Int J Clin Exp Pathol 2015;8(10):12053-12063
www.ijcep.com /ISSN:1936-2625/IJCEP0013914

Original Article

Downregulation of SUMF2 gene in ovalbumin-induced rat model of allergic inflammation

Chuanfeng Fang, Xiaoxia Li, Hongyan Liang, Li Xue, Lei Liu, Chun Yang, Guangqiang Gao, Xiaofeng Jiang

Department of Clinical Diagnosis, The Fourth Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang Province, China

Received August 3, 2015; Accepted September 20, 2015; Epub October 1, 2015; Published October 15, 2015

Abstract: Sulfate-modifying factor 2 (SUMF2), a member of the formylglycine-generating enzyme family, was earlier found to play a role in the regulation of interleukin (IL)-13 expression and secretion in airway smooth muscle cells. IL-13 is a T helper 2 cytokine that plays important roles in the pathogenesis of asthma. However, there is little evidence of the potential role of SUMF2 in the cellular inflammatory responses in asthma. Here, using an ovalbumin-induced asthma rat model, we show that SUMF2 gene expression is significantly decreased in allergic asthma rats. Moreover, several pathological changes were observed in the lung tissue and IL-13 was found to be overexpressed in the ovalbumin-induced asthma model. Additional studies on the lung bronchial epithelial tissues, peripheral blood lymphocytes and bronchoalveolar lavage fluid of the OVA-induced asthma rats showed that SUMF2 mRNA and protein expression were attenuated. However, there was only a little significant correlation was found between SUMF2 and IL-13 expression. These results indicate that SUMF2 may mediate airway inflammation in allergic asthma by modulating the expression of IL-13. More data from in vivo experiments are needed to clearly understand the role of SUMF2 in asthma.

Keywords: Allergic asthma, interleukin-13, SUMF2 gene, OVA, airways inflammation, airway smooth muscle cells

Introduction

Asthma is a Th2-cell dependent, IgE-mediated allergic disease. The pathogenesis of this disease is characterized by airway hyperactivity, mucus overproduction and inflammatory cell infiltration [1-3]. Epidemiological studies have shown the worldwide incidence of asthma to be 5%-16% [4]. The clinical signs and symptoms of asthma are observed very early; they include exacerbation of anaphylaxis, increase in symptom severity, and increase in lung function defects with age.

Interleukin-13 (IL-13) exacerbates asthmatic airway obstruction by increasing hyperplasia of mucous cells in the airway and by promoting mucus secretion [5]. In addition, IL-13 increases the sensitivity of smooth muscle cells of the airway to acetylcholine, which is a causative factor for the initiation and progression of airway hyperresponsiveness. Moreover, IL-13 upregulates the expression of transforming growth factor in airway epithelial cells [6, 7]. IL-13 plays a key role in the gradual deterioration in the progress of an allergic reaction [8-10], which is an important factor in Th2-mediated cellular inflammation and allergic reaction in asthma [10]. Further, both IL-13 and IL-4 are specifically involved in enhancing the synthesis of IgE [8, 9]. In the last decade, the crucial role of IL-13 in the initiation and exacerbation of the pathogenesis of asthma has received attention [9-11]. Through its combined actions on the epithelial and smooth muscle cells, IL-13 can induce all the pathological mechanisms of asthma independently of traditional effector cells such as mast cells and eosinophils [12]. More recently, IL-13 overexpression has been shown to be associated with inflammation-induced genotoxicity in asthma that extended beyond the primary site of the lung to circulating leukocytes and erythroblasts in the bone marrow and elicited systemic effects [13]. However, the molecular mechanisms underlying these inflammation-associated effects of IL-13 are not clearly known, despite recent research into this cytokine [10, 11, 14].

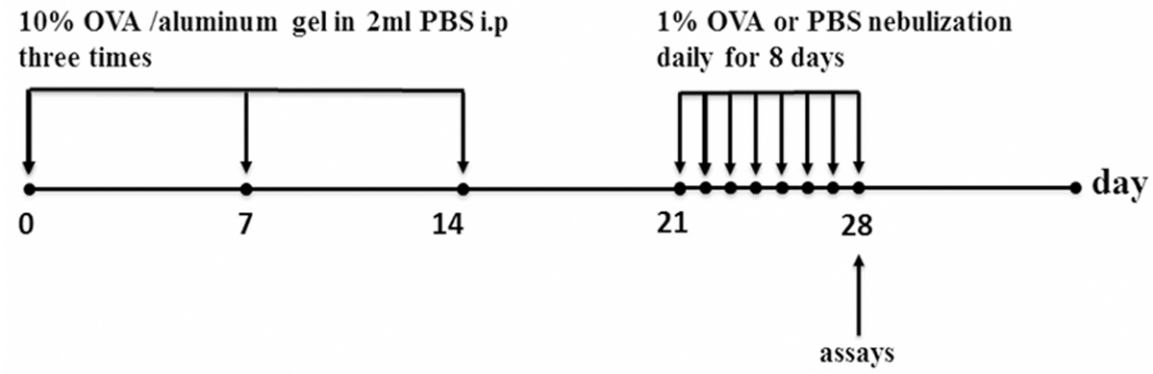

Figure 1. The protocol for OVA-induced experimental asthma. In the asthma model, rats were sensitized by three intraperitoneal injections of OVA on days 0, 7 and 14, after which they were administered the aerosolized OVA challenge on days 21-28 using a nebulizer. OVA: Ovalbumin, PBS: Phosphate-buffered saline.

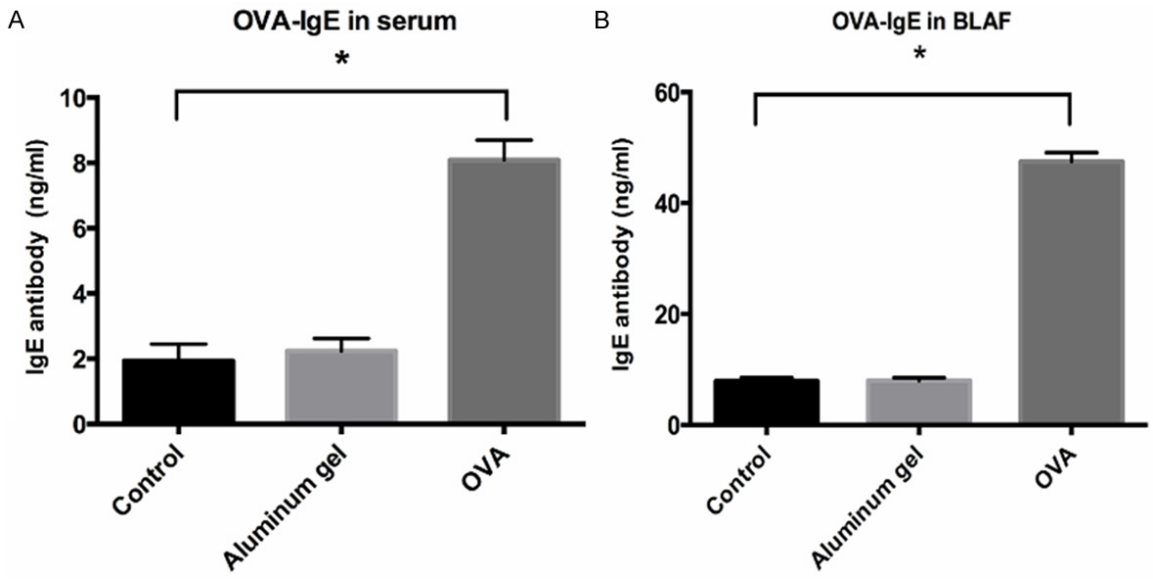

Figure 2. (A and B) Concentration of IgE in the serum and BALF in the three groups. The serum IgE (A) and OVA-specific IgE concentrations in the BALF (B) were assessed by ELISA as described in "Method" sections. The data are presented as mean ± SD for 20 rats per group. *P < 0.001 for the control versus OVA-inhalation group by the Mann-Whitney test.

SUMF2, sulfate-modifying factor 2, is a member of the formylglycine-generating enzyme (FGE) family and catalyzes the oxidation of a specific cysteine to Cα-formylglycine [15]. Despite the advances in research on the metabolic functions of pFGE (SUMF2 expression) in the last decade [15-21], the role of SUMF2 in allergic inflammation in asthma remains poorly understood. Recent studies in other groups have suggested that SUMF2 (and SUMF1) was located in the luminal space of the endoplasmic reticulum (ER), where synthesized sulfatases are post-translational modified by the formation of FGly. SUMF2 can bind to SUMF1 and sulfatase, and regulate their activities and the process of FGly formation [16, 18]. Interestingly, human IL-13 usually undergoes post-translational modification in the ER [22]. Based on these findings, we hypothesized that SUMF2 may alter the modification and secretion of IL-13 in the ER.

Our previous work with yeast two-hybridization screening experiments showed that SUMF2 interacts with IL-13 and inhibits its secretion, independently of IL-13 glycosylation, in bronchial smooth muscle cells (BSMCs) [23]. Specifically, our research revealed that the level of

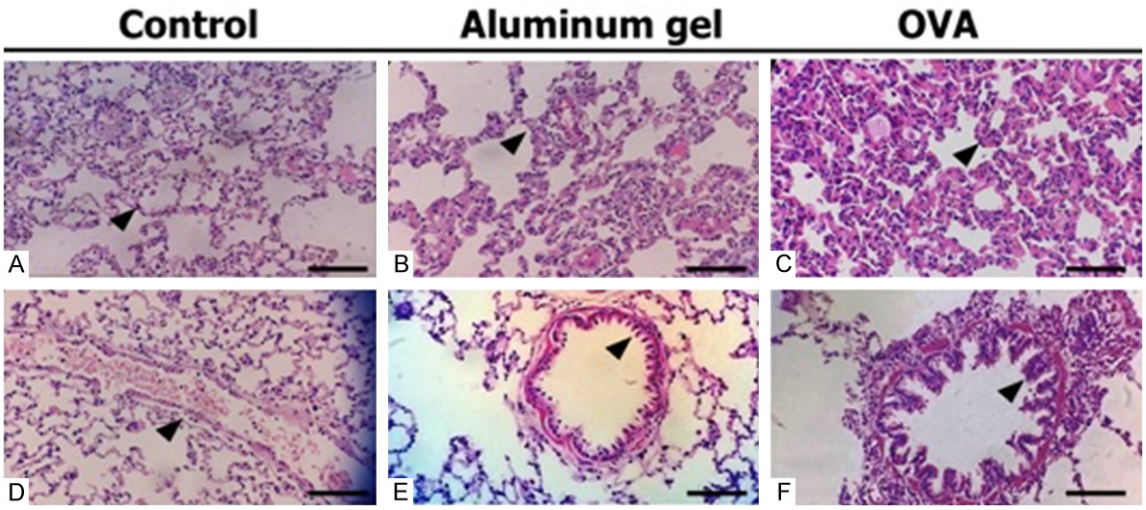

Figure 3. H&E-stained specimens in the OVA-induced asthma group. Images are presented at the original magnification of ×400. Arrows indicate the areas of cellular inflammation in the lung bronchial tissues. A-C. The arrows indicate progressive thickening of the alveolar epithelial cells in the lung. D. The arrow in panel D indicates a normal lung capillary in a control rat. E, F. The arrows indicate edema and thickening of the bronchial epithelium and significant inflammation and infiltration of inflammatory cells.

Table 1. IL-13 and IL-4 concentration in BALF by ELISA (pg/mL)

Group	N/group	IL-4	IL-13
Control group	20	27.49 ± 1.64	25.26 ± 1.29
Aluminum gel group	20	27.98 ± 1.33	25.68 ± 1.77
OVA-induced asthma group	20	75.63 ± 3.28*,#	69.17 ± 0.71*,#

Control-group: normal control rats treated with PBS; Aluminum gel group: normal control rats treated with aluminum gel; OVA-induced asthma group: normal control rats treated with ovalbumin. *P < 0.05 vs. control-group. #P < 0.05 vs. aluminum gel group.

the 12-kDa form of intracellular IL-13 was significantly increased, whereas the level of IL-13 in lymphocyte culture supernatants was significantly reduced. In addition, blocking N-glycosylation via treatment with tunicamycin eliminated the 17-kDa form of intracellular IL-13 and inhibited the secretion of IL-13 by BSMCs [23]. However, the roles of SUMF2 and IL-13 in asthmatic inflammation in the animal model of asthma are unknown. Therefore, in order to investigate this, using a rat model of acute asthma, we attempted to shed light on the interaction between SUMF2 and IL-13 expression in asthmatic rats.

Materials and methods

Rats

Sixty female Wistar rats of the same age were used in this study (Vital-River Laboratory, Beijing, China). All the animals were housed at facilities under standard environmental conditions. Food and water were provided ad libitum. Six- to eight-weeks-old Wistar rats were randomly divided into three groups (20 rats per group): the asthma group, which was administered ovalbumin (OVA)/$Al(OH)_3$; the aluminum gel group, which was administered $Al(OH)_3$; and the control group, which was administered normal saline [24, 25]. All the experimental procedures were conducted in conformity with the institutional guidelines for the care and use of laboratory animals at Harbin Medical University, China.

Administration of OVA and aluminum gel

The asthma group was sensitized on days 0, 7, and 14 by intraperitoneal injection of 200 μg OVA mixed with aluminum gel (as an adjuvant, Sigma. St Louis. USA) integrated in a total volume of 2 mL phosphate-buffered saline (PBS). Then, 250, 250, and 1000 μL volumes of axillary, bilateral inguinal, and intraperitoneal injections were administered respectively. The control group and aluminum gel group were administered equal volumes of PBS and aluminum gel respectively. Then, from the 15th day onwards, the rats were challenged with aero-

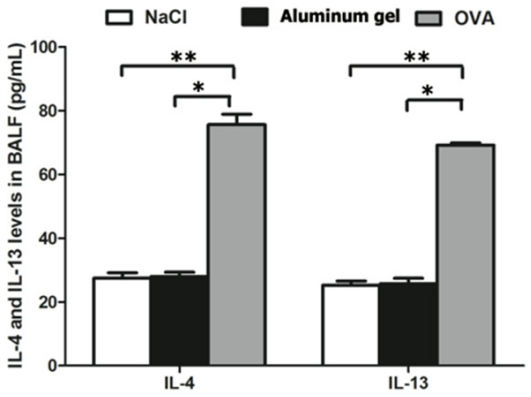

Figure 4. IL-13 and IL-4 secretion in the BALF of OVA-induced asthma rats. The OVA-induced asthma rats had significantly higher levels of IL-4 and IL-13 in the BALF, as measured by ELISA. All data are presented as the mean ± SEM (n = 20/group). $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the control group and aluminum gel group, respectively, as assessed by the Nemenyi test.

solized OVA (1% w/v, PBS) using an ultrasonic nebulizer (Yuwell Medical Device Company, Jiangsu, China) that produced an airflow rate of 4-8 L/min and particle sizes of 0.5 to 5.0 μm. This challenge was performed for 30 min every day for 8 days. Twenty-four hours after the last aerosol challenge, the abdominal aorta was sectioned under deep chloral hydrate-induced anesthesia (400 mg/kg, intraperitoneal) in all the animals, which were then sacrificed by cervical dislocation (**Figure 1**).

Bronchoalveolar lavage fluid and peripheral lymphocyte collection

In brief, tracheostomy was performed. To obtain the bronchoalveolar lavage fluid (BALF), ice-cold PBS (0.5 mL) was infused three times into the lungs and withdrawn via tracheal cannulation (total volume, 1.5 mL). The recovered BALF samples were centrifuged at 1500 rpm for 5 min at 4°C, and then the cell pellets were re -suspended in 800 μL of PBS and centrifuged for 5 min at 1500 rpm at 4°C. The cells were re-suspended in 100 μL of PBS for ELISA. The supernatant obtained from the BALF samples was stored at -70°C for biochemical analysis.

Peripheral blood samples were harvested by sectioning the abdominal aorta under deep chloral hydrate-induced anesthesia. Peripheral blood lymphocytes were collected using the Lymphocyte Separation Medium (RAT) (Catalog No: P8630; Solarbio CO., Beijing, China) [26, 27]. First, 3 mL of LSM was aseptically transferred to a 15-mL centrifuge tube. Then, 2 mL of defibrinated, heparinized blood was mixed with 2 mL of physiological saline. Next, the diluted blood was carefully layered over 3 mL of LSM (room temperature) in a 15-mL centrifuge tube, in order to create a clear blood-LSM interface. The tube was centrifuged at 400 *g* at RT for 15-30 min. This resulted in sedimentation of erythrocytes and polynuclear leukocytes, and the formation of a band of mononuclear lymphocytes above the LSM layer. The following bands were obtained, from top to bottom: plasma layer, mononuclear cell layer, LSM layer, and RBC pellet. The top layer of clear plasma was aspirated to within 2-3 mm above the lymphocyte layer. Then, the lymphocyte layer along with about half of the LSM layer below it was aspirated and transferred to a centrifuge tube. An equal volume of buffer was added to the tube. Saline solution was added and the tube was centrifuged for 10 min at a speed sufficient to sediment the cells without damaging them, i.e. 160-260 g, at room temperature (18-25°C). The sediment was then washed twice with buffer-balanced saline solution in order to remove LSM and reduce the percentage of platelets. The sedimented cells were then re-suspended in an appropriate medium for the following analyses.

ELISA

The concentrations of IgE, IL-13 and IL-4 present in the BALF supernatants were determined by ELISA (Rat IgE ELISA Kit: Catalog No: E-EL-R0517c, Elabscience Biotechnology Co., Wuhan, China; IL-13- and IL-4-specific ELISA kits: Catalog No: IL-13, SEA060Ra, and IL-4, SEA077Ra, Cloud-Clone Corp, Wuhan, China) [28]. The absorbance of the samples was measured at 450 nm using a spectrophotometer (KHB company, Shanghai, China).

Histopathological analysis

Standard histopathological analyses were performed to assess the level of IL-13 and SUMF2 expression in the samples. Briefly, we stained 4-μm paraffin sections of the lung tissue samples, using peroxidase-labeled chain avidin (SP); this was followed by de-waxing and hydration. Then, the section was exposed to 3% hydrogen peroxide to remove the endogenous

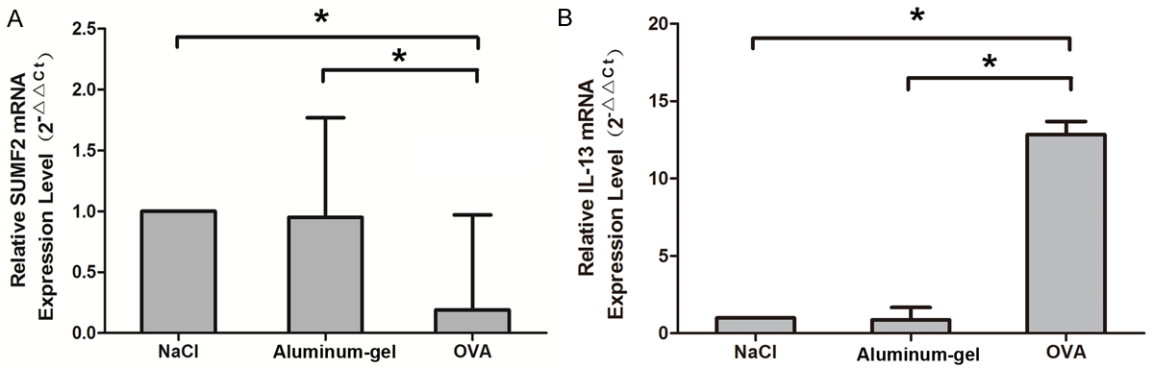

Figure 5. Analysis of the mRNA expression of SUMF2 and IL-13 in lung tissues. A. Real-time PCR analysis results showing SUMF2 gene expression in the lung bronchial tissues of the OVA-induced asthma group, aluminum gel group and control group rats (n = 20/group). B. IL-13 mRNA expression in the lung bronchial tissues in the three groups. All data are presented as the mean ± SEM. *$P < 0.05$ compared with the control group and aluminum gel group, respectively, according to the Nemenyi test.

oxidase, and subject to microwave antigen retrieval. The primary antibody was added, and the section was incubated overnight at 4°C.

First, the sections were stained with hematoxylin and eosin (H&E) to detect pathological changes in lung tissues. The primary antibodies used were rat SUMF2 and IL-13 polyclonal antibodies diluted to 1:100 (ProteinTech Company, Wuhan, China). The SP kit and diaminobenzidine (DAB) color kit were purchased from Zhongshan Golden Bridge Biotechnology Limited (Beijing, China). The staining intensity of SUMF2 and IL-13 was graded on a scale of 0 to 4 (0 = no straining, 1 = weak immunoreactivity, 2 = moderate immunoreactivity, 3 = strong immunoreactivity, and 4 = strongest immunoreactivity). The percentage of cells that showed immunoreactivity was scored on a scale of 0 to 4 (0 = no positive cells, 1 = 25% positive cells, 2 = 25%-50% positive cells, 3 ≥ 50% positive cells, and 4 ≥ 75% positive cells). The staining intensity score and the percentage of immunoreactivity were then multiplied to obtain the composite score (CS). Depending on the CS, SUMF2 and IL-13 expression was classified as high (> 6) or low (≤ 6) [29, 30].

Western blotting assay

Lung homogenates were prepared from the control as well as the OVA-sensitized rat samples. Lung tissue was homogenized on ice, frozen in liquid nitrogen and stored at -80°C. The protein concentrations were determined using a BCA kit (Cat No: P10010S; Beyotime Institute of Biotechnology, China). Samples containing 30 mg of proteins were loaded in each lane, resolved via 8%-12% SDS-PAGE, and transferred to poly vinylidene fluoride membranes. Membranes were blocked in Tris-buffered saline containing 0.1% Tween-20 solution containing 5% skim milk (TBST) for 2 h at room temperature (RT) and incubated with primary antibodies (rat monoclonal anti-SUMF2 antibody diluted 1:1000, Cat No: sc-367191, SANTA CRUZ BIOTECHNOLOGY, USA; rat monoclonal anti-IL-13 antibody diluted 1:1000, Cat No: sc-101407, SANTA CRUZ Biotech, USA). For the negative control, we chose β-actin (Cat No: BA0410, BOSTER Company, Wuhan, China). Next, the tissue sections were washed three times in TBST and incubated with peroxidase-conjugated secondary antibodies for 1 h at RT.

The blot was visualized using a chemiluminescence method, with the DAB coloration kit (Cat No. AR1024; BOSTER Company, Wuhan, China). According to manufacturer's instructions, 500-1000 μL of the DAB coloration regent was added to the blots and incubated for 5-10 min at RT in order to visualize the protein bands.

Quantitative real-time PCR for quantification of gene expression

Total mRNA from the lung tissue samples was extracted using RNAiso plus (Takara, Dalian, China), and the SUMF2 and IL-13 expression levels in these samples were evaluated using the SUMF2 primer (forward primer: 5'-GAGGAGGCTTGAAGGGTCAG-3', reverse primer: 5'-AG-

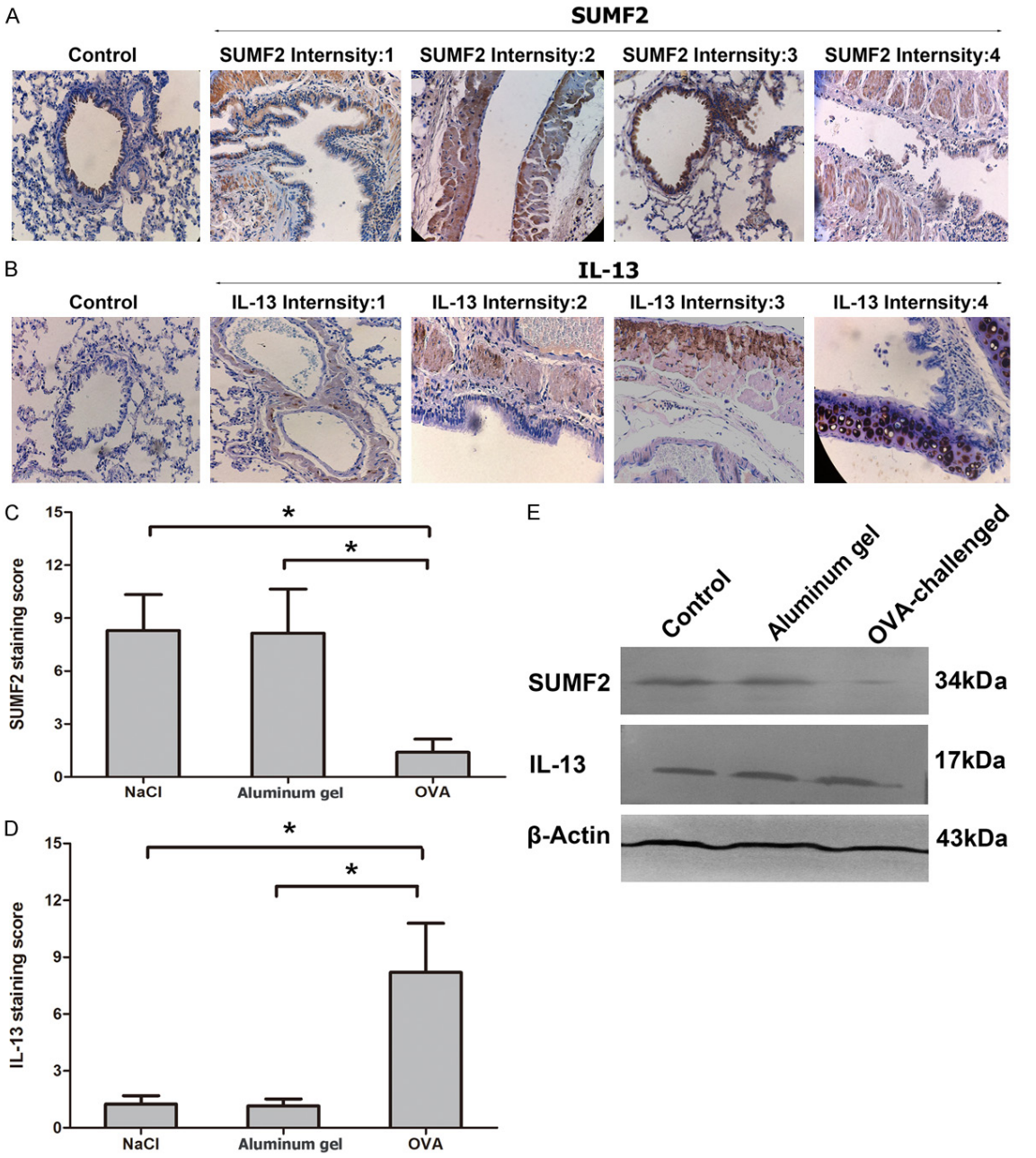

Figure 6. Protein expression of SUMF2 and IL-13. A and B. Results of SUMF2 and IL-13 immunohistochemical staining in OVA-induced asthma rats. Immunohistochemical staining of paraffin-embedded sections from allergic asthma rats in the control, aluminum gel and OVA-induced asthma groups. Representative allergic asthma lung tissues with SUMF2 and IL-13 staining scores of 0 to 4. C. Immunochemistry composite score (ICS) for SUMF2 in the OVA-induced asthma group (n = 20/group), control group and aluminum gel group. SUMF2 protein expression in lung tissues of OVA-induced asthma rats was significantly decreased compared with its expression in the normal saline group and aluminum gel group. All data are presented as the mean ± SEM. $^{*}P < 0.05$, compared with the control group and aluminum gel group, respectively, according to the Nemenyi test. D. Immunochemistry composite score (ICS) for IL-13 in the three groups. Relative IL-13 protein expression in lung tissues of OVA-induced asthma rats was significantly increased compared to the expression level in the normal saline group and aluminum gel group. All data are presented as the mean ± SEM. $^{*}P < 0.05$, compared with the control group and aluminum gel group, respectively, according to the Nemenyi test. E. Western-blotting results for the lung homogenates of OVA-induced asthma rats, with anti-SUMF2 and anti-IL-13 antibodies. β-Actin was used as an internal control.

CTTGGTATGTGGACGCTG-3'), the IL-13 primer (forward primer: 5'-TCTCGCTTGCCTTGGTGG-3', reverse primer: 5'-CATTCAATATCCTCTGGGTCCTGT-3'), and the GAPDH primer as the control

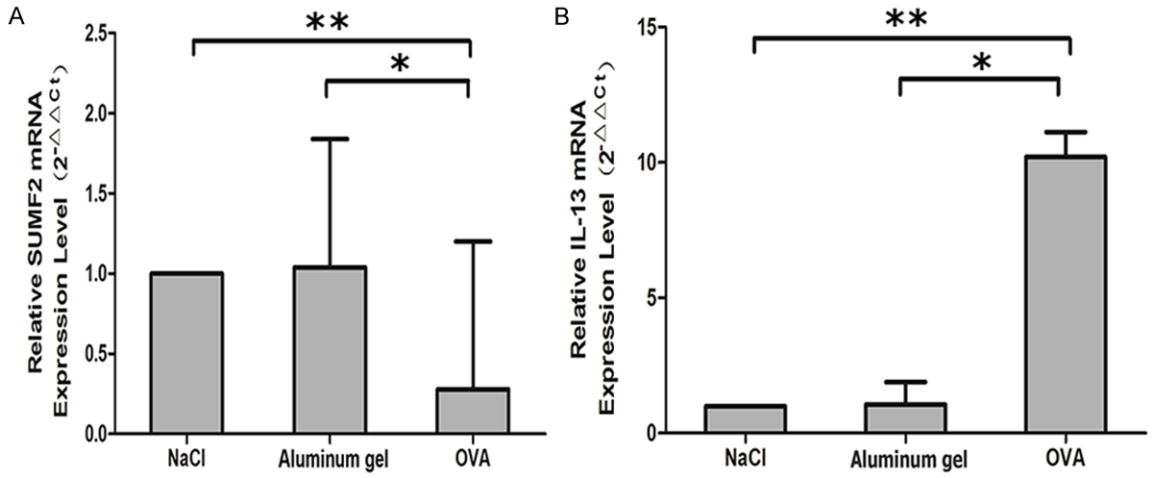

Figure 7. Relative SUMF2 and IL-13 mRNA expression in the lymphocytes of OVA-induced asthma rats. A. SUMF2 mRNA expression in the lymphocytes of OVA-induced asthma rats was significantly decreased compared with its expression in the normal saline group and aluminum gel group. B. Relative IL-13 mRNA expression in the lymphocytes of OVA-induced asthma rats was significantly increased compared to the expression level in the normal saline group and aluminum gel group. All data are presented as the mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the control group and aluminum gel group, respectively, according to the Nemenyi test.

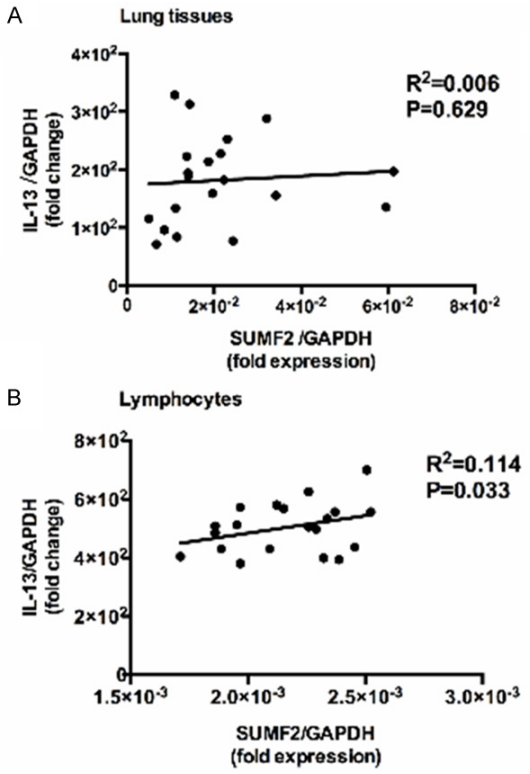

Figure 8. Results of the correlation analysis of SUMF2 and IL-13 mRNA relative expression in lung tissues and lymphocytes. A. Pearson correlation analysis of IL-13 mRNA expression and SUMF2 expression in lung tissue (relative to GADPH expression). B. Pearson correlation analysis of IL-13 mRNA expression and SUMF2 mRNA expression in lymphocytes (relative to GADPH expression).

(forward primer: 5'-GGTGCTGAGTATGTCGTGGAG-3', reverse primer: 5'-ACAGTCTTCTGAGTGGCAGTGAT-3').

The SYBER Premix Ex Taq PCR reagent from Takara Biotechnology (Dalian, China) was used to amplify the cDNA, and the products were detected using ROCHE Lightcycle480 II (Roche, Rotkreuz, Switzerland). The real-time PCR data were obtained using the 2^{-the} method, which is a convenient method for analyzing the relative changes in gene expression by real-time quantitative PCR experiments [31].

Imaging and photomicrography

Photomicrographs were taken with a Photometric Quantix digital camera running on a Windows program, and montages were assembled in Adobe Photoshop CC 2014. The images were cropped and corrected for brightness and contrast, but were not otherwise manipulated by the Image J software.

Statistical analysis

Data are expressed as mean ± standard error of the mean (S.E.M). Statistically significant differences between groups were determined by one-way ANOVA or the Nemenyi test. Statistial analyses were performed using the Graph-pad Prism software (Graph-Pad Software, Inc., USA). Statistical significance was set at $P < 0.05$.

Results

OVA-induced allergic airway inflammation asthma model

We utilized OVA with a standard protocol to establish the asthma model in Wistar rats. After the rats were sacrificed, we collected lung tissue, BALF and peripheral blood samples for immunological analyses. Next, in order to confirm that the model was successfully established, we assesssed the IgE levels in the serum and BALF, the IL-13 and IL-4 levels in the BALF, and the pathological changes after the OVA challenge, as allergic asthma is characterized by the infiltration of inflammatory cells into the airway and bronchial tissues and therefore a corresponding increase in the levels of IgE, IL-13 and IL-4.

The IgE concentrations both in the serum and BALF supernatant of OVA-induced rats were significantly higher than that in the other two groups. The IgE concentration increased from 1.94 ± 0.113 ng/ml to 8.087 ± 0.136 ng/ml in the serum (**Figure 2A**) and 9.982 ± 0.152 ng/ml to 47.51 ± 0.356 ng/ml in BALF (**Figure 2B**), respectively. ($P < 0.001$) (**Figure 2A** and **2B**).

Observation of the H&E-stained lung tissues under × 40 magnification showed that the tissues in the control and aluminum gel group rats were well defined without observable edema or thickening of the bronchial mucosa; moreover, the airways and blood vessels were not infiltrated peripherally by inflammatory cells. However, the typical pathological features of asthma were observed in the tissue samples obtained from the OVA-induced asthma model rats, which include internal edema in the bronchial structures as well as infiltration by a multitude of lymphocytes, neutrophils and eosinophils (**Figure 3A-F**).

The IL-13 and IL-4 levels in the BALF samples were significantly increased in the OVA-induced asthma group compared to the other two groups ($P < 0.05$) (**Table 1** and **Figure 4**).

Inhibition of SUMF2 in the lung tissues of OVA-induced asthmatic rats

The SUMF2 mRNA level in the OVA-induced asthma group was significantly lower than that in the other two groups (**Figure 5A**) ($P < 0.05$). Moreover, IL-13 mRNA expression was higher in the OVA-induced asthma group than in the other groups (**Figure 5B**) ($P < 0.05$).

Immunohistochemical staining for SUMF2 and IL-13 showed diffuse cytoplasmic staining for both. (**Figure 6A** and **6B**). These observations reflect the ER localization of SUMF2 and also of IL-13 in BMSCs. Moreover, the immunohistochemistry results showed that SUMF2 and IL-13 were mainly expressed in BSMCs rather than in bronchial epithelial and pulmonary capillary cells.

Moreover, the immunohistochemical scoring showed that the SUMF2 scores were decreased in the OVA-induced asthma rats (**Figure 6C**), and that the IL-13 scores were increased in the OVA-induced asthma rats (**Figure 6D**).

Finally, using western blotting, we confirmed the changes in the protein expression of IL-13 and SUMF2. As shown in **Figure 6E**, the protein expression of SUMF2 had decreased while the protein expression of IL-13 had increased in the lung tissue of the OVA-induced asthma rats (**Figure 6E**).

These findings indicate that after the OVA challenge, Th2 cells induced the overexpression of IL-13 and its secretion and release into BALF.

Inhibition of SUMF2 in the peripheral lymphocytes of OVA-induced asthmatic rats

The SUMF2 mRNA level in the peripheral blood lymphocytes of the OVA-induced asthma group was significant lower than that of the aluminum gel and control group. In contrast, IL-13 expression in the lymphocytes was considerably increased in the OVA-induced asthma group compared with the control group (**Figure 7**).

Correlation between SUMF2 and IL-13 expression in the OVA-induced asthmatic rats

Pearson correlation coefficient analysis showed that there was no significant correlation between SUMF2 and IL-13 expression in the lung tissues ($R^2 = 0.006$, $P > 0.05$), but a slightly positive correlation was observed between SUMF2 and IL-13 expression in the peripheral blood lymphocytes ($R^2 = 0.114$, $P < 0.05$) (**Figure 8A** and **8B**).

Discussion

Asthma is a common heterogeneous disease associated with both genetic and environmen-

tal factors that affects millions of individuals worldwide [13]. Its pathological features include mucus cell hyperplasia and infiltration of inflammatory cells, such as CD4+ T cells, which express the characteristic Th2 cytokines IL-4, IL-5, and IL-13 [32-35], and exacerbate the progression of allergic inflammation [36].

Our report in 2009 revealed that SUMF2 expression was related with the secretion of IL-13: briefly, SUMF2 and IL-13 were co-immunoprecipitated from BSMCs, independent of IL-13 glycosylation [23], which provided evidence that the SUMF2 gene may impact the pathogenesis of allergic asthma. However, we did not have enough evidence to elucidate the relationship between SUMF2 and IL-13 in an animal model of allergic asthma. Despite a large amount of research in the past decade on the mechanisms underlying the IL-13-related genetics, immunology and pathogenesis in allergic asthma, there are large gaps in our knowledge about IL-13 and its interaction with other molecules.

In this study, we established a classical animal model of allergic asthma to investigate SUMF2 and IL-13 expression at the mRNA and protein level. We found that SUMF2 expression was inhibited whereas IL-13 (but not IL-14) expression was increased at the mRNA and protein level. Based on our previous research findings and this study, we hypothesize that the SUMF2 gene may be inhibited as a result of the overexpression of IL-13 in asthmatic mice. Our data show that decrease in the expression of SUMF2 is accompanied by increase in the expression of IL-13 both in lung tissue and peripheral blood.

We also studied the inflammatory changes in the lung tissue induced by IL-13. In this classical OVA-induced asthma model, we found considerable evidence of allergic asthma, such as cough, dyspnea and shivering. Although other more advanced methods of establishing allergic asthma models [37] have been reported, we found more significant inflammatory cell infiltration and increased levels of inflammatory factors in our model [25].

Interestingly, we found that both SUMF2 and IL-13 protein expression in the cytoplasm of BSMCs reflected our hypothesis that IL-13 was subjected to post-translational modification, which usually occurs in the ER. Given that SUMF2 is predominantly found in the ER, we hypothesized that SUMF2 may regulate the post-translational modification and secretion of IL-13 in the ER [23]. The data in our study provide evidence in support of our hypothesis.

However, the correlation analyses did not provide any significant results; that is, no significant correlation was found between SUMF2 and IL-13 expression. One of the reasons may be because the rats were only challenged with OVA; use of different allergens may have led to different results.

Secondly, since SUMF2$^{-/-}$ transgenic mice were not used in our study as controls, intrinsic differences in SUMF2 or IL-13 expression may have been present in the normal rats. Thirdly, since the SUMF2 protein catalyzes the oxidation of cysteine in the active site of sulfatases into C-α-formylglycine (FGly) in the ER, it could interact with many other intercellular proteins. Finally, it has been reported that SUMF2 expression might impact the fever response during endotoxemia, sepsis and trauma [21].

IL-13 is a Th2 cytokine that binds to the chain of the IL-4 receptor, which induces the pathophysiological features of asthma in a manner that is independent of IgE and eosinophils. IL-13 expression in murine asthma models indicates that although IL-4 may be an immunoregulatory factor, IL-4 is not a prime effector molecule. These findings may be relevant to human asthma models too [38]. The synthesis and secretion of IL-4 may occur independently of SUMF2 gene regulation in pulmonary cells. This was observed in our previous experiments [23]. Therefore, the interaction between SUMF2 and IL-4 is not the main object of this study.

Based on our present findings, we hypothesize that allergic asthma and overexpression of IL-13 could have a specific inhibitory effect on SUMF2 transcription. Therefore, our findings may provide new insight into IL-13 and its role in allergic asthma. If SUMF2 gene suppression could be directly correlated with IL-13 secretion in vivo, therapeutic intervention targeting SUMF2 may lower the secretion of IL-13, which would be valuable in the treatment of allergic asthma. To clearly verify the correlation between SUMF2 and IL-13, a SUMF2-knockout

mouse model should be established under OVA-induced allergic asthma.

In conclusion, the findings from our study indicate the potentially critical role of the SUMF2 gene in monitoring the overexpression and secretion of IL-13 in the pathogenesis of allergic asthma. The mechanism of SUMF2 against allergic inflammation requires further study.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (#811-71657) and the Doctoral Fund of College and Universities (#20112307110021).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Xiaofeng Jiang and Hongyan Liang, Department of Clinical Diagnosis, The Fourth Affiliated Hospital of Harbin Medical University, Yiyuan Street 37#, Nangang, Harbin, Heilongjiang Province, China. Tel: +86-139-0360-6017; E-mail: Jiangxiaofeng12359@163.com

References

[1] Ghaffar O, Laberge S, Jacobson MR, Lowhagen O, Rak S, Durham SR, Hamid Q. IL-13 mRNA and immunoreactivity in allergen-induced rhinitis: comparison with IL-4 expression and modulation by topical glucocorticoid therapy. Am J Respir Cell Mol Biol 1997; 17: 17-24.

[2] Lordan JL, Bucchieri F, Richter A, Konstantinidis A, Holloway JW, Thornber M, Puddicombe SM, Buchanan D, Wilson SJ, Djukanovic R, Holgate ST, Davies DE. Cooperative effects of Th2 cytokines and allergen on normal and asthmatic bronchial epithelial cells. J Immunol 2002; 169: 407-414.

[3] Kuperman DA, Huang X, Koth LL, Chang GH, Dolganov GM, Zhu Z, Elias JA, Sheppard D, Erle DJ. Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma. Nat Med 2002; 8: 885-889.

[4] Akinbami LJ, Moorman JE, Bailey C, Zahran HS, King M, Johnson CA, Liu X. Trends in asthma prevalence, health care use, and mortality in the United States, 2001-2010. NCHS Data Brief 2012; 1-8.

[5] Yamashita N, Tashimo H, Ishida H, Matsuo Y, Tamauchi H, Terashima M, Yoshiwara I, Habu S, Ohta K. Involvement of GATA-3-dependent Th2 lymphocyte activation in airway hyperresponsiveness. Am J Physiol Lung Cell Mol Physiol 2006; 290: L1045-1051.

[6] Dai S, Yin K, Yao X, Zhou L. Inhibition of interleukin-13 gene expression by triptolide in activated T lymphocytes. Respirology 2013; 18: 1249-1255.

[7] Laporte JC, Moore PE, Baraldo S, Jouvin MH, Church TL, Schwartzman IN, Panettieri RA Jr, Kinet JP, Shore SA. Direct effects of interleukin-13 on signaling pathways for physiological responses in cultured human airway smooth muscle cells. Am J Respir Crit Care Med 2001; 164: 141-148.

[8] Corren J. Role of interleukin-13 in asthma. Curr Allergy Asthma Rep 2013; 13: 415-420.

[9] Martinez FD, Vercelli D. Asthma. Lancet 2013; 382: 1360-1372.

[10] Erle DJ, Sheppard D. The cell biology of asthma. J Cell Biol 2014; 205: 621-631.

[11] Lambrecht BN, Hammad H. The airway epithelium in asthma. Nat Med 2012; 18: 684-692.

[12] Zhang WY, Liang HY, Yang C, Xue L, Jiang XF. Sos recruitment system for the analysis of the interaction between sulfatase-modifying factor 2 subtypes and interleukin-13. Genet Mol Res 2013; 12: 5664-5672.

[13] Chapman AM, Malkin DJ, Camacho J, Schiestl RH. IL-13 Overexpression in Mouse Lungs Triggers Systemic Genotoxicity in Peripheral Blood. Mutat Res 2014; 769: 100-107.

[14] Rasmussen F, Taylor DR, Flannery EM, Cowan JO, Greene JM, Herbison GP, Sears MR. Risk factors for hospital admission for asthma from childhood to young adulthood: a longitudinal population study. J Allergy Clin Immunol 2002; 110: 220-227.

[15] Dickmanns A, Schmidt B, Rudolph MG, Mariappan M, Dierks T, von Figura K, Ficner R. Crystal structure of human pFGE, the paralog of the Calpha-formylglycine-generating enzyme. J Biol Chem 2005; 280: 15180-15187.

[16] Mariappan M, Preusser-Kunze A, Balleininger M, Eiselt N, Schmidt B, Gande SL, Wenzel D, Dierks T, von Figura K. Expression, localization, structural, and functional characterization of pFGE, the paralog of the Calpha-formylglycine-generating enzyme. J Biol Chem 2005; 280: 15173-15179.

[17] Preusser-Kunze A, Mariappan M, Schmidt B, Gande SL, Mutenda K, Wenzel D, von Figura K, Dierks T. Molecular characterization of the human Calpha-formylglycine-generating enzyme. J Biol Chem 2005; 280: 14900-14910.

[18] Zito E, Fraldi A, Pepe S, Annunziata I, Kobinger G, Di Natale P, Ballabio A, Cosma MP. Sulphatase activities are regulated by the interaction of sulphatase-modifying factor 1 with SUMF2. EMBO Rep 2005; 6: 655-660.

[19] Gande SL, Mariappan M, Schmidt B, Pringle TH, von Figura K, Dierks T. Paralog of the for-

mylglycine-generating enzyme--retention in the endoplasmic reticulum by canonical and non-canonical signals. FEBS J 2008; 275: 1118-1130.
[20] Dong C, Beecham A, Wang L, Slifer S, Wright CB, Blanton SH, Rundek T, Sacco RL. Genetic loci for blood lipid levels identified by linkage and association analyses in Caribbean Hispanics. J Lipid Res 2011; 52: 1411-1419.
[21] Ferguson JF, Meyer NJ, Qu L, Xue C, Liu Y, DerOhannessian SL, Rushefski M, Paschos GK, Tang S, Schadt EE, Li M, Christie JD, Reilly MP. Integrative genomics identifies 7p11.2 as a novel locus for fever and clinical stress response in humans. Hum Mol Genet 2015; 24: 1801-12.
[22] McKenzie AN, Li X, Largaespada DA, Sato A, Kaneda A, Zurawski SM, Doyle EL, Milatovich A, Francke U, Copeland NG, et al. Structural comparison and chromosomal localization of the human and mouse IL-13 genes. J Immunol 1993; 150: 5436-5444.
[23] Liang H, Li Z, Xue L, Jiang X, Liu F. SUMF2 interacts with interleukin-13 and inhibits interleukin-13 secretion in bronchial smooth muscle cells. J Cell Biochem 2009; 108: 1076-1083.
[24] Shin IS, Jeon WY, Shin HK, Lee MY. Effects of montelukast on subepithelial/peribronchial fibrosis in a murine model of ovalbumin induced chronic asthma. Int Immunopharmacol 2013; 17: 867-873.
[25] Jacobsen EA, Ochkur SI, Pero RS, Taranova AG, Protheroe CA, Colbert DC, Lee NA, Lee JJ. Allergic pulmonary inflammation in mice is dependent on eosinophil-induced recruitment of effector T cells. J Exp Med 2008; 205: 699-710.
[26] Caceres AI, Brackmann M, Elia MD, Bessac BF, del Camino D, D'Amours M, Witek JS, Fanger CM, Chong JA, Hayward NJ, Homer RJ, Cohn L, Huang X, Moran MM, Jordt SE. A sensory neuronal ion channel essential for airway inflammation and hyperreactivity in asthma. Proc Natl Acad Sci U S A 2009; 106: 9099-9104.
[27] Dai Y, Li F, Wu L, Wang R, Li P, Yan S, Xu H, Xia M, Bai C. Roxithromycin treatment inhibits TGF-beta1-induced activation of ERK and AKT and down-regulation of caveolin-1 in rat airway smooth muscle cells. Respir Res 2014; 15: 96.
[28] Kvarnstrom M, Ydrefors J, Ekerfelt C, Vrethem M, Ernerudh J. Longitudinal interferon-beta effects in multiple sclerosis: differential regulation of IL-10 and IL-17A, while no sustained effects on IFN-gamma, IL-4 or IL-13. J Neurol Sci 2013; 325: 79-85.
[29] Wang JY, Sun J, Huang MY, Wang YS, Hou MF, Sun Y, He H, Krishna N, Chiu SJ, Lin S, Yang S, Chang WC. STIM1 overexpression promotes colorectal cancer progression, cell motility and COX-2 expression. Oncogene 2015; 34: 4358-67.
[30] Konno R, Yamakawa H, Utsunomiya H, Ito K, Sato S, Yajima A. Expression of survivin and Bcl-2 in the normal human endometrium. Mol Hum Reprod 2000; 6: 529-534.
[31] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25: 402-408.
[32] Bentley AM, Maestrelli P, Saetta M, Fabbri LM, Robinson DS, Bradley BL, Jeffery PK, Durham SR, Kay AB. Activated T-lymphocytes and eosinophils in the bronchial mucosa in isocyanate-induced asthma. J Allergy Clin Immunol 1992; 89: 821-829.
[33] Bradley BL, Azzawi M, Jacobson M, Assoufi B, Collins JV, Irani AM, Schwartz LB, Durham SR, Jeffery PK, Kay AB. Eosinophils, T-lymphocytes, mast cells, neutrophils, and macrophages in bronchial biopsy specimens from atopic subjects with asthma: comparison with biopsy specimens from atopic subjects without asthma and normal control subjects and relationship to bronchial hyperresponsiveness. J Allergy Clin Immunol 1991; 88: 661-674.
[34] Bentley AM, Meng Q, Robinson DS, Hamid Q, Kay AB, Durham SR. Increases in activated T lymphocytes, eosinophils, and cytokine mRNA expression for interleukin-5 and granulocyte/macrophage colony-stimulating factor in bronchial biopsies after allergen inhalation challenge in atopic asthmatics. Am J Respir Cell Mol Biol 1993; 8: 35-42.
[35] Hogg JC. Pathology of asthma. J Allergy Clin Immunol 1993; 92: 1-5.
[36] Cohn L, Elias JA, Chupp GL. Asthma: mechanisms of disease persistence and progression. Ann Rev Immunol 2004; 22: 789-815.
[37] Gold M, Marsolais D, Blanchet MR. Mouse models of allergic asthma. Methods Mol Biol 2015; 1220: 503-519.
[38] Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, Donaldson DD. Interleukin-13: central mediator of allergic asthma. Science 1998; 282: 2258-2261.