



FOXA1 exacerbates LPS-induced vascular endothelial cell injury in sepsis by suppressing the transcription of NRP2

Chun Li · Likun Gou

Received: 21 March 2024 / Accepted: 17 July 2024
© The Author(s), under exclusive licence to Springer Nature B.V. 2024

Abstract Endothelial dysfunction plays a critical role in the pathogenesis of sepsis. This study aims to explore the effect and mechanism of forkhead box A1 (FOXA1) on vascular endothelial cell injury in sepsis. Human umbilical vein endothelial cells (HUVECs) were stimulated by lipopolysaccharide (LPS). Lactate dehydrogenase (LDH) release, cell viability, apoptosis, and inflammatory factors including IL-1 β , TNF- α , and IL-6 were measured using LDH kits, CCK-8 assay, flow cytometry, and ELISA respectively. RT-qPCR or Western blot determined the expression of FOXA1 or neuropilin-2 (NRP2) in cells. The binding between FOXA1 and NRP2 was confirmed using ChIP and dual-luciferase assays. Functional rescue experiments were performed to verify the effect of FOXA1 siRNA or NRP2 siRNA on cell injury. LPS treatment induced endothelial cell injury in a concentration-dependent manner. FOXA1 expression was elevated after LPS treatment. FOXA1 silencing reduced LDH release, enhanced cell viability, suppressed apoptosis, and declined inflammation factors. Mechanistically, FOXA1 bound to the NRP2 promoter to suppress the transcription of NRP2. Functional rescue experiments revealed that knockdown of NRP2 offset the protective effect of knockdown of

FOXA1 on cell injury. In conclusion, FOXA1 exacerbates LPS-insulted endothelial cell injury in sepsis by repressing the transcription of NRP2.

Keywords Sepsis · Endothelial cell injury · FOXA1 · NRP2 · Transcription factors

Introduction

Sepsis is a devastating clinical condition defined as life-threatening organ dysfunction due to dysregulated host responses to infection, and despite the considerable progress in medical care, it remains a salient clinical challenge and leads to substantial deaths in the critically ill patients worldwide (Hattori et al. 2017). Endothelial cells (ECs) play vital roles in the systemic response to bacterial infections and represent one of the earliest cell types to respond to sepsis insult (Vincent et al. 2021). Upon stimulation, ECs undergo transition towards pro-apoptotic, pro-inflammatory, pro-adhesive, and pro-coagulant states (Martin-Fernandez et al. 2021). Endothelial dysfunction is acknowledged as a critical event in the deterioration from sepsis to multiple organ failures by enhancing vascular permeability, activating the coagulation cascade reaction, promoting tissue edema, and damaging the perfusion of important organs (Bermejo-Martin et al. 2018; Ince et al. 2016). In sepsis settings, endothelial dysfunction represents a unified

C. Li · L. Gou (✉)
Department of Intensive Care Medicine, Gansu, Second People's Hospital of Lanzhou City, No. 388, Jingyuan Road, Chengguan District, Lanzhou 730030, China
e-mail: ggoulkun@163.com

explanation of the complex pathophysiology of sepsis and also a promising target for systemic therapy (Chen 2017). Therefore, it is particularly important to protect the integrity of the structure and function of VECs in sepsis, which is of great clinical significance in reducing mortality (Shapiro et al. 2010).

Forkhead box (FOX) proteins are transcription factors that participate in diverse physiological processes including development, organogenesis, metabolism, and immune regulation (Jackson et al. 2010). Forkhead box A1 (FOXA1) as the founding member of the FOX family has been associated with the development and differentiation of various tissues (Bernardo and Keri 2012). FOXA1 expression is elevated in patients with septic acute kidney injury (AKI) and lipopolysaccharide (LPS)-triggered HK2 cells, and FOXA1 addition aggravates LPS-disposed HK2 cell injury (Lu et al. 2022). FOXA1 is also reported as a pro-apoptotic factor in the pathological development of sepsis-induced AKI since FOXA1 overexpression stimulates LPS-caused HK2 cell apoptosis (Lu et al. 2020). Suppression of FOXA1 exerts protective effects against neonatal sepsis-induced systemic inflammation and elevates the survival rates of mice (Li et al. 2021). However, the transcriptional regulation of FOXA1 in sepsis-associated VEC injury remains unknown.

In this study, JASPAR database prediction suggests that FOXA1 owns a binding relationship with the promoter region of neuropilin-2 (NRP2). NRP2 is a cell surface transmembrane protein predominantly expressed in the nervous and vascular systems, which participates in embryonic development by facilitating the binding of vascular endothelial growth factor (VEGF)-VEGFR (Stevens and Oltean 2019). NRP2 deficiency increases vascular permeability during inflammation, implying the significance of NRP2 in regulating tissue swelling and alleviating edema after inflammation (Mucka et al. 2016). Importantly, NRP2 expression is inhibited in LPS-stimulated human umbilical vein endothelial cells (HUVECs), and functional NRP2 silencing exacerbates aortic endothelial dysfunction (Pan et al. 2022). Herein, this study aims to determine the regulatory mechanism of FOXA1/NRP2 in a LPS-exposed VEC injury model, thereby conferring novel targets for the clinical management of sepsis.

Materials and methods

Culture of HUVECs

HUVECs (American Type Culture Collection, Rockville, MD, USA) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (GIBCO, Grand Island, NY, USA), 1% penicillin–streptomycin, and 5 mM glucose in a humidified incubator containing 5% CO₂ at 37 °C.

Treatment of HUVECs

To determine the effect of FOXA1/NRP2 axis on sepsis-induced in vitro injury, HUVECs were treated with LPS (0, 1, 2, 5, 10 µg/mL) for 0, 2, 4, 8, and 12 h. Small interfering RNAs (siRNAs) targeting FOXA1 or NRP2 (si-FOXA1-1, si-FOXA1-2 or si-NRP2-1, si-NRP2-2) and their negative controls (si-NC) were purchased from GenePharma (Shanghai, China) and transfected into cells using the Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA). The cells were harvested 24 h later.

Cell counting kit-8 (CCK-8) assay

The CCK-8 assay was used to detect the proliferation of cells under different treatment conditions. The cells were incubated in a 96-well plate (3×10^3 cells/well) for 24 h and added with 10 µL CCK-8 reagent to each well, followed by 1 h of incubation at 37 °C. The optical density at 450 nm was detected using a microplate reader (Dojindo, Kumamoto, Japan) and used as a representative of cell viability.

Lactate dehydrogenase (LDH) release

The LDH detection kit (Jiancheng Bioengineering Research Institute, Nanjing, Jiangsu, China) was adopted to detect the release of LDH in the cell supernatant. Shortly, 1×10^5 cells/well were seeded into a 96-well plate, and the supernatant was collected in a clean tube and mixed with the reaction buffer provided by the reagent kit. The absorbance at 490 nm was measured using a microplate

(BioTek, Winooski, VT, USA), and the final result was expressed as 100%.

Flow cytometry

The cells were collected and centrifuged at 1000 g for 5 min. The supernatant was discarded and the cells were washed twice with phosphate-buffered saline (PBS). Then, the cells were re-suspended with 100 μ L 1 \times AnnexinV binding buffer to adjust the cell density to 1 \times 10⁶ cells/mL. AnnexinV-FITC binding solution and propidium iodide were added according to the protocol of AnnexinV-FITC/PI cell apoptosis detection kit (BD Biosciences, East Rutherford, NJ, USA). Cell apoptosis analysis was performed using LSRII flow cytometer (BD Biosciences) and Cell-Quest software.

Enzyme-linked immunosorbent assay (ELISA)

The supernatant was collected in a clean tube and the levels of IL-1 β (ab214025, Abcam Inc., Cambridge, MA, USA), IL-6 (ab178013, Abcam), and TNF- α (ab181421, Abcam) were detected using ELISA kits.

Chromatin immunoprecipitation (ChIP)

The EZ-Magna ChIP kit (EMD Millipore, Billerica, MA, USA) was applied for ChIP detection. The cells were cross-linked with 1% formaldehyde for 10 min. Then, the cells were harvested in lysis buffer and DNA was cut into fragments through pulsed ultrasound. After ultrasonic treatment, the sample was divided equally and incubated overnight with FOXA1 antibody (ab170933, Abcam) and non-specific IgG antibody (ab172730, Abcam) at 4 °C. The next day, each sample was incubated with 40 μ L protein A agarose beads (Thermo Fisher Scientific Inc., Waltham, MA, USA) for 3 h at 4 °C. Afterward, the immune complex was washed twice with lysis buffer and qPCR was performed on the precipitated DNA using the primers shown in Table 1, with Input used as normalization.

Dual-luciferase reporter assay

The binding site between FOXA1 and NRP2 promoter was predicted through the JASPAR database (<https://jaspar.genereg.net/>) (Castro-Mondragon et al.

Table 1 PCR primer sequences

Name	Sequence (5'–3')
FOXA1	F: GAAGACCGGCCAGCTAGAG R: GGAGTCTTCAACTCCGAGGC
NRP2	F: CATCCGAAGGTTTGACCCCA R: GAGTCTGTCCAGTCACAGCC
β -actin	F: GGCACCCAGCACAATGAAGA R: ACTCCTGCTTGCTGATCCAC
NRP2 promoter	F: CAGCTTGGCTCAACTATTTC R: ATTCTTCTGGGTGGTTTCCTC

FOXA1 forkhead box A1, *NRP2* neuropilin-2

2022). The reporting vectors for the binding site and mutation site of the NRP2 promoter were constructed by Ribo (Guangzhou, China). The cells were cultured in a 12-well plate to 50–80% confluence, and FOXA1 pcDNA3.1 or NC pcDNA3.1 was co-transfected into cells with wild-type (NRP2-WT)/mutant-type (NRP2-MUT) reporter vectors using Lipofectamine 2000. The dual-luciferase reporter gene detection system (Promega Corp., Madison, Wisconsin, USA) was used to detect luciferase 48 h after transfection. Luciferase activity was measured with a photometer (Promega) in line with the manufacturer's instructions.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

After treatment or transfection, the total RNA was extracted from cells using TRIzol (Invitrogen). The first-strand cDNA was generated using the RevertAid first-strand cDNA synthesis kit (Thermo Scientific). The reverse transcription reaction products were produced using SYBR qPCR Mix kit (Toyobo Co., Ltd., Osaka, Japan) and quantitatively analyzed in the 7500 Fast real-time PCR system (Applied Biosystems, Carlsbad, CA, USA). mRNA expression was detected using PrimeScript RT Reagent kit (TaKaRa, Tokyo, Japan), and the results were normalized with β -actin. Finally, the fold changes were detected using the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen 2001). The primers are shown in Table 1.

Western blot

After treatment and/or transfection, proteins were extracted from cells using radio-immunoprecipitation

assay buffer containing phenylmethylsulfonyl fluoride, and the protein concentration was measured using bicinchoninic acid protein assay (Pierce, Rockford, IL, USA). The protein was separated by 10–12% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride membranes. Then the membranes were blocked in Tris buffer Saline with Tween with 5% skimmed milk at room temperature for 2 h and incubated with the corresponding antibodies overnight at 4 °C. Afterwards, the membranes were washed three times with PBS buffer and incubated with the secondary antibody for 2 h. The protein bands were visualized using enhanced chemiluminescence (GE Healthcare UK Ltd, Little Chalfont, UK). The images were captured using the Bio-Rad image analysis system (Bio-Rad, Hercules, CA, USA) and analyzed using the Quantity One v4.6.2 software. The relative protein content was determined by the ratio of the grayscale value of the corresponding protein band to the grayscale value of the β -actin protein band. The primary antibodies were FOXA1 (1:1000, ab170933, Abcam), NRP2 (1:1000, ab273584, Abcam), and β -actin (1:1000, ab8227, Abcam), and secondary antibody was IgG (1:2000, ab205718, Abcam).

Statistical analysis

Data analysis and map plotting were performed using the SPSS 21.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, CA, USA). The data conformed to normal distribution and homogeneity of variance. The *t* test was adopted for comparisons between two groups. One-way or two-way analysis of variance (ANOVA) was employed for the comparisons among multiple groups, following Tukey's multiple comparison test. A value of $P < 0.05$ indicated a significant difference.

Results

LPS treatment led to VEC injury

ECs are the first line of defense in the pathological process of sepsis injury (Deutschman and Tracey 2014). To determine sepsis-associated EC injury, we treated HUVECs with different concentrations of LPS for 12 h and observed that cell viability was

reduced while cell apoptosis rate was increased with the increase of LPS concentration ($P < 0.05$, Fig. 1A, B). After treatment with different concentrations of LPS, the release of LDH and the contents of inflammatory factors IL-1 β , IL-6, and TNF- α in cells were increased ($P < 0.05$, Fig. 1E, F). Next, we used 10 μ g/mL LPS to treat HUVECs and found that as the treatment duration prolonged, the cell viability was decreased and apoptosis rate was increased ($P < 0.05$, Fig. 1C, D). Meanwhile, we found that the longer the LPS treatment duration, the more LDH was released and the higher the levels of inflammatory factors ($P < 0.05$, Fig. 1G, H).

LPS boosted FOXA1 expression

FOXA1 is highly expressed in sepsis (Lu et al. 2020, 2022). Our results unveiled that the FOXA1 expression was elevated with the increase of LPS concentration and duration ($P < 0.05$, Fig. 2A, B).

Low expression of FOXA1 alleviated LPS-insulted VEC injury

To verify the effect of FOXA1 on LPS-insulted VEC injury, we knocked down the expression of FOXA1 in VECs. Based on our previous results, we chose to treat HUVECs with 10 μ g/mL LPS for 12 h ($P < 0.05$, Fig. 3A, B). After low expression of FOXA1, the cell viability was significantly enhanced and apoptosis rate was notably decreased ($P < 0.05$, Fig. 3C, D). In addition, low expression of FOXA1 significantly inhibited LPS-triggered LDH release and inflammatory factors ($P < 0.05$, Fig. 3E, F). Briefly, low expression of FOXA1 alleviated LPS-caused VEC injury.

FOXA1 bound to the NRP2 promoter to suppress the transcription of NRP2

FOXA1 inhibits gene expression at the transcriptional level (Zhu et al. 2021). JASPAR database predicted that FOXA1 can bind to the NRP2 promoter (Fig. 4A). NRP2 expression is reduced in LPS-injured VECs (Pan et al. 2022). ChIP results revealed that FOXA1 was enriched on the NRP2 promoter, but low expression of FOXA1 significantly reduced its enrichment level ($P < 0.05$, Fig. 4B). Similarly, dual-luciferase assay also confirmed the binding of FOXA1 to the NRP2 promoter ($P < 0.05$, Fig. 4C).

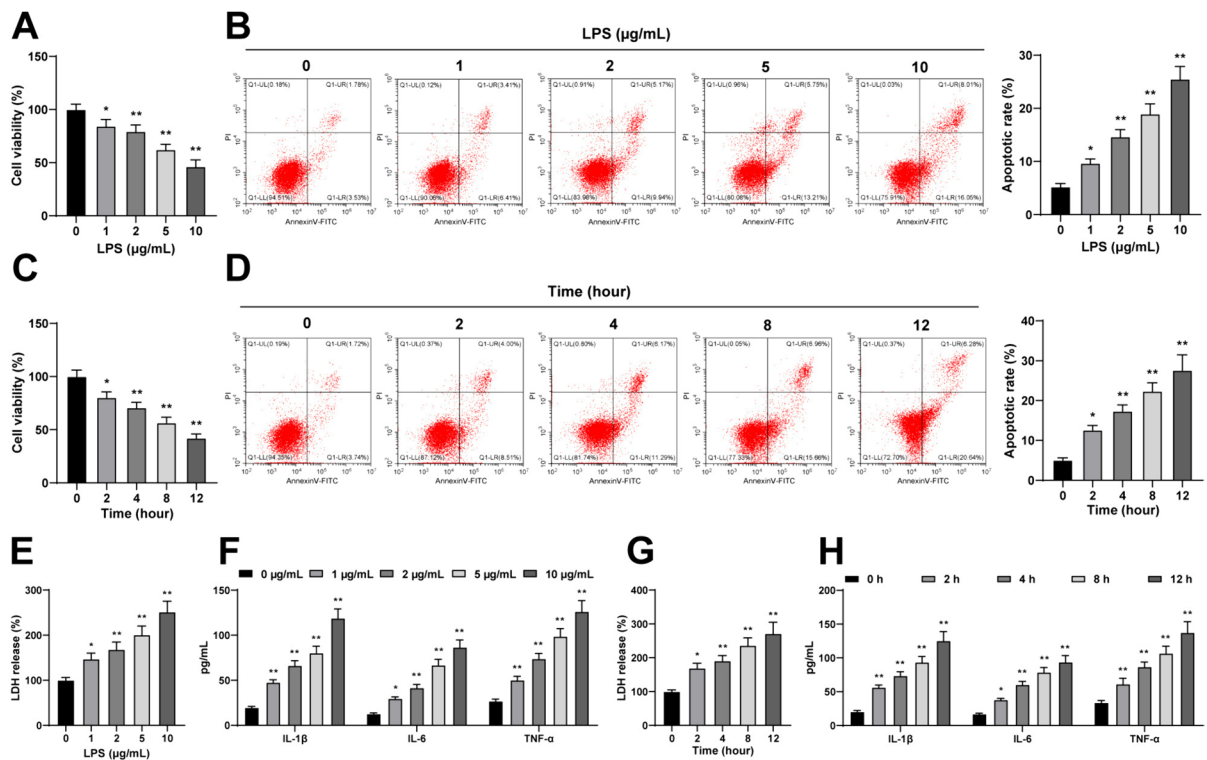


Fig. 1 LPS induced VEC injury. HUVECs were treated with different concentrations of LPS at different times to induce sepsis-induced vascular endothelial cell injury model. **A/C** Detection of cell viability by CCK-8 assay. **B/D** Detection of cell apoptosis by flow cytometry. **E/G** Detection of LDH release by kits. **F/H** Detection of inflammatory factors IL-1 β , IL-6, and TNF- α by ELISA. The cell experiments

were repeated 3 times independently. The data are expressed as mean \pm standard deviation. Comparison of data between multiple groups in panels **A–D/E/G** adopted one-way ANOVA, while comparison of data between multiple groups in panels **F/H** used two-way ANOVA, followed by Tukey's multiple comparisons test. * $P < 0.05$, ** $P < 0.01$. The control group was treated with 10 $\mu\text{g/mL}$ LPS for 0 h or 0 $\mu\text{g/mL}$ LPS for 12 h

The transcription level of NRP2 was reduced with the increase of LPS concentration and duration. However, low expression of FOXA1 enhanced the transcription of NRP2 ($P < 0.05$, Fig. 4D). The above results indicated that the binding of FOXA1 to the NRP2 promoter depressed the transcription of NRP2.

FOXA1 aggravated LPS-insulted VEC injury by suppressing NRP2 transcription

Finally, we knocked down the expression of NRP2 in cells by transfecting si-NRP2 and selected si-NRP2-2 with better transfection efficiency for subsequent experiments ($P < 0.05$, Fig. 5A, B). After low expression of NRP2, the cell viability was notably depressed and apoptosis rate was significantly elevated ($P < 0.05$, Fig. 5C, D). In addition, low expression of NRP2 significantly facilitated the release of

LDH and the contents of IL-1 β , IL-6, and TNF- α ($P < 0.05$, Fig. 5E, F). Briefly, FOXA1 aggravated LPS-insulted VEC injury through suppression of NRP2 transcription.

Discussion

Endothelial dysfunction has been recognized as a central event in the progression of sepsis into organ failure as it leads to extensive inflammatory responses, vascular leakage, and aberrant coagulation (Arina and Singer 2021). VECs have thus been considered promising targets for therapeutic interventions in sepsis (Vincent et al. 2021). Our study reveals that FOXA1 exacerbates LPS-insulted VEC injury in sepsis by depressing the transcription of NRP2.

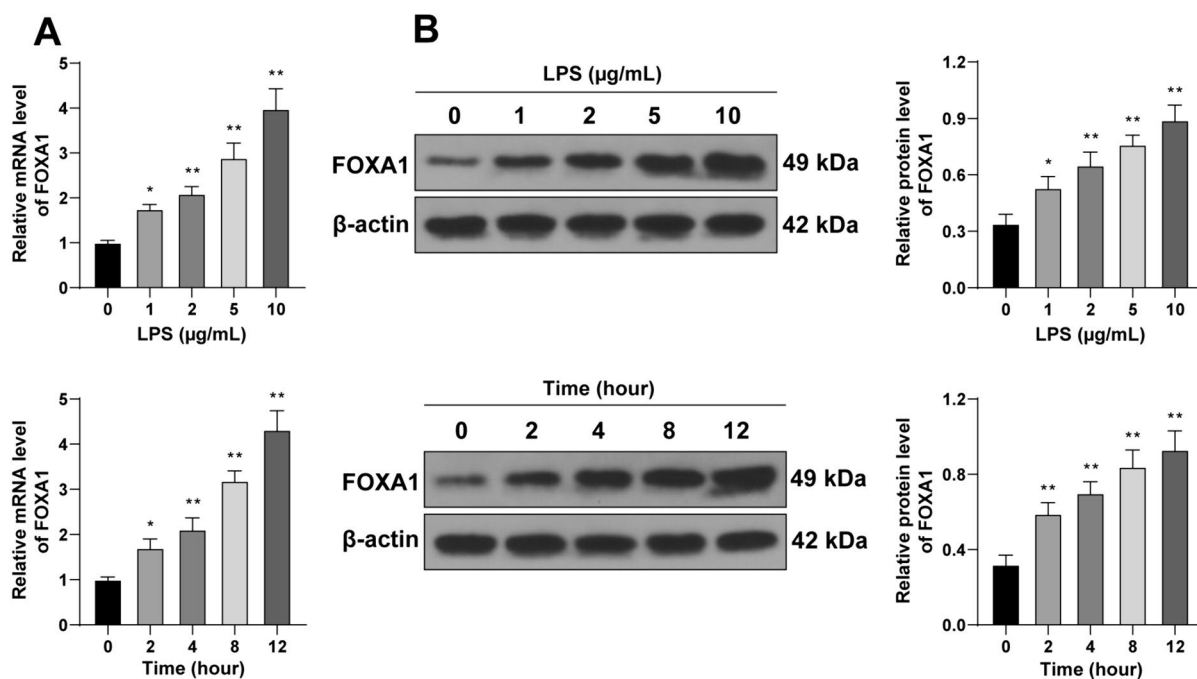


Fig. 2 LPS promoted FOXA1 expression. HUVECs were treated with different concentrations of LPS at different times to induce sepsis-induced vascular endothelial cell injury model. **A, B** Detection of FOXA1 expression in cells by RT-qPCR and Western blot. The cell experiments were repeated 3 time independently. The data are expressed as mean \pm stand-

ard deviation. Comparison of data between multiple groups in panels **A–B** adopted one-way ANOVA, followed by Tukey's multiple comparisons test. * $P < 0.05$, ** $P < 0.01$. The control group was treated with 10 $\mu\text{g/mL}$ LPS for 0 h or 0 $\mu\text{g/mL}$ LPS for 12 h

VECs are responsible for maintaining the integrity, tone, and patency of blood vessels, which play vital roles in inflammatory responses and hemostasis (Joffre et al. 2020). Sepsis affects almost all aspects of VEC functions including vascular regulation, barrier function, and inflammation (Ince et al. 2016). In the present study, we treated HUVECs with different concentrations of LPS for 12 h to mimic an in vitro model of sepsis-induced VEC injury. In vitro studies have demonstrated that a wide variety of stimuli can induce programmed cell death (such as apoptosis) of endothelial cells, and apoptosis is an important mechanism of endothelial injury or dysfunction (Winn and Harlan 2005). Inflammatory endothelial injury can lead to endothelial cell apoptosis (Jeon et al. 2017). Hence, we applied CCK-8 assay to detect endothelial viability, flow cytometry to assess apoptosis, and ELISA to detect inflammatory factors to assess cellular inflammation. LDH is also commonly detected to evaluate LPS-induced endothelial cell injury (Liu et al. 2022; Luo et al. 2024; Songjang et al. 2023;

You et al. 2021). We found that LPS treatment led to a decrease in cell viability, as well as an increase in apoptosis, LDH release, and inflammatory factors in a concentration-dependent manner. The transcription factor FOXA1 practically participates in diverse pathological processes in sepsis, such as inflammation, cell viability, and cell cycle progression (Li et al. 2021; Lu et al. 2020, 2022). We noted that FOXA1 expression was elevated with the increase of LPS concentration and duration. Therefore, we silenced FOXA1 expression HUVECs and found that FOXA1 silencing dramatically intensified cell viability, repressed apoptosis, curbed LDH release, and diminished inflammatory factors. Consistently, Lu et al. have observed an up-regulation of FOXA1 in sepsis-induced AKI and elaborated that FOXA1 promotes LPS-stimulated HK-2 cell apoptosis (Lu et al. 2020). FOXA1 suppression can restrain oxidative stress responses and diminish IL-1 β and TNF- α levels in LPS-insulted HK2 cells (Lu et al. 2022). Inhibiting FOXA1 also contributes to the positive effects of

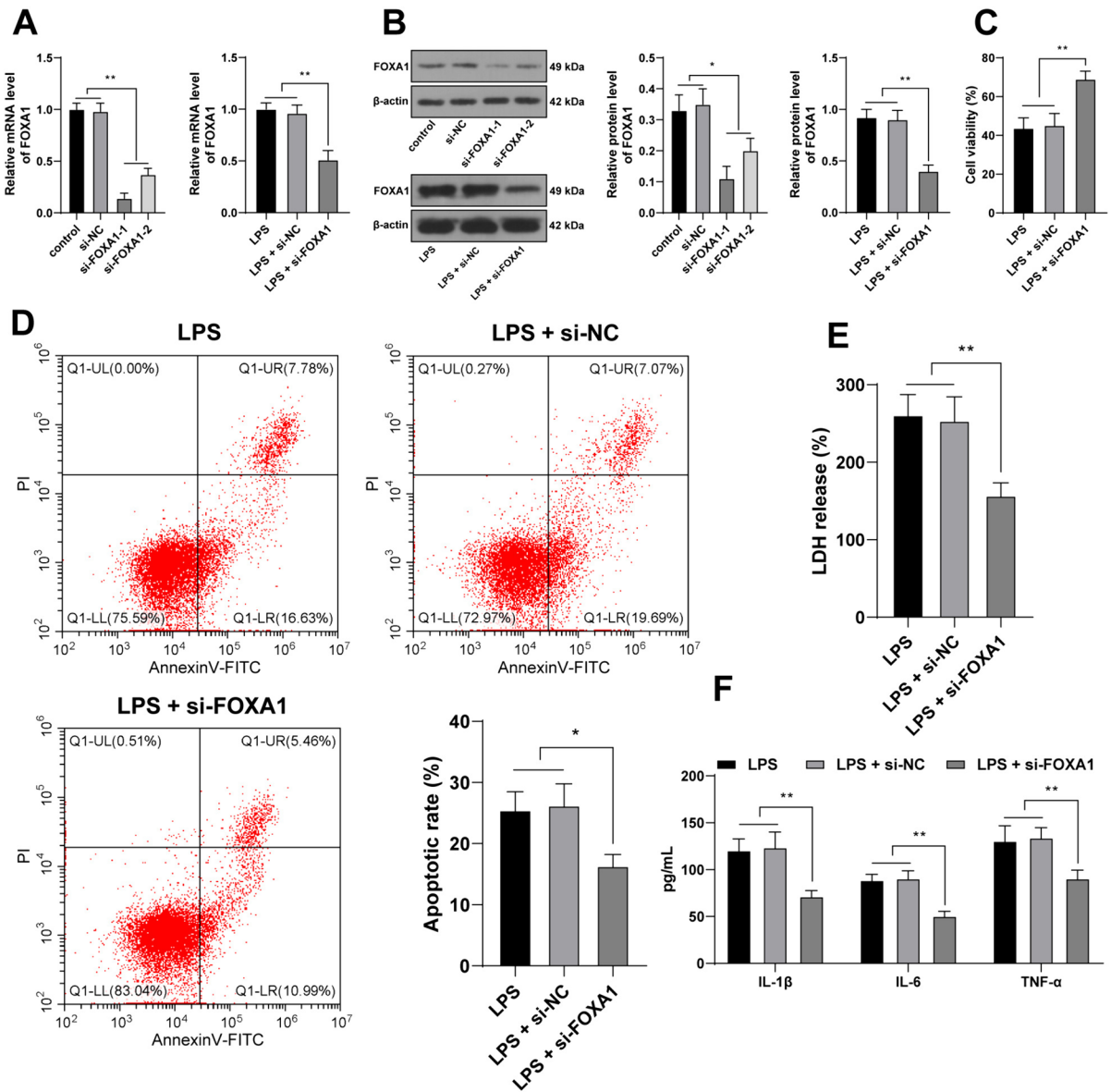


Fig. 3 Low expression of FOXA1 reduced LPS-induced VEC injury. Two FOXA1 siRNAs (si-FOXA1) were transfected into HUVECs, with si-NC as the negative control, and then HUVECs were treated with 10 μg/mL LPS for 12 h. **A**, **B** Detection of FOXA1 expression in cells by RT-qPCR and Western blot. **C** Detection of cell viability by CCK-8 assay. **D** Detection of cell apoptosis by flow cytometry. **E** Detection of LDH release by kits. **F** Detection of inflammatory factors IL-1β, IL-6, and TNF-α by ELISA. The cell experiments

were repeated 3 time independently. The data are expressed as mean ± standard deviation. Comparison of data between multiple groups in panels **A–E** adopted one-way ANOVA, while comparison of data between multiple groups in panel **F** used two-way ANOVA, followed by Tukey’s multiple comparisons test. * $P < 0.05$, ** $P < 0.01$. Control (untreated cells) was used a control for panel **A** (left)–**B** (left), and si-NC was used for the others

A

Matrix ID	Name	Score	Relative score	Sequence ID	Start	End	Strand	Predicted sequence
MA0148.1	MA0148.1.FOXA1	12.669262	0.9362692670896205	NC_000002.12:205680501-205682501	625	635	-	TATTTACTG

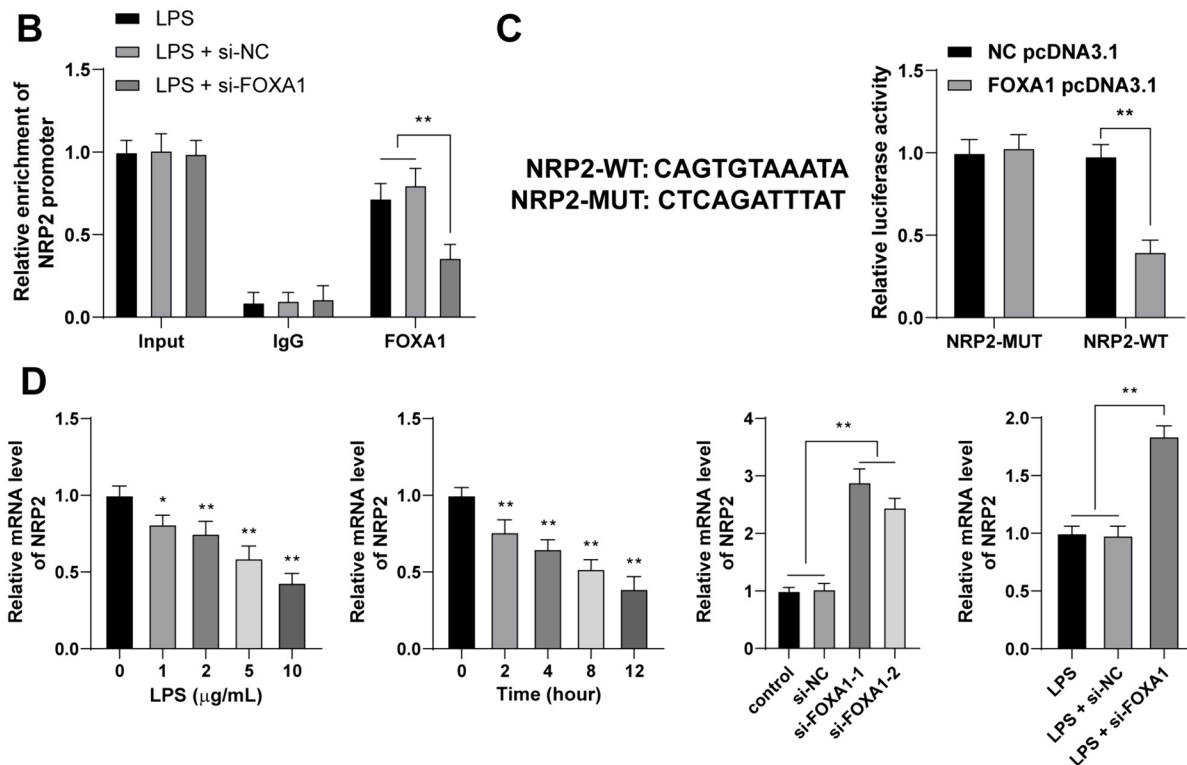


Fig. 4 FOXA1 bound to the NRP2 promoter to suppress the transcription of NRP2. **A** Prediction of the binding of FOXA1 to the NRP2 promoter through the JASPAR database. **B**, **C** Detection of the binding of FOXA1 to the NRP2 promoter by ChIP (Input was used as normalization) and dual-luciferase assays. **D** Detection of the transcription level of NRP2 in cells by RT-qPCR. The cell experiments were repeated 3 times independently. The data are expressed as mean \pm standard deviation.

tion. Comparison of data between multiple groups in panel **D** adopted one-way ANOVA, while comparison of data between multiple groups in panels **B–C** used two-way ANOVA, followed by Tukey's multiple comparisons test. $*P < 0.05$, $**P < 0.01$. In panel **D**, 10 $\mu\text{g}/\text{mL}$ LPS treatment for 0 h, 0 $\mu\text{g}/\text{mL}$ LPS treatment for 12 h, control (untreated cells), and si NC were used as a control

berberine on the survival rate and systemic inflammation in a mouse model of neonatal sepsis (Li et al. 2021). Based on these previous finding, it was indicated that low expression of FOXA1 alleviated LPS-insulted injury to VECs.

Subsequently, we focused on the downstream mechanism of FOXA1 in alleviating LPS-caused VEC injury. The prediction through the JASPAR database suggested that FOXA1 possessed a binding relationship with the NRP2 promoter region. NRP2 is a single chain transmembrane glycoprotein

abundantly expressed in lymphatic and vascular ECs (Islam et al. 2022). NRP2 in ECs is extensively implicated in the angiogenesis, lymphangiogenesis, and vasculogenesis processes by selectively binding to VEGF family members (Alghamdi et al. 2020). Existing studies focus on annotating the role of NRP2 in various cancers, while the function of NRP2 in sepsis remains largely unknown. ChIP and dual-luciferase assays confirmed the binding between FOXA1 and NRP2. NRP2 expression was declined with LPS treatment concentration and duration, but FOXA1

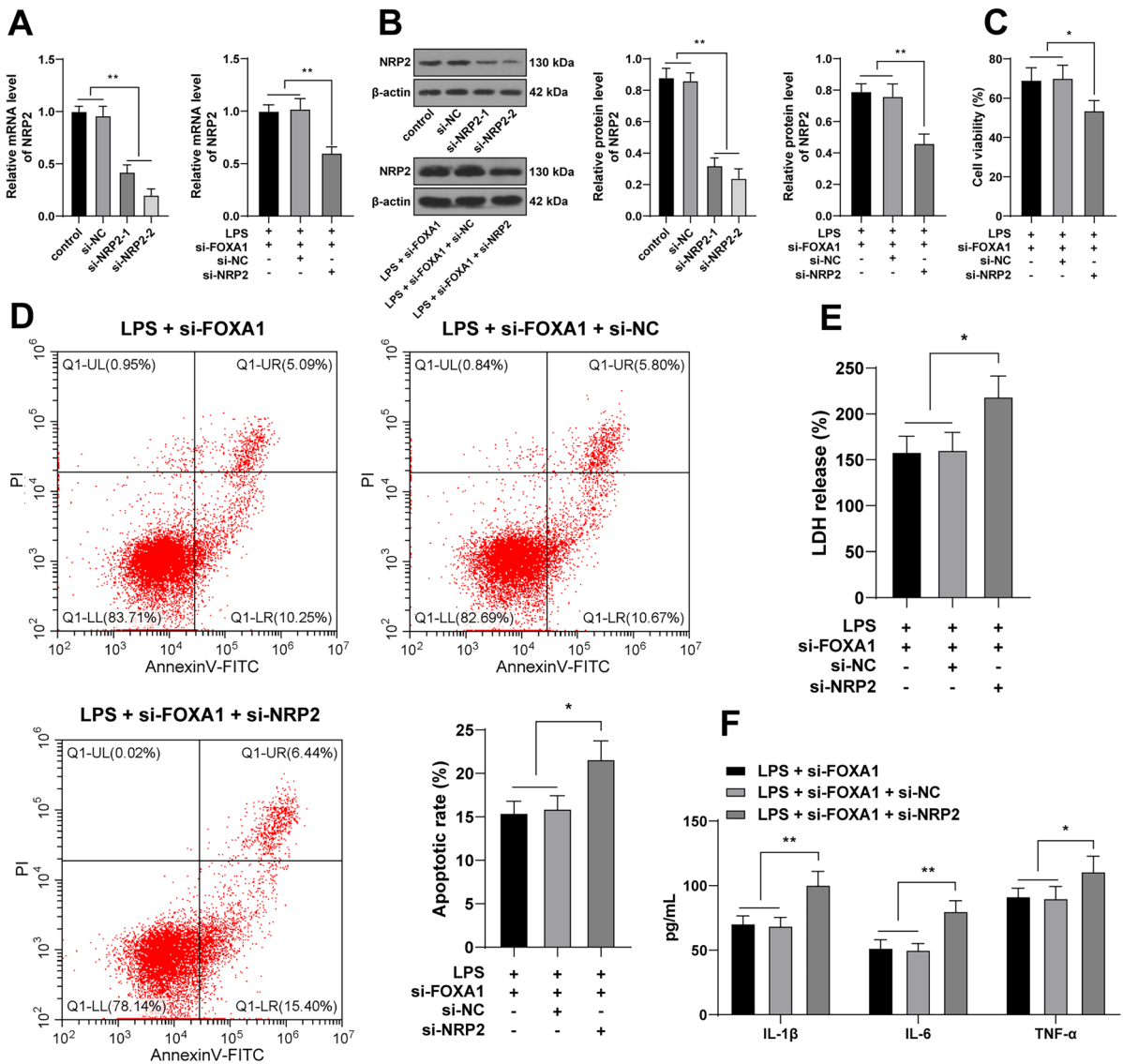


Fig. 5 FOXA1 aggravated LPS-induced VEC injury by inhibiting NRP2 transcription. Two NRP2 siRNAs (si-NRP2) were transfected into HUVECs, with si-NC as the negative control, and then HUVECs were treated with 10 μ g/mL LPS for 12 h. **A**, **B** Detection of NRP2 expression in cells by RT-qPCR and Western blot. **C** Detection of cell viability by CCK-8 assay. **D** Detection of cell apoptosis by flow cytometry. **E** Detection of LDH release by kits. **F** Detection of inflammatory factors IL-1 β , IL-6, and TNF- α by ELISA. The cell experiments

were repeated 3 time independently. The data are expressed as mean \pm standard deviation. Comparison of data between multiple groups in panels **A**–**E** adopted one-way ANOVA, while comparison of data between multiple groups in panel **F** used two-way ANOVA, followed by Tukey’s multiple comparisons test. * P <0.05, ** P <0.01. Control (untreated cells) was used a control for panel **A** (left)–**B** (left), and si-NC was used for the others

silencing lifted the transcription of NRP2, implying that FOXA1 bound to the NRP2 promoter to restrain the transcription of NRP2. NRP2 is crucial for normal vascular functions, and NRP2 deficiency can cause extensive lymphedema and magnify vascular

permeability in mice under inflammatory conditions (Mucka et al. 2016). NRP2 supports the anti-endothelial dysfunction role of ruscogenin by improving the arterial histology of septic mice, as well as enhancing viability, curbing apoptosis, and down-regulating

cytokines in LPS-injured HUVECs (Pan et al. 2022). Similarly, we found that NRP2 silencing restrained the viability of LPS-injured HUVECs, repressed cell apoptosis, boosted LDH release, and elevated IL-1 β , IL-6, TNF- α contents.

To our knowledge, we are the first to reveal that FOXA1 exacerbates sepsis-associated endothelial dysfunction by depressing the transcription of NRP2. However, this study only explored the single downstream mechanism of FOXA1 in sepsis-associated endothelial dysfunction. Moreover, we did not validate the mechanism more fully at the animal level and also did not detect the protein level of NRP2. In the future research, we will validate the proposed mechanism in animals and explore the underlying reasons for high expression of FOXA1, thus providing more theoretical knowledge for the treatment of sepsis.

Acknowledgements We appreciate the efforts and dedication of all participants.

Author contributions Guarantor of integrity of the entire study: CL, LG; study concepts: CL, LG; definition of intellectual content: CL; experimental studies: CL; data acquisition: CL; data analysis: CL, LG; manuscript preparation and editing: CL; All authors read and approved the final manuscript.

Funding There was no funding for the study.

Data availability Data will be made available on request.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

References

- Alghamdi AAA, Benwell CJ, Atkinson SJ, Lambert J, Johnson RT, Robinson SD (2020) NRP2 as an Emerging angiogenic player; promoting endothelial cell adhesion and migration by regulating recycling of alpha5 integrin. *Front Cell Dev Biol* 8:395. <https://doi.org/10.3389/fcell.2020.00395>
- Arina P, Singer M (2021) Pathophysiology of sepsis. *Curr Opin Anaesthesiol* 34:77–84. <https://doi.org/10.1097/ACO.0000000000000963>
- Bermejo-Martin JF, Martin-Fernandez M, Lopez-Mestanza C, Duque P, Almansa R (2018) Shared features of endothelial dysfunction between sepsis and its preceding risk factors (aging and chronic disease). *J Clin Med* 7:400. <https://doi.org/10.3390/jcm7110400>
- Bernardo GM, Keri RA (2012) FOXA1: a transcription factor with parallel functions in development and cancer. *Biosci Rep* 32:113–130. <https://doi.org/10.1042/BSR20110046>
- Castro-Mondragon JA, Riudavets-Puig R, Rauluseviciute I, Lemma RB, Turchi L, Blanc-Mathieu R, Lucas J, Boddie P, Khan A, Manosalva Perez N et al (2022) JASPAR 2022: the 9th release of the open-access database of transcription factor binding profiles. *Nucleic Acids Res* 50:D165–D173. <https://doi.org/10.1093/nar/gkab1113>
- Chen DC (2017) Sepsis and intestinal microvascular endothelial dysfunction. *Chin Med J (engl)* 130:1137–1138. <https://doi.org/10.4103/0366-6999.205865>
- Deutschman CS, Tracey KJ (2014) Sepsis: current dogma and new perspectives. *Immunity* 40:463–475. <https://doi.org/10.1016/j.immuni.2014.04.001>
- Hattori Y, Hattori K, Suzuki T, Matsuda N (2017) Recent advances in the pathophysiology and molecular basis of sepsis-associated organ dysfunction: novel therapeutic implications and challenges. *Pharmacol Ther* 177:56–66. <https://doi.org/10.1016/j.pharmthera.2017.02.040>
- Ince C, Mayeux PR, Nguyen T, Gomez H, Kellum JA, Ospina-Tascon GA, Hernandez G, Murray P, De Backer D, Workgroup AX (2016) The endothelium in sepsis. *Shock* 45:259–270. <https://doi.org/10.1097/SHK.0000000000000473>
- Islam R, Mishra J, Bodas S, Bhattacharya S, Batra SK, Dutta S, Datta K (2022) Role of neuropilin-2-mediated signaling axis in cancer progression and therapy resistance. *Cancer Metastasis Rev* 41:771–787. <https://doi.org/10.1007/s10555-022-10048-0>
- Jackson BC, Carpenter C, Nebert DW, Vasilio V (2010) Update of human and mouse forkhead box (FOX) gene families. *Hum Genom* 4:345–352. <https://doi.org/10.1186/1479-7364-4-5-345>
- Jeon D, Kim SJ, Kim HS (2017) Anti-inflammatory evaluation of the methanolic extract of *Taraxacum officinale* in LPS-stimulated human umbilical vein endothelial cells. *BMC Complement Altern Med* 17:508. <https://doi.org/10.1186/s12906-017-2022-7>
- Joffre J, Hellman J, Ince C, Ait-Oufella H (2020) Endothelial responses in sepsis. *Am J Respir Crit Care Med* 202:361–370. <https://doi.org/10.1164/rccm.201910-1911TR>
- Li B, Niu S, Geng H, Yang C, Zhao C (2021) Berberine attenuates neonatal sepsis in mice by inhibiting FOXA1 and NF-kappaB signal transduction via the induction of MiR-132-3p. *Inflammation* 44:2395–2406. <https://doi.org/10.1007/s10753-021-01510-2>
- Liu G, Tian R, Mao H, Ren Y (2022) Effect of lncRNA SNHG15 on LPS-induced vascular endothelial cell apoptosis, inflammatory factor expression and oxidative stress by targeting miR-362-3p. *Cell Mol Biol (noisy-Le-Grand)* 67:220–227. <https://doi.org/10.14715/cmb/2021.67.6.29>
- Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25:402–408. <https://doi.org/10.1006/meth.2001.1262>
- Lu S, Wu H, Xu J, He Z, Li H, Ning C (2020) SIKIAT1/miR-96/FOXA1 axis regulates sepsis-induced kidney injury through induction of apoptosis. *Inflamm Res* 69:645–656. <https://doi.org/10.1007/s00011-020-01350-0>

- Lu H, Chen Y, Wang X, Yang Y, Ding M, Qiu F (2022) Circular RNA HIPK3 aggravates sepsis-induced acute kidney injury via modulating the microRNA-338/forkhead box A1 axis. *Bioengineered* 13:4798–4809. <https://doi.org/10.1080/21655979.2022.2032974>
- Luo J, Fang H, Wang D, Hu J, Zhang W, Jiang R (2024) Molecular mechanism of SOX18 in lipopolysaccharide-induced injury of human umbilical vein endothelial cells. *Crit Rev Immunol* 44:1–12. <https://doi.org/10.1615/CritRevImmunol.2023050792>
- Martin-Fernandez M, Tamayo-Velasco A, Aller R, Gonzalo-Benito H, Martinez-Paz P, Tamayo E (2021) Endothelial dysfunction and neutrophil degranulation as central events in sepsis physiopathology. *Int J Mol Sci* 22:6272. <https://doi.org/10.3390/ijms22126272>
- Mucka P, Levonyak N, Geretti E, Zwaans BMM, Li X, Adini I, Klagsbrun M, Adam RM, Bielenberg DR (2016) Inflammation and lymphedema are exacerbated and prolonged by neuropilin 2 deficiency. *Am J Pathol* 186:2803–2812. <https://doi.org/10.1016/j.ajpath.2016.07.022>
- Pan D, Zhu J, Cao L, Zhu B, Lin L (2022) Ruscogenin attenuates lipopolysaccharide-induced septic vascular endothelial dysfunction by modulating the miR-146a-5p/NRP2/SSH1 axis. *Drug Des Devel Ther* 16:1099–1106. <https://doi.org/10.2147/DDDT.S356451>
- Shapiro NI, Schuetz P, Yano K, Sorasaki M, Parikh SM, Jones AE, Trzeciak S, Ngo L, Aird WC (2010) The association of endothelial cell signaling, severity of illness, and organ dysfunction in sepsis. *Crit Care* 14:R182. <https://doi.org/10.1186/cc9290>
- Songjang W, Paiyabhroma N, Jumroon N, Jiraviriyakul A, Nernpermpisooth N, Seenak P, Kumphune S, Thaisakun S, Phaonakrop N, Roytrakul S et al (2023) Proteomic profiling of early secreted proteins in response to lipopolysaccharide-induced vascular endothelial cell EA.hy926 injury. *Biomedicines* 11:3065. <https://doi.org/10.3390/biomedicines11113065>
- Stevens M, Oltean S (2019) Modulation of receptor tyrosine kinase activity through alternative splicing of ligands and receptors in the VEGF-A/VEGFR axis. *Cells* 8:288. <https://doi.org/10.3390/cells8040288>
- Vincent JL, Ince C, Pickkers P (2021) Endothelial dysfunction: a therapeutic target in bacterial sepsis? *Expert Opin Ther Targets* 25:733–748. <https://doi.org/10.1080/14728222.2021.1988928>
- Winn RK, Harlan JM (2005) The role of endothelial cell apoptosis in inflammatory and immune diseases. *J Thromb Haemost* 3:1815–1824. <https://doi.org/10.1111/j.1538-7836.2005.01378.x>
- You L, Zhang D, Geng H, Sun F, Lei M (2021) Salidroside protects endothelial cells against LPS-induced inflammatory injury by inhibiting NLRP3 and enhancing autophagy. *BMC Complement Med Ther* 21:146. <https://doi.org/10.1186/s12906-021-03307-0>
- Zhu H, Peng J, Li W (2021) FOXA1 Suppresses SATB1 transcription and inactivates the Wnt/beta-catenin pathway to alleviate diabetic nephropathy in a mouse model. *Diabetes Metab Syndr Obes* 14:3975–3987. <https://doi.org/10.2147/DMSO.S314709>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.