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ORIGINAL RESEARCH

Role of Small Airway Epithelial–Mesenchymal Transition and CXCL13 in Pulmonary Lymphoid Follicle Formation in Chronic Obstructive Pulmonary Disease

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Background: Chronic obstructive pulmonary disease (COPD) is a progressive respiratory disorder characterized by inflammation and airway remodeling. Lymphoid follicles play a crucial role in acquired immunity and the development of COPD. However, the precise mechanisms of lymphoid follicle formation in COPD and the effects of cigarette smoke (CS) exposure on this process remain unclear. Epithelial–mesenchymal transition (EMT) is implicated in the progression of COPD and may serves as a source of stromal cells that produce chemokines crucial for lymphoid follicle formation. This study aims to clarify the contributions and mechanisms of EMT in lymphoid follicle genesis in COPD, focusing specifically on the role of CXCL13.

Methods: Lung tissue samples were obtained from patients with COPD, smokers, and non-smokers. Immunohistochemistry was performed to assess the lymphoid follicles, EMT-related markers, and CXCL13 expression. In vitro experiments were conducted using CS extract (CSE)-stimulated immortalized human bronchial epithelial cells (iHBECs) to induce EMT. The expression of EMT-related markers and CXCL13 in CSE-stimulated iHBECs was analyzed using Western blotting, real-time PCR, and immunofluorescence staining. The effect of an EMT inhibitor on CXCL13 expression was also examined.

Results: Patients with COPD and lymphoid follicles exhibited significantly lower forced expiratory volume in 1 s (% predicted) values than those without lymphoid follicles. Enhanced EMT changes were observed in patients with COPD and lymphoid follicles. Increased EMT-related markers and CXCL13 expression were observed in CSE-stimulated iHBECs, and CXCL13 expression gradually increased over time. Inhibiting EMT downregulated CXCL13 expression in iHBECs.

Conclusion: Lymphoid follicles are associated with enhanced EMT in COPD. EMT may act as a key driver of the adaptive immune response in COPD by promoting a microenvironment conducive to lymphoid follicles formation through the production of CXCL13. This study provides valuable insights into the mechanisms underlying lymphoid follicle formation in COPD and identifies potential therapeutic targets.

Keywords: chronic obstructive pulmonary disease, lymphoid follicles, small airway epithelial–mesenchymal transition, CXCL13

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by persistent airway inflammation and progressive lung structure destruction. Its pathogenesis is strongly associated with smoking; however, even after the cessation of smoking or exposure to other harmful stimuli, the resultant damage continues to progress.[1] Furthermore, the precise mechanism underlying the development of COPD remains unclear, necessitating further investigation into the disease's complex biology.

Recent studies have highlighted the role of acquired immunity in COPD, particularly the regeneration of ectopic lymphoid tissue, which is crucial for the chronic and progressive nature of the disease. The formation of lymphoid follicles, often referred to as tertiary lymphoid organs, may initiate lung injury characterized by alveolar enlargement,

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breakdown of tissue elastin, and significant hypoxia—all features of cigarette smoke (CS)—induced emphysema. Additionally, lymphoid follicles are correlated with disease severity.[2–4] These follicles are near the peripheral airways and are characterized by the accumulation of B cells at their core, whereas T and dendritic cells are dispersed in the surrounding area. Chemokines secreted by stromal cells are essential for orchestrating lymphoid follicle formation.[5,6] Specifically, chemokines, such as CXCL13 and CCL19/21, play crucial roles in attracting B and T cells, respectively, thereby facilitating their aggregation into lymphoid follicles.[7,8] But the precise pathogenesis of lymphoid follicle formation in COPD and the effects of CS exposure on this process are not fully understood.

Epithelial–mesenchymal transition (EMT) is a key mechanism in airway remodeling in COPD and plays a substantial role in the transformation of stromal cells from damaged small airway epithelial cells.[9] During EMT, epithelial cells undergo phenotypic changes, losing their normal function and acquiring the characteristics of mesenchymal cells. This transition is associated with increased cell migration, tissue remodeling, and fibrosis, key features of COPD. Increased EMT activity is associated with severe airflow limitation, decreased lung function, and increased risk of exacerbations in patients with COPD.[10,11] EMT serves as a source of stromal cells, and previous studies have demonstrated that mesenchymal cells are one of the sources of factors such as CXCL13, which plays a crucial role in lymphoid follicle formation and immune responses.[12,13] However, there are currently no studies investigating the relationship between EMT and lymphoid follicle formation in COPD.

CS exposure is a well-established risk factor for both EMT and lymphoid follicle development. Nevertheless, the mechanism by which CS exposure contributes to the interplay between EMT and the formation of pulmonary lymphoid follicles in COPD remains unclear. This study aims to elucidate the contributions and underlying mechanisms of EMT in the genesis of lymphoid follicles in COPD, with a specific focus on the role of CXCL13 in this process. By understanding the role of EMT in lymphoid follicle formation, we aim to provide new insights into the pathophysiology of COPD, potentially revealing critical pathways for its progression and identifying novel therapeutic targets.

Materials and Methods

Participants and Ethical Approval

Individuals diagnosed with lung cancer or suspected of having lung cancer who underwent surgical resection of solitary lung nodules at Tianjin Medical University General Hospital were enrolled in this study. Ethical approval was granted by the Ethics Committee of Tianjin Medical University General Hospital (approval no. IRB2019-KY-133), and all participants provided written informed consent before inclusion. This study was conducted in accordance with the Declaration of Helsinki. The participants were stratified into three groups: COPD, smokers, and non-smokers. Subjects in COPD group and smoker group required a smoking history of at least 20 pack-years without any periods of cessation. All participants in these two groups are current smokers and exclusively smoke tobacco. COPD was diagnosed according to the guidelines of the Global Initiative for Obstructive Lung Disease.[14] Subjects in smoker group exhibited normal lung function. Subjects in non-smoker group denied smoking history and showed normal lung function. Characteristics of the study subjects are presented in Table 1. Exclusion criteria included patients who experienced acute COPD exacerbations within the preceding three months.

Lung Tissue and Immunohistochemistry

Lung tissue samples were collected by a pathologist from areas maximally distant from the pulmonary lesions, ensuring the absence of signs of obstructive pneumonia or tumor invasion. The samples were subsequently fixed in formalin for preservation, embedded in paraffin, and sliced into 5 μm sections for mounting on slides. The tissue sections were stained with H&E and underwent immunohistochemistry, which included endogenous peroxidase blocking, antigen retrieval, and pre-incubation in goat serum (ZSGB Biotechnology, Beijing, China). The sections were then incubated overnight at 4°C with the following primary antibodies: CD20 (Ab27093), CD4 (Ab846), CD8 (Ab4055), CXCL13 (Ab112521), E-cadherin (Ab76055), vimentin (Ab92547), S-100A4 (Ab41532), and α-SMA (Ab5694) (Abcam, Cambridge, UK). The sections were then treated with horseradish peroxidase (HRP)-conjugated secondary antibodies and visualized using the DAB Detection System kit (ZSGB Biotechnology, Beijing, China). Positive staining was identified by the presence of

Table 1 Demographics of the Study Population (n = 58)

Parameters	Non-smokers	Smokers	COPD	
			COPD with LF	COPD without LF
Patients (male, n)	**18 (6)**	**21 (19)[a]**	**8 (8)[a]**	**11 (8)[a]**
Age (years)	58.00 (45.50–63.00)	59.00 (55.00–70.50)	58.00 (48.75–69.75)	58.00 (51.00–63.00)
BMI (kg/m^2)	25.24 (22.53–28.24)	25.31 (22.01–28.33)	23.96 (20.83–26.45)	22.60 (21.30–23.88)
FEV1 (L)	2.54 (2.19–3.41)	2.58 (2.30–3.03)	1.89 (1.55–2.03)[ab]	2.06 (1.44–2.50)[ab]
FEV1 (% predicted)	94.20 (83.80–112.10)	94.00 (83.65–101.75)	56.30 (51.60–63.95)[ab]	78.10 (58.20–90.90)[abc]
FEV1/FVC	80.82 (77.50–87.74)	77.14 (74.30–80.46)[a]	58.92 (52.43–67.36)[ab]	66.37 (65.18–67.76)[ab]
DLCO (mmol/min/kPa)	6.17 (4.86–8.46)	6.45 (5.39–7.23)	5.16 (4.58–7.19)	4.97 (4.13–6.59)
DLCO (% predicted)	74.35 (63.53–83.10)	76.20 (65.23–83.40)	55.50 (45.80–75.90)[b]	64.80 (54.90–79.00)
Smoking index (pack-years)	0	50.00 (30.00–60.00)	35.00 (30.00–40.00)	60.00 (30.00–80.00)

Notes: Data are presented as the median with an interquartile range. a, $p < 0.05$, compared with non-smokers; b, $p < 0.05$, compared with smokers; c, $p < 0.05$, compared with COPD with LF.
Abbreviations: COPD, chronic obstructive pulmonary disease; LF, lymphoid follicle; BMI, body mass index; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; DLCO, diffusing capacity of the lungs for carbon monoxide.

brownish-yellow coloration in the cytoplasm, cell membrane, or both. Primary antibodies were replaced with phosphate-buffered saline (PBS) as a negative control. Twenty fields of interest (follicle or subepithelium) were randomly selected and examined under 400× magnification. E-cadherin quantification was determined by assessing staining density in the small airway epithelium. The number of Vimentin or S100A4 positive cells was quantified per millimeter of basement membrane perimeter. The calculation of α-SMA positive cells was based on the thickness of the smooth muscle layer. Imaging was performed using an Olympus BX51 microscope (Olympus, Tokyo, Japan) and analysis was conducted using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Quantification of Lymphoid Aggregates

Lymphoid follicles were defined as aggregates of contiguous B and T cells, with dense accumulations of 50 or more lymphomononuclear cells classified as lymphoid follicles, while those with fewer than 50 cells were considered lymphoid aggregates. The number of lymphoid aggregates in lung tissue sections was assessed according to established methods.[15,16] Counts of lymphoid follicles in the tissue surrounding the airways were expressed relative to the number of airways per lung section, whereas counts in the pulmonary parenchyma were normalized to the area per lung section.

CS Extract Preparation

CS extract (CSE) was prepared as previously described.[17] Smoke was slowly and uniformly collected in a controlled manner through a bubble absorption bottle under negative pressure from a series of five sequentially lit cigarettes (Baisha brand with a filter, containing 11 mg tar, 0.9 mg nicotine, and 12 mg CO per cigarette). Mainstream and sidestream smoke was dissolved in 10 mL of fetal calf serum-free cell culture medium and filtered through a 0.22 μm pore-size filter to prepare the stock solution (100% CSE). Cell proliferation assays were performed using various CSE concentrations (0.25, 0.5, 1, 2.5, 5, and 10%) with a Cell Counting Kit-8 to determine the optimal experimental concentration of CSE; a concentration of 5% CSE was the optimal concentration for further experiments.

Cell Culture

Immortalized human bronchial epithelial cells (iHBECs; Bio-Rad, Hercules, CA, USA) were cultured in Keratinocyte Serum-Free Medium (Gibco, Waltham, MA, USA) supplemented with 0.2 ng/mL recombinant epithelial growth factor, 25 μg/mL bovine pituitary extract, 250 ng/mL puromycin, and 25 μg/mL G418 (Life Technology, Carlsbad, CA, USA). Cells were cultivated in a humidified incubator at 37°C and 5% CO2 and seeded in six-well plates at a density of 0.5×10^5 cells/well. After 24 h, the cells were treated with CSE and incubated for an additional 24, 48, and 72 h. After treatment, the cells were harvested, and total proteins were extracted for immunoblotting analyses. Protein quantification was

performed using a BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA). In parallel experiments, cells were treated with SB431542 (5 μmol/mL, Sigma-Aldrich, St. Louis, MO, USA) and CSE and incubated for the same periods. SB431542, a transforming growth factor beta (TGF-β) receptor inhibitor, effectively inhibits the induction of EMT.[18] The control group was treated with PBS instead of the inhibitor.

Immunofluorescence Staining

CXCL13 expression in iHBECs was analyzed using immunofluorescence microscopy. Cells were cultured in glass-bottom dishes, fixed with 4% paraformaldehyde, permeabilized with 0.2% Triton X-100, and blocked with 5% bovine serum albumin to avoid nonspecific antibody binding. The cells were incubated overnight with a primary anti-CXCL13 antibody (Ab112521, Abcam, Cambridge, UK) at 4°C. The cells were then treated with an Alexa Fluor 594-conjugated anti-rabbit IgG secondary antibody (AB_2307325, Jackson ImmunoResearch, West Grove, PA, USA) for 1 h at 37°C. Nuclear staining was performed using 4′,6-diamidino-2-phenylindole (Sigma-Aldrich, St. Louis, MO, USA). Finally, CXCL13 expression was detected and assessed using a TCS SP8 microscope (Leica Microsystems, Wetzlar, Germany).

Western Blot Analysis

Proteins were resolved using sodium dodecyl-sulfate polyacrylamide gel electrophoresis and transferred to a polyvinylidene fluoride membrane (Bio-Rad, Hercules, CA,USA). After blocking with 5% milk, the membrane was incubated with anti-α-SMA antibodies (Ab5694, Abcam, Cambridge, UK) at 4°C overnight and visualized with HRP-conjugated substrates (Millipore, Burlington, MA, USA). Blot images were captured using an Amersham Imager 680 (GE Health, Chicago, IL, USA) and analyzed quantitatively using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Real-Time PCR

Total RNA was extracted from the cultured cells using TRIZOL reagent (Invitrogen, Waltham, MA, USA), and cDNA was synthesized using a reverse transcription kit (Applied Biosystems, Waltham, MA, USA). Quantitative real-time PCR was conducted to measure target gene expression using gene-specific primers on a QuantStudio 5 system, with GAPDH as the internal control. The primer sequences used for amplification were as follows: for GAPDH, forward 5′-AAATGGTGAAGGTCGGTGTGAAC-3′ and reverse 5′-CAACAATCTCCACTTTGCCACTG-3′; for CXCL13, forward 5′-ACTCCACCTCCAGGCAGAATG-3′ and reverse 5′- AAGTTTGTGTAATGGGCTTCCAGA-3′.

Statistical Analyses

Statistical analyses were performed using SPSS version 21.0 and GraphPad Prism 9 software. Data were presented as the mean ± standard deviation or medians with interquartile ranges depending on the distribution of the data. Comparisons between two groups were performed using the Student's *t*-test or Mann–Whitney *U*-test, as appropriate. The Student's *t*-test is appropriate when the data follow a normal distribution, whereas the Mann–Whitney *U*-test is employed when the data do not adhere to a normal distribution. Comparisons among more than two independent groups were conducted using analysis of variance followed by Bonferroni post hoc tests and the Kruskal–Wallis test followed by Dunn's post hoc tests based on the data distribution. Statistical significance was set at $p < 0.05$.

Results

Demographics of the Study Population

This study enrolled 58 participants, which included 19 patients with COPD, 21 smokers with normal lung function, and 18 non-smokers with normal lung function. Lymphoid follicles were identified in nine individuals, predominantly in those with COPD (n = 8) and in only one smoker. The demographic details are presented in Table 1.

Lymphoid Follicles in Patients with COPD

The lymphoid follicles were primarily adjacent to the peripheral airways and exhibited B cell infiltration and aggregation at the central. $CD4^+$ and $CD8^+$ T cells were distributed around the lymphoid follicles (Figures 1a–c). CXCL13 was present within lymphoid follicles (Figure 1d). Patients with COPD and lymphoid follicles exhibited a significantly lower forced expiratory volume in 1 s (% predicted) (FEV1/pred) than those without lymphoid follicles. However, no significant differences were observed in the absolute value of FEV1, FEV1/forced vital capacity, age, body mass index, diffusing capacity of the lungs for carbon monoxide, or smoking index between the two groups (Table 1).

Enhanced EMT Changes in Patients with COPD and Lymphoid Follicles

Immunohistochemistry was performed to assess the expression of E-cadherin—a marker for adherens junctions of epithelial tissues—vimentin, α-SMA protein, and S100A4—markers for mesenchymal cells in the lung tissue from each group (Figure 2a). Smokers and patients with COPD exhibited more pronounced EMT changes than non-smokers. Notably, patients with COPD and lymphoid follicles displayed more pronounced changes in the expression of EMT-related markers than those without lymphoid follicles. E-cadherin expression was significantly decreased, whereas vimentin, S100A4, and α-SMA expression were significantly increased (Figure 2b–e). These results suggest a strong association between lymphoid follicles and enhanced EMT in patients with COPD.

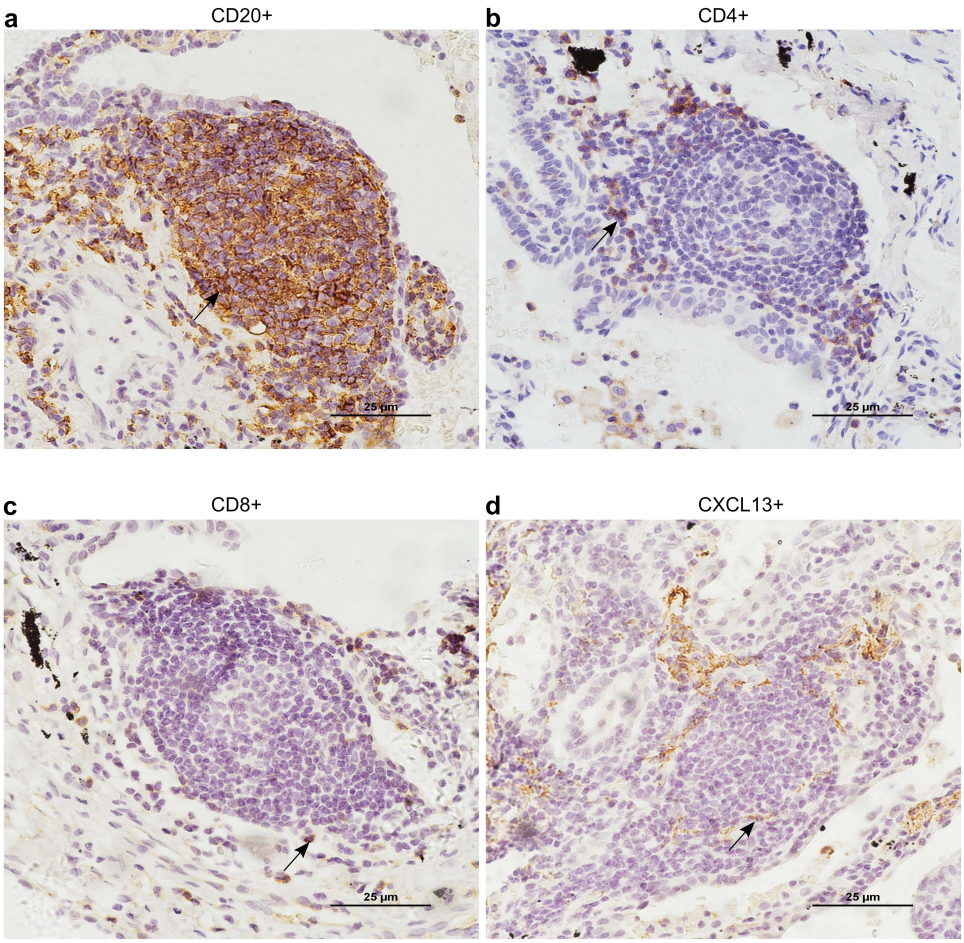

Figure 1 Immunohistochemical analysis of lung lymphoid follicles in patients with chronic obstructive pulmonary disease revealed the presence of (**a**) $CD20^+$ B, (**b**) $CD4^+$ T, (**c**) $CD8^+$ T, and (**d**) $CXCL13^+$ cells (stained with 3.39-diaminobenzidine, brown). Tissue sections were counterstained with Mayer's hematoxylin (blue). Arrows indicate positive cells. Scale bar: 25 μm.

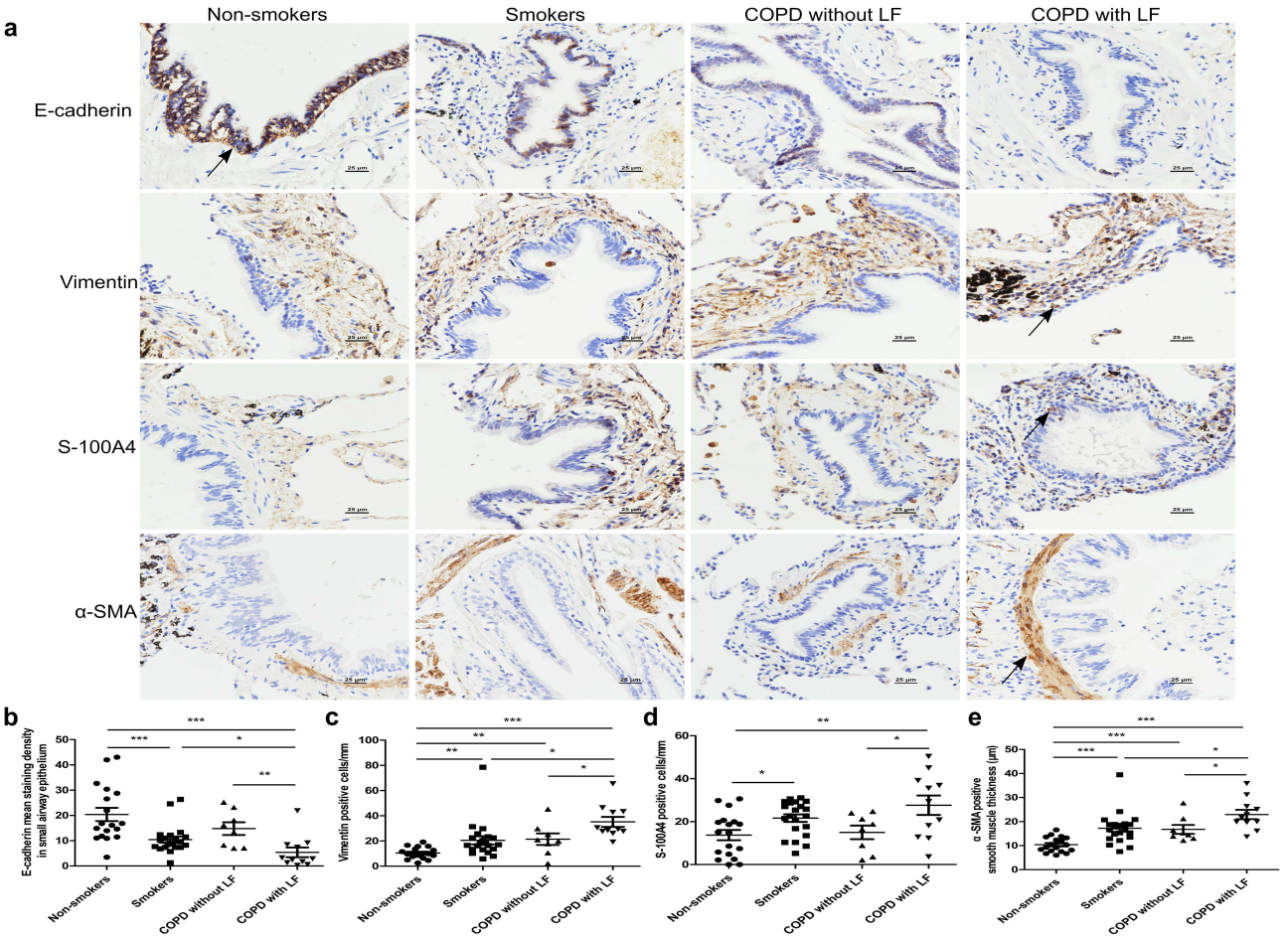

Figure 2 Expression of epithelial–mesenchymal (EMT)-related markers in lung tissue from each group. (**a**) Immunohistochemical detection of E-cadherin, vimentin, α-SMA, and S100A4 in non-smokers, smokers, patients with COPD without lymphoid follicles (LF), and patients with COPD and LF (stained with 3.39-diaminobenzidine, brown). Sections were counterstained with Mayer's hematoxylin (blue). Arrows indicate positive cells. Scale bar: 25 μm. (**b**) Quantification of E-cadherin staining density in small airway epithelium. (**c** and **d**) Vimentin and S100A4-positive cell counts per millimeter of basement membrane perimeter. (**e**) Measurement of α-SMA-positive smooth muscle layer thickness. Horizontal lines represent mean values. Data are presented as mean ± standard deviation (SD). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Enhanced CXCL13 Expression Accompanies EMT in CSE-Stimulated iHBECs

iHBECs were stimulated with CSE and collected at different time intervals (24, 48, and 72 h). Western blotting was performed to assess the α-SMA levels in iHBECs to confirm the occurrence of EMT (Figure 3a). The RNA levels of CXCL13 in iHBECs were investigated at different time points. CXCL13 expression gradually increased with the progression of CSE-induced EMT, with a noticeable difference between the 24 and 72-h time points (Figure 3b). Additionally, immunofluorescence staining for CXCL13 in iHBECs confirmed a significant increase in CXCL13-positive cells after 72 h of CSE stimulation compared to that at 24 h (Figure 3c). These data revealed a correlation between CSE-induced EMT in iHBECs and increased CXCL13 levels.

Downregulation of CXCL13 Expression After EMT Inhibition in iHBECs

The EMT inhibitor SB431542 was used to investigate the induction of CXCL13 expression during EMT. A significant decrease in CXCL13 expression was observed over time following EMT inhibition (Figure 4a and b). Additionally, immunofluorescence staining revealed a marked reduction in the proportion of CXCL13-positive cells at 72 h compared to that at 24 h after the administration SB431542 (Figure 4c).

Discussion

The importance of adaptive immunity in COPD pathogenesis has garnered increasing interest.[19] Lymphoid follicles comprise cells that produce a spectrum of cytokines and facilitate cellular activation, proliferation, and interaction. Once

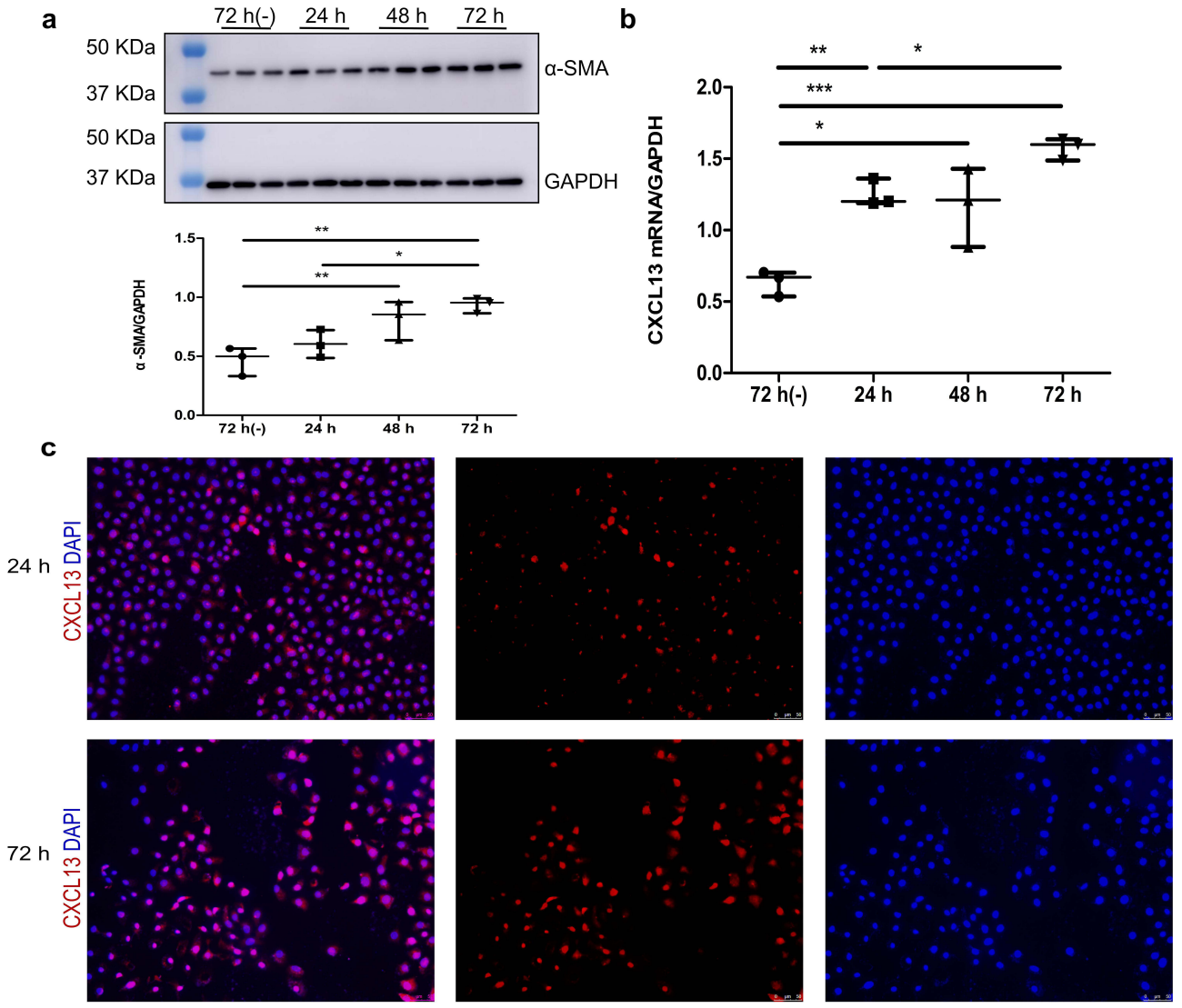

Figure 3 CXCL13 expression in cigarette smoke extract (CSE)-stimulated immortalized human bronchial epithelial cells (iHBECs). (**a**) Western blot analysis of α-SMA expression in iHBECs after CSE treatment. (**b**) Temporal changes in CXCL13 mRNA levels. (**c**) Time-lapse immunofluorescent staining of CXCL13 (Alexa Fluor 594, red) with nuclear counterstaining using 4′,6-diamidino-2-phenylindole (blue). Scale bar: 50 μm. n = 3 in each group. Data are presented as the median with an interquartile range. Comparisons of the four groups were performed using the Kruskal–Wallis test followed by Dunn's post hoc tests (α-SMA/GAPDH: H = 9.462, $p < 0.05$; CXCL13 mRNA/GAPDH: H = 9.359, $p < 0.05$). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

established, lymphoid follicles persist and actively contribute to immune defense against infections, autoimmunity, and inflammation.[20,21] Patients with COPD and lymphoid follicles exhibited significantly lower FEV1/pred values than those without, supporting the notion that patients with lymphoid follicles have more severe COPD than patients who do not have lymphoid follicles.[3] However, the precise mechanism underlying lymphoid follicle formation in COPD remain unclear. Nonetheless, this study revealed that EMT might contribute to lymphoid follicle formation in COPD by promoting CXCL13 expression. As the first study to explore the relationship between EMT and lymphoid follicle formation, our work provides insight into the development of lymphoid follicle in COPD, laying the groundwork for future research on COPD pathophysiology and potential therapeutic targets.

Airway remodeling is a key factor in COPD progression and persistent airflow limitation. EMT plays a significant role in airway remodeling in smokers with COPD,[10,22,23] and CSE induces EMT in the small airway epithelium.[24] Similarly, our findings revealed that the smoker group exhibited more pronounced EMT changes than the non-smoker group, demonstrating that CSE reduces the expression of markers of adherens junctions in epithelial tissues and increases

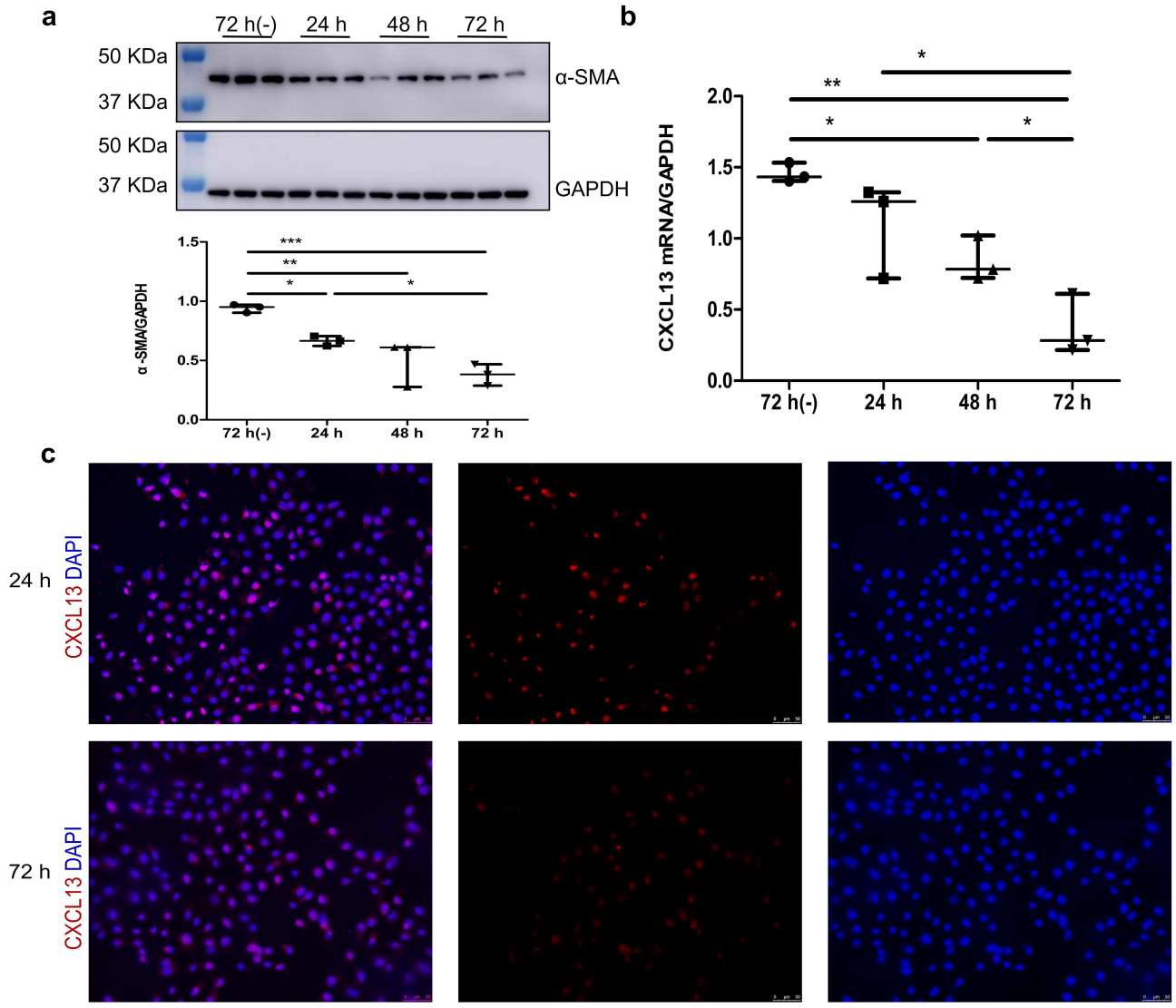

Figure 4 CXCL13 expression in CSE+SB432542-stimulated iHBECs. (**a**) Western blot analysis of α-SMA expression in iHBECs after CSE+SB431542 treatment. (**b**) Temporal changes in CXCL13 mRNA levels. (**c**) Time-lapse immunofluorescent staining of CXCL13 (Alexa Fluor 594, red) with nuclear counterstaining using 4′,6-diamidino-2-phenylindole (blue). Scale bar: 50 μm. n = 3 in each group. Data are presented as the median with an interquartile range. Comparisons of the four groups were performed using the Kruskal–Wallis test followed by Dunn's post hoc tests (α-SMA/GAPDH: H = 9.462, $p < 0.05$; CXCL13 mRNA/GAPDH: H = 9.462, $p < 0.05$). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

the expression of those associated with mesenchymal cells. These results further support the role of CSE in inducing EMT in human bronchial epithelial cells.

Our study suggests that patients with COPD and lymphoid follicles display more substantial changes in EMT markers expression than those without lymphoid follicles. This result suggests a correlation between lymphoid follicle formation and changes in EMT marker expression in patients with COPD. The previous studies had confirmed that CXCL13, which binds to the CXCR5 receptor on B cells, plays a crucial role in orchestrating lymphoid follicle formation by attracting B cells and facilitating their aggregation into lymphoid follicles and emphysema in COPD.[7] A 24-week exposure to CS induces emphysema and lymphoid follicle development in mice, with concurrent CXCL13 upregulation. Notably, anti-CXCL13 antibody treatment markedly attenuated lymphoid follicle formation and airway inflammation.[15] Stromal cells, predominantly fibroblasts or their progenitors, are principal CXCL13 producers.[21,25] In conditions such as *Helicobacter pylori*-induced gastritis and atherosclerosis, α-SMA-positive stromal cells secrete CXCL13.[12,13] The proliferation of interstitial cells surrounding the small airways is crucial for the thickening of the airway walls, and EMT is a source of

interstitial cells, including myofibroblasts.[26] Our study demonstrated that the EMT in CSE-stimulated iHBECs was associated with elevated CXCL13 expression. Furthermore, inhibiting EMT in iHBECs suppressed this increased in CXCL13 expression. EMT-induced CXCL13 creates a microenvironment conducive to B cells aggregation, essential for the lymphoid follicle development. Therefore, it is plausible to suggest that EMT may contribute to lung lymphoid neogenesis via CXCL13 and serve as a key driver of the adaptive immune response in COPD. This highlights EMT as not just a structural change in epithelial cells but also a functional contributor to follicular formation through CXCL13 production. CSE plays a crucial role in our study. Research indicates that smoking cessation significantly improves adaptive immunity and reduces pulmonary inflammation in COPD patients.[27,28] Therefore, encouraging promoting cessation is a vital strategy for preventing disease progression.

Our study have some limitations. This study underscores the possible involvement of EMT in lymphoid follicle formation, which is potentially linked to upregulating CXCL13 production. However, direct confirmation of this hypothesis and exploration of the associated mechanisms, such as specific signaling pathways, transcription factors, and cytokines, were not conducted in this study. Second, the TGF-β1 receptor inhibitor, SB431542, used in this study only blocked a subset of pathways associated with EMT. Additional inhibitors targeting other relevant pathways should be used in future studies to investigate the role of EMT comprehensively.

Conclusion

The enrichment of lymphoid follicles in the lung tissues of COPD is associated with disease progression and plays an important role in the adaptive immunity of COPD. Our study indicates that EMT may play a crucial role in the development and formation of lymphoid follicles in COPD by inducing CXCL13 production. EMT could serve as a key driver of the adaptive immune response in COPD by promoting a microenvironment conducive to follicular formation through the production of CXCL13. These findings substantially enhance our understanding of the underlying cellular and molecular mechanisms involved in adaptive immunity in COPD and provide valuable insights and potential targets for future therapeutic strategies. However, future investigations, including animal studies, are essential to validate our observations and elucidate the underlying mechanisms in greater detail.

Abbreviations

COPD, Chronic obstructive pulmonary disease; EMT, epithelial–mesenchymal transition; CS, cigarette smoke; CSE, cigarette smoke extract; iHBECs, immortalized human bronchial epithelial cells; TGF-β, transforming growth factor beta; LF, lymphoid follicle; BMI, body mass index; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; DLCO, diffusing capacity of the lungs for carbon monoxide.

Ethics Approval and Informed Consent

The present study was approved by the Ethics Committee of Tianjin Medical University General Hospital (IRB2019-KY-133). Written informed consent of the participants for this research study were collected at the time of sample collection.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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