Epithelial–mesenchymal transition changes in nonsmall cell lung cancer patients with early COPD

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EMT is a fundamental underlying pathological mechanism in COPD airways leading to epithelial malignancy and small airway fibrosis. It is a novel therapeutic target, and has implications for early management of COPD and protection against lung cancer. https://bit.ly/3v1x96G

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Abstract

Background Epithelial–mesenchymal transition (EMT) might be central to lung cancer development in smokers and COPD. We illustrate EMT changes in a broader demographic of patient groups who were diagnosed with nonsmall cell lung cancer (adenocarcinoma and squamous cell carcinoma). These included COPD current and ex-smokers, patients with small airway disease and normal lung function smokers compared to normal controls.

Methods We had access to surgically resected small airway tissue from 46 subjects and assessed for airway wall thickness and immunohistochemically for the EMT biomarkers E-cadherin, N-cadherin, S100A4, vimentin and epidermal growth factor receptor (EGFR). All tissue analysis was done with a computer and microscope-assisted Image-Pro Plus 7.0 software.

Results Airway wall thickness significantly increased across all pathological groups ($p<0.05$) compared to normal controls. Small airway epithelial E-cadherin expression markedly decreased ($p<0.01$), and increases in N-cadherin, vimentin, S100A4 and EGFR expression were observed in all pathological groups compared to normal controls ($p<0.01$). Vimentin-positive cells in the reticular basement membrane, lamina propria and adventitia showed a similar trend to epithelium across all pathological groups ($p<0.05$); however, such changes were only observed in reticular basement membrane for S100A4 ($p<0.05$). Vimentin was higher in adenocarcinoma *versus* squamous cell carcinoma; in contrast, S100A4 was higher in the squamous cell carcinoma group. EGFR and N-cadherin expression in both phenotypes was markedly higher than E-cadherin, vimentin and S100A4 ($p<0.0001$).

Conclusion EMT is an active process in the small airway of smokers and COPD diagnosed with nonsmall cell lung cancer, contributing to small airway remodelling and cancer development as seen in these patients.

Introduction

Over 3 million people died from COPD in 2019 and it is currently the third most common cause of death globally [1]. The main features of COPD include a gradual narrowing of the airways and small airway fibrosis and obliteration, resulting in persistent airflow limitation and difficulty breathing when performing physical activities [2]. Lung cancer is the primary cause of cancer-related death, and there were over 2 million newly diagnosed cases worldwide in 2020 [3]. About 80–85% of lung cancer is nonsmall cell lung cancer (NSCLC), with the highest number of cancer-related deaths globally. Adenocarcinoma is the most frequently occurring histological type of NSCLC, accounting for 40% of cases, followed by squamous cell carcinoma, which makes up approximately 25–30% of cases [4, 5]. Studies have shown that

smokers with COPD, and the presence of emphysema in particular, have a four- to six-fold more significant risk of developing lung cancer than smokers with normal lung function [6, 7]. Lung cancer is a common cause of death in COPD patients, especially those with severe disease [8, 9]. COPD and lung cancer have common characteristics, including elevated mortality rates and risk factors such as cigarette smoking. Several mechanisms have been elucidated for the correlation between COPD and lung cancer, such as genetic mutations, chronic inflammation and dysregulated activation of bronchioalveolar stem cells but especially epithelial–mesenchymal transition (EMT) [10, 11].

EMT is the process of epithelial cells undergoing multiple molecular changes to obtain a mesenchymal phenotype with the potential to migrate through the reticular basement membrane (Rbm) to the sub-epithelial lamina propria (LP) [12, 13]. It is a widely recognised mechanism in embryonic development (type-1 EMT), while pathological forms promote fibrosis (type-2 EMT) and epithelial malignancy (type-3 EMT), mostly seen in cancer development, invasion and metastasis [14, 15]. Furthermore, we have reported EMT activity as central to fibrotic small airway remodelling and epithelial malignancies in COPD patients [11, 16–18]. Hence, EMT could play a vital role in the connection between COPD and lung cancer, which can be triggered by smoking.

To explore this further, we investigate here the EMT-related changes and airway wall thickness in the small airways of broader demographic patient groups who are smokers with lung cancer and with or without COPD. We also made an attempt to differentiate EMT activity based on the type of lung cancer in these patients.

Materials and methodology

Participants

We had access to surgically resected lung tissues away from the primary tumour mass from 35 participants who consented to clinical samples at Royal Hobart Hospital (table 1). The thoracic surgeon (A.H.) performed the surgeries according to appropriate guidelines. There were 22 patients with adenocarcinoma and 13 patients with squamous cell carcinoma. 19 participants had mild–moderate COPD classified as Global Initiative for Obstructive Lung Disease (GOLD) 1 and 2, of which nine were current smokers with COPD (COPD-CS) and 10 were ex-smokers (>1 year smoking cessation) with COPD (COPD-ES). In addition, seven participants were normal lung function smokers (NLFS) and nine participants had small airway disease (SAD). The Tasmanian Health & Medical Human Research Ethics Committee approved the study (Ethics ID: H0012374), while tissue from 11 healthy non-smoking individuals who had died due to causes unrelated to pulmonary diseases were obtained from the James Hogg Lung Registry at the University of British Columbia (Ethics ID: H00–50110) for the normal control (NC) group.

Immunohistochemistry

Resected small airway tissue sections were cut at 3 µm from the paraffin-embedded blocks as previously reported [19–21]. Tissue sections were dewaxed and rehydrated in distilled water using xylene, absolute ethanol and 70% ethanol (v/v) in distilled water, respectively. Heat-induced epitope retrieval was used with

TABLE 1 Patient demographics[#]

Groups	NC	NLFS	SAD	COPD-CS	COPD-ES
Subjects, n	11	7	9	9	10
GOLD, n					
GOLD 1				3	6
GOLD 2				6	4
Sex, n					
Female	6	4	7	5	4
Male	5	3	2	4	6
Age, median (range), years	47 (35–87)	72 (52–79)	59 (42–84)	63 (59–78)	68.5 (56–85)
Smoking, pack-years	NA	20.69±21.44	35.67±20.54	32.25±15.81	28.8±17.78
Post-BD FEV_1 % pred	NA	107.5±22.98	86.4±21.8	82.14±12.52	83.68±11.29
Post-BD FEV_1/FVC %	NA	79.7±6.45	73.84±3.21	66.39±3.55	63.39±4.83
Post-BD $FEF_{25–75\%}$, $L{\cdot}s^{-1}$	NA	86.64±16.4	46.6±12.78	35.63±6.26	39.84±11.52

Data presented as mean±SD, unless otherwise indicated. NC: normal control; NLFS: normal lung function smoker; SAD: small airway disease; COPD-CS: current smoker with COPD; COPD-ES: ex-smoker with COPD; GOLD: Global Initiative for Chronic Obstructive Lung Disease; Post-BD: post-bronchodilator; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; $FEF_{25–75\%}$: forced expiratory flow at 25–75% FVC. [#]: adenocarcinoma n=22; squamous cell carcinoma n=13.

a Decloaking Chamber (Biocare Medical, Queensland, Australia) at 110°C for 15 min with heat retrieval citrate solution (pH 6; S2369, Dako, Victoria, Australia). Endogenous enzyme blocking was performed with 3% hydrogen peroxide in distilled water (v/v). Immunochemical staining was carried out with epithelial junctional marker E-cadherin (1:50 dilution; M3612, Dako) and mesenchymal markers mouse monoclonal N-cadherin (1:100 dilution; ab98952, Abcam, Victoria, Australia), rabbit polyclonal S100A4 (1:1000 dilution; A5114, Dako), mouse monoclonal vimentin (1:200 dilution; M7020, Dako) and epidermal growth factor receptor (EGFR) (1:150 dilution; ab32077, Abcam), followed by an enzyme-conjugated polymer backbone that carries secondary antibodies (Dako REAL EnVision detection system, K5007), with 3,3′-diaminobenzidine (DAB+) as the chromogen for visualisation. In addition, for correlation purposes, we incorporated an overlapping group of smokers and COPD tissues from a previous study [16] in which small airways had been stained in the same way for α-smooth muscle actin (α-SMA), collagen-1 and fibronectin.

Quantification of biomarkers expression in small airway

All quantification was done using a Leica camera (ICC50W, Leica, Sydney, NSW, Australia), microscope (DM500, Leica) and computer-assisted Image-Pro Plus 7.0 software (Media Cybernetics, Rockville, MD, USA). Non-overlapping images of small airways in each tissue section were taken at a ×40 bright field. Eight images were randomly selected from the total number of images using an online random number generator program (www.calculatorsoup.com) for quantification. The number of cells positive for S100A4 and vimentin in small airway epithelium and Rbm were quantified and normalised per millimetre of Rbm length. Additionally, the percentage of epithelial cells with positive expression of EGFR and EMT markers (N-cadherin, E-cadherin, S100A4 and vimentin) was measured. The number of positive cells in the LP and adventitia are presented as cells·mm^{-2} of the area, respectively.

Measurement of small airway wall thickness

The small airway images of each subject were taken at ×40 bright field, and eight of the total images were randomly selected (same as mentioned above). The airway sub-epithelial regions were divided into the Rbm, which refers to the area between the lower margin of the epithelium and the upper margin of the LP; the LP, which is the area between the lower limit of the Rbm and the upper margin of the muscle layer; and the adventitia, which is the region between the lower margin of the muscle layer and the margin of the alveolar tissue interface. The thickness of each layer was determined using Image-Pro Plus 7.0 software by drawing a line along the outer margins of each layer and using the software's automated distance and area calculator to calculate the distance and area.

Statistics

GraphPad version 9.0 (GraphPad Software Inc., La Jolla, CA, US) was used for statistical analysis. Nonparametric ANOVAs were performed using the Kruskal–Wallis test; specific group differences without correction for multiple comparisons were assessed using a one-way ANOVA test with Dunn's multiple comparison test. Correlation analysis was performed with regression analyses using Spearman's rank test. A p-value <0.05 was deemed to be statistically significant.

Results

Small airway wall thickness

The thickness of small airway wall sub-epithelial area was significantly increased in all pathological groups compared to NC. Specifically, markedly thickened Rbm (median 11.23 µm, range 7.20–15.20 µm, p<0.001), LP (median 57.95 µm, range 39.72–78.99 µm, p<0.001) and adventitia (median 107.20 µm, range 56.91–186.20 µm, p<0.001) were observed in COPD-CS compared to NC (Rbm: median 4.74 µm, range 3.20–11.91 µm; LP: median 21.74 µm, range 10.00–48.37 µm; adventitia: median 41.23 µm, range 24.01–88.82 µm). In addition, the Rbm thickness was notably high in SAD (median 12.89 µm, range 7.67–17.84 µm, p<0.001) compared to NC. NLFS and COPD-ES groups also showed significantly thicker Rbm (p<0.005), LP (NLFS p<0.005; COPD-ES p<0.05) and adventitia (p<0.005) (figure 1).

EMT marker expression in small airway epithelium

Representative images of EMT marker expression in small airways of COPD-CS and NC are shown in figure 2. Intense positive mesenchymal marker expression (brown) was seen in COPD-CS (figure 2d, f, h, j) compared to NC (figure 2c, e, g, i), while the epithelial marker was lost in COPD-CS (figure 2b) *versus* NC (figure 2a). Epithelial marker E-cadherin expression significantly decreased across all pathological groups compared to NC (p<0.01) (figure 3a). In contrast, a significant increase in mesenchymal marker N-cadherin was observed in SAD (p<0.05) and COPD-CS (p<0.01) compared to NC (figure 3b). Even though the increase in N-cadherin expression was not noteworthy in every pathological group compared to NC, the ratio of N-cadherin to E-cadherin was substantially increased by over 20-fold in all pathological

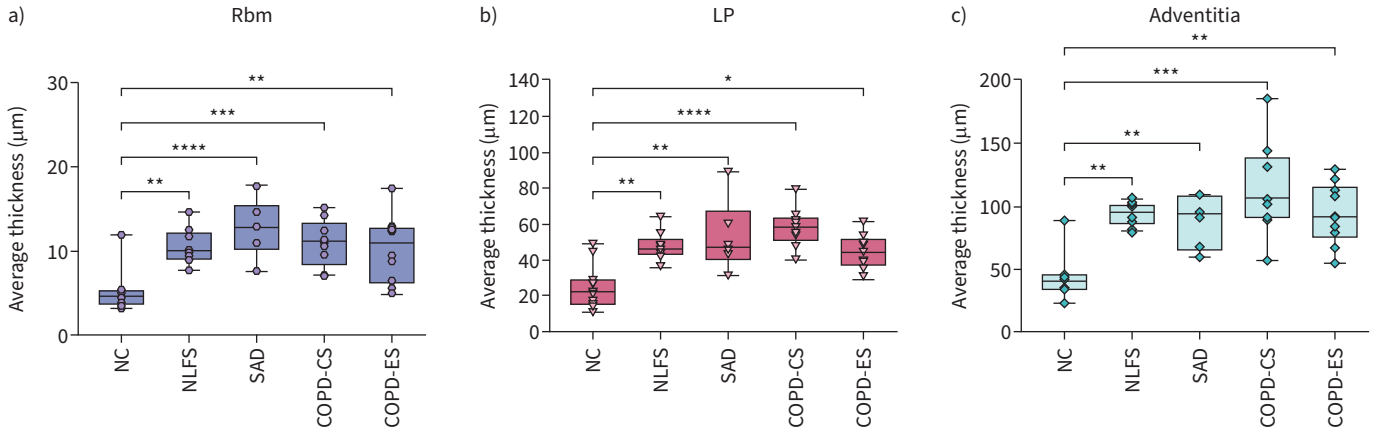

FIGURE 1 Small airway sub-epithelial layer thickness across all pathological groups compared to normal control (NC). a) Reticular basement membrane (Rbm) thickness; b) lamina propria (LP) thickness; c) adventitia thickness. NLFS: normal lung function smoker; SAD: small airway diseases; COPD-CS: current smoker with COPD; COPD-ES: ex-smoker with COPD. *: p<0.05; **: p<0.01; ***: p<0.005; ****: p<0.001.

groups, particularly the COPD-CS group, which showed a 24-fold increase (figure 4a). Epithelial vimentin and S100A4 expression were significantly upregulated in all pathological groups compared to NC (p<0.05 and p<0.05, respectively) (figure 3c, d). In addition, the epithelial expression percentage ratio of both vimentin and S100A4 to E-cadherin increased by 1.67–4.79-fold across the pathological groups (figure 4b, c). Similarly, epithelial EGFR expression was markedly increased in all pathological groups compared to NC (p<0.05) (figure 3e); the expression ratio to E-cadherin also showed a 19–40-fold increase across all the pathological groups compared to NC (figure 4d).

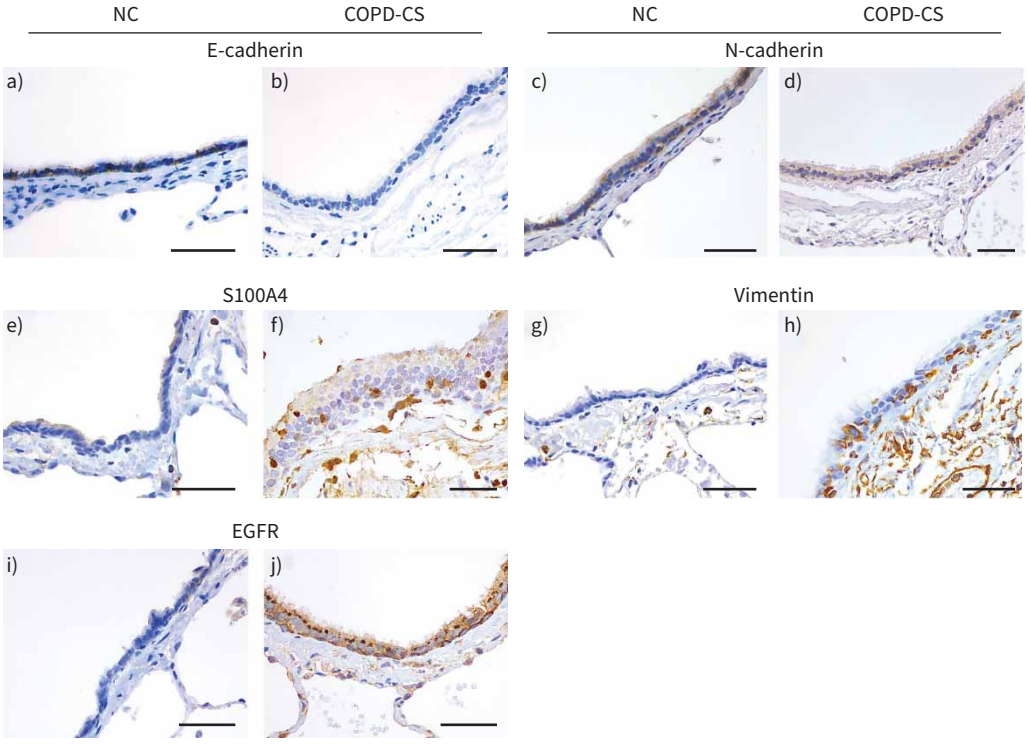

FIGURE 2 Representative images of epithelial–mesenchymal transition marker expression in COPD current smoker (COPD-CS) and normal control (NC) small airways. a, b) E-cadherin expression; c, d) N-cadherin expression; e, f) S100A4 expression; g, h) vimentin expression; i, j) epidermal growth factor receptor (EGFR) expression. Bright field ×40. Scale bars: 50 µm.

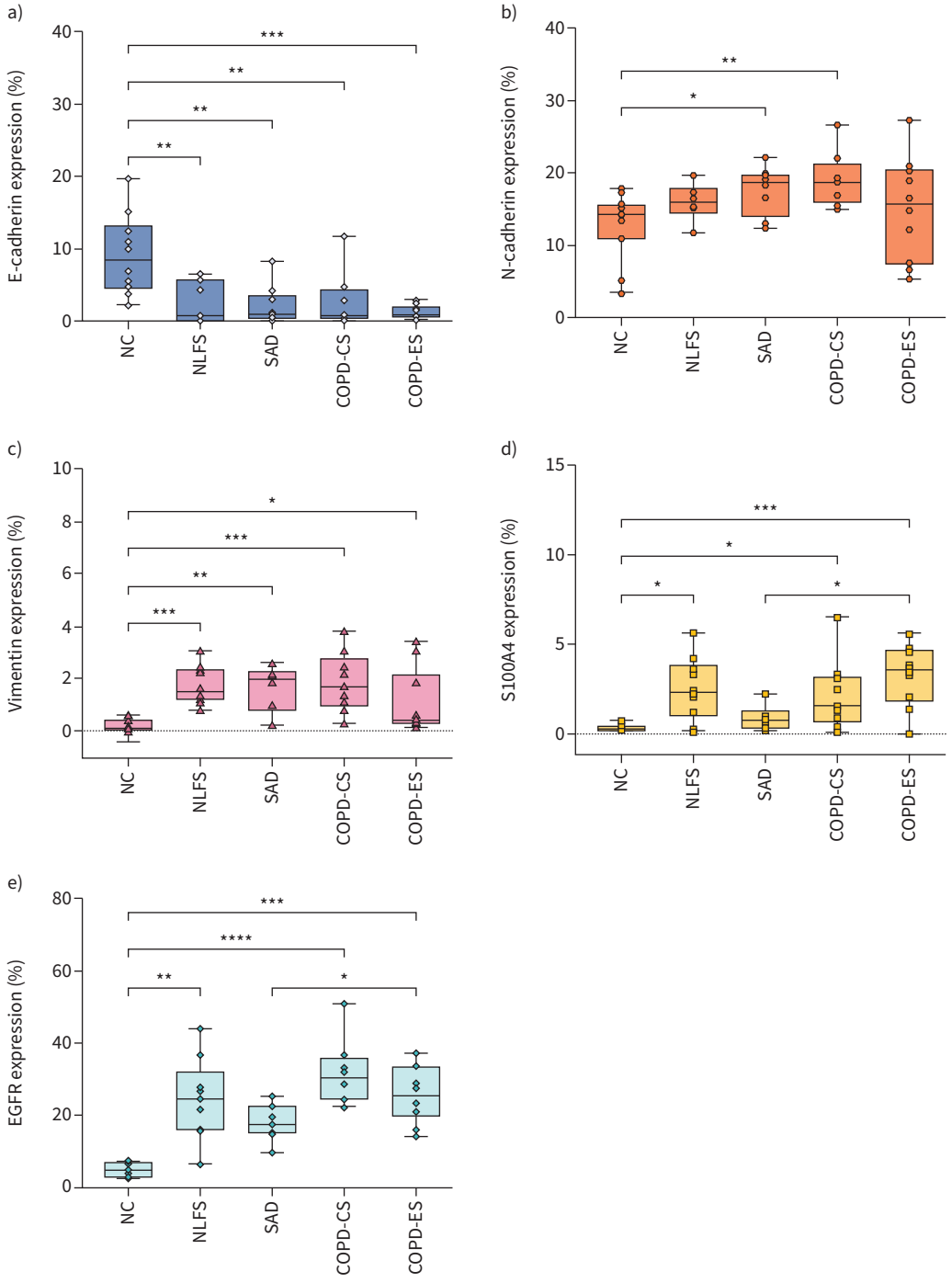

FIGURE 3 Epithelial–mesenchymal transition marker expression in small airway epithelium. a) E-cadherin expression percentage; b) N-cadherin expression percentage; c) vimentin expression percentage; d) S100A4 expression percentage; e) epidermal growth factor receptor (EGFR) expression percentage. NC: normal control; NLFS: normal lung function smoker; SAD: small airway disease; COPD-CS: current smoker with COPD; COPD-ES: ex-smoker with COPD. *: p<0.05; **: p<0.01; ***: p<0.005; ****: p<0.001.

Mesenchymal marker expression in Rbm, LP and adventitia

Similar to the epithelium, Rbm cells were positive for vimentin and S100A4 across all pathological groups compared to NC (p<0.01 and p<0.05, respectively) (figure 5a, b). Rbm vimentin and S100A4 expression negatively correlated with E-cadherin expression (r= −1.32, p<0.005 and r= −0.45, p<0.05, respectively).

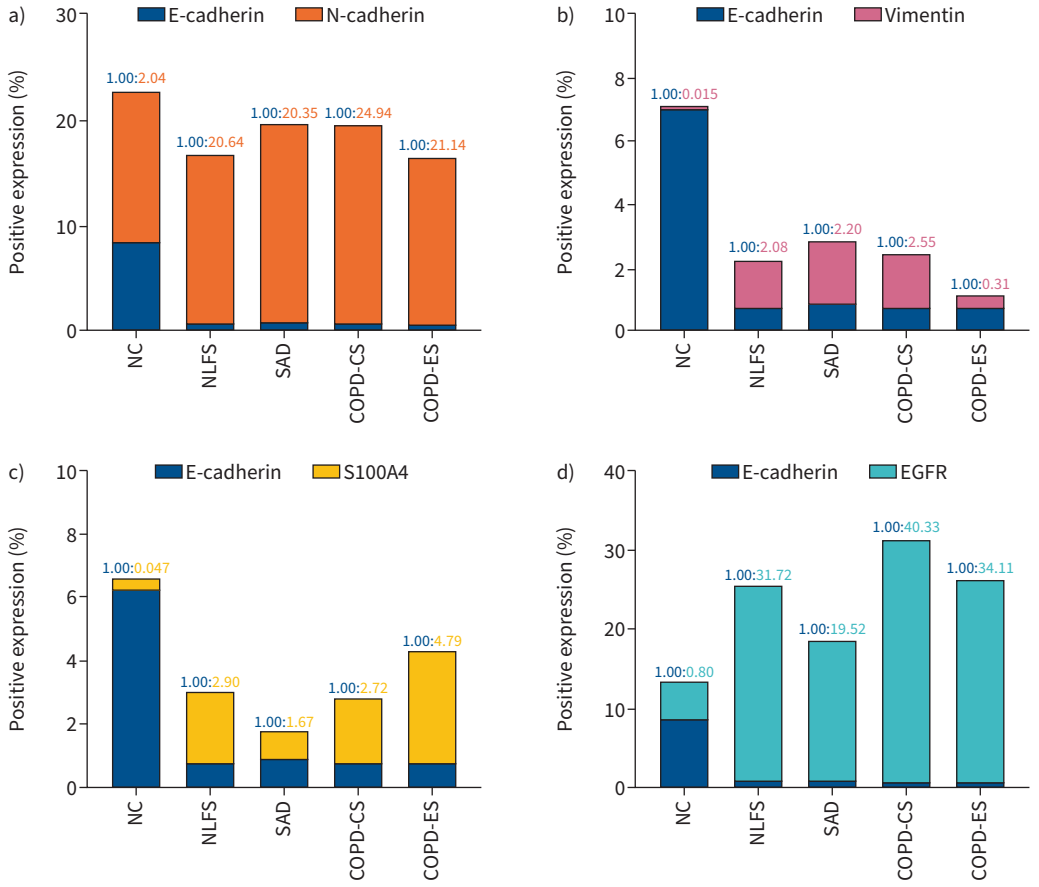

FIGURE 4 Epithelial–mesenchymal transition marker expression ratio in small airway epithelium between a) E-cadherin and N-cadherin; b) E-cadherin and vimentin; c) E-cadherin and S100A4; and d) E-cadherin and epidermal growth factor receptor (EGFR). NC: normal control; NLFS: normal lung function smoker; SAD: small airway disease; COPD-CS: current smoker with COPD; COPD-ES: ex-smoker with COPD.

In addition, compared to NC, vimentin cell counts (cells·mm^{-2}) in LP and adventitia showed a significant increase across all pathological groups ($p<0.01$ and $p<0.05$, respectively) (figure 5c, e); however, this increase was not observed for S100A4 (figure 5d, f). Interestingly, a reverse trend was observed with vimentin-positive cells in Rbm, LP and adventitia in COPD-ES, which, however, was not observed with S100A4.

Correlation between EMT marker expression and small airway thickness in COPD

We observed a positive correlation between mesenchymal markers and small airway wall thickness in the COPD group. Specifically, epithelial vimentin expression and Rbm S100A4 expression showed a significant positive correlation with Rbm thickness ($r'=0.523$, $p<0.01$ and $r'=0.537$, $p<0.01$, respectively). Epithelial vimentin expression also positively correlated with LP thickness ($r'=0.505$, $p<0.01$) and epithelial S100A4 expression positively correlated with adventitial thickness ($r'=0.429$, $p<0.05$).

Correlation between EMT markers and airway wall remodelling factors (α-SMA, fibronectin and collagen-1) in COPD

We used expression data for small airway wall remodelling factors in sub-epithelial layers, including α-SMA, collagen-1 and fibronectin, from our previous study [16] for correlation analysis. The expression of these markers was positively correlated with EMT markers. Significantly, epithelial EGFR expression was positively correlated with α-SMA expression in Rbm ($r'=0.685$, $p<0.02$). Epithelial N-cadherin and Rbm S100A4 expression was positively correlated with collagen-1 expression in LP ($r'=0.411$, $p<0.05$ and $r'=0.375$, $p<0.05$, respectively), and epithelial vimentin expression was positively correlated with adventitial collagen-1 expression ($r'=0.435$, $p<0.03$).

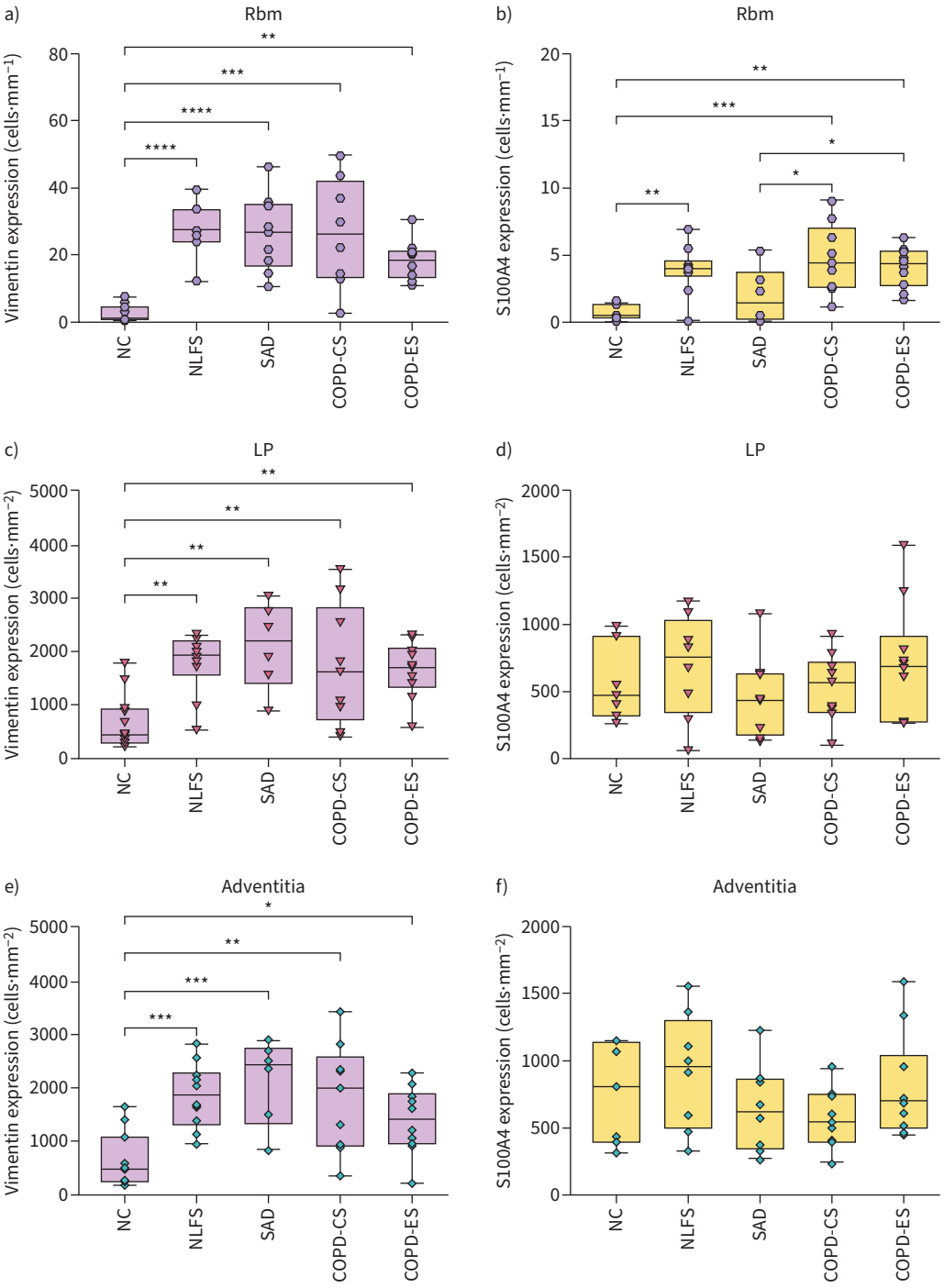

FIGURE 5 Epithelial–mesenchymal transition marker expression in small airway sub-epithelial layers. a) Vimentin-positive cells per mm of reticular basement membrane (Rbm) length; b) S100A4-positive cells per mm of Rbm length; c) vimentin-positive cells per mm^2 of lamina propria (LP) area; d) S100A4-positive cells per mm^2 of LP area; e) vimentin-positive cells per mm^2 of adventitia area; f) S100A4-positive cells per mm^2 of adventitia area. NC: normal control; NLFS: normal lung function smoker; SAD: small airway disease; COPD-CS: current smoker with COPD; COPD-ES: ex-smoker with COPD. *: p<0.05; **: p<0.01; ***: p<0.005; ****: p<0.001.

Correlation between EMT marker expression, smoking history and lung physiology parameters in patients with COPD

We observed a positive correlation between smoking pack-years and Rbm thickness (r′=0.456, p=0.0249) (figure 6a) and similar significant positive correlations of smoking pack-years with Rbm vimentin and

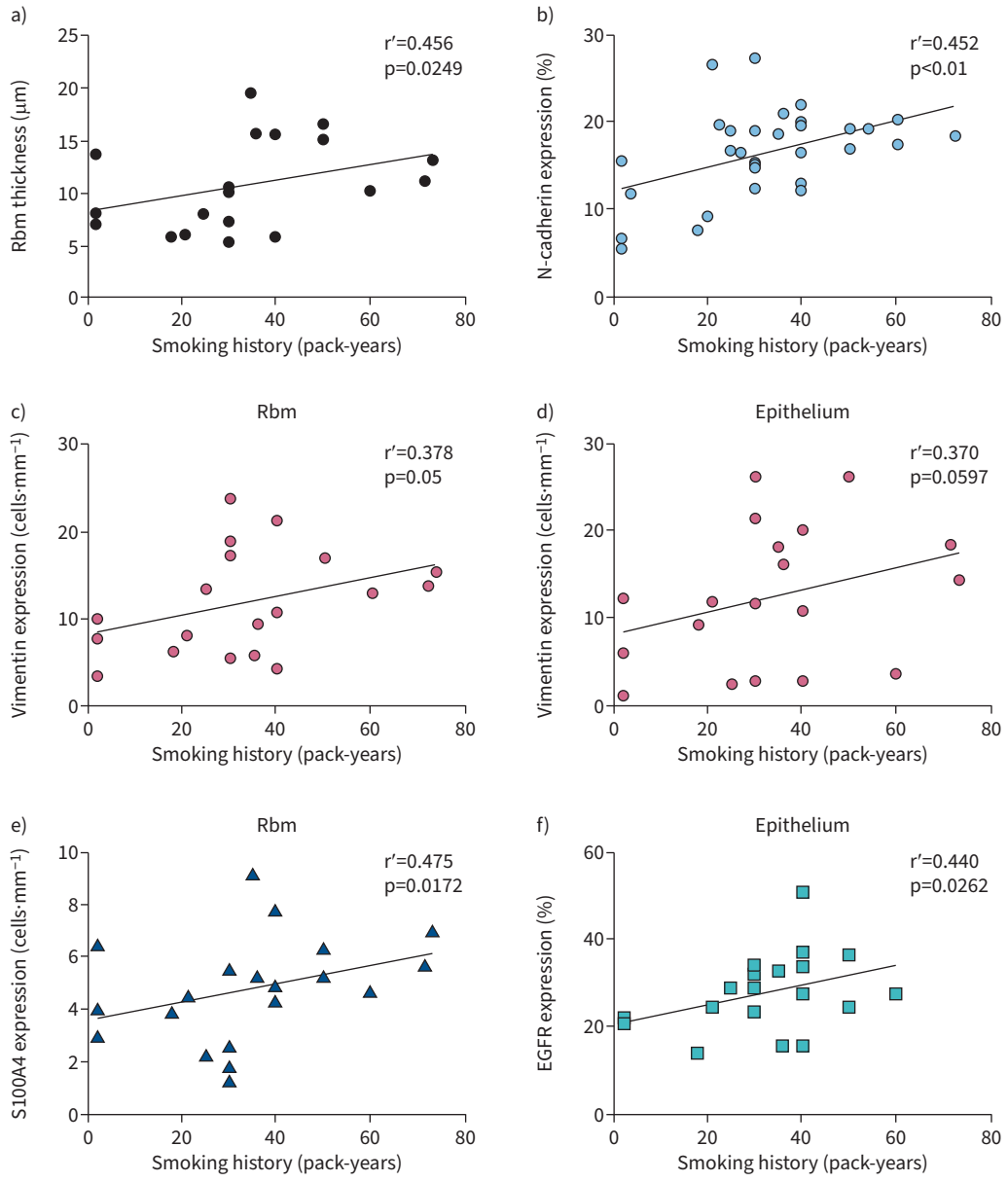

FIGURE 6 Correlation between epithelial–mesenchymal transition marker expression and smoking history. a) Reticular basement membrane (Rbm) thickness *versus* smoking history; b) epithelial N-cadherin expression percentage *versus* smoking history; c) Rbm vimentin expression cells per mm of Rbm length *versus* smoking history; d) epithelial vimentin expression cells per mm of Rbm length *versus* smoking history; e) Rbm S100A4 expression cells per mm of Rbm length *versus* smoking history; f) epithelial epidermal growth factor receptor (EGFR) expression percentage *versus* smoking history.

S100A4 expression ($r'=0.378$, $p=0.05$ and $r'=0.475$, $p=0.0172$, respectively) (figure 6c, e), and epithelial N-cadherin and EGFR expression ($r'=0.452$, $p<0.01$ and $r'=0.440$, $p<0.05$, respectively) in the COPD groups (figure 6b, f). We also observed a close to statistically significant positive correlation between epithelial vimentin expression and pack-years (figure 6d). EMT marker expression was negatively correlated with lung function (as assessed by forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC), particularly Rbm and LP S100A4 expression ($r'=-0.410$, $p<0.05$ and $r'=-0.552$, $p<0.01$, respectively), and EGFR expression was negatively correlated with forced expiratory flow at 25–75% of FVC ($FEF_{25–75\%}$) ($r'=-0.415$, $p<0.05$). We also found that airway thickness in NLFS and COPD-CS, particularly Rbm and adventitial thickness, negatively correlated with FEV_1/FVC ($r'=-0.646$, $p<0.005$ and $r'=-0.649$, $p<0.005$, respectively), and negatively correlated with $FEF_{25–75\%}$ ($r'=-0.530$, $p<0.05$ and $r'=-0.531$, $p<0.005$, respectively).

Correlation between EMT marker expression and cancer morphology type

Adenocarcinoma and squamous cell carcinoma phenotypes were more commonly seen in the COPD-CS group (figure 7a, b). Airway expression of all mesenchymal markers was significantly higher in both cancer phenotypes than in NC, particularly N-cadherin and EGFR (figure 7c). EGFR and S100A4 expression was relatively higher in squamous cell carcinoma than in adenocarcinoma, particularly in COPD-CS (figure 7d, e).

Discussion

In the current study, we observed EMT changes and small airway fibrotic remodelling in a more detailed subgroup of patients. Specifically, the thickness of sub-epithelial layers, *i.e.* the Rbm, LP and adventitia, was markedly increased in both the current smoker groups, with and without COPD (COPD-CS and NLFS), compared to NC. Although sub-epithelial layers were significantly thickened in the COPD-ES group compared to NC, they showed a reverse trend compared to the COPD-CS group, though still higher than in the NLFS group. This indicates the potential of smoking cesstion to reverse small airway wall thickening. This work provides a basis for investigating the effects of longitudinal smoking cessation on EMT, angiogenesis, transforming growth factor-β/Smad, Wnt/β-catenin signalling and inflammatory pathways. Such studies will be highly informative in understanding what gets switched off on smoking cessation and what does not, given that ex-smokers still get lung cancer and continue to have fibrotic changes.

We investigated EMT biomarker expression in each sub-epithelial layer and correlated it with the thickness in these patients. EMT markers in the layers are a sign of cell migration, *i.e.* type 3 EMT activity. We observed a substantial increase in vimentin expression in all sub-epithelial layers of the small airway wall in all pathological groups. The most significant increase was observed in the COPD-CS and NLFS groups compared to the NC group. Additionally, we found a robust positive correlation between vimentin expression and Rbm and LP thickness.

Our findings indicated a significant increase in S100A4 or fibroblast-specific protein 1 levels within the small airway epithelium and Rbm of both COPD groups and NLFS compared to NC. S100A4 expression positively correlated with Rbm and adventitial thickness. The correlation between vimentin, S100A4 and airway thickness is further supported by the positive correlation with the expression of the airway wall remodelling factors α-SMA, fibronectin and collagen-1 in sub-epithelial layers. Indeed, the airway epithelium of COPD patients exhibits heightened cellular expression of mesenchymal markers such as S100A4 and vimentin [17]. We have previously shown that EMT increases myofibroblasts, leading to the formation and progression of fibrosis in COPD patients [16, 22]. EMT-mediated fibrosis is also one of the causes of lung cancer [23]. Therefore, the prevalence of lung cancer among COPD patients is primarily attributed to the presence of airway fibrosis and remodelling [24, 25]. Additionally, elevated levels of S100A4 and vimentin are inversely correlated with lung function, indicating a potential physiological impact [26].

Furthermore, the other main characteristic of EMT is the decrease in E-cadherin levels. E-cadherin is crucial for preserving the connection between epithelial cells and arranging the cytoskeleton [27]. By contrast, N-cadherin is an intercellular junctional mesenchymal marker. The presence of N-cadherin suggests the occurrence of EMT, and its expression has been linked to the progression of different types of carcinomas [28–30]. We observed a marked decrease in E-cadherin across all pathological groups, along with an increase in N-cadherin, with a particularly high increase in the COPD-CS group compared to the NC group. The ratio of N-cadherin to E-cadherin in the COPD-CS group was approximately 24-fold higher, indicating that small airway epithelium lost the epithelial characteristics and transformed to the mesenchymal phenotype in all pathological groups. The negative correlations of epithelial vimentin and S100A4 expression with E-cadherin expression further indicate that mesenchymal transition is progressing (data not shown). In addition, we observed that N-cadherin was significantly elevated whereas E-cadherin was significantly downregulated in both carcinoma phenotypes compared to NC, and N-cadherin was higher than the other mesenchymal markers, vimentin and S100A4. Cancer-related EMT involves increased expression of N-cadherin and decreased expression of E-cadherin. This shift in cadherin expression is linked to augmented migratory and invasive characteristics, leading to lower patient survival rates [28, 31].

Cigarette smoking is a significant risk factor for developing lung cancer and COPD. Our results show that vimentin and S100A4 were markedly increased in the NLFS group compared to NC and even higher in the COPD-CS group. The expression of N-cadherin, vimentin and S100A4 was also positively correlated with smoking history, which further indicates that EMT progression is escalated by smoking and COPD.

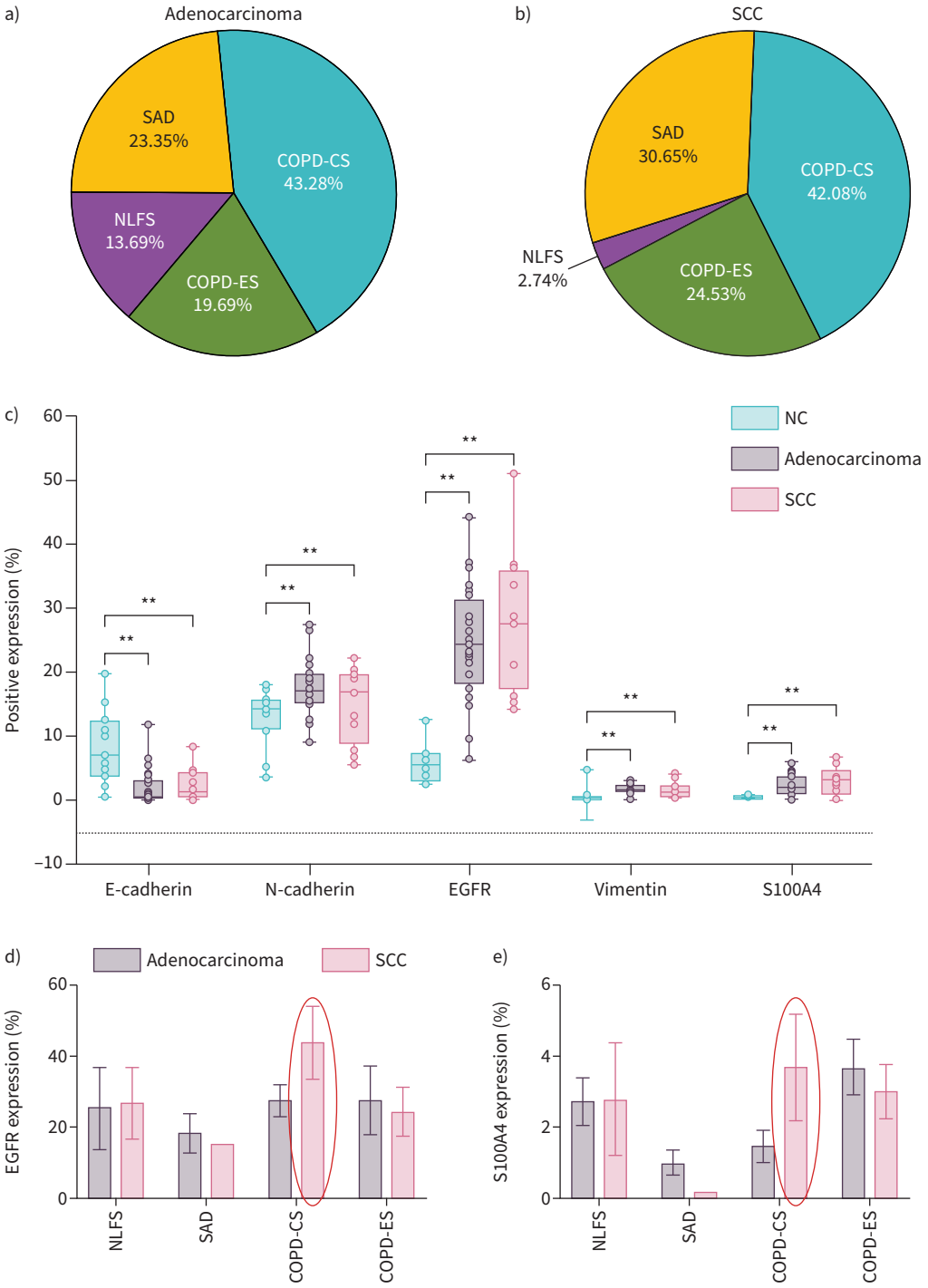

FIGURE 7 Epithelial–mesenchymal transition (EMT) marker expression and cancer morphological types. a) The proportion of each subgroup with adenocarcinoma phenotype. b) The proportion of each subgroup with squamous cell carcinoma (SCC) phenotype. a and b indicate that the major proportion in both cancer types is current smokers with COPD (COPD-CS). c). EMT marker expression in the small airway epithelium in adenocarcinoma and SCC compared to normal controls (NC). d) Epidermal growth factor receptor (EGFR) expression in each subgroup in adenocarcinoma *versus* SCC. e) S100A4 expression of each subgroup in adenocarcinoma *versus* SCC. Red circles in d and e indicate EGFR and S100A4 were relatively higher in SCC in COPD-CS than in adenocarcinoma. NLFS: normal lung function smoker; SAD: small airway disease; COPD-ES: ex-smoker with COPD. **: p<0.01.

More EMT activity was seen in squamous cell carcinoma compared to adenocarcinoma, especially in the COPD-CS group. However, EMT activity was relatively lower in COPD-ES compared to COPD-CS. This indicates that smoking cessation could potentially reduce EMT progression and, therefore, prevent cancer development.

EGFR is implicated in the pathogenesis of NSCLC [32, 33]. Our results demonstrated significantly elevated EGFR levels in smokers with and without COPD, particularly in COPD-CS, which had a strong positive correlation with smoking history. EGFR expression was higher in both NSCLC morphological types and significantly higher in COPD-CS patients with squamous cell carcinoma. The heightened expression of EGFR in NSCLC is related to lower survival rates [32], as well as frequent lymph node metastasis and inadequate response to chemotherapy.

Enhanced understanding of EMT mechanisms linked to disease provides an opportunity for precise therapy aimed at inhibiting COPD progression and preventing cancer metastasis. Inhaled corticosteroids have become a standard treatment in more severe COPD and have an anti-EMT effect in COPD airways [34, 35], further reducing the risk of lung cancer in COPD patients [36]. This supports the administration of inhaled

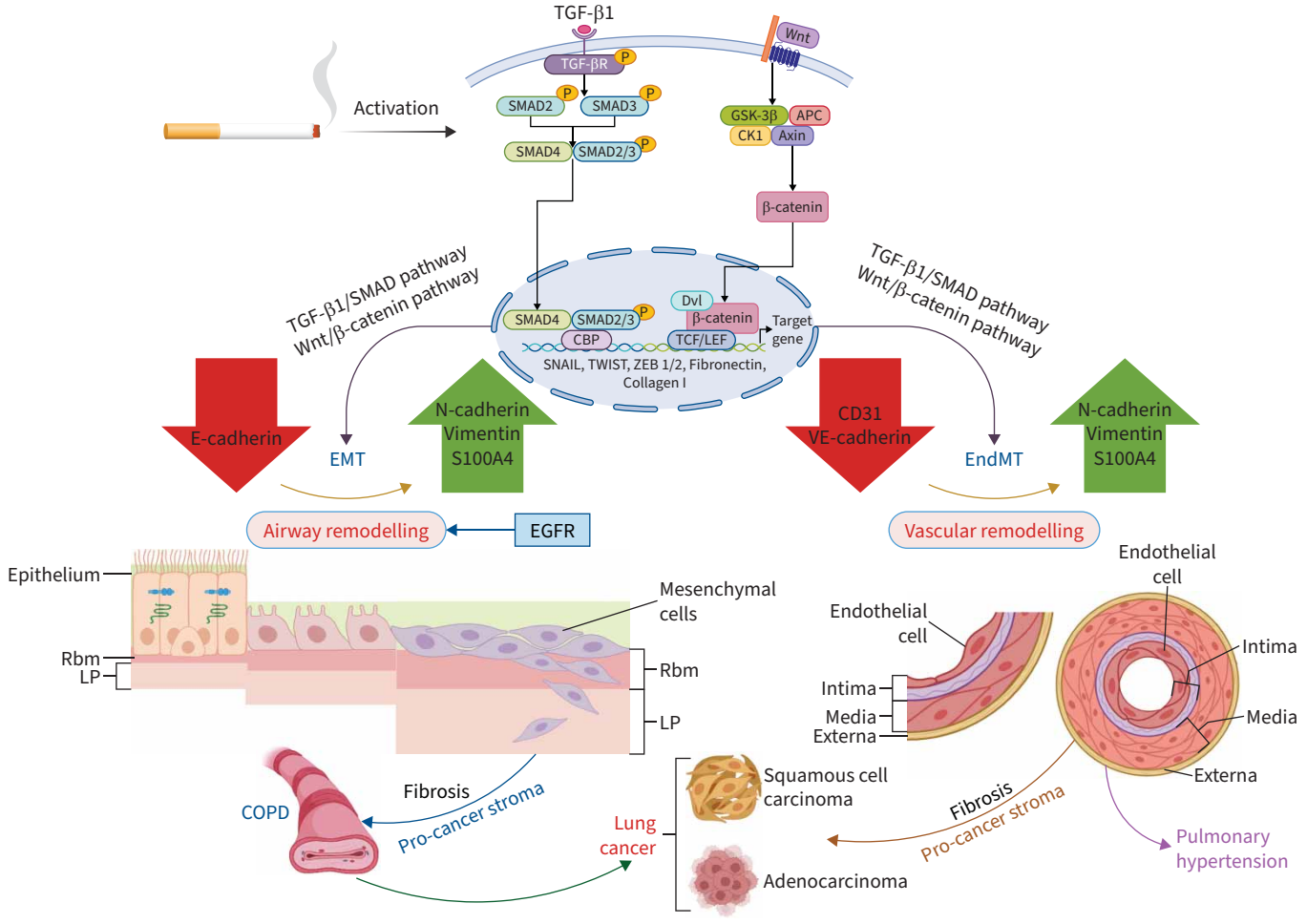

FIGURE 8 Cigarette smoking activates the transforming growth factor-β (TGF-β)/SMAD, Wnt/β-catenin and epidermal growth factor receptor (EGFR) signalling pathways. Both pathways initiate the epithelial–mesenchymal transition (EMT) and endothelial–mesenchymal transition (EndMT) processes in the airways and vasculature. During EMT, there is a reduction in the expression of the epithelial junctional protein E-cadherin, resulting in a loss of cell–cell adhesion among epithelial cells. Simultaneously, these cells acquire mesenchymal phenotype and proteins, including N-cadherin, vimentin and S100A4, leading to the formation of fibrotic tissue and pro-cancer stroma. Similarly, in EndMT, endothelial cells lose endothelial junctional proteins such as VE-cadherin and CD31, transforming into mesenchymal cells such as fibroblasts, resulting in vascular remodelling, fibrosis and cancer. EMT and EndMT in COPD can both create the pro-cancer stroma that promotes lung cancer development, including both squamous cell carcinoma and adenocarcinoma. Rbm: reticular basement membrane; LP: lamina propria.

corticosteroids or other medications with comparable effects at an early stage of COPD [37]. This could help to not only reduce airway inflammation but also prevent EMT activation, which results in fibrosis and potential malignant consequences. In addition to EMT, we believe endothelial–mesenchymal transition can also contribute to the formation of pro-cancer stroma and fibrosis (figure 8). During endothelial–mesenchymal transition, endothelial cells, like epithelial cells, can gain a mesenchymal phenotype leading to fibrotic or malignant changes [25]. Further work in this direction would be of great value.

There are limitations to this study. First, the sample size was relatively small, which limits the correlation evaluation. For example, we can see the negative correlation trend between EMT marker expression and lung function; however, a larger sample size could improve the outcomes and better evaluate EMT activity-mediated lung physiology changes. Second, the clinical and pathological stage of lung cancer was unavailable. However, our findings have indicated that escalated EMT-mediated fibrosis and small airway remodelling in early COPD contributes to NSCLC development.

In conclusion, this study investigated EMT activities in lung cancer patients with COPD. We found that EMT is an active process in the small airway of smokers with COPD diagnosed with lung cancer, contributing to the small airway remodelling and cancer development seen in these patients. This is the first study to show such changes in broadly phenotyped individuals. EMT markers and EGFR can be used as the therapeutic target in lung cancer patients with COPD, and smoking cessation can assist in reducing EMT progression [38–40].

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Ethics statement: This study was approved by the Tasmanian Health and Medical Human Research Ethics Committee (identifier H0012374) and the University of British Columbia, with approval from the Providence Health Care Research Ethics Board.

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