HSPB8 attenuates lipopolysaccharide-mediated acute lung injury in A549 cells by activating mitophagy

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Abstract. Sepsis is a life-threatening multiple organ failure disease caused by an uncontrolled inflammatory response and can progress to acute lung injury (ALI). Heat-shock protein B8 (HSPB8) serves a cytoprotective role in multiple types of diseases; however, to the best of our knowledge, the regulatory role of HSPB8 in sepsis-induced ALI remains unclear. A549 human alveolar type II epithelial cells were treated with lipopolysaccharide (LPS) for 24 h to simulate a sepsis-induced ALI model. Cell transfection was performed to overexpress HSPB8, and cells were treated with mitochondrial division inhibitor-1 (Mdivi-1) for 2 h before LPS induction to assess the underlying mechanism. Protein expression was evaluated using western blotting and an immunofluorescence assay. Cytokines were examined using ELISA assay kits and antioxidant enzymes were examined using their detection kits. Cell apoptosis was detected using flow cytometry. The mitochondrial membrane potential was detected by JC-1 staining. HSPB8 was upregulated in A549 cells treated with LPS and HSPB8 overexpression attenuated LPS-induced inflammatory cytokine levels, oxidative stress and apoptosis in A549 cells. LPS inhibited mitophagy and reduced the mitochondrial membrane potential in A549 cells, which was partly inhibited by HSPB8 overexpression. Furthermore, Mdivi-1 decreased the inhibitory effect of HSPB8 on the inflammatory response, oxidative stress and apoptosis in LPS-treated A549 cells. In conclusion, HSPB8 overexpression attenuated the LPS-mediated inflammatory response, oxidative stress and apoptosis in A549 cells by promoting mitophagy, indicating HSPB8 as a potential therapeutic target in sepsis-induced ALI.

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Introduction

Sepsis is a life-threatening multiple organ failure caused by an uncontrolled inflammatory response in response to infection and has become a leading cause of mortality in intensive care units (1,2). The lungs are the most vulnerable organs in patients with sepsis, with >50% of these patients developing acute lung injury (ALI) (3,4). ALI often progresses into its severe form acute respiratory distress (ARDS), which is associated with a high mortality rate globally (5). Epidemiological data demonstrate that >210,000 individuals in the US are diagnosed with sepsis every year and the fatality rate of severe ARDS [the ratio of the partial pressure of oxygen in the blood to the fraction of inspired oxygen delivered (FIO_2) <100] approaches 40% in 50 countries in 2014 (6). Although current medical technologies and pharmacological approaches including corticosteroids, omega fatty acids, statins, have achieved notable improvements, effective therapeutic drugs that shorten the duration of ventilation and improve the mortality rate are limited (7). Therefore, it is necessary to fully elucidate the molecular mechanism associated with initiation and development of sepsis-induced ALI to identify effective intervention targets.

Heat-shock proteins (HSPs) are a family of structurally conserved proteins expressed at high levels in various tissues, such as heart, muscle and brain, that protect the cell from numerous stressors and stimuli (8). HSPs prevent and reduce apoptosis, oxidative stress and human inflammatory diseases (9,10). HSPB8, also known as HSP22, is a HSP expressed at high levels in the myocardium, endothelium and motoneurons, and has been shown to exert protective effects on sepsis-induced myocardial dysfunction, diabetes-induced endothelial injury, and motoneuron diseases (11-14). However, to the best of our knowledge, the regulatory role of HSPB8 in lung disease has been rarely investigated. In lung ischemia-reperfusion injury, HSPB8 was demonstrated to inhibit cell apoptosis and lipid peroxidation, indicating that increasing HSPB8 expression can protect lung cells from external damage (15). Notably, the levels of HSPB8 are elevated following sepsis, and the upregulation of HSPB8 improves sepsis-induced myocardial dysfunction and alleviates cognitive dysfunction in sepsis-associated encephalopathy, suggesting a role of HSPB8 in response to sepsis-associated diseases (13,16). To the best of our knowledge, however, the specific roles of HSPB8 in sepsis-induced ALI and whether HSPB8 overexpression can attenuate sepsis-induced ALI have not been reported.

In the present study, an *in vitro* sepsis-induced ALI model was developed using lipopolysaccharide (LPS)-induced A549 cells (17). To the best of our knowledge, the present study was the first to assess the regulatory role of HSPB8 in the LPS-induced inflammatory response, oxidative stress and apoptosis in A549 cells, and its mechanism of action. The present study aimed to provide a potential novel approach for therapeutic intervention in sepsis-induced ALI.

Materials and methods

Cell culture, treatment and transfection. A549 human alveolar type II epithelial cells were cultured in Ham's F-12K medium (both iCell Bioscience, Inc.) supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin/streptomycin in a 5/95% CO_2/air incubator at 37˚C. A549 cells were treated with 1 μg/ml LPS (MilliporeSigma) for 24 h at 37˚C to simulate sepsis-induced ALI. To assess the molecular mechanism associated with mitophagy, A549 cells were treated with 10 μM mitochondrial division inhibitor-1 (Mdivi-1; Abcam) (18,19) for 2 h at 37˚C before LPS stimulation.

The full length of HSPB8 was cloned into the pcDNA3.1 plasmid to construct the HSPB8 overexpression vector (oe-HSPB8; Shanghai GenePharma Co., Ltd.). The empty pcDNA3.1 vector was used as a negative control (oe-NC; Shanghai GenePharma Co., Ltd.). A549 cells were transfected with 15 nM oe-NC or oe-HSPB8 using Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) at 37˚C for 6 h according to the manufacturer's instructions. At 48 h post-transfection, the transfection efficacy was determined by western blotting.

Western blotting. Total protein was isolated from cells using RIPA lysis buffer (Beyotime, Shanghai, China) containing protease inhibitor cocktail (Roche Applied Science). Total protein was quantified using a BCA Protein Assay kit (Pierce; Thermo Fisher Scientific, Inc.), and 30 μg/lane protein was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 12% gel and transferred onto a polyvinylidene fluoride membrane (MilliporeSigma). The membranes were blocked with 5% skimmed milk at room temperature for 2 h, and probed with primary antibodies against HSPB8 (1:2,000; cat. no. 15287-1-AP; Wuhan Sanying Biotechnology), Bcl-2 (1:1,500; cat. no. 26593-1-AP; Wuhan Sanying Biotechnology), Bax (1:8,000; cat. no. 50599-2-Ig; Wuhan Sanying Biotechnology), caspase 3 (1:800; cat. no. 19677-1-AP; Wuhan Sanying Biotechnology), Parkin (1:2,000; cat. no. 14060-1-AP; Wuhan Sanying Biotechnology), cytochrome c oxidase (COX) IV (1:10,000; cat. no. 11242-1-AP; Wuhan Sanying Biotechnology), PTEN-induced kinase 1 (PINK1; 1:600; cat. no. 23274-1-AP; Wuhan Sanying Biotechnology), LC3II/I (1:2,500; cat. no. 14600-1-AP; Wuhan Sanying Biotechnology), Beclin-1 (1:1,000; cat. no. 11306-1-AP; Wuhan Sanying Biotechnology), p62 (1:10,000; cat. no. 18420-1-AP; Wuhan Sanying Biotechnology) and β-actin (1:5,000; cat. no. 20536-1-AP; Wuhan Sanying Biotechnology) at 4˚C overnight. Subsequently, the membranes were incubated with HRP-conjugated secondary antibody (1:5,000; cat. no. SA00001-2; Wuhan Sanying Biotechnology) for 2 h at room temperature. The bands were visualized using an Amersham ECL Western Blotting Detection Kit (Amersham; Cytiva) on a Bio-Rad ChemiDoc XRS+ System (Bio-Rad Laboratories, Inc.) and quantified using ImageJ version 1.52 software (NIH, USA). COX IV was used to normalize protein expression of Parkin and β-actin was used as the loading control for other proteins.

ELISA. The levels of TNF-α, IL-1β and IL-6 in culture medium were measured using Human TNF-alpha Quantikine ELISA Kit (cat. no. STA00D), Human IL-1 beta/IL-1F2 Quantikine ELISA Kit (cat. no. SLB50) and Human IL-6 Quantikine ELISA Kit (cat. no. S6050B) according to the manufacturer's instructions (R&D Systems, Inc.), respectively. The absorbance was measured at 450 nm using a microplate reader (Bio-Rad Laboratories, Inc.).

Measurement of reactive oxygen species (ROS), malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT). To assess ROS production, A549 cells were incubated with 5 μM dichlorofluorescin-diacetate (MilliporeSigma) for 1 h at 37˚C in the dark. Images were captured under a fluorescence microscope (Olympus Corporation). Commercial kits from Nanjing Jiancheng Bioengineering Institute were used to measure MDA content (cat. no. A003-4-1), and the activities of SOD (cat. no. A001-3-2) and CAT (cat. no. A007-1-1) in the culture medium according to the manufacturer's instructions.

Flow cytometry. Cell apoptosis was examined using an Annexin V-FITC cell apoptosis kit (Nanjing KeyGen Biotech Co., Ltd.) according to the manufacturer's instructions. In brief, cells were washed with pre-chilled PBS and resuspended with 500 μl 1X binding buffer. Subsequently, 5 μl Annexin V-FITC and 10 μl PI were added to the suspension. After mixing for 10 min at room temperature in the dark, apoptotic cells (early + late apoptotic cells) were detected using a FACS Canto™ II flow cytometer (Becton, Dickinson and Company) and the data were analyzed using the Cell Quest software (version 5.1; BD Biosciences).

Immunofluorescence assay. A549 cells ($5x10^4$ cells/ml) were seeded on glass coverslips and stained with 100 μM MitoTracker Deep Red (Invitrogen; Thermo Fisher Scientific, Inc.) at 37˚C for 10 min to assess the mitochondrial morphology. A549 cells were washed with PBS, fixed with 4% paraformaldehyde at room temperature for 10 min, permeabilized with 0.2% Triton-X 100 for 5 min and blocked with 10% normal goat serum (Solarbio Life Sciences, Beijing, China) at 37˚C for 1 h. Cells were incubated with primary antibodies against Parkin (1:100; cat. no. 14060-1-AP; Wuhan Sanying Biotechnology) at 4˚C overnight, followed by incubation with CoraLite488-conjugated Goat Anti-Rabbit IgG secondary antibody (1:500; cat. no. SA00013-2; Wuhan Sanying Biotechnology) at room temperature for 1 h in the dark. The nuclei were counterstained with 4',6-diamidino-2-phenylindole at room temperature for 5 min. Images were captured under a fluorescence microscope (Olympus Corporation).

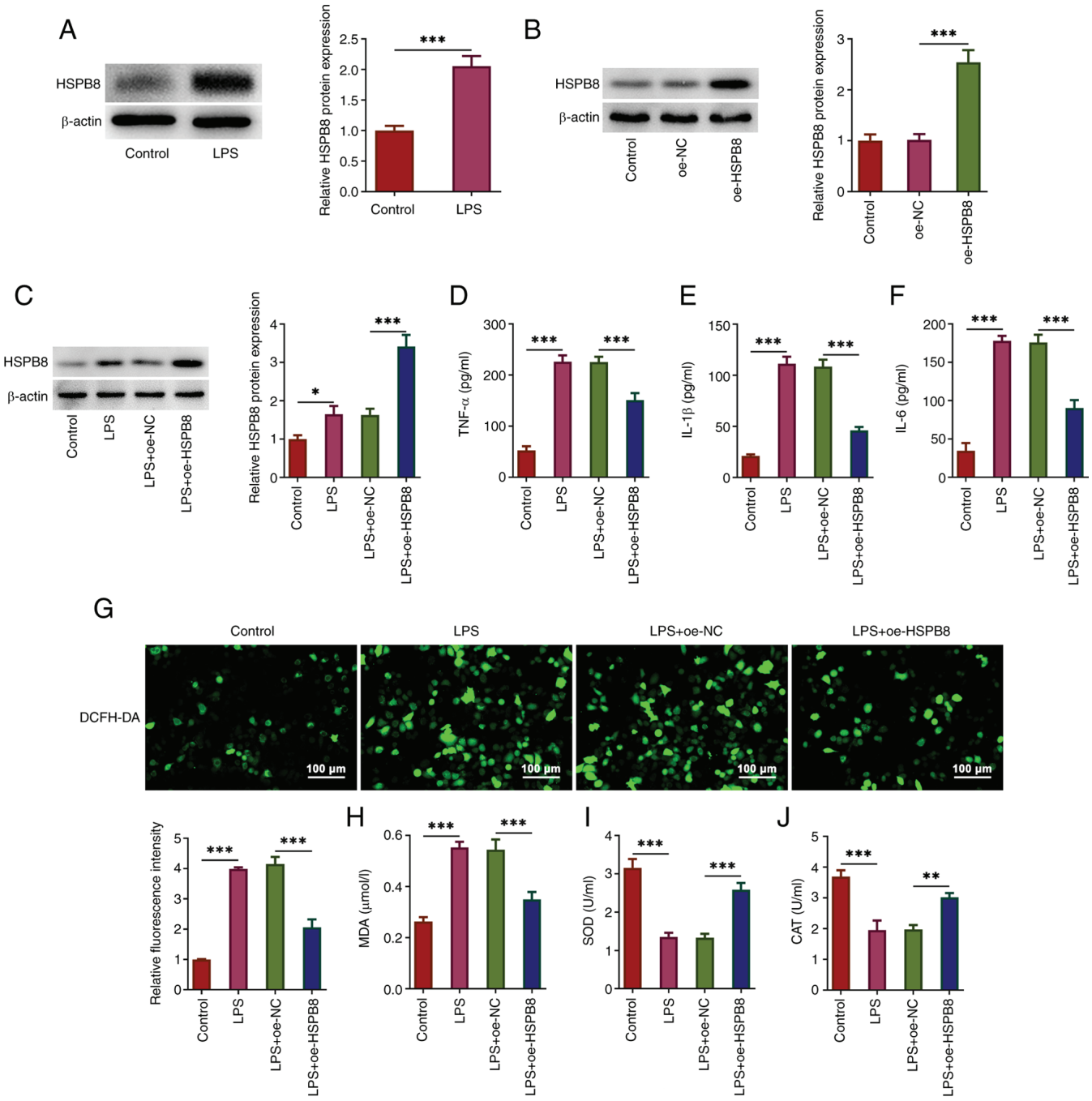

Figure 1. HSPB8 overexpression attenuates LPS-induced inflammatory cytokine levels and oxidative stress in A549 cells. (A) A549 cells were stimulated by LPS to simulate sepsis-induced acute lung injury. The protein expression levels of HSPB8 were assessed by western blotting. (B) A549 cells were transfected with oe-NC or oe-HSPB8, and the HSPB8 expression was assessed. (C) A549 cells and HSPB8-overexpressing A549 cells were treated with LPS. The protein expression levels of HSPB8 were assessed using western blotting. The concentration of (D) TNF-α, (E) IL-1β and (F) IL-6 in culture medium of A549 cells was detected using ELISAs. (G) Production of intracellular reactive oxygen species was detected by DCFH-DA staining. Scale bar, 100 μm. (H) MDA content, and (I) SOD and (J) CAT activity were examined using commercial kits. *P<0.05, **P<0.01 and ***P<0.001. CAT, catalase; DCFH-DA, 2'-7'-dichlorodihydrofluorescein diacetate; HSP, heat-shock protein; LPS, lipopolysaccharide; MDA malondialdehyde; NC, negative control; oe, overexpression; SOD, superoxide dismutase.

Measurement of the mitochondrial membrane potential. The mitochondrial membrane potential was detected by JC-1 staining (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. In brief, A549 cells were seeded into 6-well plates ($2x10^5$ cells/well) and treated with 1 μg/ml LPS at 37˚C for 24 h, then 10 mg/ml JC-1 staining solution was added to each well for 10 min at 3˚C in the dark. Images were captured under a fluorescence microscope (Olympus Corporation). Red fluorescence indicated normal mitochondrial membrane potential and green fluorescence indicated decreased mitochondrial membrane potential.

Statistical analysis. All data are presented as the mean ± standard deviation. All experiments were repeated at least three times. Data were analyzed using Student's unpaired t-test or one-way analysis of variance followed by Tukey's post hoc test. GraphPad

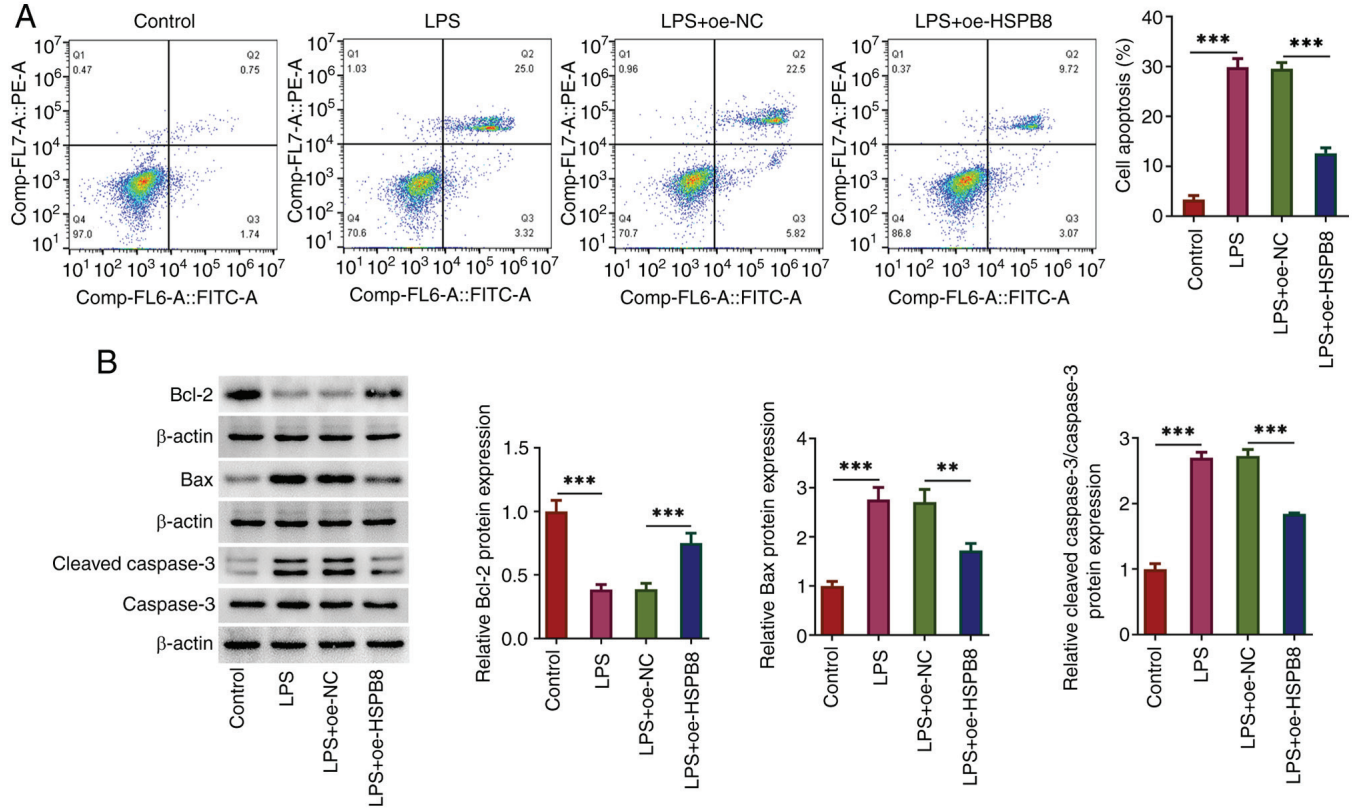

Figure 2. HSPB8 overexpression decreases LPS-induced apoptosis in A549 cells. (A) Flow cytometry was used to assess the cell apoptosis rate. (B) Expression levels of apoptosis-related proteins were evaluated using western blotting. **P<0.01 and ***P<0.001. HSP, heat-shock protein; LPS, lipopolysaccharide; NC, negative control; oe, overexpression.

Prism (version 8.0; Dotmatics) was used for data analysis. P<0.05 was considered to indicate a statistically significant difference.

Results

HSPB8 overexpression attenuates LPS-induced inflammatory cytokine production and oxidative stress in A549 cells. HSPB8 protein expression was significantly increased in A549 cells following LPS stimulation compared with that in the control group (Fig. 1A). To assess the role of HSPB8 overexpression in attenuating LPS-induced ALI *in vitro*, A549 cells were transfected with oe-HSPB8 to overexpress HSPB8 before LPS stimulation. Successful transfection was demonstrated by a significant increase in HSPB8 protein expression in the oe-HSPB8 group compared with the oe-NC group (Fig. 1B). The LPS group exhibited a significant increase in HSPB8 protein expression compared with the control group. HSPB8 protein expression in the LPS + oe-HSPB8 group was significantly increased compared with that in the LPS + oe-NC group (Fig. 1C).

ELISAs demonstrated that LPS treatment significantly increased the production of TNF-α, IL-1β and IL-6 in A549 cells compared with that in the control group. The LPS + oe-HSPB8 group exhibited significantly decreased TNF-α, IL-1β and IL-6 levels compared with the LPS + oe-NC group (Fig. 1D-F), suggesting that HSPB8 could alleviate LPS-induced inflammatory cytokine production in A549 cells. Furthermore, increased ROS fluorescence intensity was observed in the LPS group, with decreased fluorescence intensity in the LPS + oe-HSPB8 group (Fig. 1G). MDA content was significantly increased, and SOD and CAT activities were significantly decreased in the LPS group compared with the control group, indicating the occurrence of oxidative stress in LPS-induced A549 cells. However, this was mitigated by oe-HSPB8 transfection, with significantly decreased MDA levels, and significantly increased SOD and CAT activity compared with the LPS + oe-NC group (Fig. 1H-J). These results suggested that HSPB8 could partly decrease LPS-induced oxidative stress in A549 cells.

HSPB8 overexpression decreases LPS-induced apoptosis in A549 cells. The impact of HSPB8 on LPS-induced apoptosis in A549 cells was assessed. According to the results of the flow cytometry analysis, LPS significantly promoted cell apoptosis compared with the control group, which was partly blocked by HSPB8 overexpression compared with the LPS + oe-NC group (Fig. 2A). LPS significantly decreased the expression levels of the anti-apoptotic protein Bcl-2, and significantly increased the expression levels of the pro-apoptotic proteins Bax and cleaved-caspase 3 compared with those in the control group. These changes were significantly reversed by oe-HSPB8 transfection (Fig. 2B). Therefore, oe-HSPB8 transfection exerted anti-apoptotic activity in A549 cells exposed to LPS.

HSPB8 overexpression activates mitophagy in LPS-exposed A549 cells. The potential regulatory mechanism of HSPB8 was subsequently investigated. MitoTracker was used to indicate the location of mitochondria. Parkin fluorescence signal was markedly reduced following LPS treatment and partly restored by oe-HSPB8 transfection (Fig. 3A). Compared to the control group,

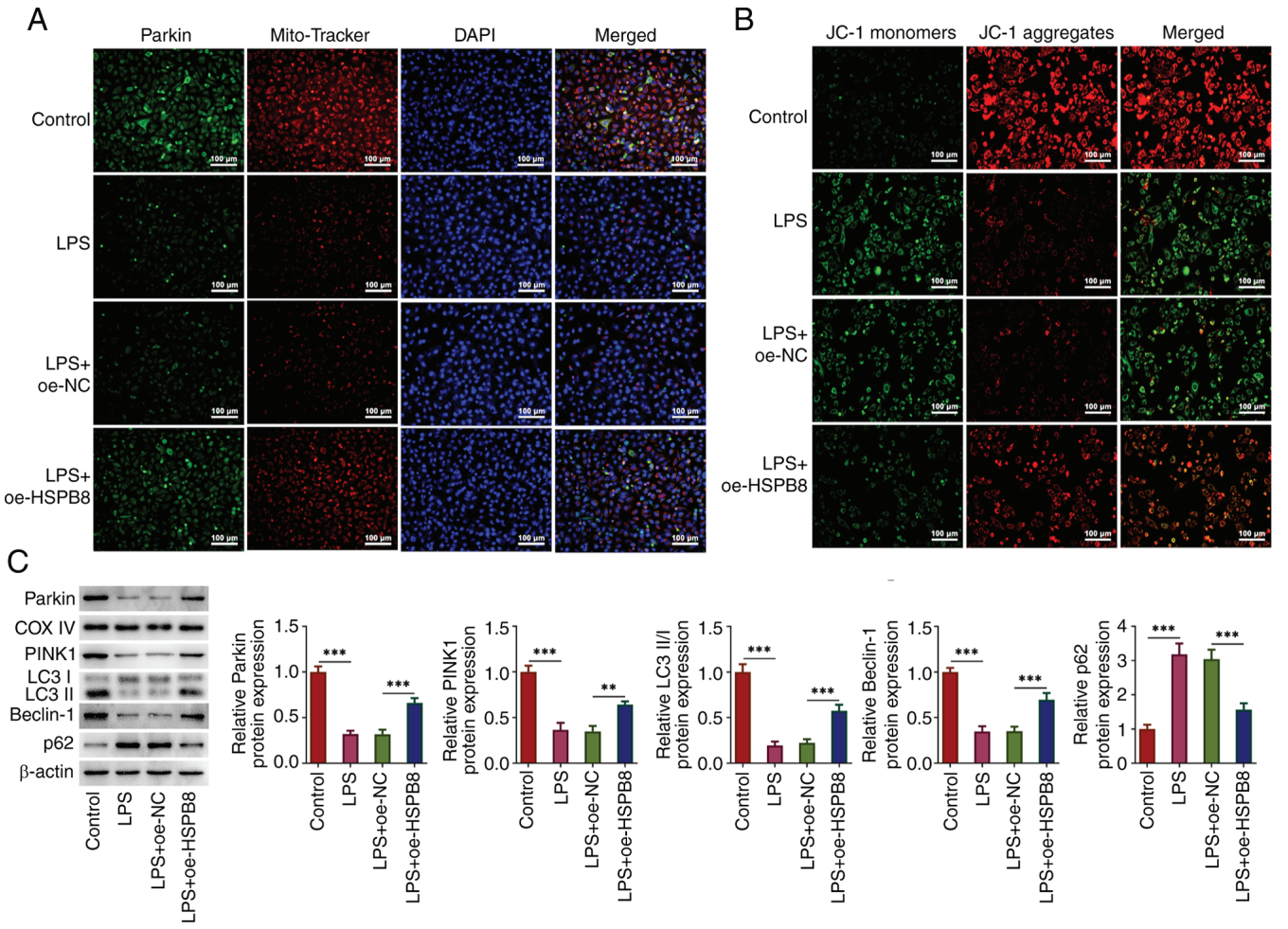

Figure 3. HSPB8 overexpression activates mitophagy in LPS-treated A549 cells. (A) Representative double staining with MitoTracker and Parkin. Scale bar, 100 μm. (B) Mitochondrial membrane potential was detected by JC-1 staining. Scale bar, 100 μm. (C) Expression levels of mitophagy-related proteins were examined using western blotting. **P<0.01 and ***P<0.001. HSP, heat-shock protein; LPS, lipopolysaccharide; NC, negative control; oe, overexpression; COX IV, cytochrome c oxidase; PINK1, PTEN-induced kinase 1.

the low red immunofluorescent intensity in LPS group revealed that LPS caused a reduction of the mitochondrial membrane potential, which was partly restored by oe-HSPB8 transfection as the red immunofluorescent intensity was enhanced compared with the LPS + oe-NC group (Fig. 3B). Furthermore, the protein levels of Parkin, PINK1, LC3II/I and Beclin-1 were significantly decreased, and the protein expression levels of p62 were significantly increased in the LPS group compared with the control group; however, this was mitigated by oe-HSPB8 transfection, with significantly decreased p62 expression, and significantly increased protein levels of Parkin, PINK1, LC3II/I and Beclin-1 compared with the LPS + oe-NC group (Fig. 3C). This suggested that mitophagy was inhibited in LPS-induced A549 cells, whereas oe-HSPB8 transfection activated mitophagy.

Mdivi-1 decreases the inhibitory effects of HSPB8 on the inflammatory response, oxidative stress and apoptosis in LPS-treated A549 cells. Finally, the role of mitophagy in the protective mechanism of HSPB8 against LPS-mediated cell injury was confirmed using the mitophagy inhibitor Mdivi-1. The inhibitory effects of oe-HSPB8 transfection on TNF-α, IL-1β and IL-6 in LPS-treated A549 cells were significantly inhibited by additional treatment with Mdivi-1, evidenced by elevated concentrations of TNF-α, IL-1β and IL-6 in the Mdivi-1 + LPS + oe-HSPB8 group compared with the LPS + oe-HSPB8 group (Fig. 4A-C).

MDA levels were significantly increased, and SOD and CAT activity was significantly decreased in the Mdivi-1 + LPS + oe-HSPB8 group compared with the LPS + oe-HSPB8 group (Fig. 4D-F). An increase in ROS fluorescence was observed in the Mdivi-1 + LPS + oe-HSPB8 group compared with the LPS + oe-HSPB8 group (Fig. 4G), suggesting that Mdivi-1 partly reversed the inhibitory effect of HSPB8 on oxidative stress in LPS-exposed A549 cells. Furthermore, the cell apoptosis rate in the Mdivi-1 + LPS + oe-HSPB8 group was significantly increased compared with that in the LPS + oe-HSPB8 group (Fig. 4H), accompanied by significantly increased Bax and cleaved-caspase 3 expression, and significantly decreased Bcl-2 protein expression in the Mdivi-1 + LPS + oe-HSPB8 group compared with the LPS + oe-HSPB8 group (Fig. 4I), demonstrating that Mdivi-1 decreased the anti-apoptotic effect of oe-HSPB8 transfection.

Discussion

Sepsis is a life-threatening condition with a complex pathological mechanism. The sepsis-mediated inflammatory

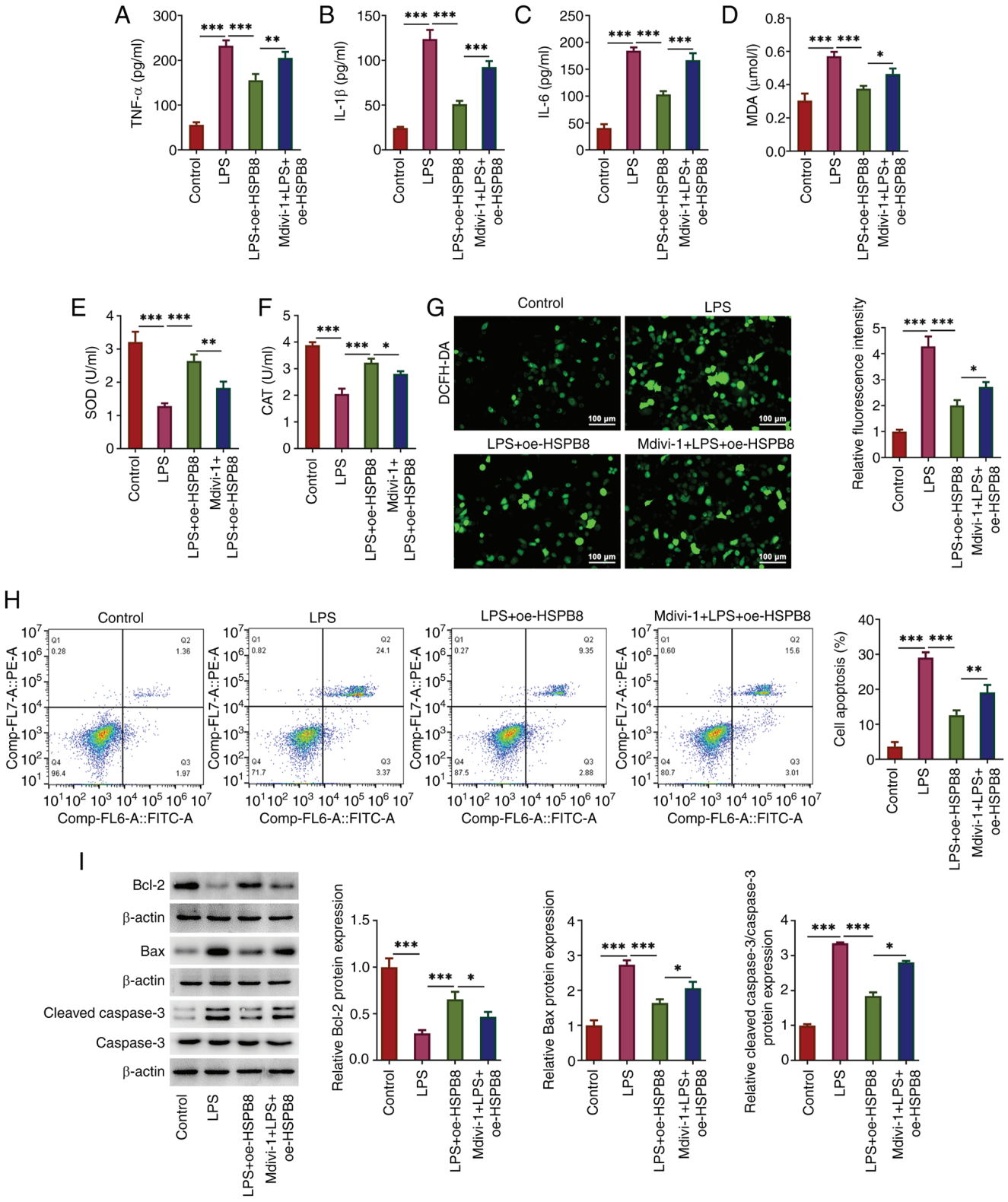

Figure 4. Mdivi-1 decreases the inhibitory effects of HSPB8 on the inflammatory response, oxidative stress and apoptosis in LPS-exposed A549 cells. The concentration of (A) TNF-α, (B) IL-1β and (C) IL-6 in the culture medium of A549 cells was detected using ELISAs. (D) MDA content, and (E) SOD and (F) CAT activities were measured using commercial kits. (G) Production of intracellular ROS was detected by DCFH-DA staining. Scale bar, 100 μm. (H) Flow cytometry was performed to investigate the cell apoptosis rate. (I) Expression levels of apoptosis-related proteins were assessed using western blotting. *P<0.05, **P<0.01 and ***P<0.001. CAT, catalase; DCFH-DA, 2'-7'-dichlorodihydrofluorescein diacetate; HSP, heat-shock protein; LPS, lipopolysaccharide; MDA malondialdehyde; Mdivi-1, mitochondrial division inhibitor-1; oe, overexpression; ROS, reactive oxygen species; SOD, superoxide dismutase.

response causes alveolar epithelial cell damage, epithelial barrier dysfunction and fluid extravasation into the alveolar space, ultimately leading to alveolar epithelial cell death and ALI progression (20). LPS is the primary component of the outer membrane of Gram-negative bacteria. As a primary pathogenic factors of sepsis, LPS could trigger the

inflammatory cascade, inducing necrosis and apoptosis of epithelial cells (21). Accordingly, LPS-induced A549 cells were used in the present study to establish an *in vitro* cellular model that simulates sepsis-induced ALI.

It is widely recognized that oxidative stress and inflammatory responses serve roles in facilitating sepsis-induced ALI, and decreasing excessive production of ROS and proinflammatory cytokines can attenuate pathological injury of lung tissues and improve the survival rate of mice receiving a lethal dose of LPS (22-24). Furthermore, as a major form of cell death, apoptosis is involved in the pathogenesis of sepsis-induced ALI (25). Decreasing cell apoptosis is an option to improve sepsis-associated pulmonary epithelial barrier dysfunction (26). At present, numerous potential targets have been demonstrated to exert protective effects against sepsis-stimulated ALI due to anti-inflammatory, anti-oxidation and anti-apoptotic properties. For example, topiroxostat has been reported to inhibit oxidative stress, inflammation and apoptosis, and decrease lung damage in a rat model of sepsis (27). Protocatechuic acid could effectively counteract sepsis-mediated lung injury by reducing the inflammatory response, oxidative stress and apoptotic events, with the potential to alleviate sepsis-induced ALI (28). HSPB8 serves a role in cytoprotection and resistance to oxidative stress and inflammation (13,14). Yu *et al* (14) reported that HSPB8 attenuated diabetes-induced endothelial injury by decreasing mitochondrial ROS generation. Yu *et al* (13) reported that HSPB8 alleviated LPS-induced myocardial injury by inhibiting inflammation, oxidative stress and apoptosis in cardiomyocytes. In agreement with the aforementioned studies, the present study demonstrated that LPS treatment increased HSPB8 expression in A549 cells. oe-HSPB8 transfection mitigated the LPS-induced inflammatory response, oxidative stress and apoptosis in A549 cells, suggesting that HSBP8 may attenuate sepsis-induced ALI. This is in line with a previous study, which has reported that increased HSPB8 expression can protect lung cells from external damage (15). A previous study reported that HSPB8 deficiency increased mitochondrial oxidative stress and mitochondrial damage, while HSPB8 overexpression inhibited mitochondrial oxidative stress and impairment in A549 cells (29), further confirming that HSPB8 exerts a cytoprotective function in A549 cells under both normal and inflammatory conditions.

Mitophagy removes damaged mitochondria via autophagy and serves a role in maintaining mitochondrial homeostasis and cell survival (30). Mitophagy is associated with sepsis-induced ALI (31-33). The PINK1/Parkin signaling is essential to maintain mitochondrial quality control through activating mitophagy. During this process, PINK1 can promote the translocation of Parkin from the cytoplasm to mitochondria for mitophagy activation (34). Hydrogen has been demonstrated to relieve sepsis-induced ALI by promoting PINK1/Parkin-mediated mitophagy (35). Kahweol (a natural diterpene extracted from coffee beans) treatment can alleviate oxidative stress and the inflammatory response in sepsis-induced ALI by increasing mitophagy and improving mitochondrial homeostasis (34). Previous studies have reported that HSPB8 was directly involved in mitophagy and attenuated myocardial ischemia-reperfusion injury through mitophagy (36,37). Consistently, in the present study, mitophagy was inhibited in LPS-treated A549 cells, demonstrated by decreased expression levels of Parkin, PINK1, Beclin-1 and LC3II/I, and increased p62 expression. oe-HSPB8 transfection increased mitophagy through enhancing Parkin, PINK1, Beclin-1 and LC3II/I, and reducing p62 expression and inhibited the inflammatory response, oxidative stress and apoptosis in LPS-induced A549 cells, suggesting that the protective role of HSPB8 against sepsis-induced ALI impacts mitophagy. The present study demonstrated that the inhibitory effects of oe-HSPB8 transfection on LPS-induced oxidative stress, the inflammatory response and apoptosis in LPS-induced A549 cells were mitigated by treatment with Mdivi-1, suggesting that HSPB8 alleviated sepsis-induced ALI by activating mitophagy.

The present study had a number of limitations. Firstly, the present study only assessed the protective mechanism of oe-HSPB8 transfection; in future, this should be confirmed by HSPB8 knockdown to achieve comprehensive understanding of the molecular function of HSPB8 in lung injury. Secondly, the present study only assessed the effect of oe-HSPB8 transfection on A549 cells under inflammatory conditions; the effect of oe-HSPB8 transfection under normal conditions should also be evaluated. Furthermore, *in vivo* studies are required to validate the present findings and develop drugs targeting HSPB8 for clinical treatment of sepsis-related ALI.

In summary, the present study reported the regulatory role of HSPB8 in sepsis-induced ALI. oe-HSPB8 transfection could attenuate the LPS-mediated inflammatory response, oxidative stress and apoptosis in A549 cells by promoting mitophagy. HSPB8 may serve as a potential therapeutic target in sepsis-induced ALI, and drugs targeting HSPB8 may be potential candidates for the clinical treatment of sepsis-associated ALI.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

XZ contributed to study conception and design. Data collection and analysis were performed by XZ, MW, MS and NY. The manuscript was drafted by MW and revised by XZ. XZ and MW confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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