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Original Research

Epithelial SERPINB2 Overexpression Contributes to Impaired Fibrinolysis in Chronic Rhinosinusitis With Nasal Polyps via tPA Downregulation

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Abstract

Background: Chronic rhinosinusitis with nasal polyps (CRSwNP) is characterized by persistent tissue remodeling, but the mechanisms underlying impaired fibrinolysis were not fully understood. SERPIN family B member 2 (SERPINB2/plasminogen activator inhibitor type 2 [PAI-2]) is a known inhibitor of tissue plasminogen activator (tPA). However, its role in CRSwNP pathogenesis remains unclear. This study investigated whether SERPINB2 contributes to fibrinolytic dysfunction in CRSwNP. **Methods**: tPA and SERPINB2 expression levels were assessed in nasal polyp and control turbinate tissues using qRT-PCR, Western blot, and immunofluorescence. Primary human nasal epithelial cells were stimulated with IFN-γ, IL-13, or IL-17A to evaluate cytokine-mediated regulation. The direct effects of SERPINB2 on tPA expression were examined using recombinant protein treatment and siRNA-mediated knockdown. tPA enzymatic activity and fibrinolytic function were measured using a fluorometric substrate assay and D-dimer ELISA, respectively. **Results**: tPA expression was significantly reduced in nasal polyp tissues compared to control turbinate and inversely correlated with SERPINB2 levels. Immunofluorescence analysis revealed decreased tPA-positive and increased SERPINB2-positive cells in the nasal epithelium. Both the Th1 cytokine IFN-γ and the Th2 cytokine IL-13 downregulated tPA while upregulating SERPINB2 in primary nasal epithelial cells, whereas IL-17A showed no significant effect. Notably, recombinant SERPINB2 dose-dependently suppressed epithelial tPA expression, while SERPINB2 knockdown rescued cytokine-induced tPA downregulation. Functionally, SERPINB2 inhibited tPA enzymatic activity in a dose-dependent manner and significantly impaired fibrinolytic function. **Conclusions**: This study identifies a novel SERPINB2-tPA regulatory axis in nasal epithelial cells. The convergent regulation by both Th1 and Th2 cytokines suggests that fibrinolytic dysfunction occurs across different CRSwNP inflammatory endotypes. These findings provide mechanistic insights into fibrin accumulation in nasal polyps and identify SERPINB2 as a potential therapeutic target for the prevention of polyp formation and recurrence.

Keywords: rhinosinusitis; nasal polyps; plasminogen activator inhibitor 2; tissue plasminogen activator; fibrinolysis

1. Introduction

Chronic rhinosinusitis with nasal polyps (CRSwNP) is an inflammatory condition characterized by outgrowth of sinonasal tissue [1]. According to the degree of eosinophilic infiltration, CRSwNP can be further divided into eosinophilic CRSwNP (eCRSwNP) and non-eosinophilic CRSwNP (neCRSwNP) [2]. The eCRSwNP phenotype is characterized by predominant Th2 immune responses, while neCRSwNP displays a heterogeneous inflammatory profile involving Th1, Th2, and Th17 pathways [3]. Regardless of these distinct immunological patterns, both subtypes demonstrate substantial subepithelial fibrin accumulation [4]. Fibrin degradation is facilitated by plasmin, which is generated from plasminogen upon cleavage by either urokinase plasminogen activator (uPA) or tissue plasminogen activator (tPA) [5]. Previous reports have shown significant downregulation of tPA, but not uPA, in both eCRSwNP and neCRSwNP patients [6]. Exposure to both Th1 and Th2 inflammatory mediators has been shown to downregulate tPA in cultured nasal epithelial cells [7]. However, the mechanisms underlying their regulation in CRSwNP remain poorly understood. Elucidating the mechanisms driving fibrin accumulation in CRSwNP could lead to improved therapeutic strategies.

SERPIN family B member 2 (SERPINB2), also known as plasminogen activator inhibitor type 2 (PAI-2), is a coagulation factor that inactivates uPA and tPA [8]. Previous studies have shown that SERPINB2 is dramatically elevated in bronchial epithelial cells of asthmatic patients and is positively associated with pivotal clinical markers, such as FEV1, FeNO, eosinophil counts, and asthma severity [9]. Single-cell RNA sequencing analysis has identified SERPINB2 as one of the core upregulated genes in human bronchial epithelial cells after segmental allergen challenge [10]. We have previously shown that SERPINB2 upregulated by IL-13 promotes the expression of 15-lipoxygenase-

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1 (15LO1), CCL26 and iNOS through activation of the STAT6 pathway in nasal polyp epithelial cells [11]. We also reported that elevated SERPINB2 in allergic rhinitis patients regulates MUC5AC expression via STAT6 signaling in nasal epithelial cells [12]. Based on these findings, we hypothesize that elevated SERPINB2 in nasal epithelial cells may suppress the expression and activity of tPA, thereby contributing to fibrin deposition in nasal polyps.

To test this hypothesis, SERPINB2 and tPA expression were analyzed in nasal polyp (NP) tissues from CRSwNP patients and turbinate tissues from healthy controls. Their colocalization was examined by immunofluorescence. Using air-liquid interface (ALI) cultures of primary nasal epithelial cells, the regulatory effects of SERPINB2 on tPA were examined through cytokine stimulation, recombinant protein treatment, and RNA interference. tPA enzymatic activity and fibrinolytic function were measured by fluorometric assays and D-dimer ELISA. Our results demonstrated that increased SERPINB2 in nasal polyp epithelial cells suppresses tPA expression and reduces tPA activity in CRSwNP.

2. Materials and Methods

2.1 Participants

The Ethical Committee of Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine granted approval for this investigation, and all subjects provided written informed consent prior to participation. The study cohort comprised 20 eCRSwNP patients, 25 neCRSwNP patients, and 20 healthy controls (HCs) (**Supplementary Table 1**). CRSwNP classification adhered to the EPOS2020 criteria. Eosinophilic CRSwNP was defined as tissue eosinophil counts of $\geq$10 per high-power field ($\times$400), while counts below this threshold were classified as non-eosinophilic CRSwNP. Control specimens consisted of inferior turbinate (IT) or middle turbinate (MT) biopsies obtained during septoplasty or skull base surgeries in patients without sinonasal pathology. Additional methodological details are provided in the **Supplementary Material**.

2.2 Multiplex Immunohistochemistry Staining

Multiplex immunohistochemistry (mIHC) was performed using the TSA Fluorescence Staining Kit on formalin-fixed paraffin-embedded tissue sections. Briefly, tissue sections underwent deparaffinization, antigen retrieval, and sequential antibody staining. Primary antibodies against tPA and SERPINB2 were sequentially applied, followed by HRP-conjugated secondary antibodies and TSA-fluorophore amplification. Microwave treatment was performed between each cycle to strip antibodies. Nuclei were counterstained with DAPI. Confocal images were acquired using Zeiss LSM 780 confocal laser scanning microscope. SERPINB2^{+} and tPA^{+} cells within the epithelial compartment were manually counted in 5 randomly selected fields (200$\times$ magnification) per sample.

2.3 Primary NEC ALI Culture and Short Interfering RNA Transfection

Turbinate epithelial scrapings (IT/MT) from control subjects served as the source for primary NEC isolation. All primary NECs were isolated and cultured following established protocols. Cell identity was confirmed by characteristic epithelial morphology under microscopy and tested negative for mycoplasma. Cultures were maintained under ALI conditions following established protocols. Inflammatory stimuli (IFN-γ, IL-13, IL-17A) or SERPINB2 recombinant protein were administered via the basolateral compartment for 48 hours to recapitulate the subepithelial inflammatory microenvironment. Basolateral supernatants were subsequently collected for tPA quantification by ELISA. Gene silencing was achieved through DsiRNA delivery via Lipofectamine RNAiMAX-mediated transfection. Comprehensive protocols are provided in the **Supplementary Material**.

2.4 Quantitative Real-Time PCR

Gene expression was quantified using SYBR Green-based qRT-PCR on a Roche LightCycler 480 II system. β-Glucuronidase (GUSB) served as the endogenous reference gene for normalization. Relative mRNA levels were calculated using the $2^{-\Delta\Delta Ct}$ method. Primer sequences and detailed protocols are provided in **Supplementary Table 2**.

2.5 Western Blotting

Fresh tissue samples and cell lysates underwent 10% SDS-PAGE separation followed by immunoblotting with antibodies against tPA, SERPINB2, and GAPDH. Band intensities were quantified using ImageJ software (version 1.54j; National Institutes of Health, Bethesda, MD, USA). Detailed procedures are provided in the **Supplementary Material**.

2.6 ELISA

tPA protein levels in culture media were measured with quantitative sandwich ELISA kits (DTPA00, R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

2.7 tPA Enzymatic Activity Assay

tPA enzymatic activity was measured fluorometrically using the synthetic substrate Z-GGR-AMC (carbobenzoxy-Gly-Gly-Arg-7-amino-4-methylcoumarin). Recombinant human tPA (5 nM) was pre-incubated with increasing concentrations of recombinant SERPINB2 (0, 6.25, 25, or 100 nM) for 30 min at 37 °C to allow SERPINB2-tPA complex formation. Following pre-incubation, Z-GGR-AMC substrate was added to a final concentration of 100 µM. Fluorescence (Ex/Em 360/460 nm) was continuously monitored

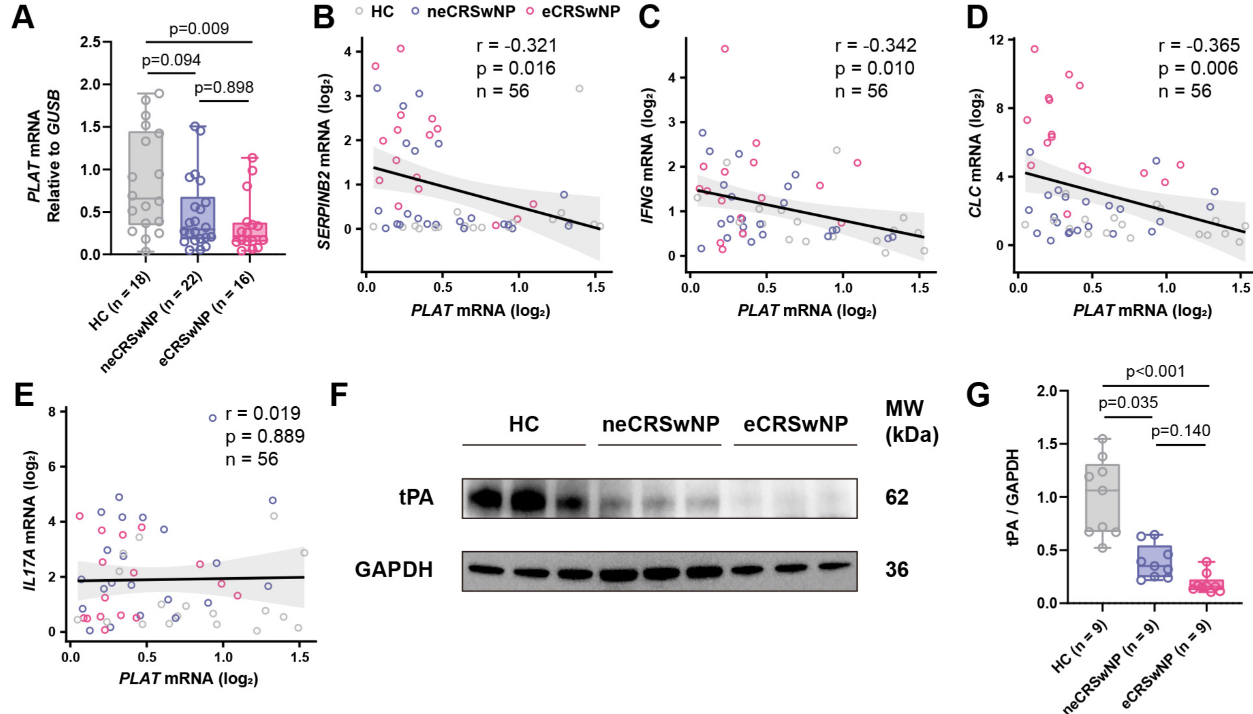

Fig. 1. tPA expression is reduced in CRSwNP tissues and inversely correlates with SERPINB2. (A) RT-qPCR analysis of *PLAT* mRNA expression in inferior turbinate controls (n = 18), neCRSwNP (n = 22), and eCRSwNP (n = 16) tissues. (B–E) Spearman correlation analyses between *PLAT* mRNA levels and (B) *SERPINB2*, (C) *IFNG*, (D) *CLC* or (E) *IL17A* mRNA expression in all tissue samples (n = 56). (F) Representative Western blot images showing tPA protein levels in control, neCRSwNP, and eCRSwNP tissues. (G) Densitometric quantification of tPA protein levels normalized to GAPDH. HC, healthy control; neCRSwNP, non-eosinophilic CRSwNP; eCRSwNP, eosinophilic CRSwNP; SERPINB2, SERPIN family B member 2; tPA, tissue plasminogen activator; CRSwNP, chronic rhinosinusitis with nasal polyps.

for 60 min at 37 °C using a microplate reader. The rate of AMC release, reflecting tPA proteolytic activity, was quantified as relative fluorescence units (RFU) over time.

2.8 Fibrinolysis Functional Assay

To assess the functional impact of SERPINB2 on fibrinolysis, human serum was treated with recombinant tPA (50 nM), recombinant SERPINB2 (0, 6.25, 25, or 100 nM), or both and incubated at 37 °C for 300 s. The higher tPA concentration (50 nM vs. 5 nM in the enzymatic assay) was used to overcome endogenous serum protease inhibitors and ensure measurable fibrinolysis. D-dimer levels, a specific marker of fibrin degradation, were quantified by ELISA according to the manufacturer's instructions.

2.9 Statistical Analysis

Statistical analyses and figure generation were performed using GraphPad Prism software (version 8.0; GraphPad Software, San Diego, CA, USA). Parametric data are presented as mean ± SEM; nonparametric data are presented as median (IQR). Comparisons between two groups were performed using unpaired *t*-tests or Mann-Whitney U tests based on distribution normality. Multiple-group analyses were conducted using one-way ANOVA or Kruskal-Wallis tests. Variable associations were examined using Spearman rank correlation. Statistical significance was defined as $p < 0.05$.

3. Results

3.1 tPA Expression Is Reduced in Nasal Polyp Tissues and Inversely Correlates With SERPINB2

To investigate tPA expression in CRSwNP, we first examined *PLAT* mRNA levels in nasal polyp tissues from patients with eCRSwNP (n = 16) and neCRSwNP (n = 22), compared to HCs (n = 18). qRT-PCR analysis showed that *PLAT* mRNA levels were significantly reduced in eCRSwNP compared to control subjects, while neCRSwNP exhibited a trend toward reduction that did not reach statistical significance (Fig. 1A). Subgroup analysis showed no significant difference in *PLAT* expression between asthmatic and non-asthmatic patients within the eCRSwNP group (**Supplementary Fig. 1A**), or between non-asthmatic eCRSwNP and neCRSwNP patients (**Supplementary Fig. 1B**), suggesting that tPA downregulation in CRSwNP is not confounded by asthma comorbidity. Correlation analysis revealed inverse correlations between *PLAT* mRNA and *SER-*

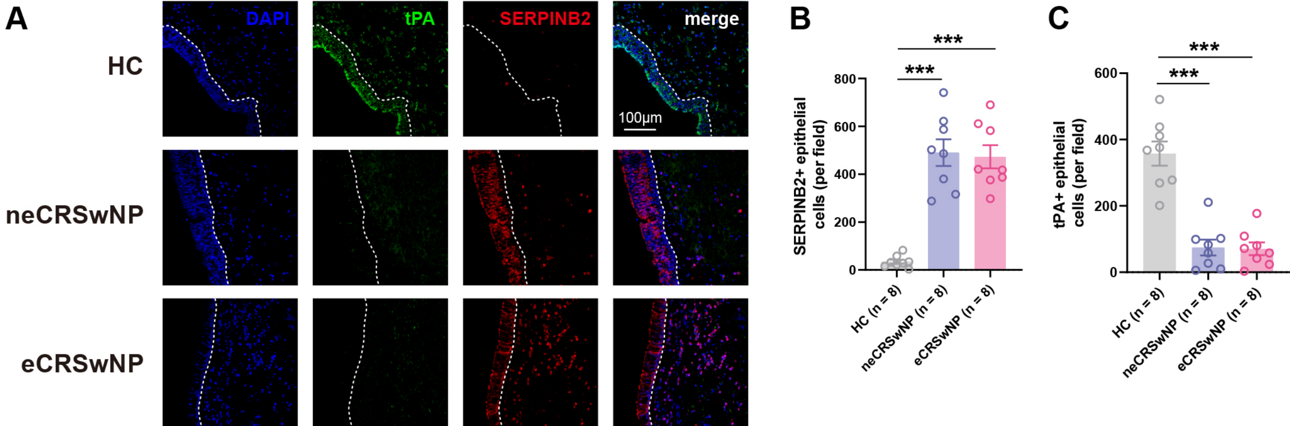

Fig. 2. Multiplex immunohistochemistry confirms reciprocal tPA and SERPINB2 expression in nasal polyp epithelium. (A) Representative multiplex immunohistochemistry (mIHC) images showing tPA (green), SERPINB2 (red), and DAPI (blue, nuclei) staining in healthy controls, neCRSwNP, and eCRSwNP tissues. Scale bars: 100 µm. (B) Quantification of SERPINB2$^+$ cells within the epithelial compartment per field (200× magnification). (C) Quantification of tPA$^+$ cells within the epithelial compartment per field (200× magnification). ***$p < 0.001$.

PINB2 (Fig. 1B), *IFNG* (Fig. 1C), and *CLC* (Fig. 1D) expression in nasal polyp tissues, while no significant correlation was observed between *PLAT* and *IL17A* mRNA levels (Fig. 1E), suggesting that tPA downregulation is associate with both Th1 and Th2, but not Th17, inflammatory mediators. At the protein level, WB analysis confirmed significant downregulation of tPA in both eCRSwNP and neCRSwNP tissues compared to control subjects (Fig. 1F,G).

3.2 Nasal Polyp Epithelium Exhibits Decreased tPA$^+$ and Increased SERPINB2$^+$ Cells

To further characterize tPA and SERPINB2 expression at the cellular level, we performed mIHC on nasal tissue sections. Consistent with the mRNA findings described above, mIHC analysis revealed a significant increase in SERPINB2$^+$ epithelial cells in both eCRSwNP and neCRSwNP tissues compared to control subjects (Fig. 2A,B). Conversely, tPA$^+$ epithelial cells were markedly reduced in both polyp endotypes relative to control subjects (Fig. 2A,C). No significant differences in either tPA$^+$ or SERPINB2$^+$ epithelial cell counts were observed between eCRSwNP and neCRSwNP groups (Fig. 2B,C). These findings confirm reciprocal changes in tPA and SERPINB2 expression within the nasal polyp epithelium, corroborating the inverse correlation observed at the mRNA level.

3.3 IFN-γ and IL-13 Downregulate tPA While Upregulating SERPINB2 in Primary Nasal Epithelial Cells

To investigate whether key inflammatory cytokines regulate tPA and SERPINB2 expression in nasal epithelium, we isolated and cultured primary human NECs from healthy donors and stimulated them with Th1 (IFN-γ, 10 ng/mL), Th2 (IL-13, 20 ng/mL), and Th17 (IL-17A, 10 ng/mL) cytokines for 48 hours. qRT-PCR analysis revealed that IFN-γ and IL-13 significantly downregulated *PLAT* mRNA expression compared to vehicle controls (Fig. 3A). Conversely, both IFN-γ and IL-13 markedly upregulated *SERPINB2* mRNA levels (Fig. 3B). In contrast, IL-17A treatment had no significant effect on either *PLAT* or *SERPINB2* mRNA expression (Fig. 3A,B). Consistent with these mRNA findings, ELISA analysis of culture supernatants showed significant reductions in secreted tPA protein following IFN-γ and IL-13 treatment (Fig. 3C), while IL-17A had no significant effect. Western blot analysis of cell lysates confirmed decreased intracellular tPA protein (Fig. 3D,E) and increased SERPINB2 protein (Fig. 3D,F) in IFN-γ- and IL-13-treated cells compared to vehicle controls. These findings demonstrate that Th1 and Th2 cytokines, but not the Th17 cytokine IL-17A, suppress tPA and induce SERPINB2 expression in nasal epithelial cells, providing a mechanistic link between Th1/Th2 inflammation and the reciprocal tPA/SERPINB2 expression pattern observed in CRSwNP tissues.

3.4 Recombinant SERPINB2 Suppresses Epithelial tPA Expression Dose-Dependently

To investigate whether SERPINB2 directly regulates tPA expression, we treated primary NECs with recombinant human SERPINB2 protein (0, 10, 25, or 50 nM) for 48 hours. qRT-PCR showed dose-dependent suppression of *PLAT* mRNA with increasing SERPINB2 concentrations (Fig. 4A). ELISA revealed corresponding reductions in secreted tPA protein (Fig. 4B). Western blot confirmed decreased intracellular tPA protein in cell lysates (Fig. 4C,D). These results demonstrate that exogenous SERPINB2 dose-dependently suppresses tPA expression, suggesting a potential contribution to impaired fibrinolysis in CRSwNP.

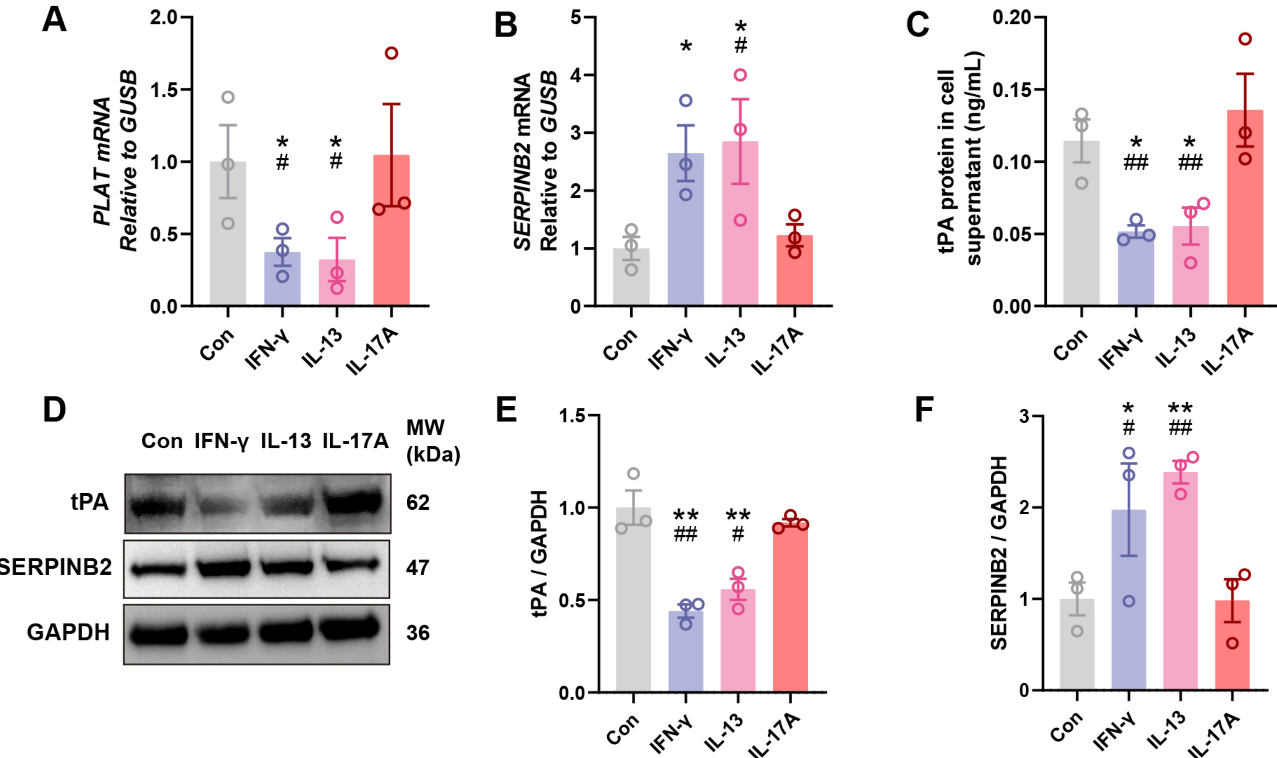

Fig. 3. IFN-γ and IL-13, but not IL-17A, coordinately suppress tPA and induce SERPINB2 in primary nasal epithelial cells. (A) RT-qPCR analysis of *PLAT* mRNA expression following cytokine treatment. (B) RT-qPCR analysis of *SERPINB2* mRNA expression following cytokine treatment. (C) ELISA quantification of secreted tPA protein in culture supernatants following cytokine treatment. (D) Representative Western blot images showing tPA, and SERPINB2 protein levels in cell lysates from vehicle-, IFN-γ-, IL-13-, or IL-17A-treated nasal epithelial cells. (E) Densitometric quantification of tPA protein levels normalized to GAPDH. (F) Densitometric quantification of SERPINB2 protein levels normalized to GAPDH. Data are presented as mean ± SEM (n = 3 independent experiments). *$p < 0.05$ vs. control; **$p < 0.01$ vs. control; $^{\#}p < 0.05$ vs. IL-17A; $^{\#\#}p < 0.01$ vs. IL-17A.

3.5 SERPINB2 Knockdown Rescues Th1 and Th2 Cytokine-Induced tPA Downregulation

To test whether SERPINB2 mediates cytokine-induced tPA suppression, we performed siRNA-mediated SERPINB2 knockdown in primary nasal epithelial cells. Knockdown efficiency was confirmed by Western blot analysis in three independent ALI culture samples (**Supplementary Fig. 2**). Critically, SERPINB2 knockdown rescued IFN-γ- and IL-13-induced *PLAT* mRNA suppression (Fig. 5A,B). This rescue was confirmed at the protein level for both secreted (Fig. 5C,D) and intracellular (Fig. 5E–G) tPA. Together, these loss-of-function data demonstrate that SERPINB2 is required for cytokine-mediated tPA suppression, establishing a causal link between Th1/Th2 inflammatory signaling and impaired tPA expression in CRSwNP.

3.6 SERPINB2 Inhibits tPA Enzymatic Activity and Impairs Fibrinolytic Function In Vitro

To validate the inhibitory effect of SERPINB2 on tPA activity and fibrinolytic function, we performed *in vitro* enzymatic activity and fibrinolysis assays. Using a fluorogenic substrate assay (Fig. 6A), we observed that tPA alone (Sub+tPA) produced robust enzymatic activity, with fluorescence signals reaching approximately 4000 RFU at 300 seconds. Co-incubation with SERPINB2 dose-dependently inhibited tPA activity. At concentrations of 6.25 nM, 25 nM, and 100 nM, SERPINB2 reduced fluorescence signals to approximately 1900, 1100, and 600 RFU, respectively, corresponding to inhibition rates of 55%, 74%, and 86%, respectively. Consistent with these findings, a D-dimer release assay (Fig. 6B) demonstrated that SERPINB2 significantly impaired fibrinolytic function. While tPA alone generated approximately 290 ng/mL D-dimer at 300 seconds, SERPINB2 at 6.25 nM, 25 nM, and 100 nM reduced D-dimer levels to approximately 160, 90, and 0 ng/mL, respectively, representing inhibition rates of 45–69%. These results demonstrate that SERPINB2 directly inhibits tPA enzymatic activity in a dose-dependent manner, thereby impairing fibrinolytic function and potentially contributing to edema formation and fibrin accumulation in CRSwNP.

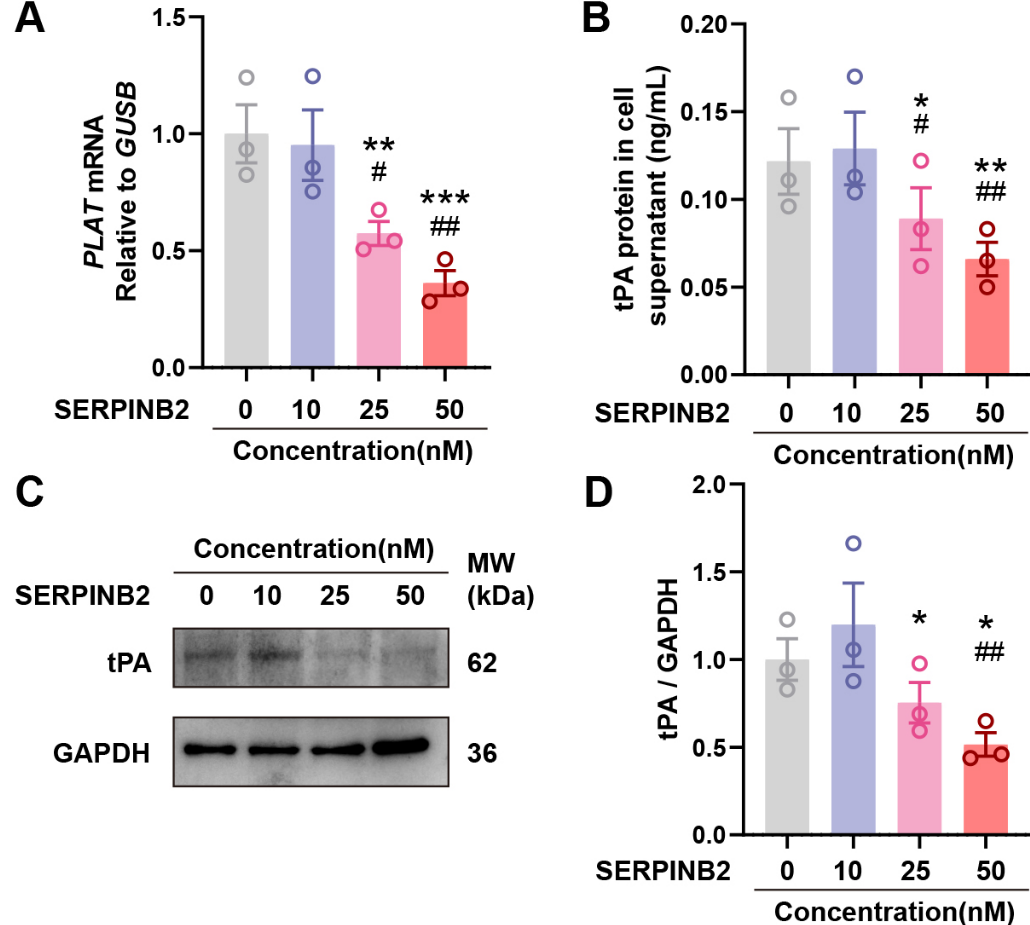

Fig. 4. SERPINB2 dose-dependently suppresses tPA expression in primary nasal epithelial cells. (A) RT-qPCR analysis of *PLAT* mRNA expression following SERPINB2 treatment. (B) ELISA quantification of secreted tPA protein in culture supernatants following SERPINB2 treatment. (C) Representative Western blot images showing tPA protein levels in cell lysates from vehicle- or SERPINB2-treated nasal epithelial cells. (D) Densitometric quantification of tPA protein levels normalized to GAPDH. Data are presented as mean ± SEM (n = 3 independent experiments). *$p < 0.05$ vs. 0 nM; **$p < 0.01$ vs. 0 nM; ***$p < 0.001$ vs. 0 nM; $^{\#}p < 0.05$ vs. 10 nM; $^{\#\#}p < 0.01$ vs. 10 nM.

4. Discussion

In this study, we identified a novel SERPINB2-tPA regulatory axis in NECs that contributes to fibrin deposition in CRSwNP. Our findings demonstrate that SERPINB2 expression is significantly elevated in NP tissues and inversely correlates with tPA levels, with epithelial cells serving as the primary cellular source of this dysregulation. Importantly, we uncovered a dual inhibitory mechanism whereby SERPINB2 not only directly inhibits tPA enzymatic activity through classic SERPIN-mediated protease inhibition, but also suppresses tPA expression at the transcriptional level. This regulation is driven by both Th1 (IFN-γ) and Th2 (IL-13) cytokines, suggesting that irrespective of inflammatory endotype, CRSwNP patients may experience convergent impairment of local fibrinolytic capacity. The functional consequence of this SERPINB2-tPA imbalance is severely compromised fibrin degradation, which may create a permissive microenvironment for persistent tissue remodeling and polyp formation. These findings provide a mechanistic explanation for the fibrin accumulation observed in nasal polyps and identify SERPINB2 as a potential therapeutic target for the prevention of polyp development and recurrence.

The inverse relationship between SERPINB2 and tPA observed in nasal polyp tissues parallels findings in other fibroproliferative disorders [13,14], suggesting a conserved pathogenic mechanism. Similar dysregulation has been reported in infectious pulmonary disease, chronic obstructive pulmonary disease, and allergic asthma [15–17], where impaired fibrinolysis contributes to excessive extracellular matrix deposition and progressive fibrosis. In the tumor microenvironment, elevated SERPINB2 has been associated with fibrin-rich matrices that promote cancer cell invasion and metastasis [18,19]. However, our study reveals features unique to upper airway disease. Unlike PAI-1, SERPINB2 is highly expressed in nasal polyp epithelium, highlighting

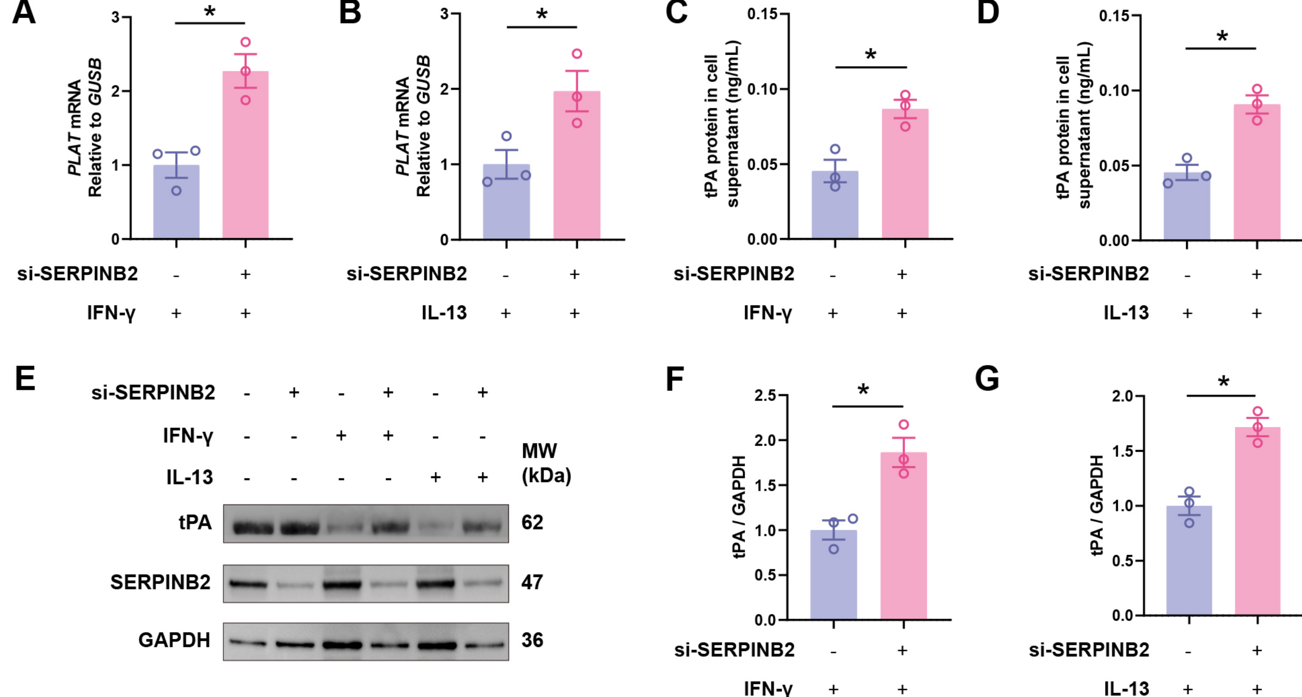

Fig. 5. SERPINB2 knockdown rescues Th1 and Th2 cytokine-induced tPA downregulation. (A,B) RT-qPCR analysis of *PLAT* mRNA expression in IFN-γ-treated (A) and IL-13-treated (B) epithelial cells following SERPINB2 knockdown. (C,D) ELISA quantification of secreted tPA protein in culture supernatants from IFN-γ-treated (C) and IL-13-treated (D) cells. (E) Representative Western blot images showing tPA protein levels in cell lysates across different treatment conditions. (F,G) Densitometric quantification of tPA protein levels normalized to GAPDH for IFN-γ (F) and IL-13 (G) treatment conditions. Data are presented as mean $\pm$ SEM (n = 3 independent experiments). *$p < 0.05$.

the central role of the airway epithelium in local hemostatic balance. The predominant epithelial localization also suggests that environmental triggers (allergens, pathogens, and pollutants) that directly stimulate epithelial cells may initiate the fibrinolytic dysfunction cascade [20]. Furthermore, the convergent regulation by both Th1 and Th2 cytokines distinguishes CRSwNP from other conditions where fibrinolytic impairment is typically associated with a specific inflammatory profile. This finding may explain why nasal polyps develop across different CRSwNP endotypes and why anti-inflammatory therapies targeting a single cytokine pathway show variable efficacy in preventing polyp recurrence.

Perhaps the most significant finding of our study is the discovery that SERPINB2 employs a dual mechanism to drive fibrinolytic dysfunction in CRSwNP. Excessive fibrin deposition is a pathological hallmark of CRSwNP, contributing to tissue remodeling and polyp recurrence [21–23]. The fibrinolytic system, primarily controlled by tPA and its inhibitors, normally maintains tissue homeostasis by clearing fibrin matrices [24]. However, the mechanisms underlying fibrinolytic impairment in nasal polyps have remained poorly understood. Our findings demonstrate that SERPINB2 disrupts this balance through two complementary pathways. Beyond its known function as a direct tPA inhibitor via SERPIN-mediated enzymatic inactivation, our data reveal a novel transcriptional regulatory role. Recombinant SERPINB2 dose-dependently suppressed tPA expression, while SERPINB2 knockdown rescued cytokine-induced tPA downregulation, providing strong evidence for this previously unrecognized mechanism. This dual inhibitory action—suppressing tPA gene expression while simultaneously inactivating residual tPA enzymatic activity—creates a synergistic anti-fibrinolytic effect that severely compromises local fibrin clearance in polyp tissues. Whether these two mechanisms interact through feedback loops remains an open question. SERPINB2-mediated inhibition of tPA activity may secondarily alter the local cytokine milieu and reinforce SERPINB2 expression in a feed-forward manner; conversely, tPA may reciprocally regulate SERPINB2 transcription through plasmin-dependent signaling. Elucidating the temporal hierarchy and potential interdependence of these two mechanisms represents an important direction for future investigation.

The molecular mechanisms underlying SERPINB2-mediated transcriptional suppression of tPA warrant further investigation. Potential mechanisms include: (i) SERPINB2 acting through cell surface receptor-mediated transduction signaling to modulate downstream tran-

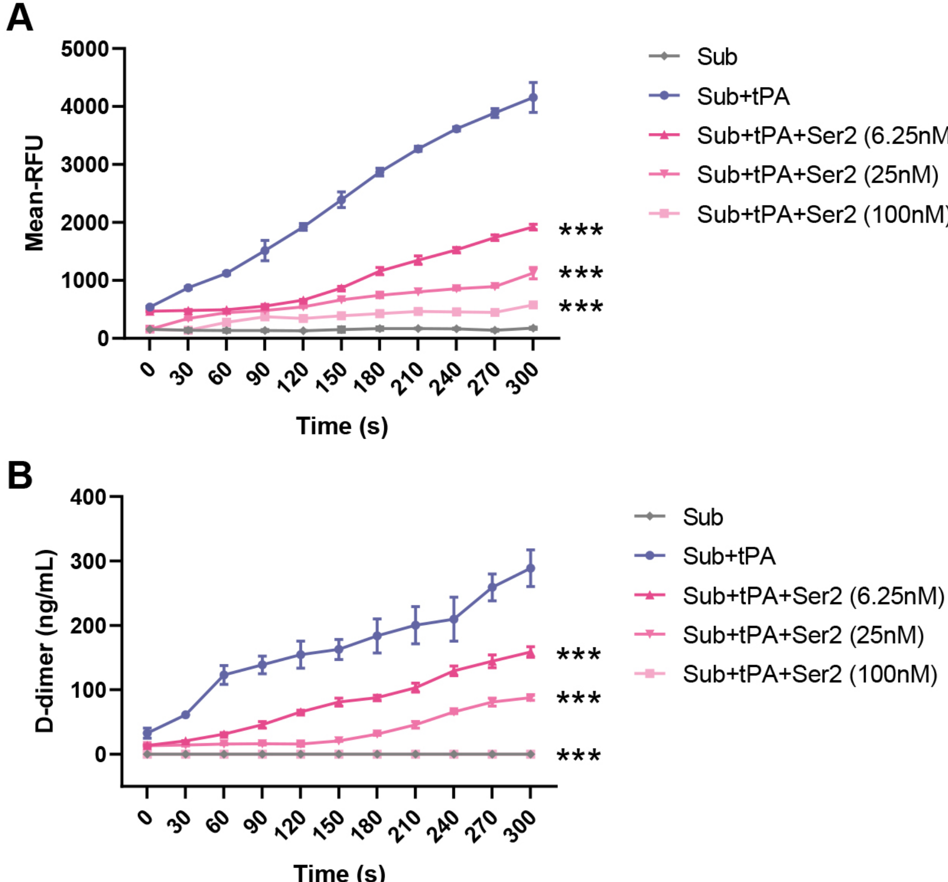

Fig. 6. SERPINB2 inhibits tPA enzymatic activity and impairs fibrinolytic function *in vitro*. (A) Time-course analysis of tPA enzymatic activity measured by fluorometric substrate assay. (B) Fibrinolytic function assessed by D-dimer release assay. ***$p < 0.001$ vs. Sub+tPA group. RFU, relative fluorescence units.

scriptional activity, consistent with the demonstrated GPER/SERPINB2 signaling axis [25]; (ii) intracellular SERPINB2 interacting with transcription factors at the *PLAT* promoter, supported by evidence that IRF1 functions as an upstream transcriptional regulator in type 1 inflammatory environments [26]; and (iii) epigenetic modifications of the tPA gene locus, which remain speculative and require dedicated investigation. These possibilities align with the emerging recognition that SERPINs exert functions beyond classical protease inhibition [27,28]. The convergent suppression of tPA by IFN-γ and IL-13 is particularly noteworthy: despite engaging distinct upstream pathways (STAT1 vs. STAT6) [11,29], both cytokines coordinately upregulate SERPINB2 and downregulate tPA, implying the existence of shared downstream transcriptional repressors at the PLAT locus, potentially within the JAK/STAT signaling network that operates in CRSwNP [30]. Identifying these convergence points may reveal broadly applicable therapeutic targets across CRSwNP endotypes.

Our findings have important translational implications for the management of CRSwNP. First, the epithelial expression of SERPINB2 makes it potentially accessible as a disease biomarker through minimally invasive nasal brushing or lavage fluid sampling. Elevated SERPINB2 levels could serve as a surrogate marker of impaired fibrinolytic capacity and identify patients at high risk for polyp recurrence. Second, our knockdown experiments provide proof-of-concept that targeting SERPINB2 can restore tPA expression and fibrinolytic function. While therapeutic development would require extensive validation, potential strategies include neutralizing antibodies or small molecule inhibitors targeting the SERPINB2-tPA interaction. Ultimately, defective fibrin clearance and subsequent fibrin overload represent a shared pathogenic pathway underlying edematous changes across CRSwNP endotypes. Interventions targeting fibrinolytic dysfunction could provide treatment benefits independent of endotype by disrupting the self-reinforcing cascade linking fibrin deposition, tissue remodeling, and polyp formation.

5. Limitations

Several limitations of this study should be acknowledged. First, this study relies primarily on *in vitro* ALI cultures and cross-sectional clinical tissue samples, and lacks *in vivo* functional validation. Future studies employing animal models with SERPINB2 knockout or epithelial-specific

overexpression to assess fibrinolytic activity, fibrin deposition, and polyp formation would provide stronger causal evidence. Second, our *in vitro* studies utilized healthy control-derived ALI cultures, which may not fully reflect the altered responsiveness of the polyp epithelium or the complex multicellular interactions in nasal polyp tissues. Third, our fibrinolysis assay utilized a serum-based system, which measures tPA-mediated plasminogen activation but does not fully recapitulate the tissue fibrin microenvironment of nasal polyps; future studies incorporating 3D fibrin gel models or *ex vivo* tissue explants would provide additional spatial and contextual information. Fourth, the clinical findings of this study are based on cross-sectional observations; longitudinal studies correlating SERPINB2 and tPA dynamics with disease progression and treatment response are needed to establish their clinical translational relevance.

6. Conclusions

This study identifies a novel SERPINB2-tPA regulatory axis that drives fibrinolytic dysfunction in CRSwNP. We demonstrate that SERPINB2 employs a dual inhibitory mechanism—suppressing tPA expression at the transcriptional level while directly inhibiting its enzymatic activity—resulting in severely impaired local fibrinolysis. The convergent upregulation of SERPINB2 by both the Th1 cytokine IFN-γ and the Th2 cytokine IL-13 suggests that fibrinolytic impairment occurs across different inflammatory endotypes in CRSwNP. These findings provide mechanistic insights into the fibrin accumulation and persistent tissue remodeling in nasal polyps, and identify SERPINB2 as a promising therapeutic target for the prevention of polyp formation and recurrence.

Availability of Data and Materials

The data presented in this study are available on request from the corresponding author.

Author Contributions

Conceptualization, WZ, and ZL; methodology, YZ, JZ, SZ, and ZL; validation, YZ, JZ, and SZ; formal analysis, YZ, and ZL; investigation, YZ, JZ, SZ, and ZL; resources, WZ, and ZL; data curation, YZ, JZ, and SZ; writing—original draft preparation, YZ, and ZL; writing—review and editing, YZ, WZ, and ZL; visualization, YZ; supervision, WZ, and ZL; funding acquisition, WZ, and ZL. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was approved by the Ethical Committee of Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, with written informed consent obtained from all participants. Approval Number: 2022-069. The study was carried out in accordance with the guidelines of the Declaration of Helsinki.

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Conflicts of Interest

The authors declare no conflicts of interest.

Declaration of AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the authors used Claude to improve the grammar, readability, and organization of the manuscript text. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/FBL50406.

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