

RESEARCH ARTICLE

WILEY

Journal of Cellular Biochemistry

Elevated S100A4 in asthmatics and an allergen-induced mouse asthma model

Xiaolin Huang¹ | Dongming Qu² | Yue Liang³ | Qinghua Huang² | Mengze Li² | Changchun Hou² 

¹The Department of Intensive Care Unit, The Second Affiliated Hospital of Guangxi Medical University, China

²The Department of Respiratory Medicine, The Second Affiliated Hospital of Guangxi Medical University, China

³The Department of Respiratory Medicine, The Eighth People's Hospital of Nanning City, Nanning, China

Correspondence

Changchun Hou, The Department of Respiratory Medicine, The Second Affiliated Hospital of Guangxi Medical University, Nanning 530021, China.
Email: houchangchun305@163.com

Funding information

National Natural Science Foundation of China, Grant/Award Number: 81600020; Natural Science Foundation of Guangxi, Grant/Award Number: 2016GXNSFBA380100

Abstract

The elevated S100A4 level has been found in some inflammatory diseases. However, the expression and role of S100A4 in asthma is unknown. The expression of S100A4 in induced sputum and plasma from healthy control and asthmatics were assessed by ELISA. Then an allergen-induced asthma mouse model treatment with anti-S100A4 antibody was used to explore the role of S100A4 in the pathogenesis of asthma. The S100A4 levels in sputum not in plasma in asthmatics were significantly increased than those of healthy controls and were negatively correlated with some lung function parameters and were positively correlated with sputum eosinophilia and lymphocyte. The expression of S100A4 in the lung as well as in BALF were also significantly higher in the asthma mouse model and treatment with anti-S100A4 antibody exhibited reductions in inflammatory cell accumulation, inflammatory mediators, and airway hyper-responsiveness. We further showed that LY294002, a specific inhibitor of PI3K, markedly decreased S100A4 expression in lung and S100A4 secretion in BALF in asthmatic mice. In conclusion, these data demonstrated that S100A4 may be involved in the pathogenesis of airway inflammation in asthma.

KEYWORDS

asthma, mouse model, S100A4

1 | INTRODUCTION

Asthma is one of the most common forms of respiratory disease. It is an obstructive airway disease that is characterized by airway inflammation, eosinophilic infiltration, immune hyper-responsiveness, and mucus hypersecretion.^{1,2} It has been demonstrated that a number of inflammatory mediators and cytokines play a critical role in the pathogenesis of this lung disease. Damage-associated molecular patterns (DAMPs) can activate the innate immune response by interacting with pattern recognition

receptors.³ Increased levels of several DAMPs, including HMGB1, HSP70, and S100A9 have been observed in induced sputum and serum of asthma patients in previous studies including ours,⁴⁻⁶ and have been implicated in the pathogenesis of asthma. S100A4 is a member of the S100 calcium-binding family. Once secreted through the action of specific chemokines, necrosis or stress, S100A4 can function as DAMPs.⁷ Several studies have demonstrated the role of S100A4 in promoting angiogenesis, cell motility and cell survival, and its ability to enhance tumor progression and metastasis.⁸ S100A4 released by tumor or stromal cells could induce the production of MMPs by endothelial and tumor cells.⁹ In addition, recent studies have demonstrated elevated S100A4 levels in several inflammatory diseases like

Huang and Qu have contributed equally to this study.

arthritis, neuronal injury and kidney fibrosis.^{10,11} Increased expression of S100A4 was found in resident chondrocytes in the cartilage of osteoarthritis patients. S100A4 stimulated production and release of MMP-13 from chondrocytes as well as cartilage explants in a RAGE-mediated signaling manner.¹² Importantly, stimulation of T cells with S100A4 lead to increased production of cytokines, particularly granulocyte colony-stimulating factor (GM-CSF) and eotaxin-2, both of which are involved in the pathogenesis of eosinophilic airway inflammation in asthma.¹³ Based on current knowledge, we hypothesized that S100A4 could be involved in the pathogenesis of asthma. To determine this, we measured S100A4 protein levels in induced sputum, plasma, lung tissue and BALF in the asthmatic mouse models. We then investigated the therapeutic effects of anti-S100A4 antibody administration by examining airway responsiveness, airway inflammation, and inflammatory mediators.

2 | MATERIALS AND METHODS

2.1 | Patient samples

Samples were collected from 133 patients (99 patients from the Department of Respiratory Medicine of the first affiliated hospital of Guangxi Medical University and 33 patients from the Eighth People's Hospital of Nanning City). Sixty-eight healthy controls (NS) were negative for respiratory diseases like asthma, COPD, and pneumonia. Asthmatic patients ($n = 65$) were initially diagnosed at the two hospitals mentioned above based on the Global Initiative for Asthma (GINA) guidelines.¹ Briefly, Asthma was defined as a reversible airflow obstruction with post-bronchodilator FEV1/FVC $\geq 70\%$ and with one or more of the following parameters: a increase in (FEV1) of $\geq 12\%$ with post-bronchodilator and post-bronchodilator FEV1 increase ≥ 200 mL or a bronchodilator response of $\geq 15\%$ or a fall in FEV1 of $\geq 15\%$ in the bronchial challenge test. Exclusion criteria for all patients included respiratory tract infection based on purulent sputum and X-ray results, oral steroid treatment or an exacerbation during the month before admission or a history of other lung diseases.

The ethics committee of Guangxi Medical University approved the study. All study participants provided informed consents.

2.2 | Pulmonary function tests

Pulmonary function tests before sputum induction was performed using the Jaeger Masterscope[®] spirometry system. Two reproducible measurements of FEV1, FEV1/FVC, and FEV1pred were obtained before and after inhalation of 400 mg Salbutamol.

2.3 | Sputum induction and plasma collection

Procedure for sputum induction and processing has been previously described.⁶ Sputum induced by inhalation of hypertonic saline was weighed and diluted 4 $\times$ in 0.1% dithiothreitol (Sigma-Aldrich, St. Louis) in water. The suspension was then shaken and incubated in a shaking water bath at 37°C. Samples were then centrifuged at 750g for 10 minutes and the supernatants were stored at -80°C . Blood samples were collected in ethylenediamine tetraacetic acid (EDTA)-anticoagulated tubes and stored at 4°C. Blood samples were then centrifuged at 1000g at 4°C for 15 minutes and plasma was stored in microfuge tubes at -80°C until measurements were performed.

2.4 | Animals

Balb/c female mice (6-8 week-old) were purchased from the Guangxi Medical University Animal Center and were housed under specific pathogen-free conditions in the animal center. The animal care and use committee of the Guangxi Medical University approved all animal experiments. Eight mice per group were used in this study. Ovalbumin-induced allergic airway inflammation mouse model.

The allergen-induced asthma murine model was produced as described in detail previously.¹⁴ Mice were divided into the PBS control group (PBS), ovalbumin (OVA) group, OVA+isotype antibody group, and the OVA+anti-S100A4 antibody group. Female mice were treated on days 0, 7, and 14 by intraperitoneal injections of 20 mg OVA (Sigma, St. Louis, MO; grade V) absorbed in 0.5 mg aluminum hydroxide and then challenged with OVA (40 mg per mouse) or PBS intranasally at day 21, 22, and 23. Anti-S100A4 neutralizing antibody (sigma; 100 $\mu\text{g}/\text{body}$) or a control IgG antibody (100 $\mu\text{g}/\text{body}$) was injected intraperitoneally 30 minutes before challenge.

In addition, we investigated the potential role of the PI3K/AKT pathway in regulating S100A4 expression and secretion in the OVA-induced murine asthmatic model. Female mice were divided into the control group (Control), ovalbumin (OVA) group, LY294002 (a specific inhibitor of PI3K) group, and OVA+LY294002 group. LY294002 (5 mg/kg, Sigma) or Control (polyethylene glycol, Sigma) was administered intra-tracheally 1 hour before each aerosol challenge.

2.5 | AHR measurement

Twenty-four hours after the final aerosol challenge, airway hyper-responsiveness (AHR) to methacholine (MCh; Sigma) was determined using body plethysmography

(Buxco Electronics). The procedure has been described previously in detail.¹⁴

2.6 | Broncho-alveolar lavage fluid and lung tissue collection

48 hours after the last challenge, mice were euthanized for collection of broncho-alveolar lavage fluid (BALF) and lung tissues. Total and differential cell counts were determined by hematoxylin and eosin (H&E). The right lung was removed and frozen in liquid nitrogen for enzyme-linked immunosorbent assays (ELISA) and Western blot analysis. For IHC, the left lung was fixed using 4% formaldehyde, then paraffin-embedded and stained with H&E to assess S100A4 expression and inflammatory infiltrates.

2.7 | Immunohistochemistry

IHC was performed as described previously.¹⁴ Deparaffinized sections (5-mm thick) blocked in goat serum were incubated with the anti-S100A4 antibody (1:300 dilution; Sigma). Sections were then washed and incubated with antirabbit HRP secondary antibody. Diaminobenzidine (Zymed Laboratories) was used as the substrate for immunoperoxidase reaction. All sections were stained with hematoxylin (JianCheng, China) for 2 minutes.

2.8 | Real-time PCR

Total RNA was extracted from lung tissues using Trizol reagent (Invitrogen) according to the manufacturer's protocol. RNA was then reverse-transcribed with a commercially available kit (Format, Thermo Fisher Scientific). Quantitative real-time PCR was performed using the ABI 7900 real-time PCR System and SYBR Premix EX Taq (Takana, Japan). The following primer sequences were used:

β -actin: 5-AAGAGAGGCATCCTCACCT-3

and 5-TACATGGCTGGGGTGTGAA-3;

S100A4 : 5-ATTTCTGCCAGAGCCGCTTCTACT-3

and 5-CAGTTTGTATCCGGCAAAGTAGTA-3. β -actin

was used as the control.

2.9 | Western blot analysis

The Western blot analysis procedure has been described previously.¹⁴ Lung tissues were incubated in lysis buffer (Biyuntian; China) and amount of total protein extracted was determined using the BCA Protein Assay Kit (Biyuntian). Protein samples were loaded in equal amounts and electrophoresed using a sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE). Proteins were

then transferred onto nitrocellulose membranes (PALL) and incubated with rabbit polyclonal Abs against S100A4 (Sigma) or rabbit Abs against β -actin (Cell Signaling Biotechnology). β -actin expression was used as the internal control. Protein bands were detected using the ECL system (Pierce).

2.10 | Enzyme-linked immunosorbent assay

Protein levels of IL-4, IL-5, IL-13, eotaxin-2, GM-CSF, and S100A4 in BALF and induced sputum were determined using ELISA. ELISA kits were purchased from R&D Systems (Minneapolis, MN) except for the S100A4 ELISA kit, which was purchased from CUSABIO Biotechnology Company (Wuhan, China).

2.11 | Statistical analysis

Data were analyzed using the SPSS 13.0 software. Results were expressed as means $\pm$ SE. Comparisons between groups were performed using Student *t* test or ANOVA followed by least significant difference (LSD) post hoc comparison. Frequency data were analyzed by χ^2 test. Correlations analysis was performed using Spearman's rank correlation. *P*-values less than 0.05 were considered statistically significant.

3 | RESULTS

3.1 | Clinical study participant characteristics

Clinical characteristics of all study participants are shown in Table 1. The two groups were similar for age, gender, the number of cigarettes smoked and body mass index (BMI). Participants with asthma had higher counts of cells in the sputum, percentage of neutrophils and eosinophils, and significantly lower lung function and percentage of macrophages compared with healthy controls.

3.2 | Increased sputum S100A4 levels correlate with number of sputum eosinophils and severity of asthma

Compared with NS (198.62 ± 11.92 ng/mL; Figure 1A), S100A4 levels in induced sputum were significantly higher in patients with asthma (416.92 ± 35.40 ng/mL, $P = 0.000$). However, there were no differences in plasma S100A4 levels between the healthy control group (56.72 ± 5.15 ng/mL; Figure 1A) and the asthma group (65.64 ± 5.63 ng/mL; $P = 0.244$).

TABLE 1 The clinical characteristics of all participants

Characteristic	Health control (NS)	Asthma	P value
n	68	65	
Sex (M/F)	42/26	38/27	0.698
Age, yr	53.57 ± 1.11	51.67 ± 1.58	0.316
BMI	23.12 ± 0.55	22.14 ± 0.39	0.152
Never/current/ex-smokers	42/22/20	41/8/19	
Smoking pack years	10.49 ± 1.97	8.59 ± 1.67	0.166
Family asthma history (Y/N)	4/64	15/50	0.000
Allergic rhinitis (Y/N)	5/63	21/44	0.000
ICS use (Y/N)	0/68	0/67	
Post bronchodilator			
FEV1 (L)	3.16 ± 0.09	2.17 ± 0.11	0.000
FEV1 pred%	96.21 ± 1.44	74.71 ± 3.01	0.000
FEV1/FVC%	88.48 ± 0.71	78.31 ± 1.55	0.000
Daily score		5.88 ± 0.36&	
Nighttime score		1.02 ± 0.16&	
Sputum cell counts			
TCC (10 ⁶ /mL)	3.87 ± 0.14	4.46 ± 0.17	0.007
Macrophages%	56.88 ± 1.46	43.39 ± 2.14	0.000
Neutrophils%	39.16 ± 1.39	44.39 ± 2.11	0.037
Lymphocytes%	2.83 ± 0.35	5.13 ± 0.62	0.001
Eosinophils%	1.03 ± 0.16	7.11 ± 0.95	0.000

Abbreviations: BMI, body mass index; FEV1, forced expiratory volume in 1s (FEV1); FVC, forced vital capacity; TCC, total cell count.

Data are means ± SEM.

Interestingly, sputum S100A4 levels for all participants showed a significant positive correlation with sputum eosinophil counts and percentages, as well as sputum lymphocyte counts and percentages (Table 2). In addition, sputum S100A4 levels positively correlated with eotaxin-2 and GM-CSF levels in sputum, which are important eosinophil chemo-attractants and pro-survival cytokines. Additionally, sputum S100A4 levels correlated negatively with FEV1, FEV1% pred, and FEV1/FVC%, which are generally considered to reflect the severity of asthma.

3.3 | Higher expression of S100A4 in OVA-induced asthma mouse model

Immunohistochemistry analysis showed weak S100A4 expression in a number of inflammatory cells including macrophages and granulocytes in mice from the control group (Figure 2A). However, mice in the asthma mouse model group had moderate expression levels of S100A4 in inflammatory and vascular smooth muscle cells, with prominent S100A4 expression in granulocytes (Figure 2B and 2C). In addition, S100A4 was weakly stained in a number of epithelial cells in asthmatic mice. Compared to control mice, the number of positive stained S100A4 cells were significantly increased in asthmatic mice.

Both mRNA and protein expression of S100A4 were significantly increased in lung tissues from asthmatic mice compared to PBS control mice (Shown in Figure 3A and 3B). Furthermore, S100A4 levels were higher in BALF from asthmatic mice compared to PBS control mice. Blocking S100A4 activity by administering anti-S100A4 antibodies significantly decreased S100A4 levels in BALF (Figure 3C).

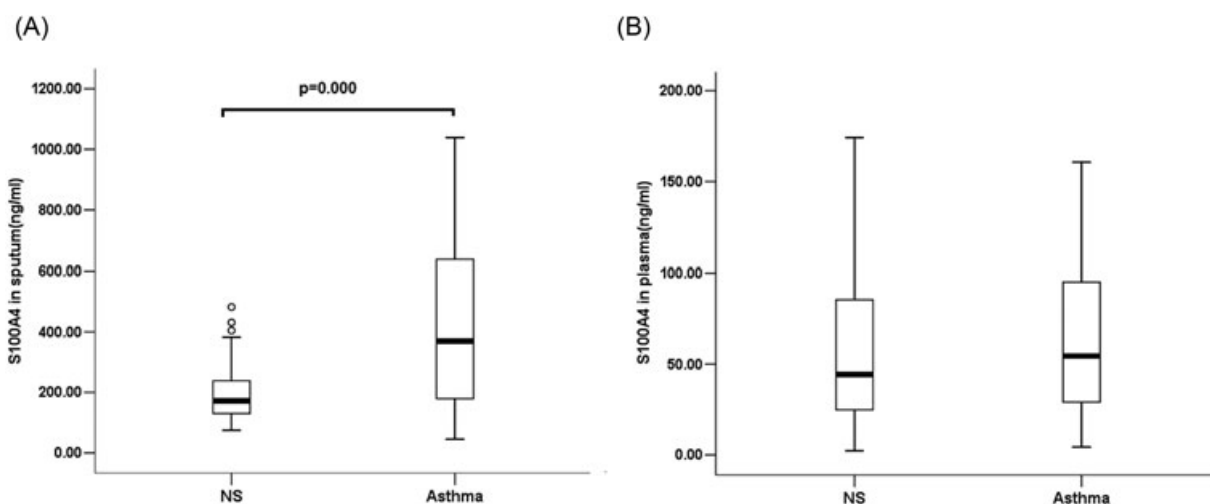


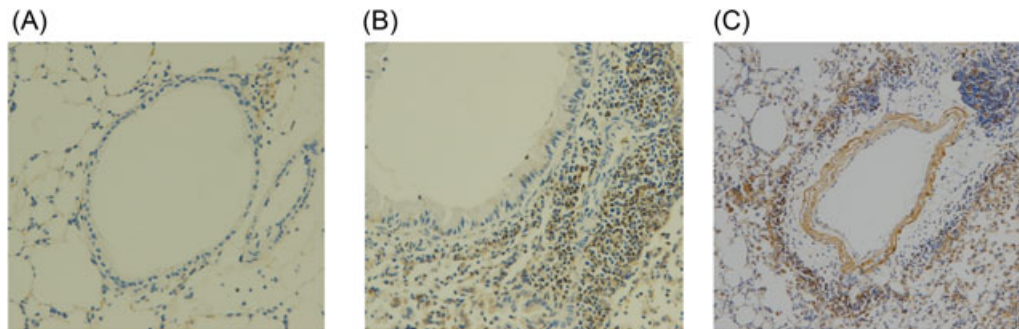
FIGURE 1 Expression levels of S100A4 in plasma and induced sputum from asthmatics. Lines inside the boxes represent median values, while the boxes represent the 25% to 75% and the lines outside the boxes are 10% and 90%. NS, healthy control

TABLE 2 Correlations between sputum S100A4 level and eotaxin-2, GM-CSF and clinical characteristics and in all study subjects (N = 133)

	S100A4	P value		S100A4	P value
Eotaxin-2	0.462	0.000	TCC (10^6 /mL)	0.169	0.056
GM-CSF	0.455	0.000	Lymphocyte %	0.176	0.045
FEV1(L)	-0.487	0.000	Lymphocyte counts	0.221	0.012
FEV1 pred%	-0.383	0.000	Eosinophils %	0.527	0.000
FEV1/FVC%	-0.440	0.000	Eosinophils counts	0.520	0.000

Abbreviations: FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity.

Data are Spearman rank correlation coefficients between variables.

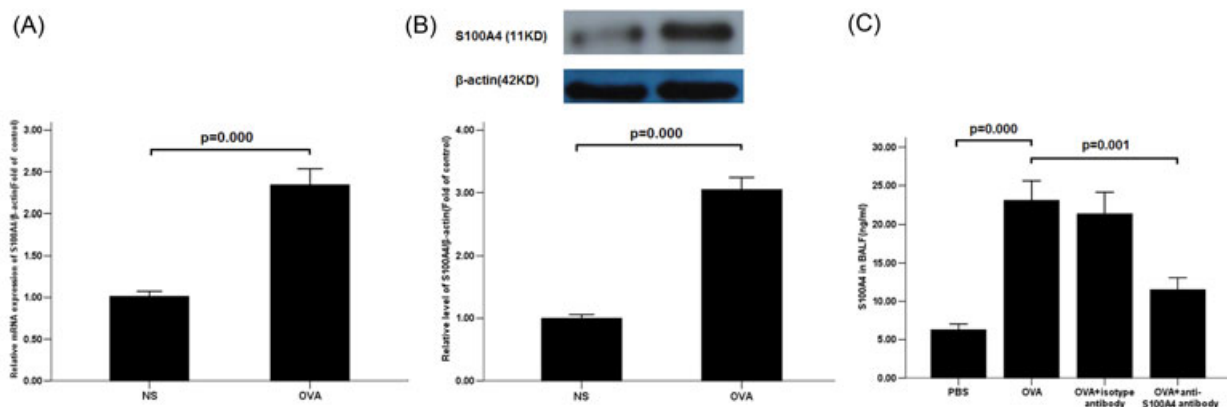
**FIGURE 2** Immunohistochemistry analysis of S100A4 in lung tissue sections from OVA-induced murine asthmatic models. A, PBS control group (PBS). B, OVA-induced asthmatic model group (OVA) (Bronchial area; S100A4 [dark brown], 200 $\times$) C, OVA-induced asthmatic model group (OVA) (perivascular area; S100A4 [dark brown], 200 $\times$)

3.4 | Treatment with anti-S100A4 antibody suppresses antigen-induced airway inflammation

H&E-stained histological analysis showed dramatic inflammation responses in the perivascular and peribronchial areas in OVA-challenged mice (Figure 4B). The massive eosinophil and lymphocyte recruitment into the peribronchial and perivascular areas in OVA-challenged mice were suppressed in the OVA+anti-S100A4 antibody administered group

(Figure 4D). In OVA-induced asthmatic mice, total cell numbers, as well as the number of eosinophils in BALF, were dramatically increased (Figure 5A); while anti-S100A4 antibody administration reduced the number of eosinophils and lymphocytes in BALF (Figure 5A).

As expected, OVA-challenged mice had a dramatic increase in AHR but were reduced when treated with an anti-S100A4 antibody (Figure 5B). In the BALF of OVA-induced asthmatic mice, there was an elevated production of IL-4, IL-5, and IL-13 as well as eotaxin-2 and GM-CSF,

**FIGURE 3** S100A4 expression in lung tissues and BALF in the allergen-induced murine asthmatic model. A, S100A4 mRNA expression in lung tissues by Real-Time PCR. B, S100A4 protein levels in lung tissues. C, S100A4 levels in BALF (NS, control group; OVA, OVA-induced acute asthmatic model group; Data are mean $\pm$ SEM, n = 8/group). BALF, broncho-alveolar lavage fluid

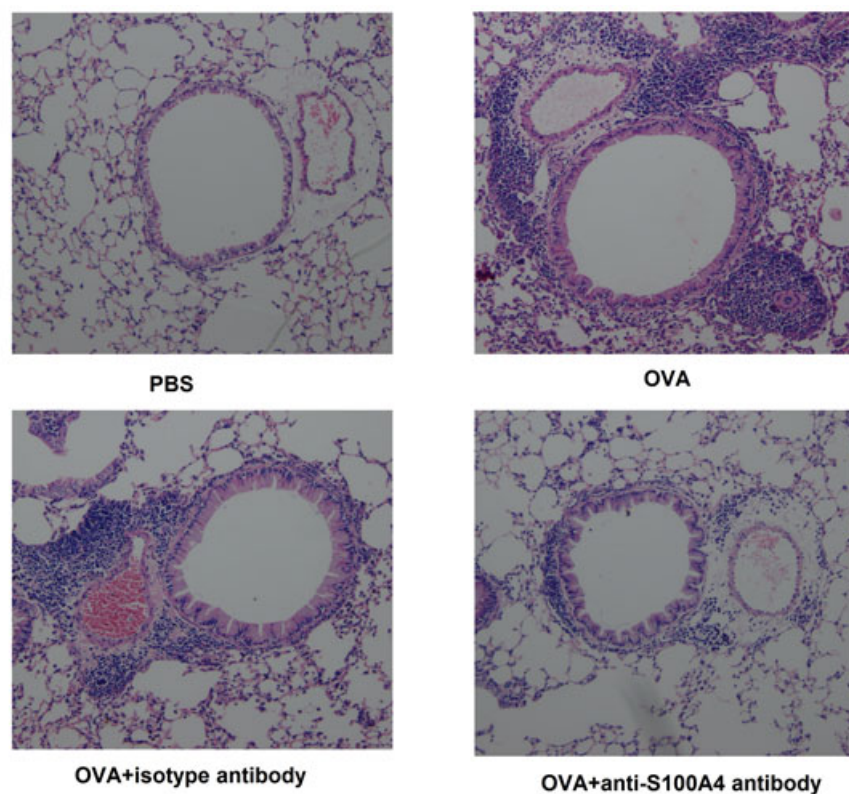


FIGURE 4 Representative images of H&E-stained lung tissue sections from mice (*100). (PBS, control mice; OVA, OVA-induced acute asthmatic mice). H&E, hematoxylin and eosin

but all were significantly reduced after anti-S100A4 antibody administration (Figure 5C and 5D). Additionally, the levels of S100A4 in BALF of all mice were positively correlated to the levels of eotaxin-2, GM-CSF and eosinophil, and lymphocyte counts in BALF (Table 3).

3.5 | Inhibition of the PI3K/AKT pathway decreased S100A4 expression and secretion in OVA-induced murine asthmatic mice

Although it is well documented that S100A4 is over-expressed in cancer cells, immune cells, and vascular cells in a number of non-cancer human disorders, the exact molecular mechanisms for this increase in expression in asthma remains to be deciphered. Several recent publications have demonstrated that the PI3K/AKT signaling pathway may play a role in the upregulation of S100A4 in breast cancer cells and pulmonary epithelial cells.^{15,16} Hence we investigated whether the PI3K/AKT pathway mediates S100A4 expression and secretion in lung tissue using LY294002, a specific inhibitor of PI3K. As shown in Figure 6A and 6E, OVA challenge induced marked infiltration of inflammatory cells into the peribronchiolar and perivascular regions and increased phosphorylation of AKT. LY294002 (5 mg/kg) significantly attenuated leukocyte infiltration compared with the OVA group. This was accompanied by a decrease in

AKT phosphorylation. Both mRNA and protein expression of S100A4 was significantly decreased in lung tissues from OVA+LY294002 mice compared to mice in the OVA group (Figure 6B and 6C). Treatment with LY294002 led to an obvious decrease in S100A4 levels in the BALF of mice compared to mice in the OVA-challenged group (Figure 6D). Collectively, the data suggest that the PI3K/AKT signaling pathway was involved in the upregulation and secretion of S100A4 in allergen-induced murine asthmatic models.

4 | DISCUSSION

Our results suggest that S100A4 may play an important role in the pathogenesis of airway inflammation in asthma. First, we found that S100A4 levels were increased in the induced sputum of patients with asthma. Moreover, we observed that sputum S100A4 levels were significantly elevated in asthmatics and positively correlated with sputum eosinophil and lymphocyte counts, as well as sputum eotaxin-2 and GM-CSF levels. More importantly, sputum S100A4 levels showed a negative correlation with FEV1, FEV1% pred, and FEV1/FVC%.

Using an OVA-induced acute asthma mouse model, we confirmed that S100A4 expression levels were elevated and treatment with anti-S100A4 antibody suppressed TH2-mediated airway inflammation, AHR, eotaxin-2, and

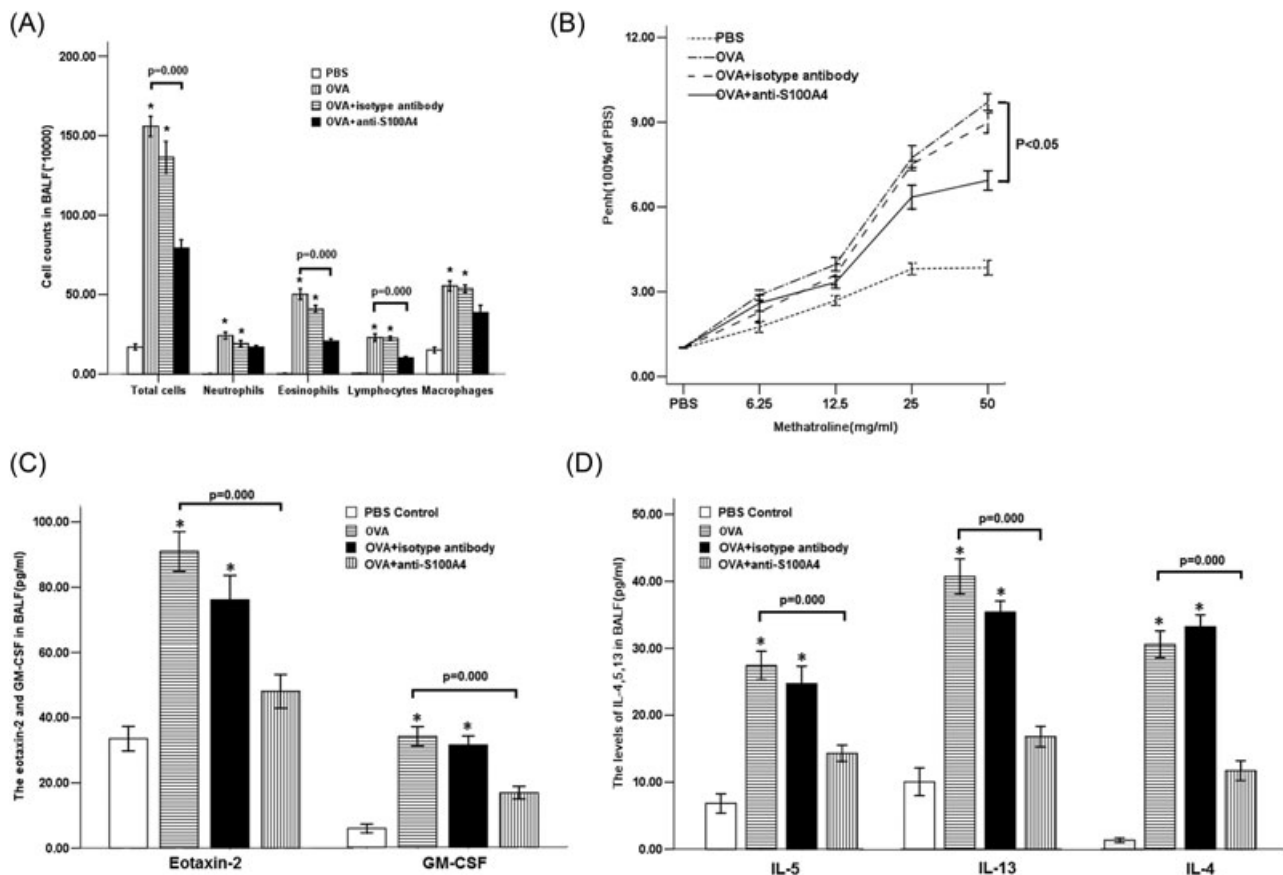


FIGURE 5 Effect of anti-S100A4 antibody on OVA-induced acute airway inflammation. A, The change of inflammatory cells in BALF. B, Effect of anti-S100A4 on OVA-induced airway hyperreactivity. C, Levels of eotaxin-2 and GM-CSF in BALF detected by ELISA ($n = 8$). D, Levels of IL-4, IL-5 and IL-13 in BALF detected by ELISA ($n = 8$). (BALF, broncho-alveolar lavage fluid; PBS, control mice; OVA, OVA-induced acute asthmatic mice; * $P < 0.05$ versus control group; Data are mean $\pm$ SEM, $n = 8$ /group)

GM-CSF levels in BALF. To the best of our knowledge, this is the first report to investigate the role of S100A4 in the development of asthmatic airway inflammation.

S100A4, a member of the S100 calcium-binding family, has been investigated in several cancers for its ability to induce cell motility, invasion and angiogenesis.¹⁷ However elevated S100A4 levels have also been recently found in a number of other diseases including several inflammatory diseases, and kidney and cardiac fibrosis.¹⁰ Rheumatoid

arthritis (RA) is a chronic inflammatory joint disease. Expression of S100A4 in cells from RA synovial tissue and plasma were found to be increased compared to healthy controls.¹⁸ S100A4 also has been implicated in the development of several lung diseases. Expression of S100A4 was significantly increased in a dose- and time-dependent manner in lung tissues of bleomycin-induced murine models for pulmonary fibrosis and localized mainly in lung fibroblasts.¹⁸ S100A4, used as a marker for EMT, stained weakly in a number of biopsy epithelial cells from patients with stable lung allografts.¹⁹ S100A4 mRNA and protein expression was elevated in pulmonary arteries of mice treated with hypoxia. S100A4 protein was specifically localized in vascular smooth muscle cells and fibroblasts as demonstrated by immunohistochemistry analysis.²⁰ To the best of our knowledge, this is the first report to demonstrate that sputum S100A4 levels from asthmatics were significantly increased compared to healthy subjects and were correlated with the patients' disease severity.

It is unclear what type of cells in the lungs are responsible for S100A4 secretion; however, cell types like

TABLE 3 Correlations between S100A4 level in BALF and other variables (including eotaxin-2, GM-CSF, and eosinophil and lymphocyte counts in BALF in all mice ($N = 32$))

	S100A4	P value
Eotaxin-2	0.725	0.000
GM-CSF	0.668	0.000
Eosinophil counts	0.634	0.000
Lymphocyte counts	0.605	0.000

Data are Spearman rank correlation coefficients between variables. Abbreviations: BALF, broncho-alveolar lavage fluid; GM-CSF, granulocyte/macrophage colony-stimulating factor.

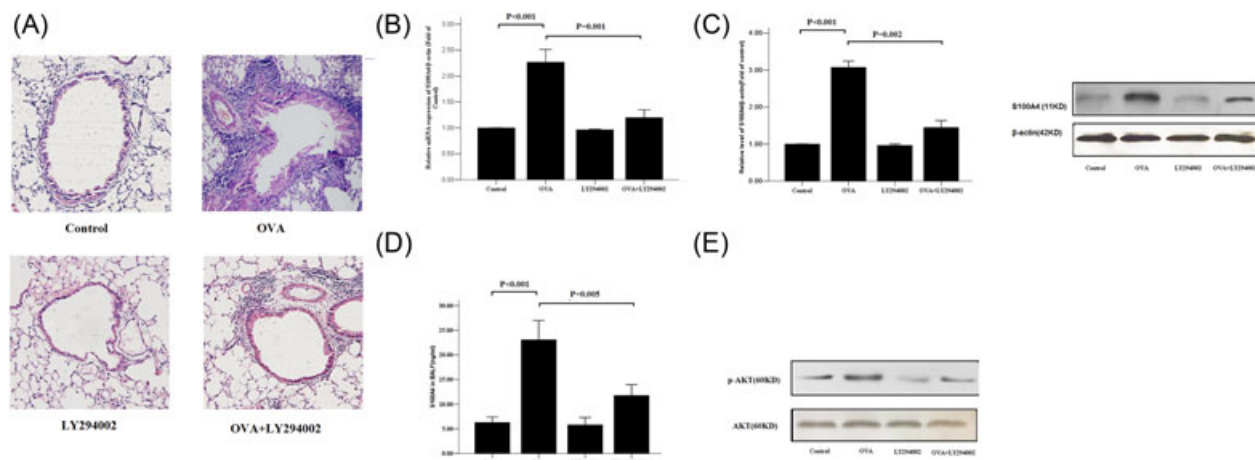


FIGURE 6 Inhibition of PI3K/AKT pathway decreased S100A4 expression and secretion in an OVA-induced murine asthmatic model. A, Representative images of H&E-stained lung tissue sections from mice ($\times 100$). B, S100A4 mRNA expression in lung tissues by Real-Time PCR. C, S100A4 protein levels in lung tissues detected by Western Blot. D, Levels of S100A4 in BALF detected by ELISA ($n = 8$). E, Quantification of p-AKT expression in lung tissues from mice. (OVA, OVA-induced acute asthmatic model group; Data are mean $\pm$ SEM, $n = 8$ /group). BALF, bronchoalveolar lavage fluid

pulmonary artery smooth muscle cells,²¹ fibroblasts,²⁰ and several cancer cells¹⁰ have been identified to secrete S100A4. S100A4 immuno-staining in lung tissues in our study showed that S100A4-stained cells were mainly infiltrating inflammatory cells especially granulocyte and vascular smooth muscle cells. Furthermore, the number of S100A4-positive cells in the interstitial and perivascular regions were significantly increased in OVA-induced asthmatic mice compared to PBS control mice. This suggests that inflammatory cells, especially granulocytes and vascular smooth muscle cells are potential sources of S100A4 in the airway and lungs. However, further studies need to be performed to confirm the cell types responsible for S100A4 secretion and determine the mechanism underlying the secretion process.

Extracellular S100A4 can affect numerous cell types by binding to their receptors. Epidermal growth factor receptor (EGFR)²² and the Receptor for Advanced Glycation End products (RAGE)²³ have been shown to be the extracellular receptors for S100A4. S100A4 can enhance EGFR/ErbB2 receptor signaling pathway to induce cell proliferation and promote tumor progression.²² In addition, extracellular S100A4 can also interact with RAGE to increase plasmin levels and thus induce tube formation in endothelial cells.²³ More importantly, S100A4 plays a crucial role in leukocyte migration, which has been associated with the pathogenesis of inflammatory diseases. S100A4 can induce the secretion of cytokines, particularly eotaxin-2 and GM-CSF from T lymphocytes¹³ and hence promote the invasion of T lymphocytes into primary tumors. The expression of S100A4 is induced during macrophage or granulocyte differentiation of HL-60 cells and is expressed in

macrophages and polymorphonuclear leukocytes. In addition, S100A4 has been shown to affect the motility of leukocytes.²⁴ Based on these observations above we hypothesized that the potential role of S100A4 in asthmatic airway inflammation maybe partially attributed to its ability to promote infiltration of inflammatory cells. In our present study, we found that S100A4 levels in the sputum and BALF were positively correlated to the number of eosinophils and lymphocytes. Consistent with these findings,¹³ S100A4 levels in the sputum and BALF were positively correlated with the levels of GM-CSF and eotaxin-2, both of which play critical roles in promoting survival, maturation, and chemotaxis of eosinophils. We hypothesized that S100A4 could indirectly promote mobility and infiltration of eosinophils into the airways of asthma patients by interacting with GM-CSF and eotaxin-2. Interestingly human eosinophils express S100A4 and RAGE (S100A4 receptor),²⁵ which suggests that S100A4 may have a direct effect on the function of eosinophils. We plan to investigate this in future studies. Furthermore, by blocking S100A4 activity, the increased number of eosinophils and lymphocytes and the increased levels of GM-CSF, eotaxin-2 and TH2 cytokines decreased significantly in the acute asthma mouse model. These results indicate the important role of S100A4 in the upregulation of eosinophilic airway inflammation.

It is well documented that S100A4 is involved in promoting cancer progression, metastasis, and various nontumor disorders. Increased expression of S100A4 has been reported in various diseases and different types of cells. However, the exact molecular mechanisms mediating S100A4 expression and secretion remains to be deciphered. Akt plays a key role in cell survival,

proliferation, protein synthesis, growth, and migration. Liu et al.¹⁵ demonstrated that hepatitis B X-interacting protein could activate the PTEN/PI3K/AKT signaling pathway to subsequently induce S100A4 expression. MK-2206, a pan Akt inhibitor or Akt knockdown reversed cigarette smoke-induced S100A4 expression in pulmonary epithelial cells.¹⁶ Consistent with these studies, we found that inhibition of PI3K/AKT signaling by LY294002 suppressed S100A4 expression in lung tissue and S100A4 secretion in BALF. Expression of S100A4 has been reported to be affected by methylation, β -catenin, and extracellular factors such as IL-7, ATP, and 5-HT.^{21,26,27} However, more studies are needed to investigate the mechanisms that mediate S100A4 expression and secretion in asthma.

In our present study, we observed that S100A4 levels in induced sputum were negatively and significantly correlated with FEV1, FEV1% pred and FEV1/FVC%, which are well-known parameters that reflect disease severity. The induced sputum test is a simple and noninvasive procedure to assess airway inflammation.²⁸ Our findings suggest that S100A4 levels in sputum may be a novel biomarker to determine disease severity of asthma. In addition, our results indicate that S100A4 may participate in the development of asthma. However, further studies are needed to confirm our findings.

In conclusion, we demonstrated that elevated sputum S100A4 levels in asthmatics were associated with disease severity and anti-S100A4 antibody administration resulted in decreased eosinophilic allergic airway inflammation in an OVA-induced acute asthma model. Our data suggest that S100A4 may participate in the pathogenesis of Asthma. However further studies are needed to determine the role and mechanism of S100A4 in asthma.

ACKNOWLEDGMENTS

This study was supported by the National Natural Science Foundation of China (No: 81600020) and the Natural Science Foundation of GuangXi (No: 2016GXNSFBA380100).

ORCID

Changchun Hou  <http://orcid.org/0000-0001-6246-2241>

REFERENCES

- GINA. The Global Strategy for Asthma Management and Prevention; 2016.
- Galli SJ, Tsai M, Piliponsky AM. The development of allergic inflammation. *Nature*. 2008;454(7203):445-454.
- Matzinger P. Tolerance, danger, and the extended family. *Annu Rev Immunol*. 1994;12:991-1045.
- Hou C, Zhao H, Liu L, et al. High mobility group protein B1 (HMGB1) in Asthma: comparison of patients with chronic obstructive pulmonary disease and healthy controls. *Mol Med*. 2011;17:807-815.
- Changchun H, Haijin Z, Wenjun L, et al. Increased heat shock protein 70 levels in induced sputum and plasma correlate with severity of asthma patients. *Cell Stress Chaperones*. 2011;16(6):663-71.
- Lee TH, Jang AS, Park JS, et al. Elevation of S100 calcium binding protein A9 in sputum of neutrophilic inflammation in severe uncontrolled asthma. *Ann Allergy Asthma Immunol*. 2013;111(4):268-275.
- Donato R, R. cannon B, Sorci G, et al. Functions of S100 Proteins. *Curr Mol Med*. 2013;13(1):24-57.
- Boye K, Mælendsmo GM. S100A4 and metastasis: a small actor playing many roles. *Am J Pathol*. 2010;176(2):528-535.
- Schmidt-Hansen B, Örnås D, Grigorian M, et al. Extracellular S100A4(mts1) stimulates invasive growth of mouse endothelial cells and modulates MMP-13 matrix metalloproteinase activity. *Oncogene*. 2004;23(32):5487-5495.
- Schneider M, Hansen JL, Sheikh SP. S100A4: a common mediator of epithelial-mesenchymal transition, fibrosis and regeneration in diseases? *J Mol Med*. 2008;86(5):507-522.
- Senolt L, Grigorian M, Lukanidin E, et al. S100A4 is expressed at site of invasion in rheumatoid arthritis synovium and modulates production of matrix metalloproteinases. *Ann Rheum Dis*. 2006;65(12):1645-1648.
- Yammani RR, Carlson CS, Bresnick AR, Loeser RF. Increase in production of matrix metalloproteinase 13 by human articular chondrocytes due to stimulation with S100A4: role of the receptor for advanced glycation end products. *Arthritis Rheum*. 2006;54(9):2901-2911.
- Grum-Schwensen B, Klingelhöfer J, Grigorian M, et al. Lung metastasis fails in MMTV-PyMT oncomice lacking S100A4 due to a T-cell deficiency in primary tumors. *Cancer Res*. 2010;70(3):936-947.
- Hou C, Kong J, Liang Y, et al. HMGB1 contributes to allergen-induced airway remodeling in a murine model of chronic asthma by modulating airway inflammation and activating lung fibroblasts. *Cell Mol Immunol*. 2015;12(4):409-423.
- Liu S, Li L, Zhang Y, et al. The oncoprotein HBXIP uses two pathways to up-regulate S100A4 in promotion of growth and migration of breast cancer cells. *J Biol Chem*. 2012;287(36):30228-30239.
- Jiang B, Guan Y, Shen H, et al. Akt/PKB signaling regulates cigarette smoke-induced pulmonary epithelial-mesenchymal transition. *Lung Cancer*. 2018;122:44-53.
- Boye K, Mælendsmo GM. S100A4 and metastasis: a small actor playing many roles. *Am J Pathol*. 2010;176(2):528-535.
- Klingelhöfer J, Šenolt L, Baslund B, et al. Up-regulation of metastasis-promoting S100A4 (Mts-1) in rheumatoid arthritis: putative involvement in the pathogenesis of rheumatoid arthritis. *Arthritis Rheum*. 2007;56(3):779-789.
- Lawson WE, Polosukhin VV, Zoia O, et al. Characterization of fibroblast-specific protein 1 in pulmonary fibrosis. *Am J Respir Crit Care Med*. 2005;171(8):899-907.
- Ward C, Forrest IA, Murphy DM, et al. Phenotype of airway epithelial cells suggests epithelial to mesenchymal cell transition in clinically stable lung transplant recipients. *Thorax*. 2005;60(10):865-871.

21. Lawrie A, Spiekerkoetter E, Martinez EC, et al. Interdependent serotonin transporter and receptor pathways regulate S100A4/Mts1, a gene associated with pulmonary vascular disease. *Circ Res*. 2005;97(3):227-235.
22. Klingelhöfer J, Möller HD, Sumer EU, et al. Epidermal growth factor receptor ligands as new extracellular targets for the metastasis-promoting S100A4 protein. *FEBS J*. 2009;276(20):5936-5948.
23. Semov A, Moreno MJ, Onichtchenko A, et al. Metastasis-associated protein S100A4 induces angiogenesis through interaction with Annexin II and accelerated plasmin formation. *J Biol Chem*. 2005;280(21):20833-20841.
24. Takenaga K, Nakamura Y, Sakiyama S. Expression of a calcium binding protein pEL98 (mts1) during differentiation of human promyelocytic leukemia HL-60 cells. *Biochem Biophys Res Commun*. 1994;202(1):94-101.
25. Curran CS, Bertics PJ. Human eosinophils express RAGE, produce RAGE ligands, exhibit PKC-delta phosphorylation and enhanced viability in response to the RAGE ligand, S100B. *Int Immunol*. 2011;23(12):713-728.
26. Liu Y, Geng YH, Yang H, et al. Extracellular ATP drives breast cancer cell migration and metastasis via S100A4 production by cancer cells and fibroblasts. *Cancer Lett*. 2018;430:1-10.
27. Yammani RR, Long D, Loeser RF. Interleukin-7 stimulates secretion of S100A4 by activating the JAK/STAT signaling pathway in human articular chondrocytes. *Arthritis Rheum*. 2009;60(3):792-800.
28. Fahy JV, Wong H, Liu J, Boushey HA. Comparison of samples collected by sputum induction and bronchoscopy from asthmatic and healthy subjects. *Am J Respir Crit Care Med*. 1995;152(1):53-58.

How to cite this article: Huang X, Qu D, Liang Y, Huang Q, Li M, Hou C. Elevated S100A4 in asthmatics and an allergen-induced mouse asthma model. *J Cell Biochem*. 2018;1-10.
<https://doi.org/10.1002/jcb.28245>