MicroRNA-216a-5p in lipopolysaccharide-induced endothelial injury

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Abstract. MicroRNAs (miRNAs/miRs) are a type of non-coding RNA that are closely associated with disease development and treatment. The present study aimed to investigate the role of miR-216a-5p in lipopolysaccharide (LPS)-induced endothelial injury *in vitro*. The EdU assay was performed to detect EdU-positive cells, while flow cytometric analysis was performed to detect apoptotic cells. Reverse transcription-quantitative PCR and western blot analyses were performed to detect the expression levels of miR-216a-5p, Toll-like receptor 4 (TLR4), MyD88 and nuclear factor (NF)-κB(p65) and phosphorylated (p)-NF-κB(p65). Furthermore, p-NF-κB(p65) nuclear expression level was detected via cellular immunofluorescence. The dual-luciferase reporter assay was performed to verify the association between miR-216a-5p and TLR4. The results demonstrated that the number of EdU-positive cells significantly decreased, the apoptotic rate significantly increased, and TLR4, MyD88 and NF-κB(p65) mRNA expression levels were significantly upregulated.TLR4, MyD88 and p-NF-κB(p65) protein expression levels were significantly upregulated and p-NF-κB(p65) nuclear concentration was significantly enhanced in the small interfering RNA-miR-216a-5p and LPS groups (P<0.001, respectively) compared with the negative control group. However, the addition of miR-216a-5p significantly increased the number of EdU-positive cells, significantly decreased the apoptotic rate and significantly downregulated the mRNA expression levels of TLR4, MyD88 and NF-κB(p65), as well as the protein expression levels of TLR4, MyD88 and p-NF-κB(p65). In addition, the p-NF-κB(p65) nuclear concentration was significantly decreased in the miR-216a-5p group (P<0.001, respectively) compared with the LPS group. Taken together, the results suggest that overexpression of miR-216a-5p suppresses the effects of LPS induced endothelial injury.

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Key words: microRNA-216a-5p, endothelial injury, lipopolysaccharide, TLR4/MyD88/NF-κB(p65)

Introduction

Vascular endothelial cells create a monolayer lining on the surface of vascular intima. This is crucial in maintaining the normal tension of blood vessels and the normal state of blood vessels, and is a bridge between the blood and the vascular muscular layer that participates in the development of blood vessels. Endothelial cell apoptosis is the initiating factor of vascular endothelial injury, which constitutes the pathological foundation of various cardiovascular diseases, such as atherosclerosis (1).

MicroRNAs (miRNAs/miRs) are a class of endogenous non-protein coding, single-stranded small RNAs of 22-25 nucleotides in length (2). In 1993, Lee *et al* (3) discovered the first miRNA that regulates temporal expression of cells in *Caenorhabditis elegans*, known as Lin-4. Subsequently, novel miRNAs have been identified in animals, plants and microorganisms, which are involved in the regulation of coding proteins, and regulate all pathological and physiological processes *in vivo* (4).

Apoptosis is considered the initiating factor of several cardiovascular diseases. Notably, miRNAs can mediate the function of vascular endothelial cells by regulating their apoptosis, and thus are involved in regulating the progression of cardiovascular diseases (5). Research on vascular endothelial cells have demonstrated the significant change in the expression profile of miRNAs in human umbilical vein endothelial cells (HUVECs) stimulated by oxidized low density lipoprotein (OX-LDL) (6). It can inhibit Bcl-2 expression, promote the production of reactive oxygen species induced by OX-LDL via the mitochondrial apoptosis pathway, resulting in the apoptosis of endothelial cells (7).

miR-216a-5p is a newly discovered miRNA (8-10). Current literature focuses on the role of miR-216a-5p in tumors (9,11). In addition, it has been reported that miR-216a-5p can effectively improve the damage of bronchial cells caused by H_2O_2 stimulation (12). However, the role of miR-216a-5p

in vascular endothelial injury and its molecular mechanism remain unclear. In the present study, a model of vascular endothelial cell injury induced by lipopolysaccharide (LPS) was established to investigate the role of the knockdown and overexpression of miR-216a-5p in vascular endothelial injury, and determine its molecular mechanism.

Materials and methods

Cells and reagents. HUVECs were purchased from the American Type Culture Collection. Cells were maintained with 5% CO_2 at room temperature in DMEM (Hyclone; Cytiva) supplemented with FBS (Hangzhou Sijiqing Biological Engineering Materials Co., Ltd.). LPS was obtained from *Escherichia coli* (Sigma-Aldrich; Merck KGaA). Small interfering RNA (si)-miR-216a-5p, si-negative control (NC), miR-NC and miR-216a-5p were purchased from Nanjing KeyGen Biotech Co., Ltd. The PCR kit was purchased from Takara Bio, Inc. Antibodies against Toll-like receptor 4 (TLR4; cat. no. ab13556; 1:500), MyD88 (cat. no. ab219413; 1:500) and phosphorylated (p)-nuclear factor (NF)-κB(p65) (cat. no. ab76302; 1:500) were purchased from Abcam, while the secondary antibody was purchased from OriGene Technologies, Inc.

Cell transfection. Cell transfection was performed using Lipofectamine® RNAiMAX reagent (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Subsequent experiments were performed 48 h post-transfection.

LPS treatment. Cells with routine culture and those transfected with miR-216-5p for 48 h, after continued to culture for 24 h, the cells were seeded into 6-well plates at a cell density of $1x10^5$ cells/well or seeded into 96-well plates at the cell density of $2x10^3$ cells/well. Following incubation for 24 h at room temperature, 1.0 mg/l LPS was added to each well and cells were cultured for an additional 48 h.

Cell treatment protocols. Cells were classified into six treatment groups as follows: No treatment (control), transfection with blank inhibitor vector (si-NC), LPS treatment alone (LPS), transfection with miR-216a-5p inhibitor (si-miR-216a-5p), transfection with miR-NC (miR-NC) and transfection with miR-216a-5p+LPS treatment (LPS+miR-216a-5p) groups. The sequences as following: si-NC: 5'-CAGUACUUUUGUGUAGUACAA-3'; si-miR-216a-5p, 5'-UCACAGUUGCCAGCUGAGAUUA; miR-NC, F: 5'-UUCUCCCAACGUGUCACGUTT-3'; R, 5'-ACGUGACACGUUCGGAGAATT-3'; miR-216a-5p F, 5'-UAAUCUCAGCUGGCAACUGUGA-3'; miR-216a-5p R, 5'-ACAGUUGCCAGCUGAGAUUAUU-3'.

EdU staining. Following treatment, cell proliferation was assessed in each group via EdU staining (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Cells were observed under a fluorescence microscope (x200).

Apoptosis analysis. Cell apoptosis was detected using the Annexin V-PI Apoptosis Detection kit (Thermo Fisher Scientific, Inc.). Cells were stained with Annexin V and PI, according to the manufacturer's instructions. Cells were incubated for 30 min at room temperature in the dark and detected via flow cytometric analysis by BD FACSAria (BD Biosciences) and ModFit software version 3.2 (BD Biosciences).

Reverse transcription-quantitative (RT-q)PCR. Total RNA was extracted from cells using TRIzol® (cat. no. 15596-026, Invitrogen; Thermo Fisher Scientific, Inc.) reagent to detect the expression levels of miR-216a-5p, TLR4, MyD88 and NF-κB(p65). The miScriptII RT reverse transcription kit (Takara Bio, Inc.) was used to reverse transcribe total RNA into cDNA. The extracted RNA was reverse transcribed into cDNA using the RevertAid first strand cDNA synthesis kit to detect the expression levels of TLR4, MyD88 and NF-κB(p65). The synthesized cDNA was amplified and quantified using the Taqman Real-time PCR Master Mixes SYBR-Green kit (Takara Bio, Inc.). The quantitative system and reaction conditions of RT and amplification were performed according to the manufacturer's instructions. Relative expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method (13). U6 was the internal reference for miR-216a-5p, while GAPDH was the internal reference for TLR4, MyD88 and NF-κB (p65). The primer sequences used for qPCR are listed in Table I.

Western blotting. Cells in each group were treated with corresponding treatments, and the protein samples were extracted by the whole protein extraction kit (cat. no. KEP250; Nanjing KeyGen Biotech Co., Ltd.) for electrophoresis. Protein concentration was measured via the BCA methods. The collected lysate samples (20 μg/well) were separated via SDS-PAGE on 12% gels, transferred onto nitrocellulose membranes and blocked with 50 g/l skimmed milk for 2 h at room temperature. The membranes were incubated with rabbit anti-mouse TLR4 (cat. no. ab13556), MyD88 (cat. no. ab219413), p-NF-κB(p65) (cat. no. ab76302) (all 1:500) and rabbit anti-mouse GAPDH (cat. no. ab9485) (1:500) polyclonal antibodies for 12 h at 4˚C. Following the primary incubation, membranes were incubated with secondary antibody Goat Anti-Rabbit IgG (cat. no. ab150077; 1:1,000) at room temperature for 1 h. Protein bands were then visualized using an ECL reagent (Thermo Fisher Scientific, Inc.), using Tanon 5200 detection system (Tanon Science & Technology Co., Ltd.) for imaging. Protein band densitometry was quantified using ImageJ software for Windows V 1.52v (National Institutes of Health) and normalized to GAPDH.

Cell immunofluorescence assay. Following treatment and once HUVECs reached confluence (70-80%), cells were fixed with 4% paraformaldehyde for 30 min at room temperature and blocked with 10% normal goat serum (Sigma-Aldrich; Merck KGaA) for 30 min at room temperature. Cells were incubated with p-NF-κB(p65)1 antibody (1:200) in the wet box overnight at 4˚C. Subsequently, cells were incubated with goat anti-rabbit IgG at 37˚C for 30 min. Nuclei were stained with 5 μg/ml DAPI for 10 min at room temperature and subsequently sealed with glycerin-buffered saline (Sigma-Aldrich; Merck KGaA). Cells were observed under a fluorescence inverted phase contrast microscope (magnification, x200).

Dual-luciferase reporter assay. The sequences of wild-type (WT) and mutant (MUT) TLR4 mRNA in 3'-untranslated

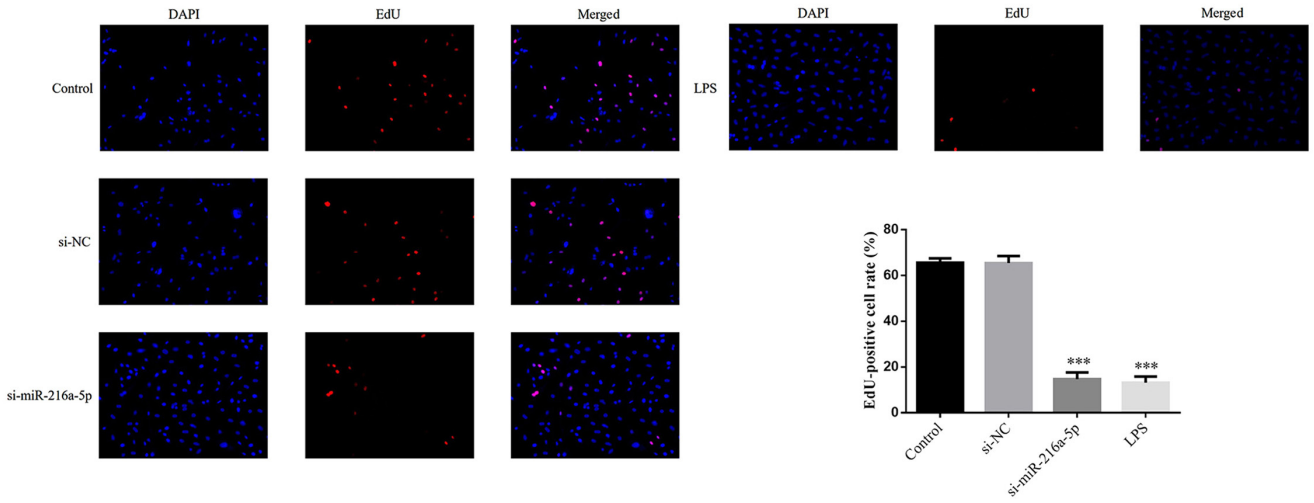

Figure 1. Effect of miR-216a-5p knockdown and LPS intervention on endothelial cell proliferation. Control, cells were cultured under normal conditions; si-NC, cells were transfected with si-NC; si-miR-216a-5p, cells were transfected with si-miR-216a-5p; LPS, cells were treated with LPS (1.0 mg/l). Magnification, x200. ***P<0.001 vs. control. miR, microRNA; LPS, lipopolysaccharide; si, small interfering RNA; NC, negative control.

Table I. Primer sequences of different genes.

Gene name	Sequence (5'-3')
microRNA-216a-5p	F: ATCCAGTGCGTGTCGTG R: TGCTTAATCTCAGCTGGCA
U6	F: CTCGCTTCGGCAGCACA R: ACGCTTCACGAATTTGCGT
Toll-like receptor 4	F: TGGATACGTTTCCTTATAAG R: GAAATGGAGGCACCCCTTC
MyD88	F: ACCTGGCTGGTTTACACGTC R: CTGCCAGAGACATTGCAGAA
NF-κB(p65)	F: ATGCTTACTGGGTGCCAAAC R: GGCAAGTCACTCAGCCTTTC
GAPDH	F: AGGTCGGTGTGAACGGATTTG R: 5'-TGTAGACCATGTAGTTGAGG TCA-3'

F, forward; R, reverse.

regions (UTRs) were synthesized and cloned into fluorescent reporter plasmids (cat. no. KGAF040; Nanjing KeyGen Biotech Co., Ltd.), named WT and MUT. The fluorescent reporter plasmids was subsequently transfected using Lipofectamine® 3000 (Thermo Fisher Scientific, Inc.) into HUVECs, with miR-216a-5p mimic sequence or miR-NC, respectively for 24 h at room temperature. Transfected cells were seeded into 96-well plates at the density of $1x10^4$ cells/well and cultured for 48 h. Luciferase activities were measured using the Promega dual-luciferase reporter assay system (Promega Corporation). Firefly luciferase activity was normalized to *Renilla* luciferase activity.

Statistical analysis. Statistical analysis was performed using the SPSS 17.0 software (SPSS, Inc.). All experiment repeated three times. Data are presented as the mean ± SD. Tukey's following one-way ANOVA were used for pairwise comparison between multiple groups. P<0.05 was considered to indicate a statistically significant difference.

Results

Effect of miR-216a-5p knockdown and LPS intervention on endothelial cell proliferation. No significant difference in the number of EdU-positive cells was observed in the si-NC group compared with the control group (P>0.05; Fig. 1). The results demonstrated that transfection with si-NC caused no injury to HUVECs. Notably, the number of EdU-positive cells significantly decreased in the si-miR-216a-5p and LPS groups (P<0.001; Fig. 1). Taken together, the results suggest that miR-216a-5p knockdown or LPS stimulation can decrease the proliferation of HUVECs.

Effect of miR-216a-5p knockdown and LPS intervention on endothelial cell apoptosis. No significance difference in cell apoptosis was observed between the control and si-NC groups (P>0.05; Fig. 2), suggesting that transfection with si-NC caused no injury to HUVECs. However, cell apoptosis was notably increased in the si-miR-216a-5p and LPS groups (P<0.001; Fig. 2). Collectively, the results suggest that miR-216a-5p knockdown or LPS intervention promote the apoptosis of HUVECs.

Effect of miR-216a-5p knockdown and LPS intervention on gene expression. No significant differences were observed in the expression levels of miR-216a-5p, TLR4, MyD88 and NF-κB(p65) between the control and si-NC groups (P>0.05; Fig. 3). Moreover, the results revealed no influence of si-NC transfection in HUVECs on miR-216a-5p, TLR4, MyD88 and NF-κB(p65) mRNA expression levels. Furthermore, miR-216a-5p expression was notably decreased in the si-miR-216a-5p and LPS groups, while the mRNA expression levels of TLR4, MyD88 and NF-κB(p65) were significantly increased (P<0.001; Fig. 3). Taken together, the results suggest that miR-216a-5p knockdown or LPS intervention affect

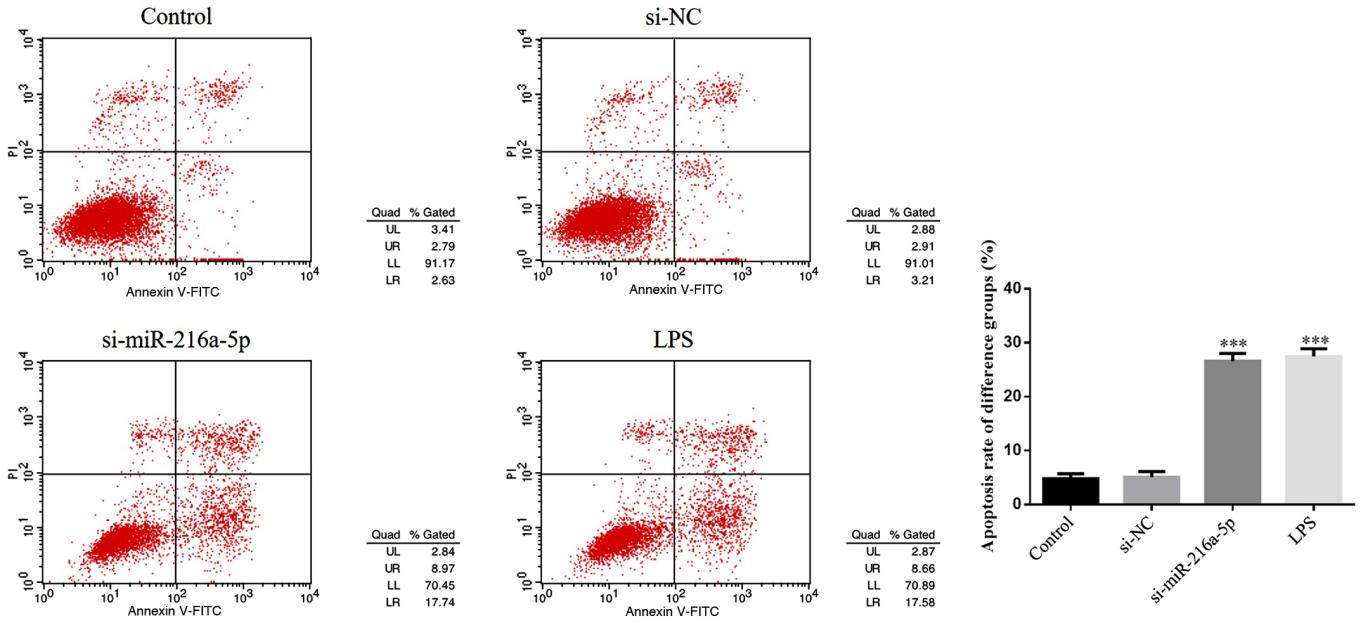

Figure 2. Effect of miR-216a-5p knockdown and LPS intervention on endothelial cell apoptosis. Control, cells were cultured under normal conditions; si-NC, cells were transfected with si-NC; si-miR-216a-5p, cells were transfected with si-miR-216a-5p; LPS, cells were treated with LPS (1.0 mg/l). ***P<0.001 vs. control. miR, microRNA; LPS, lipopolysaccharide; si, small interfering RNA; NC, negative control.

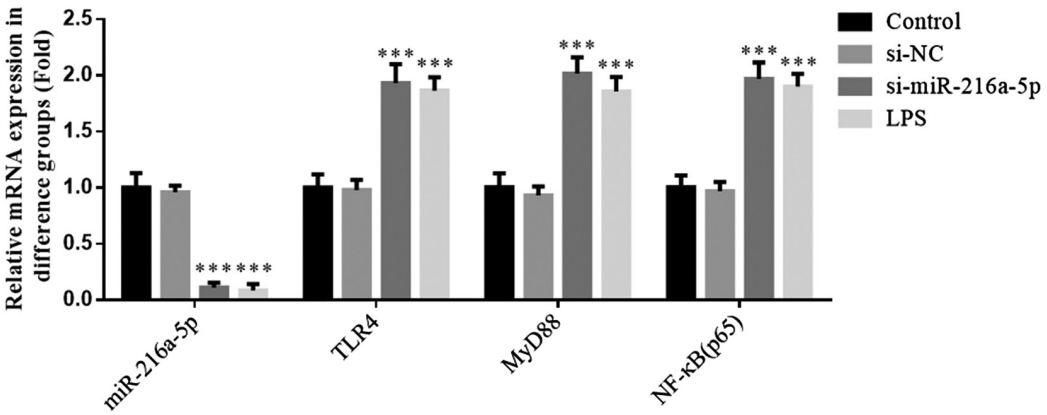

Figure 3. Effect of miR-216a-5p knockdown and LPS intervention on gene expression. Control, cells were cultured under normal conditions; si-NC, cells were transfected with si-NC; si-miR-216a-5p, cells were transfected with si-miR-216a-5p; LPS, cells were treated with LPS (1.0 mg/l). ***P<0.001 vs. control. miR, microRNA; LPS, lipopolysaccharide; si, small interfering RNA; NC, negative control.

the expression levels of miR-216a-5p, TLR4, MyD88 and NF-κB(p65).

Effect of miR-216a-5p knockdown and LPS intervention on protein expression. No significant differences in the protein expression levels of TLR4, MyD88 and NF-κB(p65) were observed between the control and si-NC groups (P>0.05; Fig. 4). The protein expression levels of TLR4, MyD88 and NF-κB(p65) were significantly increased in the si-miR-216a-5p and LPS groups (P<0.001; Fig. 4). Collectively, these results suggest that miR-216a-5p knockdown or LPS intervention affect the protein expression levels of miR-216a-5p, TLR4, MyD88 and NF-κB(p65).

Effect of miR-216a-5p knockdown and LPS intervention on the protein transportation of p-NF-κB(p65) to the nucleus. No significant differences in the protein transportation of p-NF-κB(p65) to the nucleus were observed between the control and si-NC groups (P>0.05; Fig. 5). Notably, the protein transportation of p-NF-κB(p65) to the nucleus was significantly increased in the si-miR-216a-5p and LPS groups (P<0.001; Fig. 5). Taken together, the results suggest that miR-216a-5p knockdown or LPS intervention affect protein transportation of p-NF-κB(p65) to the nucleus.

miR-216a-5p reverses the effect of LPS in inhibiting HUVEC proliferation. In the present study, plasmids were used for transfection, and the results (Fig. S1) showed that the plasmid transfection rate of miR-NC (78.82±2.67%) and miR-216a-5p (79.17±2.82%) were higher compared with the control group. RT-qPCR demonstrated that the miR-216a-5p mRNA expression level of the miR-216a-5p group was significantly upregulated compared with the control group, (P<0.001, Fig. S2). No significant difference in the number

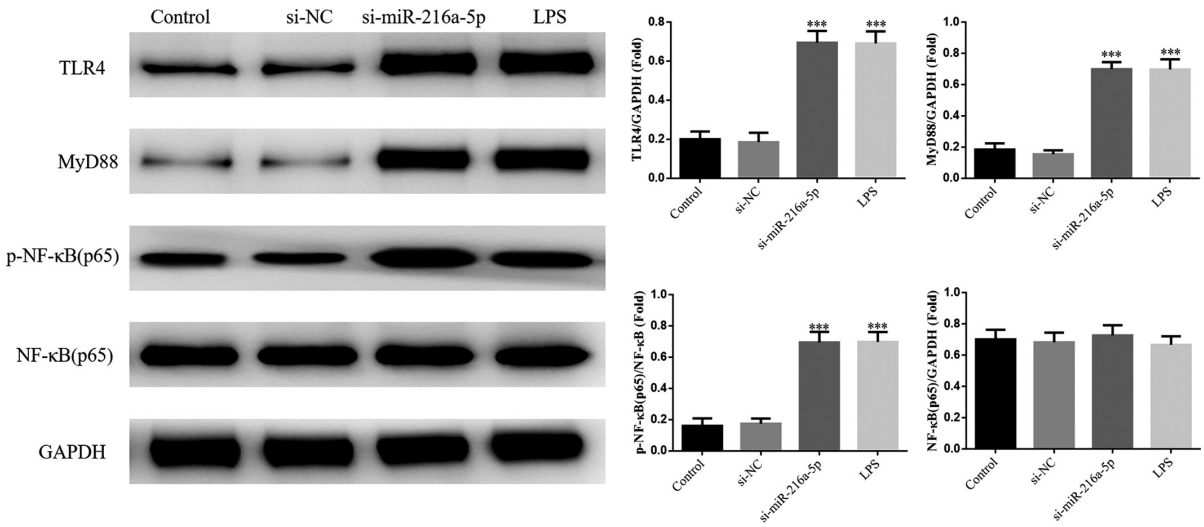

Figure 4. Effect of miR-216a-5p knockdown and LPS intervention on protein expression. Control, cells were cultured under normal conditions; si-NC, cells were transfected with si-NC; si-miR-216a-5p, cells were transfected with si-miR-216a-5p; LPS, cells were treated with LPS (1.0 mg/l). ***P<0.001 vs. control. miR, microRNA; LPS, lipopolysaccharide; si, small interfering RNA; NC, negative control; TLR4, Toll-like receptor 4; p-, phosphorylated; NF-κB, nuclear factor-κB.

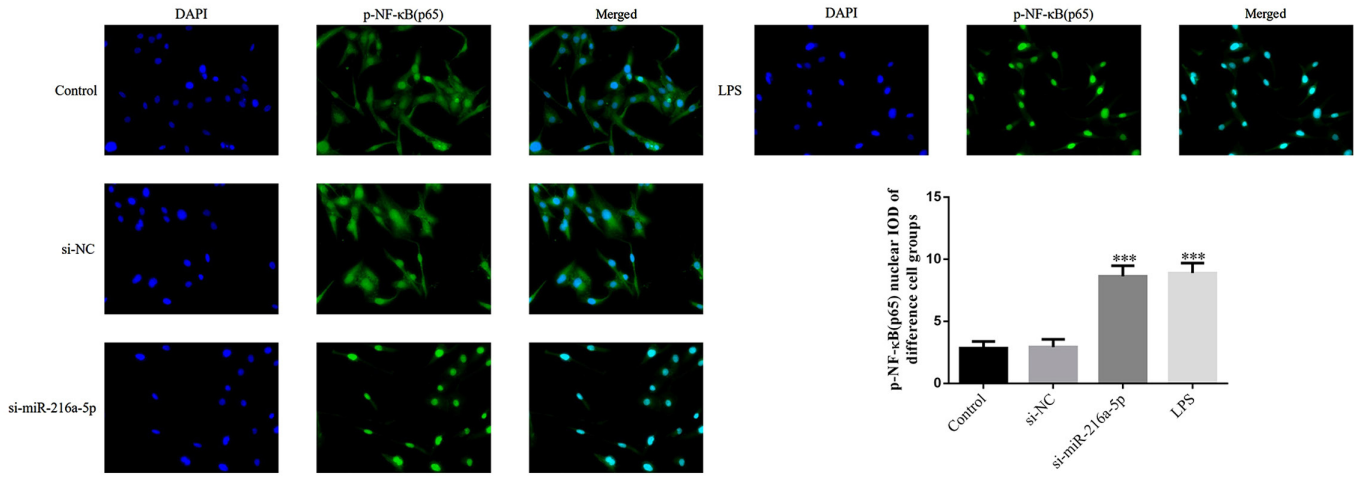

Figure 5. Effect of miR-216a-5p knockdown and LPS intervention on the protein transportation of p-NF-κB(p65) IOD to the nucleus. Control, cells were cultured under normal conditions; si-NC, cells were transfected with si-NC; si-miR-216a-5p, cells were transfected with si-miR-216a-5p; LPS, cells were treated with LPS (1.0 mg/l). Magnification, x200. ***P<0.001 vs. control. miR, microRNA; LPS, lipopolysaccharide; si, small interfering RNA; NC, negative control; p-NF-κB, phosphorylated nuclear factor-κB.

of EdU-positive cells was observed between the miR-NC and control groups (P>0.05; Fig. 6). The number of EdU-positive cells was significantly decreased following treatment with LPS (P<0.001; Fig. 6), suggesting that LPS intervention decreases the proliferation of HUVECs. The number of EdU-positive cells was significantly increased in the LPS + miR-216a-5p group compared with the LPS group (P<0.001; Fig. 6).

miR-216a-5p reverses the effect of LPS in inducing HUVEC apoptosis. There was no significant difference in cell apoptosis between the control and miR-NC groups (P>0.05; Fig. 7), suggesting that transfection with miR-NC causes no injury to HUVECs. Notably, cell apoptosis was decreased in the LPS group (P<0.001; Fig. 7), suggesting that LPS intervention promotes the apoptosis of HUVECs. Cell apoptosis was significantly decreased in the LPS + miR-216a-5p group compared with the LPS group (P<0.001; Fig. 7).

Effect of miR-216a-5p on gene expression induced by LPS. No significant differences in the expression levels of miR-216a-5p, TLR4, MyD88 and NF-κB(p65) were observed between the control and miR-NC groups (P>0.05; Fig. 8). miR-216a-5p expression was decreased, while the expression levels of TLR4, MyD88 and NF-κB(p65) were significantly increased in the LPS group (P<0.001; Fig. 8). The aforementioned results suggest that LPS intervention affects the expression levels of miR-216a-5p, TLR4, MyD88 and NF-κB(p65). miR-216a-5p expression was significantly increased, while the expression levels of TLR4, MyD88 and NF-κB(p65) were significantly decreased following transfection of HUVECs with miR-216a-5p compared with the LPS group (P<0.001; Fig. 8).

Effect of miR-216a-5p on protein expression induced by LPS. No significant differences were observed in the protein expression levels of TLR4, MyD88 and p-NF-κB(p65) between the

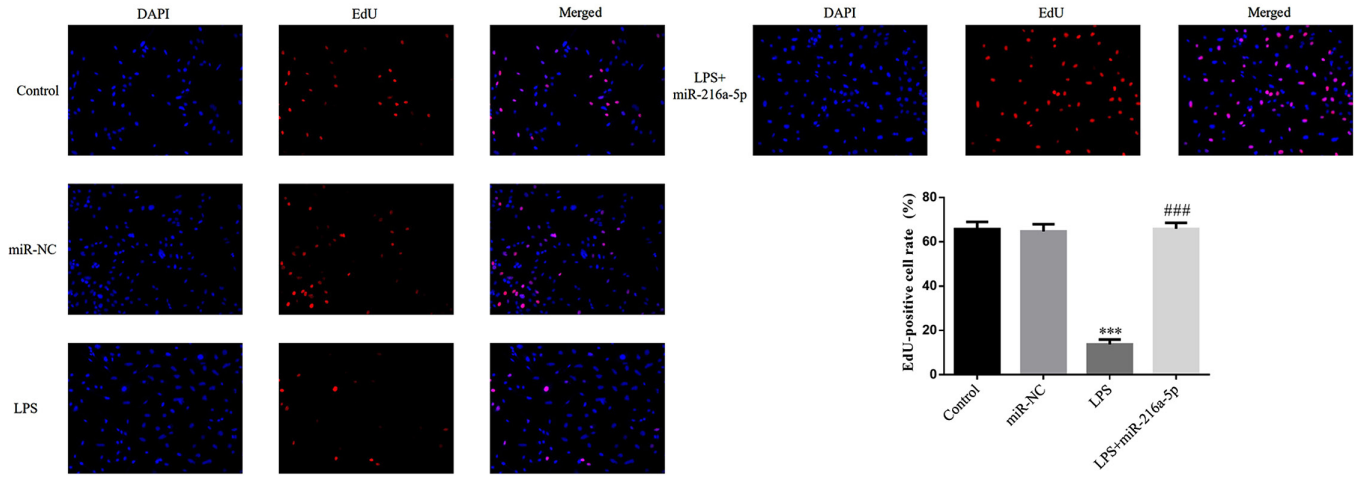

Figure 6. miR-216a-5p reverses the effect of LPS on inhibiting the proliferation of human umbilical vein endothelial cells. Control, cells were cultured under normal conditions; miR-NC, cells were transfected with miR-NC; LPS, cells were treated with 1.0 mg/l LPS; LPS+miR-216a-5p, cells transfected with miR-216a-5p were treated with 1.0 mg/l LPS. Magnification, x200. ***P<0.001 vs. control; ###P<0.001 vs. LPS group. miR, microRNA; LPS, lipopolysaccharide; NC, negative control.

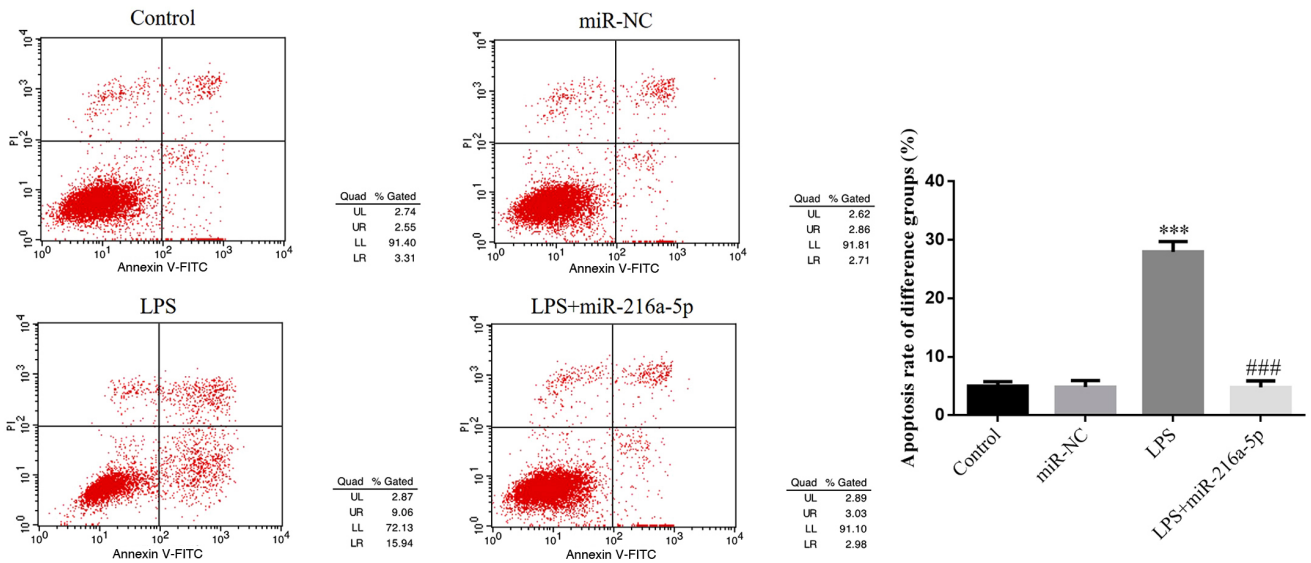

Figure 7. miR-216a-5p reverses the effect of LPS in inducing human umbilical vein endothelial cell apoptosis. Control, cells were cultured under normal conditions; miR-NC, cells were transfected with miR-NC; LPS, cells were treated with 1.0 mg/l LPS; LPS+miR-216a-5p, Cells transfected with miR-216a-5p were treated with 1.0 mg/l LPS. ***P<0.001 vs. control; ###P<0.001 vs. LPS group. miR, microRNA; LPS, lipopolysaccharide; NC, negative control.

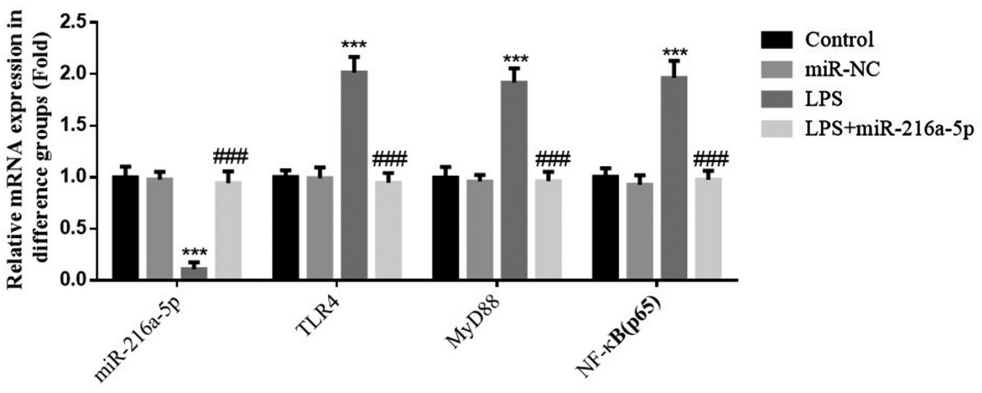

Figure 8. Effect of miR-216a-5p on gene expression induced by LPS. Control, cells were cultured under normal conditions; miR-NC: Cells were transfected with miR-NC; LPS, cells were treated with 1.0 mg/l LPS; LPS+miR-216a-5p, cells transfected with miR-216a-5p were treated with 1.0 mg/l LPS. ***P<0.001 vs. control; ###P<0.001 vs. the LPS group. miR, microRNA; LPS, lipopolysaccharide; HUVECs, human umbilical vein endothelial cells; NC, negative control; TLR4, Toll-like receptor 4; p-, phosphorylated; NF-κB, nuclear factor-κB.

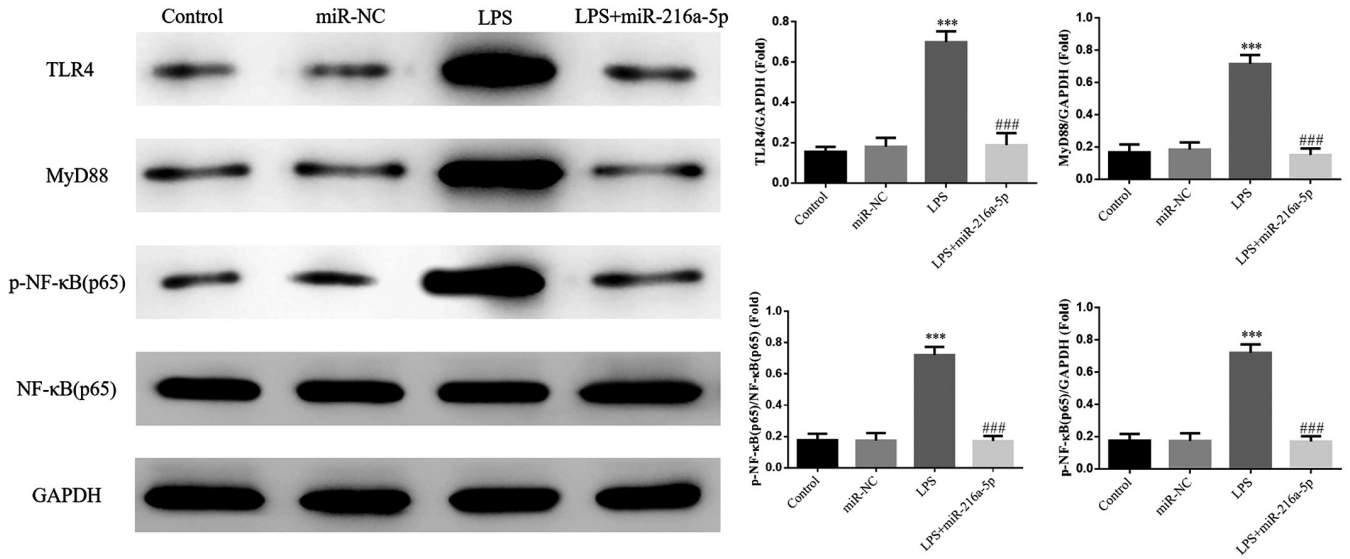

Figure 9. Effect of miR-216a-5p on protein expression induced by LPS. Control, cells were cultured under normal conditions; miR-NC, cells were transfected with miR-NC; LPS, cells were treated with 1.0 mg/l LPS; LPS+miR-216a-5p, cells transfected with miR-216a-5p were treated with 1.0 mg/l LPS. ***P<0.001 vs. control; ###P<0.001 vs. the LPS group. miR, microRNA; LPS, lipopolysaccharide; NC, negative control; TLR4, Toll-like receptor 4; p-, phosphorylated; NF-κB, nuclear factor-κB.

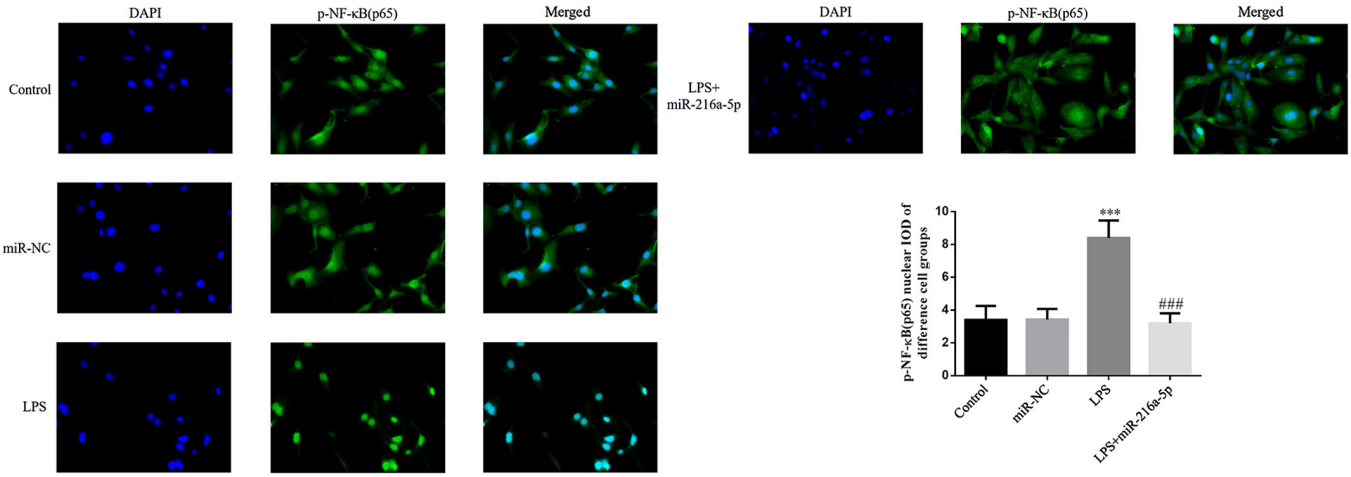

Figure 10. Effect of miR-216a-5p on the protein transportation of p-NF-κB(p65) IOD to the nucleus induced by LPS. Control, cells were cultured under normal conditions; miR-NC, cells were transfected with miR-NC; LPS, cells were treated with 1.0 mg/l LPS; LPS+miR-216a-5p, Cells transfected with miR-216a-5p were treated with 1.0 mg/l LPS. Magnification, x200. ***P<0.001 vs. control; ###P<0.001 vs. LPS group. miR, microRNA; LPS, lipopolysaccharide; NC, negative control; p-NF-κB, phosphorylated nuclear factor-κB.

miR-NC and control groups (P>0.05; Fig. 9). Furthermore, the protein expression levels of TLR4, MyD88 and p-NF-κB(p65) were significantly increased in the LPS group (P<0.001; Fig. 9), suggesting that LPS intervention affects the protein expression levels of TLR4, MyD88 and NF-κB(p65). Notably, the protein expression levels of TLR4, MyD88 and p-NF-κB(p65) were significantly decreased in the LPS + miR-216a-5p compared with the LPS group (P<0.001; Fig. 9). In addition, no significant difference in the protein expression of NF-κB(p65) was observed between the groups.

Effect of miR-216a-5p on the protein transportation of p-NF-κB(p65) to the nucleus induced by LPS. No significant difference in protein transportation of p-NF-κB(p65) to the nucleus was observed between the miR-NC and control groups (P>0.05; Fig. 10). Treatment with LPS significantly increased the protein transportation of p-NF-κB(p65) to the nucleus (P<0.001; Fig. 10), suggesting that LPS affects the protein transportation of p-NF-κB(p65) to the nucleus. In addition, protein transportation of p-NF-κB(p65) to the nucleus significantly decreased in the LPS + miR-216a-5p group compared with the LPS group (P<0.001; Fig. 10).

Association between miR-216a-5p and TLR4. According to the results of the dual-luciferase reporter assay, the relative fluorescence value was lower in cells transfected with miR-216a-5p and WT reporter plasmids, and the difference was statistically significant compared with the other groups (P<0.001; Fig. 11). The aforementioned results suggest that miR-216a-5p can bind to the 3'-UTR of TLR4 and target this gene.

	Predicted consequential pairing of target region (top) and miRNA (bottom)	Site type	Context++ score	Context++ score percentile	Weighted context++ score	Conserved branch length	PCT
Position 7168-7174 of TLR4 3' UTR	5' ...UAUUGGUUUUGUUCU-UGAGAUUU...	7mer-m8	-0.06	67	0.00	2.944	< 0.1
hsa-miR-216a-5p	3' AGUGUCAACGGUCGACUCUAAU						

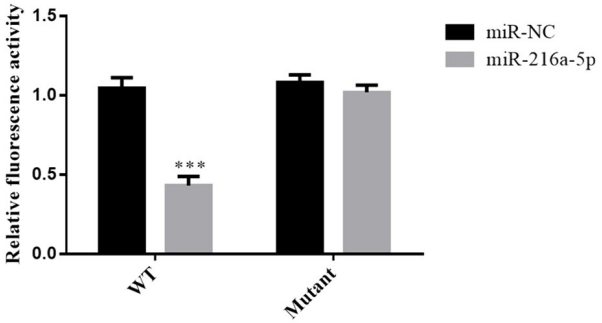

Figure 11. Association between miR-216a-5p and TLR4. There was no significant difference between miR-NC and miR-216a-5p groups in WT cell; there were significantly difference between miR-NC and miR-216a-5p in TLR4 mutant cell. ***P<0.001 vs. the miR-NC group. miR, microRNA; TLR, Toll-like receptor; NC, negative control; WT, wild type.

Discussion

The results of the present study demonstrated that miR-216a-5p significantly promoted the apoptosis of HUVECs. Furthermore, miR-216a-5p knockdown notably inhibited the proliferation, and significantly promoted the apoptosis of HUVECs, similar to the results following treatment with LPS. Taken together, the results suggest that miR-216a-5p may play a role in LPS-induced injury of HUVECs. It has been reported that miRNAs may have crucial effect on LPS-induced cell injury (14). According to the present study, transfection of HUVECs with miR-216a-5p overexpression vector reversed the induced apoptosis and inhibited proliferation induced by LPS. The expression levels of the corresponding genes and proteins were detected, and TLR4/NF-κB(p65) was identified as playing a key role in this process.

TLRs are key components of the innate immune system, which play a key role in the pathogenesis of inflammation. It has been reported that TLR-mediated signaling pathways exhibit a close association with the development of diabetes mellitus, diseases belonging to the cardiovascular and nervous systems, and disorders of the liver and kidney (15-17). Simultaneously, TLRs are involved in the process of multiple organ injury caused by inflammation (18,19). For example, TLRs can interact with their associated signaling molecules to activate the expression of cytokines, thus, participating in renal injury caused by immune response (20). Overactivation of TLRs may induce injury of the organism (21). In addition, TLRs participate in the MyD88-dependent signaling pathway and activate NF-κB(p65), resulting in the release of inflammatory factors (22). Organisms under high oxidative stress and strong inflammatory reaction may experience the generation and release of several oxygen free radicals and inflammatory factors, further stimulating protein transportation of p-NF-κB(p65) to the nucleus, and thus inducing serious organ damage (23,24). TLR4 can activate NF-κB(p65) via activation of the MyD88-dependent signaling pathway, which triggers the activation of inflammatory cytokines and results in apoptosis (25). In the present study, treatment with LPS increased the expression levels of TLR4, MyD88 and NF-κB(p65), resulting in aggravated cell injury. Following simultaneous transfection with miR-216a-5p under the same condition, the protein expression levels of MyD88 and p-NF-κB(p65) decreased, which in turn decreased cell apoptosis and increased cell proliferation. It was suggested that the decreased proliferation and increased apoptosis of HUVECs induced by LPS intervention can be attributed to the down-regulated miR-216a-5p expression. Simultaneous transfection with miR-216a-5p notably recovered HUVECs proliferation and decreased cell apoptosis.

In conclusion, overexpression of miR-216a-5p may exert a positive role in alleviating LPS-induced vascular endothelial injury by regulating the TLR4/MyD88/NF-κB(p65) signaling pathway.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

WL and QY conceived the study and established the initial design of the study. WL, WX, YL, KH, XZ and YW performed the experiments and analyzed the data. WL prepared the manuscript. WL and QY confirmed the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patients consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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