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SERPINB10 promotes macrophage M2 polarization and airway inflammation in asthma

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Abstract

Background Macrophage M2 polarization plays a critical role in type 2 airway inflammation in asthma. We previously reported that serine peptidase inhibitor, clade B, member 10 (SERPINB10) promotes airway eosinophilic inflammation in asthma.

Objective To investigate the role of SERPINB10 in macrophage M2 polarization and airway inflammation in asthma.

Methods The expression of *SERPINB10* was detected in bronchoalveolar lavage (BAL) cells from 15 control subjects and 36 asthma patients. *Serpinb10* knockout mice and wild type mice were sensitized and challenged with ovalbumin (OVA). Macrophage polarization and airway inflammation were evaluated. An adoptive transfer experiment of *Serpinb10*-deficient macrophages to macrophage-depleted mice was performed to assess the effect of *Serpinb10* deficiency in macrophages on the airway inflammation in the model. The role of SERPINB10 in the activation of IL-4 receptor (IL-4R) signaling pathway and macrophage M2 polarization was investigated in cell cultures.

Results *SERPINB10* expression was markedly elevated in BAL cells from asthmatic patients, and was significantly correlated with fractional exhaled nitric oxide and *CD206*, a marker for macrophage M2 polarization. In the OVA-induced allergic airway inflammation mouse model, *Serpinb10* deficiency significantly inhibited airway inflammation, mucous cell metaplasia and airway hyperresponsiveness. Moreover, *Serpinb10* deficiency suppressed the expression of M2 markers including *Cd206*, *Arg1* in mouse lung tissues and the protein levels of M2 macrophage effector cytokines including Ccl17 and Ccl22 in BAL fluid. Adoptive transfer of *Serpinb10*-deficient bone marrow-derived macrophages (BMDMs) to wild type mice depleted macrophages significantly suppressed the airway inflammation and mucous cell metaplasia. Mechanistically, SERPINB10 suppresses the degradation of IL-4Rα in macrophages, thereby upregulating the phosphorylation of Stat6 and Akt and leading to macrophage M2 polarization.

Conclusions SERPINB10 promotes macrophage M2 polarization by suppressing IL-4Rα degradation and upregulating IL-4R signaling. SERPINB10 is a potential therapeutic target for asthma.

Keywords Asthma, SERPINB10, Airway inflammation, Macrophage polarization, IL-4Rα

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Introduction

Asthma is a chronic inflammatory airway disease, characterized by, airway inflammation, mucus hypersecretion, and airway hyperresponsiveness, and affects more than 300 million people worldwide [1–3]. Asthma has long been associated with type 2 immune responses, in which a variety of cells including macrophages are involved [4]. Macrophages are classically activated or alternatively activated, also called M1 or M2 polarization, respectively [5]. M2 macrophages contribute to the pathogenesis of asthma by promoting type 2 airway inflammation [6].

Allergens stimulate dendritic cells to present antigen to T cells and promote Th2 cells differentiation. Th2 cytokines including IL-4 drive macrophage M2 polarization [7]. M2 macrophages express CCL17 and CCL22, which orchestrate Th2-dominant immune responses in asthma via CCR4-mediated recruitment of Th2 lymphocytes and eosinophils to the airways. This cascade exacerbates key pathological hallmarks of asthma, including airway hyperresponsiveness, mucus hypersecretion, and eosinophilic inflammation [7–9]. Although M2 macrophages are crucial in type 2 airway inflammation of asthma, the mechanism underlying macrophage M2 activation remains not fully understood.

Serine protease inhibitors (SERPIN) is a family of proteins which regulates the activity of a variety protease including serine protease [10]. It is involved in biological processes such as phagocytosis, apoptosis, and cell migration. SERPINB2 has long been associated with chronic inflammatory airway diseases including asthma [11]. SERPINB2 has been implicated in macrophage M2 polarization in breast cancer and enteric infection [12, 13]. SERPINB2 is also reported to regulate immune response in kidney injury by regulating phagocytosis and migration of macrophages [14]. Our previous study revealed that *SERPINB10* expression was increased in bronchial brushing samples and could serve as a potential marker of airway eosinophilic inflammation in asthma patients. *Serpinb10* deficiency suppressed airway eosinophilia in a mouse model [15]. However, whether *SERPINB10* is expressed in macrophages and its role in macrophage M2 polarization is unclear. We hypothesize that SERPINB10 is involved in allergic airway inflammation via promoting macrophage M2 polarization in asthma.

In this study, we demonstrate that *SERPINB10* expression was elevated in BAL cells from asthma patients and was positively correlated with FeNO and *CD206* expression. *Serpinb10* deficiency attenuated OVA-induced airway inflammation and expression of *Cd206*, *Arg1* in mouse lung tissues and the protein levels of Ccl17, Ccl22 in BALF. Adoptive transfer of *Serpinb10*-deficient macrophages to macrophage-depleted wild type mice suppressed the lung inflammation and mucus production. Mechanistically, SERPINB10 promotes macrophage M2 polarization by suppressing IL-4R degradation.

Materials and methods

Subjects

Thirty-six asthma patients and 15 healthy control subjects were recruited from Tongji Hospital. None of the subjects had a smoking history or received corticosteroid therapy. Clinical data analysis of subjects is shown in Table S1. Bronchoscopy was conducted as previously described [16]. All subjects had signed written informed consent. The study obtained approval from the ethics committee of Tongji Hospital, Huazhong University of Science and Technology (TJ-IRB20231161).

Murine model of allergic airway inflammation

Serpinb10$^{-/-}$ mice and wild type (WT) mice were purchased from Animal Center of Hubei Province (Wuhan, China). On days 0, 7 and 14, mice underwent sensitization through intraperitoneal injection of 100 μg OVA (Sigma-Aldrich, USA) in 100 μL saline mixed with $Al(OH)_3$ or an equivalent volume of saline intraperitoneally. On days 21, 22, and 23, mice were challenged intranasally with the OVA solution (1 mg in 50 μL saline). 24 h after the final challenge, pulmonary resistance was measured and BAL cell counts were performed [17]. The animal experiments were approved by the Animal Care and Use Committee of the Tongji Hospital (TJH-202309026).

Macrophage depletion and adoptive transfer

Adoptive transfer experiments is the transplantation of ex vivo-modified immune cells (e.g., T cells or macrophages) into recipient mice to evaluate their function in disease models [18]. In order to deplete mouse lung macrophages, we injected clodronate liposomes (200 μL) into the lungs of both *Serpinb10*$^{-/-}$ and WT mice on days 18 and 19 [17]. *Serpinb10*$^{-/-}$and WT BMDMs were treated with 20 ng/mL IL-4 for 48 h. On day 20, the aforementioned macrophages were administered into the lungs of *Serpinb10*$^{-/-}$ and WT mice pretreated with clodronate liposomes at a dose of 1×10^6 cells/50 μL per mouse.

Assessment of airway inflammation and mucus secretion

The intensity of peribronchial inflammation and the density of PAS-positive cells in the airway of mouse lung sections were scored as previously described using HE-staining and PAS staining, respectively [16, 19].

Cell culture and treatment

THP-1 cells were cultured in RPMI 1640 medium with 10% FBS (Biological Industries, Israel) and stimulated

by PMA. Then, THP-1 cells were transfected with NC siRNA or SERPINB10 siRNA utilizing Lipofectamine 3000 (Invitrogen, CA). 48h before cell harvest for qPCR and western blotting, IL-4 (20 ng/mL, PeproTech) was added.

Culture and treatment of primary macrophages

BMDMs were derived from *Serpinb10*$^{-/-}$ and WT mice and cultured with M-CSF. After 7 days, BMDMs were treated with 20 ng/mL IL-4. Primary lung macrophages were derived from both *Serpinb10*$^{-/-}$ and WT mice. Using sterile collagenase I, the mice lung tissue was converted into single cell suspension. Then cells were treated with 20 ng/mL IL-4.

ELISA

According to the manufacturer's instructions, the concentration of Ccl17, and Ccl22 in the BALF was determined using ELISA kits.

Immunofluorescence staining

Immunofluorescence staining of BMDMs was performed following the protocol of the Universal IF Toolkit (Abbkine, Wuhan, China). Briefly, BMDM cell slides were fixed in 4% paraformaldehyde, and incubated with immunostaining permeabilization buffer and then with goat serum blocking buffer at 25℃. The cells were then incubated with primary antibodies overnight at 4 °C. The cells were washed with antibody wash buffer, and incubated with fluorescent secondary antibodies for 1 h in the dark. Subsequently, DAPI was re-stained for 15 min and the slides were mounted with superKine™ enhanced antifade mounting medium.

Western blotting

RIPA buffer was used to extract proteins. Western blotting of the proteins was performed with the specified antibodies. Image J software was utilized to evaluate densitometry.

Quantitative RT-PCR

Total RNA was extracted using the TRIzol reagent. A reverse transcriptase kit was used to acquire cDNA. SYBR Premix Ex Taq was used for quantitative RT-PCR. All of the above reagents were purchased from Takara (Tokyo, Japan). The primers are listed in Table S2.

Statistical analysis

Normally distributed data are presented as means ± SD, and parametric tests analysis were used. In cases of non-normal distribution, median values and their interquartile ranges are computed, and non-parametric statistical approaches are applied. For evaluating correlations, Spearman's rank correlation coefficient is utilized. Statistical analysis was carried out with GraphPad Prism version 9.5 (GraphPad Software, USA). Statistical significance is set at $P < 0.05$.

Results

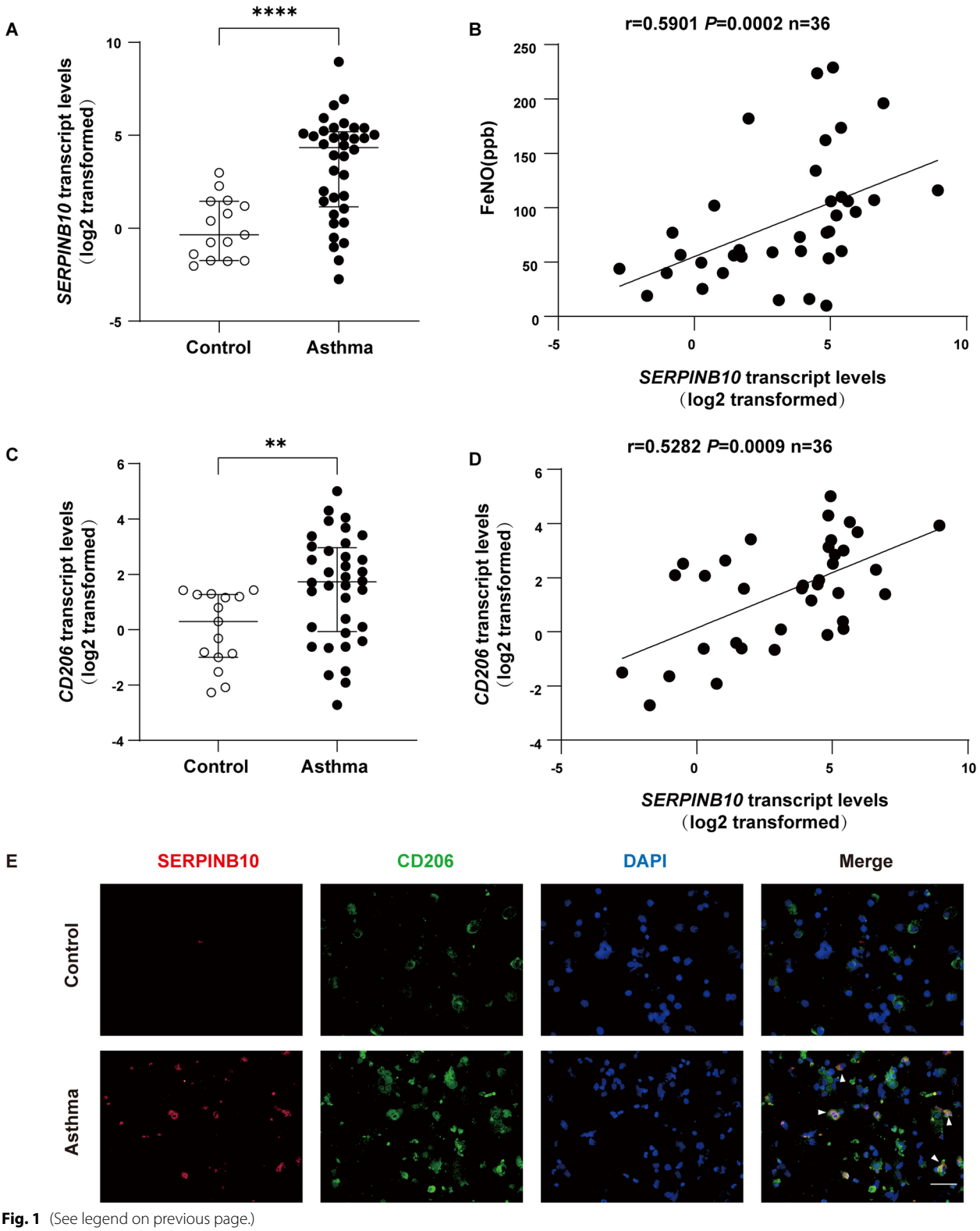
SERPINB10 expression is increased and correlated with *CD206* expression in BAL cells from asthma patients

The characteristics of subjects were summarized in Table S1. There were no statistical differences between the two groups in terms of age, sex, and body mass index. Compared to the controls, the predicted FEV_1% of asthma patients were significantly lower. FeNO and blood eosinophil count were significantly higher in asthma patients than control subjects.

SERPINB10 transcript levels in BAL cells were significantly higher in asthma patients compared to control subjects (Fig. 1A). Moreover, *SERPINB10* expression is positively correlated with FeNO, a measurement reflecting airway eosinophilia, in asthma patients (Fig. 1B). This suggests that SERPINB10 expression in BAL cells is upregulated and associated with airway eosinophilia in asthma. The transcript levels of *CD206*, a marker for macrophage M2 polarization, in BAL cells were significantly higher in asthma patients compared to control subjects (Fig. 1C). Interestingly, the transcript levels of *SERPINB10* and *CD206* were positively correlated with each other (Fig. 1D). SERPINB10 and CD206 expression in BAL cells were also examined using immunofluorescence staining. BAL cells which were positive for

(See figure on next page.)

Fig. 1 *SERPINB10* expression is increased and correlated with CD206 expression in BAL cells from asthma patients. **A** *SERPINB10* transcript levels in BAL cells from control subjects ($n = 15$) and asthma patients ($n = 36$) were determined by quantitative PCR. **B** The correlation between *SERPINB10* transcript levels and FeNO in asthma patients ($n = 36$) was analyzed by Spearman correlation assay. **C** *CD206* transcript levels in BAL cells from control subjects ($n = 15$) and asthma patients ($n = 36$) were determined by quantitative PCR. **D** The correlation between *SERPINB10* and *CD206* transcript levels in asthma patients ($n = 36$) was analyzed by Spearman correlation assay. **E** Representative images for co-immunostaining of SERPINB10 and CD206 in BAL cells from control subjects and asthma patients. White arrow heads indicate the cells which are positive for both SERPINB10 and CD206. The nuclei were stained with DAPI. scale bar: 50 μm. * $P < 0.05$, ** $P < 0.01$, and **** $P < 0.0001$



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both SERPINB10 and CD206 staining were observed in asthma patients (Fig. 1E, indicated by arrow heads), but barely seen in control subjects. Our findings suggest that SERPINB10 expression is associated with macrophage M2 polarization in asthma.

Serpinb10 deficiency attenuates eosinophilic airway inflammation and mucous cell metaplasia in OVA-induced mouse model

To explore the role of SERPINB10 in macrophage polarization and airway inflammation in asthma. *Serpinb10*-deficient (*Serpinb10*$^{-/-}$) and wild-type (WT) mice were sensitized and challenged with OVA (Fig. 2A). H&E staining revealed airway inflammation was reduced in OVA-challenged *Serpinb10*$^{-/-}$ mice (Fig. 2B, D). Moreover, the number of eosinophils in BAL cells were notably reduced in OVA-challenged *Serpinb10*$^{-/-}$ mice when compared with OVA-challenged WT mice (Fig. 2G). Our data suggest that *Serpinb10* deficiency attenuates eosinophilic airway inflammation in OVA-induced mouse model. Moreover, the number of PAS-staining-positive mucous cells were significantly decreased in OVA-challenged *Serpinb10*$^{-/-}$ mice when compared with OVA-challenged WT mice (Fig. 2C, E). Methacholine-induced airway resistance was lower in OVA-challenged *Serpinb10*$^{-/-}$ mice when compared with OVA-challenged WT mice (Fig. 2F). Our data indicate that *Serpinb10* deficiency suppresses mucous cell metaplasia and airway hyperresponsiveness in the mouse model.

Serpinb10 deficiency suppresses macrophage M2 polarization

We next examined the effect of *Serpinb10* deficiency on macrophage M2 polarization in the mouse model. The expression of M2 markers including *Cd206*, *Arg1* and *Fizz1* in mouse lungs was determined by quantitative PCR and western blotting. The mRNA levels of *Cd206*, *Arg1* and *Fizz1* were decreased in OVA-challenged *Serpinb10*$^{-/-}$ mice compared to WT mice (Fig. 3A-C). The protein levels of Cd206 and Arg1 were also reduced in OVA-challenged *Serpinb10*$^{-/-}$ mice compared to WT mice (Fig. 3D-F). Moreover, we measured the protein levels of Ccl17 and Ccl22, the chemokines released by M2 macrophages, in BAL fluid using ELISA. The protein levels of Ccl17 and Ccl22 were markedly decreased in OVA-challenged *Serpinb10*$^{-/-}$ mice compared to WT mice (Fig. 3G, H). Our data indicate that *Serpinb10* deficiency suppresses macrophage M2 polarization in the mouse model.

Adoptive transfer of *Serpinb10*-deficient BMDMs suppresses allergic airway inflammation and mucous cell metaplasia in mice

We further investigate that whether *Serpinb10* deficiency only in macrophages can suppress allergic airway inflammation in the mice model. BMDMs from *Serpinb10*$^{-/-}$ or WT mice were intratracheally transferred into recipient mice depleted with lung macrophages (Fig. 4A). The lung macrophages in recipient mice were eliminated by intratracheal administration with clodronate liposomes (Supplementary Fig. 1). OVA-challenged WT recipient mice transferred with *Serpinb10*$^{-/-}$ BMDMs showed reduced airway inflammation and less PAS-positive mucous cells when compared with OVA-challenged WT recipient mice transferred with WT BMDMs (Fig. 4B-D). Our findings suggest that *Serpinb10* deficiency in macrophages suppresses allergic airway inflammation and mucous cell metaplasia in mice. In addition, there were no significant difference in airway inflammation and mucous cell metaplasia between OVA-challenged WT recipient mice transferred with WT BMDMs and OVA-challenged *Serpinb10*$^{-/-}$ recipient mice transferred with WT BMDMs.

SERPINB10 promotes macrophages M2 polarization in vitro

We next investigate the role of Serpinb10 in macrophages M2 polarization in primary culture of mouse BMDMs. IL-4 stimulation increased the protein levels of M2 markers including Cd206 and Arg1 in WT BMDMs. However, IL-4-induced Cd206 and Arg1 expression were significantly reduced in *Serpinb10*$^{-/-}$ BMDMs (Fig. 5A-C). Consistently, immunostaining of Cd206 in IL-4-stimulated BMDMs showed that Cd206-positive cells were significantly less in cultured *Serpinb10*$^{-/-}$ BMDMs compared to WT BMDMs

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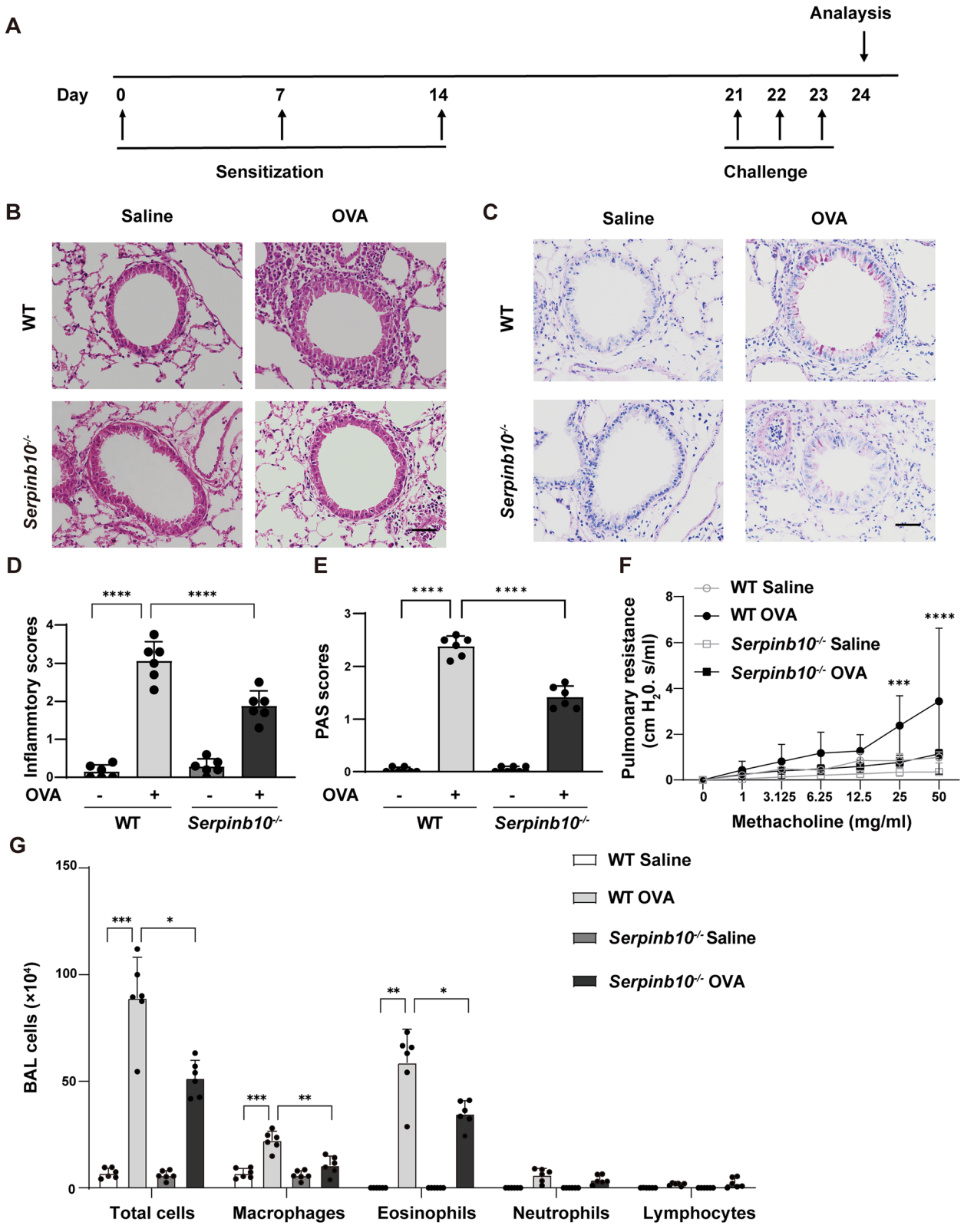
Fig. 2 *Serpinb10* deficiency attenuates eosinophilic airway inflammation and mucous cell metaplasia in OVA-sensitized and challenged mice. **A** Schematic overview of mouse experiments. **B** Representative images of H&E staining of lung sections from WT or *Serpinb10*$^{-/-}$ mice sensitized and challenged with saline or OVA. Scale bar: 50 μm. **C** Representative images of PAS staining of lung sections from WT or *Serpinb10*$^{-/-}$ mice sensitized and challenged with saline or OVA. Scale bar: 50 μm. **D** Lung inflammatory scores were calculated as described in Methods. **E** PAS scores were calculated as described in Methods. **F** Pulmonary resistance in response to gradient concentrations of intravenous methacholine in WT mice and *Serpinb10*$^{-/-}$ mice sensitized and challenged with saline or OVA. **G** BAL cell differentiation and counting for macrophages, eosinophils, lymphocytes and neutrophils. $n = 6$ mice per group. Data are mean ± SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$



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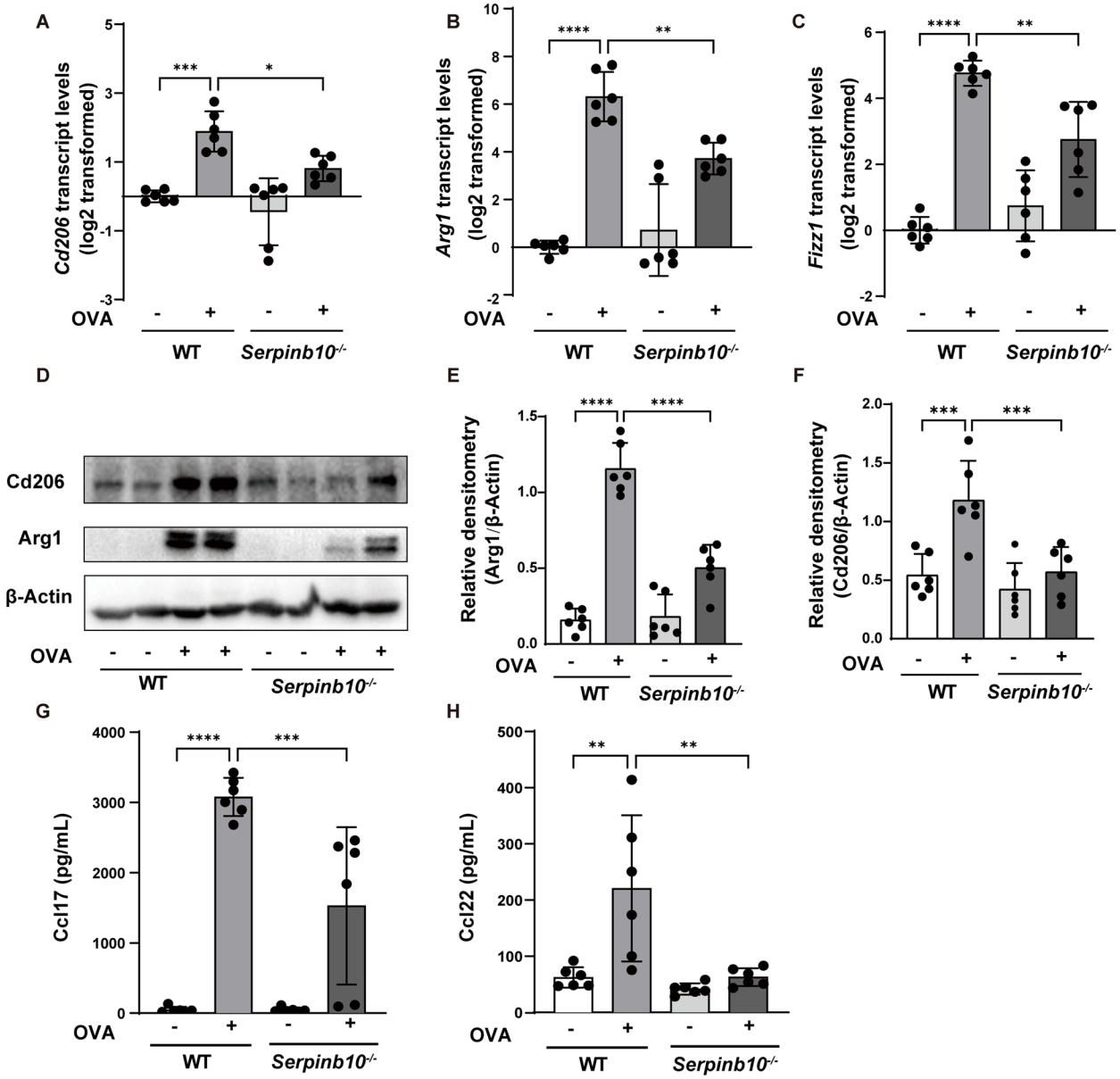

Fig. 3 *Serpinb10* deficiency suppresses macrophage M2 polarization in the mouse model. **A**-**C** *Cd206* (*A*), *Arg1* (*B*) and *Fizz1* (*C*) mRNA levels in mouse lungs were determined by quantitative PCR. **D** Representative images of western blotting for Cd206, Arg1 using mouse lungs. **E**, **F** Densitometry assay was performed using ImageJ, and the protein levels of Cd206 (*E*), Arg1 (*F*) were indexed to β-Actin. **G**, **H** The protein levels of Ccl17 (*G*), Ccl22 (*H*) in BALF were determined by ELISA. $n = 6$ mice per group. Data are mean ± SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$

(Fig. 5D). The transcript levels of *Arg1, Ym1,* and *Ccl22* were significantly lower in IL-4-stimulated *Serpinb10*$^{-/-}$ BMDMs when compared with IL-4-stimulated WT BMDMs (Fig. 5E-H). In human THP-1 cells, *SERPINB10* knockdown inhibited IL-4-induced expression of M2 marker such as ARG1 (Supplementary Fig. 2A-D). These data suggest that SERPINB10 promotes macrophages M2 polarization.

SERPINB10 suppresses IL-4Rα degradation and upregulates IL-4R signaling in macrophages

IL-4R activation and downstream Stat6 or Akt phosphorylation play a key role in macrophage M2 polarization. We further assessed the effect of *Serpinb10* deficiency on Stat6 or Akt phosphorylation in IL-4-stimulated

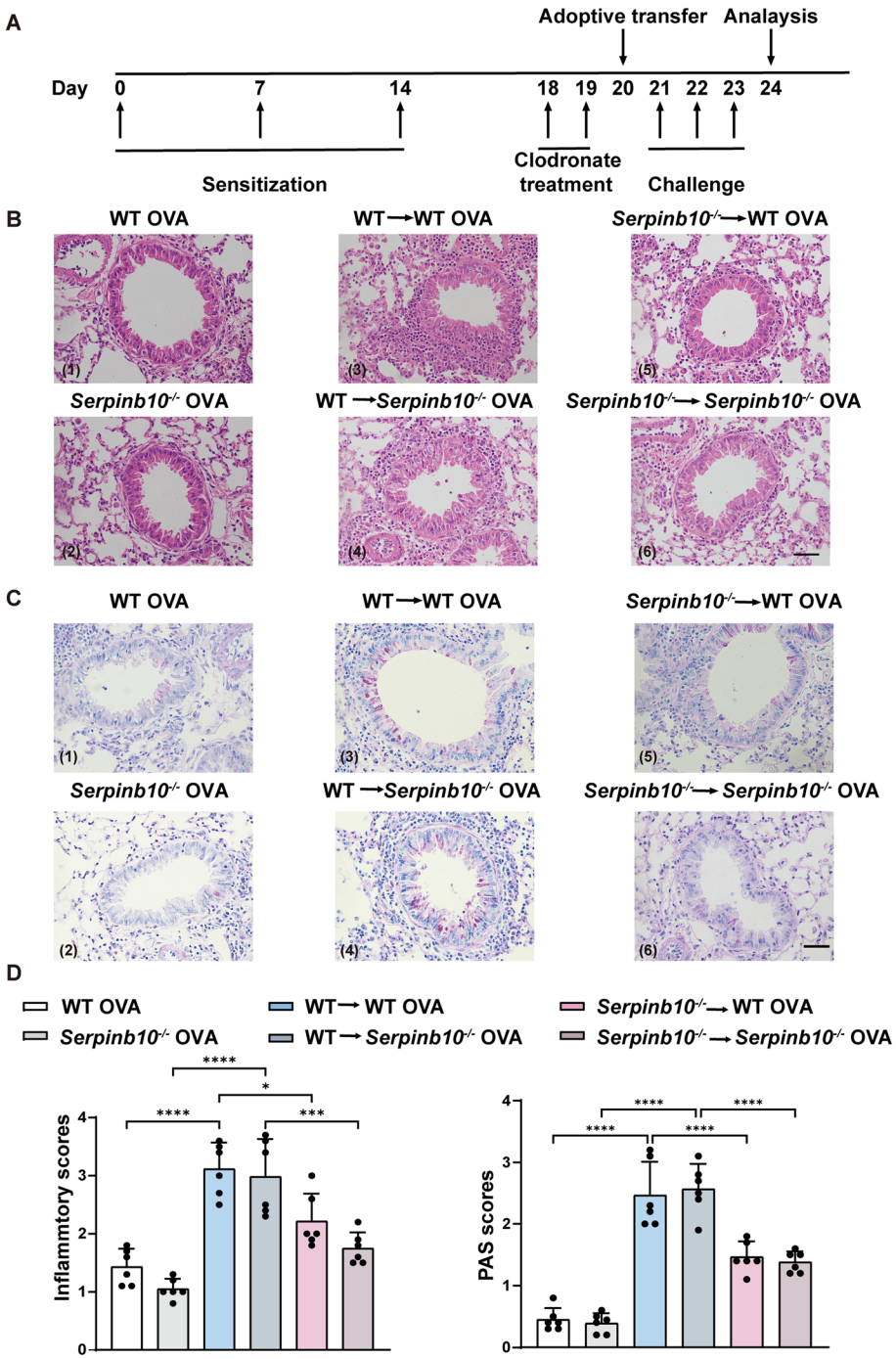

Fig. 4 Adoptive transfer of *Serpinb10*-deficient BMDMs suppresses allergic airway inflammation and mucous cell metaplasia in mice. **A** Schematic overview of the mouse experiments. **B** Representative images of H&E staining of lung sections from OVA-challenged WT mice (1) and *Serpinb10*$^{-/-}$ mice (2) depleted of macrophages, OVA-challenged WT mice (3) and *Serpinb10*$^{-/-}$ mice (4) depleted of macrophages and transferred with WT BMDMs, OVA-challenged WT mice (5) and *Serpinb10*$^{-/-}$ mice (6) depleted of macrophages and transferred with *Serpinb10*$^{-/-}$ BMDMs. Scale bar, 50 μm. **C** Representative images of PAS staining of mouse lung sections. Scale bar, 50 μm. **D** Lung inflammatory scores and PAS scores were calculated as described in Methods. $n = 6$ mice per group. Data are mean ± SD. *$P < 0.05$, ***$P < 0.001$, ****$P < 0.0001$

BMDMs. The protein levels of phosphorylated Stat6 in WT BMDMs were upregulated after IL-4 stimulation for 0.5 h, 1 h and 3 h. However, the levels of phosphorylated Stat6 were significantly reduced in *Serpinb10*$^{-/-}$ BMDMs compared to WT BMDMs (Fig. 6A, B). The phosphorylation of Akt were also decreased in *Serpinb10*$^{-/-}$ BMDMs compared to WT BMDMs (Fig. 6C, D). IL-4 stimulation increased IL-4Rα protein expression in human THP-1 cells. Transfection with *SERPINB10* siRNA substantially reduced IL-4-induced IL-4Rα protein expression (Fig. 6E, F). Similarly, IL-4Rα expression was decreased in *Serpinb10*$^{-/-}$ BMDMs compared to WT BMDMs (Supplementary Fig. 3).

We speculated that SERPINB10 may regulate IL-4Rα protein levels by suppressing the proteasomal degradation of IL-4Rα. The degradation of the IL-4Rα protein was analyzed in the presence of cycloheximide (CHX). The degradation rate of IL-4Rα protein in THP-1 cells transfected with *SERPINB10* siRNA was significantly higher than control cells (Fig. 6G and H), indicating that SERPINB10 inhibits IL-4Rα degradation. Together, SERPINB10 suppresses IL-4Rα degradation and upregulates downstream STAT6 and AKT phosphorylation, leading to macrophage M2 polarization (Fig. 7).

Discussion

In the present study, we demonstrated that *SERPINB10* expression is enhanced and correlated with FeNO and macrophage M2 markers in BAL cells from asthmatic patients. *Serpinb10* deficiency alleviated airway inflammation, and macrophage M2 polarization in the mice model of allergic airway inflammation. Furthermore, adoptive transfer of *Serpinb10* deficient macrophages to WT mice depleted lung macrophages suppresses airway inflammation. Mechanistically, SERPINB10 inhibits the degradation of the IL-4Rα protein, thereby upregulating the IL-4R signaling and promoting macrophage M2 polarization.

Our previous study demonstrated that epithelial SERPINB10 is of great significance in promoting eosinophilic airway inflammation by upregulating the expression of chemokines including CCL26 [15]. Here, we demonstrated enhanced expression of SERPINB10 in BAL cells from asthmatic patients. Draijer and colleagues reported that the number of CD206^{+} M2 macrophages was increased in BAL cells from asthma patients [20]. We also observed increased CD206 expression in BAL cells from our asthma patients. Intriguingly, our analysis revealed a robust correlation between SERPINB10 and CD206 mRNA levels. Immunostaining demonstrated that the number of SERPINB10^{+} and CD206^{+} cells in BAL cells of asthma patients were increased. Our findings suggest a link between upregulated SERPINB10 expression and macrophage M2 polarization.

In the OVA-induced murine model, we observed that *Serpinb10* deficiency significantly alleviated the peribronchial infiltration of inflammatory cells, decreased the number of mucus cells, and diminished the airway hyperresponsiveness. Furthermore, the expression of M2 macrophage markers including *Arg1* and *Cd206* were significantly decreased in the lung tissues of OVA-induced *Serpinb10*-deficient mice. Additionally, the protein levels of M2 macrophage effector molecules Ccl17 and Ccl22 were also decreased in OVA-sensitized and challenged *Serpinb10*-deficient mice.

Adoptive transfer experiments are used to clarify the role of a gene in a specific cell type in animal disease models [21]. We conducted adoptive transfer experiments to verify the effect of macrophage *Serpinb10* deficiency on asthmatic airway inflammation. Compared to adoptive transfer of WT macrophages, transfer of *Serpinb10*$^{-/-}$ macrophages obviously mitigated airway inflammation and reduced the number of mucus cells in WT mice depleted of lung macrophages. Our in vitro data showed that *Serpinb10* deficiency inhibited IL-4-induced polarization in BMDMs and primary mouse lung macrophages. Silencing the *SERPINB10* gene suppressed the level of M2 markers in THP-1 cells. This suggests that SERPINB10 promotes macrophage M2 polarization.

IL-4 binds to IL-4R expressed on the membrane of macrophage, activating the IL-4R signaling pathway, which has a pivotal impact on macrophage M2 polarization [22].The downstream signaling pathways of IL-4R mainly include the JAK1-STAT6 pathway and the PI3K-AKT pathway [23].Our findings indicate that *Serpinb10* deficiency suppresses the IL-4-induced phosphorylation levels of Stat6 and Akt in BMDMs.

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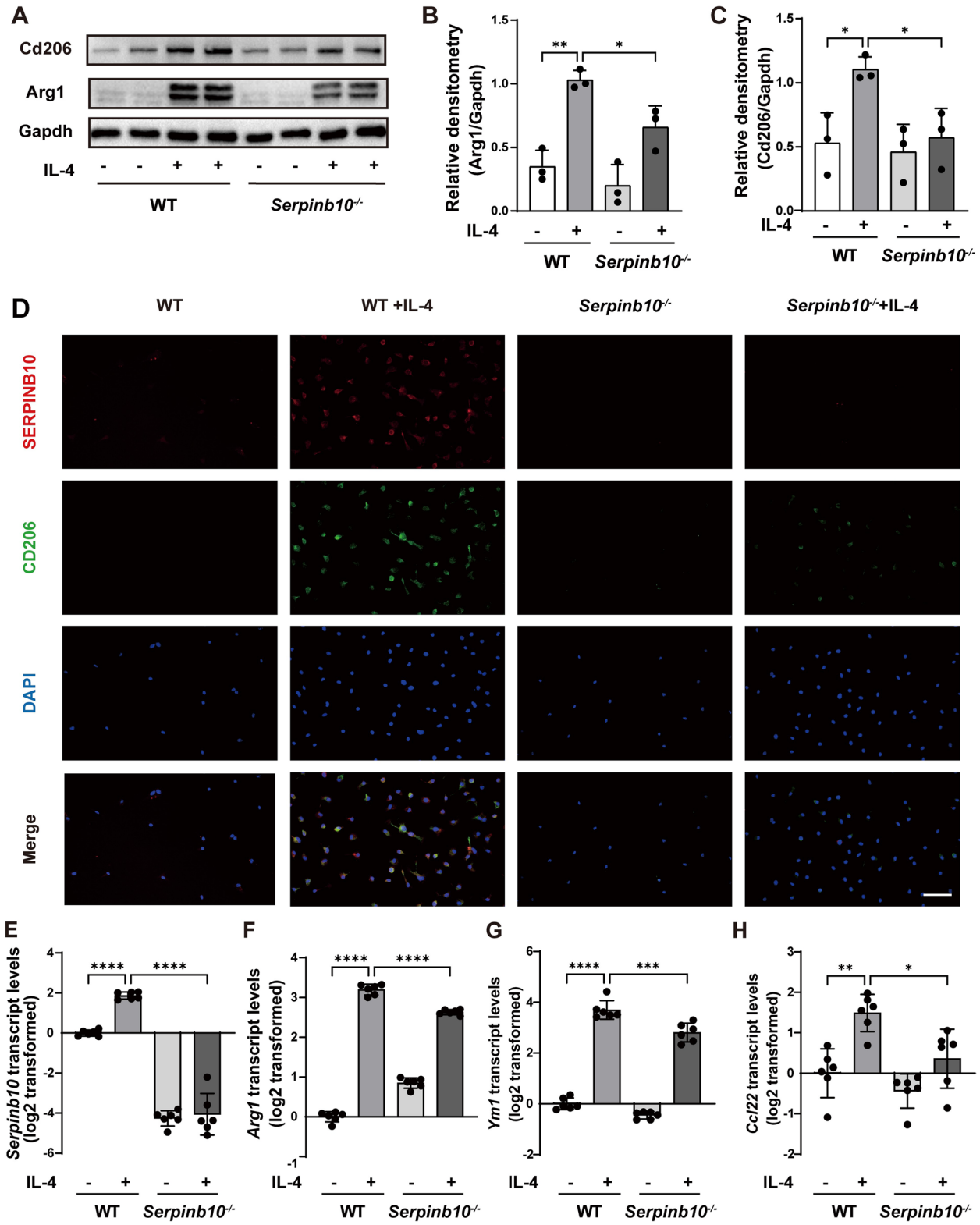
Fig. 5 *Serpinb10* deficiency suppressed M2 polarization in vitro. **A** Cd206, Arg1 protein levels in baseline and IL-4 stimulated WT and *Serpinb10*$^{-/-}$ BMDMs were measured by western blotting. **B**, **C** Densitometry assay was performed using ImageJ, and the protein levels of Cd206 (*B*), Arg1(*C*) were indexed to Gapdh. **D** Representative images for co-immunostaining of Serpinb10 and Cd206 in baseline and IL-4 stimulated WT and *Serpinb10*$^{-/-}$ BMDM. The nuclei were stained by DAPI. scale bar: 50 μm. **E**–**H** *Serpinb10* (*E*), *Arg1* (*F*), *Ym1* (*G*) and *Ccl22* (*H*) mRNA expression in baseline and IL-4 stimulated WT and *Serpinb10.*$^{-/-}$ BMDMs were determined by quantitative PCR. Data are mean ± SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$



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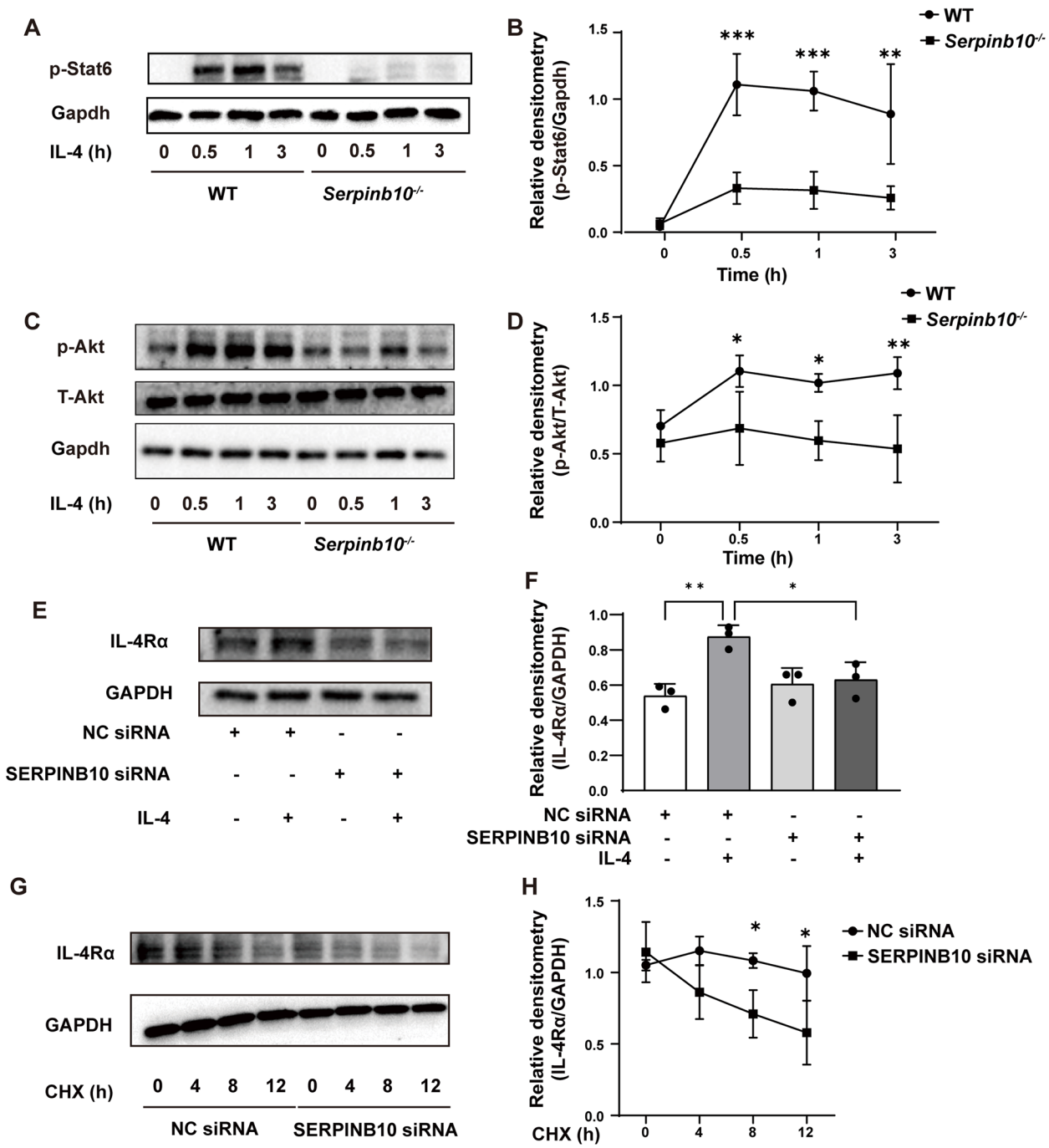

Fig. 6 SERPINB10 suppresses IL-4Rα degradation and upregulates IL-4R signaling in macrophages. **A** Representative image of western blotting for phosphorylated Stat6 (p-Stat6) at different time points after IL-4 stimulation in BMDMs. **B** Densitometry assay was performed using Image J to quantify the protein levels of p-Stat6. **C** Representative image of western blotting for phosphorylated Akt (p-Akt) at different time points after IL-4 stimulation in BMDMs. **D** Densitometry assay was performed using Image J to quantify the protein levels of p-Akt. **E** Representative image of western blotting for IL-4Rα in THP-1 cells after transfection with negative control or *SERPINB10* siRNA and stimulated with or without IL-4. **F** Densitometry assay was performed using Image J to measure the protein levels of IL-4Rα. **G** Representative image of western blotting for IL-4Rα in THP-1 cells transfected with negative control or *SERPINB10* siRNA and treated with cycloheximide for different times as indicated. **H** Densitometry assay was performed using Image J to measure the protein levels of IL-4Rα. Data are mean ± SD. **P* < 0.05, ***P* < 0.01, and *****P* < 0.0001

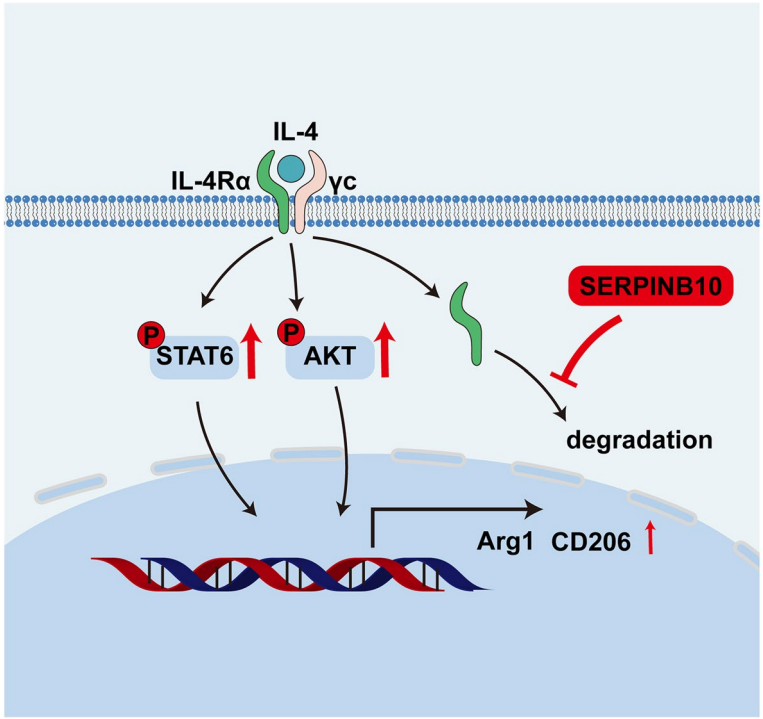

Fig. 7 Diagram of the mechanisms by which SERPINB10 promotes macrophage M2 polarization. SERPINB10 suppresses IL-4Rα degradation and upregulates downstream Stat6 and Akt phosphorylation, thereby promoting macrophage M2 polarization in asthma

Since SERPINB10 regulates the phosphorylation of both STAT6 and AKT, belonging to JAK1-STAT6 pathway and the PI3K-AKT pathway, respectively, we determined the role of SERPINB10 in IL-4Rα expression, the common upstream receptor for both pathways. IL-4Rα is a common subunit of type I and type II IL-4R. IL-4Rα is involved in regulating airway inflammation in asthma [24].We found that *Serpinb10* deficiency reduced the protein level of IL-4Rα in BMDMs. Silencing the expression of SERPINB10 decreased IL-4Rα protein expression in THP-1 cells.

Protein degradation pathways are important mechanisms for regulating protein expression levels [22]. IL-4Rα is degraded through various pathways including ubiquitination and proteasome degradation. SERPIN family member is involved in protein degradation [25]. For example, the protection of SERPINB2 on cell apoptosis is due to its interaction with proteasome subunits [26, 27]. We observed the degradation of IL-4Rα protein in THP-1 cells in the presence of CHX, and discovered that silencing the expression of SERPINB10 accelerated the degradation of IL-4Rα protein.

This study has several limitations. Firstly, the sample size of our cohort was relatively small. Second, although we performed adoptive transfer experiment using *Serpinb10*-deficient macrophages, mouse model using macrophage-specific *Serpinb10* knockout mice is warranted in future study. Third, the mechanism by which SERPINB10 alleviates the degradation of IL-4Rα needs further investigation.

In summary, our findings suggest that SERPINB10 upregulates the protein level of IL-4Rα by inhibiting its protein degradation, thereby upregulating the IL-4R signaling, promoting macrophage M2 polarization and allergic airway inflammation. SERPINB10 is a potential therapeutic target for asthma.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12931-025-03252-3.

Supplementary Material 1: Supplementary Figure 1. Depletion of lung macrophages.

Supplementary Material 2: Supplementary Figure 2 SERPINB10 promotes macrophages M2 polarization in vitro. (A, B) The protein levels of SERPINB10(A), ARG1(B) in THP-1-derived macrophages after transfection with negative control or *SERPINB10* siRNA with or without IL-4 treatment for 48h were detected by Western blotting. (C, D) Densitometry assay was performed using ImageJ to measure the protein levels of CD206(C), ARG1(D). Data are mean ± SD. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.

Supplementary Material 3: Supplementary Figure 3 Serpinb10 upregulates Il-4Rα in vitro. (A) western blotting results for Il-4Rα in BMDM. (B) Densitometry assay was performed using Image J to measure the protein levels of IL-4Rα. Data are mean ± SD. **P*<0.05, ***P*<0.01, and *****P*<0.0001.

Supplementary Material 4: Table S1. Subject characteristics.

Supplementary Material 5: Table S2. Primers for quantitative PCR.

Acknowledgements
We thank all the participants in this study; Xiaoling Rao, Shiqiang Wang, and Meifang Shen for bronchoscopy support; and Wang Ni, Kun Zhang, and Bohua Fu for spirometry measurement.

Authors' contributions
LY and GZ conceived and designed research; LZ, WW, GC, CH, WK, WG, and HJ performed experiments; LZ, WW, GC, CH, WK, and GZ analyzed data; LZ, WW, GC, CH, WK, WG, HJ, LY, and GZ interpreted results of experiments; LZ and GZ prepared figures; LZ and GZ drafted manuscript; LZ, LY and GZ edited and revised manuscript. All authors read and approved of the final manuscript.

Funding
This work was supported by National Natural Science Foundation of China (grant 82170036), Natural Science Foundation of Hubei Province (grant 2024AFB622), Key Research and Development Program of Hubei Province (grant 2024BCB042), and Innovation and Translation Project of Tongji Hospital (grant 2023CXZH006).

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This study was approved by the institutional ethics board of Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 18 December 2024 Accepted: 21 April 2025
Published online: 10 May 2025

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