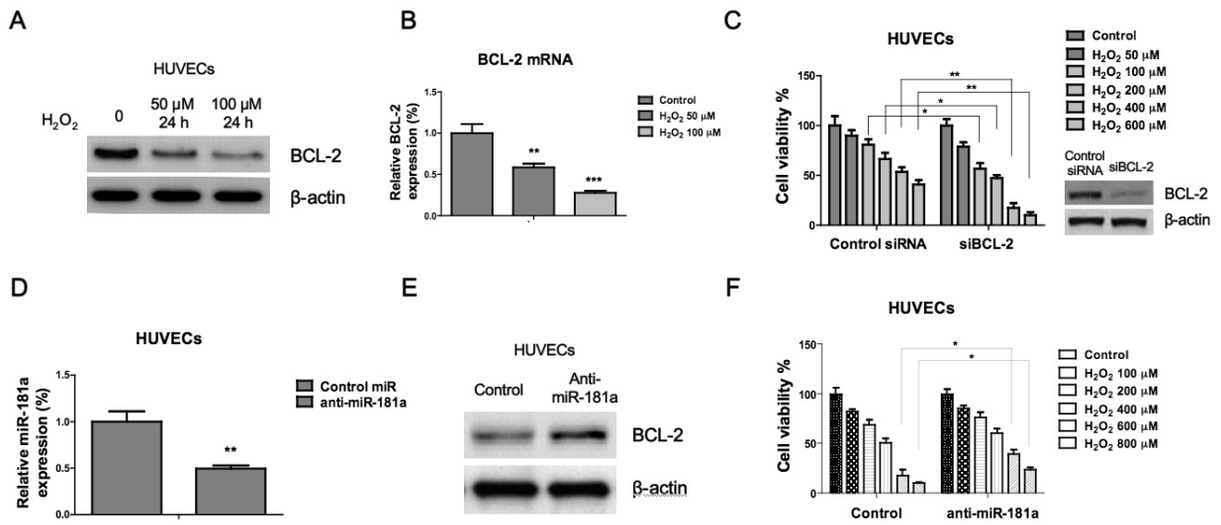

Figure 4. Regulation of Bcl-2 by siRNA or antisense miR-181a modulates sensitivities of HUVECs to H_2O_2. ***A,*** HUVECs were treated with 0, 50 or 100 μM H_2O_2 for 24 hrs. Cell lysates were prepared for Western blotting analysis. β-actin was used as a loading control. ***B,*** HUVECs were treated with 0, 50 or 100 μM H_2O_2 for 24 hrs. Total RNA was isolated and subjected for qRT-PCR analysis. ***C,*** HUVECs were transfected with control siRNAs or siBcl-2 for 48 hours, followed by the treatments of H_2O_2 at 0, 50, 100, 200, 400 or 600 μM H_2O_2 for 24 hrs. Cells viabilities were analyzed by MTT assay. ***D,*** HUVECs were transfected with control antisense or anti-miR-181a for 48 hours, followed by the measurements of the expressions of miR-181a by qRT-PCR. (E) HUVECs were transfected with control antisense or anti-miR-181a for 48 hours, followed by the measurements of the expressions of Bcl-2 by Western blot. β-actin was used as a loading control. ***F,*** HUVECs were transfected with control antisense or anti-miR-181a for 48 hours, followed by the treatments of H_2O_2 at 0, 100, 200, 400, 600 or 800 μM for 24 hrs. Cells viabilities were analyzed by MTT assay. Columns, mean of three independent experiments; bars, SE. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

idative stress. To support this, we transfected antisense of miR-181a or control antisense to HUVECs (Figure 4D). Inhibition of endogenous miR-181a in HUVECs resulted in significantly increased Bcl-2 protein expression (Figure 4E), suggesting that the regulation of Bcl-2 by miR-181a is likely to be of biologic significance. On the basis of previous results illustrating that HUVECs were sensitive to H_2O_2 with low Bcl-2 expression (Figure 4C), we predicted that inhibition of miR-181a could facilitate HUVECs resistant to H_2O_2 through the upregulation of Bcl-2. Our data demonstrated that transfection of antisense miR-181a decreased the H_2O_2 sensitivity (Figure 4F), suggesting the endogenous regulation of Bcl-2 expression by miR-181a is likely to be one mechanism for the modulation of oxidative stress sensitivity.

Restoration of BCL-2 in miR-181a Overexpressing Cells Confers Endothelial Cells Resistant to H2O2

We next investigated whether miR-181a affects H_2O_2 sensitivity through direct suppression of Bcl-2. Therefore, we transfected pre-miR-181a or pre-miR-181a with Bcl-2 overexpression vector into HUVECs. Our data showed transfection of Bcl-2 into miR-181a overexpressing cells could restore the expression of Bcl-2 (Figure 5A). Then we treated cells with indicated concentrations of H_2O_2 for 24 hrs. Transfection with the pre-miR-181a alone decreased the cell viabilities in response to H_2O_2 (Figure 5B). Consequently, restoration of Bcl-2 rescued the cell viabilities of these cells to H_2O_2 (Figure 5B). Taken together, our results clearly demonstrate that miR-181a plays an important role in the oxidative stress-induced cell death of HUVECs through the direct suppression of Bcl-2.

Reverse Correlation of miR-181a and BCL-2 in Human Atherosclerosis Plaques

To further verify whether Bcl-2 is a target of miR-181a in human atherosclerosis plaques, we analyzed the expressions of Bcl-2 in atherosclerosis plaque samples. Figure 6A showed significantly down-regulated expressions of Bcl-2 mRNAs in atherosclerotic plaques compared with heathy vessels. Moreover, the expression be-

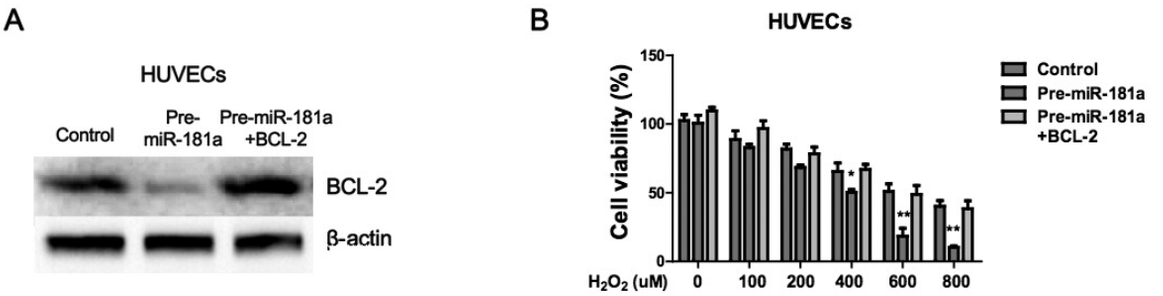

Figure 5. Restoration of Bcl-2 in HUVECs rescues the miR-181a mediated cell sensitization to H_2O_2. ***A,*** HUVECs were transfected with control microRNAs or pre-miR-181a for 48 hours, cells with pre-miR-181a were then transfected with a vector containing wild-type Bcl-2 for 24 hours. Cells were collected and the protein expression of Bcl-2 was analyzed by Western blot. β-actin was used as a loading control. ***B,*** HUVECs were transfected with control microRNAs or pre-miR-181a for 48 hours, cells with pre-miR-181a were then transfected with a vector containing wild-type Bcl-2 for 24 hours, followed by the treatments of H_2O_2 at 0, 100, 200, 400, 600 or 800 μM for 24 hrs. Cells viabilities were analyzed by MTT assay. Columns, mean of three independent experiments; bars, SE. *: $p < 0.05$; **: $p < 0.01$.

tween miR-181a and Bcl-2 in atherosclerosis plaque samples exhibited inverted correlation patterns of miR-181a and Bcl-2: a strong negative correlation between miR-181a and Bcl-2 expressions ($p < 0.05$) was found in human atherosclerotic plaques (Figure 6B), demonstrating that Bcl-2 is a target of miR-181a in human atherosclerotic plaques.

Discussion

In the present study, a distinct miR-181a expression pattern in human atherosclerotic plaques and healthy blood vessels was demonstrated. MiR-181a was found to be highly expressed in atherosclerotic plaques, intriguing us to investigate the roles of miR-181a in the pathological progress of atherosclerosis.

MiRNAs have been demonstrated to play a key role in mitochondrial metabolism, therefore modulating the oxidative stress sensitivity[20]. Other miRNAs, such as miR-663, miR-19a and miR-23b have also been studied and shown to be involved in the modulation of EC inflammation and proliferation, respectively[21]. Our study focused on the potential cellular properties of HUVECs regulated by miR-181a in response to H_2O_2. The miR-181a level is induced by H_2O_2 treatments. In addition, overexpression of miR-181a renders HUVECs sensitive to H_2O_2, suggesting miR-181a might target anti-apoptotic molecules during the oxidative stress-induced apoptosis. Although miR-181a has been wildly studied in cancer development, the function of miR-181a in atherosclerosis remains unclear. In this work, we illustrated that the miR-181a contributed to the formation of human atheroscle-

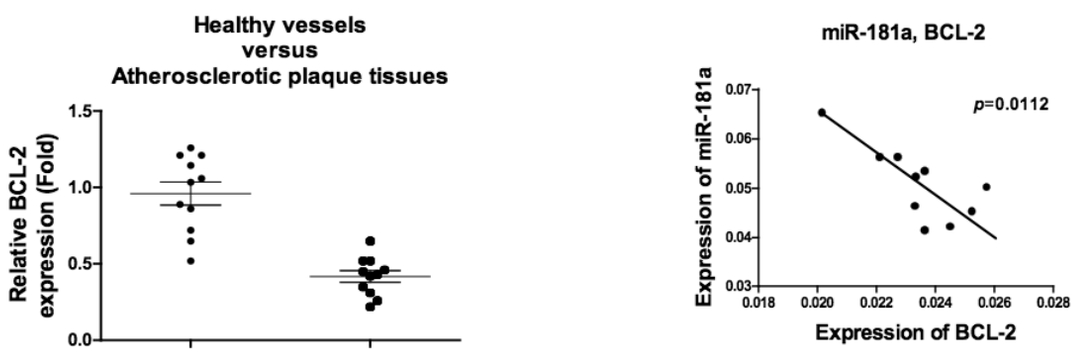

Figure 6. Bcl-2 is downregulated in atherosclerotic plaques and inversely correlated with miR-181a. ***A,*** The mRNA levels of Bcl-2 were measured in healthy human vessels and atherosclerotic plaques by qRT-PCR. Each group has 10 samples. ***B,*** The correlation of Bcl-2 and miR-181a levels in atherosclerotic plaque tissues was analyzed.

rotic plaques due to its high expression. Another investigations reported that miR-181a could protect against the angiotensin II-induced osteopontin expression in vascular smooth muscle cells[22], suggesting miR-181a plays a role to prevent atherosclerosis through the inhibition of angiotensin II.

Endothelial dysfunction is an early feature of both atherosclerosis and vascular diseases in humans[12]. Accumulation of oxidative stress produced ROS impaired biological functions of endothelium, leading to endothelial dysfunction[23]. Therefore, an imbalance of reduced production of anti-ROS molecules or increased production of reactive oxygen species may promote endothelial dysfunction. The Bcl-2 protein is specifically considered an important anti-apoptotic protein which determines the commitment of cells to apoptosis[14,15]. In atherosclerotic lesions, apoptosis is triggered by inflammatory processes through cell-cell contact and cytokines and oxidized lipids[24]. In the late stage, especially when the plaque is formed, apoptosis lead to plaque rupture and thrombosis[24]. Thus, preventing apoptosis by restoration of Bcl-2 levels in atherosclerotic plaque could be selected to development therapeutic strategies against atherosclerosis. Our results revealed the expression of Bcl-2 is lower in atherosclerotic plaque due to its inhibition by miR-181a, suggesting inhibition of miR-181a might contribute to anti-atherosclerosis therapy.

Conclusions

We demonstrated an essential role of miR-181a in the development of atherosclerosis through the regulation of the endothelial dysfunction. Moreover, we showed the inhibition of endogenous miR-181a could prevent the H_2O_2-induced HUVECs apoptosis, providing a mechanism for the development of new antioxidant drugs for the treatment of atherosclerosis.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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