Int J Clin Exp Med 2020;13(4):2194-2204
www.ijcem.com /ISSN:1940-5901/IJCEM0105638

Original Article

Effects of miR-23b-3p on the biological characteristics of LPS-stimulated umbilical vein endothelial cells via targeting Fas

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Received November 28, 2019; Accepted December 29, 2019; Epub April 15, 2020; Published April 30, 2020

Abstract: Objective: Aiming to explore the protective action of miR-23b-3p/Fas axis on cell damage in LPS-stimulated human umbilical vein endothelial cells (HUVEC). Methods: *In vitro* cell damage model was constructed by stimulating HUVECs with LPS and the treated cells were divided into different groups. Targeting the relationship between miR-23b-3p and Fas was further verified through dual luciferase reporter assay. Expressions of Fas and Bax, Bcl-2 and cleaved-Caspase3 relating to apoptosis were detected through western blot. MTT assay and flow cytometry were employed to estimate cell viability and apoptosis. Cell migration and invasive ability were valued through wound-healing and transwell assay. ELISA was conducted to examine the expression of inflammatory factors TNF-α, IL-1β and INF-γ. Results: Dual luciferase reporter assay exhibited that miR-23b-3p suppresses the expression of Fax by targeting the 3'UTR of Fas ($P<0.05$). Overexpression of miR-23b-3p protected against LPS-stimulated cell damage by promoting cell proliferation and suppressing cell invasion, migration, apoptosis and down-regulating the level of inflammatory factors TNF-α, IL-1β and INF-γ ($P<0.05$). Simultaneously, the protective effect of miR-23b-3p mimic could be set-off by Fas overexpression. Conclusion: MiR-23b-3p targets Fas, further to suppress LPS-induced cell apoptosis, migration and release of inflammatory factors in HUVEC.

Keywords: MiR-23b-3p, Fas, umbilical vein endothelial cells, cell apoptosis, cell migration

Introduction

Atherosclerosis (AS) is a kind of chronic inflammatory disease of blood vessels, which is a frequent clinical complication of cardiovascular and cerebrovascular diseases, and the mortality of AS is higher than other cardiovascular diseases [1, 2]. In addition to causing vascular disease, AS can also affect other organs [3]. At present, there are many explanations for the complex pathogenesis of AS, including the endothelial cell damage theory. Some studies have confirmed that the variation of vascular endothelial cells play an important effect in the onset of AS. The damage of vascular endothelial cells is one of the early manifestation of AS and the proliferation of vascular endothelial cells is able to promote the formation of atheromatous plaques [4].

MircroRNA (miRNA) is composed of single stranded RNA molecules, which regulate gene expression mainly by binding with target genes [5, 6]. Recently, the function of miRNA in AS has become a research hotspot. More and more studies show that miRNA is closely participating in the occurrence and development of AS by acting on multiple targets [7, 8]. MiR-23b-3p is a single stranded RNA molecule with 21 nucleotides, and it often forms gene clusters with miR-27b and miR-24 [9]. MiR-23b-3p has also been proven to regulate the proliferation, differentiation and apoptosis of neurons, and also participate in the pathological mechanism of various diseases [10]. In the study of the inhibitory effect and mechanism of miR-23b-3p on the injury of coronary artery endothelial cells induced by homocysteine, it was found that miR-23b-3p can down-regulate the production of apo (a) in endothelial cells through signaling pathways, thus playing a protective role for vascular endothelial cells [11]. However, it has not been stated whether miR-23b-3p can regulate

LPS-induced umbilical vein endothelial cell damage.

Lipopolysac charide (LPS) is involved in the formation of AS, especially the initial process of inflammation [12, 13]. LPS activates immune cells when it enters the body, further leading to the releasing of pro-inflammatory cytokines, thus inducing inflammatory responses. In this process, a variety of cells, including vascular endothelial cells, will continue to secrete a large number of pro-inflammatory cytokines, accelerating the development of AS [14, 15].

Fas, also known as CD95 or Apo-1, is regarded as a potential target gene of miR-23b-3p, with FasL as its ligand [16]. It has been shown that Fas/FasL complex regulates the apoptotic pathway of human cardiac micro-vascular endothelial cells in ischemia-reperfusion injury [17]. Therefore, miR-23b-3p plays a momentous regulatory role in LPS induced damage in vascular endothelial cells by targeting Fas.

Methods

Cell culture, transfection and the construction of an in vitro model

HUVEC cells used in this experiment were acquired from ATCC cell bank (USA), and were then kept in DMEM medium with 10% fetal bovine serum (FBS) (GIBCO, USA), 100 U/ml penicillin (GIBCO, USA) and 100 μg/mL streptomycin (GIBCO, USA) in cell incubator at 37°C in 5% CO_2.

We have 6 treatment groups: control group, LPS stimulation group, NC group, miR-23b-3p mimic group, oe-Fas group (transfected with Fas overexpression plasmid), and miR-23b-3p + oe-Fas group (co-transfection of miR-23b-3p mimic and Fas overexpression plasmid). MiR-23b-3p mimic, NC and Fas overexpression plasmids were synthesized and provided by Shanghai Jima Pharmaceutical Co., Ltd.

Logarithmic growth HUVEC cells were inoculated into 24 well plates and were transfected as indicated when the cell density reached 80%. Cell transfection was conducted using lipofectamin 2000 kit (Invitrogen) following the instructions.

After dilution separately with 250 μL of opti MEM (GIBCO) medium and incubation at room temperature for 5 minutes, 4 μg target plasmid and 10 μL lipofectamin 2000 were mixed gently. After 20 minutes of stewing, the mixture was added into the cell culture plate. Shaking the plate gently until well mixed, then the cells continued cultured. After 8 hours of transfection, the culture medium was replaced with complete culture medium for 24 h incubation. Then, the cells were starved in serum-free medium for 6 h, and were then stimulated with or without 1 μg/mL LPS (Shanghai Jiwei Biotechnology Co., Ltd.) for 12 h, and the treated cells were collected for subsequent experiments.

Dual luciferase reporter assay

The relationship between miR-23b-3p and Fas was initially predicted through bioinformatics website and was verified by dual luciferase reporter assay. Correlative binding sites of miRNA-23b-3p and Fas were analyzed and fragment sequences containing the binding sites were obtained. Synthetic wild type (WT) or mutant (MUT) 3'-UTR of miRNA-23b-3p gene containing putative Fas targeting site was inserted into the psiCHECK2 vector. Mimic NC or miRNA-23b-3p mimic was co-transfected with Luciferase Report vector into 293T cells (obtained from Shanghai cell bank of Chinese Academy of Sciences). Luciferase activity was measured with Luciferase test kit (Promega, Madison, WI, USA) and targeting effect was exhibited in the way of relative luciferase activity. After 48 hours of incubation, the cells were cleaved using 1× passive lysate and activity of firefly luciferase was measured by dual luciferase reporter assay system (Promega, USA). Luciferase activity of murine kidney was used as internal reference. Relative luciferase activity was analyzed as firefly luciferase activity/murine kidney activity.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Trizol-one step method (Sigma, USA) was used to extract total RNA in HUVEC cells and RT-PCR was processed using two-step method with the RT-PCR kit (Invitrogen, 11615-010 Taq DNA Polymerase) as follows: pre-denaturation at 94°C for 5 minutes, denaturation at 94°C for 30 s, annealing for 30 s at 56°C, total for 35 PCR cycles, extension at 72°C for 10 min. U6 and GAPDH were used as internal reference for

Table 1. Primer sequences of qRT-PCR

Genes	Sequences of related Primer
Fas	
Forward	5'-CAATTCTGCCATAAGCCCTGTC-3'
Reverse	5'-GTCCTTCATCACACAATCTACATCTTC-3'
miR-23b-3p	
Forward	5'-TGGTGAAGAATGCTGAGATGTC-3'
Reverse	5'-GGTGATGCTCTGCTGAACG-3'
GAPDH	
Forward	5'-GCACCGTCAAGGCTGAGAAC-3'
Reverse	5'-TGGTGAAGACGCAGTGGA-3'
U6	
Forward	5'-GCTTCGGCAGCACATATACTAAAAT-3'
Reverse	5'-CGCTTCACGAATTTGCGTGTCAT-3'

miR-23b-3p and Fas mRNA, respectively. $2^{-\Delta\Delta Ct}$ represents relative expression level of target genes: $\Delta\Delta CT = \Delta Ct_{experimental\ group} - \Delta Ct_{control\ group}$, $\Delta Ct = Ct_{target\ gene} - Ct_{internal\ reference}$. Ct is amplification cycle number of qPCR. RT-PCR was carried out accordance strictly with the instructions. Related primer sequences are showed in **Table 1**.

Western blotting (WB)

After cell transfection and LPS stimulation for 12 h, HUVEC cells were washed with PBS twice and total protein from HUVEC cells were lysed by Ripa lysate (R0010, Solarbio) containing PMSF. The lysate was collected and incubated 30 minutes on ice, supernatant was collected after lysate was centrifuged 10 min at 12000 rpm/min in 4°C. Protein concentration was examined using BCA kit (23225, Pierce) and equal 30 μg protein sample was separated by SDS-PAGE gel (P0012A, Biyun Tian Biotechnology Research Institute, Shanghai, China) (80 V, 2 h) and then was transferred to PVDF membrane (ISEQ00010, Millipore, Billerica, MA, USA) through wet transfer method (110 V, 2 h). PVDF membranes were blocked with TBST buffer (Dalian Meilun Biotechnology Co., Ltd.) containing 5% nonfat milk powder for 1.5 h and then were treated with primary antibody respectively: anti-Fas (1:5000, ab133619, Abcam, UK), anti-Bax (1:1000, ab32503, Abcam, UK), anti-Bcl-2 (1:500, ab185002, Abcam, UK), anti-Cleaved caspase3 (1:500, ab32042, Abcam, UK), anti-GAPDH (1:10000, ab181602, Abcam, UK) at 4°C overnight. Washed the membranes for at last 3 times, 15 min per time. Then, the membranes were incubated with corresponding secondary antibody (Beijing Zhongshan Biotechnology Company). Equal amounts of liquid A and liquid B from the ECL fluorescence detection kit (No.: BB-3501, Ameshame company, UK), were mixed in the darkroom, and were added to the membrane. After exposure, development and fixation, the blots on X film gradually appear. Relative protein level is gray value of corresponding protein band/gray value of GAPDH which are analyzed through Quantity one v4.6.2.

MTT assay for cell viability detection

MTT assay was conducted in 96 well plates and each group was set with 3 replicates. After treatment as indicated, the cells were mixed with 20 μL 5 mg/mL MTT solution (C0009, Beyotime Biotechnology, Shanghai, China) and then were cultured for another 4 h. Carefully washed off the supernatant, 150 μL DMSO (Shanghai Biotechnology Co., Ltd.) was added to each well. Then, gently mixed to prevent bubbles. Cell absorbance was measured at 490 nm using a micro-plate Reader. Cell viability = (experimental group - blank control group)/(normal control group - blank control group) ×100%.

Flow cytometry

Annexin V-FITC/PI double staining kit (No. 556547, Shanghai shuojia biology, China) was used to detect the apoptosis of HUVEC cells as following: Dilute 10× binding buffer to 1× binding buffer with double distilled water. After the cells were digested and collected, they were centrifuged at 1200 rpm/min at room temperature for 5 min and the supernatant was discarded. After re-suspended with precooled 1× PBS, cells were centrifuged at 1200 rpm/min for 10 minutes, washed, and were mixed with 300 μL 1× binding buffer suspension. After that, cell suspension and 5 μL annexin V-FITC were mixed and were incubated in dark at room temperature for 15 min. Then, 5 μL PI was added into the mixture without light for 5 min in an ice bath. FITC was detected at the wavelength of 480 nm and 530 nm and PI signal was detected at the wavelength greater than 575 nm using flow cytometry (cube6, partec, Germany).

Wound-healing assay

After 48 hours of HUVEC cell transfection, cell scratches at the bottom of the cell plate perpendicular to the cell plate were made using 10 μL sterile pipette tip, then the culture medium

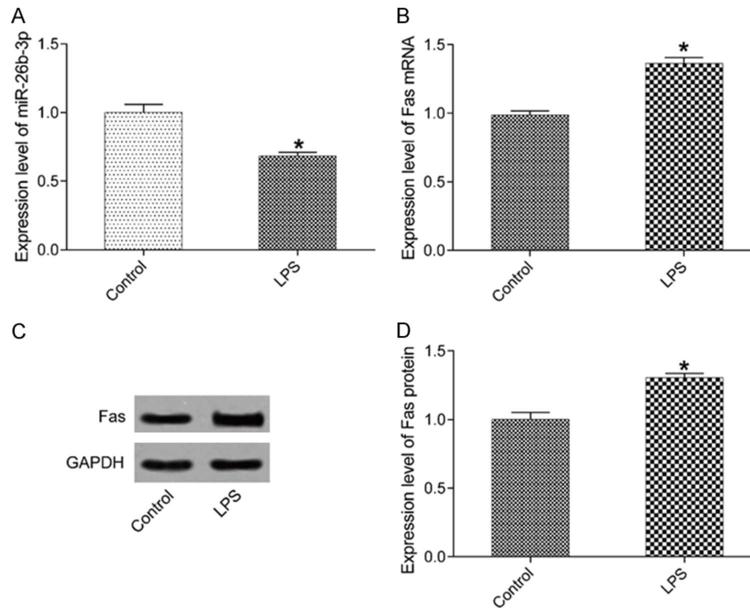

Figure 1. Expressions of miR-23b-3p and Fas in HUVEC. A: Expression of miR-23b-3p was valued by qRT-PCR; B: Expression of Fas was valued through qRT-PCR; C: WB was used to examine the expression of Fas; D: Histogram analysis of Fas expression detected through WB; Control group: normal HUVEC. LPS group: HUVEC treated with 1 μg/mL LPS for 12 h. *P vs control group, *P<0.05; HUVEC: Human Umbilical vein endothelial cells; LPS: Lipopolysaccharide.

was discarded, and 1 mL PBS solution preheated at 37°C was added into the plate. After gently shaking and twice washing, scratched cells were moved. Then serum-free DMEM medium (GIBCO, USA) and 1 μg/mL LPS were added into the medium, and the cells were cultured again in the 5% CO_2 incubator. Twelve hours later after scratching, cell migration was observed and was photographed under microscope, and cell migration distance was analyzed with Image J software.

Transwell invasion assay

The transwell chamber was pre-coated with 50 μL Matrigel (sigma, Germany) overnight at room temperature one day before the experiment and then was washed twice for further use. The transfected cells were digested with trypsin (Thermo Fisher Scientific, USA) and blown into cell suspension, washed twice with PBS and counted. Then, 100 μL cell suspension (5×10^4 cells) was inoculated into 24 transwell wells. Six-hundred μL DMEM complete medium with 20% fetal bovine serum was added in the lower chamber. At the same time, LPS with a final concentration of 1 μg/mL was added into the inner and outer chambers for 6 h. Then, removed the inner chamber, wash twice with PBS, wipe off the cells on the upper layer of the inner chamber fibrous membrane with cotton swab gently, and then put the inner chamber into 4% paraformaldehyde (Leagene, Beijing, China) for 20 minutes. After being twice washed, the inner chamber placed into 0.05% gentian violet (Solarbio, Beijing, China) for 5 minutes, and 5 stochastic fields were selected to calculate the cells number through the membrane under the microscope.

Detection of related inflammatory factors by ELISA

HUVEC cells were inoculated into 24 well plates, and the supernatant of cell culture medium was collected after the cells were treated according to the requirements of each group. Contents of TNF-α, IL-1β and INF-γ in cell culture supernatant were detected following the instructions of the ELISA Kit (R&D, UK), and then the standard curve was drawn and the contents of inflammatory factors in the serum of each group were calculated.

Statistical analysis

Data analysis was conducted using Graphpad 7.0 version (San Diego, California), and all measurement data are expressed as mean ± standard deviation. One way ANOVA combined with Bonferroni post hoc test were used for comparing two groups. P<0.05 means the difference is statistically significant.

Results

Expression of miR-23b-3p was suppressed and Fas was elevated in LPS-induced HUVEC injury model

Expressions of miR-23b-3p and Fas in normal umbilical vein endothelial cells HUVEC and LPS stimulated model cells were detected through qRT-PCR (**Figure 1A**, **1B**). The level of miR-23b-3p was clearly decreased and Fas was largely

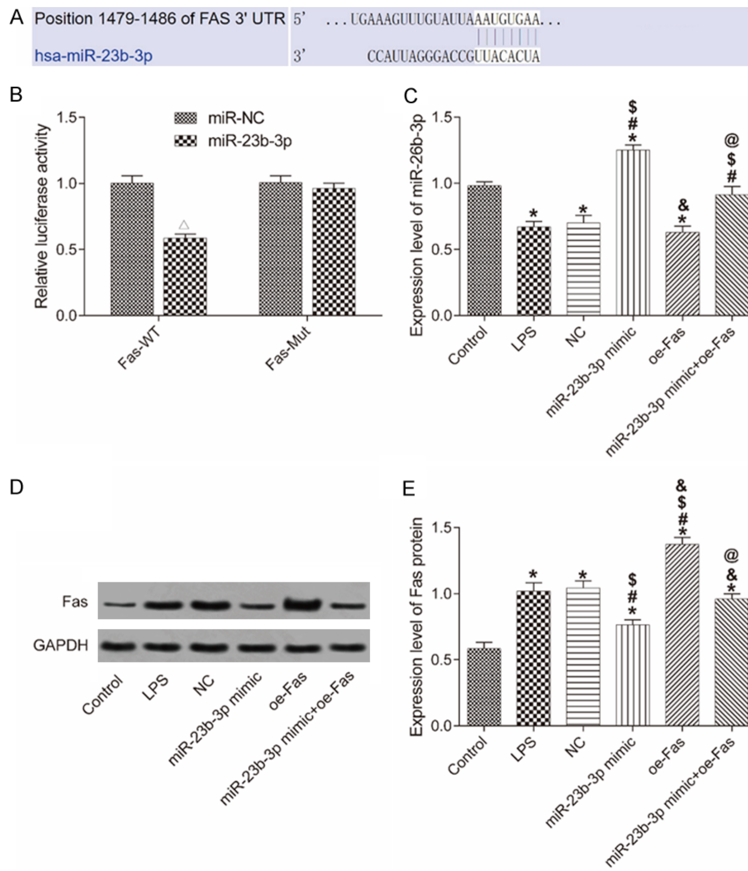

Figure 2. MiR-23b-3p can target and regulate the down-regulation of Fas expression. A: The complementary binding site between miR-23b-3p and Fas were first predicted and analyzed by bioinformatics website; B: Dual Luciferase Report assay was conducted to verify the targeting relationship between miR-23b-3p and Fas; C: Expression of miR-23b-3p in each group detected through qRT-PCR; D: Expression of Fas in each group detected through WB; E: Histogram analysis of Fas expression; $^{\Delta}$P<0.05 vs NC group; *P<0.05 vs control group; $^{\#}$P<0.05 vs LPS group; $^{\$}$P<0.05 vs NC group; $^{\&}$P<0.05 vs miR-23b-3p mimic group; $^{@}$P<0.05 vs oe-Fas group; LPS: Lipopolysaccharide.

increased in LPS group compared with the control group (P<0.05). Correspondingly, the results of western blot in **Figure 1C**, **1D** also showed that expression level of Fas was much higher in LPS group than the control group (P<0.05).

MiR-23b-3p can target and regulate the down-regulation of Fas expression

Bioinformatics website analysis showed that there are binding sites between miR-23b-3p and Fas (**Figure 2A**). Dual luciferase reporter assay showed that, the intensity of luciferase activity in co-transfection group between miR-23b-3p mimic and Fas-WT was decreased significantly compared with miR-NC group (P<0.05), while it made no difference on the intensity of luciferase activity in miR-23b-3p mimic and Fas-Mut co-transfection groups (P>0.05) (**Figure 2B**).

In order to further explore whether miR-23b-3p regulates the expression of Fas, qRT-PCR was employed to detect miR-23b-3p production in each group (**Figure 2C**). The expression of miR-23b-3p in LPS stimulated HUVEC cells was significantly down-regulated compared with the control (P<0.05). Compared with NC group, the expression of miR-23b-3p in miR-23b-3p mimic group and miR-23b-3p mimic + oe-Fas group was both significantly increased (P<0.05), but no significant difference was shown in miR-23b-3p expression among LPS group, NC group and oe-Fas group (P>0.05).

The content of Fas in cells of each group was detected by WB (**Figure 2D**, **2E**). The results showed that the expression of Fas in other groups was all significantly higher than the control group (P<0.05). Compared with LPS group, the expression of Fas protein in miR-23b-3p mimic group was down-regulated, while that in oe-Fas group was up-regulated (P<0.05). Besides, the expression of Fas in miR-23b-3p mimic + oe-Fas group was significantly up-regulated compared with miR-23b-3p mimic group, but was strongly down-regulated compared with that in oe-Fas group (P<0.05). There was no visible difference in Fas protein expression among the LPS group, NC group and miR-23b-3p mimic + oe-Fas group (P>0.05).

The results state that miR-23b-3p regulates LPS stimulated HUVEC cells through targeting Fas.

Cell viability detection through MTT

Cell viability was detected through MTT (**Figure 3**). The results showed that cell viability in other

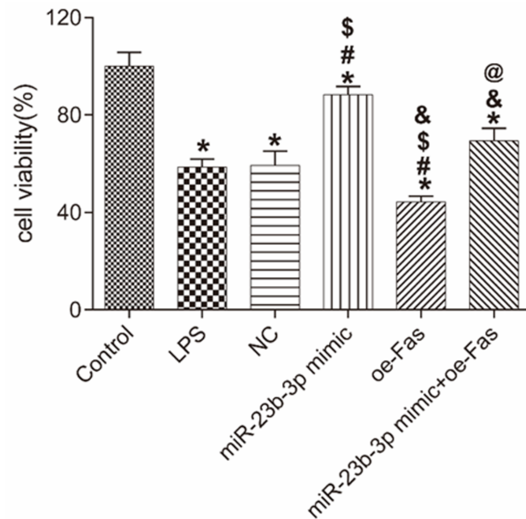

Figure 3. Cell viability detection through MTT. *P<0.05 vs control group; #P<0.05 vs LPS group; $P<0.05 vs NC group; &P<0.05 vs miR-23b-3p mimic group; @P<0.05 vs oe-Fas group; LPS: Lipopolysaccharide.

groups was strongly decreased compared with the control group (P<0.05). Besides, compared with LPS group, cell viability was significantly elevated in miR-23b-3p mimic group and was strongly suppressed in oe-Fas group (P<0.05). At the same time, cell viability in miR-23b-3p mimic + oe-Fas group was clearly inhibited compared with that in miR-23b-3p mimic group, but was enhanced compared with that of oe-Fas group (P<0.05). There was no distinct difference in cell viability among the LPS group, NC group and miR-23b-3p mimic + OE Fas group (P>0.05).

Effects of miR-23b-3p/Fas overexpression on cell apoptosis in HUVEC injury model

Cell apoptosis detection was conducted by flow cytometry (**Figure 4A**). The results showed that the proportion of apoptotic cells in other groups all increased significantly compared with the control group (P<0.05). Comparing with NC group, cell apoptosis in miR-23b-3p mimic was clearly suppressed, while that in the oe-Fas group was largely promoted (P<0.05). Moreover, the proportion of apoptotic cells in miR-23b-3p mimic + oe-Fas group was higher than that in miR-23b-3p mimic group, but was lower than that in the oe-Fas group (P<0.05).

Corresponding detection of cleaved Caspase-3, Bax and Bcl-2 which are all interrelated to cell apoptosis was conducted through WB (**Figure 4B**). Similarly, the expression of cleaved caspase-3 and Bax was elevated and expression of Bcl-2 was suppressed in other groups compared to the control group (P<0.05). Comparing to NC group, expression of cleaved Caspase-3 and Bax was down-regulated and expression of Bcl-2 was up-regulated in miR-23b-3p mimic group, but adverse consequences were found in oe-Fas group (P<0.05). Beyond that, expression of cleaved Caspase-3 and Bax was elevated and Bcl-2 was suppressed in miR-23b-3p mimic + oe-Fas group compared with that in miR-23b-3p mimic group (P<0.05). By contrary, expression of cleaved Caspase-3 and Bax was decreased and Bcl-2 was increased in miR-23b-3p mimic + oe-Fas group compared to the oe-Fas group (P<0.05).

These above results showed that miR-23b-3p mimic suppressed cell apoptosis, but overexpressed Fas promoted cell apoptosis in LPS stimulated HUVEC cell injury model.

Effects of miR-23b-3p/Fas overexpression on cell migration and invasion in HUVEC injury model

Cell migration and invasion ability were detected through wound healing (**Figure 5A**) and transwell (**Figure 5B**), respectively. Related results exhibited that the migration and invasion ability in LPS group was enhanced significantly compared to the control group (P<0.05), but there was no distinct difference between LPS group and NC group (P>0.05). Besides that, comparing with NC group, cell migration and invasion ability of miR-23b-3p mimic group reduced significantly (P<0.05), while that of oe-Fas group enhanced significantly (P<0.05). Additionally, cell migration and invasion ability of miR-23b-3p mimic + OE Fas group was strengthened significantly compared with miR-23b-3p mimic group, which was weakened significantly compared with oe-Fas group (P<0.05).

Detection of inflammatory factors by ELISA

Expressions of inflammatory factors TNF-α, IL-1β and INF-γ were checked through ELISA (**Figure 6**). The results exhibited that the expression levels of TNF-α, IL-1β, INF-γ in other groups were all significantly higher than that in the control group (P<0.05). Comparing with NC group, expressions of TNF-α, IL-1β and INF-γ in miR-

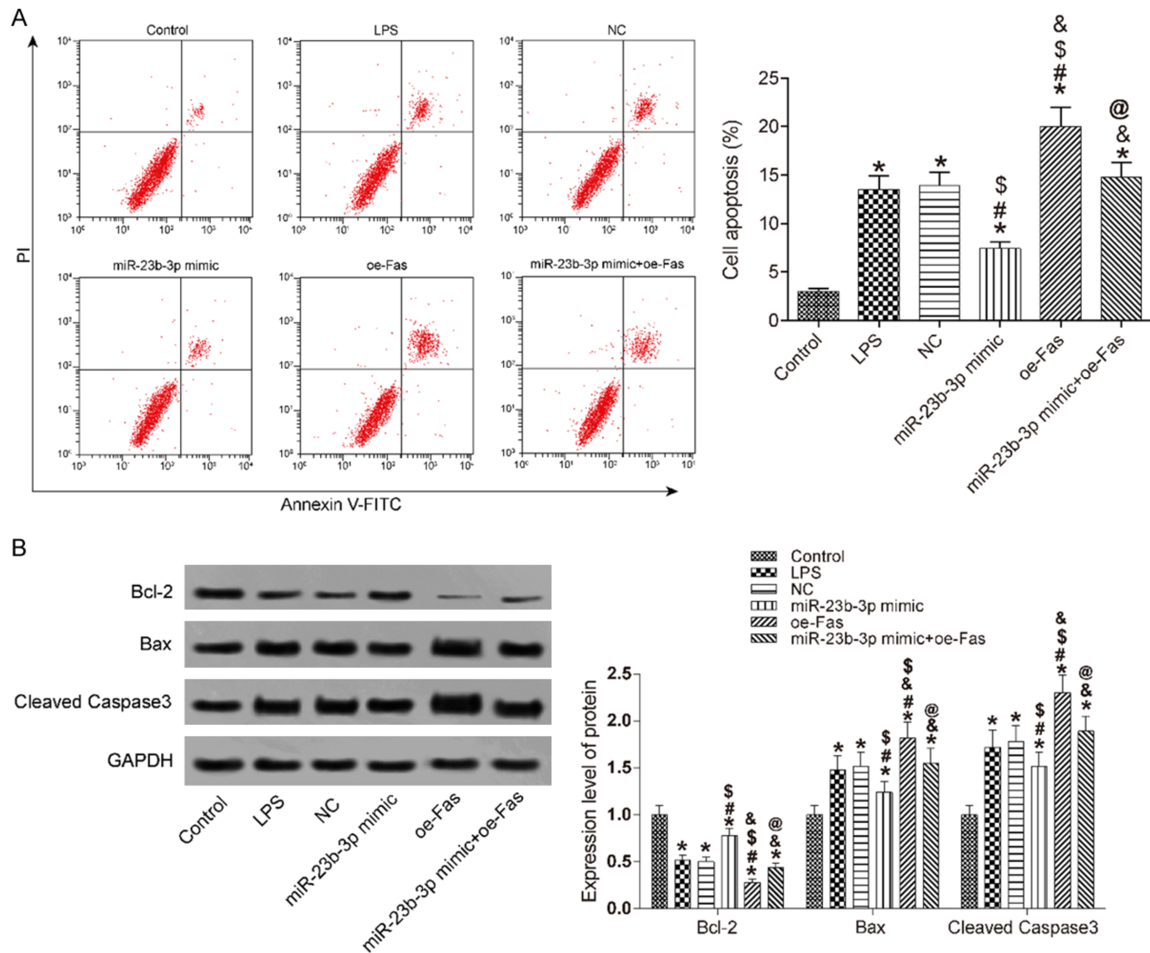

Figure 4. Detection of cell apoptosis. A: Apoptosis detection by flow cytometry and statistical results of apoptosis; B: WB detection of apoptosis related factors through WB and corresponding column chart. *P<0.05 vs control group, #P<0.05 vs LPS group, $P<0.05 vs NC group, &P<0.05 vs miR-23b-3p mimic group, @P<0.05 vs oe-Fas group; LPS: Lipopolysaccharide.

23b-3p mimic were all down-regulated, while expressions of TNF-α, IL-1β and INF-γ were strongly up-regulated in oe-Fas group (P<0.05). In addition, expressions of TNF-α, IL-1β and INF-γ in miR-23b-3p mimic + oe-Fas group were largely elevated compared with the miR-23b-3p mimic group (P<0.05), which were decreased compared with that of oe-Fas group (P<0.05). Thus, we concluded that overexpression of Fas aggravated inflammatory responses in LPS stimulated HUVEC cell injury model, however overexpression of miR-23b-3p successfully suppressed inflammatory response.

Discussion

In the process of atherosclerosis (AS), endothelial cells secrete a variety of cytokines under the stimulation of other factors, which gradually induces chemotaxis and adhesion of inflammatory cells, leading to the formation of atheromatous plaques [18]. In this study, we explored the protective effects of miR-23b-3p and its target gene Fas on lipopolysaccharide (LPS) stimulated vascular endothelial cells, hoping to offer a new idea to the diagnosis and treatment of AS.

MiRNA is able to target multiple genes at the same time. Previous studies have confirmed that miR-23b-3p can use ETS1 as the target gene to down-regulate the expression level of apo (a) in endothelial cells through Akt/eNOS signaling pathway, so as to protect the coronary artery endothelial cells [11]. Thus, it is suggested that miR-23b-3p and its target genes play an

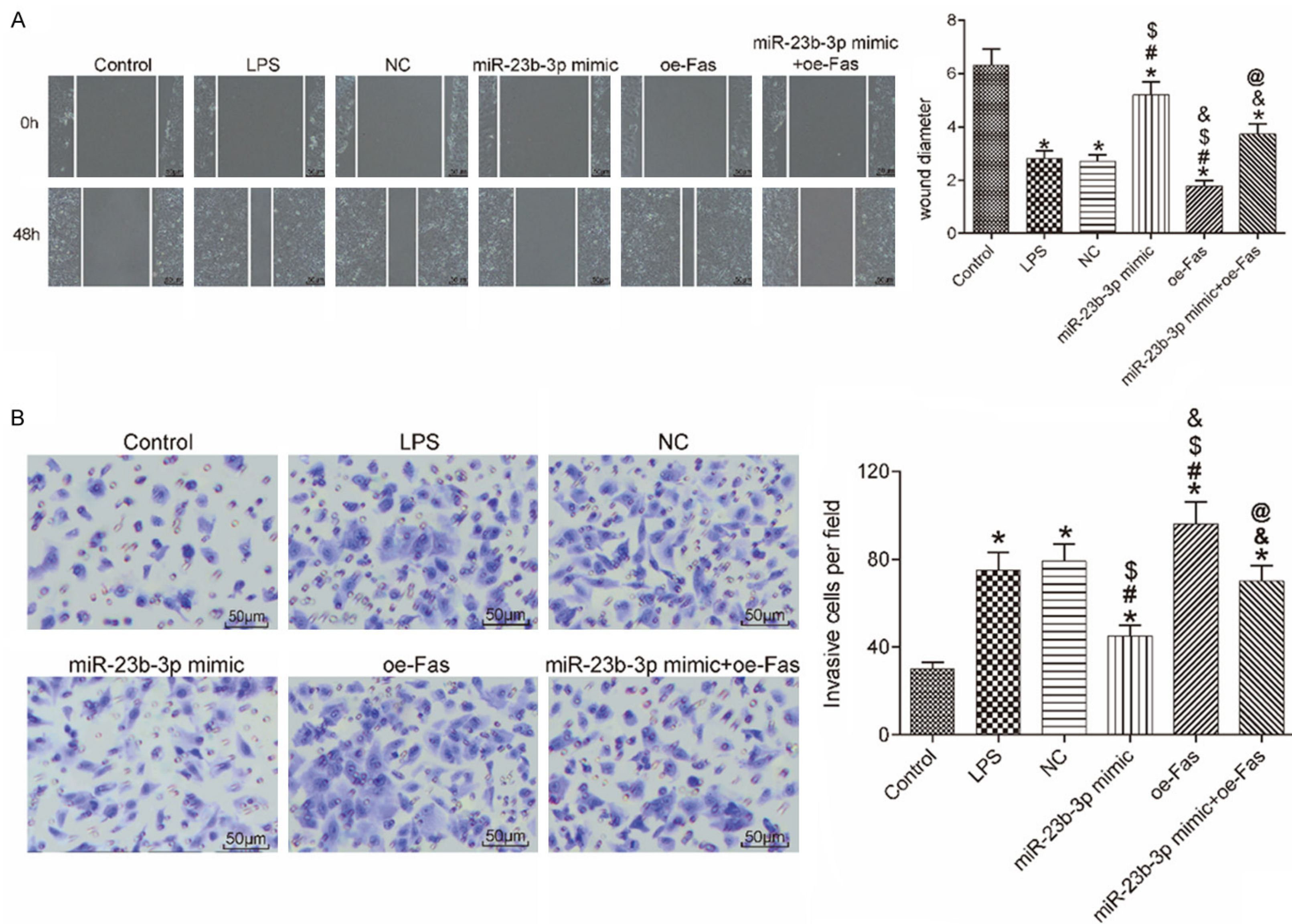

Figure 5. Detection of cell migration and invasion ability. A: Detection of cell migration by wound healing assay; B: Detection of cell invasion by transwell assay; *P<0.05 vs control group; #P<0.05 vs LPS group; $P<0.05 vs NC group; &P<0.05 vs miR-23b-3p mimic group; @P<0.05 vs oe-Fas group; LPS: Lipopolysaccharide.

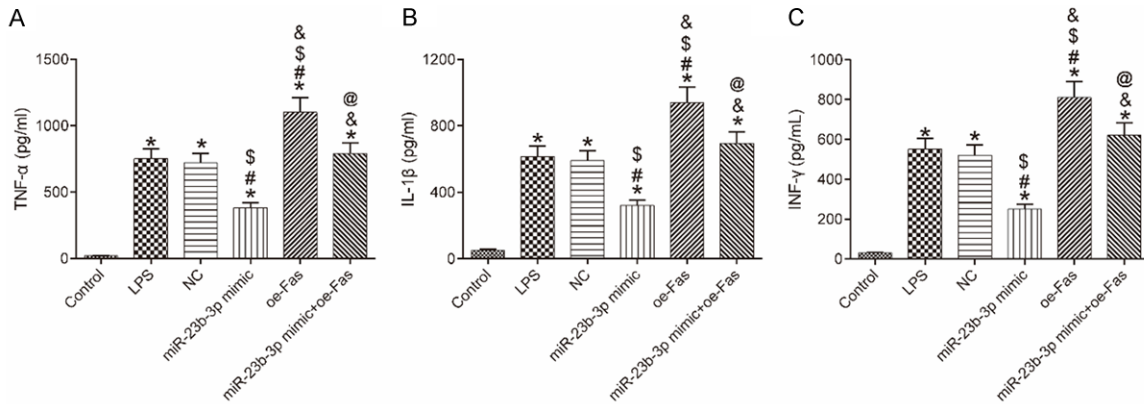

Figure 6. Expressions of TNF-α, IL-1β and INF-γ. A-C. Expressions of TNF-α, IL-1β and INF-γ in different groups; *P<0.05 vs control group; #P<0.05 vs LPS group; $P<0.05 vs NC group; &P<0.05 vs miR-23b-3p mimic group; @P<0.05 vs oe-Fas group; LPS: Lipopolysaccharide.

important role in vascular endothelial cells. In our study, Fas was found to act as a target gene of miR-23b-3p. Fas and its ligand FasL belong to the tumor necrosis factor receptor family. At present, Fas/FasL mediated apoptosis is widely studied [19]. Specifically, Fas is a typical apoptotic receptor. Fas can activate the intracellular death domain by binding with its ligand FasL, thus activating Fas related death domain and downstream caspase families, and finally inducing apoptosis [20, 21].

In the process of AS formation, proliferation and migration ability of endothelial cells are regulated by miRNA [22]. Similarly, we revealed that overexpression of miR-23b-3p can protect LPS stimulated HUVEC cells by promoting cell proliferation, inhibiting migration and invasion ability and reducing cell apoptosis, while overexpression of Fas showed opposite results. It has also been reported that in addition to inducing apoptosis, the activation of Fas pathway can also induce non-apoptotic effects in cells, such as regulating cell cycle and promoting migration [23]. In addition, studies have pointed out that up-regulation of Fas and FasL expression in tumor cells not only can induce apoptosis of most cells, but also enhance the migration ability of these cells by activating the non-apoptotic Fas pathway, which finally leads to tumor metastasis [24, 25].

In addition, some other studies have found that the binding of Fas and its ligands can not only transmit death signals, but also regulate non-apoptotic reactions including inflammatory reactions [26, 27]. At present, relevant research shows that Fas/FasL can mediate inflammatory response through MyD88 dependent pathway or recruitment of centralized granulocytes and some other non-apoptosis related pathways [28]. It has also been found that Fas/FasL mediated apoptosis has a bearing on the activation of p38 MAPK pathway, which can regulate inflammatory factors through complex cascade reactions [29-31]. In this study, we also demonstrated that miR-23b-3p reduced the release of inflammatory factors induced by LPS by targeting Fas.

In conclusion, miR-23b-3p can play a protective role in HUVEC by suppressing LPS induced apoptosis, migration and release of inflammatory factors via targeting Fas. Although some progress has been made about the effects of miRNA in AS, there are still some unsolved problems. For example, due to the cell type specificity, the same miRNA may play different, or even completely opposite roles in different types of cells. In addition, the same miRNA may also play different roles in different stages of AS. Thus, we look forward to further exploration in these aspects in the future.

Disclosure of conflict of interest

None.

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