

## Original Article

# Urokinase-type plasminogen activator protects human umbilical vein endothelial cells from apoptosis in sepsis

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**Abstract:** The aim of this study was to investigate the effect of urokinase-type plasminogen activator (uPA) on cell viability, apoptosis, and inflammatory cytokine levels in septic human umbilical vein endothelial cells (HUVECs). Lipopolysaccharides (LPS) were added to construct septic HUVECs, then the septic HUVECs were treated by uPA, and cell viability, apoptosis, TNF- $\alpha$ , IL-6, GMCSF and uPAR levels were evaluated by CCK-8, AV/PI, qPCR, western blot and ELISA, respectively. Subsequently, uPA shRNA was transferred into septic HUVECs, and the cells viability, cell apoptosis and the expressions of TNF- $\alpha$ , IL-6, GMCSF, as well as uPAR were assessed by the same methods. uPA promoted viability while reducing apoptosis in septic HUVECs. However, uPA had no effect on the regulation of TNF- $\alpha$  or IL-6 expression in septic HUVECs. In addition, uPA elevated the expressions of GMCSF and uPAR in septic HUVECs. After the transfection of uPA shRNA, cell viability was decreased, apoptosis was enhanced, and GMCSF and uPAR expressions were reduced, while TNF- $\alpha$  or IL-6 expression did not vary in septic HUVECs. In conclusion, uPA promotes cell viability, represses apoptosis, and has no effect on regulating inflammatory cytokines in septic HUVECs.

**Keywords:** Urokinase-type plasminogen activator, septic HUVECs, cell viability, apoptosis, inflammatory cytokine

## Introduction

Sepsis is an intricate systemic disorder characterized by rapid organ dysfunction with original causes of various infections, affecting 19 million patients worldwide each year and leading to a high mortality rate approximately ranging from 30% to 50% [1-3]. Host response to infection is the mainstay of the pathogenesis of sepsis, in which several critical processes have been identified, including the trigger of pro-inflammatory and anti-inflammatory factors induced by nuclear factor  $\kappa$ B and neutrophils and generation of inflammatory cytokines that result in microthrombi and immunosuppression [4, 5]. Management of sepsis patients requires timely interventions, which consist of rapid fluid resuscitation, source removal, antibiotic treatment, fluid resuscitation, and vasoactive drugs [4]. However, although mortality rate has declined, the high in-hospital incidence, high morbidity rate and many inefficient withdrawn novel drugs, for example the recombinant human activated protein C (drotrecogin

alfa), still makes sepsis a huge problem in emergency medicine [5-7].

The plasminogen activator (PA) system, an essential system in cell differentiation, migration and reconstruction, mainly contains urokinase-type plasminogen activator (uPA), uPA receptor (uPAR), tissue type plasminogen activator (tPA), plasminogen activator inhibitor-1 (PAI-1), and PAI-2. uPA commonly is expressed in neutrophils, monocytes, macrophages, and activated T cells, and is reported to be capable of regulating inflammation, immune responses, and human endothelial cell functions in cancers and several inflammatory diseases [8-10]. The therapeutic effect of uPA has been demonstrated in several diseases, such as ischemic brain injury and central nervous system (CNS) axon injury [11, 12]. Moreover, studies also report that uPA is beneficial in inflammation by activating T cells and may act as an antibiotic agent in mice model infected with *staphylococcus aureus* [13, 14]. However, the therapeutic role of uPA in sepsis remains largely unknown.

## uPA in septic HUVECs

**Table 1.** Antibodies for western blot

Antibody	Company (country)	Dilution
Primary Antibody		
Rabbit polyclonal to GM-CSF	Abcam (USA)	1:1000
Rabbit polyclonal to TNF alpha	Abcam (USA)	1:1000
Rabbit polyclonal to IL6	Abcam (USA)	1:1000
Rabbit polyclonal to uPA Receptor	Abcam (USA)	1:1000
Rabbit monoclonal [E17] to Bcl-2	Abcam (USA)	1:1000
Rabbit monoclonal [EPR16891] to GAPDH	Abcam (USA)	1:1000
Secondary Antibody		
Goat Anti-Rabbit IgG H&L (HRP)	Abcam (USA)	1:2000

**Table 2.** Primers for qPCR

Gene	Forward Primer	Reverse Primer
IL-6	5' CTTCCGGTCCAGTTGCCTTCTC 3'	5' AGGTGAGTGGCTGTCTGTGT 3'
TNFA	5' TGTTCTCTCAGCCTCTTCTCCTT 3'	5' CTCTCAGCTCCACGCCATTG 3'
uPA	5' GTCGCTCAAGGCTTAACCTCAA 3'	5' TCAGCAAGGCAATGTCGTTGT 3'
uPAR	5' GCTATCGGACTGGCTTGAAGA 3'	5' GTGGAAGGTGTCGTTGTTGTG 3'
GMCSF	5' GCGTCTCCTGAACCTGAGTAGA 3'	5' GTGCTGCTTGTAGTGGCTGG 3'
GAPDH	5' GAGTCCACTGGCGTCTTCCAC 3'	5' ATCTTGAGGCTGTTGCATACTTCT 3'

Thus, the aim of this study was to investigate the effect of uPA on cell viability, apoptosis, and inflammatory cytokine levels in septic human umbilical vein endothelial cells (HUVECs).

### Materials and methods

#### Cell culture

Human umbilical vein endothelial cells (HUVECs) were purchased from American Type Culture Collection (ATCC, USA) and cultured in 90% DMEM-F12 (Gibco, USA) medium complemented with 10% fetal bovine serum (FBS) (Gibco, USA), then 5 units/ml heparin (Sigma, USA), 30 µg/ml endothelial cell growth factor (Sigma, USA) and 100 units/ml penicillin-streptomycin (Gibco, USA) were added in the medium as well. Cells were incubated under 95% air and 5% CO<sub>2</sub> at 37°C.

#### Determination of the effect of uPA on septic HUVECs cells viability, apoptosis and inflammation

HUVECs were cultured in normal medium, medium with 200 ng/ml lipopolysaccharides (LPS) (Sigma-Aldrich, USA) and medium with 200 ng/ml LPS as well as 10 µg/ml recombinant human uPA (Sigma-Aldrich, USA) for 24 h,

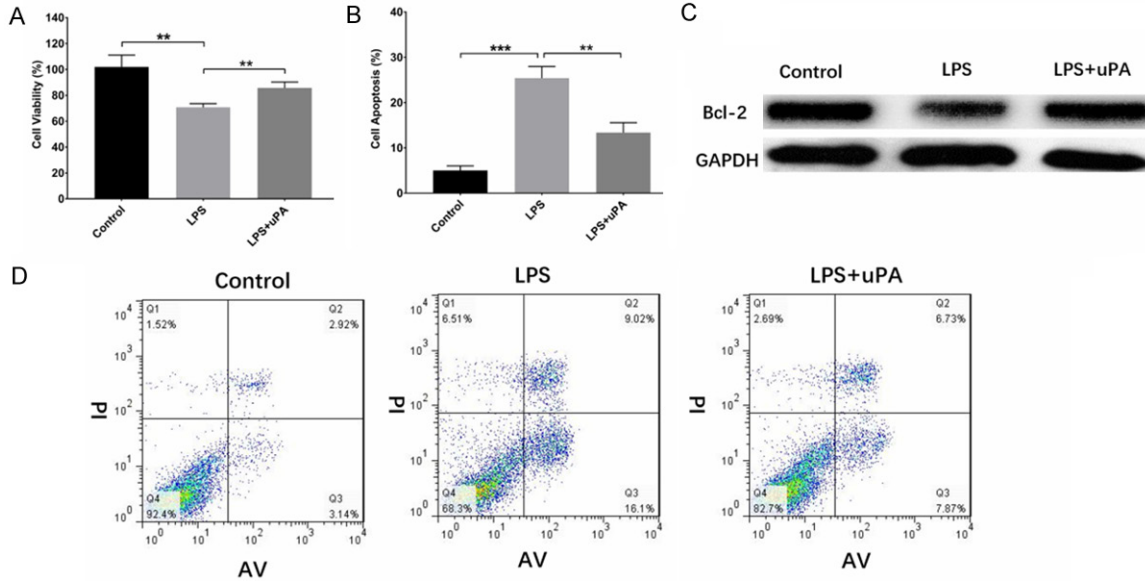
and accordingly divided into 3 groups: Control group, LPS group and LPS+uPA group. Subsequently, cell viability was measured by Cell Counting Kit-8 (CCK-8) (Medchemexpress, USA), apoptosis rate was measured by Annexin V/Propidium Iodide (AV/PI) (Thermo, USA), and anti-apoptosis marker Bcl-2 expression was measured by western blot. Inflammatory markers (TNF-α and IL-6) expression in cells were measured by quantitative polymerase chain reaction (qPCR) and western blot while their expressions in cell supernatant fluid were measured by enzyme-

linked immunosorbent assay (ELISA) (eBioscience, USA). In addition, granulocyte-macrophage colony stimulating factor (GMCSF) and uPAR expression in cells were measured by qPCR and western blot.

#### Determination of the effect of ShuPA on septic HUVEC functions

Blank shRNA and uPA shRNA plasmids were constructed using pGPH1 (Shanghai GenePharma Company, China), and then transferred into HUVECs with or without LPS using HilyMax (Dojindo, Japan) and cells were categorized into 4 groups: NC group (blank shRNA), ShuPA group (uPA shRNA), NC+LPS group (blank shRNA+200 ng/ml LPS) and ShuPA+LPS group (uPA shRNA+200 ng/ml LPS). Cells were cultured for 24h after transfection. The following assays were conducted: uPA mRNA expression was measured by qPCR, cell viability was measured by CCK8 (Medchemexpress, USA), apoptosis rate was measured by AV/PI (Thermo, USA) and anti-apoptosis marker Bcl-2 expression was measured by western blot. TNF-α and IL-6 expression in cells were measured by qPCR and western blot while their expression in cells supernatant were measured by ELISA (eBioscience, USA). GMCSF and uPAR expression in cells were measured by qPCR and western blot.

## uPA in septic HUVECs



**Figure 1.** uPA promoted cell viability and inhibited apoptosis in septic HUVECs. Viability was decreased in LPS group compared to controls while it was enhanced in the LPS+uPA group compared with LPS group (A). Apoptosis was elevated in LPS group compared with controls but was decreased in LPS+uPA group compared to LPS group (B, D). The anti-apoptosis protein Bcl-2 expression showed the same trend (C). Comparison between two groups was determined by t test.  $P < 0.05$  was considered significant.  $**P < 0.01$ ,  $***P < 0.001$ . uPA, urokinase-type plasminogen activator; HUVECs, human umbilical vein endothelial cells; LPS, lipopolysaccharides.

### Western blot

After extraction of total protein, a BCA kit (Pierce Biotechnology, USA) was used to assess the total protein concentration by making a standard curve. Then electrophoresis was conducted in the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) for the isolation of total protein, after which the sample was transferred to polyvinylidene fluoride (PVDF) membrane (Millipore, Bedford, USA). Subsequently, the PVDF membrane was blocked and then primary antibody was added, which was incubated under  $4^{\circ}\text{C}$  overnight and then washed by wash buffer, subsequently the secondary antibody was added, which was incubated under  $37^{\circ}\text{C}$  for 1 h. Afterward, the bands were visualized using enhanced chemiluminescence (ECL) kit (Millipore, USA). Antibodies used in western blot were listed in **Table 1**.

### qPCR

The cells were collected after being digested using 0.25% Trypsin (Gibco, USA), and then the total RNA was extracted by TRIzol Reagent

(Invitrogen, USA). Subsequently, reverse transcription of RNA to cDNA was performed by using ReverTra Ace<sup>®</sup> qPCR RT Master Mix (Toyobo, Japan). Then the qPCR was conducted using the QuantiNova SYBR Green PCR Kit (Qiagen, Germany), and finally, setting GAPDH as an internal reference, the results were determined by the  $2^{-\Delta\Delta\text{Ct}}$  formula. Primers used in qPCR are listed in **Table 2**.

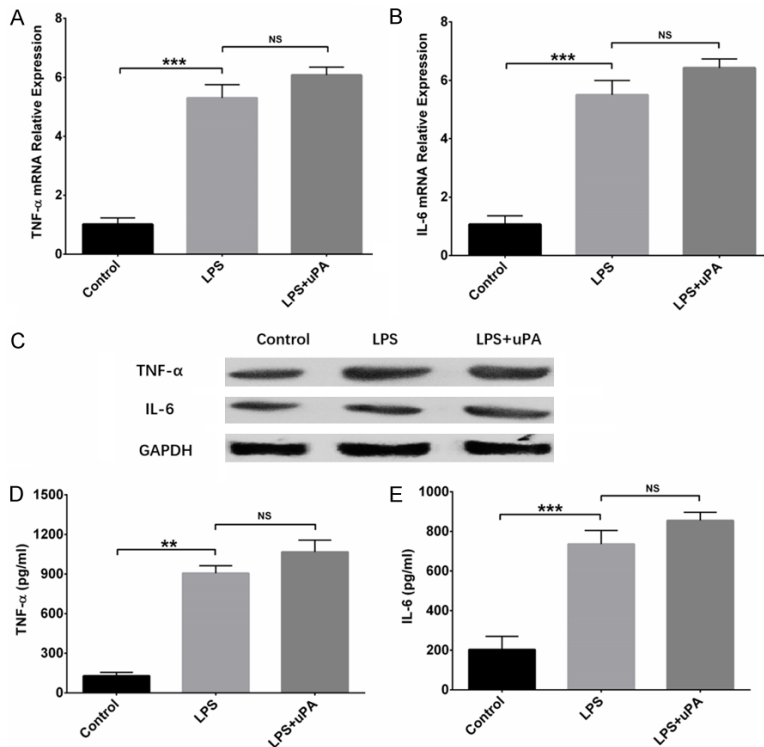
### ELISA

TNF- $\alpha$  and IL-6 expression in cell supernatant were measured using commercial ELISA kits (eBioscience, USA) according to the instructions of the manufacturer.

### CCK8

Each plate was incubated with 5%  $\text{CO}_2$  in  $37^{\circ}\text{C}$  for 2 h after being added with the  $10\ \mu\text{L}$  CCK-8 reagent (Medchemexpress, USA) and  $90\ \mu\text{L}$  RPMI 1640 medium. Afterward, a microplate reader (BioTek, USA) was used for assessing the optical density (OD) value to evaluate the cell viability capacity. Cell viability was calculated as OD value in experimental group/OD value in control (or NC) group  $\times 100\%$ .

## uPA in septic HUVECs



**Figure 2.** uPA had no impact on pro-inflammatory cytokine levels in septic HUVECs. TNF- $\alpha$  (A) and IL-6 (B) mRNA expressions in LPS group were both higher compared with controls and did not differ in the LPS+uPA group compared with the LPS group. TNF- $\alpha$  and IL-6 expressions in HUVECs (C) and TNF- $\alpha$  as well as IL-6 levels in cell supernatant fluid presented the same trends (D, E). Comparison between two groups was determined by t test.  $P < 0.05$  was considered significant. \* $P < 0.05$ , \*\*\* $P < 0.001$ . uPA, urokinase-type plasminogen activator; HUVECs, human umbilical vein endothelial cells; LPS, lipopolysaccharides; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; IL-6, interleukin-6.

was detected by t test.  $P < 0.05$  was considered as significant.

## Results

### *Effect of uPA on cell viability and apoptosis in septic HUVECs*

Cell viability was decreased in LPS group compared to the control group ( $P < 0.01$ ), and was increased in LPS+uPA group compared with the LPS group ( $P < 0.01$ ) (**Figure 1A**). As for apoptosis, it was elevated in LPS group compared with the control group ( $P < 0.001$ ) but was decreased in LPS+uPA group compared to the LPS group ( $P < 0.01$ ) (**Figure 1B, 1D**). The anti-apoptosis protein Bcl-2 expression was down-regulated in the LPS group compared to the control group, while it was up-regulated in LPS+uPA group compared to LPS group (**Figure 1C**). These results indicated that uPA played a protective role in septic HUVECs.

### *AV/PI*

First, the cells were digested using 0.25% trypsin (Gibco, USA) at 37°C and were subsequently washed by 1 mL precooled PBS twice, after which the cells were centrifuged at 1000 rpm for 5 mins. Second, the cells were re-suspended by 100  $\mu$ L binding buffer; later on, 5  $\mu$ L AV (Thermo, USA) and 10  $\mu$ L PI (Thermo, USA) were added and incubated for 15 mins, which were subsequently added in the flow cytometry tube with 385  $\mu$ L binding buffer, and then the flow cytometry assay was conducted.

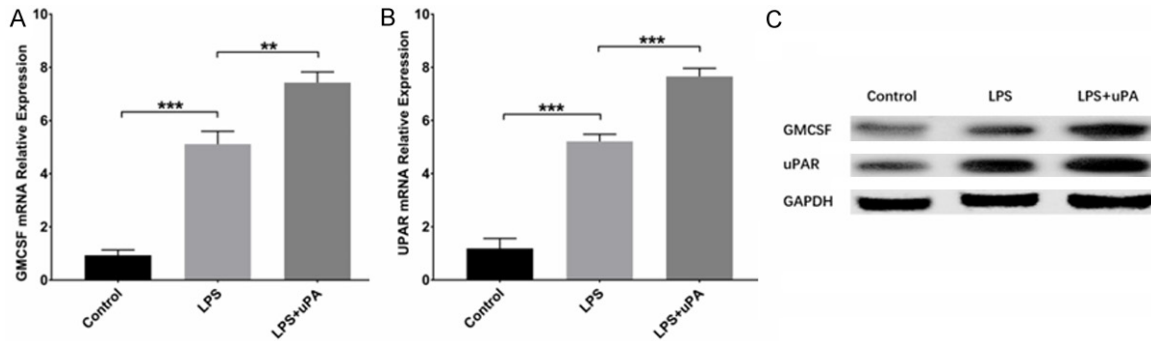
### *Statistics*

Statistical analysis was conducted using SPSS 22.0 Software (IBM, USA) and GraphPad Software 6.01 (GraphPad Software, USA). Data were presented as mean  $\pm$  standard error (SEM), and comparison between two groups

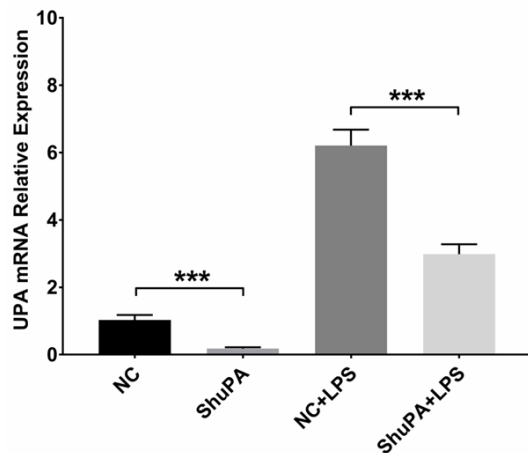
### *Effect of uPA on inflammatory cytokines levels in septic HUVECs*

The mRNA expression of TNF- $\alpha$  and IL-6 were both increased in the LPS group compared with controls (both  $P < 0.001$ ), while they were not different in LPS+uPA group compared with LPS group (both  $P > 0.05$ ) (**Figure 2A, 2B**). As to their protein expression, the TNF- $\alpha$  and IL-6 protein expression in HUVECs were up-regulated in LPS group compared to the control group, but they were similar between the LPS+uPA group and LPS group (**Figure 2C**). In addition, the TNF- $\alpha$  and IL-6 protein levels in cell supernatant fluid were elevated in the LPS group compared with controls (all  $P < 0.001$ ), while no difference was found between the LPS+uPA group and LPS group (all  $P > 0.05$ ) (**Figure 2D, 2E**). These results suggest that uPA has no effect in regulating inflammatory cytokine expression in septic HUVECs.

## uPA in septic HUVECs



**Figure 3.** uPA elevated GMCSF and uPAR levels in septic HUVECs. GMCSF (A) and uPAR (B) mRNA levels both were increased in LPS group compared to controls and were also elevated in LPS+uPA group compared with the LPS group. GMCSF and uPAR protein levels disclosed the same trends as their mRNA expression (C). Comparison between two groups was determined by t test.  $P < 0.05$  was considered significant. \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . uPA, urokinase-type plasminogen activator; GMCSF, granulocyte-macrophage colony stimulating factor; uPAR, uPA receptor; HUVECs, human umbilical vein endothelial cells; LPS, lipopolysaccharides.



**Figure 4.** uPA mRNA expression. uPA mRNA expression was down-regulated in the ShuPA group compared with NC group and was also decreased in ShuPA+LPS group versus the NC+LPS group. Comparison between two groups was determined by t test.  $P < 0.05$  was considered significant. \*\*\* $P < 0.001$ . uPA, urokinase-type plasminogen activator; LPS, lipopolysaccharides.

### Effect of uPA on GMCSF and uPAR expression in septic HUVECs

Subsequently, we evaluated the expressions of GMCSF and uPAR in septic HUVECs using western blot and qPCR, which showed that the GMCSF mRNA level was up-regulated in LPS group compared to controls ( $P < 0.001$ ), and it was also elevated in LPS+uPA group compared to the LPS group ( $P < 0.01$ ) (Figure 3A). Similarly, the uPAR mRNA expression was higher in LPS group compared with controls ( $P < 0.001$ ) and

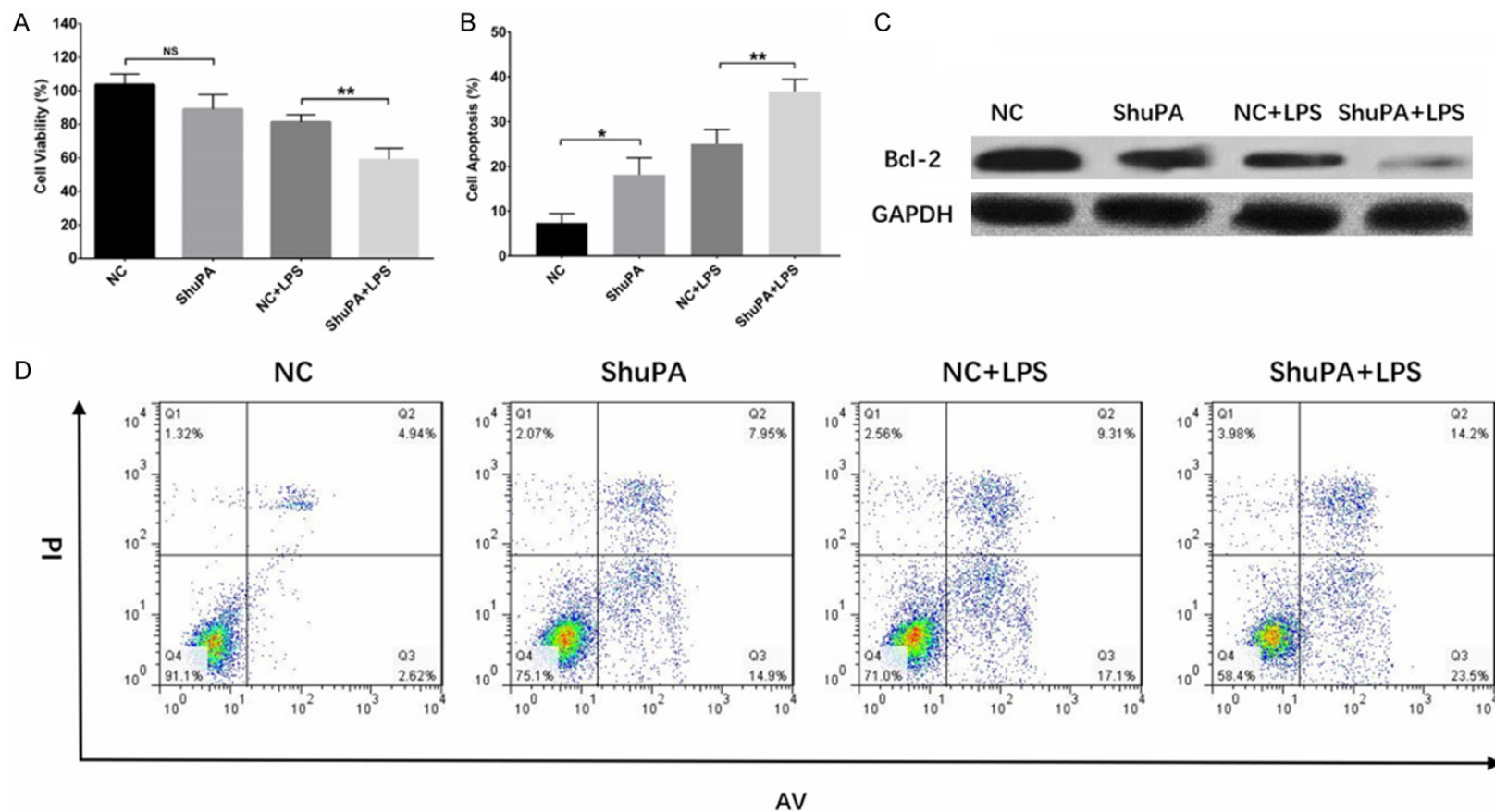
was increased in the LPS+uPA group compared to the LPS group ( $P < 0.001$ ) (Figure 3B). The GMCSF and uPAR protein levels were both elevated in the LPS group compared with controls, and they were higher in the LPS+uPA group than the LPS group (Figure 3C). Our results indicated that uPA may regulate GMCSF and uPAR expression in septic HUVECs.

### Effect of ShuPA on cell viability and apoptosis in septic HUVECs

Since we found uPA had therapeutic effects on septic HUVECs, we further investigated the effect of uPA shRNA on proliferation, apoptosis, and inflammatory cytokines in HUVECs. uPA mRNA expression was down-regulated in ShuPA group compared to the NC group ( $P < 0.001$ ) and was also decreased in Shu+LPS group compared with NC+LPS group ( $P < 0.001$ ), suggesting that the transfections were successful (Figure 4). After transfections, cell viabilities in ShuPA group and NC group were similar ( $P > 0.05$ ), but the cell viability was decreased in ShuPA+LPS group compared to in NC+LPS group ( $P < 0.01$ ) (Figure 5A). Cell apoptosis was increased in ShuPA group compared to the NC group ( $P < 0.05$ ) and was elevated in ShuPA+LPS group compared with the NC+LPS group as well ( $P < 0.01$ ) (Figure 5B, 5D). The level of anti-apoptosis protein Bcl-2 was decreased in the ShuPA group compared with NC group, and it was also reduced in ShuPA+LPS group compared with NC+LPS group (Figure 5C), suggesting that inhibition of uPA had an exacerbating effect in septic HUVECs.

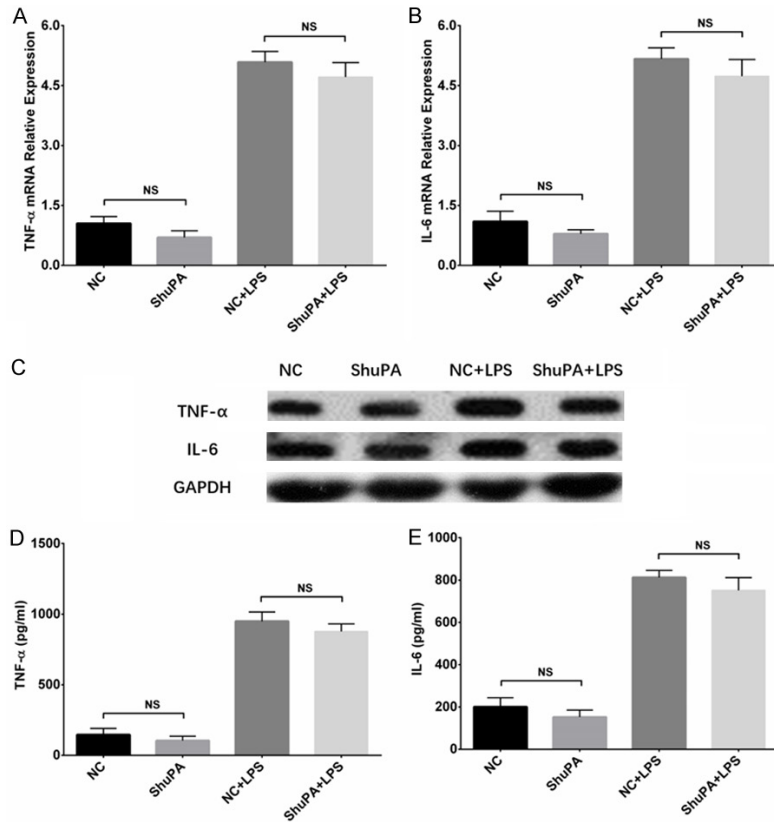


### uPA in septic HUVECs



**Figure 5.** ShuPA decreased cell viability and up-regulated apoptosis in septic HUVECs. Viability in ShuPA group was similar to that in the NC group while it was decreased in ShuPA+LPS group versus the NC+LPS group (A). Apoptosis was higher in the ShuPA group compared with the NC group and was elevated in the ShuPA+LPS group compared with the NC+LPS group (B, D). The level of anti-apoptosis protein Bcl-2 displayed the same trend (C). Comparison between two groups was determined by t test. P<0.05 was considered significant. \*P<0.05, \*\*P<0.01; NS, not significant. HUVECs, human umbilical vein endothelial cells; LPS, lipopolysaccharides.

## uPA in septic HUVECs



**Figure 6.** ShuPA down-regulated pro-inflammatory cytokine levels in septic HUVECs. The TNF- $\alpha$  mRNA level (A), IL-6 mRNA level (B), TNF- $\alpha$  protein level in HUVECs (C), IL-6 protein level in HUVECs, TNF- $\alpha$  protein level in cells supernatant fluid (D) or IL-6 protein expression in cells supernatant fluid (E) did not vary between the NC group and ShuPA group, nor between the NC+LPS group and ShuPA+LPS group. Comparison between two groups was determined by t test.  $P < 0.05$  was considered significant. \* $P < 0.05$ , \*\* $P < 0.01$ ; NS, not significant. HUVECs, human umbilical vein endothelial cells; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; LPS, lipopolysaccharides; IL-6, interleukin-6.

### Effect of ShuPA on inflammatory cytokine expressions in septic HUVECs

After transfections, the level of TNF- $\alpha$  mRNA in the ShuPA group was similar to that in NC group ( $P > 0.05$ ) and was also similar in the ShuPA+LPS group compared with NC+LPS group ( $P > 0.05$ ) (Figure 6A). Additionally, the IL-6 mRNA expressions between ShuPA group and NC group, and between ShuPA+LPS group and NC+LPS group were similar (all  $P > 0.05$ ) (Figure 6B). The TNF- $\alpha$  or IL-6 protein expression in HUVECs in ShuPA group was similar to that in NC group and was also not different in ShuPA+LPS group compared with NC+LPS group (Figure 6C). The TNF- $\alpha$  and IL-6 protein in the supernatant fluid were similar between the ShuPA group and the NC group (all  $P > 0.05$ ), and between the

ShuPA+LPS group and the NC+LPS group (all  $P > 0.05$ ) (Figure 6D, 6E). This indicated that uPA shRNA may have no effect on inflammatory cytokine levels in septic HUVECs.

### Effect of ShuPA on GMCSF and uPAR expression in septic HUVECs

GMCSF mRNA expression was lower in the ShuPA group compared with NC group ( $P < 0.05$ ) and was also decreased in ShuPA+LPS group compared to the NC+LPS group ( $P < 0.01$ ) (Figure 7A). As for the uPAR mRNA level, it was reduced in the ShuPA group compared to NC group ( $P < 0.05$ ) and was reduced in ShuPA+LPS group compared to the NC+LPS group as well ( $P < 0.01$ ) (Figure 7B). In addition, the GMCSF and uPAR protein expression in HUVECs cell supernatant fluid were lower in the ShuPA group compared to the NC group, and was also decreased in the ShuPA+LPS group versus the NC+LPS group (Figure 7C). These results suggest-

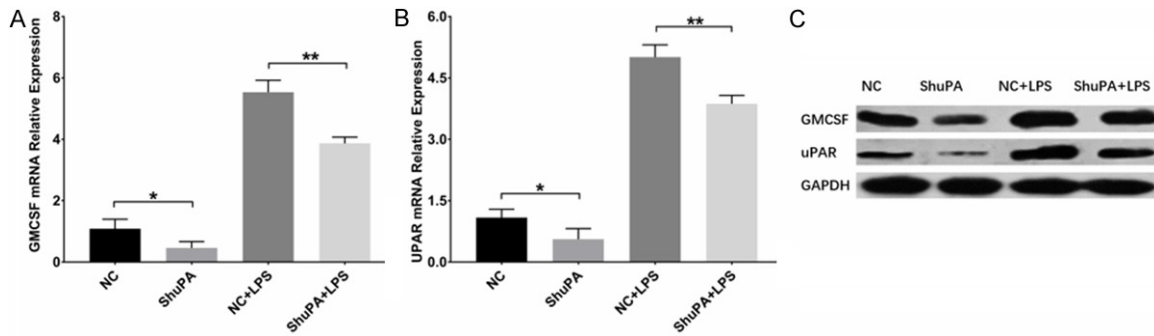
ed that uPA shRNA can modulate the levels of GMCSF and uPAR in HUVECs.

### Discussion

In this study, the results disclosed that: (1) uPA presented a therapeutic effect by enhancing cell viability and reducing apoptosis in septic HUVECs; (2) uPA had no effect on the expression of pro-inflammatory cytokines TNF- $\alpha$  and IL-6 in septic HUVECs; (3) uPA regulated GMCSF and uPAR expression in septic HUVECs.

It is reported that uPA has the ability to regulate cell functions in various diseases. For example, high molecular weight-uPA (HMW-uPA) and amino-terminal fragment (ATF) promotes breast cancer proliferation via the mitogen-activated protein kinase (MAPK) pathway [15]. The study

## uPA in septic HUVECs



**Figure 7.** ShuPA down-regulated GMCSF and uPAR levels in septic HUVECs. GMCSF (A) and uPAR (B) mRNA levels both decreased in the ShuPA group versus the NC group, and also decreased in the ShuPA+LPS group versus the NC+LPS group. GMCSF and uPAR protein expression presented with the same trends as their mRNA levels (C). Comparison between two groups was determined by t test.  $P < 0.05$  was considered significant. \* $P < 0.05$ , \*\* $P < 0.01$ . GMCSF, granulocyte-macrophage colony stimulating factor; HUVECs, human umbilical vein endothelial cells; LPS, lipopolysaccharides; uPAR, uPA receptor.

conducted by Cui et al reveals that uPA may enhance cells proliferation of outer root sheath keratinocytes [16]. There is also a study elucidating that Sphingosine kinase (SphK) 1 promotes cell proliferation and invasion by increasing the MMP-2/9 and uPA through MAPK pathway products in colon cancer cells, which suggests that the uPA might play a critical role in enhancing colon cancer proliferation [17]. uPA may also play a regulatory role in promoting cell proliferation in multiple diseases. Nonetheless, very limited studies report the impact of uPA in sepsis; for instance, an animal experiment discloses that plasmin, which is activated by uPA and uPAR, harms septic mice by enhancing inflammatory cytokine production, causing tissue damage, decreased neutrophil function, and impaired ability to kill bacteria [18]. However, the effect of uPA in mediating endothelial cells functions in sepsis has not been investigated. CoSince uPA has a critical role in mediating immunity, inflammation, and cell function in various diseases and has also been found to participate in pathology of sepsis, we deduced that uPA might impact the function of septic endothelial cells. Herein, we invested its effect on cell viability and apoptosis in septic HUVECs and found that uPA promoted proliferation and inhibited apoptosis, indicating that uPA may have a therapeutic effect on septic endothelial cells. This may result from: (1) uPA probably escalated cell proliferation and reduced apoptosis through the similar mechanisms of uPA in mediating cancer or normal cell functions via various pathways such as the MAPK pathway [15-17]; (2) uPA dis-

plays a capability of promoting angiogenesis by regulating proline-rich homeodomain (PRH) and vascular endothelial growth factor (VEGF) receptor, which is a mechanism that ameliorates sepsis [19].

Moreover, we also found that uPA had no impact on pro-inflammatory cytokine levels in septic HUVECs, which suggested that uPA might not be involved in the regulation of inflammatory cytokine expressions in septic cells. Nonetheless, the regulatory role of uPA in inflammation is controversial. A previous experiment elucidates that uPA promotes leukocyte infiltration in corneal inflammation by boosting inflammatory cytokine secretion from corneal fibroblasts [8]. Another study reveals that uPA interacts with uPAR to enhance the release of inflammatory cytokines, chemokines, and growth factors which subsequently promotes the development of rheumatoid arthritis, a disease closely related to inflammation [9]. An earlier *in vivo* and *in vitro* experiment reports that uPA stimulates the activation of neutrophils and promotes inflammation via regulating multiple pathways which consist of Akt and c-Jun N-terminal kinase, nuclear translocation of the transcriptional regulatory factor NF-kappa B, and pro-inflammatory cytokines levels: pathways that include IL-1 $\beta$ , macrophage-inflammatory protein-2, and TNF- $\alpha$  [20]. In contrast, a study showed that uPA has an anti-inflammatory effect. uPA ameliorated inflammatory osteoclastogenesis caused by LPS by modulating the plasmin/PAR-1/Ca(2+)/CaMKK/AMPK axis in mice models with bone destruction [21].



Therefore, the conflicting roles of uPA in mediating inflammation found by previous studies may explain our results [8, 9, 20-22].

In addition, we discovered that uPA could modulate GMCSF and uPAR expression in septic HUVECs. GMCSF, a cytokine closely related to immunity and inflammation, is a well-known immunostimulant used in sepsis, which is capable of enhancing the production and antibacterial function of immune cells, such as neutrophils and monocytes [23]. Therefore, GMCSF could be regulated by uPA in septic HUVECs due to its critical role in immune and inflammatory responses. As for the regulatory effect of uPA on uPAR expression in septic HUVECs, it may arise from uPAR being the receptor of uPA, so that an increase of uPA elevated uPAR production in septic HUVECs, and vice versa.

In conclusion, uPA promotes cell viability, represses apoptosis and has no effect on inflammatory cytokines in septic HUVECs.

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#### Disclosure of conflict of interest

None.

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#### References

- [1] Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche JD, Coopersmith CM, Hotchkiss RS, Levy MM, Marshall JC, Martin GS, Opal SM, Rubenfeld GD, van der Poll T, Vincent JL and Angus DC. The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA* 2016; 315: 801-810.
- [2] Fleischmann C, Scherag A, Adhikari NK, Hartog CS, Tsaganos T, Schlattmann P, Angus DC, Reinhart K; International Forum of Acute Care T. Assessment of global incidence and mortality of hospital-treated sepsis. current estimates and limitations. *Am J Respir Crit Care Med* 2016; 193: 259-272.
- [3] Martin GS, Mannino DM, Eaton S and Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med* 2003; 348: 1546-1554.
- [4] Cecconi M, Evans L, Levy M and Rhodes A. Sepsis and septic shock. *Lancet* 2018; 392: 75-87.
- [5] Hotchkiss RS and Karl IE. The pathophysiology and treatment of sepsis. *N Engl J Med* 2003; 348: 138-150.
- [6] Kaukonen KM, Bailey M, Suzuki S, Pilcher D and Bellomo R. Mortality related to severe sepsis and septic shock among critically ill patients in Australia and New Zealand, 2000-2012. *JAMA* 2014; 311: 1308-1316.
- [7] Angus DC. The search for effective therapy for sepsis: back to the drawing board? *JAMA* 2011; 306: 2614-2615.
- [8] Sugioka K, Mishima H, Kodama A, Itahashi M, Fukuda M and Shimomura Y. Regulatory mechanism of collagen degradation by keratocytes and corneal inflammation: the role of urokinase-type plasminogen activator. *Cornea* 2016; 35 Suppl 1: S59-S64.
- [9] Dinesh P and Rasool M. uPA/uPAR signaling in rheumatoid arthritis: shedding light on its mechanism of action. *Pharmacol Res* 2018; 134: 31-39.
- [10] Lian S, Xia Y, Nguyen TT, Ung TT, Yoon HJ, Kim NH, Kim KK and Jung YD. Docosahexaenoic acid inhibits tumor promoter-induced urokinase-type plasminogen activator receptor by suppressing PKCdelta- and MAPKs-mediated pathways in ECV304 human endothelial cells. *PLoS One* 2016; 11: e0163395.
- [11] Yepes M. Urokinase-type plasminogen activator promotes synaptic recovery in the ischemic brain. *J Transl Neurosci* 2018; 3.
- [12] Merino P, Diaz A, Jeanneret V, Wu F, Torre E, Cheng L and Yepes M. Urokinase-type plasminogen activator (uPA) binding to the uPA receptor (uPAR) promotes axonal regeneration in the central nervous system. *J Biol Chem* 2017; 292: 2741-2753.
- [13] Jin T, Bokarewa M and Tarkowski A. Urokinase-type plasminogen activator, an endogenous antibiotic. *J Infect Dis* 2005; 192: 429-437.
- [14] Mondino A and Blasi F. uPA and uPAR in fibrinolysis, immunity and pathology. *Trends Immunol* 2004; 25: 450-455.
- [15] Gandhari M, Arens N, Majety M, Dorn-Beineke A and Hildenbrand R. Urokinase-type plasminogen activator induces proliferation in breast cancer cells. *Int J Oncol* 2006; 28: 1463-1470.
- [16] Cui ZH, Yang T, Gao QG and Wang Y. Urokinase plasminogen activator (uPA) is a positive regulator of outer root sheath keratinocyte proliferation. *Cell Biol Int* 2004; 28: 571-575.

## uPA in septic HUVECs

- [17] Liu SQ, Huang JA, Qin MB, Su YJ, Lai MY, Jiang HX and Tang GD. Sphingosine kinase 1 enhances colon cancer cell proliferation and invasion by upregulating the production of MMP-2/9 and uPA via MAPK pathways. *Int J Colorectal Dis* 2012; 27: 1569-1578.
- [18] Guo Y, Li J, Hagstrom E and Ny T. Beneficial and detrimental effects of plasmin(ogen) during infection and sepsis in mice. *PLoS One* 2011; 6: e24774.
- [19] Stepanova V, Jayaraman PS, Zaitsev SV, Lebedeva T, Bdeir K, Kershaw R, Holman KR, Parfyonova YV, Semina EV, Beloglazova IB, Tkachuk VA and Cines DB. Urokinase-type plasminogen activator (uPA) promotes angiogenesis by attenuating proline-rich homeodomain protein (PRH) transcription factor activity and de-repressing vascular endothelial growth factor (VEGF) receptor expression. *J Biol Chem* 2016; 291: 15029-15045.
- [20] Abraham E, Gyetko MR, Kuhn K, Arcaroli J, Strassheim D, Park JS, Shetty S and Idell S. Urokinase-type plasminogen activator potentiates lipopolysaccharide-induced neutrophil activation. *J Immunol* 2003; 170: 5644-5651.
- [21] Kanno Y, Ishisaki A, Kawashita E, Kuretake H, Ikeda K and Matsuo O. uPA Attenuated LPS-induced Inflammatory Osteoclastogenesis through the Plasmin/PAR-1/Ca(2+)/CaMKK/AMPK Axis. *Int J Biol Sci* 2016; 12: 63-71.
- [22] Kanno Y, Maruyama C, Matsuda A and Ishisaki A. uPA-derived peptide, A6 is involved in the suppression of lipopolysaccharide-promoted inflammatory osteoclastogenesis and the resultant bone loss. *Immun Inflamm Dis* 2017; 5: 289-299.
- [23] Hamilton JA. Colony-stimulating factors in inflammation and autoimmunity. *Nat Rev Immunol* 2008; 8: 533-544.