FGF-FGFR$_1$ system and Tissue Remodeling

During Chronic Lung Disease

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6.1 Summary

An important feature of chronic obstructive pulmonary disease (COPD) is airway remodeling of which the molecular mechanisms are poorly understood. We assessed the role of fibroblast growth factors (FGF-1 and FGF-2) and receptor, FGFR-1 in bronchial airway wall remodeling 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 as compared to 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 assay 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 FGF/FGFR-1 system in the remodeling of bronchial airways in COPD.

6.2 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 ten percent of all smokers develop COPD. One of the key pathological features of COPD is thickening of 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 remodeling (3). A variety of growth factors and cytokines including platelet-derived growth factor-B (PDGF-B) and epidermal growth factor (EGF), 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 cocultures from 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 the airway wall remodelling. A number of studies have demonstrated that members of 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 affinity 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 the cell proliferation and the expression of FGFR-1 in cultured human ASM cells stimulated with FGF-1 and FGF-2.

6.3 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 fifteen subjects with forced one-second expiratory volume (FEV₁) <75% of predicted value (15) before bronchodilatation, FEV₁/FVC ratio <75%, a reversibility in FEV₁ ≤12% of predicted after 400 µg inhaled salbutamol, and with a carbon monoxide diffusion capacity (K\textsubscript{CO}) ≤80% of predicted value or to the Non-COPD group (n = 16) consisting of sixteen subjects with FEV₁ >85% before bronchodilatation, FEV₁/FVC ratio >85% and the 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 of 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 4.1.
Immunohistochemistry

Serial sections of 4 μm were deparaffinized, 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), FGF-1 and FGFR-1 (kind gift from Dr. J. Walters) as described previously (18, 19). Color was developed using New Fuchsin or 3,3-diaminobenzidine as chromogens. Slides were counter stained with Mayer's hematoxylin. 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 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: 736x574) from each subject were analysed using 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 interactively drawing areas 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 is expressed as the number of positive nuclei divided by total nuclei (labelling index, LI). In case of 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 4.1) representing none (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 immunocytochemically characterised (α-SMA and smooth muscle myosin heavy chain staining) and used for experiments at passage 4-5.
Figure 6.1. 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 to D show representative examples of staining intensities used for visual scoring, 0-3 respectively. Original magnification: x100.

ASM cell Proliferation assays

Cells were seeded at a density 1×10^4 cells/well in 96-wells plates, cultured until confluence, subsequently serum deprived to synchronise 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, 1μCi/well of [3H]-thymidine (Amersham, Roosendaal, the Netherlands) was added. The cells were harvested on glass fiber filters and radioactivity was assessed using a Microplate Scintillation β-counter (Topcount, Packard, Meridan, USA). The mean CPM of quadruple wells and subsequently from three different cell batches was expressed as fold change compared to controls. In a parallel series of
experiments, cells in quadruple were stimulated for 24 and 48 hours and processed for cell counting in the Caseyl® 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 (10ng/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 β-actin cDNAs were employed (22, 23). The PCR products were separated on 1.5% agarose gel, digitally photographed and the intensity of the bands was quantified in relation to β-actin band using Molecular Analyst (V 1.5) image analysis program (Biorad Laboratories, Hercules, USA) and values were expressed as a ratio to the controls.

Statistical Analysis
Data were analysed for statistical significance using the unpaired, two-tailed Students’ t-test as well as the non-parametric Mann-Whitney test, where appropriate. The data were expressed as mean ± SEM. Staining for different compartments were correlated with FEV₁ and Kco using Pearson’s correlation analysis. Differences with p ≤ 0.05 were considered to be statistically significant.

6.4 Results

Clinical Parameters
The clinical and lung function characteristics of all subjects included in the study are listed in Table 6.1 (16). The COPD group demonstrated an elevated residual volume (RV), whereas the CO-diffusion (Kco) was reduced as compared to controls (p<0.005). The subjects in the two groups did not differ significantly in age, total lung capacity (TLC), reversibility in FEV₁, smoking status (pack-years) or steroid use (Table 6.1).

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 cell. 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.

Microphotographs showing the expression patterns of FGF-1 and FGF-2 are presented in Figure 4.2, panels A, C and E, G (non-COPD), and B, D and F, H (COPD), respectively. Video image analysis revealed that the expression levels for FGF-1 in the bronchial epithelium (Figure 4.3, panel A) were increased significantly (stained/total epithelial area: 0.32±0.04 vs. 0.20±0.03, p<0.04) in COPD cases as compared to non-COPD. In ASM cells no difference was found for FGF-1 (0.16±0.04 vs. 0.14±0.03, p=0.77).

Table 6.1. Subject characteristics and clinical parameters

<table>
<thead>
<tr>
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<th>Non-COPD</th>
<th>COPD</th>
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<tr>
<td>FEV₁ (%pred.)</td>
<td>97±1.6</td>
<td>54±3.3 *</td>
</tr>
<tr>
<td>dFEV₁ (% change from pred.)</td>
<td>3±0.6</td>
<td>4±0.9</td>
</tr>
<tr>
<td>FEV₁/FVC (%pred.)</td>
<td>100±2.1</td>
<td>58±2.3 *</td>
</tr>
<tr>
<td>TLC (%pred.)</td>
<td>104±1.9</td>
<td>103±3.6</td>
</tr>
<tr>
<td>RV (%pred.)</td>
<td>117±5.4</td>
<td>141±10 ^</td>
</tr>
<tr>
<td>Kco (%pred.)</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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**Abbreviations:** Forced expiratory volume in 1 second (FEV₁), forced vital capacity (FVC), total lung capacity (TLC), residual volume (RV), reversibility of FEV₁ after 400 μg salbutamol (dFEV₁) and carbonmonooxide diffusion constant (Kco) are given as percentage of predicted. * P < 0.005 versus non-COPD. The patients in these two groups participated in a larger project, part of which has been published previously (16, 17).

FGF-2 expression however was clearly up-regulated in bronchial epithelium of COPD cases (0.35±0.03 vs. 0.20±0.04, p<0.008, figure 4.3, panel B) and ASM nuclei (LI ASM nuclei, 0.84±0.07 vs. 0.32±0.06, p<0.0001, figure 4.3, panel C). The distribution of total nuclei/total ASM tissue area remained unchanged in both the 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 increase in their size but without their apparent proliferation.
Figure 6.2. Immunohistochemical localization of FGF-1 and FGF-2 in central airways. Photomicrographs of central bronchial tissue sections from patients without COPD (A, C, E and G) and with COPD (B, D, F and 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. Original magnification: x200. 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 in airway smooth muscle (ASM) cells. Original magnification: x400. Scale bar = 50 μm.
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 Figure 4.4, panels A and C (non-COPD), and B and D (COPD). Graphic representations of the data as assessed by video image analysis for FGFR-1 immunostaining is shown in Figure 4.5, panel A. 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 as compared to non-COPD (fold increase 1.65, p<0.01, Figure 4.5, panel B). Elevated staining of FGFR-1 in COPD as compared to non-COPD patients was observed in smooth muscle of subepithelial microvessels (1.6 fold increase, p<0.05, Figure 4.5, panel B).

Bronchial airways were also stained with smooth muscle specific antibody, α-SMA (Figure 4.4, panel E) as well as with cell proliferation marker, Ki-67 (Figure 4.4, panel F). 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 found very rarely an ASM cell stained with Ki-67 and this was the case in both COPD and non-COPD groups.

**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 4.6. 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 packyears (r=0.49, p<0.01). Moreover, we found 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 significant correlation was found between FGF-1 and FGF-2 localization.
Figure 6.3 Quantitative analysis of FGF-1 and FGF-2 expression. Graphic representations of FGF-1 expression using video image analysis (A) in Bronchial epithelium (EPI) and Airway Smooth muscle cells (ASM), and FGF-2 expression (B) in Bronchial epithelium depicted as a ratio of stained area divided by tissue area in non-COPD (white bars) and COPD groups (gray bars). (C) FGF-2 expression in ASM cells presented as Labeling Index (LI) of total ASM nuclei. Values are mean ± SEM from 13-15 patients in each group. *P <0.05 versus the non-COPD group.
Figure 6.4  Immunohistochemical localization of FGFR-1, α-SMA and Ki67 in central airways. Photomicrographs of central bronchial tissue sections from patients without COPD (A) and with COPD (B) showing FGFR-1 staining (red new-fuchsine) in bronchial epithelium. Panels C (non-COPD) and D (COPD) show representative staining in airway smooth muscle (ASM) cells. Representative staining in bronchial airways for α-SMA (E) and for cell proliferation marker, Ki-67 immunoreactivity (F) in COPD cases. Original magnification: x200. Scale bar = 50 μm.
Figure 6.5 Quantitative analysis of FGFs and FGFR-1 expression. Graphic representations of FGFR-1 expression using video image analysis (A) in Bronchial epithelium (EPI) and Airway Smooth muscle cells (ASM), depicted as a ratio of stained area divided by tissue area in non-COPD (white bars) and COPD groups (gray bars). (B) Graphic representations 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 (gray bars). * P <0.05 versus the non-COPD group.
In order to further investigate the role of fibroblast growth factors on airway smooth muscle remodelling, isolated human airway smooth muscle cells were stimulated in vitro with increasing concentrations of FGF-1 or FGF-2. Both FGF-1 and FGF-2 resulted in significantly increased cell numbers at a concentration of 10 ng/ml after 48 h of incubation. Therefore, we opted for this concentration of both the growth factors in our further experiments. Figure 4.7, panel B shows the fold increase in cell number after 48 hours of stimulation with 10 ng/ml FGF-1, FGF-2 and the combination of the two over the control. Significantly increase in ASM cell numbers (fold increase) after 48 h of incubation with FGF-1 (1.37±0.08, p<0.01) of 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 time dependent $[^3H]$TdR uptake at a concentration of 10 ng/ml of FGF-1 or FGF-2 is presented in figure 4.7, panel C. After 24 of stimulation, we found significantly increased $[^3H]$TdR uptake with FGF-1 and FGF-2, but after 48 hours only with FGF-2. The combined incubation with 10 ng/ml of each 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 marked increase in $[^3H]$TdR uptake (Figure 4.7, panel C).

To examine whether human ASM cells express FGFR-1 and if 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 of FGF-1 or FGF-2 for various time-periods and compared the expression pattern with controls. FGFR-1 mRNA could be detected in ASM cells using RT-PCR for all treatments at all different time-points (Figure 4.8).

A photograph showing the representative example, after agarose gel-electrophoresis with PCR products for FGFR-1 (497 bp) and β-actin (625 bp), is shown in Figure 4.8B. 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 4.8B). 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.

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Figure 6.6 Correlation analysis of FGFs and FGR-1 expression. Correlation was made for Packyears with FGF-1 in bronchial epithelium, FGF-2 in ASM with forced vital capacity and FGFR-1 and FGFR-1 in ASM with forced vital capacity. Correlation coefficient (r) was obtained using linear regression (Pearson's) analysis and significance level P value, P < 0.05. Abbreviations: Forced expiratory volume in 1 second (FEV1), FEV1/FVC (forced vital capacity).
Figure 6.7  Assessment of human ASM cell proliferation in relation to FGF-1 and FGF-2.  Panel A: A graphic representation of dose-dependent increase in cell number of human ASM cells after 48 hours stimulation with increasing concentrations of FGF-1 or FGF-2.  Panel B: Fold induction in ASM cells relative to control after stimulation with 10 ng/ml of FGF-1, FGF-2 or a combination of both ligands for 48 h.  Panel C: Time course of $[^3H]$-thymidine uptake in ASM cells after stimulation with 10 ng/ml of FGF-1 and FGF-2 a combination of both ligands. Data is represented as mean fold increase in relation to control from three independent experiments performed in quadruplicate. Values are mean ± SEM and *P < 0.05 versus the control group.
Figure 6.8  RT-PCR analysis of FGFR-1 mRNA expression in human ASM cells. Agarose gel electrophoresis of RT-PCR products of cDNA synthesised from human ASM cells treated with FGF-1 or FGF-2 (10ng/ml) (n=3). Representative example of an agarose gel-electrophoresis (Panel A) with PCR products for FGFR-1 (497 bp) and β-actin (625 bp). The different lanes marked on top denote: pGEM marker (M), Control cells at 1, 8, 24h (C₁, C₈, C₂₄), FGF-1 or FGF-2 stimulated ASM cells for 1, 2, 4, 8, 24 and 48 h. Bar diagram showing quantitative analysis of FGFR-1 mRNA expression (Panel B). Intensity of the bands was analysed using digital image analysis software and FGFR-1/β-actin ratio was calculated as described in the text. Values are mean±SEM from 4 independent measurements.

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 as compared to control (p<0.05). Whereas, FGF-2 stimulation resulted in elevated levels for FGFR-1 mRNA at 4 h (1.32±0.14 fold, P<0.05) and at 48 h (1.21±0.13, ns) of incubation.
6.5 Discussion

In this study we have shown that COPD is associated with an increased expression of FGF-1, FGF-2 and FGFR-1 in the bronchial epithelium and an 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 packyears 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 the 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. Pulmonary expression patterns of FGF-1, FGF-2 and FGFR-1, found in our study are in agreement with results by Hughes and Hall (24) on the expression of these growth factors in the normal lungs. In additional, 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 like 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, ischemic heart disease and interstitial lung fibrosis (12, 33-35). Barrios and co-workers (12) showed FGF-1 and FGFR-1 expression in experimentally induced pulmonary fibrosis. Bocq and colleagues showed that FGF-1 expression in the 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 misbalance 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 show increased FGF-2 and FGFR-1 expression but not FGF-1 in airway smooth muscle cells using immunohistochemistry. By interactively counting of ASM nuclei using video image analysis we found a highly significant increase in positive cells in COPD. Singh and colleagues 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, this FGF-2 is believed to be targeted for translocation to the nucleus. Recently, the role of FGF-2 in the nucleus has been partly clarified, as has been reviewed in two recent reviews (40, 41). The basic FGF gene can produce at least five different isotypes: the conventional 18 kDa extracellular bFGF, as well as four additional high molecular weight forms which 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 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, as was evidenced by a study of Stachowiak and co-workers showing co-localization of the receptor FGFR-1 and FGF-2 in the nucleus, which could indicate a novel FGFR-1 and FGF-2 functional mechanism (42). From the pattern we observed, we assume that the positivity in the nuclei was not due to an artefact but 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 in the 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 was evidenced from increased \(^{3}\text{H}\)-thymidine incorporation. However, scarce Ki-67 positive ASM cells in COPD despite enhanced FGFs expression indicate for low turn over 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 for the role of FGFs COPD. Pearson’s correlation analysis revealed significant inverse correlation of FEV\(_1\) 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 packyears in all patients, although no significant difference was observed when comparing packyears between non-COPD and COPD patients. This suggests that responses to cigarette smoke exposure are involved in epithelial cell function. We also observed 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 myofibroblasts and inflammatory cells via growth factors and cytokines, are major actors 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 in 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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