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Original Article

Inhibition of SERPINE2/protease nexin-1 by a monoclonal antibody attenuates airway remodeling in a murine model of asthma

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Abstract: SERPINE2, also known as protease nexin-1 (PN-1), is a serine protease inhibitor produced by many cell types and has pleiotropic biological functions. It has been reported that SERPINE2/PN-1 is involved in tissue remodeling of fibrotic diseases including idiopathic pulmonary fibrosis and cardiac fibrosis. However, the potential role of SERPINE2/PN-1 in asthmatic airway remodeling has remained barely investigated so far. In this study, BALB/c male mice were sensitized and challenged by ovalbumin to generate murine models of airway remodeling. Anti-SERPINE2 monoclonal antibody was intraperitoneally injected into these mice during the ovalbumin challenge while IgG antibody was used as a vehicle control. The results revealed that the expression of SERPINE2/PN-1 was significantly upregulated in the lung extracts of ovalbumin-challenged mice, and this upregulation was inhibited by dexamethasone. Sustained ovalbumin stimulation increased the thickness of airway wall and α-SMA positive areas in lung, which was attenuated by the treatment with SERPINE2 antibody. In addition, SERPINE2 antibody partially blocked the phosphorylation of ERK, and reduced the upregulation of MMP-9 and TIMP-1 expressions in asthmatic mice. These findings suggest that SERPINE2/PN-1 may play a role in the pathologic development of airway remodeling. Monoclonal antibody against SERPINE2 may have the potential as an effective pharmacotherapy for asthmatic airway remodeling.

Keywords: Asthma, airway remodeling, SERPINE2, protease nexin-1, monoclonal antibody

Introduction

Asthma is a common long-term allergic inflammatory disease of airways characterized by intermittent and reversible airway obstruction. More than 300 million people are suffered from asthma, with about 400,000 deaths per year attributed to this disease globally [1], which emphasizes the urgency to develop counteracting strategies. Airway remodeling is an important feature of chronic asthma and well accepted as a major pathological process which determines the progressive airflow limitation. Even though the combination of an inhaled corticosteroid and a β_2-adrenergic agonist is effective on respiting clinical symptoms in asthmatic patients, remodeling of airway structure is difficult to be ameliorated or reversed. Therefore, more studies focusing on airway remodeling are required to develop new therapeutic targets for asthma.

Remodeling of airway in asthma is defined as a number of structural changes, including thickening of reticular basement membrane, epithelial detachment, mucus gland hyperplasia, subepithelial fibrosis, inflammatory cells infiltrate, bronchial smooth muscle hypertrophy, and vascular changes. Extracellular signal-regulated kinase (ERK) is a member of the mitogen-activated protein kinase (MAPK) family, which has been reported to play an important role in airway remodeling partially through upregulating the transcription of matrix metallopeptidases (MMPs), including MMP-9 [2-4]. MMP-9 is one of the crucial enzymes responsible for extracellular matrix (ECM) turnover. It has been reported that the expression and secretion of MMP-9

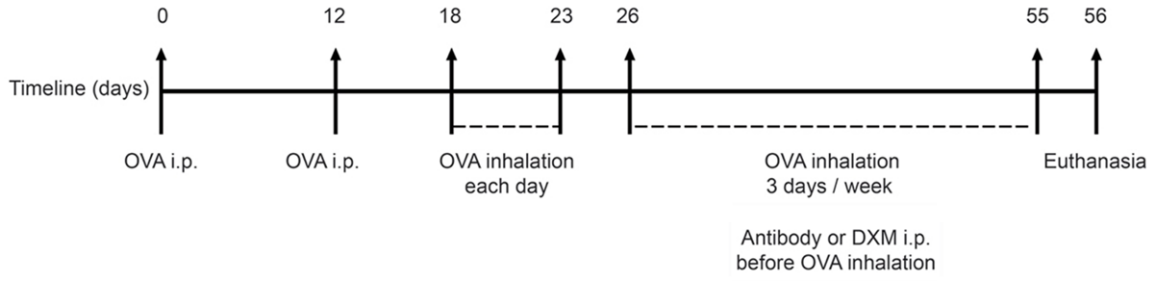

Figure 1. A schematic description for murine models of allergic airway remodeling and SERPINE2 antibody intervention.

are elevated in patients with asthma [5], and MMP-9 deficient mice display less subepithelial fibrosis after chronic allergy stimulation compared with wild type mice.

Recently, the crucial role of serine proteinase inhibitors (serpins), especially plasminogen activator inhibitor-1 (PAI-1), in airway remodeling has been indicated [6, 7]. Pharmacological inhibition of PAI-1 significantly reduces airway remodeling in murine models of chronic asthma [8-10]. Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 2 (SERPINE2), also known as PN-1, is a member of serpin superfamily which can inhibit urokinase, plasmin and thrombin. SERPINE2/PN-1 is most highly expressed during murine alveogenesis and synthesized by multiple cell types, including alveolar inflammatory cells, lung fibroblasts [11] and airway epithelial cells [12].Previously, *SERPINE2* is recognized as a candidate susceptibility gene for chronic obstructive pulmonary diseases (COPD) in humans [12, 13]. While little doubt exists that common mechanisms underlie COPD and asthma, the potential role of SERPINE2 in asthma has barely been investigated yet. Interestingly, a recent study demonstrated that 5 single-nucleotide polymorphisms (SNPs) of *SERPINE2* gene are associated with asthma phenotypes in humans [14], indicating that, like its closest relative PAI-1 [15], SERPINE2/PN-1 may be implicated in the pathogenesis of asthma.

Therefore, in this study, we investigated the expression of SERPINE2/PN-1 in lung tissues in a murine model of chronic asthma. Next, we examined whether a monoclonal antibody against SERPINE2/PN-1 affected airway remodeling and explored the potential molecular mechanisms involved in this process.

Materials and methods

Reagents

OVA was purchased from Sigma (Saint Louis, MO, USA). Dexamethasone (DXM) was obtained from SINOPHARM (Beijing, China). Mouse IgG2b negative control antibody and mouse monoclonal SERPINE2 antibody (clone 8C4.1) were purchased from EMD Millipore (Billerica, MA, USA). Rabbit monoclonal β-Tubulin, P-p44/42 MAPK (ERK 1/2) (Thr202/Tyr204) and p44/42 MAPK (ERK 1/2) were obtained from Cell Signaling Technology (Boston, MA, USA). Mouse monoclonal α-SMA antibody was purchased from Servicebio (Wuhan, Hubei, China). Goat polyclonal MMP-9 antibody and goat polyclonal TIMP-1 antibody were obtained from R&D Systems (Minneapolis, MN, USA).

Animals

All animal protocols were approved by the Animal Care and Use Committee of the Central South University and were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. Male BALB/c mice (8-10 weeks of age) were obtained from the Center of Laboratory Animals, Central South University (Changsha, Hunan, China). Animals were housed in a specific-pathogen-free laminar-flow atmosphere under controlled temperature, humidity and light with a standard rodent chow diet. Animal modeling was performed after the mice had been acclimated for 1 week.

Mouse experimental design

All mice were randomly divided into 7 groups: 1) control group; 2) OVA group; 3) OVA+DXM group;

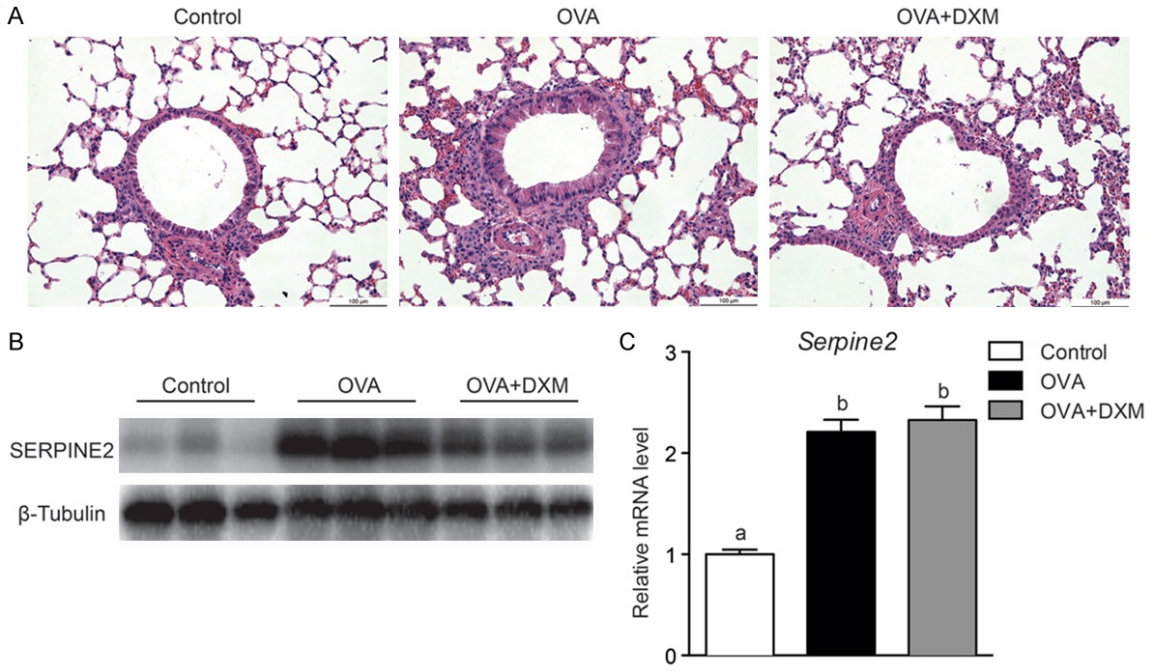

Figure 2. The expression of SERPINE2/PN-1 is upregulated in lung during the development of airway remodeling and reduced by DXM treatment. (A) Histological examination of lung tissues from mice in control, OVA and OVA+DXM groups by H&E staining. (B, C) Representativeimmunoblot of SERPINE2 protein (B, n=3) and quantitative PCR analyses of *Serpine2* mRNA level (C, n=4) in lung tissues from mice in control, OVA and OVA+DXM groups. Original magnification: ×200. Scale bars = 100 μm. Data in (C) is presented as mean ± SEM. A different letter denotes a significant difference between groups at $P<0.05$.

4) OVA+low dose of SERPINE2 antibody group (OVA+low Ab); 5) OVA+middle dose of SERPINE2 antibody group (OVA+mid Ab); 6) OVA+high dose of SERPINE2 antibody group (OVA+high Ab); 7) OVA+IgG control antibody group (OVA+IgG). As shown in **Figure 1**, BALB/c mice were sensitized by intraperitoneal injections of 20 μg of OVA and 1 mg of $Al(OH)_3$ suspended in 0.2 ml saline on days 0 and 12. Subsequently, these mice were challenged with 5% OVA aerosol for 30 min each day on days 18-23 and three days per week on days 26-55 to construct a murine model with airway remodeling. Whereas mice in control group were sensitized with sterile saline and challenged with saline aerosols. From day 26 to day 55, IgG2b control antibody, anti-SERPINE2 monoclonal antibody (0.001, 0.01 or 0.1 mg/kg body weight) or DXM (2 mg/kg body weight) were intraperitoneally injected into the OVA-challenged mice 30 min before each OVA challenge. DXM was used as a positive control of effective treatment in asthma. An equal quantity of sterile saline was administered to mice in control and OVA groups. All mice were sacrificed on day 56 (namely, 24 h after the last aerosol challenge and intraperitoneal injection) and lung tissues were harvested for further analysis.

Quantitative real-time PCR

Total RNA was extracted from lung tissues using Trizol Reagent (TaKaRa, Shiga, Japan) according to the manufacturer's instructions. RNA was reverse transcribed to cDNA using All-in-One™ First-Strand cDNA Synthesis Kit (GeneCopoeia, MD, USA) and qPCR was performed using All-in-One™ qPCR Mix (GeneCopoeia, MD, USA) on a 7900HT Fast Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). Fold change was determined using the DDCt method with samples normalized to the reference gene *Actb*. Primer sequences used for quantitative PCR were as follows: *Serpine2* forward: ATGTAAACGGAGTTGGTAAAGTGCT, reverse: TCAGATTTGGGGAAAGCAGATTA; *Mmp9* forward: CTCGGGAAGGCTCTGCTGTT, reverse: AACTCACACGCCAGAAGAATTTG; *Timp1* forward: CCCAGAAATCAACGAGACCACC, reverse: ACGCCAGGGAACCAAGAAGC;

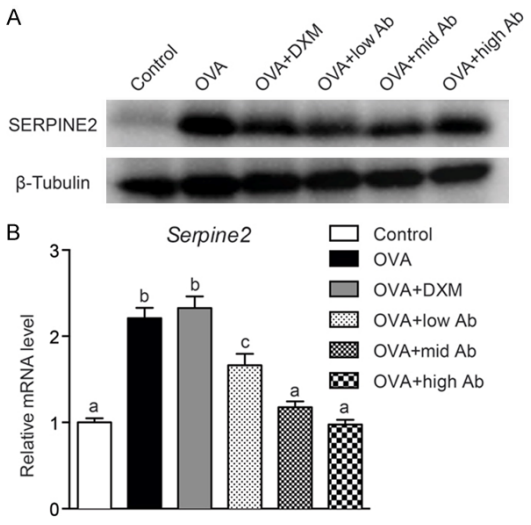

Figure 3. The administration of SERPINE2 antibody inhibits the upregulation of SERPINE2 in OVA-challenged mice. (A, B) Representative immunoblot of SERPINE2 protein (A, n=3) and quantitative PCR analyses of *Serpine2* mRNA level (B, n=4) in lung tissues from mice in control, OVA, OVA+DXM and OVA+low/mid/high Ab groups. Data in (B) is presented as mean ± SEM. A different letter denotes a significant difference between groups at $P<0.05$.

Actb forward: GTGACGTTGACATCCGTAAAGA, reverse: GTAACAGTCCGCCTAGAAGCAC.

Western blot assay

Lung tissues were lysed with RIPA buffer (50 mM Tris-HCl, pH 7.5, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride) supplemented with protease inhibitor cocktail (Roche, Basel, Switzerland) and phosphatase inhibitors (10 mM NaF, 60 mM β-glycerolphosphate, pH 7.5, 2 mM sodium orthovanadate, 10 mM sodium pyrophosphate). Protein extracts were electrophoresed in 10% sodium dodecyl sulphate-polyacrylamide (SDS-PAGE) gels and transferred to polyvinylidene fluoride membranes. After blocking with 5% non-fat milk in TBS-T for 1 h at room temperature, membranes were incubated overnight at 4°C with primary antibodies against SERPINE2 (1:1000), β-Tubulin (1:1000), p-ERK (1:1000) and total ERK (1:1000). After three washes, membranes were incubated with the secondary antibody conjugated with horseradish peroxidase at room temperature for 1 h. The blots were developed with Super Signal chemiluminescent substrate (Pierce Chemical Co, Rockford, IL, USA) and exposed to film.

Histology

Briefly, mice were euthanized 24 h after the last OVA challenge or treatment with saline aerosol. Lung tissues were fixed in 4% paraformaldehyde overnight at 4°C and routinely processed for histological inclusion in paraffin. Lung tissue sections (5 μm) were stained with hematoxylin and eosin (H&E), and the morphological changes in lung were observed under a light microscope (Olympus, Tokyo, Japan). All experiments were performed with lung tissues from 4 mice in each group, and 3 bronchioles with 150-200 μm inner diameter were selected and counted on each slide. The perimeter of basement membrane (Pbm) and total area of airway wall (Wat) were measured with the Image Pro Plus 6.0 software (MediaCybernetics Co., Bethesda, MD, USA) and the bronchial wall thickness was calculated as the ratio of Wat to Pbm (Wat/Pbm) for subsequent statistical analysis.

Immunohistochemistry

To determine the expression of α-SMA, MMP-9 and TIMP-1 in the lung tissue of mice, immunohistochemistry analyses were carried out. Briefly, lung sections (5 μm) were deparaffinized with xylene, rehydrated in ethanol and blocked with 3% bovine serum albumin for 30 min at room temperature. Subsequently the sections were routinely stained with primary antibodies against α-SMA (1:2000), MMP-9 (1:50) or TIMP-1 (1:50) overnight at 4°C. The reaction products were visualized by treating the slide with 3,3'-diaminobenzidine (DAB) and counterstaining with hematoxylin. The immunohistochemical changes of the lung were observed under a light microscope (Olympus, Tokyo, Japan) and the average optical density (AOD) was measured with the Image Pro Plus 6.0 software for subsequent statistical analysis. All experiments were performed with lung tissues from 4 mice in each group.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 6. Data are expressed as mean ± SEM. A one-way analysis of variance (ANOVA) was applied for multiple group com-

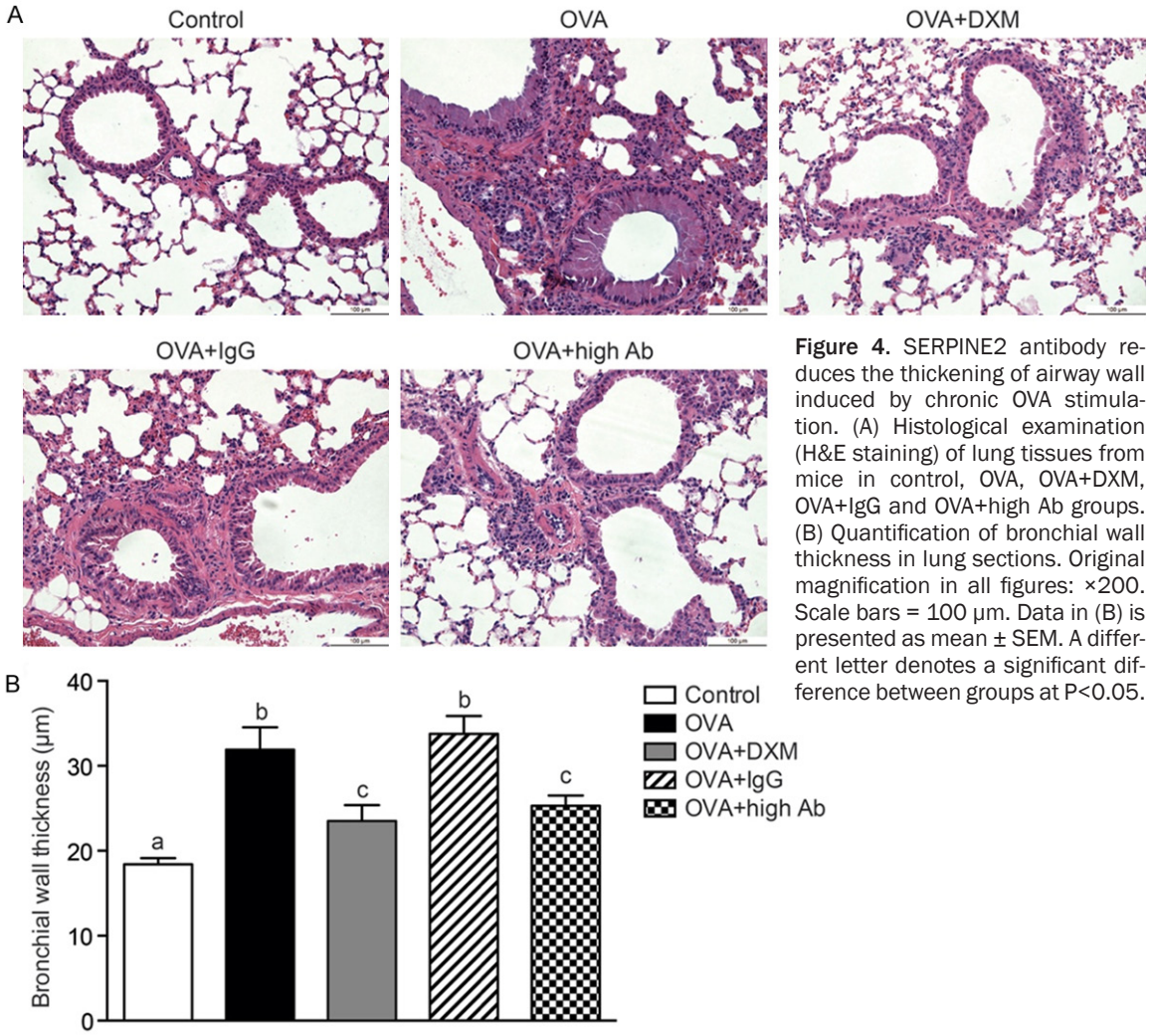

Figure 4. SERPINE2 antibody reduces the thickening of airway wall induced by chronic OVA stimulation. (A) Histological examination (H&E staining) of lung tissues from mice in control, OVA, OVA+DXM, OVA+IgG and OVA+high Ab groups. (B) Quantification of bronchial wall thickness in lung sections. Original magnification in all figures: ×200. Scale bars = 100 μm. Data in (B) is presented as mean ± SEM. A different letter denotes a significant difference between groups at P<0.05.

parisons involving one independent variable. A *P* value <0.05 was considered to be statistically significant. The data presented is representative of 2-3 independent experiments.

Results

Expression of SERPINE2/PN-1 significantly increased in lung during airway remodeling

Murine models of chronic asthma were induced by sustained OVA stimulation (**Figure 1**). Typical pathological changes were observed in the bronchia of OVA-challenged mice instead of mice in control group, including epithelial detachment, subepithelial fibrosis, inflammatory cell infiltration and smooth muscle hypertrophy, which were ameliorated by DXM (**Figure 2A**), an effective steroid on reducing airway structural changes in asthma [16]. Interestingly, a dramatic elevation of SERPINE2 protein level was observed in the lung extracts of OVA-challenged mice, and DXM treatment largely suppressed this elevation (**Figure 2B**). *Serpine2* mRNA level also displayed a significant upregulation after OVA stimulation (**Figure 2C**). Taken together, these data suggested that SERPINE2/PN-1 may be involved in the pathological development in OVA-induced airway remodeling.

Treatment with SERPINE2 antibody blocked the upregulation of SERPINE2/PN-1 induced by OVA

To further investigate whether the increased SERPINE2/PN-1 expression plays a role in airway remodeling, a specific monoclonal antibody against SERPINE2 was intraperitoneally injected into asthmatic mice. Markedly, the induction of SERPINE2 protein in lung tissues

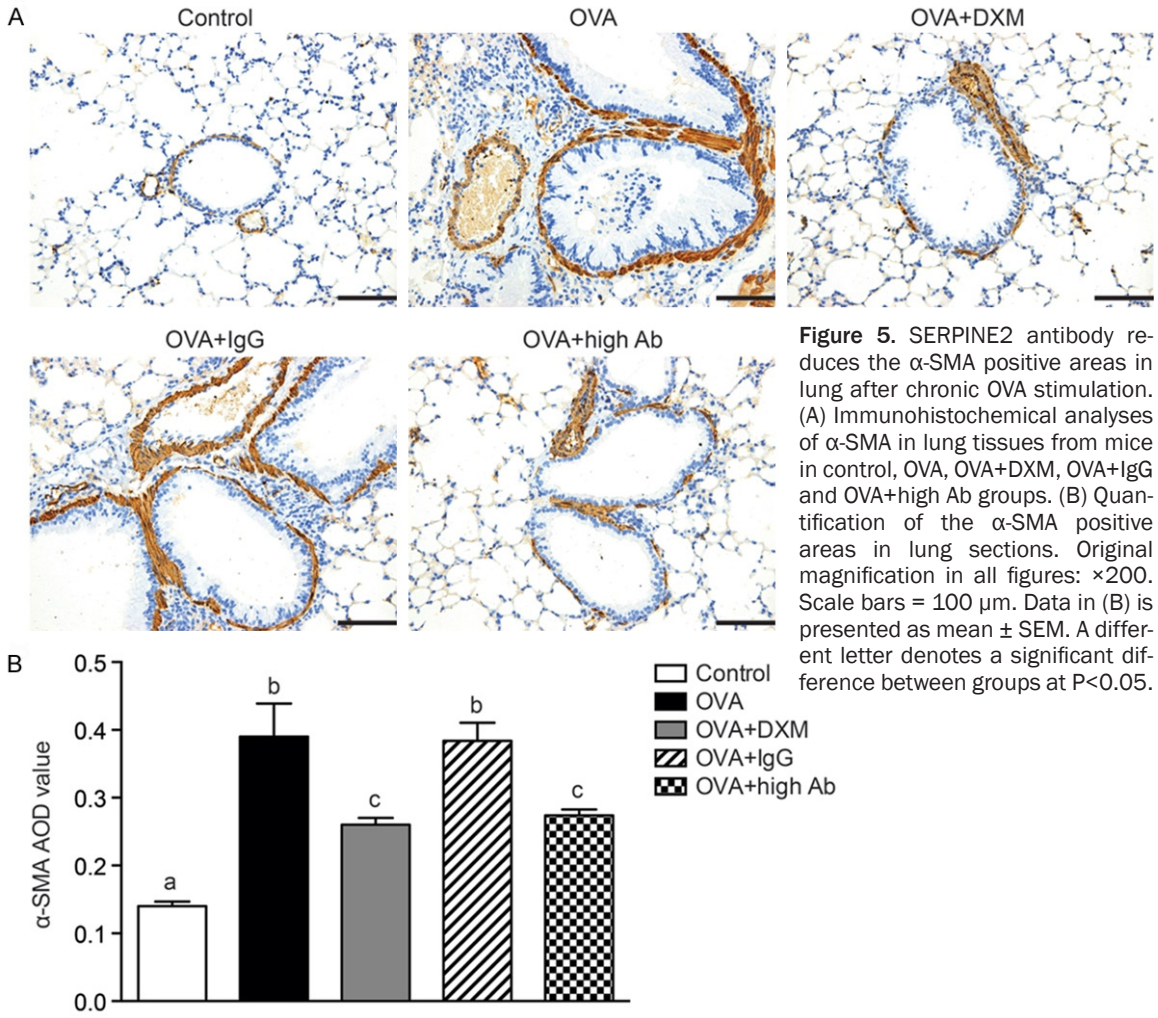

Figure 5. SERPINE2 antibody reduces the α-SMA positive areas in lung after chronic OVA stimulation. (A) Immunohistochemical analyses of α-SMA in lung tissues from mice in control, OVA, OVA+DXM, OVA+IgG and OVA+high Ab groups. (B) Quantification of the α-SMA positive areas in lung sections. Original magnification in all figures: ×200. Scale bars = 100 μm. Data in (B) is presented as mean ± SEM. A different letter denotes a significant difference between groups at $P<0.05$.

from asthmatic mice was significantly blocked by treatment of SERPINE2 antibody (**Figure 3A**). Consistently, the OVA-induced upregulation of *Serpine2* mRNA level was reduced by SERPINE2 antibody at different doses (**Figure 3B**).

Treatment with SERPINE2 antibody attenuated the thickening of airway walls

Structural changes in asthma lead to the thickening of airway walls [17]. Indeed, bronchial walls were thickened in asthmatic mice compared with control mice (**Figure 4A**). Importantly, similar with DXM treatment, injection of high-dose SERPINE2 antibody significantly attenuated the thickening of bronchial walls in asthmatic mice, while IgG control antibody did not show such an effect (**Figure 4A**). Furthermore, quantification of bronchial wall thickness consistently supported the effect of SERPINE2/PN-1 antibody on bronchial wall thickness (**Figure 4B**).

Treatment with SERPINE2 antibody reduced α-SMA positive areas in lung of OVA-challenged mice

Smooth muscle hypertrophy in airway is a major pathological feature of chronic asthma. As a marker of smooth muscle, α-SMA is considered as an index of airway remodeling [18]. Thus, we further measured α-SMA positive areas in lung tissues. Compared with mice in control group, the α-SMA-stained smooth muscle layers significantly increased in OVA-challenged mice (**Figure 5A**). Intraperitoneal injection of SERPINE2 antibody or DXM reduced the α-SMA positive areas while IgG control antibody did not (**Figure 5A**). Quantification of α-SMA staining also indicated that SERPINE2 antibody

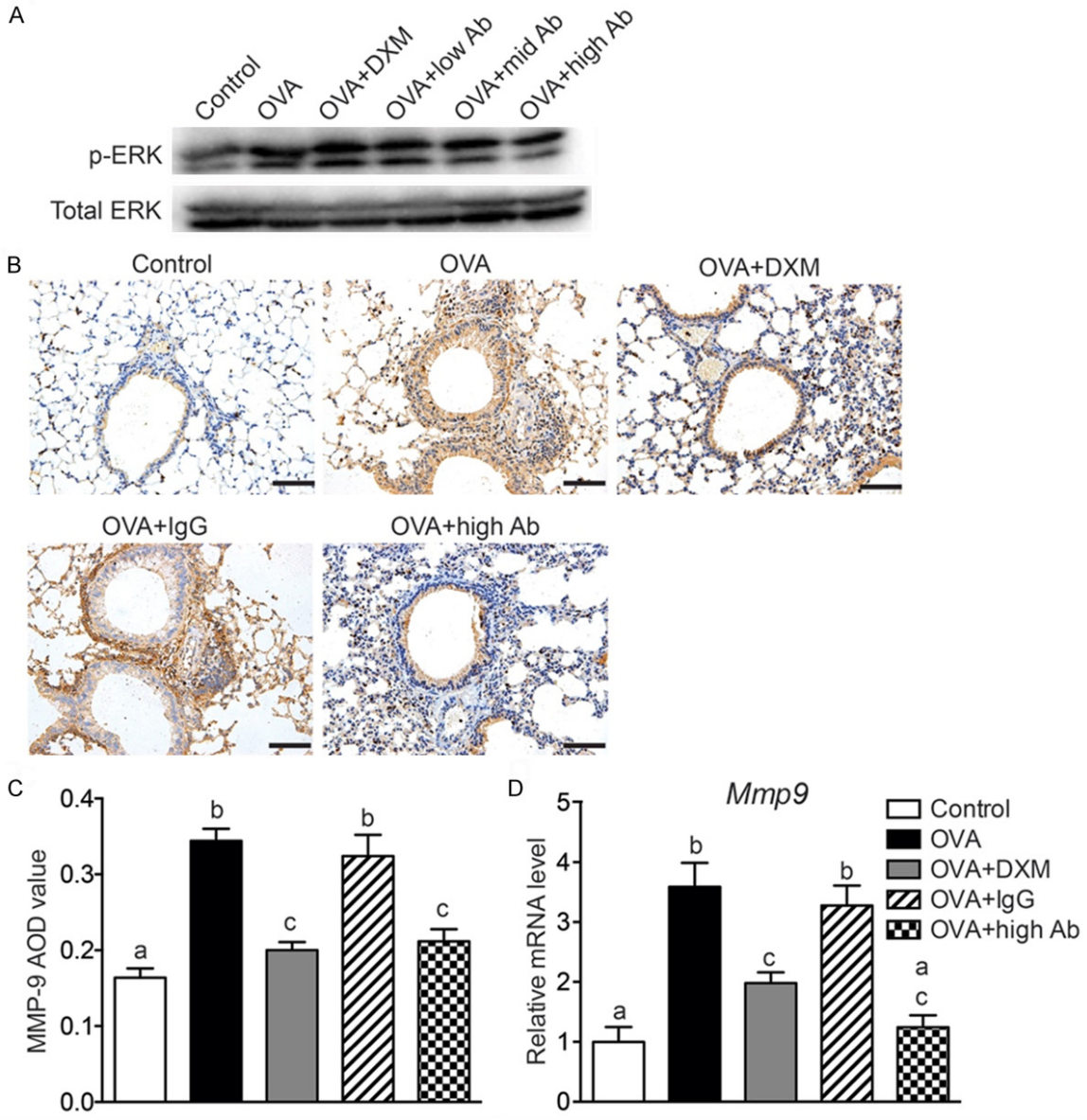

Figure 6. SERPINE2 antibody partially blocks the phosphorylation of ERK and the upregulation of MMP-9 expression in lung of OVA-challenged mice. (A) Representative immunoblot of phosphorylated ERK in lung tissues from mice in control, OVA, OVA+DXM and OVA+low/mid/high Ab groups. (B) Immunohistochemical analyses of MMP-9 protein level in lung tissues from mice in control, OVA, OVA+DXM, OVA+IgG and OVA+high Ab groups (n=4). (C) Quantification of MMP-9 positive staining areas in lung sections. (D) Quantitative PCR analyses of *Mmp9* mRNA levels in lung tissues from mice in control, OVA, OVA+DXM, OVA+IgG and OVA+high Ab groups (n=4). Original magnification in all figures: ×200. Scale bars = 100 μm. Data in (C and D) is presented as mean ± SEM. A different letter denotes a significant difference between groups at $P<0.05$.

inhibited smooth muscle hypertrophy in lung of OVA-challenged mice, to a slightly less extend compared with DXM treatment (**Figure 5B**).

SERPINE2 antibody partially inhibited the activation of ERK and the upregulation of MMP-9 and TIMP-1 in OVA-challenged mice

ERK pathway is an important mediator for asthmatic airway remodeling [2, 3]. SERPINE2/PN-1 has been reported to activate ERK pathway and subsequently promote ECM degradation in breast cancer cell lines [19, 20]. To test the possibility that SERPINE2/PN-1 affects airway remodeling through ERK pathway, we subsequently detected p-ERK protein level in lung tissues of asthmatic mice treated with SERPINE2 antibody or not. As shown in **Figure 6A**, OVA stimulation clearly induced the phosphoryla-

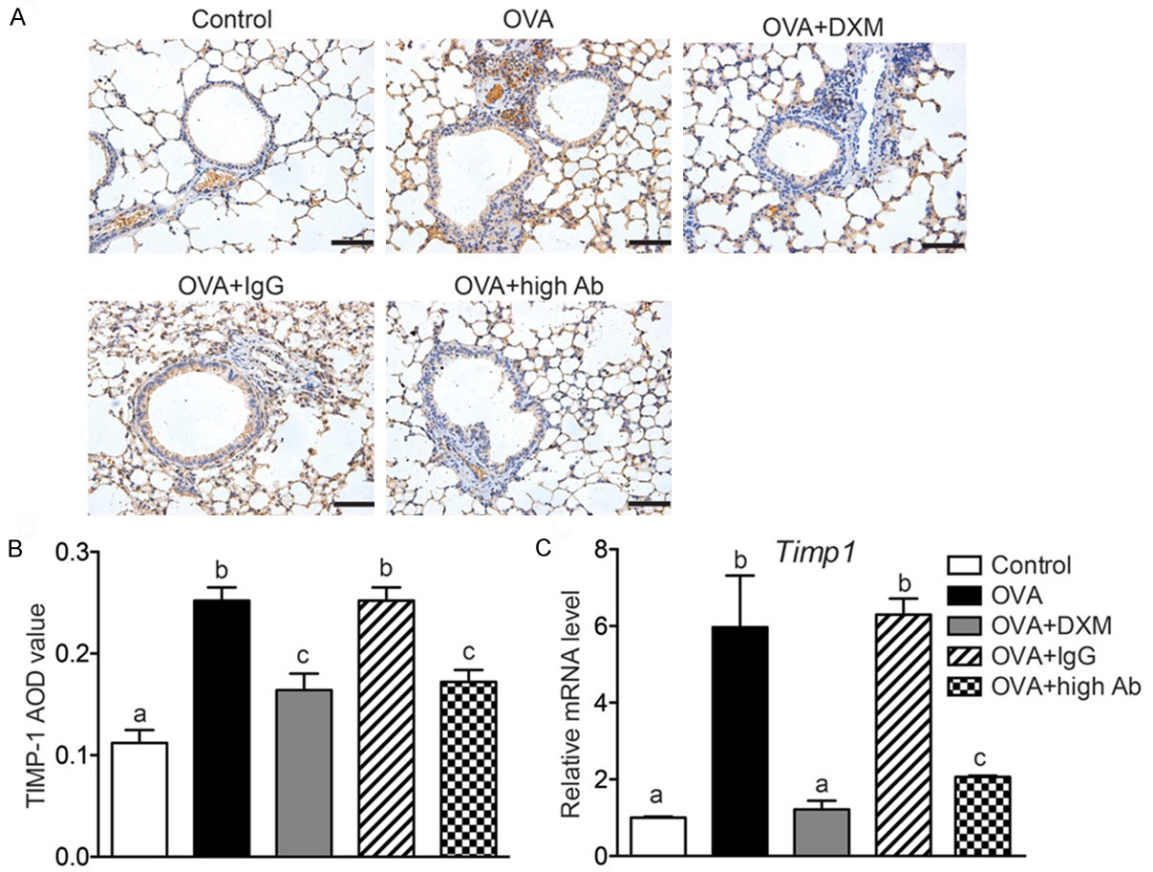

Figure 7. SERPINE2 antibody reduces the upregulation of TIMP-1 expression in lung of OVA-challenged mice. (A) Immunohistochemical analyses of TIMP-1 protein level in lung tissues from mice in control, OVA, OVA+DXM, OVA+IgG and OVA+high Ab groups (n=4). (B) Quantification of TIMP-1 positive staining areas in lung sections. (C) Quantitative PCR analyses of *Timp1* mRNA levels in lung tissues from mice in control, OVA, OVA+DXM, OVA+IgG and OVA+high Ab groups (n=4). Original magnification in all figures: ×200. Scale bars = 100 μm. Data in (B and C) is presented as mean ± SEM. A different letter denotes a significant difference between groups at $P<0.05$.

tion of ERK in lung, which was partially inhibited by high dose of SERPINE2 antibody.

It has been reported that MMP-9 expression level and activity are elevated in patients with asthma [5]. Additionally, activation of ERK pathway has been demonstrated to upregulate the transcription of MMP-9 [21]. Indeed, we found that both protein and mRNA levels of MMP-9 in lung tissues were upregulated by OVA (**Figure 6B-D**). Interestingly, this upregulation of MMP-9 was again blocked by SERPINE2 antibody (**Figure 6B-D**). Meanwhile, the expression of TIMP-1, the endogenous inhibitor of MMP-9, was also elevated by OVA and reduced by SERPINE2 antibody (**Figure 7A-C**), which might be a represent of an endogenous protective mechanism to adaptively regulate the MMP-9 activity in lung.

Discussion

Progressive airway remodeling is a major challenge in asthma therapy. Herein, we found that the expression level of SERPINE2/PN-1 was significantly upregulated in lung tissues from mice with airway remodeling. Antibody-mediated SERPINE2/PN-1 inhibition attenuated airway structural changes including thickening of bronchial wall and smooth muscle hypertrophy. Additionally, the inactivation of ERK pathway and the downregulation of MMP-9 and TIMP-1 may be involved in the protective effect of SERPINE2 antibody on airway remodeling.

SERPINE2/PN-1 is a serpin commonly reported to regulate fibrinolysis and coagulation functions through inactivation of multiple serine proteases, including thrombin, urokinase and

plasmin. To date, the expression and biological functions of SERPINE2/PN-1 have been most extensively studied in the nervous and reproductive systems, while the potential role of SERPINE2/PN-1 in asthma and airway remodeling remains unknown. In this study, we found that SERPINE2/PN-1 protein level was upregulated in lung tissues by chronic OVA stimulation, which was greatly suppressed by DXM treatment, supporting its involvement in the pathogenesis of asthmatic airway remodeling. But *Serpine2* mRNA level elevated in OVA-challenged mice was not blocked by DXM, indicating that posttranscriptional regulation of SERPINE2/PN-1 might be more important in this process. Previously, SERPINE2/PN-1 overexpression has been reported to promote ECM production in pancreatic tumors [22]. Increased SERPINE2/PN-1 expression and SERPINE2/PN-1-mediated deposition of ECM were observed in several fibrotic diseases including idiopathic pulmonary fibrosis and cardiac fibrosis induced by surgical transverse aortic constriction [11, 23]. These findings collectively suggest that tissue remodeling observed in asthma and other diseases may share a common pathophysiologic mechanism, in which SERPINE2/PN-1 may play an important role.

Moreover, our current study demonstrated that treatment with a monoclonal antibody against SERPINE2/PN-1 ameliorated airway structural changes in asthmatic mice, including bronchial wall thickening and smooth muscle hypertrophy. In recent years, neutralizing antibodies have shown novel therapeutic potentials in various diseases by specifically targeting the endogenous antigen [24-26]. Indeed, a significant decrease of SERPINE2 protein and mRNA levels was observed in lung tissues from mice treated with SERPINE2 antibody, confirming that SERPINE2 antibody attenuated airway remodeling through effective inhibition of SERPINE2/PN-1. Recently, the crucial role of serpins in airway remodeling has been strongly indicated. PAI-1, the phylogenetically closest relative of SERPINE2/PN-1 in serpin superfamily, is upregulated in lung after allergy stimulation [27]. Lee SH *et al.* showed that a specific PAI-1 inhibitor Tiplaxtinin attenuated airway inflammation and collagen deposition in a murine model of chronic asthma [8]. IMD-4690, another novel PAI-1 inhibitor, reduced allergic airway remodeling in mice [9]. TM5275, an orally effective small molecule inhibitor of PAI-1, was also demonstrated to reduce airway inflammation and subepithelial fibrosis [10]. Although the underlying mechanism has not been clearly elucidated, all these findings collectively suggest that serpins such as SERPINE2/PN-1 and PAI-1 may be promising therapeutic targets for asthma patients with airway remodeling. Investigations focusing on developing novel inhibitors that could be translated into clinical practice are needed in the future.

The molecular mechanism through which SERPINE2/PN-1 may contribute to the development of airway remodeling has not identified yet. We found that the protective effect of SERPINE2 antibody on airway remodeling is associated with the inhibition of ERK pathway and downregulation of MMP-9 expression. Activation of ERK is known to promote airway remodeling [2, 3] and upregulate the transcription of MMP-9 [21]. It has been demonstrated that expression of MMP-9, the most studied member of MMPs, increased in sputum samples from patients with asthma and positively correlated with the severity of asthma [5]. In addition, MMP-9 deficient mice display less peribronchial fibrosis upon chronic OVA stimulation [28]. Meanwhile, upregulation of TIMP-1 expression is also observed in mice with airway remodeling, and MMP-9/TIMP-1 imbalance is an important pathological factor in chronic asthma [29]. In line with these previous studies, we observed an elevated expression of MMP-9 and TIMP-1 induced by OVA in mice. SERPINE2 antibody largely suppressed the elevation of MMP-9 and TIMP-1, suggesting the MMP-9/TIMP-1 balance may have a role in the potential contribution of SERPINE2/PN-1 to airway remodeling. Recent studies revealed that genetic SERPINE2/PN-1 deficiency in mice and humans is associated with lymphocyte accumulation and activated immune responses in lung [30], indicating that SERPINE2/PN-1 may also be implicated in regulating the activities of immune cells, thus contributing to asthmatic airway remodeling. It's worth noting that SERPINE2/PN-1 has pleiotropic biological functions through inhibition of multiple serine proteases. Therefore, SERPINE2/PN-1 may regulate a number of intracellular signaling except for ERK pathway and be involved in various pathological processes in lung, depending on its preferential anti-protease activities.

When generalizing the present results, several limitations must be considered. Firstly, the inhibition of SERPINE2/PN-1 was performed by using the SERPINE2 monoclonal antibody. More technical tools should be considered in future studies to clearly elucidate the potential role of SERPINE2/PN-1 in asthmatic airway remodeling, including genetic engineering animal models, novel pharmacological inhibition of SERPINE2/PN-1 and the treatment with recombinant SERPINE2 protein *in vivo*. In addition, it has been reported that SERPINE2/PN-1 is detectable in bronchoalveolar lavage fluid [11], and *SERPINE2* expression level is inversely correlated with post-bronchodilator FEV_1 and diffusing capacity of the lung for carbon monoxide (DLCO) in COPD patients [12], indicating SERPINE2/PN-1 might be a potential biological markers for evaluating the pulmonary function loss in certain diseases. Thus, further studies are required to investigate the association between SERPINE2/PN-1 expression and the pulmonary function or the severity of asthma in humans.

In conclusion, the present study provides evidence that SERPINE2/PN-1 may play a role in asthmatic airway remodeling. Particularly, a monoclonal antibody against SERPINE2/PN-1 effectively ameliorates airway remodeling, which may be mediated through the ERK pathway and MMP-9. Taken together, these findings indicate that SERPINE2/PN-1 may be a promising target for developing therapeutic strategies and SERPINE2 antibody might have a therapeutic potential for patients with asthma.

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Disclosure of conflict of interest

None.

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