In vitro/vivo Mechanism of Action of S100A12 in Sepsis-induced ARDS

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Research

Abstract

Objective

Sepsis is a multiple organ dysfunction elicited by the dysregulated host immune response to microbial infection. Acute respiratory distress syndrome (ARDS) is a serious and acute inflammatory lung injury resulting from sepsis and other serious diseases. The present study aims to investigate the role of S100A12, a pro-inflammatory factor, in the pathophysiologic mechanism underlying the process in sepsis-induced ARDS.

Methods

The blood samples and clinical base line of study subjects were collected. C57BL/6 mice underwent cecal ligation and puncture (CLP) to establish sepsis models. Hematoxylin and Eosin (H&E) Staining was performed to observe pathological changes. Enzyme-Linked Immunosorbent Assay (ELISA) and specific commercial assay kits were employed to analyze the levels of inflammatory cytokines and antioxidant capacity, respectively. Western blot, immunohistochemistry (IHC) staining and reverse-transcriptase quantitative real-time PCR (RT-qPCR) were performed to determine target gene and protein expression. TUNEL Assay and Flow Cytometry were performed to assay cell apoptosis.

Results

The levels of S100A12 and sRAGE are upregulated in the serum of patients with Sepsis-induced ARDS and sepsis mice. Furthermore, higher cell apoptosis rate was observed in lung tissue of sepsis mice. In addition, S100A12 resulted in excessive mucins and the secretion of inflammatory cytokines secretion, and promoted the expression of chemokines and cell adhesion molecules via activating NLRP3 inflammasome pathway in NHBE cells. Finally, S100A12 increased oxidative stress status and cell apoptosis in NHBE cells.

Conclusion

The present study provides evidence that S100A12 is closely related to pathogenesis of sepsis-induced ARDS. Hence, S100A12 may be a useful biomarker of pulmonary injuries for clinical diagnosis of sepsis-induced ARDS.

Introduction

Sepsis is a multiple organ dysfunction elicited by the dysregulated host immune response to microbial infection, which has been classified as a serious disease threatening human health (1). It is well known that sepsis is a systemic inflammatory response resulting in significant mortality worldwide (2). Recent studies have reported that lung is one of the most vulnerable organs and approximately 45% patients developed into acute lung injury after the onset of sepsis (3). Acute respiratory distress syndrome (ARDS) is a serious and acute inflammatory lung injury results from sepsis and other serious disease and

characterized by refractory hypoxemia and progressive respiratory failure (4). Furthermore, it is generally accompanied with alveolar interstitial edema syndrome or diffuse alveolar inflammatory response (5).

It has also been reported that numerous cytokines and chemokines accelerate the pathological and physiological progress of ARDS (6), suggesting that inflammatory response is closely related to the pathogenesis of ARDS. Chemokines are a group of cytokines superfamily, which have been classified into 4 subfamilies (CXC, CX3C, CC, and C). Growing evidence suggested that Chemokines and chemokine receptors are crucial regulatory factors in acute lung injury (7). Cxc Chemokine Receptor 4 (CXCR4) / stromal-derived factor-1 (SDF-1), also known as Cxc Chemokine Ligand 1 (CXCL1), chemokine axis was associated with inflammation of injured tissues (8). In addition, mucus hypersecretion by goblet cells plays an important role in infection and inflammation in lung injury (9). Mucins, produced in the airway epithelia, are the main component of mucus. Mucins were divided into two categories including transmembrane or membrane-bound mucins (e.g., MUC1 and MUC4) and secreted/gel-forming mucins (e.g., MUC5AC and MUC5B). Dysregulation of mucins contributes to uncontrolled inflammation which results in abnormal airway function. Thus, MUC5AC and MUC1 are identified as markers of damage to lung epithelial cells (10). However, pharmacologic therapies and several other therapies still have numerous limitations to decrease mortality rate and ameliorate outcomes of ARDS, which have prompted interest in investigating the fundamental cellular mechanism that related to the onset and development of ARDS, and in developing novel and effectively therapeutic strategies for patients with ARDS (11, 12).

The S100 family, to which the calcium binding pro-inflammatory protein-S100A12 is belonged, is associated with various host immune signaling and exaggerated cytokines production and secretion, resulting in systemic inflammatory response as well as secondary organ failure (13). Increased serum levels of S100A12 are related to numerous acute and chronic inflammatory disorders. It is documented that S100A12 was significantly elevated in neonatal sepsis and may be considered as a novel biomarker for it (14). In addition, the levels of S100A12 in serum were upregulated in the early stage of the development of acute lung injury (ALI) and significantly decreased on postoperative day 1, making it possible that S100A12 be a useful marker of sepsis (15). However, the underlying mechanism of the pro-inflammatory effects of S100A12 on ARDC caused by sepsis remains unclear.

The present study aims to investigate the role of S100A12 in the pathophysiologic mechanism underlying the process in sepsis-induced ARDS. Our study provides evidences to present a novel therapeutic target in clinical therapeutic strategy of sepsis-induced ARDS.

Materials And Methods

Study subjects and collection of blood samples

A total of 30 sepsis-associated ARDS patients and 30 normal controls (≥ 18 years) hospitalized in Jianhu Hospital Affiliated to Nantong University were recruited. Patients with ARDS during sepsis were also included in accordance with the ACCP/SCCM Consensus ("Sepsis-2") Criteria (16). Main

clinicopathological characteristics of the participants are summarized in Table 1. All corresponding blood samples were collected according to the protocol approved by the Ethics Committee at Jianhu Hospital Affiliated to Nantong University and written informed consents were obtained from all participants.

Animals

All animal care and experiment procedures were conducted in accordance with the guidelines approved by the Animal Ethical Committee of Jianhu Hospital Affiliated to Nantong University. Eight-week-old wild-type (WT) mice (C57BL/6 background) were obtained from Oriental Bio Service Inc. (Nanjing) and housed in a room on a 12 h light/dark cycle at 22 °C ± 2 °C with 55–65% relative humidity and fed on a standard chow diet ad libitum.

Mice model of polymicrobial sepsis

Mice were anesthetized with 10% chloral hydrate and underwent cecal ligation and puncture (CLP) as described previously with minor modification (17). Briefly, incision (2–3 cm) was made in the midline abdominal wall to expose the cecum. Subsequently, the cecum was ligated one centimeter from the tip with 4 – 0 silk suture. A 21-gauge needle was used for one puncture site where a small amount of fecal contents were squeezed. Finally, the incision was sutured with 4 – 0 silk suture. The sham-operated mice were defined as normal, with only laparotomy performed. After CLP procedures, mice were resuscitated with 1 ml of pre-warmed saline through subcutaneously injection.

Hematoxylin and Eosin (H&E) Staining

Lung tissues were placed in 4% paraformaldehyde for 24 h, dehydrated for another 12 h, paraffn-embedded, and sliced at 5 µm thickness. Then, the lung tissue sections were dewaxed with xylene and rehydrated with gradient ethanol. Sequentially, hematoxylin and eosin were used to stain the slides. Finally, the sections were sealed with gum and observed under a light microscope (Nikon Eclipse Ti; Nikon Corporation, Tokyo, Japan) at the 200 × magnification to capture the pathological changes including hyperemia, alveolar congestion, presence of exudates, etc.

Enzyme-Linked Immunosorbent Assay (ELISA)

The amount of pro-inflammatory cytokines such as S100A12, sRAGE, TNF-a,IL-1β and IL-6 in serum, bronchoalveolar lavage fluid (BALF) and NHBE cells supernatants of each group was detected by enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA) following the manufacturer's instructions. The optical density (OD) value at 450–650 nm was measured by a microplate reader. Western blot analysis

Homogenized mice lung tissues and NHBE cells were lysed with IPA lysis buffer supplemented with 1 mmol/L PMSF and other protease inhibitors to abtain samples. Protein samples (25 µg) were fractionated by 10% SDS-PAGE gels and then transferred onto PVDF membranes. After blocked with 5% skim milk, the membranes were incubated with primary antibodies against S100A12 (1 : 1,000), sRAGE (1 : 1,000), SDF-1(1 : 1,000), CXCR4 (1 : 1,000), ICAM-1 (1 : 1,000),VCAM-1(1 : 1,000), MCP-1 (1 : 1,000), bcl2

(1 : 1,000), bax (1 : 1,000), cleaved caspase (1 : 500), caspase3 (1 : 500), NLRP3 (1:1000), ASC (1:1000), caspase1 (1:1000), and GAPDH (1 : 2,000) overnight at 4 °C, then the membranes were washed with PBS and incubated with appropriate HRP-conjugated secondary antibody at room temperature for 2 h. The protein bands were measured with chemiluminescence reagent (Thermo Fisher Scientifc, Inc.). Finally, we determined the relative intensity for target proteins via dividing the absolute intensity of target proteins by the absolute intensity of GAPDH.

Immunohistochemistry (IHC) staining

All paraffin sections were immersed in 3% H_2O_2 in PBS for 10–15 min to block endogenous peroxidase activity, after deparaffinization and rehydration. Subsequently, sections were incubated with 5% Bovine Serum Albumin (BSA) at room temperature for 40 min to block non-specific binding. For immunostaining, anti-S100A12 monoclonal antibody (1:100) and anti-sRAGE monoclonal antibody (1:100) were applied at 4 °C overnight, which were purchased from Millipore (Billerica, MA). After PBS washing, the sections were incubated with biotin-conjugated secondary antibody (1:100) at room temperature for 2 h and then were visualized with DAB. The spread and intensity of S100A12 and sRAGE positive immunoreactivity of each group were observed and captured under a light microscope at the 200 × magnification (Nikon Eclipse Ti; Nikon Corporation, Tokyo, Japan).

TUNEL Assay

The apoptotic cells in lung tissue were detected via TUNEL assay. The TUNEL assay kit (Merck Millipore, Darmstadt, Germany) was employed to determine the presence of apoptotic cells following the manufacturer's protocol. The lung tissue sections were stained with TUNEL reagents and observed under an optical microscope (Olympus Corp., Tokyo, Japan) at 200 × magnification to count the positive cells.

Cell culture

The normal human bronchial epithelial (NHBE) cell line was obtained from American Type Culture Collection (Manassas, VA, USA) and was cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Thermo Fisher Scientific, U.S.A.), 100 μg/mL streptomycin and 100 units/mL penicillin (Invitrogen, Carlsbad, CA, USA) at 37 °C in a 5% CO2 humidified atmosphere. The cells were stimulated with Recombinant human S100A12 (50, 100, 200, 500 ng/ml), purchased from MBL (Aichi, Japan), before use in subsequent experiments.

RNA extraction and Reverse-transcriptase quantitative real-time PCR (RT-qPCR)

Total RNA was prepared from NHBE cells with Trizol reagent (Ambion, USA) according to the manufacturer's recommendations. The cDNAs were synthesized by reverse transcription of 1 μg total RNAs using PrimeScript RT Master Mix Kit (TaKaRa, Otsu, Shiga, Japan). Subsequently, qPCR assay was employed to detect the target gene expression of each word with equal amount of cDNA using SYBR® Green Master Mix (Applied Biosystems, Foster City, CA, USA). Gene expression levels were carried out

using the $2^{-\Delta\Delta Ct}$ method and normalized to the expression of GAPDH. Gene specific primer sequences for qPCR amplification were designed as follows:

MUC5AC	Forward	5'- CCTGCAAGCCTCCAGGTAG – 3'
	Reverse	5'- CTGCTCCACTGGCTTTGG – 3'
MUC1	Forward	5'- TCCAATATTAAGTTCAGGCCAGGA – 3'
	Reverse	5'- CACATCACTCACGCTGACGT – 3'
SDF-1	Forward	5'- AAAGAAGCGACAGAAGAAGAG – 3'
	Reverse	5'- AAGAGGGAGGAGCGAGTT – 3'
CXCR4	Forward	5'- CTACAGCAGCGTTCTCATC – 3'
	Reverse	5'- TTTTCAGCCAGCAGTTTC – 3'
ICAM-1	Forward	5'- AGACCTATGTCCTGCCATCG – 3'
	Reverse	5'- GGTGCCCTCCTCATTTTCCT – 3'
VCAM-1	Forward	5'- GAACTGGAAGTCTACATCTC – 3'
	Reverse	5'- CAGAGAATCCGTGGAGCTGG – 3'
MCP-1	Forward	5'- CCCTAAGGTCTTCAGCACCT – 3'
	Reverse	5'- ACTGTCACATGGTCACTCC – 3'
GAPDH	Forward	5'- GAAGGTGAAGGTCGGAGTCA – 3'
	Reverse	5'- GACAAGCTTCCCGTTCTCAG – 3'

Flow Cytometry

The Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) apoptosis detection kit ((Biosea, Beijing, China) was used to analyze cell apoptosis in vitro, following the manufacturer's protocol. NHBE cells were seeded in 6-well plates and then incubated with or without 100 ng/mL LPS and S100A12 (50, 100, 200, 500 ng/ml) for 24 h. NHBE cells (1×10^6 cells/mL) were collected, washed with cold PBS and re-suspended with 1 × binding buffer. Then, NHBE cells were stained with Annexin 5 μL V-FITC and 10 μL PI staining solution. The Annexin V-FITC-positive and PI-negative cells were defined as apoptotic cells, and the percentage of apoptotic cells in each group was conducted and analyzed using a flow cytometer (Beckman, Coulter, USA).

Analyses for Total Antioxidant Capacity, ROS, MDA, and Antioxidant Enzymes

Total antioxidant capacity of cell lysates was analyzed. The amount of reactive oxygen species (ROS) and malondialdehyde (MDA), and antioxidant enzymes activities, such as superoxide dismutase (SOD),

lactate dehydrogenase (LDH), glutathione peroxidase (GSH-Px), were detected using the specific commercial assay kits, according to the manufacturer's recommendations. Subsequently, absorbance was measured by a microplate reader (Thermo Fisher Scientific, USA)

Statistical Analysis

All experimental data are expressed as the mean ± standard deviation (SD) and statistically analyzed with SPSS 17.0 software. Students' t-tests were used to analyze the parameters among groups, and one-way analysis of variance (ANOVA) test followed by Bonferroni's post hoc test was performed to analyze the statistical difference among multi-groups. P-value < 0.05 was considered to indicate the statistical significance.

Results

Background factors and variations of S100A12 and sRAGE

The study subjects were allocated into two groups (n = 30): healthy and sepsis-lung injury group, according to whether patients had sepsis-induced ARDC or not. As shown in table 1, there were no significant differences in gender, age, BW and smoking history between healthy people and patients with sepsis-induced ARDS enrolled in this study. However, significant changes were found in PACHE II score, PaO_2/FiO_2, MAP, Lactic acid level between the two groups, indicating that sepsis may be a contributor to serious pulmonary injury. Furthermore, increased levels of S100A12 and sRAGE were observed in serum of sepsis-lung injury group, compared with healthy group (Fig. 1).

The levels of S100A12 and sRAGE is upregulated in the serum and BALF of sepsis mice

To further confirm the S100A12 and sRAGE levels in sepsis-associated ARDS, C57BL/6 mice were selected and underwent CLP to establish sepsis models. HE staining was performed to observe the pathological changes of lung tissues. As shown in Fig. 2A, lung sections belonging to mice with sepsis confirmed alveolar congestion, hemorrhage, edema and inflammatory cell infiltration, compared with normals. In addition, ELISA kits, western blot and IHC staining were employed to detect the levels of S100A12 and sRAGE in serum and BALF. These results suggested that the levels of S100A12 and sRAGE of sepsis model mice were higher than the controls (Fig. 2B-C, Fig. 3A-B). Finally, TUNEL assay was used to analyze cell apoptosis in lung tissues. Compared with normal, higher cell apoptosis rate was observed in lung tissue of mice with sepsis (Fig. 3C). The evidence above implies that acute inflammation in mice with sepsis lead to pulmonary injury and even the acceleration in the development of ARDS.

S100A12 resulted in excessive mucins secretion and inflammatory response in NHBE cells

MUC5AC and MUC1 are identified as biomarkers of damage to lung epithelial cells. To assess the role of S100A12 in NHBE cells, MUC5AC and MUC1 mRNA levels were detected using RT-qPCR under S100A12 challenge (50, 100, 200, 500 ng/ml). The results showed that LPS treatment (positive control) significantly elevated the expression of MUC5AC and MUC1, when compared with normal NHBE cells. Interestingly, S100A12 treatment group have the same trend in a dose-dependent manner. In addition, sRAGE mRNA level was also determined by RT-qPCR under the same conditions. The result showed that both LPS and S100A12 treatment increased sRAGE expression (Fig. 4A). Due to the cytotoxicity of 500 ng/ml S100A12, the doses of 50, 100 and 200 ng/ml of S100A12 were selected for the next experiment.

To further examine the effect of S100A12 on inflammatory response, ELISA kits were employed to analyze the levels of pro-inflammatory cytokines in culture supernatants, including Tumor necrosis factor-α (TNF-α), Inter leukin-1β (IL-1β) and IL-6. Consistent with above results, S100A12 treatment enhanced the amount of TNF-α, IL-1β and IL-6 in a dose-dependent manner in comparison with the controls (Fig. 4B). The results suggested that S100A12 led to abnormal mucins expression, which further resulted in uncontrolled inflammation, indicating S100A12 played an important role in impaired airway function.

S100A12 promoted the expression of chemokines and cell adhesion molecules in NHBE cells

Chemokines and cell adhesion molecules are crucial regulative factors in inflammation. RT-qPCR and western blotting were performed to detect the mRNA level and protein expression of chemokine receptor4 (CXCR4), stromal-derived factor-1 (SDF-1), Intercellular adhesion molecule-1 (ICAM-1), Vascular cell adhesion molecule-1 (VCAM-1) and Monocyte chemotactic protein 1 (MCP-1) levels, respectively. As shown in Fig. 5, SDF-1, CXCR4, ICAM-1, VCAM-1 and MCP-1 expressions were notably increased by LPS and S100A12 stimulation (50, 100 and 200 ng/ml) in a dose-dependent manner. The results suggested that S100A12 enhanced the expression of chemokines and cell adhesion molecules to aggravate inflammatory response.

S100A12 increased oxidative stress status and activate NLRP3 inflammasome pathway in NHBE cells

To further investigate the effect of S100A12 in ARDA, the levels of ROS and MDA production and the activities of SOD, LDH and GSH-Px were detected using specific commercial test kits. In comparison with control, the levels of ROS and MDA, and the activities of LDH were increased by LPS and S100A12 treatment (50, 100 and 200 ng/ml) in a dose-dependent manner, while the activities of SOD and GSH-Px were decreased (Fig. 6A-B). In addition, the effect of S100A12 on NLRP3 inflammasome pathway was analyzed with western blotting. As shown in Fig. 6C, the expression levels of NLRP3, apoptosis-associated speck-like protein containing caspase-recruitment domain (ASC) and caspase1 were upregulated significantly in a dose-dependent manner under LPS and S100A12 stimulation (50, 100 and

200 ng/ml). The results implied that S100A12 increased oxidative stress status and activates NLRP3 inflammasome pathway in NHBE cells, indicating S100A12 played a crucial role in airway inflamamtion.

S100A12 promoted cell apoptosis in NHBE cells

To further confirm the role of S100A12 in pulmonary injury of ARDA, Flow Cytometry was used to evaluate the cell apoptosis rate. As shown in Fig. 7A-B, the number of apoptotic cells was markedly increased after LPS and S100A12 treatment (50, 100 and 200 ng/ml). Furthermore, the expressions of marked proteins related to cell apoptosis, such as Bcl2, Bax, cleaved caspase3 and caspase3, were detected by western botting. As shown in Fig. 7C, Bcl2 expression level was significantly reduced by LPS and S100A12 challenge (50, 100, 200, 500 ng/ml). In contrast, the expression levels of Bax and cleaved caspase3 were significantly upregulated by LPS and S100A12 treatment in a dose-dependent manner. There is no significant difference of caspase3 expression between controls and treatment groups. The results suggested that S100A12 promoted cell apoptosis and aggravated airway damage.

Discussion

Sepsis is defined as an organ dysfunction threatened health and life of population, which caused by unbalanced response to infection in host (18). An earlier study reported that lung is one of the most vulnerable organs, sepsis patients trend to develop into ARDS and, hence, sepsis is the most common cause of ARDS (19). ARDS is an acute and serious disorder, characterized by dysregulated airway inflammation and pulmonary trauma, contributing to high mortality rate. In the present study, abnormal levels of S100A12 and sRAGE were observed in clinical subjects with sepsis-induced ARDS and sepsis mice model. Hence, NHBE cell line was selected and treated with different doses of S100A12 as to further confirm the biochemical mechanisms underlying the effect of S100A12 in pathogenesis of sepsis-induced ARDS. The present study provides in vivo and vitro evidence that S100A12 played an important role in the development of sepsis-induced ARDS.

Our results showed that S100A12 level was upregulated in sepsis-induced ARDS patients and sepsis model mice, and that elevated levels of inflammatory cytokines, such as TNF-α IL-1β and IL-6, which were observed in NHBE cells under S100A12 treatment, implied S100A12 involved in the pathogenesis of sepsis-induced ARDS. Accumulating evidence indicates that dysregulation of mucins contributes to uncontrolled inflammation which resulted in abnormal airway function (20). It is well established that mucus hypersecretion and production are primary reasons for airflow obstruction and inflammatory response (10). As the disease progressed, infection and inflammation in turn further promoted mucus hypersecretion. Thus, a vicious circle was created, promoting the pathogenetic process of ARDS. In accordance with above study, S100A12 increased MUC5AC and MUC1 expression in NHBE cells. Moreover, significant upregulation of SDF-1, CXCR4, ICAM-1, VCAM-1 and MCP-1 was observed when NHBE cells were exposed to S100A12, which is consistent with our results. Previous study reported that SDF-1/CXCL12 chemokine axis was activated and induced adhesion molecules expression in injury site, and further caused excessive inflammation (21). Furthermore, the effect of S100A12 on the nucleotide-

binding oligomerization domain (Nod) -like receptors (NLRs) inflammasome pathway was evaluated in our study. Numerous studies have reported that NLRs are responsible for proinflammatory cytokines maturation and secretion, and that they are central to innate immune responses (22). The NLRP3 inflammasome, the most known about NLRs, can be activated in response to cellular stresses during cellular or tissue injury (23). In the present study, S100A12 increased the expression of NLRP3 and other components of inflammasome complex, including ASC and caspase-1, indicating S100A12 involved in mechanisms of inflammatory response via activation of NLRP3 inflammasome signaling.

It is well established that RAGE is defined as the receptor of advanced glycation end products (AGEs) (24). Previous studies have demonstrated that the level of sRAGE, the soluble form of RAGE, may be applied as a useful biomarker in diagnosis of lung injury (25). Our results suggested that S100A12 induced increased level of sRAGE in serum of patients and mice with sepsis-associated ARDS, which may reflect the severity of lung injury. Based on results of H&E staining and flow cytometry assay, higher cell apoptosis rate was observed under S100A12 treatment. In addition, our findings demonstrated that S100A12 increased levels of ROS and MDA, and decreased activities of SOD and GSH-Px under S100A12 stimulation for impairing antioxidant balance. Impaired antioxidant defense is closely related to the development of lung injury (26). Collectively, these findings support that S100A12 induced excessive inflammation and antioxidant unbalance and further caused pulmonary injury and airway dysfunction in sepsis-induced ARDS. Further studies are still needed to evaluate the influence of S100A12 on pathogenesis of sepsis-induced ARDS.

Conclusion

In summary, the levels of S100A12 and sRAGE were elevated in serum of clinical patients and mice with sepsis-induced ARDS. Moreover, S100A12 induced excessive inflammation and antioxidant unbalance via various pathways to cause cell apoptosis. Our findings provide evidence that S100A12 is closely related to pathogenesis of sepsis-induced ARDS. Hence, S100A12 may be a useful biomarker of pulmonary injuries for clinical diagnosis of sepsis-induced ARDS.

Abbreviations

ARDS	Acute respiratory distress syndrome
CXCR4	Cxc Chemokine Receptor 4
SDF-1	stromal-derived factor-1
CXCL1	Cxc Chemokine Ligand 1
ALI	Acute lung injury
NLRP3	NOD-like receptor protein 3
CLP	Cecal ligation and puncture
BALF	Bronchoalveolar lavage fluid

Declarations

Ethics approval and consent to participate

Study protocol was approved by the Ethics Committee at Jianhu Hospital Affiliated to Nantong University and written informed consents were obtained from all participants.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding and first authors on reasonable request.

Competing interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Authors' contributions

Zhenen zhang participated in conception and design, searching and selecting papers, data extraction, analyzing data, writing and approving final paper. Ye Shen participated in the selection of title, keywords, and approving final paper. Nannan Han participated in searching and selecting papers and approving final paper. All authors read and approved the final manuscript.

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Table

Table.1 Baseline characteristics of subjects of study population (n=30).

Characteristic	Healthy (n=30)	Sepsis-lung injury (n=30)	P-value
Male/Female	11/19	18/12	0.41
Age (years)	58.36± 11.84	65.03± 10.02	0.54
BW (kg)	68.53 ± 8.86	65.27 ± 6.14	0.93
APACHE II score[a]	15.39 ± 1.26	24.75 ± 2.47	<0.001
PaO_2/FiO_2[b] (mmHg)	395 ± 28.95	118.36 ± 22.32	<0.001
MAP (mmHg)	82.53 ± 0.65	52.13 ± 6.05	<0.001
Lactic acid (mmol/L)	1.1 ± 0.16	5.02 ± 2.25	<0.001
Smoking history Nonsmoker/Smoker	9/21	12/18	0.32

BW = Body Weight; SBP = Systolic Blood Pressure;
DBP = Diastolic Blood Pressure; MAP = Mean Arterial Pressure
[a] Acute Physiology and Chronic Health Evaluation II score
[b]Partial pressure of O_2/Fraction of inspiration O_2

Figures
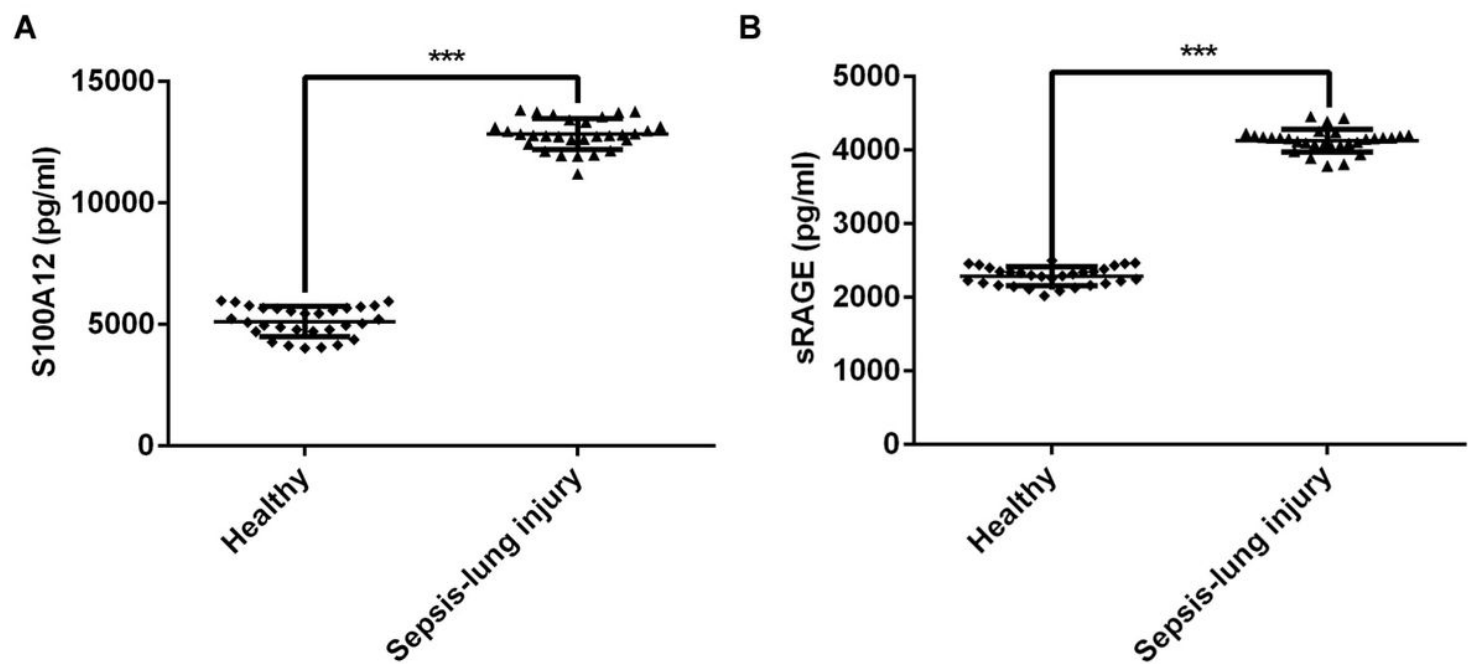
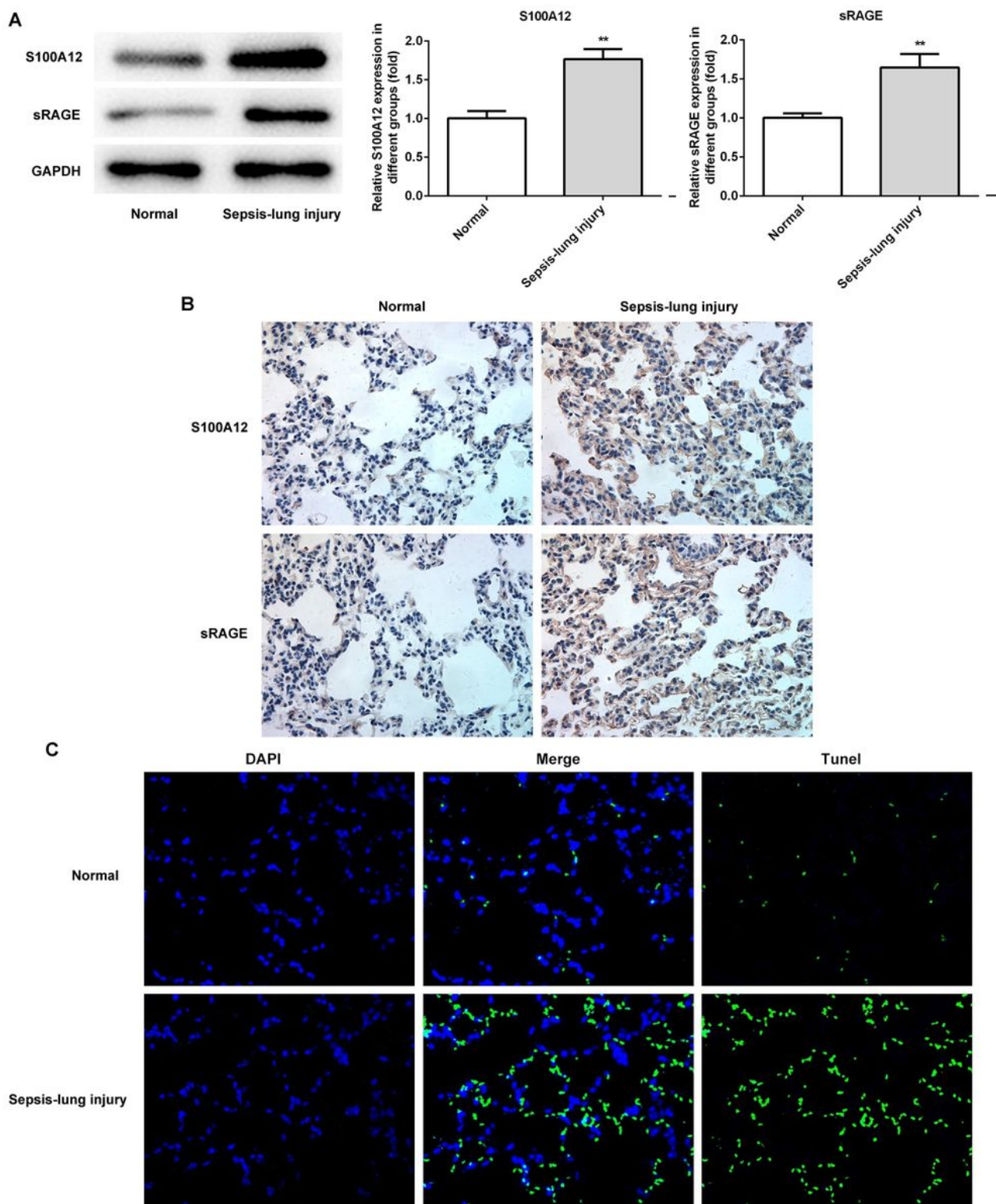# Figure 1

variations of S100A12 and sRAGE in clinical patients with sepsis-induced ARDS. (A) S100A12, (B) sRAGE levels in serum were detected with ELISA kits. The data were expressed as means ± SD, ***P < 0.001 vs. Healthy.
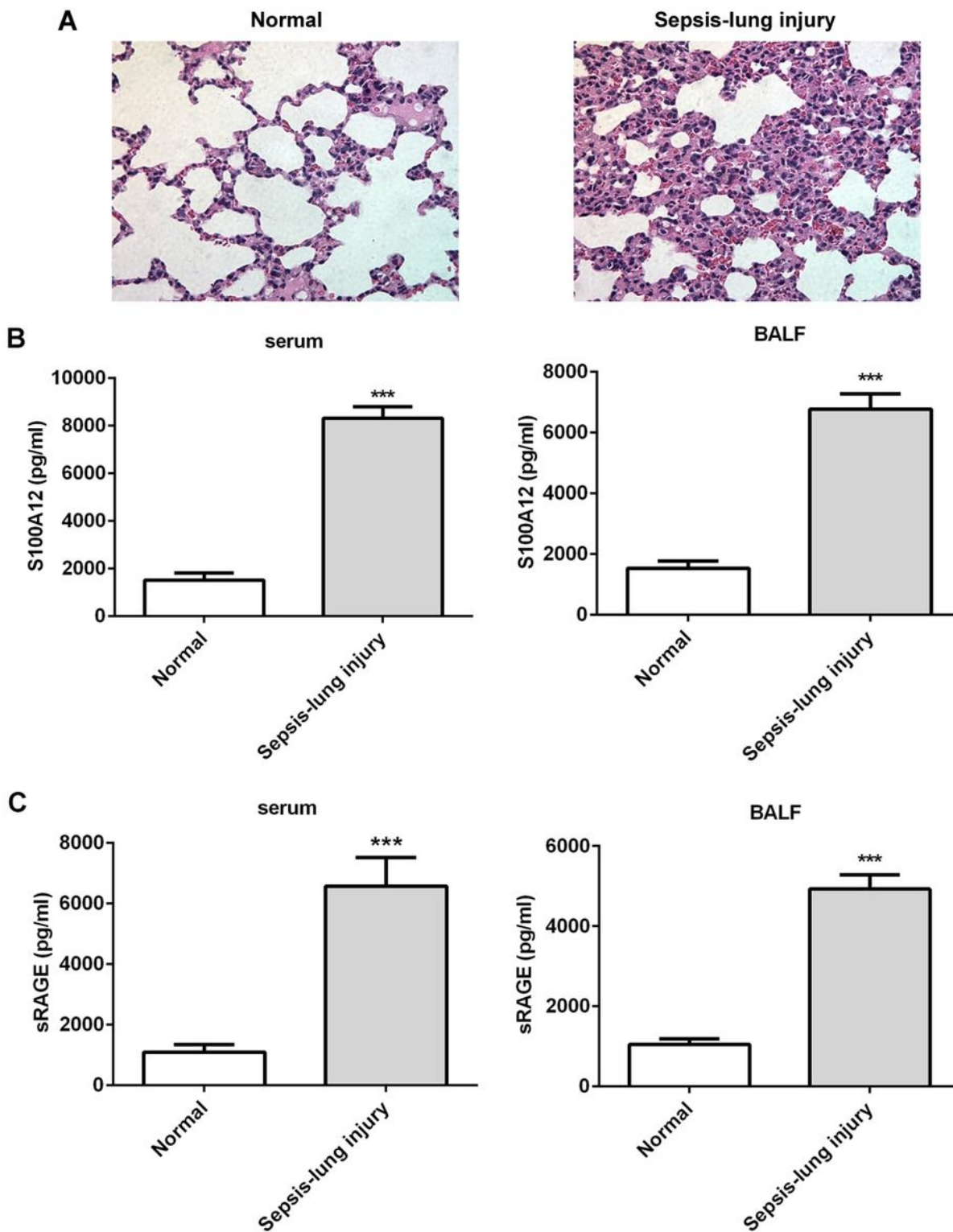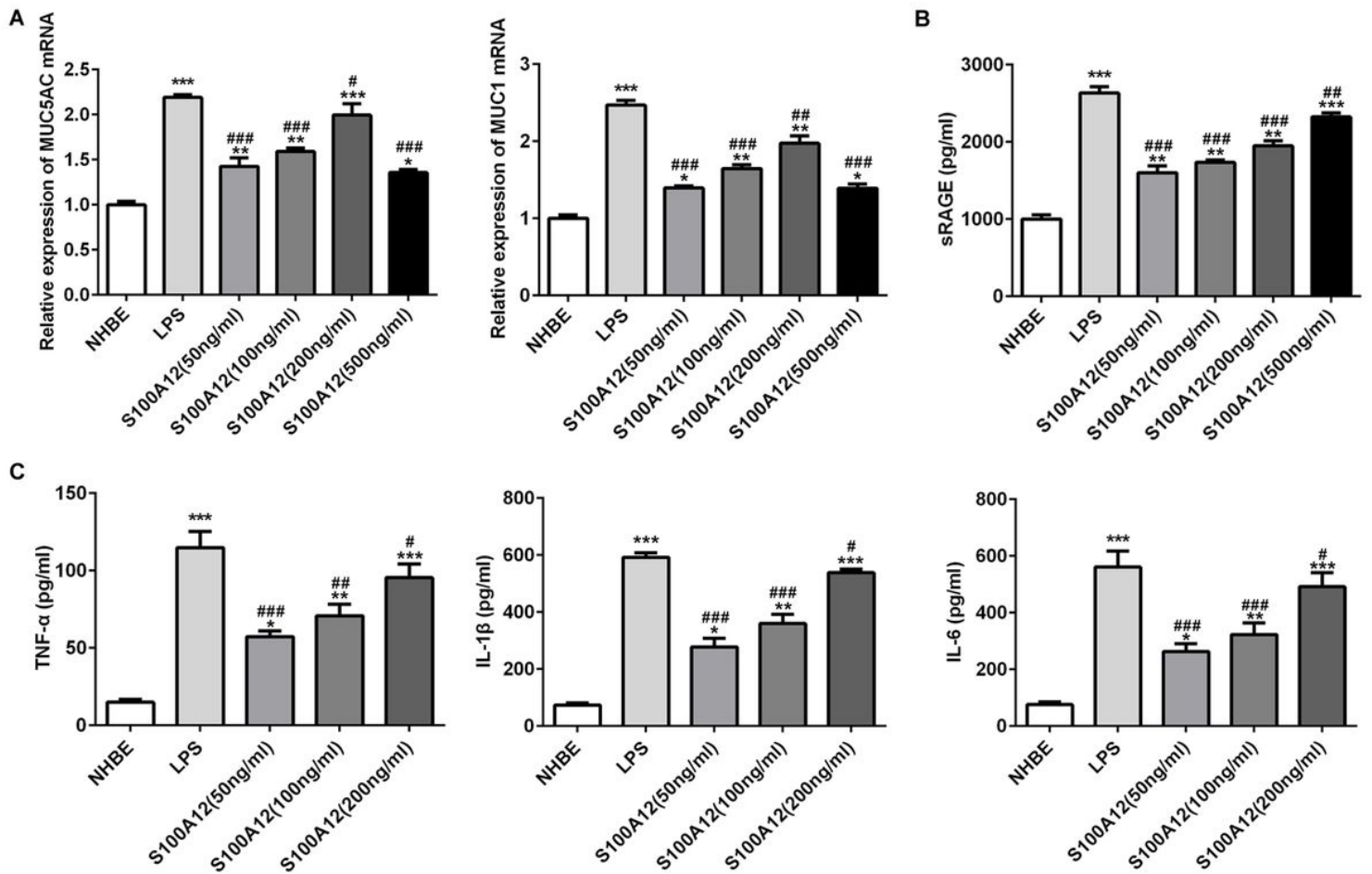
Figure 2

The levels of S100A12 and sRAGE were upregulated in the serum and BALF of sepsis mice. (A) Representative images of hematoxylin and eosin (H&E) staining lung tissues from different groups (×200 magnification). (B) S100A12, (C) sRAGE levels in serum and BALF were detected with ELISA kits. The data were expressed as means ± SD, ***P < 0.001 vs. Normal.
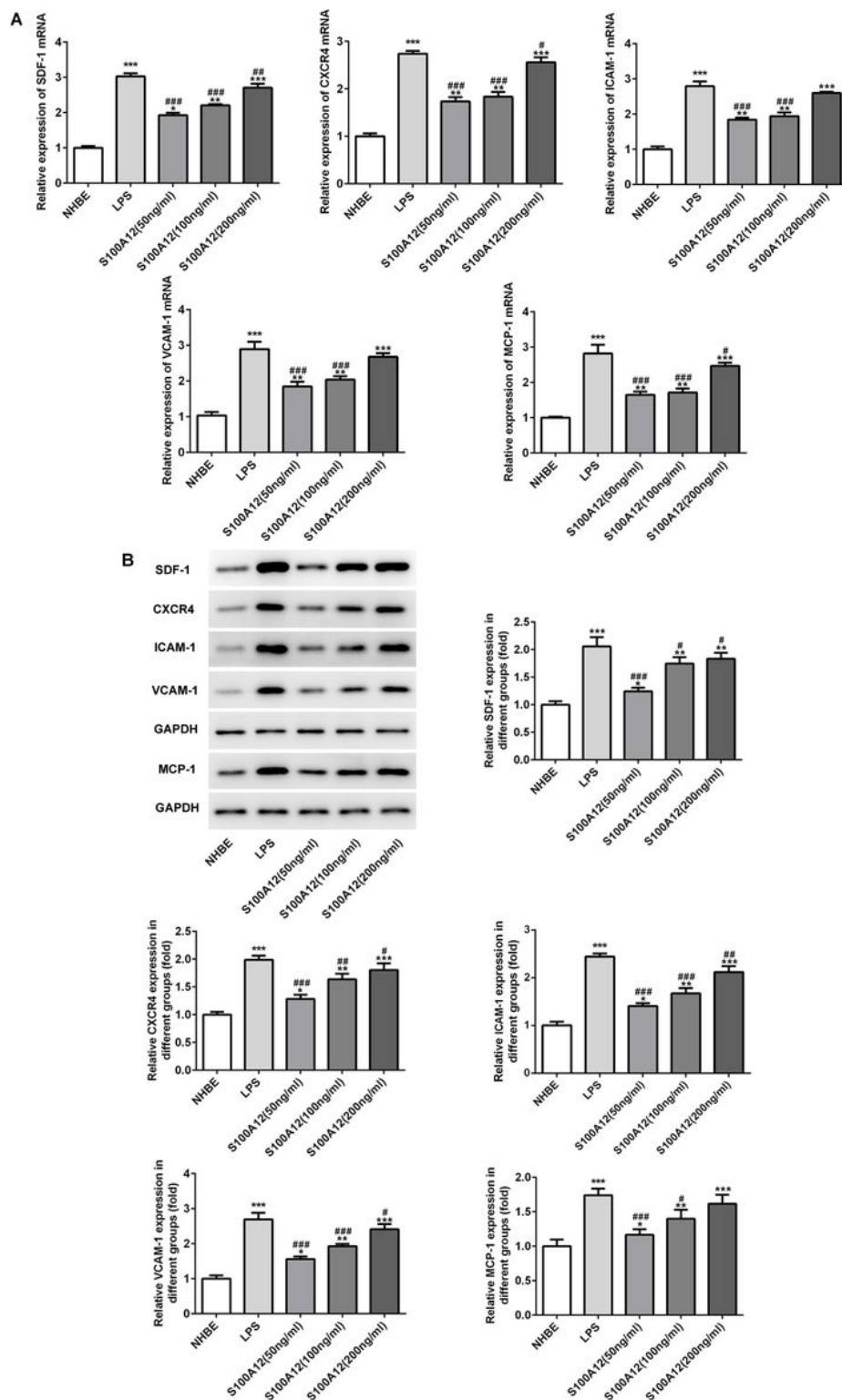
Figure 3

S100A12 induced pulmonary injury and cell apoptosis in lung tissue of sepsis mice. (A) The expressions of S100A12 and sRAGE were detected by western blot. (B) The expressions of S100A12 and sRAGE were analyzed by Immunohistochemistry (×200 magnification). (C) Cell apoptosis was analyzed by TUNEL staining (×200 magnification). The data were expressed as means ± SD, **P < 0.01 vs. Normal.
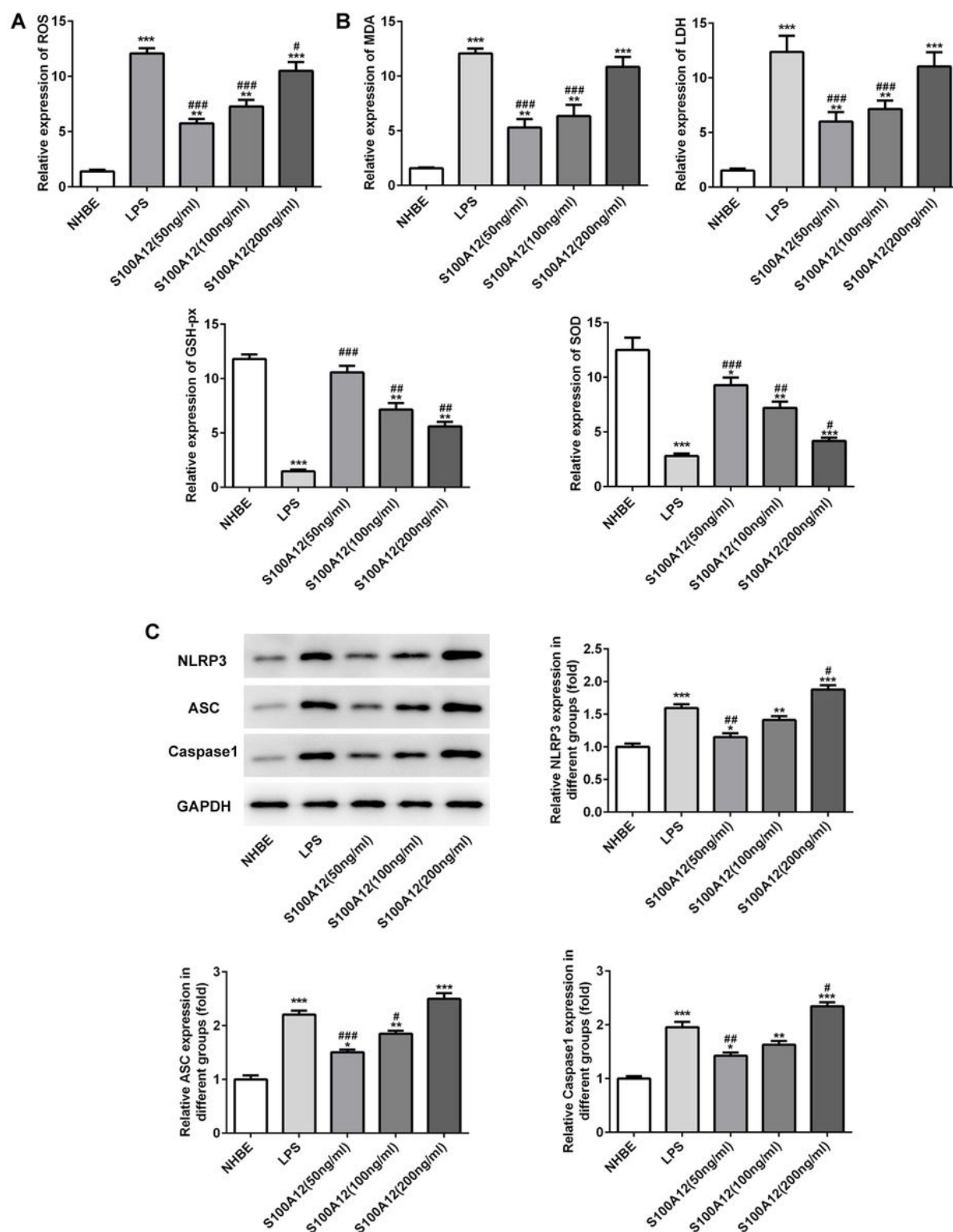
Figure 4

S100A12 resulted in excessive mucins secretion and inflammatory response in NHBE cells. (A) The mRNA levels of MUC5AC, MUC1 were analyzed by RT-qPCR. (B) The amount of sRAGE, TNF-α, IL-1β and IL-6 were detected by ELISA kits. The data were expressed as means ± SD, ***P < 0.001 vs. NHBE. #P < 0.05, ##P < 0.01, ###P < 0.001 vs. NHBE.

Figure 5

S100A12 promoted the expression of chemokines and cell adhesion molecules in NHBE cells. (A) The mRNA levels of SDF-1, CXCR4, ICAM-1, VCAM-1 and MCP-1 were measured by RT-qPCR. (B) The expressions of SDF-1, CXCR4, ICAM-1, VCAM-1 and MCP-1 were measured by western blot. The data were expressed as means ± SD, ***$P < 0.001$ vs. NHBE. #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ vs. NHBE.

Figure 6

S100A12 increased oxidative stress status and activate NLRP3 inflammasome pathway in NHBE cells. (A) The amount of reactive oxygen species (ROS) and malondialdehyde (MDA), and activities of superoxide dismutase (SOD), lactate dehydrogenase (LDH), glutathione peroxidase (GSH-Px) were detected using the specific commercial assay kits. (B) The expressions of NLRP3, ASC and Caspase1

were measured by western blot. The data were expessed as means ± SD, ***P < 0.001 vs. NHBE. #P < 0.05, ##P < 0.01, ###P < 0.001 vs. NHBE.
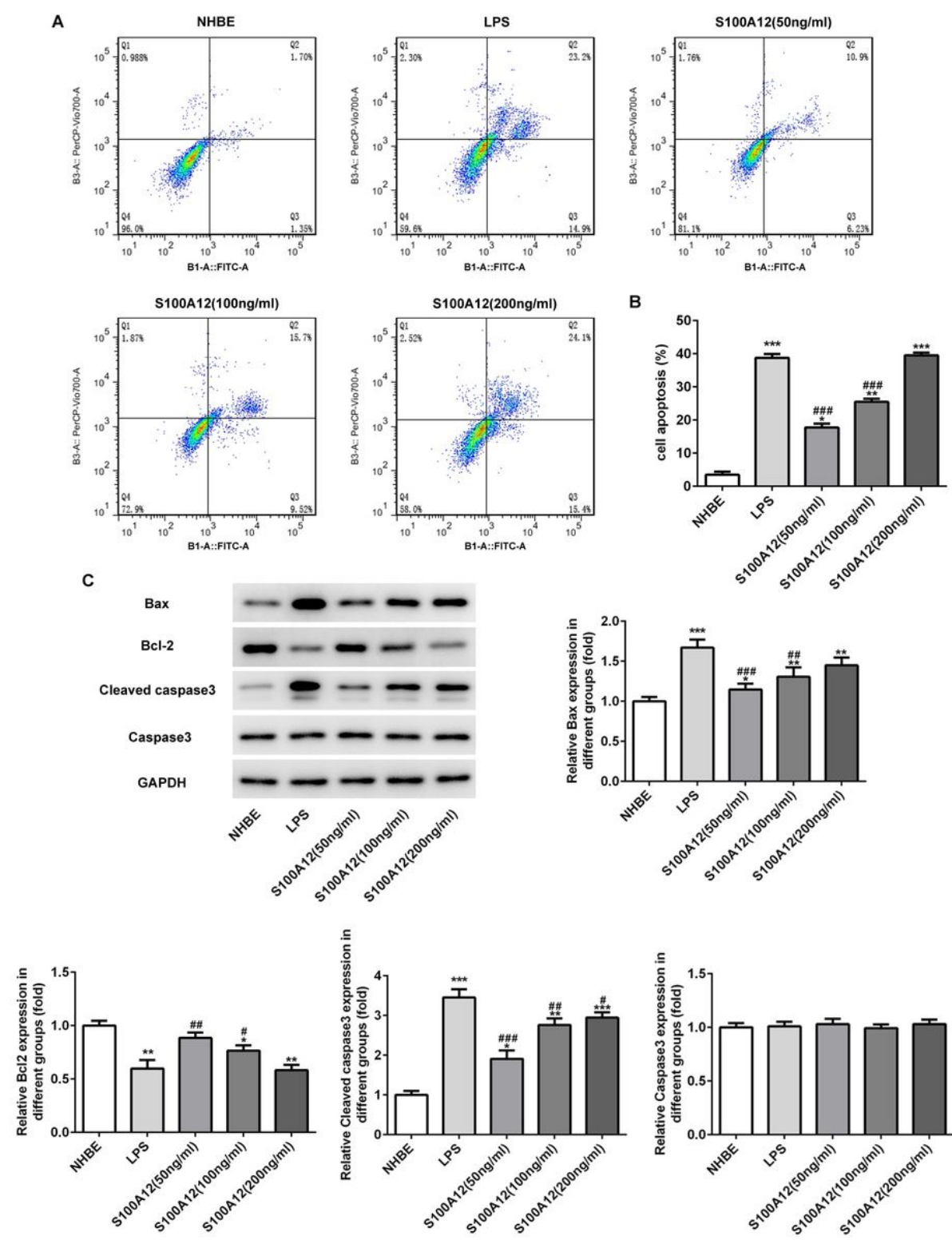

Figure 7

S100A12 promoted cell apoptosis in NHBE cells. (A-B) Cell apoptosis was detected by flow cytometry assay. (C) The expressions of Bax, Bcl-2, Cleaved caspase3 and Caspase3 were measured by western

blot. The data were expressed as means ± SD, ***P < 0.001 vs. NHBE. #P < 0.05, ##P < 0.01, ###P < 0.001 vs. NHBE.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementarymaterial.docx