MiR-551b-5p Contributes to Pathogenesis of Vein Graft Failure via Upregulating Early Growth Response-1 Expression

Ran Dong[1], Kui Zhang[1], Yue-Li Wang[2], Feng Zhang[3], Jian Cao[1], Ju-Bing Zheng[1], Hong-Jia Zhang[1,4,5,6]

[1]Department of Cardiac Surgery, Beijing Anzhen Hospital, Capital Medical University, Beijing 100029, China
[2]Department of Ultrasonics, Beijing Anzhen Hospital, Capital Medical University, Beijing 100029, China
[3]Department of Bioinfomatics, Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing 100101, China
[4]Beijing Laboratory for Cardiovascular Precision Medicine, Beijing 100029, China
[5]The Key Laboratory of Remodeling-related Cardiovascular Disease, Ministry of Education, Beijing 100029, China
[6]Beijing Aortic Disease Center, Cardiovascular Surgery Center, Beijing 100029, China

Abstract

Background: Vein graft failure (VGF) is a serious complication of coronary artery bypass graft, although the mechanism remains unclear. The study aimed to investigate the effects of microRNAs (miRNAs) on the endothelial dysfunction involved in VGF.

Methods: Human umbilical vein endothelial cells (HUVECs) were subjected to mechanical stretch stimulation to induce endothelial dysfunction. Genome-wide transcriptome profiling was performed using the Human miRNA OneArray® V4 (PhalanxBio Inc., San Diego, USA). The miRNA-messenger RNA (mRNA) network was investigated using gene ontology and Kyoto Encyclopedia of Genes and Genomes. The miR-551b-5p mimic and inhibitor were applied to regulate miR-551b-5p expression in the HUVECs. The 5-ethynyl-2'-deoxyuridine assay, polymerase chain reaction (PCR), and Western blotting (WB) were used to assess HUVECs proliferation, mRNA expression, and protein expression, respectively. The vein graft model was established in early growth response (*Egr*)-1 knockout (KO) mice and wide-type (WT) C57BL/6J mice for pathological and immunohistochemical analysis. Endothelial cells isolated from the veins of WT and *Egr-1* KO mice were subjected to mechanical stretch stimulation; PCR and WB were conducted to confirm the regulatory effect of *Egr-1* on Intercellular adhesion molecule (*Icam-1*). One-way analysis of variance and independent *t*-test were performed for data analysis.

Results: Thirty-eight miRNAs were differentially expressed in HUVECs after mechanical stretch stimulation. The bioinformatics analysis revealed that *Egr-1* might be involved in VGF and was a potential target gene of miR-551b-5p. The mechanical stretch stimulation increased miR-551b-5p expression by 2.93 ± 0.08 fold ($t = 3.07$, $P < 0.05$), compared with the normal HUVECs. Transfection with the miR-551b-5p mimic or inhibitor increased expression of miR-551b-5p by 793.1 ± 171.6 fold ($t = 13.84$, $P < 0.001$) or decreased by $26.3\% \pm 2.4\%$ ($t = 26.39$, $P < 0.05$) in the HUVECs, respectively. HUVECs proliferation and *EGR-1* mRNA expression were significantly suppressed by inhibiting miR-551b-5p expression ($P < 0.05$). The lumens of the vein grafts in the *Egr-1* KO mice were wider than that in the WT mice. *Icam-1* expression was suppressed significantly in the *Egr-1* KO vein grafts ($P < 0.05$).

Conclusions: Increased miR-551b-5p expression leads to endothelial dysfunction by upregulating *Egr-1* expression. *EGR-1* KO can improve the function of a grafted vein through suppressing *Icam-1*.

Key words: Early Growth Response Protein 1; Endothelial Dysfunction, miR-551b-5p; Vein Graft Failure

Introduction

Coronary artery bypass graft (CABG) is an effective therapy for coronary artery disease. The saphenous vein is the most commonly used vein graft in CABG. However, the success rate of CABG surgery is limited by vein graft failure (VGF). As shown in a study of Goldman *et al*.,[1] the saphenous vein graft patency rate at the 1st year is 89%, and it drops to 61% at the 10th year. The mechanism underlying the actions of VGF is a hot topic in the field of cardiac surgery but is not well understood. VGF can be categorized

© 2017 Chinese Medical Journal ¦ Produced by Wolters Kluwer - Medknow

Access this article online

Quick Response Code:

Website:
www.cmj.org

DOI:
10.4103/0366-6999.208246

into the early, delayed, and late stages. After CABG, up to 12% of saphenous vein grafts occlude in the first 6 months, and 3.4% occlude as early as 2nd to 3rd weeks.[2,3] Plenty of studies have revealed that endothelial dysfunction is a critical factor in VGF in the early stage.[4-6] During the early stage, VGF is associated with inflammation and acute thrombosis induced by endothelial dysfunction. High arterial blood pressure increases mechanical stretch, resulting in changes in endothelial structures and functions. The expression of cellular factors and adhesion molecules is increased via the nuclear factor kappa-B signaling pathway, which promotes an inflammatory response.[7] Activation of the mitogen-activated protein kinase/early growth response (EGR) signaling pathway increases EGR-1 expression.[8] EGR-1 upregulates monocyte chemoattractant protein 1 via the extracellular signal-regulated kinase (ERK) pathway to promote macrophage adhesion, thrombosis, and atherosclerotic plaque formation.[9,10] Therefore, endothelial dysfunction induced by mechanical stretch is the initial event in VGF.

A microRNA (miRNA or miR) is a type of small noncoding RNA molecule that is 22 nucleotides in length. MiRNAs play important roles in cell differentiation, metabolism, cell growth, and many other activities. Recently, increasing evidences have suggested that miRNA expression might have critical effects on VGF pathology and might be involved in neointimal formation, vascular smooth muscle cell proliferation, intimal hyperplasia, endothelial cell functions, cytokine responsiveness, and vascular inflammation.[11] Although miRNAs have emerged as new regulators of endothelial dysfunction,[12,13] the effects and mechanisms of miRNAs in endothelial dysfunction remain unclear.

Therefore, this study investigated the effects of miRNAs on endothelial dysfunction induced by mechanical stretch and identified novel target genes regulated by miRNAs to illustrate the regulatory mechanism.

METHODS

Ethical approval
All animals were managed according to the guidelines of Capital Medical University, Beijing Anzhen Hospital. The experimental protocols were approved by the Ethics committee of Capital Medical University, Beijing Anzhen Hospital. The animal experiments were approved by the Beijing Institute of Heart, Lung, and Blood Vessel Diseases.

Cell culture, transfection, and mechanical stretch stimulation
Human umbilical vein endothelial cells (HUVECs) were purchased from Genechem Company (Shanghai, China). The HUVECs were incubated in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, California, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, California, USA) at 37°C with 95% air and 5% CO_2. This study used a miR-551b-5p mimic and inhibitor to up- and down-regulate miR-551b-5p expression in HUVECs, respectively. A total of 100 nmol/L of

a miR-551b-5p mimic (Ribobio, CA, China) was introduced using the riboFECT™ CP transfection kit (Ribobio, CA, China) according to the manufacturer's protocol.

Similarly, a total of 100 nmol/L of a miR-551b-5p inhibitor (Ribobio, CA, China) was introduced using the riboFECT™ CP transfection kit (Ribobio, CA, China) according to the manufacturer's protocol.

HUVECs were plated onto collagen-coated plates with silicone elastomer bottoms (Flexcell, Mckeesport, PA, USA). A computer-controlled cyclic stress unit (Flexcell 5100, McKeesport, PA, USA) was used to subject the HUVECs to mechanical stretch for 3 h (60 cycles/min, 15% elongation). Normal HUVECs were negative control (NC) group, HUVECs treated with 15% elongation stimulation were stimulation (ST) group, HUVECs transfected with miR-551b-5p mimic were mimic group, and HUVECs transfected with miR-551b-5p inhibitor were inhibitor group.

Microarray profiling
The Human miRNA OneArray® V4 (PhalanxBio Inc., San Diego, USA) was used to perform a Human genome-wide miRNA microarray analysis. Briefly, the miRNA ULS™ Labeling Kit (Kreatech Diagnostics, Amsterdam, Netherlands) was used to prepare fluorescent targets from 2.5 μg total RNA samples. The OneArray® Hybridization Chamber was used to hybridize the labeled targets with Phalanx hybridization buffer. After overnight hybridization at 37°C, nonspecific binding targets were eliminated by three washing steps. After drying by centrifugation, the slides were scanned using Axon GenePix® 4000B scanner (Molecular Devices, Sunnyvale, CA, USA). GenePix 4.1 software (Molecular Devices, CA, USA) was used to analyze the Cy5 fluorescence intensities of each spot. The signal intensity of each spot was processed using the R program. Spots that passed the criteria were normalized using the 75% scaling normalization method. Normalized spot intensities were converted into gene expression $\log_2$ ratios for control (normal HUVECs) and treatment group (HUVECs which been stimulated with mechanical stretch. Spots with $\log_2$ ratios ≥ 1 or $\log_2$ ratios ≤ -1 and $P < 0.05$ were selected for further analysis.

Animals and vein graft model
Six (donor mice: $n = 3$, receptor mice: $n = 3$) male *Egr-1* knockout (KO) mice (Jie Du Laborator, Beijing Institute of Heart, Lung and Vessel Disease) and six (donor mice: $n = 3$, receptor mice: $n = 3$) wide-type (WT) C57BL/6J mice (Vital River Company, Beijing, China) aged 8–12 weeks were used to produce surgical models. *Egr-1* KO mice with a C57BL/6J × 129 background were backcrossed with the C57BL/6J strain for at least 10 generations. Before experimentation, the mice were kept for 1 week at 24°C on a 12 h light/dark cycle and fed a normal diet.

The vein graft model was previously described by Zou *et al.*[14] First, one mouse (donor mouse) was sacrificed, and the inferior vena cava was harvested for vein graft. Second,

another mouse was anesthetized by intraperitoneal injection of 1% pentobarbital sodium. The right common carotid artery was dissected from the surrounding tissue as far as possible, ligated at the midpoint with sutures, and snipped. Distal and proximal segments were combined inside a cuff, and the arterial flow was blocked using clamps placed at the cuff handles. The sutures were removed, and the distal and proximal arteries were everted over the cuffs' tubular body and fixed with 8-0 sutures. Consequently, a prepared vein segment (from the donor mouse) was grafted over the cuffs and fixed into position with sutures. Finally, we sutured the incision using 3-0 Dacron (Hangzhou AiPu Medical Products Co., Zhejiang, China).

Patency of the vein grafts was tested after the operation. If the grafted vessel pulsed vigorously and no bleeding was observed, surgery was considered to be successful. If no pulsation occurred within a few minutes of blood flow restoration or if clot formation was suspected, surgery was considered a failure.[15] The vein grafts were harvested one week after surgery. Afterward, the endothelial cells were isolated from the veins of WT and *Egr-1* KO mice and then subjected to mechanical stretch stimulation for further study.

5-Ethynyl-2'-deoxyuridine assay

The 5-ethynyl-2'-deoxyuridine (EdU) assay (Ribobio, Guangzhou, China) was performed according to the manufacturer's protocol. Briefly, HUVECs were cultured in 6-well plates at a density of 5×10^5 cells per well, transfected with the miR-551b-5p inhibitor or mimic for 24 h, and then exposed to 50 μmol/L of EdU for an additional 2 h at 37°C with 95% air and 5% CO_2. The cells were fixed with 4% formaldehyde for 30 min at room temperature and treated with 50 μl of glycine for 5 min. After washing three times with phosphate-buffered saline, the cells in each well were incubated with 100 μl of 1 × Apollo® reaction buffer for 30 min at room temperature. The DNA contents of the cells were stained with 100 μl of 1 × Hoechst for 30 min. The mean percentage of cells positive for EdU was determined in 4 or 5 different fields using a Leica DMI4000B fluorescence microscope (Leica, Wetzlar, Germany; original magnification ×200). The counting was repeated three times.

Quantitative real-time polymerase chain reaction analysis

Total RNA was extracted from the HUVECs using the Trizol reagent (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions. MiR-551b-5p primers were purchased from the RiboBio Company. U6 primers were forward 5'-TGA CTT CAA CAG CGA CAC CCA-3' and reverse 5'-CAC CCT GTT GCT GTA GCC AAA-3'. All reactions involved an initial denaturation step at 95°C for 15 s, followed by 45 cycles of 95°C for 5 s and 60°C for 30 s. Data were analyzed based on the 2-ΔΔCT method.

Real-time reverse transcription polymerase chain reaction analysis

Total RNA was extracted from the HUVECs and endothelial cells which were isolated from the veins of WT and *Egr-1*

KO mice using the Trizol reagent (Sigma-Aldrich, St. Louis, MO, USA). The RNA purity was determined using the absorbance at 260 and 280 nm ($A260/280$). After assessing the RNA concentration, a first-strand cDNA synthesis kit (Fermentas, MA, USA) was used to reverse transcribe the total RNA into complementary DNA (cDNA). MiR-551b-5p primers were purchased from the RiboBio Company. The *Egr-1* primers were forward 5'-CAG CAG CCT TCG CTA ACC-3' and reverse 5'-CCA CTG GGC AAG CGT AA-3'. The intercellular adhesion molecule (*Icam-1*) primers were forward 5'-TGA CTT CAA CAG CGA CAC CCA-3' and reverse 5'-CAC CCT GTT GCT GTA GCC AAA-3'. The Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) primers were forward 5'-TGA CTT CAA CAG CGA CAC CCA-3' and reverse 5'-CAC CCT GTT GCT GTA GCC AAA-3'. All reactions involved an initial denaturation step at 95°C for 15 s, followed by 45 cycles of 95°C for 5 s and 60°C for 30 s. Specific messenger RNA (mRNA) quantification was performed by real-time PCR using SYBR Premix Ex Taq™ II (TaKaRa Bio, Dalian, China) in a TP800 Real-Time PCR System (TaKaRa Bio, Dalian, China) according to the manufacturer's guidelines.

Western blotting

The HUVECs and endothelial cells which were isolated from the veins of WT and *Egr-1* KO mice were lysed in RIPA buffer and centrifuged for 15 min at 4°C (13,800 g). We used the BCA protein assay kit (HyClone-Pierce, Utah, CA, USA) to measure the protein concentration. Then, equal amounts of protein (40 μg) were separated on an 8% SDS-polyacrylamide gel and transferred onto a polyvinylidene fluoride membrane (Millipore, MA, USA). The nonspecific sites were blocked with 5% skimmed milk powder diluted in Tris-buffered saline with 0.05% Tween. We incubated the membranes with antibodies against interleukin-1, IL-4, tumor necrosis factor-α, and transforming growth factor-β (Abcam, Cambridge, UK) at 4°C overnight. After washing, the proteins were revealed by incubating with HRP-labeled IgG (Pierce Antibodies, Thermo Fisher Scientific, Waltham, MA, USA) at room temperature for 1 h. The membranes were developed using the electrochemiluminescence system and analyzed using the Biological Electrophoresis Image Analysis System (Furi Science & Technology Company, Shanghai, China).

Statistical analysis

The results were presented as the mean ± standard error (SE). Independent *t*-test and one-way analysis of variance (ANOVA) followed by the Bonferroni/Dunn *post hoc* analysis were performed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). A $P < 0.05$ was considered statistically significant.

Results

MicroRNA expression in human umbilical vein endothelial cells after mechanical stretch stimulation

To identify the miRNAs involved in the pathogenesis of

VGF, this study subjected HUVECs to mechanical stretch and compared them with HUVECs cultured under normal conditions. Then, microarray profiling was performed to compare miRNA expression in the two groups of HUVECs. As shown in Figure 1, the differential miRNA expression patterns of HUVECs with mechanical stretch stimulation were separated from the NC group by hierarchical cluster analysis. We found 18 significantly downregulated and 20 significantly upregulated miRNAs (with a fold change ≥0.8 and $P < 0.05$) [Figure 1a and 1b].

MicroRNA target gene network

To explore potential genes regulated by the miRNAs, this study used a bioinformatics method to analyze the relationship between miRNAs and genes. According to the gene ontology (GO) analysis, the differentially expressed miRNAs were classified into three different categories. A total of 977, 250, and 430 GO terms and 32,386, 10,874, and 11,732 target genes were mapped to the biological process, cellular component, and molecular function categories, respectively. We determined the pathways associated with the differentially expressed miRNAs using Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis and found that 2123 genes were annotated for 85 pathway terms. As shown in Figure 2, the differentially expressed miRNAs had a variety of potential targets.

MiR-551b-5p promoted endothelial cells proliferation and early growth response 1 expression

This study applied mechanical stretch on HUVECs for 3 h
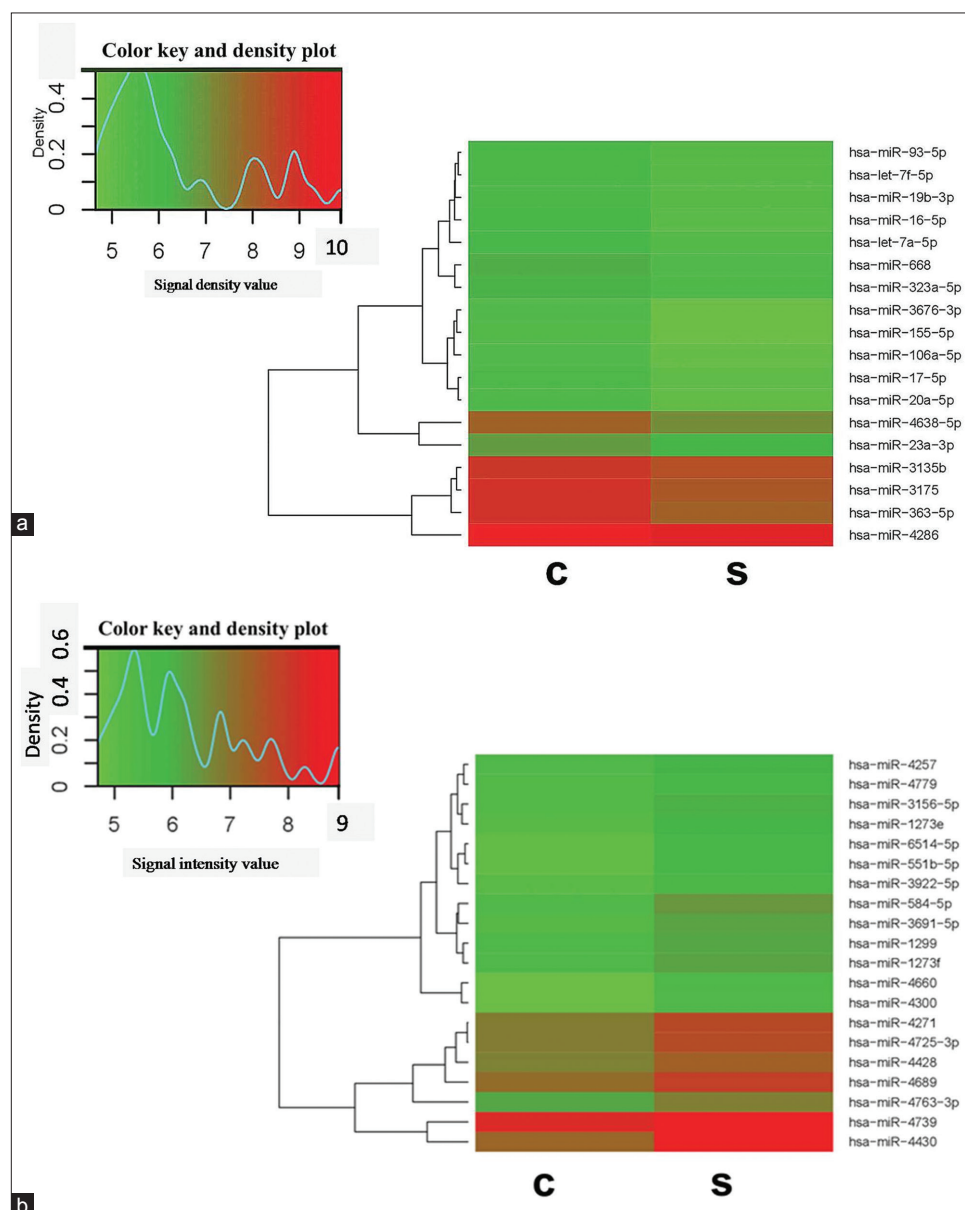
Figure 1: Differential microRNA expression in normal HUVECs and HUVECs with mechanical stretch stimulation. (a) Eighteen microRNAs' expression level was downregulated in HUVECs with mechanical stretch stimulation; (b) Twenty microRNAs' expression level was upregulated in HUVECs with mechanical stretch stimulation. C: Normal HUVECs; S: HUVECs with mechanical stretch stimulation; Red: Represents a high expression level; Green: Represents a low expression level. HUVECs: Human umbilical vein endothelial cells.

and then measured miR-551b-5p expression by quantitative polymerase chain reaction (qPCR). As shown in Figure 3a, miR-551b-5p expression in ST group was increased significantly compared with the NC group (2.93 ± 0.08 fold; $t = 3.07$, $P < 0.05$). Next, we used the miR-551b-5p mimic or inhibitor to manipulate miR-551b-5p expression in the HUVECs. HUVECs treated with the mimic and inhibitor were also subjected to mechanical stretch (15% elongation) for 3 h. MiR-551b-5p expression increased by

793.1 ± 171.6 fold ($t = 13.84$, $P < 0.001$) and decreased by $26.3\% \pm 2.4\%$ ($t = 26.39$, $P < 0.05$) following the treatment with the miR-551b-5p mimic and inhibitor, respectively [Figure 3b and 3c].

Subsequently, we assessed EGR-1 mRNA and protein expression using reverse transcription PCR (RT-PCR) and Western blotting, respectively, and found that mechanical stretch increased EGR-1 mRNA expression

Figure 2: Predicted interaction network of mRNAs and dysregulated microRNAs in HUVECs treated with mechanical stretch stimulation. (a) The red ovals represent the upregulated microRNAs and the pink rectangles represent the potential target gene; (b) The green ovals represent the downregulated microRNAs and the pink rectangles represent the potential target gene. HUVECs: Human umbilical vein endothelial cells; mRNA: Messenger RNA.

Figure 3: MiR-551b-5p regulate EGR-1 mRNA and protein expression in HUVECs with mechanical stretch stimulation. (a) Mechanical stretch stimulation increased miR-551b-5p expression in HUVECs; (b) MiR-551b-5p expression in HUVECs was upregulated after treating with miR-551b-5p mimic; (c) MiR-551b-5p expression in HUVECs was downregulated after treating with the miR-551b-5p inhibitor; (d) EGR-1 mRNA expression in NC, ST, mimic/ST, and inhibitor/ST; (e) EGR-1 protein expression in NC, ST, inhibitor/ST, and mimic/ST. Data are expressed as the mean $\pm$ standard error. *$P < 0.05$ versus NC group; †$P < 0.05$ versus ST group; ‡$P < 0.05$ versus mimic/ST group; EGR-1: Early growth response-1; HUVEC: Human umbilical vein endothelial cells; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; NC: Normal HUVECs; ST: HUVECs treated with mechanical stretch stimulation; mimic: HUVECs transfected with miR-551b-5p mimic; inhibitor: HUVECs transfected with miR-551b-5p inhibitor; mimic/ST: HUVECs treated with mechanical stretch stimulation after transfecting with miR-551b-5p mimic; inhibitor/ST: HUVECs treated with mechanical stretch stimulation after transfecting with miR-551b-5p inhibitor. mRNA: Messenger RNA.

by 3.7 ± 0.38 fold at 15% elongation stimulation (vs. NC group, $q = 12.66$ $P < 0.05$). EGR-1 mRNA expression in HUVECs with miR-551b-5p mimic transfection after a 15% elongation mechanical stretch increased by 1.9 ± 0.21 fold (vs. ST group, $q = 13.16$, $P < 0.05$) [Figure 3d]. In contrast, the miR-551b-5p inhibitor significantly suppressed EGR-1 mRNA expression by 39.7% (vs. ST group, $q = 6.89$, $P < 0.05$) [Figure 3d]. Similarly, mechanical stretch increased EGR-1 protein expression in HUVECs by 1.69 ± 0.14 fold (vs. NC group, $q = 16.51$, $P < 0.05$) [Figure 3e]. EGR-1 protein expressions in HUVECs with miR-551b-5p mimic transfection after mechanical stretch were increased by 2.36-fold (vs. NC group, $q = 6.00$, $P < 0.05$) and 1.39 fold (vs. ST group, $q = 10.51$, $P < 0.05$), respectively [Figure 3e]. The expression of EGR-1 protein in HUVECs with miR-551b-5p inhibitor after mechanical stretch was decreased to 0.74 fold (vs. ST group, $q = 16.32$, $P < 0.05$) and to 0.53 fold (vs. mimic group under ST, $q = 26.84$, $P < 0.05$), respectively [Figure 3e].

In addition, we assessed HUVECs proliferation in different group using the EdU assay. As shown in Figure 4, the EdU-positive cells in the HUVECs treated with mechanical stretch stimulation were increased by 3.9 fold compared with the NC group ($q = 20.01$, $P < 0.05$). Overexpression of miR-551b-5p further promoted HUVECs proliferation after mechanical stretch stimulation (6.9 fold vs. NC group; $q = 40.98$, $P < 0.05$). In contrast, proliferation was suppressed in the HUVECs treated with the miR-551b-5p inhibitor (33.3% of ST group; $q = 8.94$, $P < 0.05$).
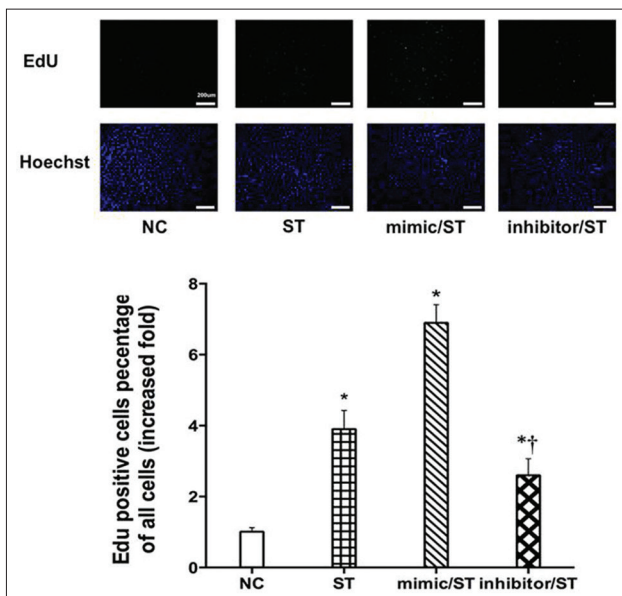

Figure 4: The miR-551b-5p inhibitor suppressed HUVECs proliferation (Scale bar: 200 μm). Data are expressed as the mean ± standard error. *$P < 0.05$ versus NC group; †$P < 0.05$ versus ST group; NC: Normal HUVECs; ST: HUVECs treated with mechanical stretch stimulation; mimic/ST: HUVECs treated with mechanical stretch stimulation after transfecting with miR-551b-5p mimic; inhibitor/ST: HUVECs treated with mechanical stretch stimulation after transfecting with miR-551b-5p inhibitor. HUVECs: Human umbilical vein endothelial cells.

Early growth response 1 upregulated intercellular adhesion molecule-1 expression to induce endothelial dysfunction

ICAM-1 expression had been shown to be a critical factor in endothelial dysfunction. To investigate whether EGR-1 could regulate ICAM-1 expression in VGF, we established a mouse vein graft model in WT and *Egr-1* KO mice. Then, we harvested the vein grafts and examined them by HE staining 1 week after surgery. As shown in Figure 5a, the lumens of vein graft were narrower than the lumens of inferior vena cava in both WT and *Egr-1* KO mice (186.21 ± 10.63 μm vs. 392.34 ± 11.29 μm, $t = 23.70$, $P < 0.05$; 296.33 ± 12.03 μm vs. 392.34 ± 11.29 μm, $t = 10.04$, $P < 0.05$). However, the lumens of the vein grafts in the *Egr-1* KO mice were wider than that in the WT mice (296.33 ± 12.03 μm vs. 186.21 ± 10.63 μm; $t = 12.42$, $P < 0.05$). Immunohistochemistry revealed that the percentages of *Icam-1*-positive cells were significantly reduced in the vein grafts from the *Egr-1* KO mice 1 week after surgery compared to the percentages from the WT mice (29.3% ± 3.2% vs. 63.4% ± 3.9%; $t = 14.85$, $P < 0.05$) [Figure 5b].

To confirm the regulatory effect of *Egr-1* on *Icam-1* at the molecular level, we isolated endothelial cells from the veins of WT and *Egr-1* KO mice and subjected them to mechanical stretch stimulation. After 3 h, mechanical stretch increased *Icam-1* mRNA expression in the WT endothelial cells but significantly suppressed *Icam-1* mRNA expression in the endothelial cells from the *Egr-1* KO mice (60.3% of WT/ST; $q = 12.67$, $P < 0.05$) [Figure 5c]. Consistently, the *Icam-1* protein level in the endothelial cells from the *Egr-1* KO mice was significantly reduced (53.7% of WT/ST; $q = 8.97$, $P < 0.05$) compared to the endothelial cells from the WT mice after mechanical stretch stimulation for 3 h [Figure 5d].

DISCUSSION

MiRNAs have been a hot topic for research in the past few decades. Many pathological processes are regulated by miRNAs. McDonald *et al.*[17] suggested that miR-21 promoted vascular smooth muscle cell and fibroblast proliferation by regulating the expression of phosphatase and tensin homolog deleted on chromosome 10, signal transducer and activator of transcription 3, and proliferation cell nuclear antigen. Ohnaka *et al.*[18] revealed that upregulation of miR-145 induced the phenotypic transformation of vascular smooth muscle cells in rabbit vein graft models. The study focused on the effects of miRNAs in endothelial dysfunction induced by mechanical stretch. The results showed that 38 miRNAs were differentially expressed in HUVECs treated with mechanical stretch stimulation. With GO and KEGG analyses, we found that the potential target genes of these miRNAs were involved in many pathological processes and were related to VGF. Therefore, the differentially expressed miRNAs might play important roles in endothelial dysfunction, resulting in VGF.

Currently, miR-551b-5p was rarely studied and was only found to be effective in the assessment of pancreatic injury
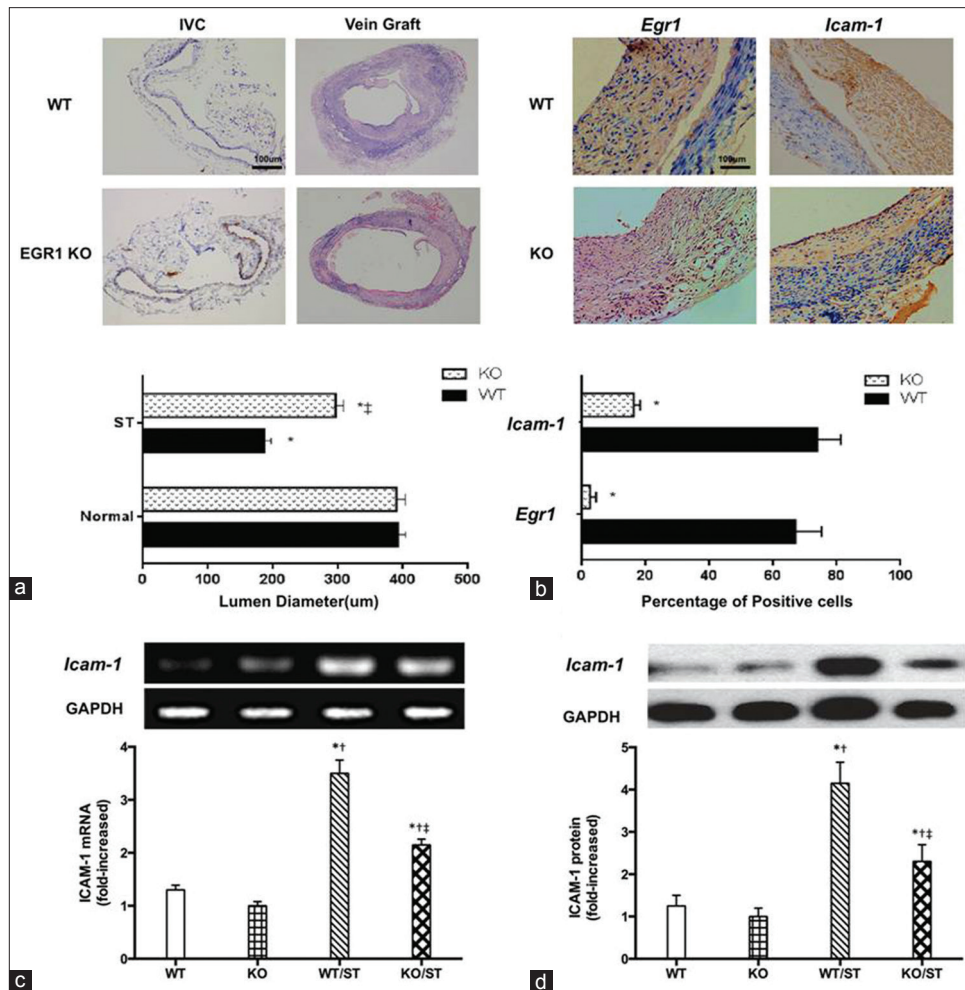
Figure 5: *Egr-1* upregulated Icam-1 expression to induce endothelial dysfunction (*n* = 3 in each group). (a) Vein grafts from *Egr-1* KO mice and WT mice were stained with HE staining (Scale bar: 100 μm). (b) Vein grafts from the *Egr-1* KO and WT mice were stained with *Egr-1* and *Icam-1* monoclonal antibodies. A brown color indicates positive staining for all primary antibodies (Scale bar: 100 μm). (c) *Egr-1* KO downregulated *Icam-1* mRNA expression, which was determined by real-time RT-PCR. (d) *Egr-1* KO downregulated *Icam-1* protein expression levels after mechanical stretch stimulation. *$P < 0.05$ versus WT group; †$P < 0.05$ versus KO group; ‡$P < 0.05$ versus WT/ST group; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; WT: Wide type mice; KO: *Egr-1* knockout mice; WT/ST: Endothelium cells isolated from the veins of WT mice and subjected to mechanical stretch stimulation; KO/ST: Endothelium cells isolated from the veins of *Egr-1* KO mice and subjected to mechanical stretch stimulation; *Egr*: Early growth response; mRNA: Messenger RNA; *Icam-1*: Intercellular adhesion molecule-1.

during the acute phase of pancreatitis.[19] However, literatures investigated the roles of miR-551b-5p in VGF were rare. In this study, miR-551b-5p was found to have a potential to regulate EGR1 expression, which is a critical factor in VGF,[10,15,16] indicating that miR-551b-5p might be involved in the process of VGF via regulating of EGR-1 expression. To identify this hypothesis, we exposed HUVECs in mechanical stretch stimulation, and found that the expression of miR-551b-5p was significantly increased, compared to normal HUVECs. Moreover, upregulation of miR-551b-5p expression was found to promote HUVECs proliferation in this study. To explore the effect of miR-551b-5p on EGR-1 expression, we transfected HUVECs with a mimic and inhibitor, and found that upregulation of miR-551b-5p enhanced EGR-1 mRNA and protein expression, whereas inhibition of miR-551b-5p suppressed EGR-1 mRNA and protein expression after stimulation by mechanical stretch.

These results suggest that miR-551b-5p might be involved in the regulation of EGR-1 expression. However, miR-551b-5p was found to increase by 793-fold, while EGR-1 only increased by 1.9-fold. The potential mechanism is uncertain. More studies need to be conducted to further investigate on this issue. Moreover, it was found that miR-551b-5p inhibitor could not completely block EGR-1 expression, which possibly due to incomplete suppression of miR-551b-5p by the inhibitor. In addition to that, there are some other microRNAs and cellular factors which might contribute to the regulation of EGR-1 expression. For instance, one study[20] found that the let-7 family miRNAs downregulated EGR-1 expression. In this study, let-7a-5p expression decreased after mechanical stretch stimulation, which might promote EGR-1 expression. Therefore, the results suggest that miR-551b-5p might be the major regulatory factor underlying EGR-1 expression in VGF.

Endothelial dysfunction plays key roles in the processes of hypertension, diabetes, atherosclerosis, and many other diseases.[21] After CABG surgery, vein grafts are stimulated by high arterial blood pressure, and endothelial cells are injured by mechanical stretch.[22] ICAM-1 has been widely known for its effect in endothelial dysfunction.[23] In this study, we also investigated the effect of *Egr-1* in regulation of *Icam-1* expression in VGF model in mice. Similar with the results of our previous study,[24] it was shown that the lumens of the vein grafts in the *Egr-1* KO mice were wider than the lumens in the WT mice and *Icam-1* expression was suppressed significantly in the *Egr-1* KO vein grafts, indicating that *Egr-1* promoted VG restenosis via upregulation of *Icam-1* expression and suppression of *Egr-1* expression could improve the function of a grafted vein.

In conclusion, mechanical stretch increased miR-551b-5p expression, which promoted HUVECs proliferation and upregulated EGR-1 expression, thereby inducing endothelial dysfunction via upregulating of ICAM-1. Given the above evidence, miR-551b-5p has the potential to be a novel therapeutic target for the treatment of CAD patients after CABG.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Goldman S, Zadina K, Moritz T, Ovitt T, Sethi G, Copeland JG, *et al.* Long-term patency of saphenous vein and left internal mammary artery grafts after coronary artery bypass surgery: Results from a Department of Veterans Affairs Cooperative Study. J Am Coll Cardiol 2004;44:2149-56. doi: 10.1016/j.jacc.2004.08.064.
2. Fitzgibbon GM, Kafka HP, Leach AJ, Keon WJ, Hooper GD, Burton JR. Coronary bypass graft fate and patient outcome: Angiographic follow-up of 5,065 grafts related to survival and reoperation in 1,388 patients during 25 years. J Am Coll Cardiol 1996;28:616-26. doi: 10.1016/0735-1097(96)00206-9.
3. Bourassa MG. Fate of venous grafts: The past, the present and the future. J Am Coll Cardiol 1991;17:1081-3. doi: 10.1016/0735-1097(91)90835-W.
4. Li FD, Sexton KW, Hocking KM, Osgood MJ, Eagle S, Cheung-Flynn J, *et al.* Intimal thickness associated with endothelial dysfunction in human vein grafts. J Surg Res 2013;180:e55-62. doi: 10.1016/j.jss.2012.06.017.
5. Momin A, Sharabiani MT, Wendler O, Angelini GD, Desai J. Arterial blood pressure and vascular function in human saphenous vein. Perfusion 2015;30:233-8. doi: 10.1177/0267659114540021.
6. Li FD, Eagle S, Brophy C, Hocking KM, Osgood M, Komalavilas P, *et al.* Pressure control during preparation of saphenous veins. JAMA Surg 2014;149:655-62. doi: 10.1001/jamasurg.2013.5067.
7. Li W, Wang C, Peng J, Liang J, Jin Y, Liu Q, *et al.* Naringin inhibits TNF-a induced oxidative stress and inflammatory response in HUVECs via Nox4/NF-? B and PI3K/Akt pathways. Curr Pharm Biotechnol 2014;15:1173-82. doi: 10.2174/13892010

8. Shin IS, Kim JM, Kim KL, Jang SY, Jeon ES, Choi SH, *et al.* Early growth response factor-1 is associated with intraluminal thrombus formation in human abdominal aortic aneurysm. J Am Coll Cardiol 2009;53:792-9. doi: 10.1016/j.jacc.2008.10.055.
9. Pagel JI, Deindl E. Disease progression mediated by egr-1 associated signaling in response to oxidative stress. Int J Mol Sci 2012;13:13104-17. doi: 10.3390/ijms131013104.
10. Harja E, Bucciarelli LG, Lu Y, Stern DM, Zou YS, Schmidt AM, *et al.* Early growth response-1 promotes atherogenesis: Mice deficient in early growth response-1 and apolipoprotein E display decreased atherosclerosis and vascular inflammation. Circ Res 2004;94:333-9. doi: 10.1161/01.res.0000112405.61577.95.
11. Chamorro-Jorganes A, Araldi E, Suárez Y. MicroRNAs as pharmacological targets in endothelial cell function and dysfunction. Pharmacol Res 2013;75:15-27. doi: 10.1016/j.phrs.2013.04.002.
12. Wang J, Chen S, Ma X, Cheng C, Xiao X, Chen J, *et al.* Effects of endothelial progenitor cell-derived microvesicles on hypoxia/reoxygenation-induced endothelial dysfunction and apoptosis. Oxid Med Cell Longev 2013;2013:572729. doi: 10.1155/2013/572729.
13. Sen A, Ren S, Lerchenmüller C, Sun J, Weiss N, Most P, *et al.* MicroRNA-138 regulates hypoxia-induced endothelial cell dysfunction by targeting S100A1. PLoS One 2013;8:e78684. doi: 10.1371/journal.pone.0078684.
14. Zou Y, Dietrich H, Hu Y, Metzler B, Wick G, Xu Q. Mouse model of venous bypass graft arteriosclerosis. Am J Pathol 1998;153:1301-10. doi: 10.1016/s0002-9440(10)65675-1.
15. Autieri MV, Kelemen SE, Gaughan JP, Eisen HJ. Early growth responsive gene (Egr)-1 expression correlates with cardiac allograft rejection. Transplantation 2004;78:107-11. doi: 10.1097/01.TP.0000132325.26299.D5.
16. Okada M, Wang CY, Hwang DW, Sakaguchi T, Olson KE, Yoshikawa Y, *et al.* Transcriptional control of cardiac allograft vasculopathy by early growth response gene-1 (Egr-1). Circ Res 2002;91:135-42. doi: 10.1161/01.RES.0000027815.75000.33.
17. McDonald RA, White KM, Wu J, Cooley BC, Robertson KE, Halliday CA, *et al.* miRNA-21 is dysregulated in response to vein grafting in multiple models and genetic ablation in mice attenuates neointima formation. Eur Heart J 2013;34:1636-43. doi: 10.1093/eurheartj/eht105.
18. Ohnaka M, Marui A, Yamahara K, Minakata K, Yamazaki K, Kumagai M, *et al.* Effect of microRNA-145 to prevent vein graft disease in rabbits by regulation of smooth muscle cell phenotype. J Thorac Cardiovasc Surg 2014;148:676-82.e2. doi: 10.1016/j.jtcvs.2013.11.054.
19. Kusnierz-Cabala B, Nowak E, Sporek M, Kowalik A, Kuzniewski M, Enguita FJ, *et al.* Serum levels of unique miR-551-5p and endothelial-specific miR-126a-5p allow discrimination of patients in the early phase of acute pancreatitis. Pancreatology 2015;15:344-51. doi: 10.1016/j.pan.2015.05.475.
20. Worringer KA, Rand TA, Hayashi Y, Sami S, Takahashi K, Tanabe K, *et al.* The let-7/LIN-41 pathway regulates reprogramming to human induced pluripotent stem cells by controlling expression of prodifferentiation genes. Cell Stem Cell 2014;14:40-52. doi: 10.1016/j.stem.2013.11.001.
21. Gimbrone MA Jr., García-Cardeña G. Vascular endothelium, hemodynamics, and the pathobiology of atherosclerosis. Cardiovasc Pathol 2013;22:9-15. doi: 10.1016/j.carpath.2012.06.006.
22. Anwar MA, Shalhoub J, Lim CS, Gohel MS, Davies AH. The effect of pressure-induced mechanical stretch on vascular wall differential gene expression. J Vasc Res 2012;49:463-78. doi: 10.1159/000339151.
23. Liao JK. Linking endothelial dysfunction with endothelial cell activation. J Clin Invest 2013;123:540-1. doi: 10.1172/jci66843.
24. Zhang K, Cao J, Dong R, Du J. Early growth response protein 1 promotes restenosis by upregulating intercellular adhesion molecule-1 in vein graft. Oxid Med Cell Longev 2013;2013:432409. doi: 10.1155/2013/432409.

155666141111114442.