Chronic obstructive pulmonary disease is associated with enhanced bronchial expression of FGF-1, FGF-2, and FGFR-1

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Abstract

An important feature of chronic obstructive pulmonary disease (COPD) is airway remodelling, the molecular mechanisms of which are poorly understood. In this study, the role of fibroblast growth factors (FGF-1 and FGF-2) and their receptor, FGFR-1, was assessed in bronchial airway wall remodelling in patients with COPD (FEV1 < 75%; n = 15) and without COPD (FEV1 > 85%; n = 16). FGF-1 and FGFR-1 were immunolocalized in bronchial epithelium, airway smooth muscle (ASM), submucosal glandular epithelium, and vascular smooth muscle. Quantitative digital image analysis revealed increased cytoplasmic expression of FGF-2 in bronchial epithelium (0.35 ± 0.03 vs 0.20 ± 0.04, p < 0.008) and nuclear localization in ASM (p < 0.0001) in COPD patients compared with controls. Elevated levels of FGFR-1 in ASM (p < 0.005) and of FGF-1 (p < 0.04) and FGFR-1 (p < 0.001) in bronchial epithelium were observed. In cultured human ASM cells, FGF-1 and/or FGF-2 (10 ng/ml) induced cellular proliferation, as shown by [3H]thymidine incorporation and by cell number counts. Steady-state mRNA levels of FGFR-1 were elevated in human ASM cells treated with either FGF-1 or FGF-2. The increased bronchial expression of fibroblast growth factors and their receptor in patients with COPD, and the mitogenic response of human ASM cells to FGFs in vitro suggest a potential role for the FGF/FGFR-1 system in the remodelling of bronchial airways in COPD.

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Keywords: chronic obstructive pulmonary disease; fibroblast growth factor; airway remodelling; airway smooth muscle; gene expression; human

Introduction

Chronic obstructive pulmonary disease (COPD) is a global health problem with increasing morbidity and mortality [1]. One of the major determining factors is tobacco smoking [2]. However, only 10% of all smokers develop COPD. One of the key pathological features of COPD is thickening of the airway walls, which is thought to be a result of a chronic smouldering inflammatory process, in which neutrophils, macrophages, and T-lymphocytes play a role, and which is associated with hyperplasia of airway smooth muscle cells and (myo-) fibroblasts, and increased deposition of extracellular matrix [3]. The bronchial epithelium and airway smooth muscle are two major cellular structures involved in airway remodelling [3]. A variety of growth factors and cytokines, including platelet-derived growth factor-B (PDGF-B), epidermal growth factor (EGF), and transforming growth factor-β (TGF-β) that are released from these sites of the airway wall, have the potential to contribute to the pathogenesis of airway remodelling [4–6]. Supporting in vitro evidence for a relationship between epithelial injury and enhanced airway remodelling is provided by studies of co-cultures of bronchial epithelial cells and myo-fibroblasts [7,8]: these studies revealed enhanced cellular proliferation and increased collagen expression resulting from the interaction of these cells with several growth factors, including basic FGF (FGF-2), insulin-like growth factor-1, PDGF-B, TGF-β, endothelin-1, and EGF.

Fibroblast growth factors (FGFs) may well play a pivotal role in regulating airway wall remodelling. A number of studies have demonstrated that members of the EGF and FGF family contribute to chronic inflammatory and tissue repair processes as well as to fibrosis in chronic airway diseases such as asthma [9,10]. Fibroblast growth factors bind to four high-affinity, transmembrane tyrosine-kinase receptors (FGFR1–4). Distinct FGF subtypes bind with different affinities...
to the various FGF receptors. Alternative splicing and regulated protein trafficking further modulate the intra-cellular events initiated by FGF ligand–receptor interaction [11]. Increased expression of FGF-1 and FGFR-1 has been shown during the development of lung fibrosis [12], and FGF-2 has been implicated in the pathogenesis of obliterative bronchiolitis in lung transplants [13].

We postulate that the FGF–FGFR system is involved in the pathogenesis of COPD. We investigated the expression patterns of FGF-1, FGF-2 and FGFR-1 in bronchial airways of (ex-) smokers with or without COPD. In addition, we examined cell proliferation and the expression of FGFR-1 in cultured human ASM cells stimulated with FGF-1 and FGF-2.

Materials and methods

Selection of specimens

The Medical Ethics Committees of the Leiden University Medical Center and Southern Hospital Rotterdam, The Netherlands approved the study. Lung tissue from the hospitals’ pathology archives was obtained from patients who underwent lobectomy or pneumonectomy. Based on lung function data, patients were assigned [6,14] to the COPD group (n = 15) consisting of 15 subjects with a forced 1-s expiratory volume (FEV1) < 75% of the predicted value [15] before bronchodilatation, an FEV1/FVC ratio < 75%, a reversibility in FEV1 ≤ 12% of the predicted value after 400 µg of inhaled salbutamol, and a carbon monoxide diffusion capacity (Kco) ≤ 80% of the predicted value, or to the non-COPD group (n = 16) consisting of 16 subjects with an FEV1 > 85% before bronchodilatation, an FEV1/FVC ratio > 85%, and a total lung capacity (TLC) of over 80% [15]. The patients in these two groups participated in a larger research project, part of which has been published previously [16,17]. Clinical data from all patients were examined for possible co-morbidity and medication usage. All pulmonary function tests were performed within 3 months prior to surgery as described earlier [16]. Lung function data and other patient characteristics are shown in Table 1.

Immunohistochemistry

Serial sections of 4 µm were dewaxed, rehydrated, and immunostained using a Multilink® labelling system (Biogenex, San Ramon, USA) and specific anti-human mouse monoclonal antibodies against α-smooth muscle actin (α-SMA; NeoMarkers, Fremont, USA), Ki-67 (Biogenex, San Ramon, USA), FGF-2 (Transduction Laboratories, Lexington, USA), and FGFR-1 and FGFR-1 (kind gift from Dr J Walters) as described previously [18,19]. Colour was developed using New Fuchsin or 3,3-diaminobenzidine as the chromogen.

Table 1. Subject characteristics and clinical parameters

<table>
<thead>
<tr>
<th></th>
<th>Non-COPD</th>
<th>COPD</th>
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<tbody>
<tr>
<td>FEV1 (L)</td>
<td>97 ± 1.6</td>
<td>54 ± 3.3*</td>
</tr>
<tr>
<td>dFEV1 (%)</td>
<td>3 ± 0.6</td>
<td>4 ± 0.9</td>
</tr>
<tr>
<td>FEV1/FVC</td>
<td>100 ± 2.1</td>
<td>58 ± 3.2*</td>
</tr>
<tr>
<td>TLC (L)</td>
<td>104 ± 1.9</td>
<td>103 ± 3.6</td>
</tr>
<tr>
<td>RV (L)</td>
<td>117 ± 5.4</td>
<td>141 ± 10*</td>
</tr>
<tr>
<td>Kco (ml)</td>
<td>94 ± 2.0</td>
<td>55 ± 5.4*</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>13/3</td>
<td>14/1</td>
</tr>
<tr>
<td>Age (years)</td>
<td>59 ± 3.5</td>
<td>64 ± 2.6</td>
</tr>
<tr>
<td>Smokers/ex-smokers/non-smokers</td>
<td>11/3/2</td>
<td>12/3/0</td>
</tr>
<tr>
<td>Pack-years</td>
<td>44 ± 8.6</td>
<td>31 ± 0.3</td>
</tr>
<tr>
<td>Steroid use (yes/no/unknown)</td>
<td>0/15/1</td>
<td>3/10/2</td>
</tr>
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FEV1 = forced expiratory volume in 1 s; FVC = forced vital capacity; TLC = total lung capacity; RV = residual volume. Reversibility of FEV1 after 400 µg of salbutamol (dFEV1) and the carbon monoxide diffusion constant (Kco) are given as a percentage of the predicted value. * p < 0.005 vs non-COPD. The patients in these two groups participated in a larger project, part of which has been published previously [16,17].

Slides were counter-stained with Mayer’s haematoxylin. Positive controls consisted of human breast carcinoma and placental tissue. The optimal dilutions for all antibodies were identified by examining the intensity of staining obtained with a series of dilutions: the optimum concentration resulted in a specific and easily visible signal on control specimens. Negative controls consisted of omission of the primary antibody.

Quantitative analyses of immunostaining

Digital images (pixel size 736 × 574) from each subject were analysed using the Leica Qwin image analysis system (Leica BV, Rijswijk, The Netherlands). Staining patterns of FGF-1, FGF-2, FGFR-1, and α-SMA were analysed by drawing areas interactively and assessing the area of positive staining divided by the total measured cellular area of the respective epithelial or ASM layer. The nuclear localization of FGF-2 in ASM was assessed by computerized counting of individual nuclei and the data are expressed as the number of positive nuclei divided by the total nuclei (labelling index, LI). For vascular expression of FGFs and FGFR-1, quantitative analysis was performed using an arbitrary visual scale with grading scores of 0, 1, 2, and 3 (Figure 1) representing no (panel A), weak (panel B), moderate (panel C), and intense (panel D) staining, respectively [6,14].

Isolation and culture of human ASM cells

Human airway smooth muscle cells were from three different non-asthmatic, non-COPD, and (ex-) smoker donors who underwent lobectomy or pneumonectomy as described previously [20,21]. ASM cells were characterized immunocytochemically (α-SMA and smooth muscle myosin heavy chain staining) and used for experiments at passage 4–5.
Figure 1. Immunohistochemical localization of FGF-2 in bronchial vessels. Representative examples of staining intensity pattern used for visual scoring. Photomicrographs depict lung tissue sections from patients without COPD (A and B) and with COPD (C and D) showing nuclear staining of FGF-2 in vascular smooth muscle cells. Panels A–D show representative examples of staining intensities used for visual scoring, 0–3 respectively. Original magnification × 100.

Figure 2. Immunohistochemical localization of FGF-1 and FGF-2 in central airways. Photomicrographs showing central bronchial tissue sections from patients without COPD (A, C, E, G) and with COPD (B, D, F, H). Panels A and B show representative examples of FGF-1 protein staining (red, New Fuchsin) in bronchial epithelium. Panels C and D show representative staining in airway smooth muscle (ASM) cells. Panels E and F show representative examples of FGF-2 protein staining (brown, 3,3-diaminobenzidine) in bronchial epithelium. Panels G and H show representative nuclear staining (shown by solid arrows) and no nuclear staining (blue nuclei) shown by open arrows in airway smooth muscle (ASM) cells. Original magnification × 400. Scale bar = 50 μm.
ASM cell proliferation assays

Cells were seeded at a density $1 \times 10^4$ cells per well in 96-well plates, cultured until confluence, subsequently serum-deprived to synchronize the growth, and incubated with either 0.1, 1.0, 10, or 50 ng/ml human recombinant FGF-1 (Promega, Madison, USA) and/or FGF-2 (Sigma-Aldrich, St Louis, USA) for 8, 24, and 48 h. Control cells received FBS-free DMEM alone. Five hours prior to the end of the treatment, 1 µCi/well of $[^3]$Hthymidine (Amersham, Roosendaal, The Netherlands) was added. The cells were harvested on glass fibre filters and radioactivity was assessed using a Microplate Scintillation $\beta$-counter (Topcount, Packard, Meridan, USA). The mean counts per minute of quadruple wells and subsequently from three different cell batches was expressed as fold change compared with controls. In a parallel series of experiments, cells in quadruple were stimulated for 24 and 48 h and processed for cell counting in the Casy®1 system (Schärfe System GmbH, Reutlingen, Germany) [20].

RNA isolation and RT-PCR

Growth-arrested ASM cells were incubated with either FGF-1 or FGF-2 (10 ng/ml) for 1, 2, 4, 8, 24, and 48 h. Total RNA was extracted, treated with Rnase-free DNase to eliminate contaminating genomic DNA, and processed for the synthesis of cDNA and PCR [20,21]. Human-specific forward and reverse primers spanning over a 497 bp fragment encoding FGFR-1 and a 625 bp fragment of $\beta$-actin cDNAs were employed [22,23]. The PCR products were separated on 1.5% agarose gels, digitally photographed, and the intensity of the bands was quantified in relation to the $\beta$-actin band using the Molecular Analyst (V1.5) image analysis program (Biorad Laboratories, Hercules, USA); values were expressed as a ratio to the controls.

Statistical analysis

Data were analysed for statistical significance using the unpaired, two-tailed Student’s $t$-test as well as the non-parametric Mann–Whitney test, where appropriate. The data were expressed as mean ± SEM. Staining for different compartments was correlated with FEV$_1$ and $K_co$ using Pearson’s correlation analysis. Differences with $p \leq 0.05$ were considered to be statistically significant.

Figure 3. Quantitative analysis of FGF-1 and FGF-2 expression. Graphic representations of (A) FGF-1 expression using video image analysis in bronchial epithelium (EPI) and airway smooth muscle cells (ASM), and (B) FGF-2 expression in bronchial epithelium depicted as a ratio of the stained area divided by the tissue area in non-COPD (white bars) and COPD groups (grey bars). (C) FGF-2 expression in ASM cells presented as labelling index (LI) of total ASM nuclei. Values are mean ± SEM from 13–15 patients in each group. *$p < 0.05$ vs the non-COPD group.
Results

Clinical parameters

The clinical and lung function characteristics of all subjects included in the study are listed in Table 1 [16]. The COPD group demonstrated an elevated residual volume (RV), whereas the CO diffusion ($K_{CO}$) was reduced compared with controls ($p < 0.005$). The subjects in the two groups did not differ significantly in age, total lung capacity (TLC), reversibility in FEV$_1$, smoking status (pack-years) or steroid use (Table 1).

Localization and quantification of FGF-1 and FGF-2

FGF-1 and FGF-2 were localized in bronchial epithelial and airway smooth muscle cells (ASM), epithelial cells of the mucous glands, and VSM cells. In addition, FGF-1 was detected in the epithelial basement membrane (BM). Interestingly, FGF-2 was observed in the cytoplasm of bronchial surface and gland epithelium, whereas in smooth muscle cells of the airway and blood vessels, the immunopositivity was nuclear. This latter, nuclear, staining pattern was exclusively observed in smooth muscle cells and it was patchy so that positive nuclei were seen next to negative ones.

Figure 4. Immunohistochemical localization of FGFR-1, α-SMA, and Ki-67 in central airways. Photomicrographs of central bronchial tissue sections from patients (A) without COPD and (B) with COPD showing FGFR-1 staining (red, New Fuchsin) in bronchial epithelium. Panels C (non-COPD) and D (COPD) show representative staining in airway smooth muscle (ASM) cells. Representative microphotographs show staining in bronchial airways for α-SMA (E) and for the cell proliferation marker Ki-67 (F) in COPD cases. Original magnification $\times$ 200. Scale bar = 50 µm

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Microphotographs showing the expression patterns of FGF-1 and FGF-2 are presented in Figures 2A, 2C and 2E, 2G (non-COPD), and 2B, 2D and 2F, 2H (COPD), respectively.

Video image analysis revealed that the expression levels for FGF-1 in the bronchial epithelium (Figure 3A) were increased significantly (stained/total epithelial area: 0.32 ± 0.04 vs 0.20 ± 0.03, p < 0.04) in COPD cases compared with non-COPD. In ASM cells, no difference was found for FGF-1 (0.16 ± 0.04 vs 0.14 ± 0.03, p = 0.77). FGF-2 expression, however, was clearly up-regulated in the bronchial epithelium of COPD cases (0.35 ± 0.03 vs 0.20 ± 0.04, p < 0.008, Figure 3B) and ASM nuclei (LI ASM nuclei, 0.84 ± 0.07 vs 0.32 ± 0.06, p < 0.0001, Figure 3C). The distribution of total nuclei/total ASM tissue area remained unchanged in both groups, indicating that the number of nuclei as well as the ASM area increased simultaneously, keeping the ratio equal in both groups (data not shown). Furthermore, it appeared that COPD was associated with an increase in FGF-2 expression in ASM cells, with perhaps an increase in their size but without their apparent proliferation.

Localization and quantification of FGFR-1

FGFR-1 immunoreactivity was detected in bronchial epithelial and airway smooth muscle cells, and the endothelium and vascular smooth muscle of bronchial small vessels. Microphotographs showing the expression pattern of FGFR-1 are presented in Figures 4A and 4C (non-COPD), and 4B and 4D (COPD). Graphic representations of the data as assessed by video image analysis for FGFR-1 immunostaining are shown in Figure 5A. The expression of FGFR-1 was up-regulated in COPD in bronchial epithelium (0.21 ± 0.03 vs 0.08 ± 0.02, p < 0.001) and ASM cells (ASM/total ASM area, 0.31 ± 0.05 vs 0.11 ± 0.03, p < 0.005). Assessing the expression of both FGF-1 and FGF-2 in VSM cells using visual scoring, only FGF-2 expression levels were found to be higher in COPD than in non-COPD (fold increase 1.65, p < 0.01, Figure 5B). Elevated staining of FGFR-1 in COPD compared with non-COPD patients was observed in the smooth muscle of subepithelial microvessels (1.6-fold increase, p < 0.05, Figure 5B).

Bronchial airways were also stained with smooth muscle specific antibody α-SMA (Figure 4E) as well as with the cell proliferation marker Ki-67 (Figure 4F). The majority of ASM and VSM cells stained positive for α-SMA in both non-COPD and COPD groups. Ki-67 immunoreactivity was mainly observed in the nucleus of basal and parabasal epithelial cells, and also in some inflammatory cells. Surprisingly, we only very rarely found an ASM cell stained with Ki-67 and this was the case in both the COPD and the non-COPD groups.

![Figure 5. Quantitative analysis of FGFs and FGFR-1 expression.](image)

Figure 5. Quantitative analysis of FGFs and FGFR-1 expression. Graphic representation of visual staining scores for FGF-1, FGF-2, and FGFR-1 (mean ± SEM) in subepithelial microvasculature (VSM) in non-COPD (white bars) and COPD groups (grey bars). °p < 0.05 vs the non-COPD group

Correlation of FGFs and FGFR-1 expression with clinical data

Pearson’s correlation of FGF-1, FGF-2, and FGFR-1 expression with clinical parameters in COPD and non-COPD patients is summarized in Figure 6. For FGF-1, FGF-2, and FGFR-1, we observed a significant inverse correlation between the epithelial expression with both FEV1 and FEV1/FVC, and a positive correlation of epithelial FGF-1 expression and pack-years (r = 0.49, p < 0.01). Moreover, we found a significant inverse correlation of FGF-2 and FGFR-1 staining in ASM cells with both FEV1 and FEV1/FVC (r = −0.71, p < 0.0001). Regarding the expression of FGFR-1 and its ligands, we observed a significant positive correlation with FGF-1 (r = 0.53, p < 0.001) and with FGF-2 (r = 0.64, p < 0.001) in ASM. In the epithelium, these values were r = 0.52 (p < 0.001) and r = 0.64 (p < 0.001), respectively. However, no
Figure 6. Correlation analysis of FGFs and FGFR-1 expression. Correlation analysis was performed for FGF-1 expression in bronchial epithelium with pack-years (A); and FGF-2 (B) and FGFR-1 (C) in ASM with forced vital capacity. Panel D shows the staining correlation for FGF-2 with FGFR-1 in ASM cells. Correlation coefficients (r) were obtained using linear regression (Pearson’s) analysis and significance level (p value, p < 0.05) was considered at absolute values of r > 0.37. FEV₁ = forced expiratory volume in 1 s; FVC = forced vital capacity; Kco = carbon monoxide diffusion constant

significant correlation was found between FGF-1 and FGF-2 localization.

Mitogenic effects of FGFs in cultured human ASM cells

In order to investigate the role of FGFs on airway smooth muscle remodelling further, isolated human airway smooth muscle cells were stimulated in vitro with increasing concentrations of FGF-1 or FGF-2. Graphic representation of the dose-dependent increase in cell number for FGF-1 and FGF-2 is shown in Figure 7A. Both FGF-1 and FGF-2 resulted in significantly increased cell numbers at a concentration of 10 ng/ml after 48 h of incubation. We therefore opted for this concentration for both growth factors in our further experiments. Figure 7B shows the fold increase in cell number after 48 h of stimulation with 10 ng/ml FGF-1, FGF-2, and the combination of the two over the control. A significant increase in ASM cell numbers (fold increase) after 48 h of incubation with FGF-1 (1.37 ± 0.08, p < 0.01) or FGF-2 (1.45 ± 0.17, p = 0.05) or both ligands (1.42 ± 0.14, p < 0.03) was observed.

A graphic representation of the time-dependent [3H]TdR uptake at a concentration of 10 ng/ml of FGF-1 or FGF-2 is presented in Figure 7C. After 24 h of stimulation, we found significantly increased [3H]TdR uptake with FGF-1 and FGF-2, but only after 48 h with FGF-2. The combined incubation with 10 ng/ml each of FGF-1 and FGF-2 resulted in significantly increased thymidine uptake that was comparable to 10 ng/ml of FGF-2 alone. Eight hours of stimulation with either FGF-1 or FGF-2 did not result in a marked increase in [3H]TdR uptake (Figure 7C).

Expression of FGFR-1 mRNA in human ASM cells

To examine whether human ASM cells express FGFR-1 and whether this expression is regulated by FGF-1 and/or FGF-2, we performed RT-PCR on cDNA templates derived from cells treated with 10 ng/ml FGF-1 or FGF-2 for various time periods and compared the expression pattern with controls. A photograph showing a representative example, after agarose gel electrophoresis with PCR products for FGFR-1 (497 bp) and β-actin (625 bp), is shown in Figure 8A. Both bands were analysed using appropriate image analysis software and FGFR-1/β-actin values of FGF-1- or FGF-2-treated ASM cells at different time points were assessed in relation to controls (Figure 8B). Stimulation with 10 ng/ml FGF-1 increased FGFR-1 mRNA expression by 1.31 ± 0.11 fold at 8 h and by 1.23 ± 0.12 fold at 48 h of incubation compared with controls (p < 0.05), whereas FGF-2 stimulation resulted in elevated levels for FGFR-1 mRNA at 24 h (1.32 ± 0.14 fold, p < 0.05) and at 48 h (1.21 ± 0.13, ns) of incubation.

Discussion

In this study, we have shown that COPD is associated with increased expression of FGF-1, FGF-2, and
FGFR-1 in the bronchial epithelium, and increased expression of FGF-2 and FGFR-1 in airway smooth muscle. Correlation analysis revealed a significant inverse correlation of FEV₁/FVC with FGF-1, FGF-2, and FGFR-1 staining in the bronchial epithelium and with FGF-2 and FGFR-1 expression in airway smooth muscle. Additionally, a positive correlation of pack-years with FGF-1 was found in bronchial epithelium, indicating that the degree of pulmonary FGF-1 expression is related to the amount of airway exposure to smoke. Our *in vitro* results indicate that FGF-1 and FGF-2 are potent mitogens for isolated human airway smooth muscle cells. Taken together, these findings strongly suggest that the FGF–FGFR system contributes to airway remodelling.

Using video image analysis, we assessed systematically the expression of FGF-1, FGF-2, and FGFR-1 in the airways of non-COPD and COPD patients. Members of the fibroblast growth factor family FGF-1, FGF-2, and FGFR-1 are constitutively expressed in normal human lungs, particularly in bronchial epithelium, alveolar macrophages, and monocytes, as well as in the intima and media of pulmonary blood vessels. The pulmonary expression patterns of FGF-1, FGF-2, and FGFR-1 found in our study are in agreement with the results of Hughes and Hall [24] on the expression of these growth factors in normal lungs. In addition, we observed FGF-1 staining and FGF-2 immunoreactivity in airway smooth muscle cells.

Several studies have commented on the importance of structural and functional abnormalities and the expression of growth factors in the bronchial airways of patients with chronic obstructive lung diseases such as COPD [25–29]. In asthma, many growth factor/receptor systems are thought to be involved in tissue remodelling, including the EGF/EGFR, TGF-β, IGF-1, and FGF/FGFR systems. The combined effects of EGF, FGF-1 and FGF-2, IGF-1, and TGF-β on epithelial cells and (myo-) fibroblasts were shown to be necessary for regulating repair of epithelial injury.
by induction of cellular proliferation and collagen synthesis [8,30,31]. These same factors could, however, also be involved in fibrosis and tissue remodelling in asthma and possibly also in COPD [32].

Fibroblast growth factor family members are implicated in tissue remodelling in a wide variety of pathophysiological conditions including pulmonary hypertension, ischaemic heart disease, and interstitial lung fibrosis [12,33–35]. Barrios et al [12] showed FGF-1 and FGFR-1 expression in experimentally induced pulmonary fibrosis. Becerril et al. [36] showed that FGF-1 expression in lung fibroblasts results in down-regulation of collagen synthesis and up-regulation of collagenases, which may protect against fibrosis [36]. Furthermore, increased FGF-2 and FGFR-1 expression in vascular smooth muscle cells in vitro in response to vascular injury has been shown to be associated with extracellular matrix remodelling, cellular proliferation, down-regulation of collagen type I, and up-regulation of collagenase, MMP-1 [37]. Our findings of up-regulated FGF-1, FGF-2, and FGFR-1 expression could indicate that such compensatory mechanisms are also active in COPD, since smoking has been suggested to have a strong effect on the imbalance of proteases/anti-proteases including elastases, collagenases, and extracellular matrix deposition in the lungs. Furthermore, FGF-1 and FGF-2 in the bronchial epithelium could be involved in proliferation and repair of epithelial cells after injury, which could be higher in COPD patients. This notion is supported by our findings of increased Ki-67 expression in the bronchial epithelium of COPD patients. Several authors also showed this expression in proliferating airway epithelial cells in biopsies of normal, asthma, and chronic bronchitis patients [38,39].

In the present study, we have shown increased expression of FGF-2 and FGFR-1, but not of FGF-1, in airway smooth muscle cells using immunohistochemistry. By interactive counting of ASM nuclei using video image analysis, we found a highly significant increase in positive cells in COPD. Singh et al. have shown that increased nuclear expression of high-molecular-weight (HMW) FGF-2 in vascular smooth muscle and endothelium precedes arterial enlargement in response to increased arterial blood flow in vivo [34]. Although the function of this FGF-2 in the cell nucleus remains unclear, it is believed to be targeted for translocation to the nucleus. Recently, the role of FGF-2 in the nucleus has been partly clarified (for reviews, see refs 40 and 41). The basic FGF gene
can produce at least five different isotypes: the conventional 18 kD extracellular bFGF, as well as four additional HMW forms that are predominantly nuclear in localization. All five isoforms are able to translocate to the nucleus upon activation of different cells. In the nucleus, FGF-2 can act as a modulator of ribosomal gene transcription via direct interaction of the regulatory subunit of the protein kinase CKII. Also, the FGF receptors can be translocated to the nucleus, which could indicate a novel FGFR-1 and FGF-2 functional mechanism [42]. From the pattern that we observed, we assume that the positivity in the nuclei was not due to an artefact but was representative of specific localization of the appropriate antigen by the antibody used. In the same section, some nuclei were distinctly positive, whereas adjoining nuclei were clearly negative. Taken together, the role of FGF-2 isoforms in the nucleus is very complex, but may well represent an important feature of functional regulation.

Our ASM cell culture experiments in vitro indicate that FGF-2 and to a lesser extent FGF-1 are potent mitogens for airway smooth muscle cells, as shown by increased [3H]thymidine incorporation. However, scarce Ki-67-positive ASM cells in COPD despite enhanced FGF expression indicate a low cell turnover and untimely proliferation due to tissue damage. Our results are in accordance with previous studies on the mitogenic activity of these molecules [43,44] and further strengthen the role of FGFs in COPD.

Pearson’s correlation analysis revealed a significant inverse correlation of FEV1 on the one hand with expression of FGF-1, FGF-2, and receptor FGFR-1 in bronchial epithelium, and on the other hand with FGF-2 and FGFR-1 in ASM. These findings may indicate that the expression of these molecules is related to airflow limitation. Additionally, we observed a positive correlation of epithelial FGF-1 expression and pack-years in all patients, although no significant difference was observed when comparing pack-years between non-COPD and COPD patients. This suggests that responses to cigarette smoke exposure are involved in epithelial cell function. We also observed a highly significant correlation of FGF-1/FGFR-1 co-localization in bronchial epithelium and FGF-2/FGFR-1 in ASM cells. These findings indicate that FGF-1 and FGF-2 are differentially expressed and may regulate locally different events in the corresponding tissues.

In vivo and in vitro data indicate that smooth muscle cells, and their cross-talk with myo-fibroblasts and inflammatory cells via growth factors and cytokines, are major factors in airway remodelling due to a variety of pathophysiological conditions [36,45–47]. In line with this general picture, our findings suggest that the FGF–FGFR system contributes to airway remodelling in COPD. Taken together, our results support the notion that increased bronchial expression of FGF-1, FGF-2, and FGFR-1 in patients with COPD could participate in regulating the process of pulmonary airway remodelling. Blockade of these pathways should be considered in the development of therapeutic interventions aimed to prevent or reverse chronic airflow limitation in COPD.

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References


