



# HMGB1 was negatively regulated by HSF1 and mediated the TLR4/MyD88/NF- $\kappa$ B signal pathway in asthma

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## ABSTRACT

**Aims:** The present study explored the function and regulatory mechanism of High mobility group box 1 (HMGB1) in asthma.

**Main methods:** OVA (ovalbumin)-induced asthmatic mice model and LPS-treated cellular model were established in this study. Airway inflammation was measured through detecting the expression of IL-4, IL-5, IL-13 and Interferon- $\gamma$  (IFN- $\gamma$ ) in serum and BALF (bronchoalveolar lavage fluid) by ELISA kits. Bioinformatics predictive analysis, ChIP assays, Luciferase reporter assay and Western blotting were used to explore the relation between HMGB1 and HSF1 (Heat shock factor 1).

**Key findings:** HMGB1 expression was increased in OVA-induced asthmatic mice. Silencing HMGB1 attenuated the increasing of IgE, inflammatory factors (IL-4, IL-5 and IL-13), and airway hyperresponsiveness that induced by OVA. In addition, our study found that HSF1 directly bind with the HMGB1 promoter and negatively regulation of HMGB1. HSF-1 were upregulated in OVA-induced asthmatic mice, and knockdown of HSF1 aggravated the OVA-induced airway inflammation and airway hyperactivity in mice may through promoting the expression of HMGB1 and the activation of the Toll-like receptor 4 (TLR4)/Myeloid differentiation primary response 88 (MyD88)/Nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) signal pathway.

**Significance:** The expression of HMGB1 could be negatively regulated by HSF1, and the TLR4/MyD88/NF- $\kappa$ B signal pathway was involved in HSF1/HMGB1-mediated regulation of asthma.

## 1. Introduction

Asthma is a common chronic inflammatory disease of airways of the lung in children [1]. It is characterized by airway hyperresponsiveness, episodic airway obstruction and reduced lung function. Asthma is caused by complex gene-environment interactions [2,3]. About 14.1% of 13–14 year age children and 11.7 % of 6–7 year age children developed into asthma patients [4]. At the same time, it is estimated that asthma caused about 397,100 death in 2015 in the world [5]. To date, the aim of asthma therapy is to minimize side-effects and achieve good asthma control. However, there is no best treatment for severe asthma sufferers.

High mobility group box 1 (HMGB1) is a cytokine mediator of inflammation that secreted by immune cells or injured cells [6,7]. It was over-expressed in inflammatory conditions, such as rheumatoid

arthritis, severe acute pancreatitis, sepsis and cystic fibrosis [6,8,9]. Moreover, recent evidence has demonstrated that HMGB1 was involved in the progress of asthma [10]. HMGB1 was upregulated in the lung tissue, serum and sputum of severe asthma sufferers, and blocking HMGB1 reversed the asthma through suppressing airway inflammation [10–12]. In addition, it has been regarded as a sensitive biomarker of clinical response to allergic asthma treatment in children [11]. Nevertheless, the regulatory mechanism of HMGB1 in asthma is not completely clear.

Heat shock factor 1 (HSF1) is a major transcription factor that regulates the heat shock response pathways [13]. In addition, HSF1 transactivates genes encoding many cytoprotective proteins that involved in DNA damage repair, heat shock and metabolism [14]. Some evidence indicates that HSF1 serves as an important mediator in the inflammation [15]. Dang Xingbo et al. found that HSF1 plays anti-

**Abbreviation:** HMGB1, High mobility group box 1; HSF1, Heat shock factor 1; HSF1<sup>-/-</sup>, knockout of HSF1; OVA, ovalbumin; LPS, lipopolysaccharide; BALF, bronchoalveolar lavage fluid; TLR4, Toll-like receptor 4; NF- $\kappa$ B, nuclear factor kappa-light-chain-enhancer of activated B cells; MyD88, Myeloid differentiation primary response 88

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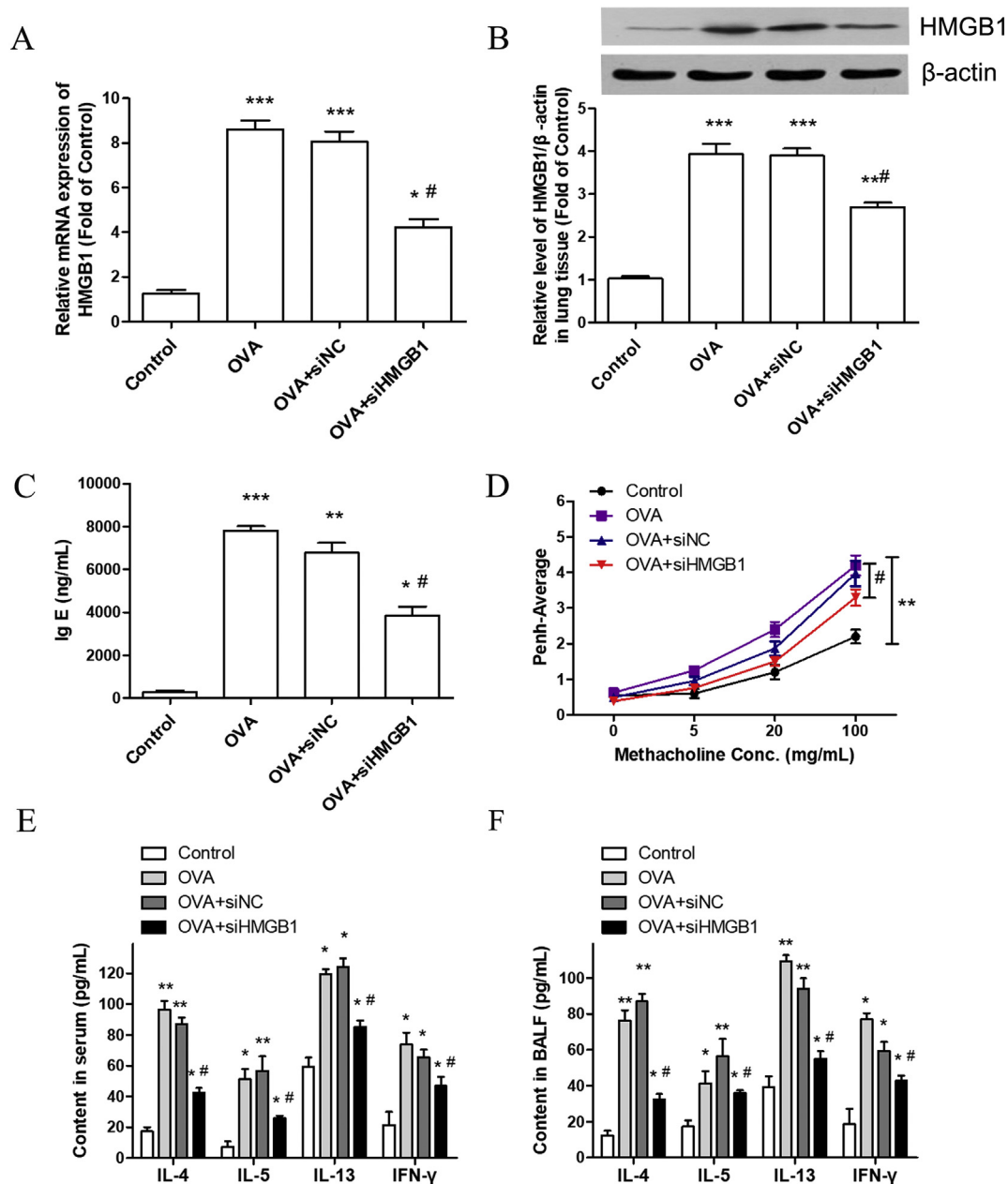


**Fig. 1.** HMGB1 expression was upregulated in OVA-induced asthmatic mice, and inhibition of HMGB1 relieved airway inflammation and hyperresponsiveness induced by OVA. A. The level of HMGB1 mRNA in the lung tissue was examined by qRT-PCR. B. The expression of HMGB1 protein in the lung tissue was detected through Western blotting. C. The content of IgE in serum samples was detected by ELISA kit. D. Airway responsiveness to methacholine was detected in Control, OVA, OVA + siNC and OVA + siHMGB1 mice groups. E–F. The levels of IL-4, IL-5, IL-13 and IFN- $\gamma$  in serum (E) and BALF (F) were measured by ELISA kit. BALF: bronchoalveolar lavage fluid. \* $P$  < 0.05, \*\* $P$  < 0.01, \*\*\* $P$  < 0.001, compared with the control group. # $P$  < 0.05, compared with OVA group.

oxidant and anti-inflammatory roles in acute lung injury [15]. Pan X et al. reported that HSF1 works as an innate repressor of HIV-induced inflammation [16]. What is more, a new study found that knockdown of HSF1 aggravated the airway inflammation and hyperresponsiveness in asthmatic mice through regulating the expression of HMGB1 [17]. Nevertheless, the relationship between HMGB1 and HSF1 in asthma needs a further investigation. Hence, the aim of this study was to investigate the function of HMGB1 in asthma, and its connection with HSF1.

## 2. Materials and methods

### 2.1. Animals and treatment

Eight week-old male BALB/c mice and wild-type of C57BL/6 mice were provided by the Beijing Weitonglihua Experimental Animal Co. (Suzhou, China). HSF1<sup>-/-</sup> (HSF1-deficient) C57BL/6 mice (aged 6–8 weeks) were provided by the Jackson Laboratory. Mice were housed with a standard 12 h light/dark cycle and ad libitum access to food and water. The murine model of asthma was constructed as described previously [18]. In brief, mice were injected intraperitoneally with 20  $\mu$ g ovalbumin (OVA) plus 0.5 mg aluminum hydroxide on days 0, 7, and 14. Then the mice were challenged by intranasal instillation of OVA (40  $\mu$ g/per mouse) alone on days 14, 15, 21, and 22. All mice were

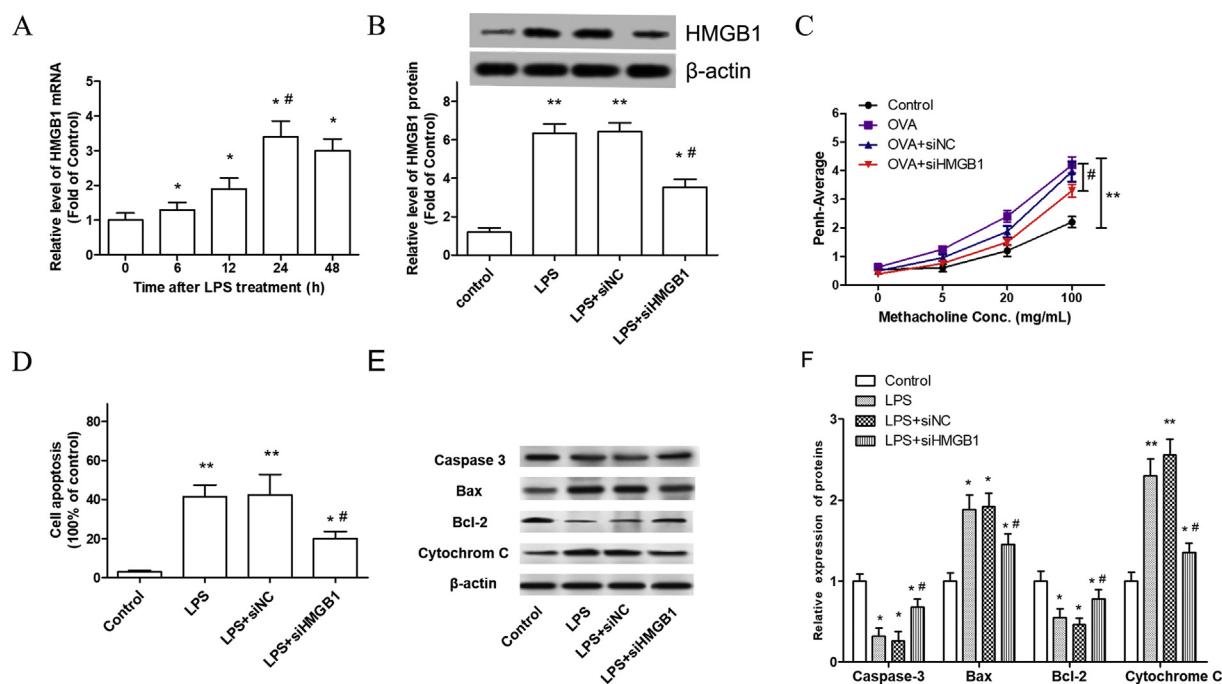


**Fig. 2.** HMGB1 expression was upregulated in LPS-stimulated PBECs. A. The level of HMGB1 mRNA in PBECs was examined after stimulated by LPS (1  $\mu$ g/mL) for different time periods (0, 6, 12, 24 and 48 h). \* $P$  < 0.05, compared with the control group, # $P$  < 0.05, compared with treated for 6 h and 12 h groups. B. The expression of HMGB1 protein in LPS-stimulated PBECs was detected after transfected with siNC or siHMGB1. C and D. Cell viability and apoptosis of LPS-stimulated PBECs were measured after transfected with siNC or siHMGB1. E and F. The expression of apoptosis markers was detected through Western blotting. PBECs were transfected with siNC (50 nM) or siHMGB1 (50 nM), and after cultured for 48 h, LPS was added and cultured for 24 h. \* $P$  < 0.05, \*\* $P$  < 0.01, compared with the control group, # $P$  < 0.05, compared with LPS group.

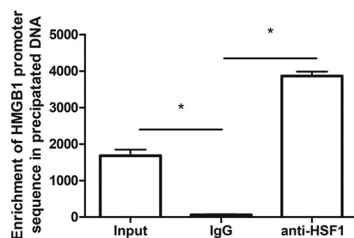
A

The promoter sequence of HMGB1: 30618596-30617597

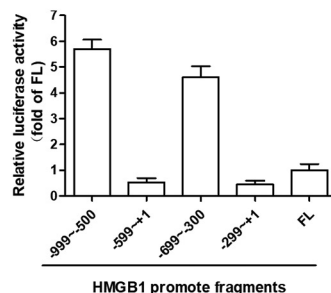
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TCCGCCCTGTGGCCGAGACGAGTTGCGACTGCGGCGACGAGGAGGGGCGGGCGGCTGCTGCTGAGCCCGCATGTGTGAGTGGCTGGGTTTG  
GGAGGCGACGTTTCTGGAAGCTGCTGGAAGCGCCGAGTGGCGGAGTGGCGCCAGCGGCGGGGTGGGCGTGATTAAACTAGTGCAGCGCT  
+1

B



C



analyzed at 24 h after the last OVA challenge. The BALB/c mice were randomly divided into four groups ( $n$  = 8): Control group (mice were sensitized with saline and challenged with OVA), OVA group, OVA + siRNA-NC group and OVA + siRNA HMGB1 group (mice were tail intravenous injected with 200 nM of siRNA-NC or 200 nM of siRNA HMGB1 1 h before the challenge). At the end of the experiment, mice were sacrificed, bronchoalveolar lavage fluid (BALF) and blood sample

were collected. All experimental animal protocols were approved by the Animal Care and Use Committee of Xi'an Children's Hospital (20190022).

## 2.2. Determination of airway responsiveness to methacholine

Airway responsiveness of mice was measured as described in

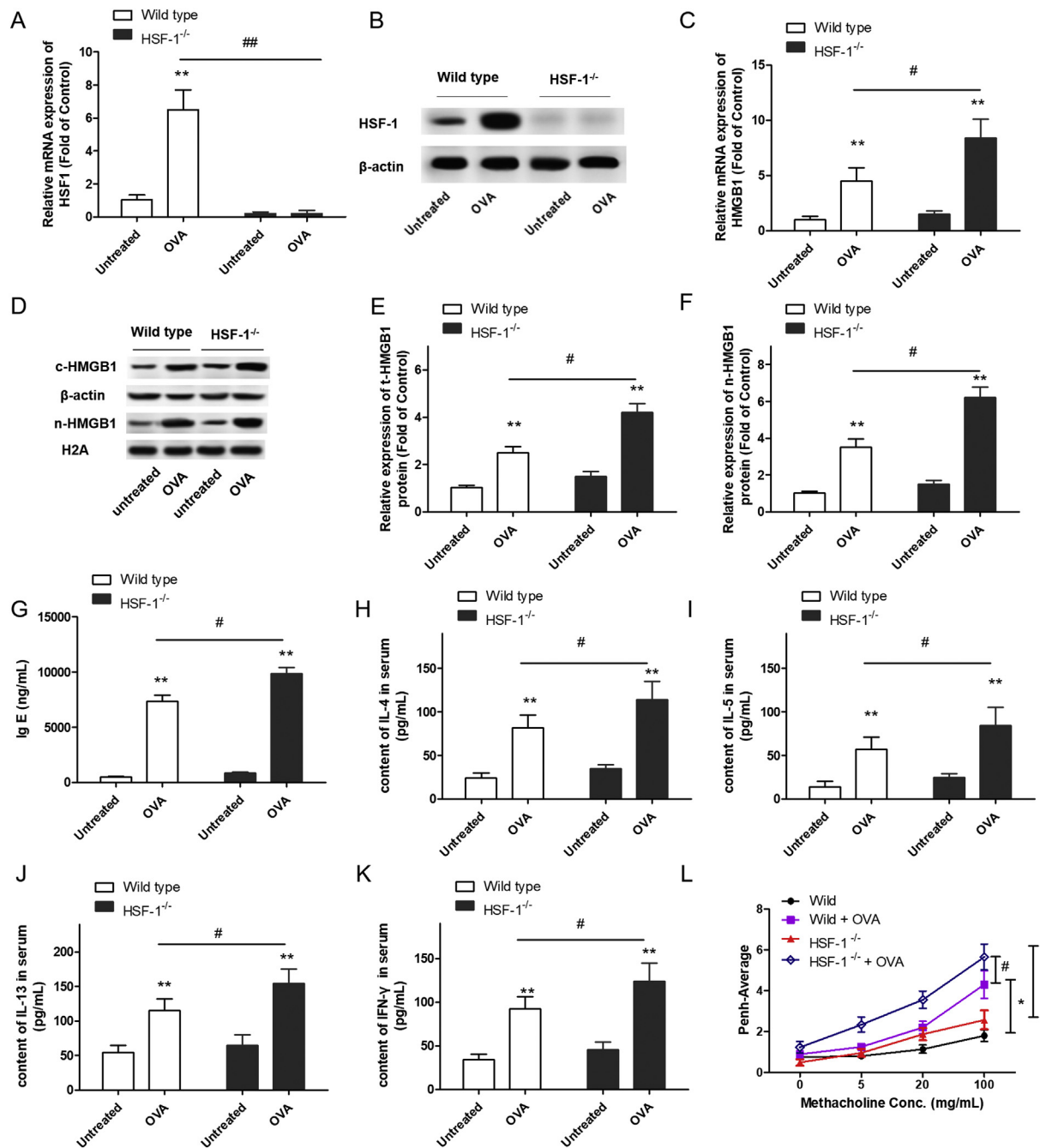


**Fig. 4.** Knockout of HSF1 promoted the expression of HMGB1 in vivo and exacerbated the inflammation of asthmatic mice. A and B. The expression of HSF1 mRNA and protein in lung tissues of mice was examined. C. The expression of HMGB1 mRNA was measured through RT-PCR. D–F. The expression of cytosolic and nuclear HMGB1 protein was detected by Western blotting. c-HMGB1: cytosolic HMGB1; n-HMGB1: nuclear HMGB1. G–K. The levels of IgE, IL-4, IL-5, IL-13 and IFN- $\gamma$  in serum were measured by ELISA kit. L. Airway responsiveness to methacholine was detected. \* $P < 0.05$ , \*\* $P < 0.01$ , compared with untreated group. # $P < 0.05$ , ## $P < 0.01$ , compared with OVA-treated wild type mice group.

previously [19]. Briefly, airway responsiveness was induced by methacholine after the final challenge with OVA. Mice were placed in a barometric plethysmographic chamber, and exposed to aerosolized saline (baseline) or increasing concentrations of aerosolized methacholine (from 0 to 100 mg/mL). Penh (enhanced pause) is a dimensionless value that represents a function of the proportion of maximal expiratory to maximal inspiratory box pressure signals and is a function of the timing of expiration, calculated as (expiratory time / relaxation time - 1)  $\times$  (peak expiratory flow / peak inspiratory flow) [20]. Penh values were recorded for 3 min after each nebulization and averaged.

### 2.3. Cells culture

Human bronchial epithelial cells (PBECS) and HEK293T cells were purchased from Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Thermo Fisher Scientific, California, USA) containing 10% fetal bovine serum (FBS), under a humidified incubator at 37 °C with 5% CO<sub>2</sub>. LPS treatment: PBECS were treated with LPS (1  $\mu$ g/mL) for 24 h.

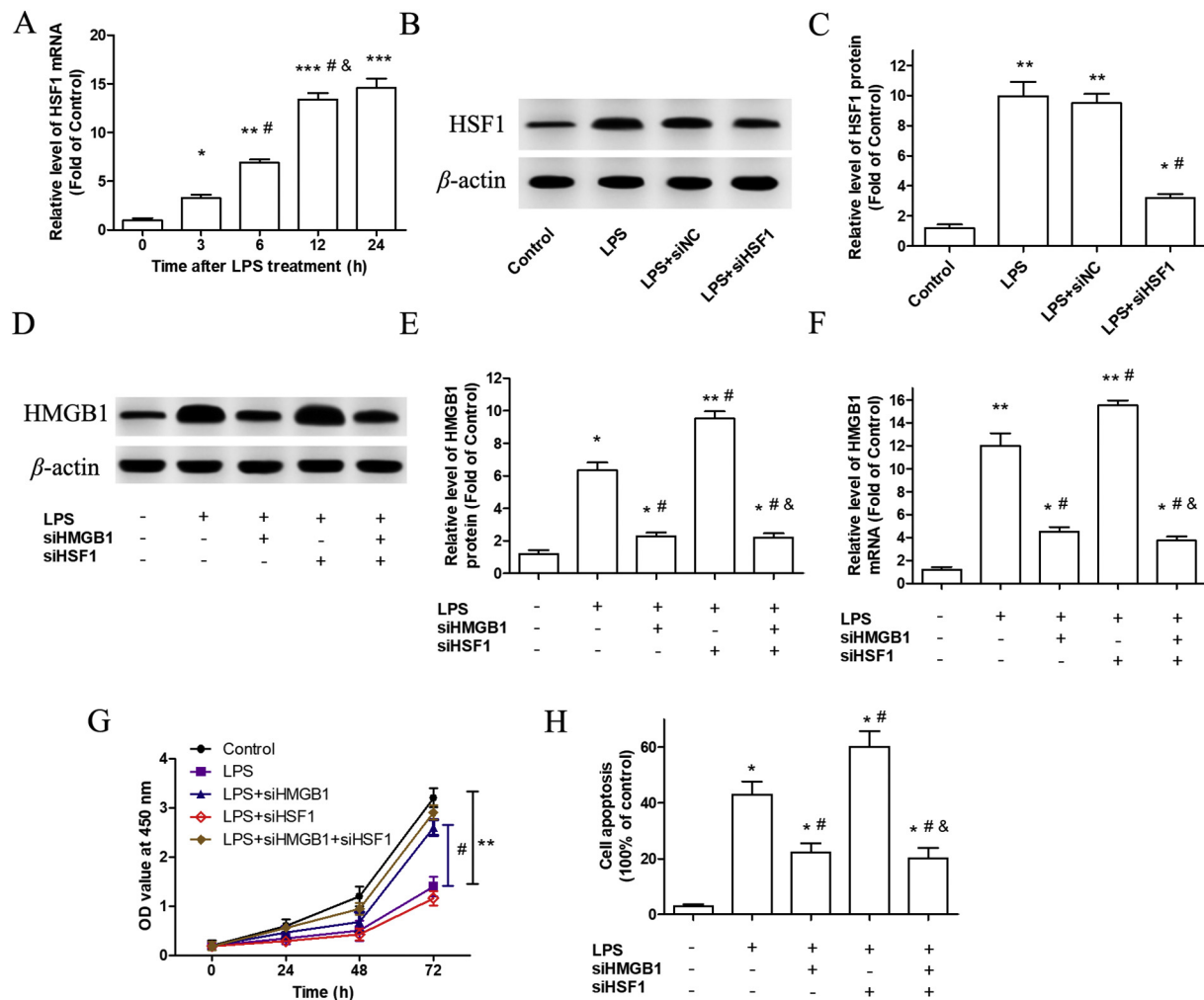


**Fig. 5.** Silence of HSF1 promoted the expression of HMGB1 in vitro. **A.** The expression of HSF1 mRNA in PBECs was examined after stimulated by LPS for different time periods (0, 3, 6, 12 and 24 h). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , compared with 0 h group. # $P < 0.05$ , compared with treated for 3 h group, & $P < 0.05$ , compared with treated for 6 h group. **B and C.** The expression of HSF1 was analyzed after transfected with siHSF1. **D–F.** The expression of HMGB1 mRNA and protein in LPS-stimulated PBECs was measured after transfected with siHMGB1 and siHSF1, respectively or both. **G and H.** Cell viability and apoptosis of LPS-stimulated PBECs were tested after transfected with siHMGB1 and siHSF1, respectively or both. PBECs were transfected with siHMGB1 (50 nM) or siHSF1 (50 nM) or both, and after cultured for 48 h, LPS was added and cultured for 24 h. \* $P < 0.05$ , \*\* $P < 0.01$  compared with the control group, # $P < 0.05$ , compared with the LPS group, & $P < 0.05$ , compared with the siHSF1 transfected group.

#### 2.4. Cell transfection

SiRNA HMGB1 (siHMGB1) and siRNA HSF1 (siHSF1) were synthesized and purchased from GenePharma (Shanghai, China). Overexpression plasmids of HSF1 (pcDNA-HSF1) were constructed and purchased from Bio-transduction Lab (Wuhan, China). pcDNA-HSF1, siHMGB1, siHSF1 or siNC were transfected into cells using lipofectamine 3000 transfection reagent according to the manufacturer's instructions (Thermo Scientific, Wilmington, DE, USA).

#### 2.5. Quantitative RT-PCR

Cells and tissues RNA were isolated using TRIzol™ Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocols. The quantitative RT-PCR was performed as previously described [21].

#### 2.6. Enzyme-linked immunosorbent assay (ELISA)

The levels of IL-4, IL-5, IL-13 and Interferon- $\gamma$  (IFN- $\gamma$ ) in serum and BALF were measured by ELISA kit according to the manufacturer's

recommendations (Abcam, Cambridge, UK). The content of Immunoglobulin E (IgE) was also measured through ELISA.

#### 2.7. Western blotting

Cells and tissues were harvested and lysed by RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China). Total cell lysates were centrifuged at 15,000 rpm for 10 min at 4 °C. The supernatant was collected, protein concentration was determined by a BCA Protein Assay kit (Beyotime Biotechnology, Shanghai, China). Then, protein was separated by electrophoresis in 10% SDS-polyacrylamide gel electrophoresis gels and transferred to nitrocellulose membranes (Millipore, Boston, MA, USA). After blocked with 5% skim milk, the blots were incubated with primary antibodies and horseradish peroxidase-conjugated secondary antibodies, respectively. The immunoreactive bands were detected using ECL substrate Kit (ab133406, Abcam, Cambridge, UK) and visualized on X-ray films. The images were subsequently analyzed using ImageJ software to quantify the protein expression. Primary antibodies used in this study including anti-HMGB1 antibody (Abcam, ab18256), anti-HSF1 antibody (Abcam, ab61382), anti-TLR4 antibody (CST, #14358), anti-MyD88 antibody (CST, #4283), anti-NF-

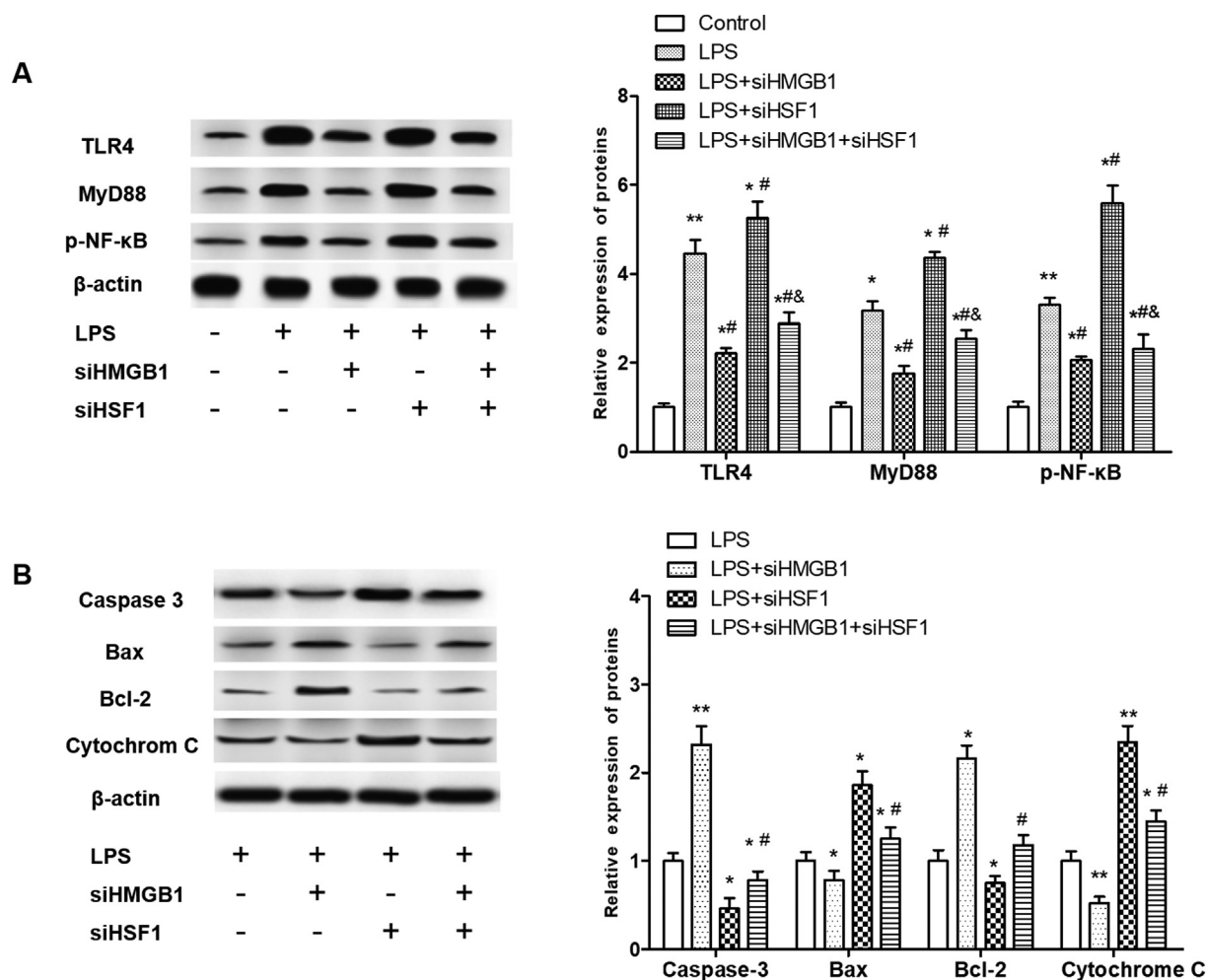


**Fig. 6.** The TLR4/MyD88/NF- $\kappa$ B signal pathway was involved in HSF1/HMGB1 mediated asthma. A. The expression of TLR4, MyD88 and p-NF- $\kappa$ B in LPS-stimulated PBECs was detected after transfected with siHMGB1 and siHSF1, respectively or both. \* $P < 0.05$  and \*\* $P < 0.01$ , compared with the control group, # $P < 0.05$ , compared with the LPS group, \$ $P < 0.05$ , compared with siHMGB1 transfected group. B. The expression of pro-caspase 3, Bax, Bcl-2, and cytochrome C in LPS-stimulated PBECs was detected. \* $P < 0.05$  and \*\* $P < 0.01$ , compared with the LPS group, # $P < 0.05$ , compared with siHMGB1 transfected group.

$\kappa$ B antibody (Abcam, ab16502) and anti- $\beta$ -actin antibody (Abcam, ab8227).

## 2.8. CCK-8 assay

Cell viability was detected by a Cell Counting Kit-8 (CCK-8, KJ800, Dojindo Laboratories, Japan). PBECs were planted into 96-well plates with a density of  $1 \times 10^4$ /well. After cultured for overnight, cells were treated with 1  $\mu$ g/mL of LPS for 24, 48 and 72 h. An hour before each time point, 10  $\mu$ L of CCK-8 reagent was added into each well. Then, a microplate reader (Elx808, Bio Tek, USA) was used to record the optical density values at 450 nm.

## 2.9. Apoptosis test

PBECs were planted into 6-well plates, and stimulated by LPS for 24 h. Cell apoptosis was detected through Annexin V-FITC/PI apoptosis kit (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's instructions.

## 2.10. Chromatin immunoprecipitation (ChIP) assay

PBECs were treated by LPS (1  $\mu$ g/mL) for 24 h, and then the cells were harvested. The ChIP assay was performed with anti-HSF1 antibody (Abcam, ab18256) using a commercial ChIP kit (Millipore,

Boston, MA, USA) in accordance with the manufacturer's instructions.

## 2.11. Luciferase reporter assay

HEK293T cells were seeded into 96-well plates. Full-length HMGB1 promoter DNA and HMGB1 promoter fragments DNA (−299 to +1, −699 to −300, −599 to +1 and −999 to −500 length) were amplified and cloned into luciferase reporter plasmids. Plasmids and pcDNA-HSF1 vectors were transfected into HEK293T cells using Lipofectamine 3000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. After 48 h, the cells were harvested and the luciferase activity was analyzed by Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA).

## 2.12. Statistical analysis

Data were analyzed with GraphPad Prism 5.0 software (GraphPad, San Diego, CA, USA) and SPSS 19.0 software (SPSS Inc., Chicago, IL, USA), and expressed as the means  $\pm$  SEM. One-way analysis of variance (ANOVA) and Student's *t*-test were used to perform the statistical analyses.  $P < 0.05$  was considered to be statistically significant. Each experiment was repeated at least three times.

### 3. Results

#### 3.1. Inhibition of HMGB1 relieved airway inflammation and responsiveness in OVA-induced asthmatic mice

The expression of HMGB1 mRNA and protein was significantly upregulated in OVA-induced asthmatic mice, while transfection with siHMGB1 effectively suppressed the expression of HMGB1 in OVA-induced asthmatic mice (Fig. 1A and B). At the same time, the level of IgE in serum was sharply increased in OVA-induced asthmatic mice, whereas inhibiting HMGB1 obviously suppressed the increasing of IgE (Fig. 1C). In addition, OVA-induced asthmatic mice exhibited a greater increase of Penh compared with the control group mice, and this was attenuated by siHMGB1 (Fig. 1D). What is more, the content of IL-4, IL-5, IL-13 and IFN- $\gamma$  in serum and BALF were significantly increased in OVA-induced asthmatic mice, which were inhibited by siHMGB1 (Fig. 1E and F). Hence, it is suggested that HMGB1 was upregulated in OVA-induced asthmatic mice, while blocking HMGB1 could relieve the airway inflammation and airway hyperactivity in asthmatic mice.

#### 3.2. HMGB1 expression was increased in LPS-stimulated cells

As displayed in Fig. 2A, the expression of HMGB1 was increased in PBECs after being treated by LPS in a time-dependent manner. SiHMGB1 transfection significantly inhibited the upregulation of HMGB1 in LPS-stimulated PBECs (Fig. 2B). Next, cell viability and apoptosis were detected by CCK-8 assay and Annexin V-FITC/PI double staining, these results suggested that blocking HMGB1 obviously inhibited the decrease of cell viability and the increase of cell apoptosis in PBECs that induced by LPS (Fig. 2C and D). Further, apoptosis related proteins were detected in PBECs after treated for LPS and silence of HMGB1. The result showed that the expression of pro-caspase 3 and Bcl-2 protein was significantly decreased after stimulated by LPS, while transfection with siHMGB1 markedly suppressed this decline (Fig. 2E and F). At the same, the expression levels of Bax and cytochrome C were increased in LPS-stimulated PBECs and this increasing was partly reversed by siHMGB1 (Fig. 2E and F).

#### 3.3. HSF1 directly binds and transcriptionally regulates the HMGB1

It has been reported that knockdown of HSF1 may negatively control of HMGB1 in asthma [17]. For exploration of the relationship between HMGB1 and HSF1 in asthma, bioinformatic analyses, ChIP assays and luciferase reporter assay were adopted in this study. As shown in Fig. 3A, there is a HSF1-binding site in the promoter region of HMGB1 via an online bioinformatic analyses tool (<http://rna.sysu.edu.cn/chipbase/>). Next, ChIP assays further confirmed that HSF1 was physically bind with the HMGB1 promoter (Fig. 3B). Moreover, luciferase reporter assay revealed the -599 to +1 and -299 to +1 length fragment of the HMGB1 promoter declined the luciferase expression, suggesting -299 to +1 region of the HMGB1 promoter is needed in the binding with HSF1 (Fig. 3C). These results suggested that HSF1 directly binds with the HMGB1 promoter, and negatively regulates the transcription of HMGB1.

#### 3.4. Knockout of HSF-1 promoted the expression of HMGB1 in vivo and exacerbated the inflammation of asthmatic mice

For further exploration the role of HSF1 in the inflammation response in the asthmatic mice and its effect on the expression of HMGB1. HSF1<sup>-/-</sup> mice and OVA-induced HSF1<sup>-/-</sup> asthmatic mice were adopted in this study. The expression of HSF1 mRNA and protein in mice lung tissues was examined, the result showed that OVA treatment significantly increased the expression of HSF1 in lung tissues of wild type mice, compared with untreated mice (Fig. 4A and B). Meanwhile, an extremely low level of HSF1 was examined in HSF1<sup>-/-</sup> mice and

OVA-induced HSF1<sup>-/-</sup> asthmatic mice, compared with wild type mice (Fig. 4A and B). Further, we found that the increased level of HMGB1 induced by OVA treatment in HSF1<sup>-/-</sup> asthmatic mice was higher than that in wild type mice (Fig. 4C-F). What is more, we found that the levels of IgE, IL-4, IL-5, IL-13 and IFN- $\gamma$  in OVA induced HSF1<sup>-/-</sup> asthmatic mice were higher than that in OVA induced wild type asthmatic mice (Fig. 4G-K). In addition, OVA-treated HSF1<sup>-/-</sup> asthmatic mice exhibited a greater increase of Penh compared with OVA-stimulated wild type asthmatic mice (Fig. 4L). In summary, these results suggested that the expression of HSF1 was increased in OVA-induced asthmatic mice and knockout of HSF1 enhanced the expression of HMGB1, the level of airway inflammation and airway hyperactivity in OVA-induced asthmatic mice.

#### 3.5. Silencing HSF1 promoted the expression of HMGB1 in vitro

Next, the function of HSF1 in LPS-stimulated PBECs was studied. LPS treatment leads to an increased expression of HSF1 in PBECs in a time-dependent manner (Fig. 5A). SiHSF1 transfection effectively suppressed the increasing of HSF1 in PBECs that induced by LPS (Fig. 5B and C). At the same time, blocking HSF1 significantly elevated the expression of HMGB1 in LPS-stimulated PBECs (Fig. 5D-F). However, transfection with siHMGB1 or co-transfection with siHMGB1 and siHSF1 significantly inhibited the expression of HMGB1 (Fig. 5D-F). What is more, silencing HSF1 obviously suppressed the cell viability and increased the cell apoptosis in LPS-treated PBECs, while siHMGB1 transfection or siHMGB1 and siHSF1 co-transfection showed a contrary effect (Fig. 5G and H). These results indicated that blocking HSF1 inhibited the cell viability and increased the cell apoptosis may through increasing the expression of HMGB1.

#### 3.6. The TLR4/MyD88/NF- $\kappa$ B signal pathway was involved in HSF1/HMGB1 mediated asthma

The TLR4-MyD88 pathway was involved in the signal transduction of mediators associated with severe asthma. As shown in Fig. 6A, the expression of TLR4, MyD88 and p-NF- $\kappa$ B was obviously increased in LPS-treated PBECs compared with the control group. While transfecting siHMGB1 into PBECs inhibited the upregulation of TLR4, MyD88 and p-NF- $\kappa$ B that induced by LPS. On the contrary, transfection with siHSF1 promoted the increasing of TLR4, MyD88 and p-NF- $\kappa$ B in LPS-stimulated PBECs. Nevertheless, co-transfection with siHSF1 and siHMGB1 significantly inhibited the upregulation of TLR4, MyD88 and p-NF- $\kappa$ B that induced by LPS. Hence, these results suggested that HSF1/HMGB1 was involved in the regulation of the TLR4/MyD88/NF- $\kappa$ B signal pathway in asthma.

Besides, we found that silence of HMGB1 inhibited the increasing expression of pro-caspase 3 and Bcl-2 in LPS-stimulated PBECs. While inhibition of HSF1 promoted the decline of pro-caspase 3 and Bcl-2 in LPS-stimulated PBECs, and effectively reversed the effect of siHMGB1 on LPS-treated PBECs (Fig. 6B). Meanwhile, the expression levels of Bax and cytochrome C in LPS-stimulated PBECs were significantly suppressed by siHMGB1 and enhanced by si-HSF1. And the inhibitory effect of siHMGB1 on Bax and cytochrome C was effectively reversed by si-HSF1 (Fig. 6B).

### 4. Discussion

HMGB1 has been implicated as an important mediator in the pathogenesis of asthma. Consistent with previous reports [12], our study verified that HMGB1 is upregulated in OVA-induced asthmatic mice and LPS-stimulated PBECs. The inflammation cytokines, which were reported to promote airway wall remodeling, mucus hypersecretion and airway hyperresponsiveness [22], were increased in OVA-induced asthmatic mice and inhibited by blocking HMGB1. Therefore, this study revealed that blocking HMGB1 could effectively relieve the asthma

through inhibiting airway inflammation and hyperresponsiveness.

HSF1, as a transcription factor, it could be activated by various environmental stress, such as heat stress, oxidative stress and other physicochemical factors [23,24]. New evidence suggested that HSF1 was high expressed in bronchial epithelial cells, alveolar epithelial cells and immune cells in OVA-induced asthmatic mice [25]. An extensive literature indicating that HSF1 plays an anti-inflammation role in many diseases, through inducing the expression of heat shock proteins indirectly, or regulating the expression of inflammatory factors directly [15,26,27]. In our study, we verified that HSF1 bind with the HMGB1 promoter and negatively regulating the transcription of HMGB1. Consistent with previous report, the expression of HSF1 was increased in OVA-induced asthmatic mice [17]. Knockdown of HSF1 aggravated the OVA-induced airway inflammation and airway hyperreactivity in mice may through promoting the expression of HMGB1. On the other hand, it is possible that HSF1 plays a protective role in OVA-induced asthmatic mice through suppressing inflammation and improving cell survival, as a result, decreased the expression of HMGB1. However, further study is needed to confirm this viewpoint.

According to previous studies, TLR4, as an important pattern recognition receptors, plays a crucial role in asthma pathophysiology, including initiate and exacerbate the progress of asthma [28,29]. Environmental pollutants, such as PM<sub>2.5</sub>, ozone and endotoxin all could exacerbate allergic inflammation through activating the TLR4/MyD88-signaling pathway [28,30–32]. While deletion of TLR4 or MyD88 attenuated the airway inflammation and hyperresponsiveness [33]. It is known that LPS could bind to TLR4 and subsequently activating MyD88-dependent pathways, and resulting in the nuclear translocation of NF- $\kappa$ B [34]. Besides, previous studies suggested that activation of the TLR4/MyD88/NF- $\kappa$ B signal pathway contributes to the upregulation of HMGB1 [35]. Meanwhile, TLR4 could be activated by HMGB1 [36,37]. Moreover, HSF1 has been reported could inhibit the TLR4/MyD88 signal pathway during the alcohol-mediated immunosuppression [27]. Another study found that HSF1 inhibited the TNF $\alpha$ -induced cardiomyocyte death through suppression of NF- $\kappa$ B pathway [26]. Our results are consistent with the previous suggestion that the TLR4/MyD88/NF- $\kappa$ B signal pathway was activated by LPS, and this activation was inhibited by blocking HMGB1 and further promoted by silencing HSF1.

In this study, LPS was adopted to establishment the cellular model. Previous studies have reported that endotoxin (LPS) enhanced the cellular response to allergen, and could augment the allergic inflammation [31,38]. Although the cellular model does not reflect the complex pathological features of human asthma, it does provide an important step in identifies the relationship between HSF1 and HMGB1 in the inflammatory response that induced by LPS, and the role of HSF1/HMGB1 in LPS-induced activation of the TLR4/MyD88/NF- $\kappa$ B signal pathway. Our study provides new insights into the pathogenesis and HSF1/HMGB1 may be served as new targets for the clinical management of asthma.

## 5. Conclusion

This study demonstrated that HMGB1 and HSF-1 were upregulated in OVA-induced asthmatic mice, and inhibiting HMGB1 could suppress the airway inflammation and hyperresponsiveness that induced by OVA. In addition, HSF1 binds with the HMGB1 promoter, and negatively regulates the transcription of HMGB1. Knockdown of HSF1 aggravated the OVA-induced airway inflammation and airway hyperreactivity in mice may through promoting the expression of HMGB1 and the activation of the TLR4/MyD88/NF- $\kappa$ B signal pathway.

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None.

## Author contributions

LQS and CCL, designed the study. LQS, LW, LZ, and JW performed the animal experiment section. LQS, XLS, NW and LZ finished the cell experiment section. LQS wrote this manuscript and CCL oversaw language edit. All authors read and approved the final manuscript.

## Declaration of competing interest

The authors declare that they are no conflicts of interest associated with the contents of this manuscript.

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