Fibroblast Growth Factors and Vascular Remodeling

4.1 Summary

Important characteristics of Chronic Obstructive Pulmonary Disease (COPD) include airway and vascular remodeling, of which the molecular mechanisms are poorly understood. We assessed the role of fibroblast growth factors (FGF) in pulmonary vascular remodeling by examining the expression pattern of FGF-1, FGF-2 and their receptor, FGFR-1 in peripheral area of the lung tissues from patients with COPD (FEV\textsubscript{1} ≤75%; n=15) and without COPD (FEV\textsubscript{1} ≥85%; n=13). Immunohistochemical staining results were evaluated by digital video-image analysis as well as by manual scoring. FGF-1 and FGFR-1 were detected in vascular smooth muscle (VSM), airway smooth muscle (ASM) and airway epithelial cells. FGF-2 was localized in the cytoplasm of airway epithelium and in the nuclei of ASM, VSM and endothelial cells. In COPD cases, an unequivocal increase in FGF-2 expression was observed in VSM (3 fold, p=0.001) and endothelium (2 fold, p=0.007) of small pulmonary vessels with a luminal diameter under 200 μm. In addition, FGFR-1 levels were elevated in the intima (1.5 fold, p=0.05). VSM cells of large (≥200 μm) pulmonary vessels showed increased staining for FGF-1 (1.6 fold, p<0.03) and FGFR-1 (1.4 fold, p<0.04) in COPD. Pulmonary vascular remodeling, assessed as the ratio of α-smooth muscle actin staining and vascular wall area with the lumen diameter, was increased in large vessels of COPD (p=0.007) and was inversely correlated with FEV\textsubscript{1} values (p<0.007). Our results suggest an autocrine role of FGF-FGFR-1 system in the pathogenesis of COPD-associated vascular remodeling.

4.2 Introduction

Chronic obstructive pulmonary disease (COPD) is a global health problem with increasing morbidity and mortality (1). One of the major causal factors is tobacco smoking (2). However, only ten percent of all smokers develop symptomatic COPD. The causes of this variability in response of the airways and lung parenchyma to tobacco smoke exposure have remained largely unclear. One of the key pathological features of COPD is thickening of airway walls as a result of inflammation, hyperplasia of airway smooth muscle cells and fibroblasts, and increased deposition of extracellular matrix (3). In addition, advanced COPD leads to pathological changes in the pulmonary circulation (4, 5). At least part of this is probably the result of alveolar hypoxia, which is well known to cause pulmonary vasoconstriction and, if the hypoxic stimulus persists, pulmonary vascular remodeling, of which increased muscularization of small arterial branches is the most striking feature (6).
With sustained vasoconstriction of pulmonary arteries, arterioles and veins, the medial vascular smooth muscle (VSM) extends distally to vessels normally devoid of smooth muscle (6). Intimal thickening due to fibrosis and emergence of smooth muscle cells within the intima of small pulmonary arterial branches has also been reported (5). Finally, loss of the pulmonary vascular bed by emphysema has been suggested to lead to the formation of new vessels (6). Thus, several phenomena acting in concert in COPD result in pulmonary vascular remodeling. Yet, little is known about the molecular mechanisms underlying these processes in the context of COPD.

A variety of growth factors and cytokines released from various sites of airway and vascular walls have the potential to contribute to the pathogenesis of vascular remodeling in COPD. In view of their important role in chronic inflammation, fibrosis and repair of various tissues, including the lung (9), fibroblast growth factors (FGFs) may well play a pivotal role in airway and vessel wall remodeling (7, 8). Fibroblast growth factors exert their biological effects via binding to four high-affinity, transmembrane tyrosine-kinase receptors designated FGFR-1 through FGFR-4 (9). Distinct FGF subtypes bind with different affinity to the various FGF receptors. Alternative splicing and regulated protein trafficking further modulate the intra-cellular events and resultant response initiated by FGF ligand-receptor interaction (9). In the lung as well as in the vascular system, FGFs have been implicated in several pathological conditions. FGF-1 and FGFR-1 were shown to be upregulated during the development of lung fibrosis (10). FGF-2 and also PDGF have been implicated in the pathogenesis of obliterative bronchiolitis after transplantation (11). Moreover, vascular remodeling in response to increased blood pressure is associated with elevated levels of basic fibroblast growth factor (12, 13).

To investigate, whether the FGF-FGFR system might be involved in the pathogenesis of COPD, we examined the expression patterns of FGF-1, FGF-2 and FGFR-1 in (ex-) smokers with or without COPD and correlated the expression with histological evidence of pulmonary vascular remodeling.

4.3 Materials and Methods

Selection of Patients' Specimens
We examined lung tissue specimens of subjects with or without COPD. Peripheral part of the lung tissue from current and ex-smokers who underwent lobectomy or pneumonectomy for lung cancer was obtained from the Pathology Laboratories of the Leiden University Medical Center, Leiden, the Netherlands, and the Zuiderziekenhuis, Rotterdam, the Netherlands.
Tissue specimens were taken distally from the lung hilus part and contain predominantly parenchyma and small airways as well as vasculature. All lung tissues were inflated by an injection syringe using formalin and fixed for approximately 24 hours after which the tissues were further dehydrated and embedded in paraffin and subsequently processed for immunohistochemical staining. Based on a number of lung function data, patients were assigned to the COPD and non-COPD groups (14, 15).

**COPD group.** Fifteen subjects were assigned to the COPD group on the basis of the following parameters: forced expiratory volume in one second (FEV₁) <75% of predicted value (16) before bronchodilatation, FEV₁/FVC ratio <75%, a reversibility in FEV₁ ≤12% of predicted after 400 µg inhaled salbutamol, and a transfer factor for carbon monoxide (diffusion capacity) per liter alveolar volume (Kco) ≤80% of predicted value.

**Non-COPD group.** Thirteen subjects were assigned to the non-COPD group on the basis of the following data; a FEV₁ >85% before bronchodilatation, FEV₁/FVC ratio >85%, and reversibility in FEV₁ ≤12% of predicted after 400 µg salbutamol inhalation. In order to exclude accompanying lung disease leading to a restrictive function disorder, the total lung capacity (TLC) of each subject included in the study was over 80% of the predicted values (16).

Clinical data of all patients were examined for possible comorbidity and medication usage. All patients were free of symptoms of upper respiratory tract infection and none received antibiotics perioperatively. None of the patients received glucocorticosteroids in the three months prior to operation; four patients received oral glucocorticosteroids perioperatively. In addition to the rigorous criteria based on lung function parameters, microscopic exclusion criteria was also applied in the selection of patients for this study. After the selection based on lung function, all the lung tissues were subsequently examined histologically by two experienced lung pathologists using following exclusion criteria: (i) presence of tumor in the lung tissue specimen submitted for this study, (ii) presence of poststenotic pneumonia in the specimen, (iii) fibrosis of lung tissue, and (iv) obstruction of the main bronchus of the resection specimen by tumor (14, 15).

**Pulmonary Function Tests**
All pulmonary function tests were performed within 3 months prior to surgery. FEV₁ and forced vital capacity (FVC) were measured by spirometry, total lung capacity and residual volume with the closed circuit helium dilution test and the Kco using the single breath-holding technique, as described by Quanjer and co-workers (16). Lung function data and other patient characteristics are shown in Table 2.1.
Sections of paraffin-embedded lung tissue were cut at 4 μm, mounted on Super Frost Plus® microscopic slides (Menzel-Gläser, Braunschweig, Germany) and processed for immunohistochemistry. Serial sections were used for immunostaining of FGF-1, FGF-2 and FGFR-1 using human specific antibodies. The optimal dilutions for all antibodies were identified by examining the intensity of staining obtained with a series of dilutions, which gave specific and easily visible signal on paraffin sections derived from the same control tissue prior to performing the staining protocol on all section. In order to avoid day to day variations in the staining intensities, the incubations of all specimens with each antibody were performed in one single run. Sections were deparaffinized and rehydrated prior to incubation with specific mouse monoclonal and affinity purified antibodies against FGF-1 (1:2000 dilution), FGF-2 (1:200 dilution) and FGFR-1 (1:2000 dilution). The mouse IgG1 antibody against human FGF-1 was raised using a synthetic peptide corresponding to the internal 61-99 amino acid sequence whereas, the mouse IgG2b antibody was raised against a synthetic peptide corresponding to the 16 amino acids from the C-terminus of human FGFR-1, as described previously (17, 18). FGF-2 was a mouse (IgG1 isotype) monoclonal antibody raised against human FGF-2 (Mol. Weight: 18-24 Kda) and it was procured from Transduction laboratories, Lexington, Ky, USA. Anti-human mouse monoclonal antibodies against α-smooth muscle actin (α-SMA), Ki-67 and FGF-2 were purchased from NeoMarkers (Clone 1A4, Fremont, CA, USA), from Biogenex (San Ramon, MO, USA) and from Transduction Laboratories (San Ramon, MO, USA), respectively. To block non-specific binding, sections were preincubated with 10% normal goat serum diluted in 5% bovine serum albumin in phosphate buffered saline (5% BSA/PBS, pH = 7.4). Subsequently, sections were incubated for overnight at 4 ºC with the primary antibodies (FGF-1 and FGFR-1) diluted appropriately or for 1 hour at room temperature in case of α-SMA (1:1000 dilution). Secondary biotinylated anti-immunoglobulins (Multilink®, 1:75 dilution, Biogenex, San Ramon, MO, USA) and tertiary streptavidin conjugated Alkaline Phosphatase (Label® 1:50 dilution, Biogenex, San Ramon, MO, USA) were used to enhance the detection sensitivity. Color was developed using New Fuchsin, while endogenous alkaline phosphatase activity was inhibited by 0.01 M levamisole.

FGF-2 and Ki-67 immunostaining was performed on serial sections after antigen retrieval by boiling in citrate buffer (10 mM citrate buffer, pH = 6.0) for 10 minutes in a microwave oven. Sections were preincubated with 10% normal goat serum in 5% BSA/PBS, followed by incubation with primary antibody (1:50 dilution) overnight at 4 ºC. Slides were rinsed in PBS,
incubated for 30 minutes with peroxidase conjugated streptavidin at a dilution of 1:50 (Biogenex, San Ramon, MO, USA). Subsequently, sections were colored using 0.025% of 3,3-diaminobenzidine (Sigma, St Louis, MO, USA) in 0.01 mol/L PBS, containing 0.03% H₂O₂. Slides were counterstained 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, which gave specific and easily visible signal on paraffin sections derived from the same control tissue. Slides were mounted and staining results were systematically investigated (see below). Negative controls consisted of omission of the primary antibody.

**Semi-quantitative Analysis**

All tissues were analyzed in a blinded fashion in random order by two independent observers, who were unaware of the clinical data of the case under study. Semi-quantitative analysis was performed using an arbitrary visual scale with grading scores of 0, 1, 2, and 3 representing no, weak, moderate and intense staining, respectively (14, 15). Errors within and between observers were assessed by correlating the expression scores using Pearson’s analysis and we found a very high correlation ranging from 0.8 to 0.9. Microphotographs in Figure 2.1, panel A to D show representative examples of staining intensities used for visual scoring, 0-3 respectively. Sections were graded for the intensity of expression signal of FGF-1, FGF-2 and FGFR-1 in the endothelium and VSM of small (50-200 μm internal diameter) and in the endothelium, VSM and adventitial area of large (>200 μm internal diameter) pulmonary arteries.

**Video Image Analysis**

In addition, video image analysis was performed for α-SMA staining using Leica Qwin system version 3.0 (Leica B.V., Rijswijk, The Netherlands). Twenty digital images (pixel size: 736x574) from each section were taken using a video camera. Internal diameter of blood vessels was derived as a mean of measured vertical and horizontal diameters. In our study, we excluded those vessels who showed the ratio of >3 for both the diameters. Based on the internal diameter, pulmonary vessels were grouped into 4 sizes (50-100 μm, 100-200 μm, 200-400 μm and >400 μm). Vascular wall (VW) area, α-smooth muscle actin (α-SMA) stained area and vessel internal diameter (ID) were measured. Measurements were expressed as percentages for staining per vessel wall (α-SMA/VW area), for VW area corrected for internal diameter (VW area/ID) and α-SMA staining, also corrected for lumen diameter (α-SMA area/ID).
Figure 4.1 Representative examples of staining intensity pattern used for visual scoring. Photomicrographs depict lung tissue sections from patients without COPD (A and C) and with COPD (B and D) showing FGFR-1 staining (red new-fuchsine) in vascular smooth muscle cells. Panels A to D show representative examples of staining intensities used for visual scoring, 0-3 respectively. Scale bar = 50 μm; original magnification: x100.

Statistical Analysis

Data were analyzed for statistical significance using the unpaired, two-tailed Students' “t”-test as well as the Mann-Whitney non-parametric test, wherever appropriate (14,15, SPSS software packet-SPSS Incorporation, Chicago, USA). The staining score data for FGF-1, FGF-2 and FGFR-1 were expressed as mean ± SEM. Furthermore, FGF-1, FGF-2 and FGFR-1 staining scores for different vessels with internal diameter > and <200 μm, were correlated with FEV₁ using Pearson’s correlation analysis. Furthermore, the individual FEV₁ values were correlated with the vascular remodeling data (VW area/ID) in both the groups. Differences with p≤0.05 were considered to be statistically significant.

4.4 Results

Clinical Parameters

The clinical and lung function characteristics of all subjects included in the study are listed in the Table 2.1. FEV₁ and FEV₁/FVC values were significantly lower in the COPD group than
in the non-COPD group (p <0.001). In the COPD group, residual volume (RV) was increased, whereas CO-diffusion (Kco) was reduced (p<0.005). The subjects in the two groups did not differ significantly with respect to age, total lung capacity (TLC), reversibility in FEV₁, smoking status (pack-years) and previous steroid usage (Table 4.1).

Localization and quantification of FGF-1 and FGF-2

FGF-1 expression was detected in the media and adventitia of large pulmonary arteries and veins, but only in the media of small vessels. No FGF-1 staining was found in the endothelial layer of any vessels. FGF-2, was localized specifically in nuclei of endothelial and vascular smooth muscle cells. Expression of FGF-1 and FGF-2 was also observed in epithelial and bronchiolar smooth muscle cells. Representative showing the expression patterns of FGF-1 and FGF-2 are presented in Figure 2.2. A summary of the semi-quantitative data of FGF-1 and FGF-2 immunostaining is given in Figure 2.3. In subjects with COPD we observed significantly increased (p<0.02) expression of FGF-1 in medial VSM of larger vessels, but the level of adventitial expression of FGF-1 remained unaltered (Figure 2.3, panel A). In contrast to FGF-1, the expression of FGF-2 was elevated in the COPD group, but only in the small vessels with a internal diameter <200 μm, where it was increased in endothelial (p<0.007) and medial smooth muscle (p<0.001) cells (Figure 2.3B).

Table 4.1 Subject characteristics and clinical parameters

<table>
<thead>
<tr>
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<th>Non-COPD</th>
<th>COPD</th>
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<tbody>
<tr>
<td>FEV₁</td>
<td>99±1.9</td>
<td>53±3.2*</td>
</tr>
<tr>
<td>dFEV₁</td>
<td>3±0.6</td>
<td>4±0.9</td>
</tr>
<tr>
<td>FEV₁/FVC</td>
<td>100±2.3</td>
<td>58±5.0*</td>
</tr>
<tr>
<td>TLC</td>
<td>104±2.0</td>
<td>108±8.8</td>
</tr>
<tr>
<td>RV</td>
<td>115±5.5</td>
<td>141±15.4*</td>
</tr>
<tr>
<td>Kco</td>
<td>94±2.0</td>
<td>55±5.4*</td>
</tr>
<tr>
<td>Sex (Male/Female)</td>
<td>11/2</td>
<td>14/1</td>
</tr>
<tr>
<td>Age (years)</td>
<td>57±3.2</td>
<td>59±5.0</td>
</tr>
<tr>
<td>Smokers/ex-smokers/non-smokers</td>
<td>9/4/0</td>
<td>12/3/0</td>
</tr>
<tr>
<td>Pack-years</td>
<td>33±4.7</td>
<td>35±5.2</td>
</tr>
<tr>
<td>Steroid use (yes/no/unknown)</td>
<td>0/12/1</td>
<td>4/9/2</td>
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</table>

**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 carbon monoxide diffusion constant (Kco) are given as percentage of predicted. FEV₁/FVC is given as actual ratio in %. * P < 0.005 versus non-COPD.
FGFR-1 immunoreactivity was detected in epithelial and bronchiolar smooth muscle cells, and in the endothelium and vascular smooth muscle of large and small vessels. No adventitial positivity for FGFR-1 was observed. Representative microphotographs showing the expression pattern of FGFR-1 are presented in Figure 2.4. A graphic representation of the data of FGFR-1 immunostaining is given in Figure 4.5. The expression of FGFR-1 was significantly elevated in medial smooth muscle cells of large vessels (p<0.04) in the COPD-group as compared to non-COPD group, whereas the staining for the receptor in the intimal endothelium remained unaltered. Moreover, in contrast to the FGF-1 expression in small vessels in COPD patients, we found significantly higher expression levels of the receptor (p<0.05) in medial smooth muscle of small vessels (Figure 4.5).

**Figure 4.2** Photomicrographs of lung tissue sections from patients without COPD (A and C) and with COPD (B and D). Panels A and B (scale bar = 50 μm; original magnification: x100) show representative examples of FGF-1 protein staining (red new-fuchsin) in vascular smooth muscle cells of a large vessel (internal diameter >200 μm). Panels C and D (scale bar = 100 μm; original magnification: x400) show representative examples of nuclear FGF-2 expression (brown 3,3-diaminobenzidine) in endothelium and vascular smooth muscle cells of vessels with internal diameter <200 μm. Arrows indicate positive nuclei.
Figure 4.3  Graphic representations of FGF-1 (panel A) and FGF-2 (panel B) expression scores (mean ± SEM) in large (internal diameter >200 μm) and small (internal diameter <200 μm) vessels in non-COPD and COPD groups. * $P < 0.05$ versus the non-COPD group.

Figure 4.4  Photomicrographs of lung tissue sections from patients without COPD (A and C) and with COPD (B and D) showing FGFR-1 staining (red new-fuchsin) in vascular smooth muscle cells from large (internal diameter >200 μm; A and B) and small (internal diameter <200 μm; C and D) blood vessels. Scale bar = 50 μm; original magnification: x100.
Figure 4.5 Graphic representations of FGFR-1 expression scores (mean ± SEM) in large (internal diameter >200 μm) and small (internal diameter <200 μm) vessels in non-COPD and COPD groups. * P <0.05 versus the non-COPD group.

Figure 4.6 Photomicrographs of lung tissue sections from patients without COPD (A and C) and with COPD (B and D) showing α-smooth muscle actin staining (red new-fuchsin) in vascular smooth muscle cells from small (internal diameter <200 μm; A and B) and large (internal diameter >200 μm; C and D) blood vessels. Scale bar = 50 μm; original magnification: x100).
**Figure 4.7** Graphic representations of vessel wall measurements (mean ± SEM) using video image analysis in non-COPD and COPD groups. Panel A: Ratio of vascular wall area/internal diameter (VW area/ID) ratio. Panel B: Ratio of α-SMA area/VW area. *P* < 0.05 versus the non-COPD group.

**Assessment of vascular remodeling**

To examine pulmonary vascular remodeling as evidenced from variations in wall thickness and muscular medial thickness, video image analysis was performed using α-SMA immunostaining. Four separate groups with vessels of internal diameters of 50-100, 100-200, 200-400 and >400 μm, respectively, were analyzed (Figure 2.6). Measurements (mean ± SEM) were expressed as VW area/ID, α-SMA area/ID or percentage α-SMA staining per vessel wall area that represents volume fraction for smooth muscle staining was shown as α-SMA/VW area. The graphic representation of vascular wall remodeling data is presented in Figure 2.7. A significant increase in VW area/ID ratio for COPD in vessels of 100-200 μm (44.2 ± 1.9 vs. 36.4 ± 2.1, p=0.007), 200-400 μm (57.9 ± 2.4 vs. 44.7± 3.2, p<0.001) and >400 μm (75.6 ± 2.6 vs. 56.8 ± 5.9, p=0.011) was found (Figure 2.7, panel A). In vessels ranging from 50 to 100 μm in internal diameter no differences in VW area/ID ratio were observed. A significantly increased α-SMA area/ID ratio was observed for COPD in the 200-400 (26.7 ± 1.9 vs. 20.3 ± 2.9, p = 0.034) and >400 μm (38.3 ± 2.0 vs. 23.5 ± 3.4, p=0.006) internal diameter vessels but not in the 50-100 and 100-200 μm vessels. Surprisingly, no significant differences were observed between COPD and non-COPD groups in the percentage vascular smooth muscle, defined as α-SMA/VW area in all vessel types (Figure 2.7, panel B). Proliferation of VSM cells as evidenced from Ki-67 positivity was observed only very occasionally (data not shown).

**Correlation with clinical data**

The staining scores of FGF-1, FGF-2 and FGFR-1 expression in COPD and non-COPD patients were analyzed using Pearson’s test. For FGF-1, we observed a weak but significant
inverse correlation \( r = -0.39, p = 0.038 \) between staining score and \( \text{FEV}_1 \) in the medial VSM of vessels > 200 \( \mu \text{m} \) in ID (Figure 2.8, panel A). Additionally, there was a significant inverse correlation of FGF-2 staining scores in both endothelium \( r = -0.44, p = 0.002 \) and medial VSM \( r = -0.55, p < 0.0001 \) of vessels <200 \( \mu \text{m} \) in internal diameter with \( \text{FEV}_1 \) (Figure 2.8, panel B and C). However, in vessels >200 \( \mu \text{m} \) in internal diameter, no significant correlation between FGF-2 expression and \( \text{FEV}_1 \) was found (data not shown). Surprisingly, staining scores for FGFR-1 were not significantly correlated with \( \text{FEV}_1 \) (data not shown). When considering the association between \( \text{FEV}_1 \) and medial hypertrophy (VW area/ID ratio), we observed a significant inverse correlation of -0.50 \( (p = 0.007) \) for vessels with internal diameter >200 \( \mu \text{m} \) (Figure 2.8, panel D). However, no significant correlation could be established between \( \text{FEV}_1 \) and VW area/ID ratio for the vessels with internal diameter <200 \( \mu \text{m} \) \( (r = -0.10, p > 0.10) \).

Figure 4.8 Correlation with \( \text{FEV}_1 \) (% predicted) of FGF-1 expression in vascular smooth muscle cells (internal diameter >200 \( \mu \text{m} \)) (A), FGF-2 expression in vascular smooth muscle cells (internal diameter <200 \( \mu \text{m} \)); B, FGF-2 expression in endothelial cells (EC) from small blood vessels (internal diameter <200 \( \mu \text{m} \)); and vascular wall area/ID ratio (VW area/ID) D). Correlation coefficient \((r)\) and significance level \((P \text{ value})\) were obtained using linear regression (Pearson's) analysis.
4.5 Discussion

In this study we have found that COPD is associated with an increase in the expression of FGF-2 in small (<200 μm) and FGF-1 in large (>200 μm) pulmonary vessels respectively whereas, FGFR-1 is increased in both vessel types. Vascular medial thickness, assessed by video image analysis, was significantly increased in COPD in pulmonary vessels of various sizes. Pearson’s correlation analysis revealed a significant inverse correlation of FEV₁ with FGF-1 staining in the media of large and with FGF-2 expression in both endothelium and VSM of small vessels. Additionally, an inverse correlation of FEV₁ with medial thickening was found in pulmonary vessels of larger caliber, indicating that the degree of pulmonary vascular remodeling is related to the severity of obstructive lung function defect.

Several studies have commented on the importance of structural and functional abnormalities in the pulmonary vasculature of COPD patients. Hypoxia is known to induce prompt and severe vasoconstriction in the pulmonary vasculature, and sustained lung tissue hypoxia, as results from obstructive lung disease such as COPD, leads to pulmonary hypertension (4, 19). Hypoxic vasoconstriction is considered to represent one of the major contributing factors of pulmonary hypertension and right-sided heart failure in COPD and other chronic pulmonary diseases (4, 19). In addition, emphysema, accompanied by loss of elastic recoil, increased pulmonary pressure and destruction of part of the pulmonary microvasculature, may contribute to the increased vascular resistance observed in COPD (5, 6).

Using Video image analysis, we assessed systematically vascular wall thickening in COPD patients and non-COPD cases. Wall thickness of vessels 200 μm or more in diameter was increased in COPD. Our results on pulmonary vascular remodeling particularly in terms of intimal and medial thickening are in agreement with several earlier reports (4, 5, 20-22). Furthermore, the degree of intimal and medial thickening correlated with the decrease in lung function and, hence, with the severity of the disease. Wright and coworkers (4, 5) also observed a correlation with the severity of disease with mild to moderate COPD with intimal thickening and in severe cases with medial thickening. Similar findings on vascular abnormalities in COPD were recently reported by Peinado and coworkers, who showed intimal but not medial thickening in the vasculature of mild COPD patients compared to non-smoking controls (20, 21).

We used expression of smooth muscle marker α-SMA (23) to investigate whether the ratio of smooth muscle (α-SMA/VW area) in the vascular wall had changed during the progression of
COPD. Surprisingly, the ratio of α-SMA stained area to VW area remained unchanged. Approximately 42% of cells in all vessels stained positive for α-SMA, indicating that the increase in wall thickness could be attributed to the deposition of extracellular matrix proteins and medial accumulation of other cells, such as inflammatory cells and fibroblasts. Recently, we found specific staining for extracellular matrix proteins, like fibronectin and collagen subtypes in the intimal vascular cells of these pulmonary vessels indicating for ongoing intimal fibrosis in COPD patients (data not shown). Taken together, the data from this study indicate that vascular remodeling in COPD could be a contributing event in the pathogenesis of pulmonary hypertension in these patients. Furthermore, the observed changes in the intimal fibrosis as well as medial thickening could narrow the vessel caliber and may eventually lead to more severe vascular obstruction in 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 airway epithelium, monocytes, and are localized in the intima and media of pulmonary vessels (24). Pulmonary expression patterns of FGF-1, FGF-2 and FGFR-1, as found in our study are in agreement with results obtained by Hughes and Hall (24) in the normal lungs. However, in the peripheral regions of the lungs of patients with COPD, we observed additionally FGF-1 in adventitia and FGF-2 immunoreactivity in the nuclei of medial smooth muscle and endothelial cells advocating for a potential role of FGF-FGFR system in vascular remodeling in COPD.

Fibroblast growth factor family members are implicated in tissue remodeling in a wide variety of pathophysiological conditions including systemic hypertension, ischemic heart disease and interstitial lung fibrosis (10, 12, 25, 26). Barrios and coworkers (10) showed FGF-1 and FGFR-1 expression in experimentally induced pulmonary fibrosis. Becerril and colleagues found 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 (27). However, 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 remodeling, cellular proliferation, down-regulation of collagen type I and up-regulation of collagenase MMP-1 (28). Our findings of upregulated FGF-1, FGF-2 and FGFR-1 expression could indicate that such compensatory mechanisms are active in COPD since smoking has been suggested to affect cellular viability in lungs.

In a recent study, Singh and colleagues demonstrated that increased nuclear expression of FGF-2 in vascular smooth muscle and endothelium precedes arterial enlargement in response
to increased arterial blood flow \textit{in vivo} (12). Though the function of high molecular weight FGF-2 in the cell nucleus remains unclear, it is believed that this form of FGF-2 translocates to the nucleus. Moreover, Stachowiak and coworkers have demonstrated co-localization of the receptor FGFR-1 and FGF-2 in the nucleus of human astrocytes suggesting for novel mechanisms for the action of FGF-2 (29). In this study we show that FGF-2 is localized in the nucleus of endothelial and VSM cells and that the expression is increased in pulmonary vessels with diameter >200 \( \mu \)m in patients with COPD indicating a role for this growth factor in vascular remodeling. We also showed that in COPD, the expression of FGF-2 was upregulated in vessels with an internal diameter of >200 \( \mu \)m. Bryant et al. (13) recently found that administration of FGF-2 could inhibit internal luminal area decrease and wall thickening in response to altered blood flow; furthermore, this inhibitory effect could be blocked by anti-FGF-2 neutralizing antibodies. Our findings suggest that FGF-2 plays an important role in the response to increased pressure in the pulmonary vasculature in COPD. Several studies on hypoxia-induced pulmonary hypertension have shown that increased smooth muscle mass develops as a result of hypertrophy and hyperplasia of pre-existing smooth muscle cells or results from differentiation of fibroblasts recruited to the media from the adventitia (23, 30). It is likely that such mechanisms of vascular smooth muscle mass increase are operational in COPD-related vascular remodeling.

\textit{In vivo} and \textit{in vitro} data indicate that smooth muscle cells, and their cross-talk with endothelium, \textit{myo}fibro\textit{blasts} and inflammatory cells via growth factors and cytokines, are major contributing factors to vascular remodeling during different \textit{...\textit{...\textit{...\textit{...*\textit{...\textit{... conditions (23, 27, 31, 32). Furthermore, inflammation, a well-established factor in peripheral as well as in central airways in COPD, could also be associated with vascular remodeling in COPD. Increased adventitial infiltration of inflammatory cells, predominantly CD8' T-lymphocytes, in muscular pulmonary and bronchiolar arteries has been reported earlier (21, 33). Taken together, our results support the notion that in COPD, increased vascular expression of FGF-1, FGF-2 and FGFR-1 could participate in an autocrine and/or in a complex growth factor-cytokine interactive manner in regulating the process of pulmonary vascular remodeling. Our data further support the hypothesis that COPD is associated with pulmonary vascular remodeling and that the FGF-FGFR system contributes to the pathogenesis and severity of the disease.

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4.6 References


