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MiR-217 inhibits apoptosis of atherosclerotic endothelial cells *via* the TLR4/PI3K/Akt/NF-κB pathway

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Abstract. – OBJECTIVE: To determine the effect of miR-217 on the apoptosis of atherosclerotic endothelial cells (AECs) through the Toll-like receptor (TLR) 4/PI3K/Akt/NF-κB pathway.

MATERIALS AND METHODS: Oxidized low-density lipoprotein (ox-LDL) was used to construct an atherosclerotic endothelial cell model, and the expression of miR-217/TLR4/PI3K/Akt/NF-κB in the cells was regulated to explore their effects on the viability, apoptosis, inflammatory factors [tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-10 (IL-10)], and endothelial-to-mesenchymal transformation (EndMT) of the endothelial cells.

RESULTS: In AECs, miR-217 expression decreased, and the PI3K/Akt/NF-κB pathway was inhibited. The Dual-Luciferase reporter assay revealed that TLR4 was the target of miR-217, and it was up-regulated in AECs, and the further study revealed that up-regulation of miR-217 protected AECs, increased their activity, reduced their apoptosis, and inhibited inflammatory response and EndMT, while TLR4 acted contrary to miR-217. Besides, it was also found that miR-217 inhibited the PI3K/Akt/NF-κB pathway, thus weakening the influence of si-TLR4 on endothelial cells. Furthermore, miR-217 inhibited EndMT by inhibiting TLR4 from activating the PI3K/Akt/NF-κB signal pathway.

CONCLUSIONS: In AECs, TLR4 expression increased, and miR-217 and the PI3K/Akt/NF-κB signaling pathway are inhibited. Additionally, miR-217 can increase the viability of AECs through the TLR4/PI3K/Akt/ NF-κB signal transduction pathway, and inhibit their apoptosis, inflammatory response, and EndMT.

Key Words:
MiR-217, Toll-like receptor 4, PI3K/Akt/NF-ÐB pathway, Endothelial cell.

Introduction

Atherosclerosis is a chronic arterial disease that gives rise to heart disease and stroke and is the leading cause of death and disability worldwide[1,2]. About 30% of people died of ischemic heart disease[3]. According to the Chinese survey of cardiovascular diseases in 2016, the number of patients suffering from ischemic heart diseases in China reaches up to 11 million, and it is expected that it will become the leading cause of disability and death for residents in 2020[4].

The endothelial cell is a vital regulator to maintain vascular balance, and its dysfunction plays a crucial role in the progression of atherosclerosis, which is mainly manifested as follows: endothelial cell apoptosis gives rise to exfoliation and dysfunction of intact endothelial monolayer, resulting in lipid accumulation and other reactions including inflammatory response, thus leading to atherosclerotic lesions[5,6]. MicroRNAs (miRNAs) are short-chain noncoding RNAs, an essential function in tumor immune regulation, chemotherapy resistance, and metastasis through targeted inhibition of messenger RNA translation, and targeting miRNAs is a promising therapeutic method[7,8]. MiR-217, located on human chromosome 2p16.1, is expressed at a high level in the plasma of atherosclerosis patients[9]. Downregulation of miR-217 can alleviate atherosclerosis by targeting sirtuin 1 and inhibiting macrophage apoptosis and inflammatory response[10]. In addition, miR-217 can inhibit endothelial cell apoptosis through the chloride intracellular channel

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(CLIC) 4[11]. Toll-like receptor 4 (TLR4), as a pivotal factor in the regulation of inflammation and stress response, directly participates in the development of atherosclerosis and protects endothelial cells[12,13], and it is also the target of miR-217[14]. Lv et al[15] have uncovered that non-muscle myosin heavy chain IIA can activate TLR4/PI3K/Akt/JNK1/2/14-3-3ε/NF-κB/MMP9 to destroy the tight connection between endothelial cells.

Therefore, we speculated that miR-217 may affect the apoptosis of atherosclerotic endothelial cells (AECs) *via* TLR4/PI3K/Akt/NF-κB, and we carried out experiments to explore it.

Materials and Methods

Source of Rats

Ten healthy Sprague-Dawley rats (8-10 weeks old and 250-300 g) purchased from Xi'an Jiaotong University Experimental Animal Center were fed at room temperature of 20-25°C and relative humidity of 40%-70% under 12 hour light-dark cycle, and they were allowed to drink freely. Each animal experiment was carried out with permission from the Laboratory Animal Care Committee of Xi'an Jiaotong University and in accordance with the guiding principles of the Council for International Organization of Medical Sciences (CIOMS).

Isolation and Culturing of Primary Endothelial Cells

Rats were killed under intraperitoneal injection of pentobarbital sodium (45 mg/kg, Sigma Chemical Co., St. Louis, MO, USA), and their aortas were completely separated. The aortas were washed with normal saline, and then washed in Dulbecco's Modified Eagle's Medium (DMEM; Life Technology, Waltham, MS, USA) to remove bloodstain, and their adipose tissues were removed, and their aortas were cut into pieces. The intravascular surface was attached to a 96-well plate, and incubated in DMEM with fetal bovine serum (10%; FBS; Life Technology, USA) and penicillin/streptomycin (1%; HyClone, South Logan, UT, USA) under 5% CO_2 at 37°C for 60 h. Upon reaching 80% confluency after being cultured, the cells were digested with 25% trypsin for passage, and those at 2nd to 4th generations were used for later experiments.

Construction of Atherosclerotic Endothelial Cell Model

Endothelial cells were treated with 100 μg/ml oxidized low-density lipoprotein (ox-LDL, Xiesheng Biotechnology Co., Ltd., Beijing, China), and an atherosclerotic endothelial cell model was constructed[16]. Normal endothelial cells were adopted as a blank control group.

Construction and Transfection of Expression Vectors

The overexpressed vectors (miR-217 mimic and sh-TLR4), underexpressed vectors (miR-217 inhibitor and si-TLR4), and empty vectors (miR-NC and TLR4-NC) were designed and synthesized by Sigma company and used for transfection with a Lipofectamine 2000 (Hyclone, South Logan, UT, USA). qRT-PCR and Western Blot (WB) assays were carried out to detect the transfection results.

Cell Grouping and Experiments

According to different transfection vectors, the cells were divided into 7 groups: MiR-217 mimic group, miR-217 inhibitor group, miR-NC group, sh-TLR4 group, si-TLR4 group, TLR4-NC group, and control group, and the viability, apoptosis, and Endothelial-to-Mesenchymal Transformation (EndMT) of endothelial cells in each group were determined.

Rescue Experiment

A control group, sh-TLR4 group, miR-217mimic+sh-TLR4 (miR rescue) group, and sh-TLR4+PI3K inhibitor (TLR4 rescue) group were set, and alpelisib produced by Novartis (Basel, Switzerland) was adopted as PI3K inhibitor to analyze the effects of the PI3K pathway on the function of TLR4.

qRT-PCR

Total RNA of cells was obtained by a TRIzol Kit (Invitrogen, Carlsbad, CA, USA), and the detection was carried out specifically with an EasyScript One-Step RT-PCR SuperMix Kit from TransGen Biotech (Beijing, China) under the kit instruction in 20 μl total volume consisting of 1 μg RNA Template, 0.4 μl Forward GSP (10 μM), 0.4 μl Reverse GSP (10 μM), 10 μl 2 times of One-Step Reaction Mix, 0.4 μl EasyScript One-Step Enzyme Mix, as well as RNase-free Water added to adjust the volume under 40°C for 30 min, followed by 40 cycles of 94°C for 5 min, 94°C for 30 s, 60°C for 30s, and 72°C for 2 kb/min, and 72°C for 10 min. Three duplicate wells were set, and the data were calculated using $2^{-\Delta CT}$ with U6 as internal reference. The forward primer of miR-217 was 5'-TACTGCATCAGGAACTGATTGGA-3', and the forward primer of U6 was 5'-GCTTCG-

GCAGCACATATACTAAAAT-3'. The universal downstream primer of 5'-CAGTGCGTGTCGTGGAGT-3' was adopted.

WB Assay

Protein was extracted from nerve cells by the freeze thaw method, and its concentration was determined using the bicinchoninic acid (BCA) method and adjusted to 4 μg/μL. The protein was isolated through 12% polyacrylamide gel electrophoresis. The initial voltage was 90V, and then the voltage was increased to 120V to move the sample to an appropriate position of the separation gel. After electrophoresis, the protein was transferred to a membrane under 100V constant voltage for 100 min, and sealed at 37°C for 60 min. Subsequently, the membrane was immersed in 5% skim milk for future immune response. The membrane was cultured with primary antibody (1:1000) at 4°C for one night, and then washed with warm phosphate-buffered saline (PBS) three times, 5 min/time. After washing, the membrane was cultured with secondary antibody (1:1000) at indoor temperature for 1 h. Afterwards, the protein was developed and immobilized through enhanced chemiluminescence (ECL) agent. The scanned protein band was processed using Quantity One software, and the relative protein level = the gray value of the band/gray value reference. The BCA protein kit, ECL kit, and trypsin were all purchased from Thermo Scientific™ (Thermo Fisher Scientific, Waltham, MS, USA), with item numbers of 23250, 35055, and 90058, respectively, and rabbit anti-mouse PI3K (monoclonal antibody), AKT (monoclonal antibody), p-AKT (polyclonal antibody), NF-κB p65 (monoclonal antibody), VE-cadherin (monoclonal antibody), CD31 (monoclonal antibody), α-SMA (monoclonal antibody), and goat anti-rabbit immunoglobulin G (IgG) secondary antibody were all purchased from the Abcam in the United States (Abcam, Cambridge, MA, USA), with item numbers of ab151549, ab179463, ab8805, ab32536, ab225442, ab134168, ab32575, and ab6721, respectively. In addition, rabbit anti-mouse p-PI3K (monoclonal antibody) was purchased from Cell Signaling Technology (Cell Signaling Technology, Danvers, MA, USA), with item number of 17366S.

Dual-Luciferase Reporter Assay

After 293T cells were cultured to logarithmic growth phase, pmirGLO-TLR4-3'UTRWt, pmirGLO-TLR4-3'UTRMut, miR-217 mimics, miR-217 inhibitor, and miR-NC were transfected in them, and their fluorescence intensity was detected 48 hours after transfection with a Dual-Luciferase reporter assay.

Cell Viability Determination by MTT

A CyQUANT™ MTT cell viability assay kit with item number V13154 was purchased from Thermo Fisher Scientific (Thermo Fisher Scientific, Waltham, MA, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was added into the cells at 12, 24, 48, 72, and 96 h after transfection, separately, and cultured at 37°C for 4 h, followed by addition of SDS-HCL and 37°C incubation for 4 h. Three duplicate cells were set, and the optical density of cells at 570 nm was determined through a Varioskan™ LUX multimode microplate reader from Thermo Fisher Scientific (Thermo Fisher Scientific, Waltham, MA, USA).

Cell Apoptosis Determination by Flow Cytometry

The cells were trypsinized through 0.25% trypsin, and then washed with PBS twice, and added with 100 μL binding buffer to prepare *10^6 cells /mL suspension. The suspension was added with AnnexinV-FITC and propidium iodide (PI) in order, cultured in the dark at indoor temperature for 5 min, and then detected by a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA). The experiment was carried out three times and the data were averaged as results. The Annexin V - FITC/PI apoptosis Assay Kit (item number: V35113) was purchased from the Invitrogen Company in the United States (Invitrogen, Carlsbad, CA, USA).

EndMT Level

A WB assay was carried out to quantify endothelial cell markers (VE-cadherin and CD31) and mesenchymal cell markers (α-SMA and N-cadherin) to evaluate the EndMT.

Determination of Inflammatory Cytokines

Endothelial cells were disrupted by ultrasound, and then centrifugated at 1000×g for 15 min to take the supernatant, and the levels of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), as well as interleukin-10 (IL-10) in the supernatant were determined by TNF-α, IL-6, as well as IL-10 ELISA kits (GD-DS1716, GD-DS1726, GD-DS1731, Guduo Biotechnology Co., Ltd., Shanghai, China), respectively, in strict according with kit instructions.

Statistical Analysis

In our study, the data were statistically processed using SPSS 19.0 (IBM, Armonk, NY, USA), and visualized into required figures through Graph-Pad Prism 8.0 (La Jolla, CA, USA). Quantitative data were expressed as the mean ± standard deviation (mean±SD), compared between groups through the Student's *t*-test and compared among groups through the one-way ANOVA. Post-hoc test was carried out using the LSD test. In addition, the post analysis was conducted using the LSD test and two-tailed test. $p<0.05$ implies a significant difference.

Results

MiR-217 Inhibits the Apoptosis of AECs

Compared with cells in the blank group, cells in the control group showed significantly down-regulated miR-217. In addition, it was found that in the miR-217 mimic group, up-regulation of miR-217 inhibited the apoptosis of the endothelial cells, strengthened their viability, upregulated VE-cadherin and CD31, downregulated α-SMA, and lowered EndMT, while downregulation of miR-217 gave rise to the opposite results, and in the miR-217inhibitor group, up-regulation of miR-217 in endothelial cells gave rise to an increase in apoptosis rate and EndMT and a decrease in cell viability (Figure 1).

MiR-217 Inhibits the Inflammatory Response of AECs

In the atherosclerotic endothelial cell model, the endothelial cells showed up-regulated TNF-α and IL-6 and down-regulated IL-10, and transfection with miR-217 mimic strongly inhibited the inflammatory response in AECs, while transfection with miR-217 inhibitor promoted the inflammatory response in them (Figure 2).

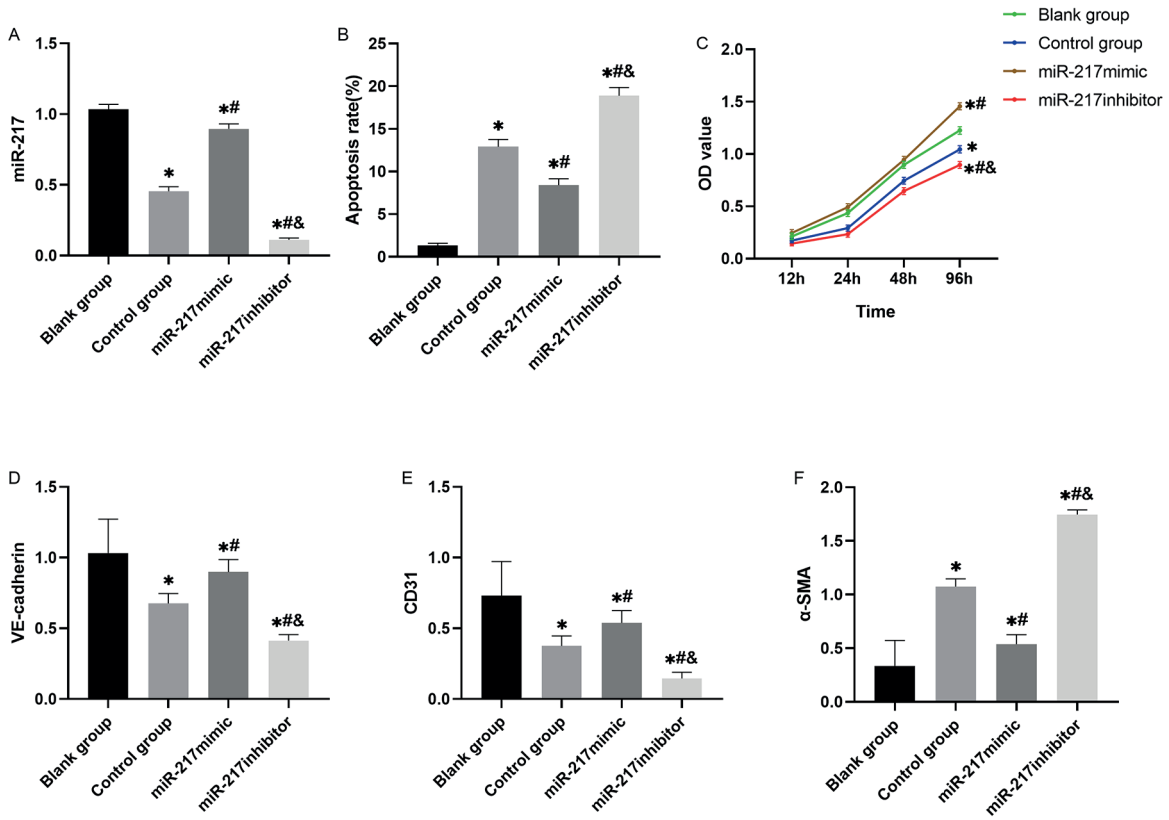

Figure 1. MiR-217 inhibits the apoptosis of AECs. **A**, Transfection results of miR-217-related vectors. **B**, Effects of miR-217 on the apoptosis of endothelial cells. **C**, Effects of miR-217 on the viability of endothelial cells. **D**, Effects of miR-217 on the expression of VE-cadherin in endothelial cells. **E**, Effects of miR-217 on the expression of CD31 in endothelial cells. **F**, Effects of miR-217 on the α-SMA expression in endothelial cells. * indicates $p<0.05$ *vs.* the blank group. # indicates $p<0.05$ *vs.* the control group. & indicates $p<0.05$ *vs.* the miR-217mimic group.

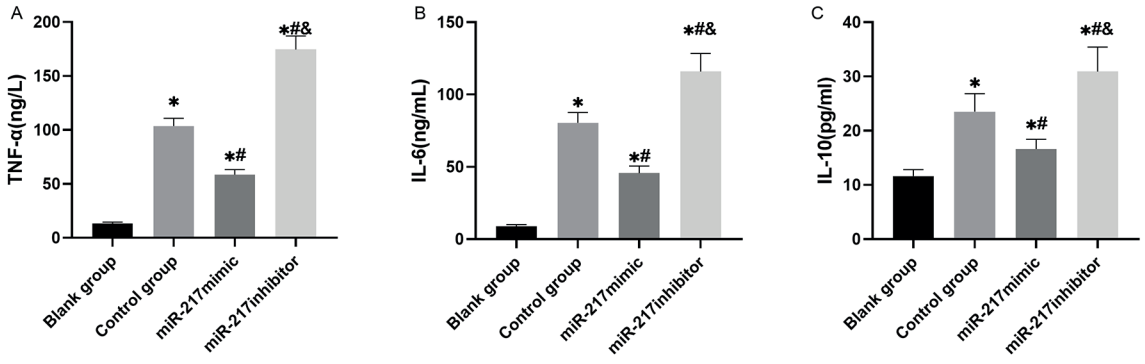

Figure 2. MiR-217 inhibits the inflammatory response of AECs. **A**, Effects of miR-217 on TNF-α level in endothelial cells. **B**, Effects of miR-217 on IL-6 level in endothelial cells. **C**, Effects of miR-217 on IL-10 level in endothelial cells. * indicates $p<0.05$ *vs.* the blank group. # indicates $p<0.05$ *vs.* the control group. & indicates $p<0.05$ *vs.* the miR-217mimic group.

TLR4 Promotes the Apoptosis of AECs

Endothelial cells in the control group showed higher TLR4 level than those in the blank group. After silence of TLR4, endothelial cells in the si-TLR4 group showed weaker apoptosis and higher cell activity than the control group, and also showed higher VE-cadherin and CD31 levels and lower α-SMA level than the control group. In addition, after up-regulation of TLR4, endothelial cells in the sh-TLR4 showed higher apoptosis, lower viability, and stronger EndMT than the control group (Figure 3).

TLR4 Promotes the Inflammatory Response of AECs

After silence of TLR4, endothelial cells in the si-TLR4 group showed decreased inflammatory response. Specifically, they showed lowered TNF-α and IL-6 levels and elevated IL-10 level, while up-regulation of TLR4 gave rise to opposite situation: endothelial cells showed severer inflammatory response, and endothelial cells in the sh-TLR4 group showed increased TNF-α and IL-6 levels and decreased IL-10 level. (Figure 4)

TLR4 Is the Target of MiR-217

The fluorescence activity determination results revealed that transfection of miR-217 mimic strongly lowered the fluorescence activity of 293T cells, while transfection of miR-217 inhibitor strongly enhanced it ($p< 0.05$) (Figure 5).

Inhibition of the PI3K/Akt/NF-κB Pathway Promotes the Apoptosis of Endothelial Cells by Interfering with TLR4

While finding the promotion of TLR4 on the apoptosis and inflammatory response of endothelial cells, we also found that after inhibition of the PI3K/Akt/NF-κB pathway, endothelial cells in the sh-TLR4 group showed lower p-PI3K and p-AKT levels, and higher NF-κB p53 level than the control group. Thus, we designed a group of cells, and silenced their TLR4 and hindered the activation of the PI3K/Akt/NF-κB pathway through PI3K inhibitor, finding that endothelial cells in the TLR4 rescue group showed increased apoptosis, lowered viability, down-regulated VE-cadherin, CD31, and IL-10, and up-regulated α-SMA, TNF-α, and IL-6 (Figure 6).

MiR-217 Inhibits the PI3K/Akt/NF-κB Pathway Via TLR4

Upregulation of miR-217 activated the PI3K/Akt/NF-κB pathway, elevated p-PI3K and p-AKT expression, and decreased NF-κB p53 expression, but after up-regulation of miR-217 and TLR4 expression, the activation of miR-217 on the PI3K/Akt/NF-κB pathway was significantly weakened, and endothelial cells in the miR rescue group showed lower p-PI3K and p-AKT levels and higher NF-κB p53 level than the miR-217 mimic group (Figure 7).

Discussion

Endothelial cells attach on the surface of the vascular lumen and form the first layer of a barrier to regulate the communication between molecules and cells inside and outside the blood vessel. Under various stimulations, endothelial cells are activated, and then lose the ability to regulate lipid homeostasis, immune function, and inflammation, eventually forming foam cells and fatty

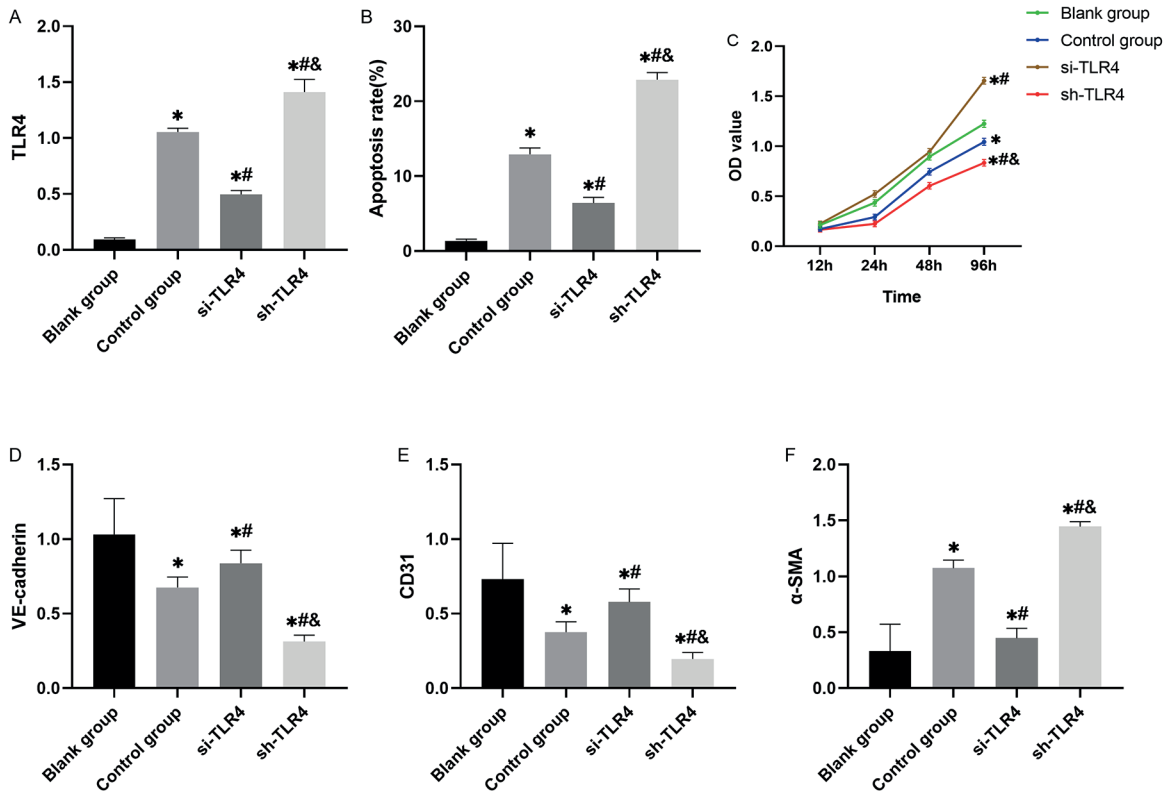

Figure 3. TLR4 promotes the apoptosis of AECs **A,** Transfection results of TLR4-related vectors. **B,** Effects of TLR4 on the apoptosis of endothelial cells. **C,** Effects of TLR4 on the viability of endothelial cells. **D,** Effects of TLR4 on the expression of VE-cadherin in endothelial cells. **E,** Effects of TLR4 on CD31 level in endothelial cells. **F,** Effects of TLR4 on α-SMA level in endothelial cells. * indicates $p<0.05$ *vs.* the blank group. # indicates $p<0.05$ *vs.* the control group. & indicates $p<0.05$ *vs.* the si-TLR4 group.

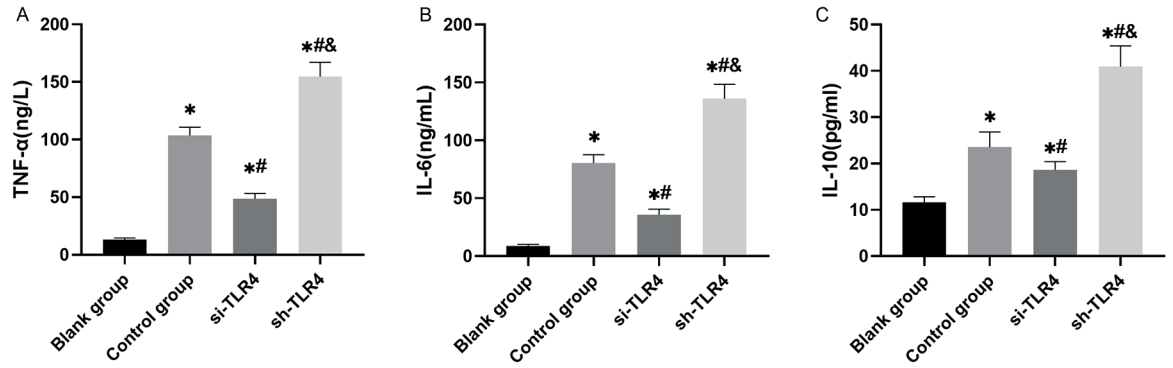

Figure 4. TLR4 promotes the inflammatory response of AECs. **A**, Effects of TLR4 on TNF-α in endothelial cells. **B**, Effects of TLR4 on IL-6 in endothelial cells. **C**, Effects of TLR4 on IL-10 in endothelial cells. * indicates $p<0.05$ *vs.* the blank group. # indicates $p<0.05$ *vs.* the control group. & indicates $p<0.05$ *vs.* the si-TLR4 group.

streaks and promoting the formation of atherosclerotic plaques[17-19]. Therefore, alleviating endothelial cell damage is an essential objective for the treatment of atherosclerosis. This study found that miR-217 inhibited the apoptosis of AECs via the TLR4/PI3K/Akt/NF-κB pathway.

In our study, in AECs, miR-217 expression decreased, and the PI3K/Akt/NF-κB pathway was

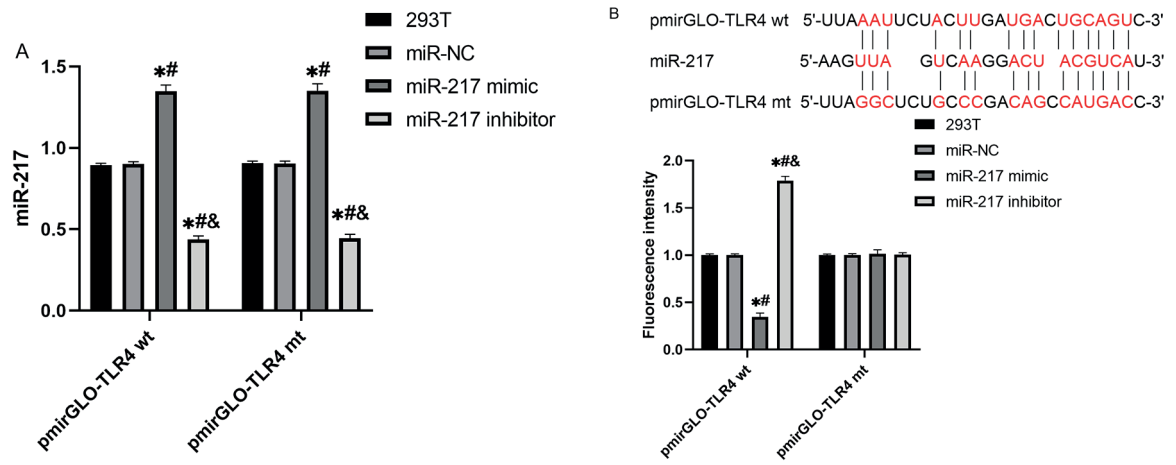

Figure 5. Verification of targeting binding site between miR-217 and TLR4. **A,** Transfection results of miR-217-related vectors. **B,** Analysis on fluorescence intensity. * indicates $p<0.05$ *vs.* the 293T group. # indicates $p<0.05$ *vs.* the miR-NC group. & indicates $p<0.05$ *vs.* the miR-217 mimic group.

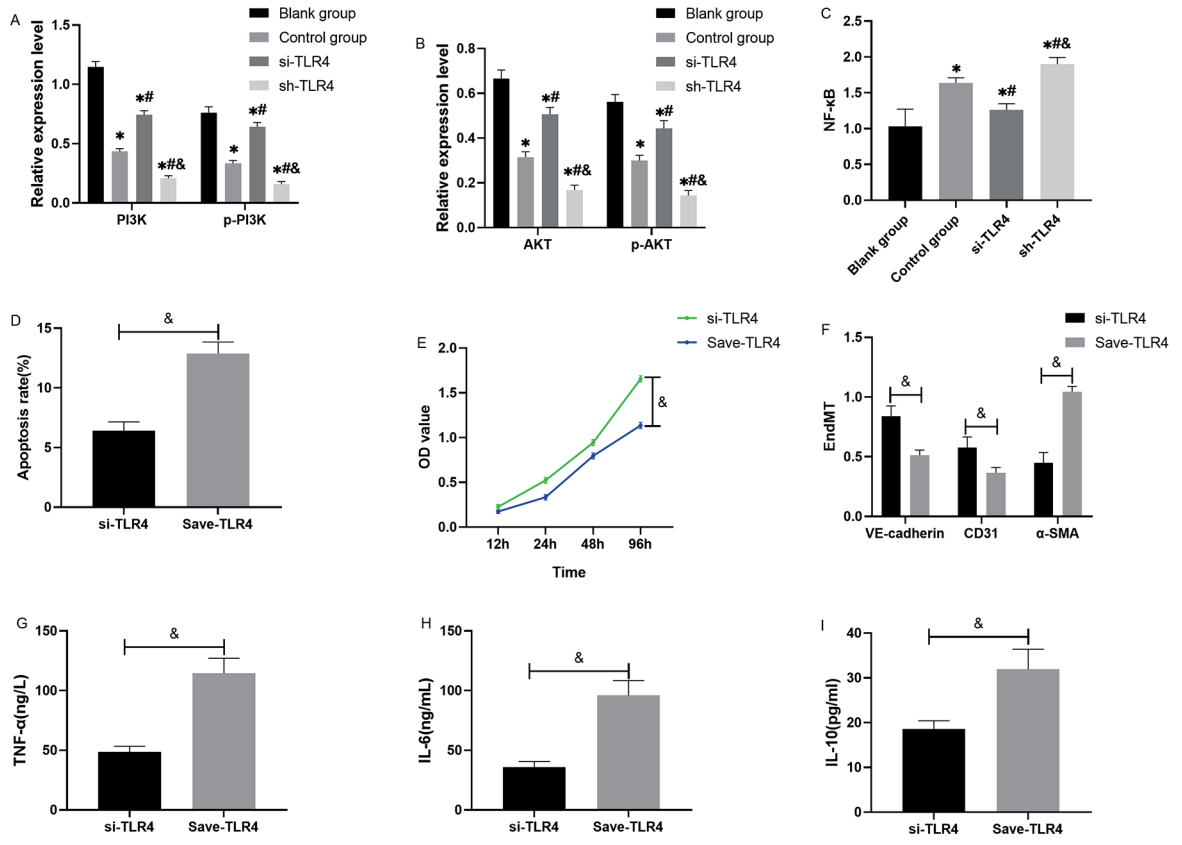

Figure 6. Inhibition of the PI3K/Akt/NF-κB pathway promotes the apoptosis of endothelial cells by interfering with TLR4. **A,** Effects of silencing TLR4 on PI3K and p-PI3K proteins. **B,** Effects of silencing TLR4 on Akt and p-Akt proteins. **C,** Effects of silencing TLR4on NF-κB protein. **D,** Inhibition of the PI3/Akt/NF-κB pathway promoted the apoptosis of endothelial cells. **E**, Inhibition of the PI3/Akt/NF-κB pathway inhibited the viability of endothelial cells. **F**, Inhibition of the PI3/Akt/NF-κB pathway promoted the EndMT. **G,** Inhibition of the PI3/Akt/NF-κB pathway increased the TNF-α level in endothelial cells. **H,** Inhibition of the PI3/Akt/NF-κB pathway increased the IL-6 level in endothelial cells. **I,** Inhibition of the PI3/Akt/NF-κB pathway increased the IL-10 level in endothelial cells. * indicates $p<0.05$ *vs.* the blank group. # indicates $p<0.05$ *vs.* the control group. & indicates $p<0.05$ *vs.* the si-TLR4 group.

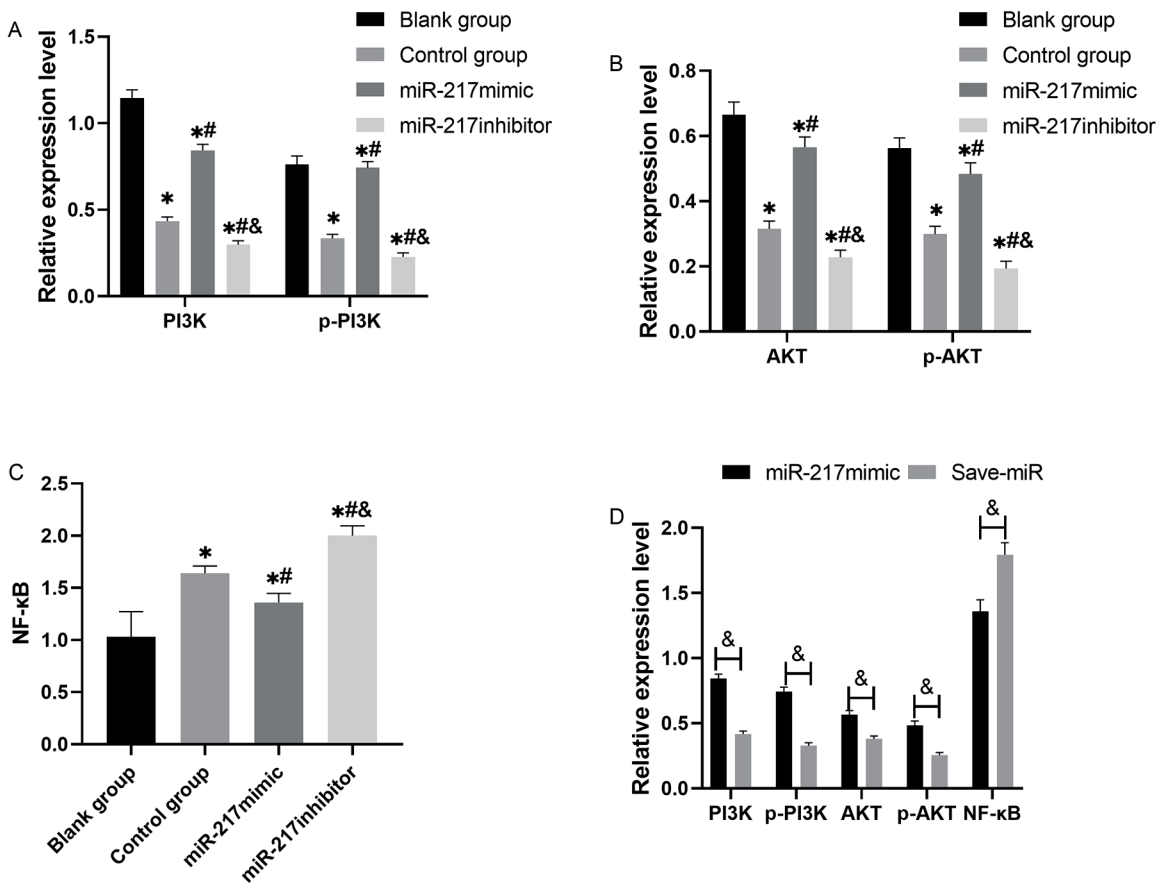

Figure 7. MiR-217 inhibits the PI3K/Akt/NF-κB pathway via TLR4. **A,** MiR-217 could activate PI3K expression. **B,** MiR-217 could activate AKT expression. **C,** MiR-217 could activate NF-κB expression. **D,** TLR4 could prevent miR-217 from activating the PI3K/Akt/NF-κB pathway. * indicates $p<0.05$ *vs.* the 293T group. # indicates $p<0.05$ *vs.* the miR-NC group. & indicates $p<0.05$ *vs*. the miR-217 mimic group.

inhibited, and TLR4 was the target of miR-217, and it was up-regulated in AECs. Further study revealed that the up-regulation of miR-217 protected AECs, increased their activity, reduced apoptosis, and inhibited inflammatory response and EndMT, while TLR4 acted contrary to miR-217. It was also found that miR-217 inhibited the PI3K/Akt/NF-κB pathway, thus weakening the influences of si-TLR4 on endothelial cells. According to our study results, miR-217 inhibits the TLR4/ PI3K/Akt/NF-κB signal transduction pathway to protect endothelial cells.

Apoptosis of endothelial cells plays a pivotal part in the development of atherosclerosis, which damages the integrity of endothelial function, depriving endothelial cells of correctly regulating lipid homeostasis, thus aggravating lipid deposition and plaque instability[20,21]. Therefore, inhibiting endothelial cell apoptosis is the key to the treatment of atherosclerosis. MiRNAs are abnormally expressed and involved in endothelial cell apoptosis[22,23]. One previous study has revealed that miR-217 can targeted inhibit Sirtuin 1 (SirT1) to promote endothelial cell senescence[24], and Zhang et al[25] have detected that miR-217 accelerates the proliferation, migration, and tube formation of endothelial cells infected by human cytomegalovirus by downregulating SirT1 and forkhead protein O3A. With further research, it was also found that miR-217 can protect endothelial cells and inhibit apoptosis[11].

Similarly, there are research reports that TLR4 is related to endothelial cell apoptosis. Yang et al[26] have found that miR-590 suppresses the apoptosis of endothelial cells in atherosclerosis through inactivating the TLR4 /NF-κB pathway. PI3K/Akt

is involved in the apoptosis of endothelial cells, and the protective effect of many cytokines or drugs on endothelial cells depends on the PI3K/Akt/NF-κB signaling pathway[27,28].

Endothelial cell apoptosis also leads to an imbalance in the regulation of inflammatory response, further aggravating endothelial dysfunction, increasing vascular permeability, extravasating pro-inflammatory cytokines, and promoting immune cells the accumulation of white blood cells and lipids in arterial intima[29,30]. Similarly, Zhang et al[10] have revealed that down-regulation of miR-217 can target sirtuin 1 to inhibit macrophage apoptosis and the expression of inflammatory factors in macrophages. However, at present, it is not clear about the inflammatory response of miR-217 to endothelial cells and its mechanism of action, but many studies report that inhibiting TLR4 can improve the production of endothelial inflammatory factors in atherosclerosis patients[31,32]. The PI3K/Akt/ NF-κB signaling pathway has similar effects[33,34], but TLR4 has contrary effects.

EndMT in another crucial cause of endothelial cell dysfunction. At the time of dysfunction, endothelial cells acquire myofibroblast-like characteristics, and accompanied by cell-to-cell contact and loss of cell polarity, endothelial cells acquire invasion and migration phenotypes, secrete extracellular matrix that accumulates in the arterial wall and increase instability of atherosclerotic plaque[35,36]. There are few studies on the relationship between miR-217 and EndMT, but much of the evidence shows that miR-217 can inhibit epithelial-mesenchymal transition (EMT) in many cancer cells, such as pancreatic cancer cells and hepatic carcinoma cells[37,38]. Zhang et al[39] have reported that miR-126 targets the PIK3R2-PI3K/Akt signaling pathway to inhibit the EndMT. This study revealed that miR-217 inhibited EndMT by inhibiting TLR4 from activating the PI3K/Akt/NF-κB signal pathway.

Conclusions

To sum up, ox-LDL can induce the expression of TLR4 in endothelial cells and inhibit miR-217 and the PI3K/Akt/ NF-κB pathway in them. In addition, miR-217 can increase the viability of AECs through the TLR4/PI3K/Akt/ NF-κB signal transduction pathway, and inhibit their apoptosis, inflammatory response, and EndMT.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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