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To cite this article: Jisu Hong, Pureun-Haneul Lee, Yun-Gi Lee, George D. Leikauf & An-Soo Jang (2020): Augmented angiogenic transcription factor, SOX18, is associated with asthma exacerbation, Journal of Asthma, DOI: [10.1080/02770903.2020.1771727](https://doi.org/10.1080/02770903.2020.1771727)

To link to this article: <https://doi.org/10.1080/02770903.2020.1771727>



Accepted author version posted online: 18 May 2020.
Published online: 27 May 2020.



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Augmented angiogenic transcription factor, SOX18, is associated with asthma exacerbation

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ABSTRACT

Background: Asthma characterized by airway hyperresponsiveness, inflammation, fibrosis, and angiogenesis. SRY-related HMG-box 18 (SOX18) is an important transcription factor involved in angiogenesis, tissue injury, wound-healing, and in embryonic cardiovascular and lymphatic vessels development. The role of angiogenic transcription factors, SOX18 and the related, prospero homeobox 1 (PROX1) and chicken ovalbumin upstream promoter transcription factor II (COUP-TFII), in asthma has had limited study.

Objective: In this study, we aimed to elucidate the role of SOX18 in the pathogenesis of bronchial asthma.

Methods: Plasma SOX18 protein was measured in control subjects, and subject with stable or exacerbated asthma. SOX18, PROX1, and COUP-TFII protein was measured by western blot, and immunohistochemistry in a murine model of ovalbumin-induced allergic asthma (OVA). SOX18, PROX1, and COUP-TFII protein was measured in lung human microvascular endothelial cells (HMVEC-L) and normal human bronchial epithelial (NHBE) cells treated with house dust mite (*Der p1*).

Results: Plasma SOX18 tended to be higher in subject with asthma compared to control subjects and increased more during exacerbation as compared to stable disease. In mice, OVA challenge lead to increased lung SOX18, PROX1, COUP-TFII, mucous gland hyperplasia and submucosal collagen. In NHBE cells, SOX18, PROX1 and COUP-TFII increased following *Der p1* treatment. SOX18 protein increased in HMVEC-L following *Der p1* treatment.

Conclusion: These results suggest that SOX18 may be involved in asthma pathogenesis and be associated with asthma exacerbation.

ARTICLE HISTORY

Received 3 January 2020

Revised 7 May 2020

Accepted 16 May 2020

KEYWORDS

Asthma; bio-markers; remodeling

Introduction

Increased size and numbers of bronchial blood vessels is an important event in the development of allergic inflammation as well as in the pathophysiology of tissue remodeling in asthma (1,2), and angiogenesis is a well-documented feature of airway remodeling in asthma (3–7). Angiogenesis is defined as the formation of new blood vessels from preexisting endothelium. Angiogenesis can be initiated by endogenous angiogenic factors released from mesenchymal cells and/or inflammatory cells (8). Under physiological conditions, angiogenesis is controlled by an equilibrium between the pro- and anti-endogenous angiogenic factors released from the extracellular matrix to become bioavailable (2).

Sry-related HMG box 18 (SOX18) is a member of SOX family transcription factors (9–11). SOX18 is

involved in the induction of angiogenesis during wound healing and tissue repair (12) and acts as a critical regulator of cardiovascular and hair follicle formation (13), and is involved in vascular cell growth (14,15). SOX18 was found in endothelial cells of pre- and postnatal developing vasculature in mice (13,16,17). SOX18 regulation of the Claudin 5 (CLDN5) promoter contributes to endothelial cell specificity of CLDN5 expression (18).

VEGF which stimulates vascular network expansion, vasodilation and plasma leakage, was increased in asthmatic serum, sputum (19,20), BALF and airway tissue (20).

Prospero homeobox 1 (PROX1) is one of the key transcription factors driving the fate and specification of lymphatic endothelial cells (21). PROX1 and its regulators COUP-TFII (Chicken ovalbumin upstream

promoter transcription factor 2 COUP-TFII aka NR2F2) and SOX18 drive lymphatic endothelial cell (LEC) specification in mice (22). LECs form the basis of the lymphatic vasculature, and absolutely require transient SOX18 and COUP-TFII activity to induce Prox1 transcription (22–24).

At present, aspect of SOX18 and other angiogenic transcription factors in asthma has had limited investigation.

Materials and methods

Experimental design

Fifty asthmatic patients were recruited and followed for 6.6 ± 3.6 years and plasma SOX18 levels determined at stable and exacerbated condition in the same patients from Jan 2013 to Dec 2017. Eight BALB/c mice were exposed to saline (control) or OVA. Detailed analyses included measurement of lung SOX18 protein, lung histology, and bronchoalveolar lavage (BAL). Additional tests were performed with normal human microvascular endothelial cells (HMVEC) and human bronchial epithelial (NHBE) cells. The study of patient was approved by Soonchunhyang University's Institutional Review Board and mice were approved by Soonchunhyang University's Institutional Animal Care and Use Committee.

Subjects

We studied 50 asthma patients who were registered in the Genome Research Center for Allergy and Respiratory Diseases in Korea (25). The biospecimens and clinical data were provided by the biobank of Soonchunhyang University, Bucheon Hospital, a member of the Korea Biobank Network. Patients were recruited from Soonchunhyang University, Bucheon Hospital. Asthma diagnoses were based on the Global Initiative for Asthma (GINA) guidelines (26). Subjects had a clinical diagnosis of asthma that was supported by one or more of the following criteria: 1) variability in the maximum diurnal peak expiratory flow $\geq 20\%$ over the course of 14 days, 2) increased FEV1 $> 12\%$ and of 200 ml after inhalation of 200 – 400 μg albuterol, or 3) decreased FEV1 $\geq 20\%$ in response to a provocative concentration of inhaled methacholine (PC20 methacholine) of $< 10 \text{ mg/mL}$. Subjects underwent standardized assessments, which included analyses of induced-sputum specimens, blood cell count with differential, IgE measurement, chest posteroanterior radiography, allergy skin prick tests, and spirometry. All data

were collected at the time of diagnosis, before administration of asthma medication.

Control subjects were matched in terms of age, gender, and BMI and were recruited from spouses of the patients or members of the general population who answered negatively to a screening questionnaire regarding respiratory symptoms and other allergic diseases, had FEV1 values over 80% predicted, PC20 methacholine over 10 mg/mL, and normal findings on simple chest radiographs.

Asthma exacerbation

Among the subjects who were followed 2 year or longer, asthma exacerbation was defined by GINA guidelines as episodes of progressive increase in shortness of breath, cough, wheezing, or chest tightness, or some combination of these symptoms, accompanied by decreases in expiratory airflow and use of systemic corticosteroids (tablets, suspension, or injection) or an increase from a stable maintenance dose, for at least 3 days, and a hospitalization or emergency department visit because of asthma, requiring systemic corticosteroids.

Cell culture

Human Lung Microvascular Endothelial Cells (HMVEC-L) and normal human bronchial primary epithelial cells (NHBE) and were purchased from Lonza (Lonza, Basel, Switzerland, cat#. CC-2527 and CC-2540, respectively). HMVEC-L cells were plated at 5000 cells/cm² in T75 flasks in Endothelial Cell Growth Medium supplemented with the EGM-2 BulletKit™ (Endothelial Cell Growth Medium-2) (Lonza, cat#: CC-3162) and cultured at 37 °C in a 5% CO2 incubator. NHBE cells were plated at 3500 cells/cm² in T75 flasks in bronchial epithelial cell growth medium supplemented with the BEGM BulletKit™ (Bronchial Epithelial Cell Growth Medium) (Lonza, cat#: CC-3170), The medium was changed every 48 h, and the cells were grown to 80–90% confluence for 5 to 6 days. HMVEC-L and NHBE cells with density 1.0×10^6 cells/ml were seeded on in 6-well plate with medium. Prior (24 h) to each test, the medium was changed to basal medium, and cells were treated with 10 $\mu\text{g/mL}$ house dust mite *Dermatophagoides pteronyssinus* product 1 (Der p1).

Animals

6-week-old female BALB/c mice (Orient Bio, Seongnam, Korea) were sensitized mice with 50 μg i.p.

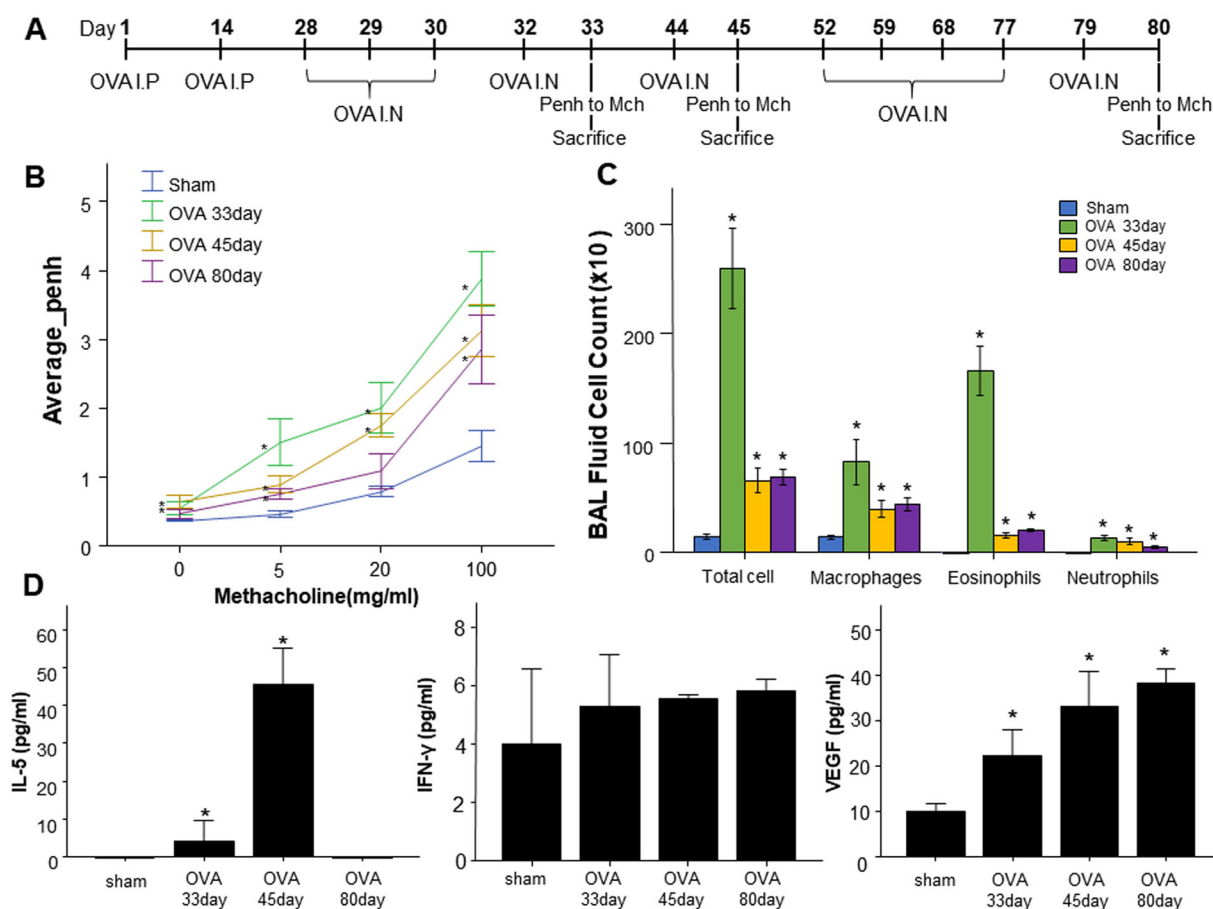


Figure 2. Ovalbumin (OVA) sensitization/challenge-induced airway hyperresponsiveness (AHR), inflammation, and VEGF and IL-5 increased in mice. (A) OVA antigen sensitization and challenge protocol. (B) Airway hyperresponsiveness analysis in chronic OVA-murine model. Airflow obstruction (Penh) was measured following increasing dose of methacholine, OVA-induced increased airflow obstruction at lower methacholine doses. Values are means ± SEM ($n = 8$ mice/group). * $p < 0.05$, OVA vs. Sham. (C) Total and differential inflammatory cell count in bronchoalveolar lavage fluid (BALF) of sham and OVA-sensitized/challenged mice. BALF was collected on 33, 45, and 80 day and cell differentials determined. OVA-induced increases in total cell number, macrophages, eosinophils, neutrophils and lymphocytes. * $p < 0.05$, OVA vs. Sham. (D) Cytokines VEGF and IL-5 increased in BALF OVA-sensitized/challenged mice. IL-5, and VEGF. * $p < 0.05$, OVA vs. Sham.

grade V chicken egg OVA (Sigma-Aldrich, St. Louis, MO) emulsified in 10 mg of hydroxyl aluminum plus 100 μ L of Dulbecco PBS. On Days 0 and 14 (Figure 2(A)), all mice received intranasal challenges with 150 μ g of grade III OVA (Sigma-Aldrich) in 50 μ L of Dulbecco PBS daily from Days 28 to 30, and on Day 32. On Day 33, airway hyperresponsiveness (AHR) was measured, BAL fluid was collected, and lung tissue was processed for protein, RNA, and hematoxylin and eosin (H&E) staining and confocal imaging. The remaining mice with established AHR were challenged with 150 μ g of grade III OVA (Sigma-Aldrich) in 50 μ L of Dulbecco PBS by intranasal on Day 44. On Day 45, AHR was measured, BALF was collected, and lung tissue was processed for protein, RNA, and hematoxylin and eosin (H&E) staining and confocal imaging. Animals were challenged 150 μ g of grade III

OVA (Sigma-Aldrich) in 50 μ L of Dulbecco PBS by intranasal weekly from Days 52 through 77, and on Day 79. On Day 80, AHR was measured, BALF was collected, and lung tissue was processed for protein, RNA, and hematoxylin and eosin (H&E) staining and confocal imaging.

AHR, BALF, morphology analysis

Mice anesthetized with 2.5 mg/kg tiletamine and xylazine (Zoletil and lumpum; Bayer Korea Co., Seoul, Korea) and AHR was assessed following challenges with 0, 5, 20, or 100 mg/mL methacholine (Sigma-Aldrich). The following day BALF was obtained, centrifuged, and the supernatant stored (-20°C). The cell pellet was resuspended for cell count and cytospin slides were made for cell differential (500 cells/mouse,

Diff-Quick-staining). A portion of the lung was fixed in 4% phosphate-buffered paraformaldehyde, embedded in paraffin, sectioned (5 μ m), and stained H&E stain.

Masson's trichrome assay

The lung tissue slides were warmed (60 °C, 30 min), immersed in xylene (10 min), followed by 100%, 95%, 90%, 80%, and 70% ethanol (5 min each), and stained with Masson's trichrome (Sigma-Aldrich, St. Louis MO, USA). Briefly, Bouin's solution was used as a mordant to intensify the color reactions. The nucleus and cytoplasm were dyed using hematoxylin and scarlet acid. Phosphotungstic/phosphomolybdic acid solution was used to change a positive to a negative charge. After staining with aniline blue, the samples were treated with 1% acetic acid. Prior to observation, the samples were mounted with a cover slip using mounting media (Amresco, OH, USA). The positive trichrome-stained area was quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA). Values are reported as a percentage of positive areas within the total sample.

Periodic acid-Schiff assay

Lung tissue was cut into 5 μ m sections and placed in periodic acid solution (Abcam, Cambridge, MA, USA) (5 min). After washing with distilled water, the specimens were immersed in Schiff's fluid (5 min). The slides washed with distilled water (5 min), placed in hematoxylin (3 min), rinsed with tap water (3 min), and bluing reagent was applied (30 s). Before observation, the slides were mounted with a cover slip using mounting media (Amresco).

Immunohistochemistry

Mouse lung sections were deparaffinized and rehydrated in an ethanol series. The sections were treated with 1.4% H₂O₂ (hydrogen peroxide) in methanol (30 min) to inhibit endogenous peroxidase, and then blocked for nonspecific binding with 1.5% goat serum and incubated with the anti-SOX18 (1:50, Santa Cruz biotechnology, Santa Cruz, CA, USA), anti-PROX1 (1:200, Thermo Fisher Scientific, Rockford, USA), or anti-COUP-TF II (1:300, Perseus Proteomics Inc, PPMX, Tokyo, Japan). The next day, sections were incubated with Avidin-Biotin Complex (Vector Laboratories, Burlingame, CA). Color reaction was developed by staining with liquid DAB + substrate kit

(Golden Bridge International Inc, Mukilteo, WA). After immunohistochemical staining the slides were counterstaining the slide with Harris's hematoxylin (1 min).

Western blot

Protein extracts of mouse lung tissue were collected as previously described (24). Protein was separated by SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were blocked for 5% Skim milk in 0.1% Tween 20 in Tris-buffered saline (TBS) (21 °C, 1 h) and incubated with anti-SOX18 (1:200, Santa Cruz), anti-PROX1 (1:1000, Thermo Fisher science), or anti-COUP TF-II (1:1000, PPMX) (4 °C overnight), followed by horseradish peroxidase (HRP)-conjugated secondary antibodies. Detection was performed using EzWestLumi plus (ATTO Corp, Tokyo, Japan). The relative abundance of protein was determined by quantitative densitometry, and the data were normalized to β -actin (Sigma-Aldrich, St. Louis, MO, USA).

Enzyme-linked immunosorbent assays

SOX18 in plasma and inflammatory mediators, interleukin 5 (IL-5) and interferon γ (IFN- γ), vascular endothelial growth factor (VEGF) in BALF were measured by ELISA (R&D System, Minneapolis, MN). To compare results from different plates, test sample ODs were adjusted relative to the positive and negative control samples supplied in each kit. The mean OD of duplicate wells was calculated. The index value of each tested plasma was defined by the following formula: index = (OD of tested plasma – OD of negative control)/(OD of positive control – OD of negative control) $\times$ 100. Lower detection limits were set at 0.057 ng/ml, 7, 2 and 3 pg/mL for SOX18, IL-5, IFN- γ , and VEGF, respectively.

Statistical analysis

Data with a normal distribution presented as mean $\pm$ standard error of the mean (SEM) or median (range) using SPSS version 22 (SPSS, Chicago, IL). Mann-Whitney U test was used to determine significant differences between groups. Paired t-test test was used to the difference between two variables for the same subject. Correlations between outcome measures were evaluated by calculating Pearson or Spearman correlation coefficients analysis. A Values of $P < 0.05$ were deemed to indicate statistical significance

Table 1. Clinical characteristics in control subjects and asthmatic patients.

		Asthmatic patients	
		Stable	Exacerbated
No of subjects	25	50	
Sex (male/female)	2/23	20/30	
Age (of initial visit), yr	58.3 ± 6.2	54.9 ± 14.1	
Onset of asthma: age, yr	–	47.06 ± 17.25	
Asthma duration, yr	–	6.63 ± 3.60	
Smoking status (NS/ES/CS)	25/0/0	32/12/6	
Cigarettes smoked, pack, yr	–	9.0 ± 15.7	
Body Mass Index, kg/ m ²	24.8 ± 2.61	25.4 ± 3.31	
Lung function	FEV1, % pred.	85.43 ± 19.82*	62.60 ± 18.14 [†]
	FVC, % pred.	84.83 ± 16.68	66.51 ± 16.76 [†]
	FEV1/FVC	74.53 ± 9.72*	68.07 ± 11.84 [†]
PC20, mg/ml	–	9.18 ± 10.37	
Total IgE, kU	106.63 ± 188.7	421.5 ± 699.19*	
Atopy	1 (4%)	21 (42%)*	
Attack average/yr	–	3.38 ± 3.24	
Duration of exacerbation during follow up, yr	–	6.63 ± 3.61	
Blood WBC/uL	5587.2 ± 1268.0	7768.8 ± 3448.9*	9873.0 ± 4917.1
Blood eosinophil, %	2.73 ± 2.26	5.09 ± 4.97*	3.83 ± 5.42
Blood neutrophil, %	56.3 ± 10.0	54.7 ± 12.9	64.6 ± 19.3 [†]

Note. Data expressed as mean ± SD. PC20 methacholine; the concentration of methacholine required to decrease the FEV1 by 20%, FEV1; forced expiratory volume in one second. FVC; forced vital capacity. BMI; body mass index. SM; smoker, ES; ex-smoker, NS; nonsmoker.

**p* < 0.01 compared with control subjects.

[†]*p* < 0.05 compared with stable asthmatics.

Results

Increased SOX18 in patients with asthma

Fifty asthmatic patients (mean age ± SD, 54.9 ± 14.1 yr) and 25 control subjects (mean age ± SD, 58.3 ± 6.2 yr) were recruited (Table 1). Initial percent-predicted FEV1 and percent-predicted FVC, and FEV1/FVC were lower in patients with asthma than in control subjects. Total IgE, atopy, and blood eosinophils were greater in patients with asthma than in control subjects. Body mass index was not different between asthmatic patients and control subjects. Duration of asthma was 6.63 ± 3.6 yr and exacerbation rate during follow up was 3.4 ± 3.2 (rate/yr). Percent-predicted FEV1, percent-predicted FVC, and FEV1/FVC were lower in exacerbated asthmatics than patients with stable asthma. Blood neutrophils were greater in exacerbated asthmatics than in stable asthma (19). Notably, plasma SOX18 tended to be greater in subjects with stable asthma (0.20 ± 0.02 ng/mL) than in healthy controls (0.18 ± 0.01 ng/mL) (Figure 1). Moreover, plasma SOX18 levels were significantly greater in exacerbated patients (0.28 ± 0.03 ng/mL, *p* < 0.01) than in subjects with stable asthma. The plasma SOX18 level was correlated with total IgE (*r* = 0.256, *p* = 0.005) in asthmatic patients and control subjects. Plasma SOX18 levels were significantly greater in exacerbated patients with positive *Der p*1 than in control subjects and stable asthmatic patients with negative *Der p*1 in allergic skin tests.

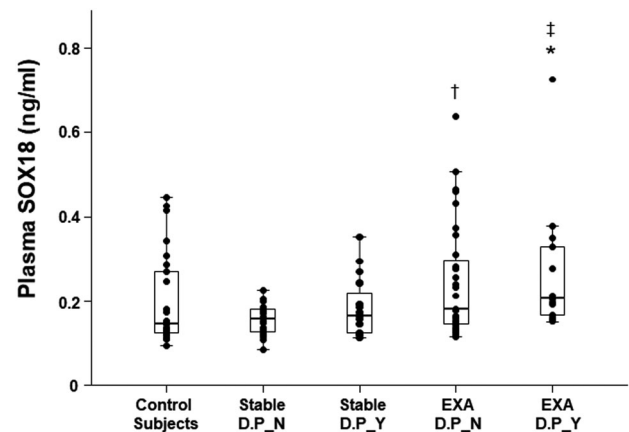


Figure 1. Plasma SOX18 level in healthy control, stable, and exacerbated (EXA) asthmatic patients. Asthmatic patients were divided into positive *Der p*1 (DP_Y) and negative *Der p*1 (DP_N) group by positivity of allergic skin tests. **P* < 0.05, DP_Y in exacerbated patients vs. control subjects. [†]*P* < 0.05, Stable DP_N vs. EXA DP_N. #*P* < 0.05, Stable DP_Y vs. EXA DP_Y.

OVA-induced inflammation, cytokines, and AHR in mice

OVA-sensitized/challenged mice had increased AHR compared to control mice (Figure 2(B)). OVA-sensitized/challenged mice had increased BALF inflammatory cells compared to control mice (Figure 2(C)). BALF IL-5 increased in OVA-sensitized/challenged mice on 33 and 45 day as compared to control mice (below limit of detection) (Figure 2(D)). BALF VEGF increased in OVA-sensitized/challenged mice on 45 day as compared

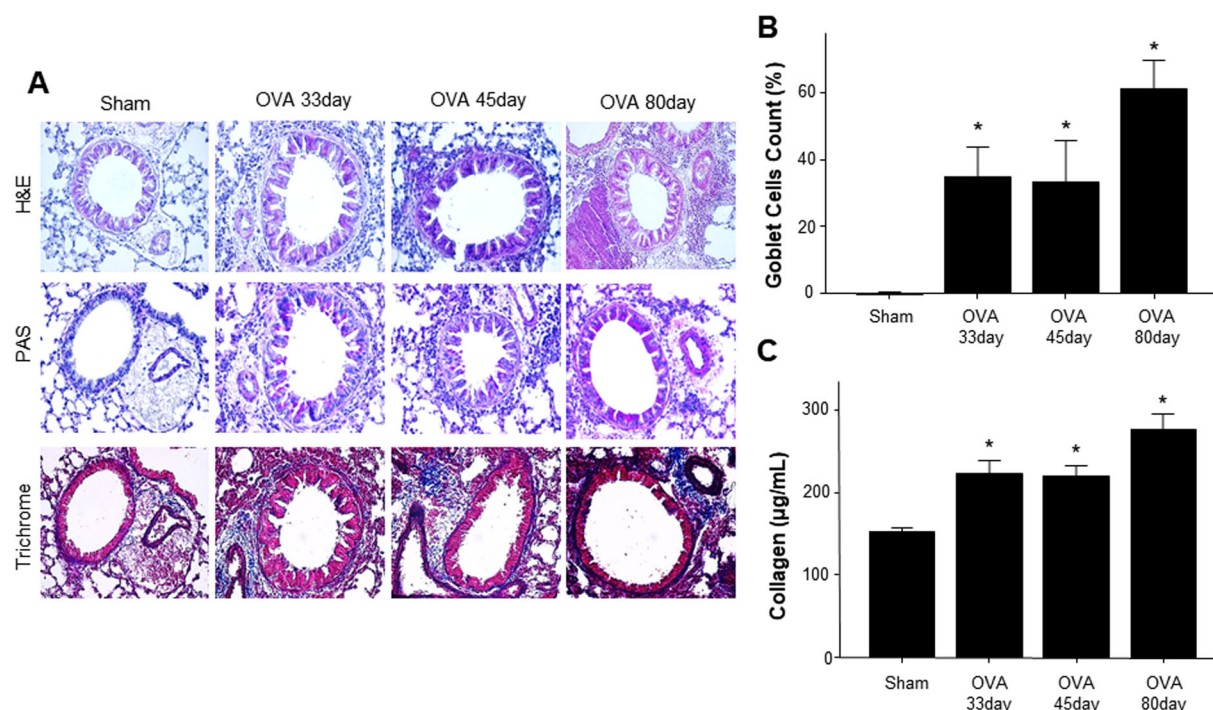


Figure 3. Lung inflammatory cell infiltration, goblet cell hyperplasia, and fibrosis increased in OVA-sensitized/challenge (OVA) mice (original magnification, $\times 400$) (A) Top Panel. Hematoxylin and eosin (H&E)-stained lung tissue contained more inflammatory cell infiltrations in OVA-sensitization/challenge mice than control mice. Middle Panel PAS-stained airways contained increased goblet cells in the OVA-mice. Lower Panel. Trichrome-stained lung tissue contained increased collagen in OVA-mice. (B) The quantitation of goblet cells counts in mouse lung. (C) The quantitation of collagen in mouse lung. * $p < 0.05$, OVA vs. Sham.

to those of control mice (below limits of detection). BALF $\text{INF-}\gamma$ was not changed in OVA-sensitized/challenged mice as compared to control mice.

OVA-induced inflammation, mucus gland hyperplasia, and fibrosis increased in mouse lung

On histologic examination, OVA-sensitized/challenged mice had numerous focal regions with inflammatory cell infiltrates and peribronchial and intraluminal areas of exudation by H&E stain (Figure 3(A)). OVA-sensitized/challenged mice had increased mucosal gland hyperplasia in PAS stain (Figure 3(A)). The quantitative value of the mucosal gland hyperplasia in PAS-stained images increased in the OVA-sensitized/challenged mice (Figure 3(B)). OVA-sensitized/challenged mice had increased fibrosis and collagen deposition (trichrome stain) (Figure 3(A)). The quantitative value of trichrome staining increased in the OVA-sensitized/challenged mice (Figure 3(C)).

OVA-induced SOX18, PROX1 and COUP-TFII protein increased in mouse lung

Lung SOX18 protein by western blotting increased in OVA-sensitized/challenged mice at 33, 45, and 80 day

compared to saline-treated mice (Figure 4(A,B)). Increased SOX18 immunohistochemical staining increased in mononuclear, endothelial, and epithelial cells in OVA-sensitized/challenged mice at 33, 45, and 80 day (Figure 5(A,B)). Lung PROX1 and COUP-TFII protein by western blotting increased in OVA-sensitized/challenged mice compared to saline-treated mice at 33, 45, and 80 day (Figure 4(A,C,D)). Increased PROX1 and COUP-TFII immunohistochemical staining was noted in mononuclear inflammatory cells, epithelial cells, and endothelial cells in OVA-sensitized/challenged mice (Figure 5). Correlation analysis (Pearson's r) were performed OVA-induced inflammation, mucus gland hyperplasia, and fibrosis and SOX18, PROX1 and COUP-TFII protein in these experimental group. SOX 18 and COUP-TFII is correlated with increased expression of mucus gland hyperplasia and collagen with fibrosis (Figure 5(C)).

House dust mite (*Der p1*)-induced SOX18, PROX1 and COUP-TFII protein increased in HMVEC-L and NHBE cells

SOX18 protein in HMVEC-L and NHBE cells increased following *Der p1* treatment (Figure 6(A,B)). PROX1 (Figure 6(C,D)) and COUP-TFII (Figure 6(C,E)) protein increased in NHBE cells following *Der p1* treatment.

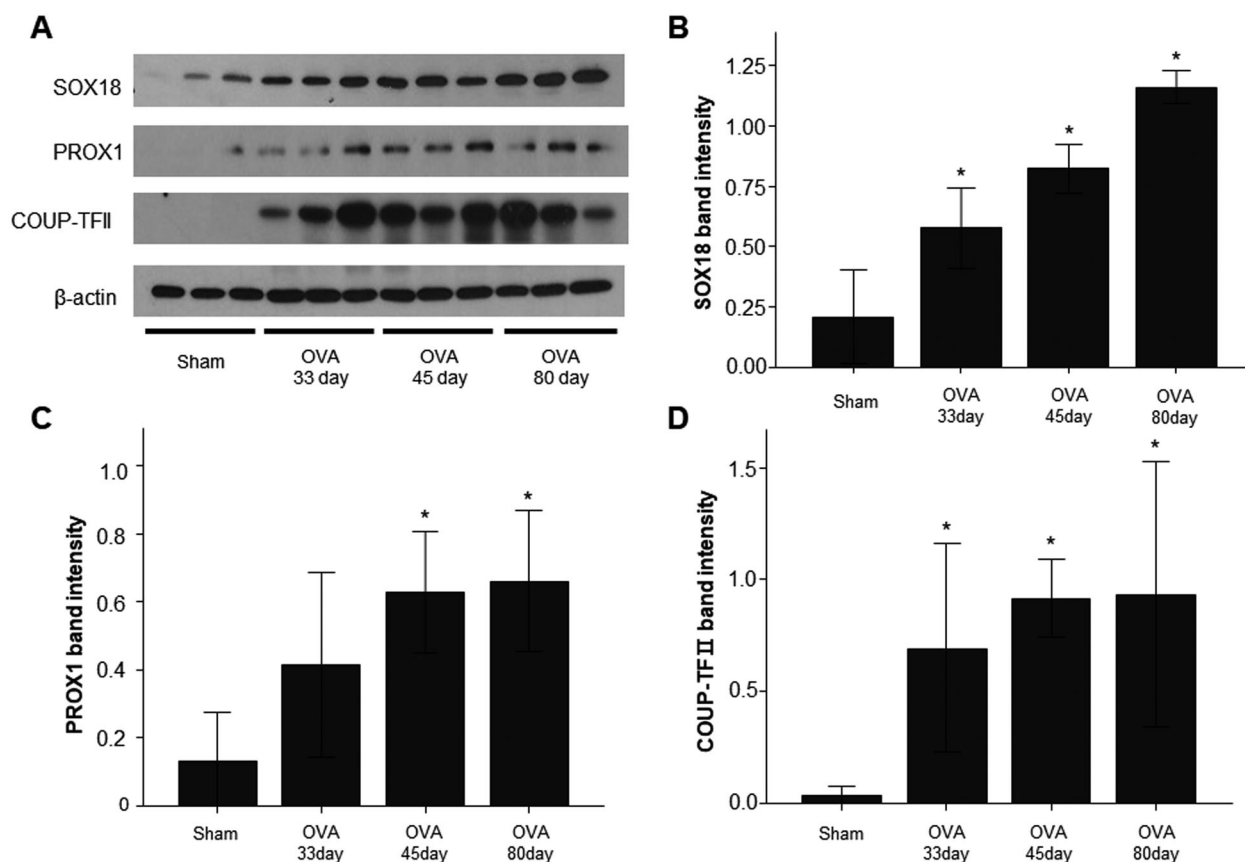


Figure 4. Lung SOX18, PROX1 and COUP-TFII protein levels increased in ovalbumin sensitized/challenged (OVA) mice. (A) Protein level of SOX18, PROX1 and COUP-TFII as determined by western blot increased in the lung of OVA mice. Densitometry was determined with immunoblots and normalized to β -actin. Values (normalized to β -actin) means $\pm$ SEM. (B–D) The quantitation of SOX18, PROX1 and COUP-TFII. * $p < 0.05$, OVA vs. Sham.

Discussion

Asthma is a chronic inflammatory disorder affecting 300 million individuals worldwide (26). One feature central to the pathophysiology of asthma is the increased microvascular network of the airway wall. Studies suggest an increased blood flow to the airway tissue to promote the chronic influx of inflammatory mediators, abnormal cell growth and proliferation, and thickening of the airway wall, all of which contribute to the pathophysiology of asthma (27,28).

Increased number, size, and density of blood vessels, as well as vascular leakage and plasma engorgement, have been reported in the airways of patients with all grades of asthma from mild to fatal (29–31). Few investigations have examined the pathophysiological mechanisms of the increased vasculature in the bronchial wall in asthma. Therefore, in this study we aimed to elucidate the role of angiogenesis, mucous gland hyperplasia, and fibrosis in chronic mouse model of asthma.

Previous studies have found increased pro-angiogenic VEGF in asthmatic serum, sputum (19,20),

BALF and airway tissue (20). VEGF and endothelial signaling play critical roles in the lung Th2 inflammation (7,32–34). Transgenic VEGF expression in mouse lung leads to eosinophilic inflammation, mucus metaplasia, and airway remodeling and VEGF receptor 2 (VEGFR2) inhibition abrogates aeroallergen-induced pulmonary inflammation (33). In our study, VEGF, IL-5, and inflammatory cells increased in BALF in mouse model of chronic asthma, suggesting that VEGF be involved in asthmatic airway inflammation.

With the findings of vascular remodeling in asthma has come the realization that blood vessel size, number, and plasma edema within the airway tissue needs to be clarified within the therapeutic approach of asthma (28–36). Neovascularization and vascular leak are prevalent in asthma (37), and as such vessels in the asthmatic lung are reminiscent of the dysfunctional, leaky vessels observed in tumors (38). Thus, the same anti-angiogenic strategies applied in oncology should provide new avenues for treating the angiogenesis related factor of airway remodeling associated with asthma.

The SOX gene family members contain high mobility group (HMG) box DNA binding regions and are

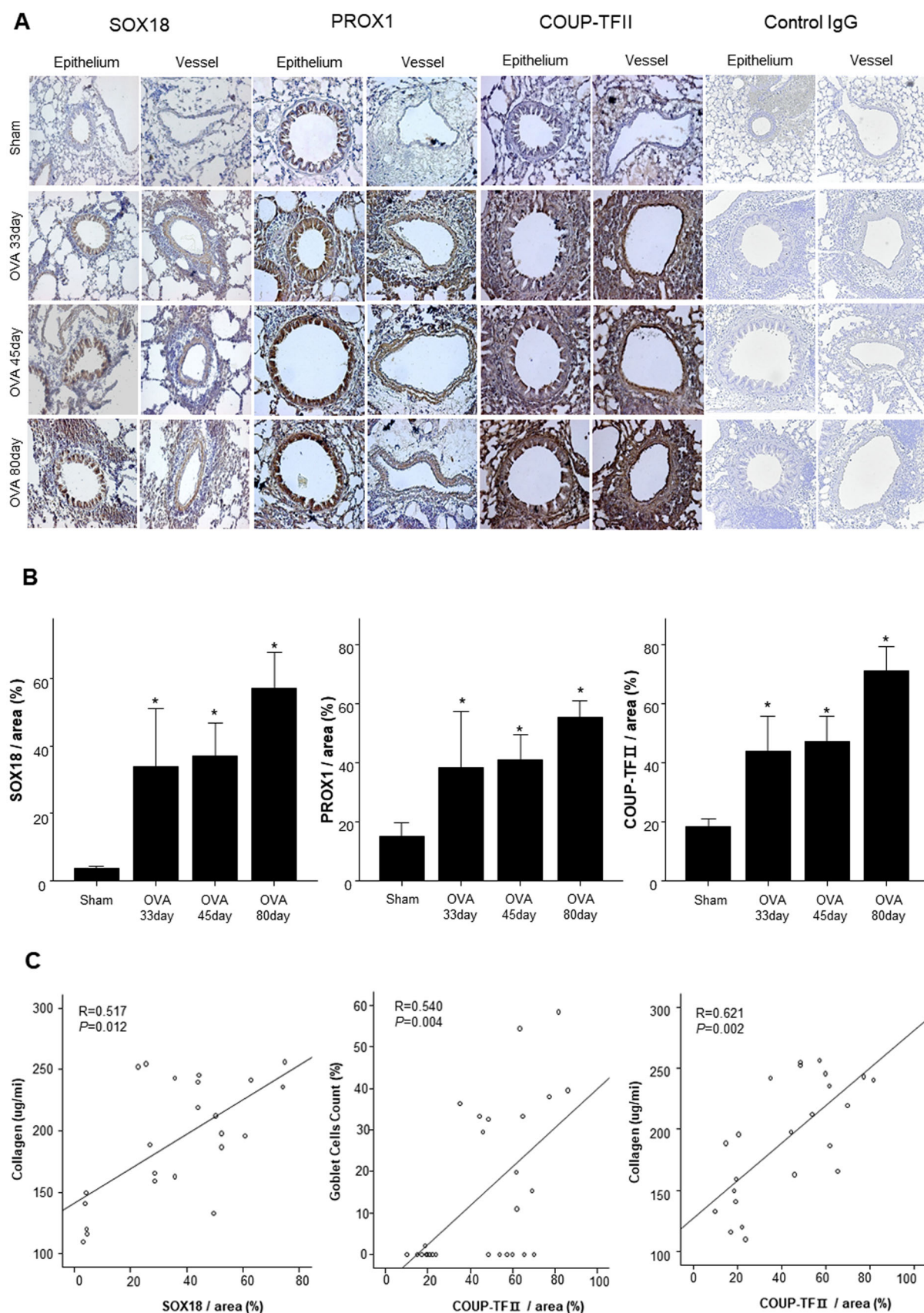


Figure 5. SOX18, PROX1, and COUP-TFII immunohistochemical staining in control mice, and ovalbumin sensitized/challenged (OVA) mice. (original magnification, $\times 400$) (A) SOX18, PROX1, and COUP-TFII expression increased in the lung of OVA-sensitized/challenged mice compared to that of control mice. (B) IHC quantification of SOX18, PROX1 and COUP-TFII in control mice, and ovalbumin (OVA) sensitized and challenged mice. (C) Correlation analysis between mucus gland hyperplasia, and fibrosis and SOX18, PROX1, and COUP-TFII protein in control mice, and ovalbumin sensitized/challenged (OVA) mice.

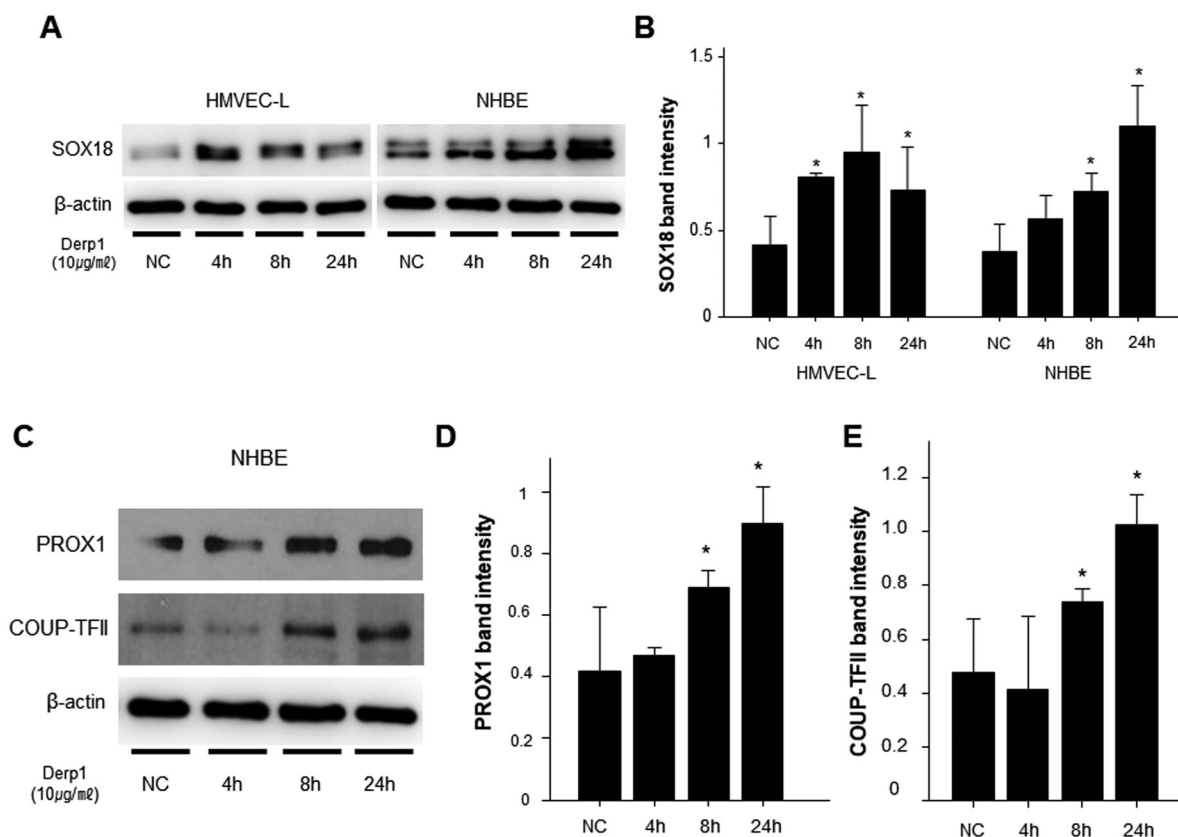


Figure 6. SOX18, PROX1, and COUP-TFII protein determined by western blot in HMVEC-L and NHBE cells treated by 10 μ g/mL *Dermatophagoides pteronyssinus* product 1 (*Der p1*). (A, B) SOX18 protein in HMVEC-L and NHBE cells increased following *Der p1* treatment. (C–E) PROX1 and COUP-TFII protein increased in NHBE cells following *Der p1* treatment.

related to the mammalian sex determining gene region of the Y (SRY). The gene SRY (sex determining region of the Y), located at the distal region of the short arm of the Y chromosome, is necessary for male sex determination in mammals. SOX genes play key roles during animal development (9,39–41), showing diverse and dynamic patterns of expression throughout embryogenesis and in a variety of adult tissue types. Mouse Sox 1, 2, and 3 are expressed at the highest level in the developing nervous system and human SOX 1–4, 10, 11, 14 and 22 genes are expressed in developing brain (42–47). Based on the sequence of its HMG box, human SOX18 along with SOX7 and SOX17, are classified as a member of the F subfamily (48,49).

SOX18 is a transcription factor involved in a range of physiological processes, including differentiation of endothelial cells during new vessel formation (43–45). SOX18 is highly expressed in various types of cancer and may serve as a prognostic factor and a promising therapeutic strategy for hepatocellular carcinoma and skin cancer, and lung cancers (48–50). Previously, SOX18 has not been investigated in asthma. In this study, SOX18 expression increased in a mouse model

of asthma. Although plasma SOX18 tended to be higher in subject with asthma compared to control subjects and increased more during exacerbation as compared to stable asthmatics suggesting that SOX18 is associated with asthma exacerbation related to angiogenesis and airway remodeling (50).

Prospero homeobox 1 (PROX1) has been identified as a master regulator of lymphangiogenesis associated with metastasis including small cell lung cancer (21). COUP-TFII aka NR2F2 is associated with numerous forms of cancer, including gastric, prostate, colon and lung cancer (51). PROX1 and its regulators COUP-TFII and SOX18 drive lymphatic endothelial cell (LEC) specification in mice (21). SOX18 is coexpressed with COUP-TFII and drives the expression of Prox1 in a subset of endothelial cells lining the wall of the cardinal vein (CV). These LECs form the basis of the lymphatic vasculature, and absolutely require transient SOX18 and COUP-TFII activity to induce Prox1 transcription (21–23). In this study, PROX1 and COUP-TFII protein were increased in the lung of mouse model of asthma, indicating that transcription factor PROX1 and COUP-TFII be involved in asthma angiogenesis in collaboration with SOX18.

Inhalation of allergens such as *Der p1* in sensitive individuals leads to a cascade of immune responses characterized by allergen-specific IgE, goblet cell hyperplasia and eosinophilia, contributing to pathogenesis of airway inflammation and respiratory symptoms (52). The authors observed association between *Der p1* exposure and SOX18, PROX1 and COUP-TFII. Following *Der p1* treatment, SOX18 protein increased in HMVEC-L and NHBE cells, and PROX1 and COUP-TFII protein increased in NHBE cells.

Conclusion

In conclusion, we have found SOX18, a crucial transcriptional regulator of angiogenesis, increase in asthma, especially following exacerbation of asthma. Similar other angiogenic transcription factors, PROX1 and COUP-TFII increased in OVA murine asthma model and in *Der p1*-treated NHBE cells. The findings provide new insights into the transcriptional regulation of angiogenesis in asthma. Therefore, the targets for angiogenesis may help modulate airway remodeling and inflammation for asthma.

Author contributions

AS designed the study and AS, JS, and GD wrote the manuscript. JS and YG performed data collection, interpretation and journal submission assistance for this manuscript. PH contributed to the conduct of the study and data collection. All authors read and approved the final manuscript.

Declaration of interest

The authors declare that they have no competing interests.

Funding

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2017R1D1A1B03027822) and Soonchunhyang University (AS), and by the National Institutes of Health grants ES015675, HL077763, and HL085655 (GDL).

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