Original Paper

Down-Regulation of MicroRNA-137 Improves High Glucose-Induced Oxidative Stress Injury in Human Umbilical Vein Endothelial Cells by Up-Regulation of AMPK α 1

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Key Words

MicroRNA-137 • HUVEC • High glucose • Oxidative stress • AMPK α 1

Abstract

Background/Aims: To investigate the effects of miR-137 on high glucose (HG)-induced vascular injury, and to establish the mechanism underlying these effects. **Methods:** Human umbilical vein endothelial cells (HUVECs) were transfected with miR-137 inhibitor or mimic, and then treated with normal or high glucose. Cell viability and apoptosis were detected by using the Cell Counting Kit-8 (CCK-8) assay and flow cytometry, respectively. Reactive oxygen species (ROS), malondialdehyde (MDA), and superoxide dismutase (SOD) were detected by fluorescent probe (DCFH-DA), thiobarbituric acid reaction, and the nitroblue tetrazolium assay, respectively. The mRNA and protein expressions of AMPK α 1 were determined by qRT-PCR and Western blotting. **Results:** Down-regulation of miR-137 dramatically reverted HG-induced decreases in cell viability and SOD levels and increases in apoptosis, ROS and MDA levels. Moreover, bioinformatics analysis predicted that the AMPK α 1 was a potential target gene of miR-137. Luciferase reporter assay demonstrated that miR-137 could directly target AMPK α 1. AMPK α 1 overexpression had the similar effect as miR-137 inhibition. Down-regulation of AMPK α 1 in HUVECs transfected with miR-137 inhibitor partially reversed the protective effect of miR-137 inhibition on HG-induced oxidative stress in HUVECs. **Conclusion:** Down-regulation of miR-137 ameliorates HG-induced injury in HUVECs by overexpression of AMPK α 1, leading to increasing cellular reductive reactions and decreasing oxidative stress. These results provide further evidence for protective effect of miR-137 inhibition on HG-induced vascular injury.

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Introduction

Diabetes mellitus (DM) is characterized by a metabolic disorder that constitutes a major global health problem [1]. Until 2030, it is estimated that great changes in nutrition and lifestyle in developing countries in Asia and the Middle East will lead to the largest increases in the prevalence of type 2 DM (T2DM) throughout the world [2, 3]. Cardiovascular disease (CVD) is one of the most important complications of T2DM [4, 5].

More and more evidence showed that oxidative stress is closely related to the pathogenesis of CVD and heart failure [6]. Oxidative stress plays an important role in the pathogenesis of beta-cell dysfunction and insulin resistance, which are the two most relevant mechanisms in the pathophysiology of T2DM and its vascular complications [1, 6-8]. Oxidative stress is induced by the persistent high glucose (HG) levels in T2DM patients, contributing to vascular injury [9]. Oxidative stress can produce reactive oxygen species (ROS) including superoxide anions, hydrogen peroxide, and hydroxyl radicals [10]. Moreover, alterations of ROS levels may cause impaired homeostasis and associated pathologies [6].

MiRNAs are small (about 22 nucleotides in length), non-coding RNAs, and miRNAs target mRNAs, resulting in inducing targeted mRNAs degradation or their protein translational repression and regulating a series of cell functions such as proliferation, differentiation, invasion and apoptosis, by binding to complementary sequences in the 3'UTRs of targeted mRNAs [11, 12]. Increasing evidence indicated that miRNAs are involved in oxidative stress [13]. Lots of miRNAs have been identified to have protective effect or damage effect, which is dependent on the role of their target genes, including miR-424 [14], miR-25 [15], miR-103 [16], miR-144 [17] and miR-122 [18]. These outcomes show a strong basis for the importance of miRNAs in the pathogenesis of CVD.

For the treatment of diabetic complications, one of the major approaches is to protect vascular cells from oxidative stress injury [19]. Recently, dysregulation of AMP-activated protein kinase (AMPK α 1) signaling has been implicated in oxidative stress-induced vascular and kidney dysfunction [20-22]. Although the effects of miR-137 have been linked to oxidative stress [23], the effects of miR-137 and its precise mechanisms remain incompletely unclear. Therefore, the aim of the present study was to investigate the effect of miR-137 on HG-induced vascular injury and clarify the mechanisms underlying these effects.

In this paper, down-regulation of miR-137 ameliorates HG-induced injury in HUVECs by overexpression of AMPK α 1, leading to increasing cellular reductive reactions and decreasing oxidative stress. Moreover, we found that AMPK α 1 was the direct target of miR-137 in HUVECs. AMPK α 1 overexpression had the similar effect as miR-137 inhibition. Down-regulation of AMPK α 1 in HUVECs partially reversed the protective effect of miR-137 inhibition on HG-induced oxidative stress in HUVECs. Therefore, our outcomes showed critical roles for miR-137 in the pathogenesis of CVD and suggested its possible application in HG-induced vascular injury treatment.

Materials and Methods

Cell culture and transient transfection

Human umbilical vein endothelial cells (HUVECs) were isolated and cultured as previously described. Briefly, HUVECs were removed from human umbilical veins after 0.125% trypsin digestion, and cultured in medium 199 containing 20% fetal calf serum, penicillin (100 U/ml), streptomycin (100 U/ml), and heparin (50 U/ml), supplemented with L-glutamine (2 mM), sodium pyruvate (1 mM) (Gibco, USA), and bFGF (5 ng/ml) (R&D Co., USA), at 37°C in 5% CO₂ on 0.1% gelatin-coated culture flasks. Endothelial cells were identified by their morphology which appears "cobblestone" mosaic appearance after reaching confluence and the presence of von Willebrand factor. Passage 3-6 HUVECs were used for experiments.

The miR-137 inhibitor, miR-negative control of inhibitor (anti-miR-NC), the miR-137 mimic, miR-negative control of mimic (miR-NC), siRNA for AMPK (si-AMPK α 1), siRNA-negative control (si-NC), pcDNA3.1-AMPK α 1 and pcDNA3.1 vector were synthesized and purified by Gene-Pharma (Shanghai, China). MiR-137 inhibitor (100 nM), mimic (50 nM), anti-miR-NC (100 nM), miR-NC (50 nM), si-NC (100 nM) and

si-AMPK α 1 (100 nM) were transfected into HUVECs by using Lipofectamine 3000 reagent (Invitrogen, USA) following the manufacturer's instructions.

Construction of Plasmids

The 3'-UTR sequences of AMPK α 1 gene, containing the putative miR-137 binding site, was amplified by PCR and cloned into the pGL3-control vector (Promega), which was named wild-type 3'-UTR (WT 3'-UTR). Point mutations in the putative miR-137 binding seed regions were carried out using the Quick-Change Site-Directed Mutagenesis kit (SBS Genetech, Beijing, China) following the manufacturer's instruction. The resultant product served as the mutated 3'-UTR (MUT 3'-UTR). Both the wild-type and mutant insert fragments sequences were confirmed by DNA sequencing.

RNA extraction and reverse transcription polymerase chain reaction

Total RNA of HUVECs was isolated by Trizol reagent (Invitrogen, USA) following the manufacturer's protocol. For quantification of miR-137, the TaqMan MicroRNA Reverse Transcription Kit and TaqMan miRNA assay (Applied Biosystems, Foster City, CA, USA) were used to perform reverse transcription and PCR according to the manufacturer's instructions. U6 small nuclear RNA was used as the internal control. The SYBR Green RT-PCR kits (TAKARA, Japan) were used to perform the qRT-PCR analyses for mRNA of AMPK α 1. AMPK α 1 expression was normalized to GAPDH. The following primers were used: miR-137 forward, 5'-GCG CTT ATT GCT TAA GAA TAC-3', reverse, 5'-CAG TGC AGG GTC CGA GGT-3'; AMPK α 1 forward, 5'-CTC ACCT CCT CCA AGT TATT-3', reverse, 5'-TCA GAT GGG CTT ATA CAGC-3'; U6 forward, 5'-CTC GCT TCG GCA GCACA-3', reverse, 5'-AAC GCT TCA CGA ATT TCGCT-3'; GAPDH forward, 5'-TCA ACG ACC ACT TTG TCA AGC TCA-3', reverse, 5'-GCT GGT GGT CCA GGG GTC TTACT-3'. Each sample was assessed in triplicate.

Cell viability assay

The viability of HUVECs was determined by using the Cell Counting Kit-8 assay (CCK-8, Sigma, USA). HUVECs (1×10^4 cells/well) were seeded in 96-well plates for 24 h. After that, cells were transfected with miR-137 inhibitor or mimic for 24 h. Then, cells were treated with HG for 24 h, and then incubated with WST-8 substrate at 37°C for 2 h. Absorbance (450 nm) of the medium was detected using a spectrophotometer by assessing the cell viability.

Detection of apoptosis by flow cytometry

After treatments, HUVECs were double-stained by using an Annexin V-FITC apoptosis detection kit (Nanjing KeyGen Biotech Co., Nanjing, China) following the manufacturer's protocols. Samples stained with Annexin V and PI were quantitatively analyzed at 488 nm emission and 570 nm excitation by Flow Cytometry (BD FACScalibu; BD Biosciences, San Jose, CA, USA).

Reactive oxygen species (ROS) detection

ROS of HUVECs was detected by the 2', 7'-dichlorofluorescein diacetate (DCFH-DA). To investigate the effects of miR-137 on HG-induced ROS production, HUVECs were transfected with miR-137 inhibitor or mimic for 24 h and then incubated with 25 mM glucose for 24 h, cells were loaded with DCFH-DA (10 μ M) in serum free medium M199 for 30 min at 37°C in 96-well bottom black plates. The fluorescence intensity of the 96-well plates was quantified by Infinite F500 Microplate Reader (TECAN, Switzerland) and is standardized to milligram protein.

Lipid peroxidation assay

The level of malondialdehyde (MDA) was examined by Lipid Peroxidation Malondialdehyde (MDA) Assay Kit (Beyotime Institute of Biotechnology, Jiangsu, China) according to the manufacturer's protocol. Briefly, after the treatment, HUVECs were lysed by RIPA lysis, and then centrifuged at 1600 $\times$ g for 10 min to discard debris. The MDA level and the protein concentration of the supernatant were detected. Lastly, the MDA level was standardized to milligram protein.

Measurement of intracellular superoxide dismutase (SOD) activities

After treatment, the medium in the 96-well plates was removed and the HUVECs were washed with PBS twice, then the cells were lysed by Freeze Thaw method for three times. The activities of SOD in cell

lysate were determined by using the SOD activity assay kit (Nanjing Institute of Jiancheng Bioengineering, Nanjing, China) according to the manufacturer's protocol.

Western blot analysis

HUVECs were lysed using protein lysis buffer and protease inhibitor cocktail. The protein concentration of cell lysates was quantified by BCA Kit (Beyotime Institute of Biotechnology, Jiangsu, China), and 50 ng of protein were separated by SDS-PAGE and then transferred onto a PVDF membrane (Millipore, USA). The membranes were blocked in 5% non-fat dry milk diluted with TBST (in mmol/L: 20 mM Tris-HCl pH7.5, 150 mM NaCl, 0.1% Tween 20) at room temperature for 1 h and probed overnight at 4°C with a monoclonal rabbit anti-AMPK α 1 (Cell Signaling Technology, USA), and then incubated for 1 h with a goat anti-rabbit IgG conjugated to horseradish peroxidase (Cell Signaling Technology, USA). Incubation with monoclonal mouse α -tubulin antibody (1:1000 dilution; Sigma, USA) was performed as the loading sample control. The proteins were visualized using ECL western blotting detection reagents (Millipore, USA). The densitometry of the bands was quantified using the Image J 1.38X software (USA).

Luciferase reporter assay

HUVECs (1×10^5 /well) were seeded in 24-well plates and incubated for 24 h before transfection. Cells were cotransfected with pGL3-AMPK α 1-3'UTR wild-type or mutant reporter plasmid, miR-137 inhibitor and anti-miR-NC or mimic and miR-NC, and pRL-TK Renilla luciferase reporter (Promega, USA) using Lipofectamine 2000. At 24 h after transfection, both firefly and renilla luciferase activities were quantified using the Dual-Luciferase reporter system (Promega, USA) according to the manufacturer's instructions. All experiments were performed in triplicate.

Statistical analysis

Experiments were repeated at least three times. Values are expressed as mean $\pm$ standard error of the mean (S.E.M.). Data were evaluated for statistical significance by analysis using one-way analysis of variance (ANOVA). $P < 0.05$ was considered to indicate a statistically significant difference. All statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Software, Inc., USA).

Results

Effect of miR-137 on HG-induced oxidative stress injury in HUVECs

To evaluate the effect of miR-137 on HG-induced cellular oxidative stress, HUVECs were transfected with miR-137 inhibitor at 0, 25, 50 or 100 nM for 24 h, and then treated with 5.5 mM or 25 mM glucose for 24 h, after which cell viability and apoptosis rate were measured. Compared with untreated controls, HG significantly suppressed cell viability and promoted cell apoptosis, whereas miR-137 down-regulation significantly increased cell viability and reduced apoptosis of HG-treated HUVECs (Fig. 1A, B). Next, cell viability and apoptosis were measured in HG-treated HUVECs transfected with 0, 25, 50 or 100 nM miR-137 mimic for 24 h. As shown in Fig. 1C and D, miR-137 significantly decreased cell viability and reduced apoptosis of HG-treated HUVECs after incubation for 24 h. We then evaluated the levels of miR-137 upon HG-induced oxidative stress in HUVECs. The results showed that miR-137 was significantly up-regulated by HG in a time-dependent manner (Fig. 1E). However, miR-137 had no effects on cell viability and apoptosis of HUVECs in normal condition (Fig. 1F, G).

We then measured ROS and MDA as indicators of the cellular oxidative state, and SOD as an indicator of cellular reductive state. HG significantly increased HUVECs ROS and MDA levels and decreased cellular SOD levels. Compared with HG-only groups, miR-137 down-regulation significantly decreased ROS and MDA levels, and increased the SOD activities (Fig. 2A). On the contrary, miR-137 up-regulation evidently increased ROS and MDA levels, and decreased the SOD activities compared to HG-only groups (Fig. 2B). These findings indicated that miR-137 down-regulation protected HUVECs from HG-induced oxidative stress in HUVECs.

Fig. 1. Effects of miR-137 on HG-induced cell viability and apoptosis in HUVECs. HUVECs were transfected with miR-137 inhibitor and anti-miR-NC (50 nM) or miR-137 mimic and miR-NC (50 nM), and then treated with 5.5 or 25 mM glucose for 24 h. (A, C, F) Cell viability of HUVECs was detected by CCK-8 assay. (B, D, G) Cell apoptosis of HUVECs was determined by flow cytometry using Annexin V/PI staining detected. (E) The level of miR-137 was detected by RT-PCR in HG-induced HUVECs. Control: without treatment; M: mannitol (isotonic group); HG: high glucose. The data shown are mean $\pm$ SEM ($n = 6$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, vs. vehicle + HG or miR-NC/anti-miR-NC + HG.

AMPK α 1 is a direct target of miR-137 in HUVECs

The online database (TargetScan 6.2) predicted that AMPK α 1 was a binding target of miR-137, we performed qRT-PCR and Western blotting to detect the expression of AMPK α 1 on mRNA and protein levels in HG-induced HUVECs transfected with miR-137 inhibitor or mimic. We found that mRNA and protein levels of AMPK α 1 was remarkably decreased after

Fig. 2. Effects of miR-137 on HG-induced oxidative stress injury in HUVECs. HUVECs were transfected with miR-137 inhibitor or mimic, and then treated with 5.5 or 25 mM glucose for 24 h. (A, B) SOD activities were detected by nitroblue tetrazolium assay. ROS production was stained by 10 μ M DCFH-DA for 30 min, whose oxidation product (DCF) fluorescence indicated ROS formation. Cells were seeded in clear bottom black 96 wells plates. Then cells were treated as above description. The DCFH-DA fluorescence intensity was measured in Multi-Mode Microplate Reader. MDA levels were detected by thiobarbituric acid reaction. The data are expressed at μ mol/mg. The data shown are mean $\pm$ SEM (n = 6). ** P < 0.01, *** P < 0.001, vs. control; ## P < 0.01, vs. vehicle + HG or miR-NC/anti-miR-NC + HG.

up-regulation of miR-137 (Fig. 3A), but was evidently increased after down-regulation of miR-137 (Fig. 3A). To further demonstrate whether AMPK α 1 was a direct target of miR-137, AMPK α 1 3'-UTR was cloned into a luciferase reporter vector and the putative miR-137 binding site in the AMPK α 1 3'-UTR was mutated (Fig. 3B). The effect of miR-137 was determined using luciferase reporter assay. The results showed that up-regulation or down-regulation of miR-137 significantly inhibited or promoted the luciferase activity of pGL3-

Fig. 3. AMPK α 1 was a direct target of miR-137. HUVECs were transfected with miR-137 inhibitor or mimic, and then treated with 5.5 or 25 mM glucose for 24 h. (A) The mRNA and protein levels of AMPK α 1 were determined by qRT-PCR and Western blot in HUVECs transfected with miR-137 inhibitor or mimic. AMPK α 1 expression was normalized to GAPDH in qRT-PCR. α -tubulin was detected as a loading control in Western blot. (B) Schematic representation of AMPK α 1 3'UTRs showing putative miRNA target site. (C) The analysis of the relative luciferase activities of AMPK α 1-WT, AMPK α 1-MUT in HUVECs. All data are presented as mean $\pm$ SEM, n = 6. ## P < 0.01 vs. miR-NC/anti-miR-NC + HG or anti-miR-NC/miR-NC.

AMPK α 1 3'-UTR WT (Fig. 3C). Mutation of the miR-137-binding site in the AMPK α 1 3'-UTR abolished the effect of miR-137, which suggested that AMPK α 1 was directly and negatively regulated by miR-137.

Up-regulation of AMPK α 1 had similar effects with miR-137 inhibition

To explore the function of AMPK α 1, HUVECs were transfected with pcDNA-AMPK α 1. Western blot analysis indicated that protein expression of AMPK α 1 was significantly increased after 24 hours in HUVECs transfected with pcDNA-AMPK α 1 (Fig. 4A). The CCK-8 assay revealed that up-regulation of AMPK α 1 enhanced HG-induced viability of HUVECs (Fig. 4B). Furthermore, AMPK α 1 overexpression evidently decreased ROS and MDA levels, and increased the SOD activities compared to HG-only groups (Fig. 4C-E). These results indicated that miR-137 inhibition up-regulated the expression of AMPK α 1, thus protecting HUVECs from HG-induced oxidative stress injury.

Overexpression of AMPK α 1 is essential for protective effect of miR-137 on HG-induced oxidative stress injury in HUVECs

Firstly, we studied the effect of si-AMPK α 1 alone on HUVECs under normal condition. Then, we found that si-AMPK α 1 could significantly decrease AMPK α 1 expression of HUVECs, and had no effects on cell viability and ROS level compared with control group (Fig. 5). Next, to determine whether miR-137 inhibition protected HUVECs from HG-induced oxidative stress injury in an AMPK α 1-dependent manner, we cotransfected HUVECs with miR-137 inhibitor and si-AMPK α 1. We found that the expression of AMPK α 1 was significantly decreased after transfection with miR-137 inhibitor and si-AMPK α 1 compared with miR-137 inhibitor and si-NC in HUVECs (Fig. 6A). Analysis by CCK-8 assay indicated that down-regulation of AMPK α 1 in cells transfected with the miR-137 inhibitor decreased HG-induced

Fig. 4. The effects of AMPK α 1 overexpression on HG-induced cell viability and oxidative stress injury in HUVECs. HUVECs were transfected with pcDNA-AMPK α 1 or pcDNA3.1, and then treated with 5.5 or 25 mM glucose for 24 h. (A) The protein expression of AMPK α 1 was determined by Western blot. α -tubulin was detected as a loading control. (B) Cell viability was assessed by CCK-8 assay. (C) ROS production was stained by 10 μ M DCFH-DA. The DCFH-DA fluorescence intensity was measured in Multi-Mode Microplate Reader. (D) MDA levels were detected by thiobarbituric acid reaction. (E) SOD activities were detected by nitroblue tetrazolium assay. All data are presented as mean $\pm$ SEM, $n = 6$. ** $P < 0.01$, *** $P < 0.001$, vs. control; ## $P < 0.01$, ### $P < 0.001$ vs. HG or HG + pcDNA3.1.

Fig. 5. The effects of si-AMPK α 1 alone on normal HUVECs. HUVECs were transfected with or without si-AMPK α 1 at 5.5 mM glucose for 24 h. The protein expression of AMPK α 1 was determined by Western blot. α -tubulin was detected as a loading control. Cell viability was assessed by CCK-8 assay. ROS production was stained by 10 μ M DCFH-DA. The DCFH-DA fluorescence intensity was measured in Multi-Mode Microplate Reader. All data are presented as mean $\pm$ SEM, $n = 6$. ### $P < 0.001$ vs. control.

the viability of HUVECs by down-regulation of miR-137 (Fig. 6B). Moreover, the results also showed that down-regulating AMPK α 1 expression could reverse the protective effect of miR-137 inhibition on HG-induced oxidative stress injury in HUVECs (Fig. 6C-E). Our results clearly demonstrated that down-regulation of miR-137 improved HG-induced decreases in cell viability and SOD levels and increases in apoptosis, ROS and MDA levels in HUVECs by up-

Fig. 6. AMPKα1 was involved in the effects of miR-137 on HG-induced cell viability and oxidative stress injury in HUVECs. HUVECs were transfected with either miR-137 inhibitor with or without si-AMPKα1, and then treated with 5.5 or 25 mM glucose for 24 h. (A) The protein expression of AMPKα1 was determined by Western blot. α-tubulin was detected as a loading control. (B) Cell viability was assessed by CCK-8 assay. (C) ROS production was stained by 10 μM DCFH-DA. The DCFH-DA fluorescence intensity was measured in Multi-Mode Microplate Reader. (D) MDA levels were detected by thiobarbituric acid reaction. (E) SOD activities were detected by nitroblue tetrazolium assay. All data are presented as mean ± SEM, n = 6. ** P < 0.01, *** P < 0.001, vs. control; ## P < 0.01 vs. HG; &P < 0.05, &&P < 0.01 vs. HG + miR-137 inhibitor + si-NC.

regulation of AMPKα1, and that overexpression of AMPKα1 was essential for the protective effect of miR-137 inhibition HG-induced oxidative stress injury in HUVECs.

Discussion

Due to contribution of HG-induced vascular injury in DM to the pathology of CVD [6, 19, 24], it is an important approach to protect vascular endothelial cells from such injury for the treatment of CVD in DM patients. In this study, we explored the effect of miR-137, a

miRNA with beneficial effects in many disease models [25-28], on cell viability of HG-induced HUVECs. The anticipated HG-induced inhibitory effect on viability of HUVECs was abolished by down-regulation of miR-137, suggesting the protective function of miR-137 inhibition in HG-induced vascular cell injury.

Oxidative stress is characterized by increasing cellular oxidation reactions and decreasing reduction reactions [7], which has a close relationship with HG-induced vascular injury [29-31]. Oxidative stress was excessively increased by impairing antioxidant defense, contributing to the development of T2DM and diabetic CVD [32]. It has been reported that high glucose can significantly cause oxidative stress of HUVECs, and restoring AMPK α /ACC (acetyl-CoA carboxylase) signaling protected HUVECs from HG-induced oxidative stress [33]. Our findings showed that miR-137 inhibition could modulate levels of ROS and the two key oxidoreductive enzymes such as MDA and SOD to reverse HG-induced oxidative stress in HUVECs.

At recent years, miRNAs are considered as the critical regulators, which contribute to regulation of multiple biological processes including oxidative stress [34-38]. It has been shown that many of the miRNA families play important roles in oxidative stress, and different miRNAs have contrary functions [16-18, 39, 40]. For example, overexpression of miR-103 significantly reduced oxidative stress induced by H₂O₂ by targeting the BCL2/Adenovirus E1B 19 kDa interacting protein 3 [16]. Gao et al. demonstrates that miR-214 protects erythroid cells against oxidative stress by ATF4 and EZH2 [39]. Inhibiting miR-144 abates oxidative stress and reduces apoptosis in hearts of streptozotocin-induced diabetic mice [17]. A previous study showed that miR-137 was up-regulated in HG-induced cardiomyocyte [23], suggesting that miR-137 may play an important role in HG-induced oxidative stress. Wang et al. demonstrated that miR-137 negatively regulated H₂O₂-induced cardiomyocyte apoptosis by targeting CDC42 [23]. However, there has been no report on whether miR-137 is differentially expressed in pathological HUVECs, or if there are any functional roles of miR-137 in regulating HG-induced vascular injury. In this paper, we demonstrated that the level of miR-137 was also markedly up-regulated in HUVECs during the process of H₂O₂-induced oxidative stress. Most importantly, we showed miR-137 down-regulation enhanced viability and reduced apoptosis in HG-induced HUVECs. Thus, this is the first report to show differential expression of miR-137 and the functional role of miR-137 in HG-induced vascular injury.

AMPK plays an important role in multiple signaling pathways that are involved in cell proliferation, migration, apoptosis and tumor metastasis [19, 28]. Increasing evidence show that AMPK has been linked to oxidative stress, insulin resistance and autophagy in DM patients [23, 24], but the exact relationship between AMPK and oxidative stress is controversial. Some studies showed that AMPK inactivation was linked to low levels of oxidative stress, and that AMPK activation reversed these effects [41, 42]. However, other studies demonstrated that AMPK inactivation was closely related to increased oxidative stress, and that AMPK reactivation decreased levels of oxidative stress [22, 43-46]. In this study, our results showed that HG-induced oxidative stress was closely related to down-regulation of AMPK α 1 expression. Moreover, AMPK α 1 expression was up-regulated by miR-137 down-regulation. Next, overexpression of AMPK α 1 had the same protective effects as miR-137 down-regulation, while the protective effects of miR-137 inhibition in this study were almost completely abolished by transfection with si-AMPK α 1. Taken together, these results demonstrated that down-regulation of miR-137 protected HUVECs from HG-induced oxidative stress injury in an AMPK signaling-dependent manner. These results support the theory that oxidative stress injury is caused by AMPK-inactivation, and that AMPK-reactivation is restorative.

Our findings showed that miR-137 inhibition protected HG-induced vascular endothelial cells via an AMPK-dependent mechanism. Down-regulation of miR-137 ameliorates HG-induced injury in HUVECs by overexpression of AMPK α 1, leading to increasing cellular reductive reactions and decreasing oxidative stress. In conclusion, this study provided the novel miR-137/AMPK α 1 axis that provides new insights into the molecular mechanisms

underlying HG-treated HUVECs, and down-regulation of miR-137 expression might be a possible therapeutic strategy for the treatment of HG-induced vascular injury in the future.

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Disclosure Statement

The authors have no competing financial interests to disclose.

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