Innate
Immunity

MALAT1 modulates miR-146's protection of microvascular endothelial cells against LPS-induced NF-κB activation and inflammatory injury

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Abstract

To investigate the role of miR-146 and its possible relationship with MALAT1 in LPS-induced inflammation in human microvascular endothelial cells (HMECs), HMEC-1 cells were treated with LPS to construct an inflammatory injury cell model, and the cell viability, TNF-α and IL-6 secretion and the expression levels of VCAM-1, SELE and ICAM-1 were analysed as markers of inflammatory injury. The regulation mechanisms of miR-146 interacted with MALAT1 and the downstream NF-κB signalling were also verified by dual-luciferase assay and knockdown technology. LPS significantly decreased the cell viability, increased levels of VCAM-1, SELE and ICAM-1 and also up-regulated miR-146a/b, TNF-α and IL-6 in a dose-dependent manner. Over-expression of miR-146a resulted in down-regulation of TNF-α and IL-6, as well as VCAM-1, SELE and ICAM-1, while inhibition of miR-146a led to opposite results. The dual-luciferase reporter assay showed both miR-146a and miR-146b directly targeted and negatively regulated the expression of MALAT1. Silencing of MALAT1 suppressed LPS-induced NF-κB activation and TNF-α and IL-6 secretion, reducing the cell inflammatory injury, but these changes were reversed after combined treatment with miR-146a inhibitor. Taken together, we demonstrate that miR-146 protects HMECs against inflammatory injury by inhibiting NF-κB activation. This process is modulated by MALAT1.

Introduction

Sepsis, characterised by an uncontrolled and systemic inflammatory response, is a persistent and serious public-health threat, with high mortality and morbidity rates, which can be as high as 70%. It is thought that about 2% of all hospital patients suffer from sepsis upon hospital admission.[1,2] The treatment of sepsis is expensive and places a significant demand on medical resources, which seriously affects quality of life and poses a huge threat to health. The most effective treatment and prevention of sepsis is based on the pathogenesis of sepsis. It is widely accepted that inflammatory cytokines such as TNF-α and IL-6, and related signalling pathways such as NF-κB signalling, play an important role in the pathogenesis of sepsis.[3–5] However, deeper insights into the development of sepsis, especially the underlying molecular mechanisms, are still unclear. The studies focusing on the underlying mechanisms of sepsis will certainly bring new hope for the treatment and prevention of sepsis.

MicroRNAs (miRNAs) are small endogenous RNAs involved in the biological process and disease.[6] In recent years, the role of miRNAs in inflammation has also been noted.[7,8] Among the miRNAs, miR-146 is considered an inflammation-associated miRNA which can regulate the proliferation of immune

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cells and inhibiting inflammatory responses.[9,10] Subsequently, it has also been reported that miR-146 is a negative feedback mediator of NF-κB pathways via directly targeting signalling components to participate in innate immune responses.[11] Furthermore, in early diabetic retinopathy, miR-146 was found to suppress NF-κB signalling and seems to be a potential therapeutic target.[12] Further studies have demonstrated that miR-146 also shows anti-inflammatory effects in sepsis.[13] However, the exact molecular mechanisms remain to be elucidated.

Recently, MALAT1, a conserved long non-coding RNA (lncRNA)-a class of non-protein-coding RNA transcripts more than 200 nucleotides long that do not contain an open reading frame- was found to be elevated in LPS-induced cardiac microvascular endothelial cells in sepsis.[14] Nevertheless, the role of lncRNAs in sepsis, including MALAT1, has not been definitively investigated. It has been widely recognised that lncRNAs exert biological functions as a competing endogenous RNA (ceRNA) for sponging miRNAs.[15–17] Furthermore, miRNAs can also target and regulate the expression of lncRNAs.[18–20] It has been demonstrated that MALAT1 could act as a ceRNA for negatively regulating miR-146 to modulate the NF-κB signalling pathway in LPS-induced acute kidney injury.[21] However, up to now, the exact relationship and the underlying regulatory network between miR-146a and MALAT1 in sepsis and inflammation injury are still unclear.

In the present study, we aimed to investigate role of miR-146 and its possible relationship with MALAT1 in LPS-induced inflammation in human microvascular endothelial cells (HMECs). This study might give deeper insights into the role of miR-146 and MALAT1 in inflammation injury, as well as provide possible new research targets in sepsis therapy.

Materials and methods

Cell culture and treatment

Human microvascular endothelial cell line (HMEC-1 from human blood vessel) was purchased from ATCC (Manassas, VA). Briefly, HMEC-1 cells were cultured in RPMI 1640 (Thermo Fisher Scientific, Waltham, MA) containing with 10% Gibco® FBS (Thermo Fisher Scientific) and 100 μg/ml penicillin-streptomycin (Sigma-Aldrich, St. Louis, MO). Cells were then cultured at 37°C and 5% CO_2. LPS (Sigma-Aldrich) was added in concentrations of 5, 10 and 15 μg/ml to induce inflammation. For inhibition of NF-κB signalling, cells were treated with JSH-23 (20 μmol/l; Sigma-Aldrich) for 24 h.

Transfection

MiR-146a/b mimics (miR-146a/b) and negative control (miR-NC), miR-146a inhibitor (anti-miR-146a) and negative control (anti-miR-NC), as well as shRNA vectors for MALAT1 (shMALAT1) and the corresponding negative control vector (shNC), were all purchased from GenePharma (Shanghai, PR China). HMEC-1 cells were transfected with miR-146a/b, anti-miR-146a/b or NC (miR-NC, anti-miR-NC), or shMALAT1 or shNC (50 nmol/L) using Lipofectamine 3000 (Invitrogen, Carlsbad, CA) in serum-free Opti-MEM medium (Gibco®) according to the manufacturer's instruction. Transfection efficiency was determined by quantitative RT-PCR 48 h after transfection.

MTT assay

Cells were cultured on 96-well plates at a density of 3×10^3 cells/well. After culturing for 72 h, 25 ml MTT solution at a concentration of 5 mg/ml was added, and cells were further cultured for 4 h at 37°C. Cells were then centrifuged at 1200 g for 5 min at room temperature, the supernatant removed and 180 ml DMSO was added. The absorbance was evaluated at 490 nm using a Synergy-HT Multi-Detection microplate reader (Bio-Tek Instruments, Inc., Winooski, VT).

Measurement of TNF-α and IL-6

The culture supernatants were collected, and the levels of TNF-α and IL-6 were measured by ELISA using commercial ELISA kits (Abcam, Cambridge, MA, USA) according to the manufacturer's instructions.

Immunofluorescence

Immunofluorescence was conducted to evaluate expression of NF-κB. Briefly, the cells were fixed, permeabilised and then incubated with anti-NF-κB Ab (Abcam) overnight at 4°C following by incubation with corresponding secondary Ab for 1 h at room temperature. DAPI was used for staining the nucleus. A TCS-SP laser scanning confocal microscope (Leica, Wetzlar, Germany) was used to take the photomicrographs.

Quantitative RT-PCR

Quantitative RT-PCR was performed to determine expression of miR-146a/b, MALAT1, VCAM-1, SELE and ICAM-1. Briefly, total RNA was extracted from the HMEC-1 cells using TRIzol™ reagent (Invitrogen™; Thermo Fisher Scientific). RNA concentration was determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). A Prime-Script™ one step quantitative RT-PCR kit (Takara Biotechnology, Dalian,

PR China) was used to convert RNA to cDNA. Quantitative RT-PCR reactions were performed using the 7500 Real Time PCR System (Applied Biosystems, Foster City, CA) using SYBR Green Master Mix (Solarbio Science & Technology Co. Ltd, Beijing, PR China) in an Exicycler™ 96 (Bioneer Corp., Daejeon, Korea). Primers used in the PCR are listed in Table 1. The relative RNA levels were calculated using the $2^{-\Delta\Delta Cq}$ method. U6 and GAPDH were used as internal controls.

Dual-luciferase reporter assay

The binding between miR-146a/miR-146b and MALAT1 was confirmed using the dual-luciferase reporter assay. The predicted binding mode for miR-146a and miR-146b and MALAT1 was obtained using bioinformatic prediction using TargetScan v5.1 (Whitehead Institute for Biomedical Research, Cambridge, MA). For the dual-luciferase reporter assay, wild type (WT) constructs of MALAT1 3′-untranslated region (3′-UTR) or mutant (MUT) were inserted into a pmirGLO Dual-Luciferase miRNA Target Expression Vector (Promega, Madison, WI). The corresponding mutant constructed by mutating the miR-146a/b seed region binding site, named as MALAT1 3′-UTR-mutated-type (MUT), was designed and synthesised by GenePharma. HEK293T cells were then transfected with the luciferase reporter plasmids and miR-146a/b mimics/miR-NC. After transfection for 48 h, luciferase activity was measured by the Dual-Luciferase Reporter System (Promega) using a Centro LB 960 microplate luminometer (Berthold Technologies, Bad Wildbad, Germany). All reactions were performed in triplicate for at least three independent experiments.

Western blotting

Western blotting was used to test the protein levels of MALAT1, VCAM-1, SELE, ICAM-1, IκBα and NF-κB p65. β-Actin served as a loading control. Proteins were extracted from HMEC-1 cells using radio-immunoprecipitation assay (RIPA) buffer (Vazyme Biotec Co., Ltd, Nanjing, PR China), and the protein amount was quantitated with protein assay reagent from Bio-Rad (Hercules, CA). Samples were then subjected to 10% SDS-PAGE, transferred to polyvinylidene difluoride membranes and blocked by 5% non-fat milk at room temperature for 1 h. Membranes were then incubated with a special primary Ab at 4°C overnight. Subsequently, membranes were incubated with a HRP-conjugated immunoglobulin G secondary Ab at 37°C for 45 min. All Abs were purchased from Abcam (Cambridge, MA, USA) and used in dilution as recommended. Protein bands were scanned with the Pierce ECL Western Blotting Substrate (Thermo Fisher Scientific) and a chemiluminescence system (Bio-Rad).

Statistical analysis

Data are expressed as the mean±standard deviation. Comparisons between two groups were performed using Student's t-test. Comparisons among three or

Table 1. Paired primer sequences used in quantitative RT-PCR.

Genes	Paired primers	Sequences (5′–3′)
TCAM-1	Sense	GGGTGGTCTTTGACCTTGAA
	Antisense	CTCCAAGGGCAACGGTAATA
SELE	Sense	TCTCCTGTGAGCAGGGTTTT
	Antisense	TAGGGGAATGAGCACACCTC
VCAM-1	Sense	GGACCACATCTACGCTGACA
	Antisense	TTGACTGTGATCGGCTTCCC
MALAT1	Sense	GCTCTGTGGTGTGGGATTGA
	Antisense	GTGGCAAAATGGCGGACTTT
GAPDH	Sense	CCAGGTGGTCTCCTCTGA
	Antisense	GCTGTAGCCAAATCGTTGT
miR-146a	RT	GTCGTATCCAGTGCAGGGTCCGAGG TATTCGCACTGGATACGACAACCCA
	Sense	CGGCCTGAGAACTGAATTCCA
	Antisense	GTGCAGGGTCCGAGGT
miR-146b	RT	GTCGTATCCAGTGCAGGGTCCGAGGTA TTCGCACTGGATACGACCAGCCT
	Sense	CGGCTGAGAACTGAATTCCAT
	Antisense	GTGCAGGGTCCGAGGT
U6	Sense	CTCGCTTCGGCAGCACA
	Antisense	AACGCTTCACGAATTTGCGT

more groups were conducted using one-way ANOVA with Tukey's post hoc test. The results were considered statistically significant at $P < 0.05$. All calculations were performed using IBM SPSS Statistics for Windows v22.0 (IBM Corp., Armonk, NY).

Results

LPS-induced up-regulation of miR-146a/b and promoted inflammatory injury of HMEC-1 cells

To investigate the LPS-induced inflammation in HMEC-1 cells, cell viability, expression of miR-146a/b, TNF-α and IL-6, as well as angiogenesis ability and injury biomarkers were measured. Results showed that LPS at concentrations of 5, 10 and 15 μg/ml significantly decreased the cell viability compared to that of the control (Figure 1a). Meanwhile, following LPS exposure, the expression of both miR-146a and miR-146b was dramatically increased compared to the control cells in a dose-dependent manner (Figure 1b). Furthermore, both TNF-α and IL-6 levels were remarkably increased in LPS-treated cells in a dose-dependent manner (Figure 1c). Determination of injury biomarkers also identified that expression of VCAM-1, SELE and

ICAM-1 was dramatically up-regulated at both protein and mRNA levels after LPS (5 μg/ml) treatment for 24 h (Figure 1d and e). All these results indicate that LPS could induce up-regulation of miR-146a, as well as TNF-α and IL-6, and promote inflammatory injury of HMEC-1 cells.

MiR-146a negatively regulated LPS-induced inflammation injury of HMEC-1 cells

Cells were then transfected with miR-146a mimics or inhibitor to investigate the role of miR-146a in LPS-induced inflammation further. As shown in Figure 2a, expression of miR-146a was significantly up-regulated in cells transfected with miR-146a mimics while being dramatically down-regulated in cells transfected with miR-146a inhibitor, suggesting the successful knock-down or overexpression of miR-146a models. In addition, the ELISA result showed that both TNF-α and IL-6 levels in the cell supernatant were significantly inhibited in cells transfected with miR-146a mimics, while they were significantly enhanced when cells were transfected with miR-146a inhibitor compared to the control (Figure 2b). Meanwhile, expression patterns of VCAM-1, SELE and ICAM-1 were remarkably decreased at both protein and mRNA levels in
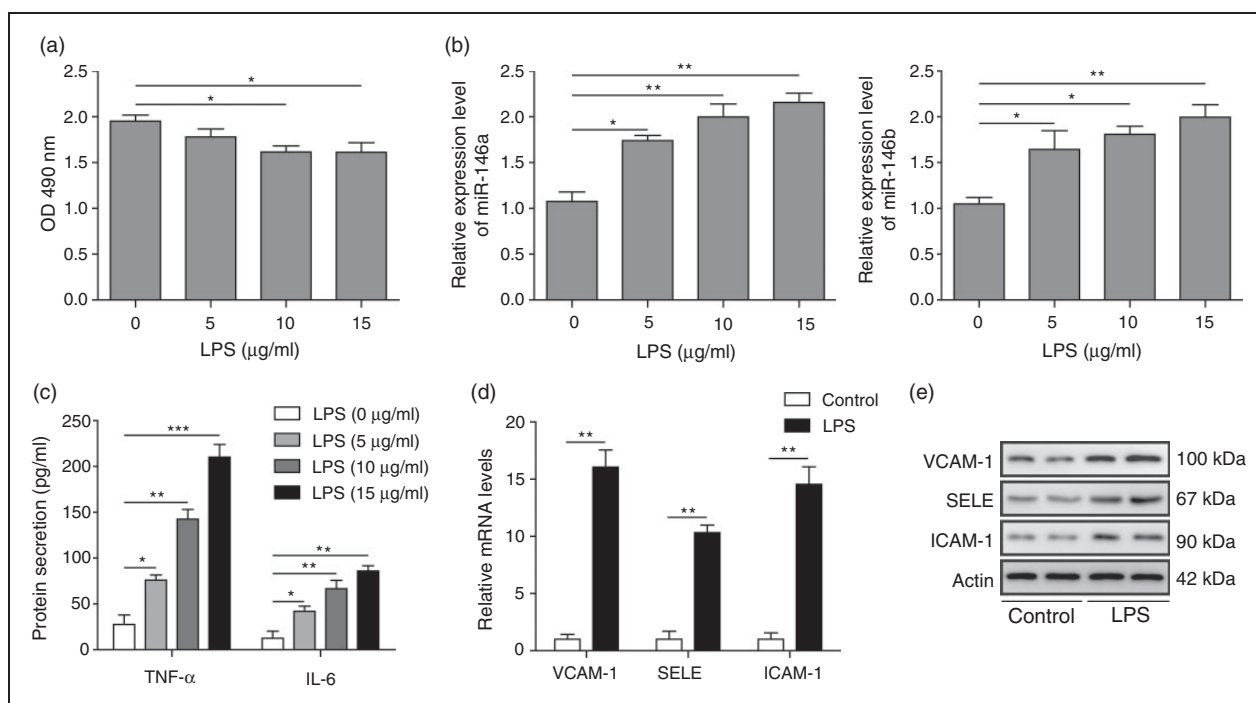

Figure 1. LPS-induced up-regulation of miR-146a/b and promoted inflammatory injury of human microvascular endothelial cells (HMEC)-1 cells. HMEC-1 cells were treated with a gradient concentration of LPS (5, 10 and 15 μg/ml) or PBS for 24 h. (a) MTT assay analysis of cell viability. (b) Quantitative RT-PCR analysis of the expression levels of miR-146a and miR-146b. (c) ELISA analysis of the release of inflammatory factors TNF-α and IL-6. (d) Quantitative RT-PCR analysis of mRNA expression levels. (e) Western blot analysis of protein levels of ICAM-1, SELE and VCAM-1 in HMEC-1 cells treated with 5 μg/ml LPS or not (PBS). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
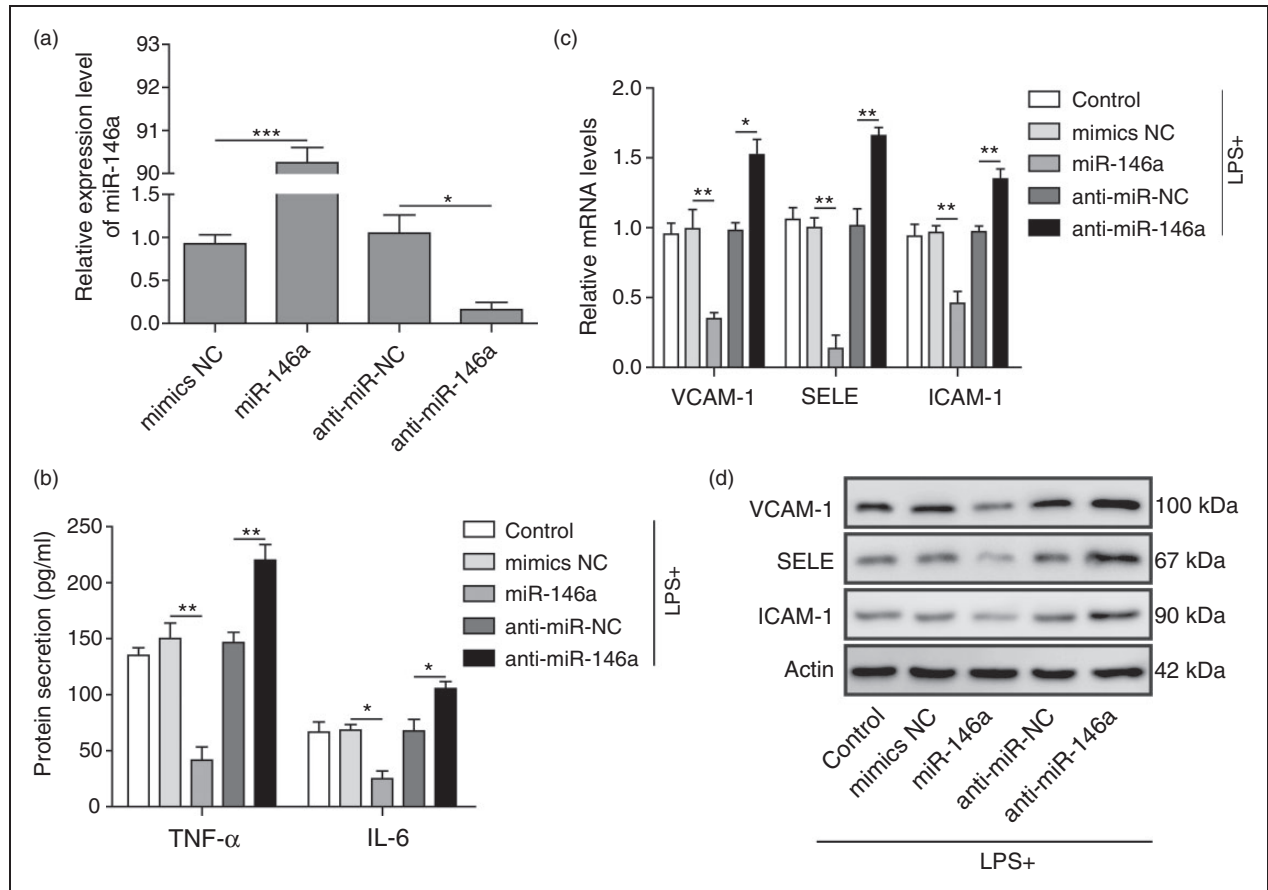
Figure 2. MiR-146a negatively regulated LPS-induced inflammation injury of HMEC-1 cells. HMEC-1 cells were transfected with miR-146a mimics or inhibitor to investigate the function of miR-146a in the processes of inflammation injury induced by LPS. (a) Quantitative RT-PCR analysis of the level of miR-146a to confirm over-expression/knockdown efficiency of mimics/inhibitor. (b) ELISA analysis of the effects of TNF-α and IL-6 release in HMEC cells after miR-146a over-expression or knockdown. (c) RT-PCR analysis of mRNA expression levels. (D) Western blot analysis of protein levels of ICAM-1, SELE and VCAM-1 in HMEC-1 cells after miR-146a over-expression or knockdown. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

cells transfected with miR-146a mimics and were significantly increased when miR-146 was suppressed (Figure 2c and d). These results suggest that miR-146a might negatively regulate LPS-induced inflammation of HMEC-1 cells.

A mutually inhibitory regulation between miR-146a/b and MALAT1

To investigate mechanisms of miR-146 involvement in LPS-induced inflammation further, expression of MALAT1 was determined using quantitative RT-PCR. In HMEC-1 cells transfected with miR-146a mimics, the expression of MALAT1 was significantly inhibited, while the knockdown of miR-146a resulted in a significant increase of MALAT1 compared to the control (Figure 3a). The predicted binding region between miR-146a/b and MALAT1 is shown in Figure 3b. Further, the dual-luciferase reporter assay

showed that luciferase activity was significantly decreased when cells were transfected with miR-146a mimics and WT MALAT1 vector (WT-MALAT1; $P < 0.05$). However, no significant difference was found in the group transfected with miR-146a mimics and mutated MALAT1 vector (MUT-MALAT1; Figure 3c), indicating that miR-146a/b directly interacted with MALAT1 at the predicted site. In addition, the classical regulatory mechanism of lncRNAs acting as ceRNA modulating the action of miRNAs has been widely recognised. Therefore, to verify whether MALAT1 acts as a ceRNA in HMEC-1 cells, the changes of MALAT1 expression were investigated (Supplemental Figure A). Moreover, quantitative RT-PCR results also revealed the negative regulation of MALAT1 on miR-146a/b expression (Supplemental Figures B and C). Taken together, these data suggest the bidirectional action of MALAT1 and miR-146a.
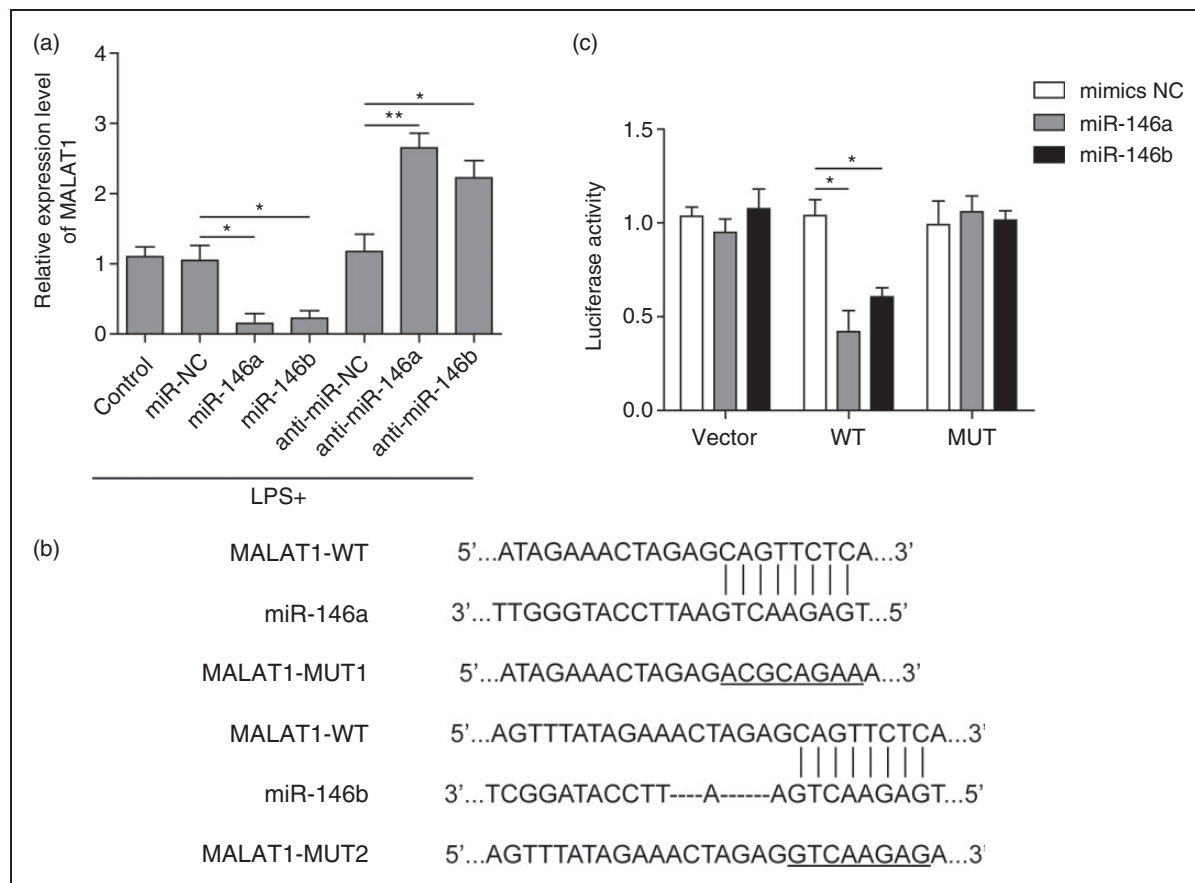
Figure 3. MiR-146a/b directly targeted and negatively regulated MALAT1. (a) Quantitative RT-qPCR analysis of the level of lncRNA MALAT1 in HMEC cells after miR-146a over-expression or knockdown. (b) Graphical representation of the predicted binding sites between MALAT1 and miR-146a/miR-146b by TargetScan software and their designed mutants. (c) The dual-luciferase reporter assay for the effects of luciferase activity of MALAT1-WT and the mutant ones (MALAT1-MUT1/MALAT1-MUT2, collectively known as MALAT1-MUT) co-transfected with miR-146a/miR-146b. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Inhibition of MALAT1 suppressed LPS-induced NF-κB activation and inflammatory factor secretion

Expression of NF-κB signalling-related proteins as well as TNF-α and IL-6 was detected when MALAT1 was silenced. We observed that expression of MALAT1 was dramatically enhanced in cells treated with LPS, while up-regulation of MALAT1 was markedly inhibited in cells transfected with shMALAT1 (Figure 4a), indicating the successful silencing of MALAT1. As shown in Figure 4b, LPS treatment significantly increased the levels of p-IκBα and p-NF-κB p65, while these positive effects were markedly repressed by MALAT1 knockdown. A similar result was also observed in immunofluorescence analysis of NF-κB. When cells were treated with LPS, the nucleus level of NF-κB was obviously enhanced, while knockdown of MALAT1 caused the inhibition of NF-κB expression in the nucleus (Figure 4c). Moreover, ELISA analysis also showed that the increased supernatant levels of TNF-α and

IL-6 induced by LPS were dramatically inhibited when cells were transfected with shMALAT1 (Figure 4d). These findings demonstrate that silencing of MALAT1 could suppress NF-κB signalling activation induced by LPS in HMEC-1 cells.

MALAT1 modulated inhibition of miR-146a on LPS-induced NF-κB activation

To certify further whether MALAT1 is involved in miR-146a protection on LPS-induced inflammation injury of HMEC-1 cells through NF-κB signalling, cells were co-transfected with miR-146a inhibitor and shMALAT1 or were transfected with miR-146a inhibitor and also treated with NF-κB signalling inhibitor JSH-23. As shown in Figure 5a, following exposure with LPS, expression of both p-IκBα and p-NF-κB p65 was significantly decreased by over-expression of miR-146a but dramatically increased by miR-146a inhibitor. However, when co-transfected with both
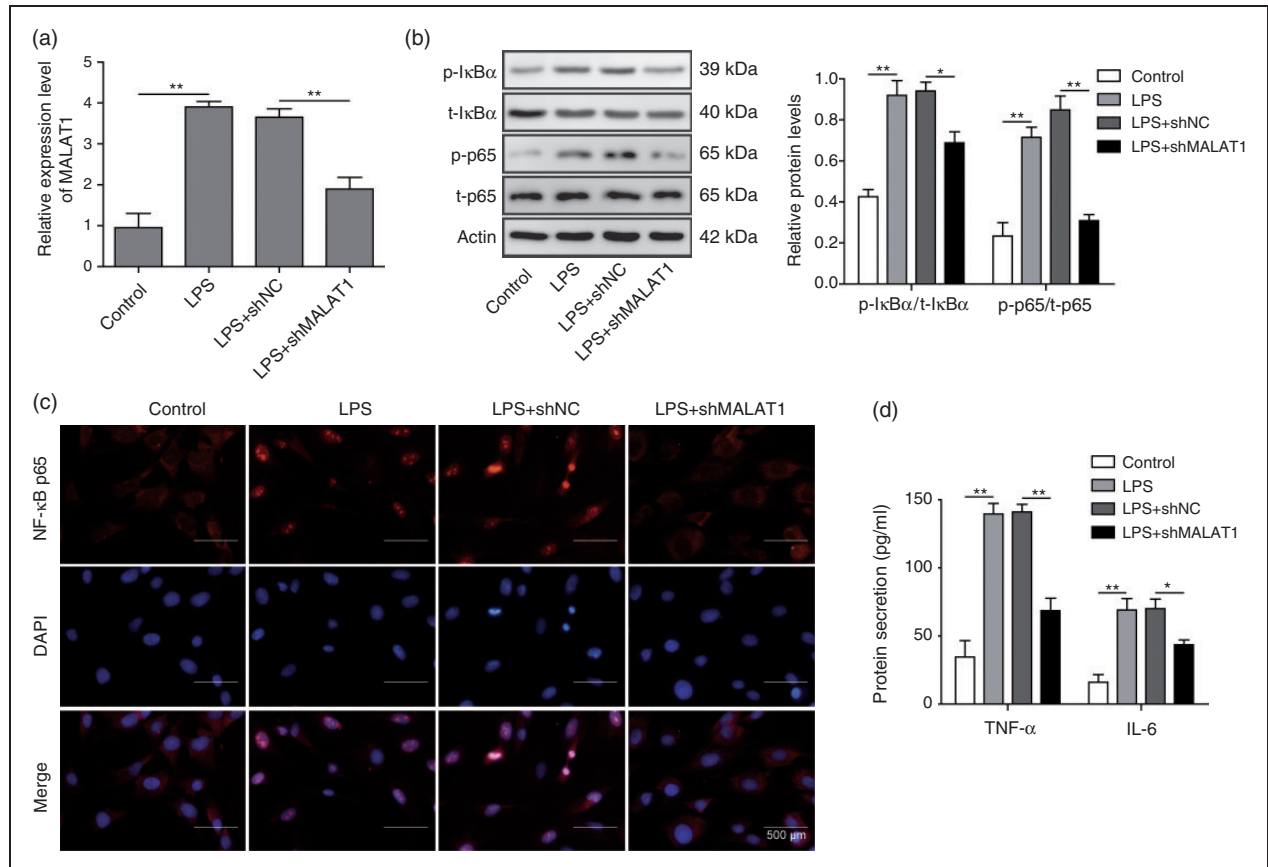
Figure 4. Inhibition of MALAT1 suppressed LPS-induced NF-κB activation and inflammatory factor secretion. (a) Quantitative RT-PCR analysis of the level of MALAT1 in MALAT1-deficient and WT HMEC-1 cells with or without LPS treatment. (b) Western blot analysis of protein levels of IκBα, NF-κB p65 and their phosphorylation levels in MALAT1-deficient and WT HMEC-1 cells with or without LPS treatment. (c) Representative images of the expression and distribution of NF-κB p65 in MALAT1-deficient and WT HMEC-1 cells with or without LPS treatment by immunofluorescence. (d) ELISA analysis of the effects of TNF-α and IL-6 release in MALAT1-deficient and WT HMEC-1 cells after LPS treatment or not. $*P < 0.05$; $**P < 0.01$.

miR-146a inhibitor and shMALAT1, the increasing effect of p-IκBα and p-NF-κB p65 by miR-146a inhibitor was dramatically recovered. Similar results were also obtained when cells were transfected with miR-146a inhibitor following exposure with NF-κB signalling inhibitor JSH-23 or shMALAT1, indicating that MALAT1 might be a target of the negative regulation of miR-146a on LPS-induced NF-κB signalling activation. Meanwhile, supernatant levels of TNF-α and IL-6 were significantly enhanced by inhibition of miR-146a. However, this effect was also recovered by co-transfection of shMALAT1 or co-treatment of JSH-23 (Figure 5b). Similarly, the increased expression of VCAM-1, SELE and ICAM-1 was remarkably decreased by co-transfection of shMALAT1 or co-treatment of JSH-23 at both protein and mRNA levels (Figure 5c and d). Altogether, these results suggest that MALAT1 could be involved in modulating the inhibition of miR-146a on LPS-induced NF-κB activation. A graph summarising these intertwined

networks is displayed in Figure 5e. Nevertheless, the exact mechanisms need to be explored further.

Discussion

Despite numerous studies on sepsis, the underlying molecular mechanisms are still elusive. Both lncRNAs and miRNAs have recently been found to be associated with inflammation. However, to the best of our knowledge, few studies have focused on the role of miR-146 and its possible relationship with LPS-induced inflammation in HMECs. In the present study, we demonstrated that miR-146 could protect HMECs against inflammatory injury by inhibiting NF-κB signalling and inflammatory factors through targeting lncRNA MALAT1.

LPS-induced inflammation in endothelial cells has been reported in many studies. It was found that LPS could induce inflammation by increasing caspase-3 activation and modulating mitochondrial function in
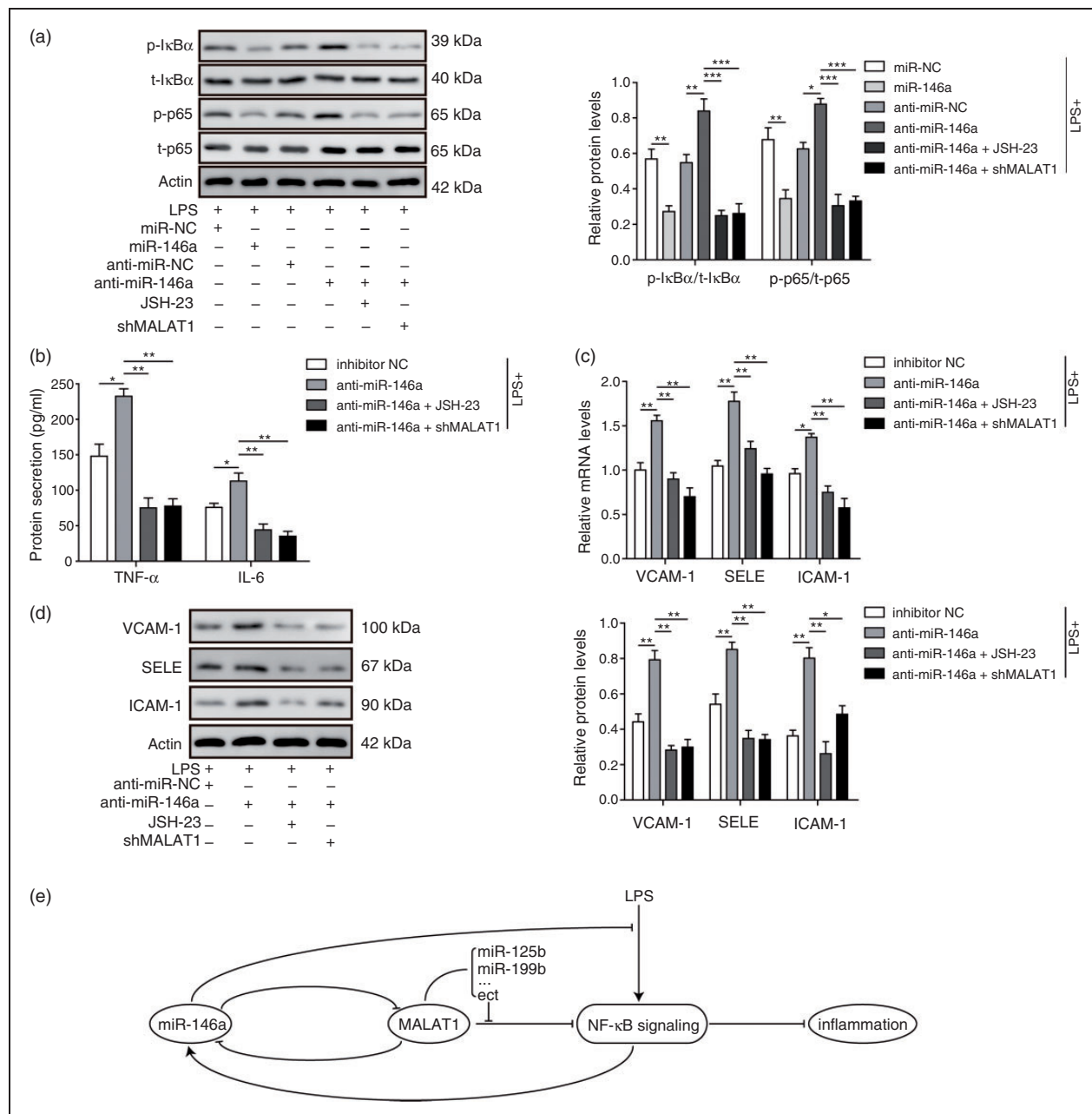
Figure 5. MALAT1 modulated MiR-146a's inhibition of LPS-induced NF-κB activation. (a) Western blot analysis of protein levels of IκBα, NF-κB p65 and their phosphorylation levels in LPS-treated HMEC-1 cells transfected with miR-146a mimics/inhibitor (miR-146a, anti-miR-146a) and their negative controls (miR-NC, anti-miR-NC) only, as well as in HMEC-1 cells co-treated with miR-146a inhibitor and shMALAT1 or JSH-23 (2 μM, an inhibitor of NF-κB pathway). (b) The HMEC-1 cells were transfected with miR-146a inhibitor only or co-treated with JSH-23 or shMALAT1 for 24 h, and then all cells in each group were exposed to LPS for another 24 h. ELISA analysis of the effects of TNF-α and IL-6 release, and (c) RT-qPCR analysis of the mRNA levels and (d) Western blot analysis of protein levels of ICAM-1, SELE and VCAM-1. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

endothelial cells.[22] Furthermore, Li et al. demonstrated that LPS could induce endothelial cell inflammatory responses through activation of the NF-κB signalling pathway.[23] It is also widely known that inflammatory factors, including TNF-α and IL-6, are up-regulated in LPS-induced inflammation.[24,25] In the present study,

we found that LPS could induce inflammatory injury in HMECs, including increasing of TNF-α and IL-6 and activation of NF-κB signalling, which is consistent with previous findings.

Both miR-146a and miR-146b can regulate inflammatory responses, and it has been recognised that

miR-146a plays a critical role in regulating the proliferation of immune cells and inhibiting inflammatory responses.[9,26] In addition, Boldin et al. demonstrated that deficiency of miR-146a could elevate the systemic response to LPS.[27] Yang et al. found that miR-146a could inhibit oxidised low-density lipoprotein-induced lipid accumulation and inflammatory response by targeting TLR4.[28] It has also been verified that NF-κB signalling can be inhibited by miR-146a in inflammation.[29] In our study, we also revealed that over-expression of miR-146a could inhibit the LPS-induced inflammation in HMEC-1 cells, and the effect was through regulation of MALAT1 and NF-κB signalling.

The interaction between miR-146a and MALAT1 has been demonstrated in several studies. On the one hand, MALAT1 could act as a ceRNA to regulate the expression of miR-146 negatively. Ding et al. demonstrated that MALAT1 could negatively regulate miR-146a in LPS-induced acute kidney injury.[21] It has also been reported that inhibition of MALAT1 led to the suppression of inflammatory responses by up-regulating miR-146a in LPS-induced acute lung injury.[30] On the other hand, there are several studies certifying the negative regulation of miRNAs on the expression of lncRNAs, including MALAT1. For example, it has been reported that miR-9 targeted MALAT1 and contributed to the degradation in the nucleus.[31] Li et al. demonstrated miR-101 and miR-127 targeted and regulated MALAT1 in esophageal squamous-cell carcinoma.[32] Therefore, these findings imply a bidirectional relationship between miRNAs and MALAT1. Similarly, our data also demonstrate a mutually inhibitory regulation between miR-146 and MALAT1 by the dual-luciferase reporter assay and quantitative RT-PCR analysis, suggesting the balance between miR-146 and MALAT1 might be a key component for inflammation development. However, deeper insights are still needed.

MALAT1 has recently been considered to be associated with inflammation process. Michalik et al. demonstrated that MALAT1 was elevated and could regulate inflammatory responses in endothelial cells.[33] It was also found that in diabetic complications, MALAT1 is up-regulated, along with increasing levels of inflammatory cytokines.[34] Meanwhile, Chen et al. revealed that in cecal ligation and a puncture-induced sepsis model, MALAT1 was up-regulated and could induce cardiac dysfunction and inflammation through interaction with miR-125b and p38 MAPK/NF-κB.[35] In addition, it has also been demonstrated that MALAT1 contributes to inflammatory response of microglia in spinal cord injury through modulating the miR-199b/IKKβ/NF-κB signalling pathway.[36] These findings indicate that MALAT1 could inhibit NF-κB signalling in an indirect manner by serving as a ceRNA of other miRNAs. Similarly, our findings also show that MALAT1 is up-regulated in LPS-induced inflammation in HMEC-1 cells, and that down-regulation of MALAT1 could reduce the LPS-induced inflammation by inhibition of NF-κB signalling. Further mechanistic research also implied that MALAT1 could be involved in miR-146's inhibition of LPS-induced NF-κB activation. However, the exact mechanisms need to be explored further. Despite the results of our research, there are many other signalling pathways involved in the development of sepsis as well as LPS-induced inflammation, such as AK/STAT signalling,[37] P2X7 receptor signalling[38] and cAMP/PKA signalling.[39] The present study only focused on the mechanisms of MALAT1/miR-146a/b/NF-κB signalling, which was also limited to *in vitro* studies. Thus, we will devote our effort to *in vivo* studies for further verification. Additionally, it is still unclear whether there are other signalling pathways involved in this process, and this needs to be explored further.

In conclusion, we conducted an *in vitro* study to investigate role of miR-146 and its possible relationship with MALAT1 in LPS-induced inflammation in HMECs. The results reveal the protective role of miR-146 in LPS-induced inflammatory injury of HMECs. Furthermore, it was shown that MALAT1 participates in miR-146's inhibition of LPS-induced NF-κB activation. Therefore, this study might give deeper insights into miR-146 and MALAT1 in the inflammation process, as well as provide possible new research targets in sepsis.

Supplemental material

Supplemental material for this article is available online.

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