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Effect of lncRNA SNHG15 on LPS-induced vascular endothelial cell apoptosis, inflammatory factor expression and oxidative stress by targeting miR-362-3p

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ABSTRACT

This study aimed to investigate the effect of lncRNA SNHG15 targeting miR-362-3p on LPS-induced vascular endothelial cell apoptosis, inflammatory factor expression and oxidative stress. For this purpose, human umbilical vein endothelial cells (HUVECs) were treated with 100 ng/mL LPS for 24 hours to establish a cell injury model. HUVECs were divided into control, LPS, LPS+si-NC, LPS+si-SNHG15, LPS+miR-NC, LPS+miR-362-3p, LPS+si-SNHG15+anti-miR-NC and LPS+si-SNHG15+anti-miR-362-3p groups. RT-qPCR was used to determine SNHG15 and miR-362-3pexpression. The cell inhibition rate was measured by the CCK-8 method; Cell apoptosis rate was detected by flow cytometry; the kits were employed to detect the intracellular SOD activity and the release of LDH; the ELISA method was applied to detcet the levels of TNF-α, IL-6 and IL-10 in the culture medium. Results showed that compared with the control group, the inhibition rate, apoptosis rate and SNHG15 expression level of HUVECs in the LPS group were increased (P<0.05), and the levels of TNF-α, IL-6 and LDH in the culture medium were increased (P<0.05), SOD activity, miR-362-3p expression level, and IL-10 level in the culture medium were reduced (P<0.05). The inhibition rate and apoptosis rate of HUVECs in the LPS+si-SNHG15 group were reduced (P<0.05), and the levels of TNF-α, IL-6 and LDH in the culture medium were reduced (P<0.05), SOD activity and IL-10 levels in the culture medium increased (P<0.05). The inhibition rate and apoptosis rate of HUVECs in the LPS+miR-362-3p group were reduced (P<0.05), and the levels of TNF-α, IL-6 and LDH in the culture medium were reduced (P<0.05), SOD activity and IL-10 level in the culture medium increased (P<0.05). miR-362-3p directly and bound to SNHG15. Compared with the LPS+si-SNHG15+anti-miR-NC group, the inhibition rate and apoptosis rate of HUVECs in the LPS+si-SNHG15+anti-miR-362-3p group were increased (P<0.05), and the levels of TNF-α, IL-6 and LDH in the culture medium were increased (P<0.05), and SOD activity and IL-10 levels in the culture medium were reduced (P<0.05). In general, silencing lncRNA SNHG15 inhibited LPS-induced vascular endothelial cell apoptosis, inflammatory factor expression and oxidative stress response by up-regulating miR-362-3p expression.

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Introduction

Sepsis is an acute inflammatory reaction and multiple organ damage caused by infectious factors, as well as one of the important causes of death in critically ill patients (1). Endothelial cells are the basic component of the vascular system, forming vascular endothelium and participating in the maintenance of vascular homeostasis. Various stimuli such as pro-inflammatory cytokines and bacterial endotoxin can induce endothelial cell inflammatory response and dysfunction, resulting in loss of vascular barrier function and increasing the severity of sepsis and the risk of death (2, 3). Therefore, elucidating the mechanism of endothelial cell injury will provide new insights for the treatment of sepsis. The length of long non-coding RNA (lncRNA) is more than 200 nucleotides, which can adsorb and inhibit microRNAs (miRNAs) and participate in regulating physiological and pathological processes such as cell differentiation, cell cycle and apoptosis (4). LncRNA and miRNA have been confirmed to play a key role in vascular integrity and inflammatory response (5). Studies have reported that lncRNA small nucleolar RNA host gene 15 (SNHG15) promotes neuronal apoptosis and oxidative stress response induced by glucose and oxygen deprivation/reoxygenation (OGD/R), and participates in the process of cerebral ischemia-reperfusion injury (6-9). Inhibition of SNHG15

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expression can inhibit myocardial apoptosis induced by hypoxia or reperfusion injury (10). The miR-362-3p is a protective miRNA, and studies have shown that miR-362-3p overexpression can promote functional recovery in rats with spinal cord injury, reduce the apoptosis rate of rat nerve cells, and inhibit neuronal inflammatory response (11). Target gene prediction suggests that miR-362-3p is a potential target of SNHG15, but it is not clear whether SNHG15 targets miR-362-3p to regulate endothelial cell injury. In this study, cell injury model was established by using lipopolysaccharide (LPS) to induce human umbilical vein endothelial cells (HUVECs) (12), and the biological functions of SNHG15 on LPS-induced apoptosis, inflammatory injury and oxidative stress of HUVECs via targeting miR-362-3p was explored, in order to provide a new target for the treatment of sepsis.

Materials and methods

Materials

HUVECs were purchased from ATCC, USA; LPS (No. L2880) was purchased from Sigma, USA; Lipofectamine 2000 was purchased from Thermo Fisher, USA; miRNA reverse transcription kit and miRNA detection kit were purchased from ABI, USA; PrimeScript reverse transcription Kit and SYBR Green PCR Master Mix were purchased from Bao Biological Company (Dalian, China); Superoxide dismutase (SOD) activity detection kit was purchased from Solarbio Science & Technology Co., Ltd. (Beijing, China); Lactate dehydrogenase (LDH) release Detection kit was purchased from Abcam, USA; Cell counting kit (CCK-8) and human tumor necrosis factor (TNF) -α, interleukin (IL-10), IL-6 detection kit were purchased from Beijing Biolab Biological Co., Ltd.(Beijing, China).

Cell culture and grouping

HUVECs were cultured in a DMEM medium containing 10% fetal bovine serum and 1% penicillomycin at 37°C in 5%CO2 in an incubator. HUVECs were cultured in a culture medium containing 100 ng/mL LPS for 24 h to establish a cell damage model, which was recorded as an LPS group. Normal cultured HUVECs were recorded as a control (con) group. Cell transfection: Logarithmic HUVECs were inoculated on 24-well plates and transfected with Lipofectamine 2000 at 50% confluence. The Lipofectamine 2000 (2μL) was diluted with 50μL of Opti-MEM, mixed gently and let stand for 5 min at room temperature. The oligonucleotide was diluted with 50 μL of Opti-MEM, mixed gently and stood for 5 min at room temperature. The diluted oligonucleotides were mixed with the diluted lipids and left for 20 min at room temperature to form the oligonucleotide -Lipofectamine 2000 complex. 100 μL of the transfection complex was added to each porocyte and cultured for 6 h, then replaced with the complete medium, and cultured for 48 h to collect cells for the next experiment. HUVECs transfected with si-NC and si-SNHG15 were recorded as a si-NC group and si-SNHG15 group, respectively. HUVECs transfected with si-NC, si-SNHG15, miR-NC, miR-362-3p mimics, si-SNHG15+anti-miR-NC, si-SNHG15+anti-miR-362-3p were incubated with culture medium containing 100 ng/mL LPS for 24 h, which were recorded as LPS+si-NC group, LPS+si-SNHG15, group, LPS+miR-NC group, LPS+miR-362-3p group, LPS+si-SNHG15 +anti-miR-NC group, LPS+si-SNHG15+anti-miR-362-3p group, respectively.

Detection of expressions of SNHG15 and miR-362-3p by RT-qPCR

Total RNA was isolated from HUVECs using TRIzol reagent. 2 μg of total RNA was used to synthesize cDNA using the PrimeScript reverse transcription kit and miRNA reverse transcription kit, respectively. Then, SYBR Green PCR Master Mix and miRNA detection kit were used for RT-QPCR. The expression levels of SNHG15 and miR-362-3p were calculated by the $2^{-\Delta\Delta CT}$ method. SNHG15forward primer 5′-GGT GAC GGT CTC AAA GTG GA-3′, reverse primer 5′-GCC TCC CAG TTT CAT GGA CA-3′; GAPDH forward primer 5′-CTC ATG ACC ACA GTC CAT GCC-3′, reverse primer 5′-GGC ATG GAC TGT GGT CAT GAG-3′ ; miR-362-3pforward primer 5′-GCC GAA ACA CAC CTA TTC AAG-3′, reverse primer 5′-TAT GGT TTT GAC GAC TGT GTG AT-3′, U6reverse primer 5′-ATT GGA ACG ATA CAG AGA AGA TT-3′, reverse primer 5′-GGA ACG CTT CAC GAA TTT G-3′.

Detection of HUVECs proliferation by CCK-8 method

Transfected HUVECs and untransfected HUVECs were inoculated on a 96-well plate at a rate of 3×10^3 / well by using LPS containing 100 ng/mL for 24 h, then culture medium containing 10% CCK-8 reagent was substituted for 2 h, followed by measurement of absorbance (A) at 450 nm with ELISA. Cell inhibition rate (%) = (1- experiment A/ control A) ×100

Detection of apoptosis rate by flow cytometry

After incubation with LPS for 24 h, cells were collected and re-suspended in 200μL 1× binding buffer containing 10μL Annexin V-FITC. After incubation at room temperature for 30 min, 300μL 1× binding buffer (containing 5μL propidium iodide) was added, after which the apoptosis of cell samples in each group was analyzed by flow cytometry immediately.

Kit detection of extracellular SOD activity and LDH release amount

After incubation with LPS for 24 h, culture supernatant was collected and centrifuged at 3000 r/min for 20 min to collect cell precipitation and culture supernatant, respectively. LDH release assay kit was used to analyze the activity of LDH in a culture medium to indicate its release amount. The extractive solution was added to the cell precipitate, the cells were ultrasonically cleaved, centrifuged at 10000r/min for 20 min to collect the supernatant for detection of the intracellular SOD activity according to the kit procedure.

Detection of levels of TNF-α, IL-6 and IL-10 in culture medium by ELISA

After incubation with LPS for 24 h, culture supernatant was collected and the levels of TNF-α, IL-6 and IL-10 were determined according to the instructions of the ELISA kit.

Double luciferase reporting assay

SNHG15 sequences with wild-type (WT) or mutated (MUT) miR-362-3p binding sites were cloned into pmirGLO vectors to generate luciferase reporter vectors WT-SNHG15 and MUT-SNHG15, respectively. The luciferase reporter vectors were co-transfected with miR-NC or miR362-3p mimics for 48 h, respectively. Then the relative luciferase activity was evaluated using a dual-luciferase reporting kit.

Statistical method

SPSS 20.0 was used for statistical analysis. Three multiple holes were set up for each test, and the experiment was independently repeated 3 times. The mean values of the two groups were compared by an independent sample T-test. The mean values of multiple groups were compared by one-way ANOVA and *LSD-t* test. The difference was considered statistically significant when $P<0.05$.

Results and discussion

Effect of SNHG15 silencing on LPS-induced apoptosis of HUVECs

Compared with con group, SNHG15 expression level, cell inhibition rate and apoptosis rate of HUVECs in the LPS group were significantly increased ($P<0.05$); Compared with the LPS+ si-NC group, the expression level of SNHG15, cell inhibition rate and apoptosis rate of HUVECs in LPS+ si-SNHG15 group were significantly decreased ($P<0.05$), as shown in Figure 1 and Table 1.

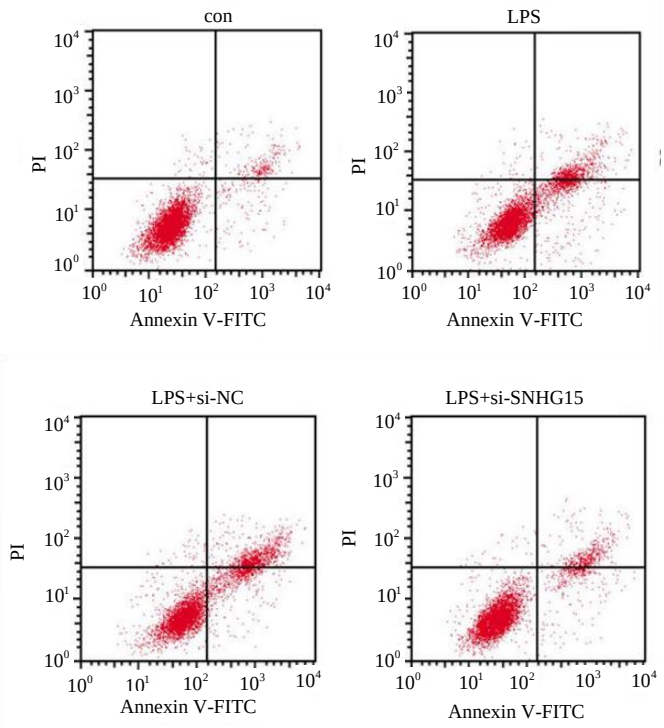

Figure 1. Effect of SNHG15 silencing on LPS-induced apoptosis of HUVECs

Effects of SNHG15 silencing on LPS-induced expression and oxidative stress injury of inflammatory cytokines in HUVECs

Compared with con group, SOD activity in HUVECs and IL-10 level in culture medium in LPS group were significantly decreased (P<0.05), the levels of TNF-α, IL-6 and LDH in culture medium were significantly increased (P<0.05); Compared with LPS+ si-NC group, SOD activity in HUVECs and IL-10 level in culture medium in LPS+ si-SNHG15 group were significantly increased (P<0.05), The levels of TNF-α, IL-6 and LDH in culture medium were significantly decreased (P<0.05), as shown in Table 2.

Table 1. Detection of LPS-induced HUVECs damage by silencing SNHG15 ($\bar{x} \pm s$, n=9)

Group	SNHG15	%	
		Inhibition rate	Apoptosis rate
con	1.00±0.00	0.00±0.00	7.99±0.48
LPS	2.77±0.08*	55.98±2.54*	23.68±1.61*
LPS+si-NC	2.75±0.13	56.16±2.88	23.51±1.79
LPS+si-SNHG15	1.29±0.07$^{\#\triangle}$	23.48±1.83$^{\#\triangle}$	13.34±0.73$^{\#\triangle}$
F	1127.822	1485.596	332.057
P	0.000	0.000	0.000

Note: compared with con group, **P*<0.05; compared with LPS group, $^{\#}P$<0.05; compared with LPS+si-NC, $^{\triangle}P$<0.05

Table 2. Effects of SNHG15 silencing on LPS-induced expression and oxidative stress injury of inflammatory cytokines in HUVECs ($\bar{x} \pm s$, n=9)

Group	pg/mL			U/L	
	TNF-α	IL-6	IL-10	SOD	LDH
con	44.67±4.34	29.58±3.46	275.34±18.65	496.05±20.86	183.67±13.81
LPS	356.22±19.16*	166.11±10.12*	90.13±5.76*	125.31±11.23*	603.50±24.65*
LPS+si-NC	357.99±24.94	164.71±15.50	94.90±11.01	119.90±12.98	596.61±39.45
LPS+si-SNHG15	139.21±12.74$^{\#\triangle}$	82.32±8.19$^{\#\triangle}$	196.01±14.55$^{\#\triangle}$	410.46±17.96$^{\#\triangle}$	273.64±22.22$^{\#\triangle}$
F	766.839	380.541	397.569	1288.691	598.275
P	0.000	0.000	0.000	0.000	0.000

Note: compared with the control group，**P*<0.05; compared with LPS group, $^{\#}P$<0.05; compared with LPS+si-NCgroup, $^{\triangle}P$<0.05

Target regulation of miR-362-3p by SNHG15

DIANA Tools predicted that SNHG15 and Mir-362-3p had complementary sequences, as shown in Figure 2. The relative luciferase activity of HUVECs in the WT-SNHG15 and miR-362-3p mimicmimics group was significantly lower than that in the WT-SNHG15 and miR-NC (P<0.05), as shown in Table 3. The expression level of miR-362-3p in HUVECs in the si-SNHG15 group was significantly higher than that in the si-NC group (P<0.05), as shown in Table 4.

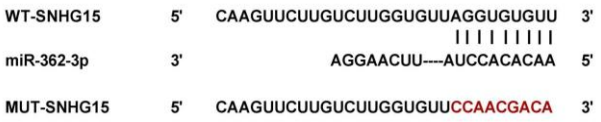

Figure 2. Complementary sequences of SNHG15 and miR-362-3p

Table 3. Double luciferase reporting assay ($\bar{x} \pm s$, n=9)

Group	WT-SNHG15	MUT-SNHG15
miR-NC	0.96±0.11	1.00±0.13
miR-362-3p	0.42±0.05*	1.02±0.11
t	13.407	0.352
P	0.000	0.729

Note: compared with the miR-NC group, **P*<0.05

Effects of miR-362-3p on LPS-induced expression and oxidative stress injury of inflammatory cytokines in HUVECs

Compared with the LPS+miR-NC group, the expression level of miR-362-3p, cell inhibition rate, apoptosis rate, SOD activity and IL-10 level in culture medium in HUVECs of LPS+ miR-362-3p group were significantly increased (P<0.05), the levels of TNF-α, IL-6 and LDH in culture medium decreased significantly (P<0.05), as shown in Figure 3 and Table 5.

Table 4. SNHG15 regulates the expression of miR-362-3p ($\bar{x} \pm s$, n=9)

Group	miR-362-3p
si-NC	1.00±0.00
si-SNHG15	2.89±0.13$^{\#}$
t	43.615
P	0.000

Note: compared with the si-NC group, $^{\#}P$<0.05

Inhibition of miR-362-3p partially reversed the effect of SNHG15 silencing on LPS-induced HUVECs injury

Compared with the LPS+ si-SNHG15 + anti-miR-362-3p group, the expression level of miR-362-3p in HUVECs in the LPS+ si-SNHG15 + anti-miR-362-3p group was significantly decreased (P<0.05), The cell inhibition rate and apoptosis rate were significantly increased (P<0.05), as shown in Figure 4 and Table 6.

Inhibition of miR-362-3p partially reversed the effects of SNHG15 silencing on LPS-induced expression of inflammatory cytokines and oxidative stress injury in HUVECs

Compared with LPS+ Si-SNHG15 + anti-miR-NC group, SOD activity of HUVECs cells and IL-10 level in culture medium in LPS+ Si-SNHG15 + anti-miR-362-3p group were significantly decreased (P<0.05), the levels of TNF-α and IL-6 in culture medium were significantly increased (P<0.05), as shown in Table 7.

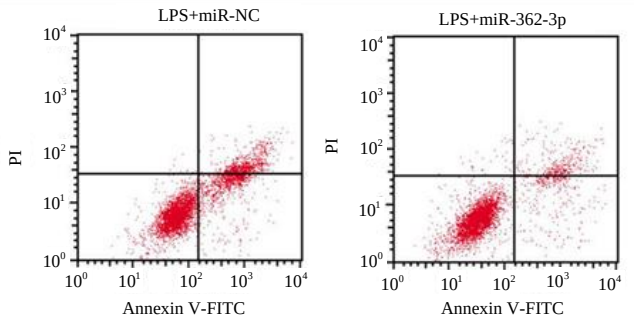

Figure 3. Effect of miR-362-3p on LPS-induced apoptosis of HUVECs

Table 5. Detection of miR-362-3p on LPS-induced expression of inflammatory factors and oxidative stress injury in HUVECs ($\bar{x} \pm s$, n=9)

Group	miR-362-3p	%		pg/mL			U/L	
		Inhibition rate	Apoptosis rate	TNF-α	IL-6	IL-10	SOD	LDH
LPS+miR-NC	1.00±0.00	55.95±3.63	23.50±1.14	356.76±26.97	167.55±14.11	94.20±5.65	125.68±17.43	595.86±33.42
LPS+miR-362-3p	3.08±0.11*	19.33±2.12*	10.79±0.68*	72.74±8.71*	51.17±3.18*	214.21±21.71*	452.26±23.22*	212.65±19.36*
t	56.727	26.134	28.725	30.064	24.139	16.049	33.745	29.766
P	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Note: compared with the LPS+miR-NC group, *P<0.05

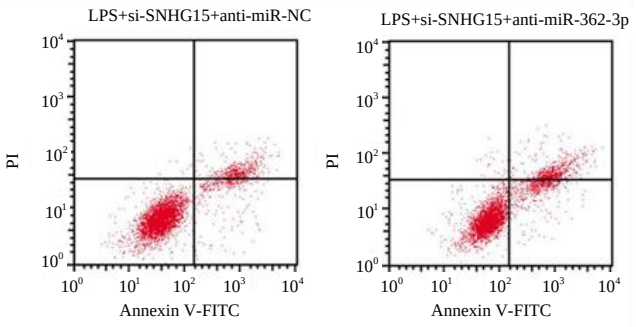

Figure 4. Effect of miR-362-3p inhibition on LPS-induced apoptosis of HUVECs treated with silenced SNHG15

Table 6. Detection of LPS-induced damage on silenced SNHG15-treated HUVECs by inhibiting miR-362-3p ($\bar{x} \pm s$, n=9)

Group	miR-362-3p	%	
		Inhibition rate	Apoptosis rate
LPS+si-SNHG15+anti-miR-NC	1.00±0.00	23.23±1.39	13.24±0.89
LPS+si-SNHG15+anti-miR-362-3p	0.28±0.03*	46.45±2.37*	20.48±0.97*
t	72.000	25.354	16.499
P	0.000	0.000	0.000

Note: compared with the LPS+si-SNHG15+anti-miR-NC group, *P<0.05

Table 7. Inhibition of miR-362-3p on LPS-induced inflammatory cytokines expression and oxidative stress in SNHG15-silenced HUVECs ($\bar{x} \pm s$, n=9)

Group	pg/mL			U/L	
	TNF-α	IL-6	IL-10	SOD	LDH
	144.50±12.89	84.37±6.05	199.26±13.98	413.87±17.77	276.70±23.00
LPS+si-SNHG15+anti-miR-NC	301.03±15.39*	140.35±10.20*	135.52±11.38*	164.13±18.42*	470.76±28.05*
LPS+si-SNHG15+anti-miR-362-3p	23.392	14.161	10.608	29.273	16.050
t	0.000	0.000	0.000	0.000	0.000

Note: compared with the LPS+si-SNHG15+anti-miR-NC group, *P<0.05

The lncRNA plays an important role in various pathways including innate immunity, mitochondrial function and apoptosis, and its misregulated expression is related to vascular endothelial cell

injury. Studies have reported that high expression of SNHG12 can inhibit the death and inflammatory response of microvascular endothelial cells in brain tissues after OGD/R, and promote angiogenesis (13). Knocking down the expression of opa interacting protein 5 antisense RNA1 (OIP5-AS1) can reduce oxidative low-density lipoprotein-induced vascular endothelial cytotoxicity and inhibit apoptosis (14). In mice with acute lung injury, overexpression of taurine up-regulated gene 1 (TUG1) can improve sepsis-induced lung injury, apoptosis and inflammation by down-regulating the expression of Mir-34B-5p, and TUG1 has a protective effect on LPS-treated lung microvascular endothelial cells (15). In this study, SNHG15 expression was significantly increased in HUVECs after LPS treatment, suggesting that dysregulation of SNHG15 expression may be related to LPS-induced injury of HUVECs. LPS is cytotoxic and can increase the expression of pro-inflammatory factors TNF-α and IL-6, induce inflammatory response and lead to sepsis (16). LPS can also promote the generation of reactive oxygen species, inhibit the activity of antioxidant enzyme SOD, destroy cell membranes and promote the release of intracellular molecule LDH to extracellular (17). In this study, silencing SNHG15 decreased the levels of TNF-α and IL-6, increased the level of IL-10, inhibited the release of LDH, and increased the activity of SOD, thus inhibiting LPS-induced oxidative stress and inflammatory response of HUVECs. In addition, SNHG15-silencing significantly attenuated LPS-induced apoptosis and proliferation inhibition of HUVECs. A number of studies have consistently shown that down-regulation of SNHG15 can improve ischemic/hypoxia-induced neuronal injury and microglial inflammation, and inhibit the progression of ischemic stroke (18, 19), which is consistent with the protective effect of SNHG15 silencing in this study. These studies suggest that SNHG15 is related to LPS-induced vascular endothelial cell injury, and silencing SNHG15 may be an important strategy based on vascular endothelial cell inhibition of sepsis progression.

The function of SNHG15 is mainly related to the regulation of miRNA expression. For example, targeting miR-211-3p by SNHG15 can promote the progression of breast cancer and lung cancer (20, 21). By interacting with miR-18a, SNHG15 is associated with neuronal cytotoxicity and apoptosis induced by hypoxia and glucose deficiency (22, 23). This study found that the expression of miR-362-3p was decreased in HUVECs after LPS treatment, and confirmed that miR-362-3p is a direct target of SNHG15, suggesting that SNHG15 plays a role in LPS-induced damage of HUVECs by targeting miR-362-3p. It has been reported that miR-362-3p overexpression can promote myocardial cell proliferation and inhibit apoptosis, thus alleviating myocardial hypoxia/reoxygenation damage (24). Down-regulation of SNHG1 can reduce autophagy and apoptosis of LPS-damaged PC12 cells by increasing the miR-362-3p level (25). In addition, the protective effect of curcumin on LPS-induced microglia apoptosis and inflammation was also related to the up-regulation of miR-362-3p expression (26-29). In this study, it was found that overexpression of miR-362-3p could reduce LPS-induced apoptosis and proliferation inhibition of HUVECs, and inhibit oxidative stress and inflammatory response, which was consistent with the protective effect of SNHG15 silencing. Further studies showed that SNHG15 silencing significantly upregulated miR-362-3p expression, and inhibition of miR-362-3p expression significantly reduced the effects of SNHG15 silencing on LPS-induced apoptosis, proliferation inhibition, oxidative stress and inflammatory response of HUVECs，which suggests that SNHG15 can regulate LPS-induced HUVECs damage by targeting miR-362-3p.

In conclusion, SNHG15 silencing protects vascular endothelial cells from LPS-induced inflammatory damage, oxidative stress and apoptosis through up-regulation of miR-362-3p. These findings provide insights into the molecular mechanisms of endothelial cell injury and provide the theoretical basis for the clinical development of potential therapeutic strategies for toxemia.

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Interest conflict

None.

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