Gremlin localization and expression levels partially differentiate idiopathic interstitial pneumonia severity and subtype

M Mylläriemi,1, *K Vuorinen,1 V Pulkkinen,1,2 H Kankaanranta,3,4 T Aine,4 K Salmenkivi,5 J Kesi-Oja,6 K Koli6 and VL Kinnula1

1Department of Medicine and Division of Pulmonary Medicine, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland
2Department of Medical Genetics, University of Helsinki, Helsinki, Finland
3Medical School, University of Tampere, and Seinäjoki Central Hospital, Finland
4Department of Respiratory Medicine, Tampere University Hospital, Finland
5Division of Pathology and Transplantation, Helsinki University Central Hospital, Helsinki, Finland
6Departments of Virology and Pathology, Haartman Institute, Helsinki, Finland

*Correspondence to: M Mylläriemi, Department of Medicine and Division of Pulmonary Medicine, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland. E-mail: marjukka.myllariemi@helsinki.fi

No conflicts of interest were declared.

Abstract

Idiopathic pulmonary fibrosis (IPF) (histopathology of usual interstitial pneumonia, UIP) and non-specific interstitial pneumonia (NSIP) are diseases characterized by loss of normal lung architecture and function. The differential diagnosis between IPF/UIP and NSIP may be difficult. The levels of bone morphogenetic protein (BMP)-4 antagonist gremlin are up-regulated in IPF/UIP. The present study was performed to clarify whether the localization or the mRNA expression of gremlin or BMP-4 could be used in the differential diagnosis or assessment of severity of IPF/UIP and NSIP. Gremlin and BMP-4 immunoreactivities were quantitated from 24 UIP and 12 NSIP lung specimens. Quantitative real-time polymerase chain reaction analyses were performed to compare gremlin and BMP-4 expression between UIP (n = 8) and NSIP (n = 5) biopsies. Immunohistochemical positivity and mRNA levels were correlated to lung function parameters. In IPF/UIP biopsies, gremlin was detected mainly in the thickened lung parenchyma, whereas in NSIP it was observed in the alveolar epithelium. BMP-4-positive (BMP-4+) cells were detected solely in the alveolar wall. The percentage of gremlin-positive area was higher in IPF/UIP (51.5 ± 0.6) than in NSIP (18.8 ± 0.7) (n = 36, p < 0.0001). Gremlin mRNA levels were higher in advanced UIP (p = 0.008) and NSIP (p = 0.007) biopsies than in the normal control lung. A negative correlation was found between the specific diffusion capacity corrected for alveolar volume (DLCO/VA) and gremlin mRNA levels (r = −0.69, p = 0.007). The highest numbers of BMP-4+ cells were found in NSIP biopsies. BMP-4 mRNA levels correlated positively with forced vital capacity (r = 0.801, p < 0.0001) and diffusion capacity. Parenchymal gremlin immunoreactivity is thus suggestive of a UIP-type interstitial pneumonia. Gremlin expression levels correlating negatively and BMP-4 levels positively with disease severity support recent observations of a fibroprotective role for the BMPs.

Copyright © 2007 Pathological Society of Great Britain and Ireland. Published by John Wiley & Sons, Ltd.

Keywords: bone morphogenetic protein; gremlin; idiopathic pulmonary fibrosis; non-specific interstitial pneumonia

Introduction

Idiopathic pulmonary fibrosis (IPF) is a progressive disease characterized by fibroproliferation and destruction of the lung parenchyma [1,2]. The histopathology of this disease, i.e. usual interstitial pneumonia (UIP), is defined by the presence of patchy areas of aggressive fibrosis, especially at the subpleural regions, and there is also low-grade inflammation. The prognosis of IPF patients is bleak compared to many other idiopathic interstitial lung diseases. The course of the disease associates with the extent of the fibroblastic lesions (so-called fibroblastic foci) in the lung [3,4]. One difficult diagnostic problem in IPF/UIP is differentiating it from non-specific interstitial pneumonia (NSIP), which generally has a more pronounced inflammatory component and a more diffuse involvement than IPF/UIP. There is a significant clinical, radiological and histopathological overlap between these diseases. Especially the fibrotic form of NSIP may be difficult to differentiate from IPF/UIP [5]. Moreover, the same lung may display both UIP and NSIP histopathology.
and some patients with fibrotic NSIP have a progressive disease with a similar behaviour as IPF/UIP [6].

We have recently shown that the bone morphogenetic protein (BMP)-4 antagonist, gremlin, is over-expressed in the lungs of patients with IPF/UIP [7]. BMPs have been identified as negative regulators of intracellular signalling of transforming growth factor-β (TGFβ). In IPF/UIP biopsies, gremlin immunoreactivity was detected both in the fibrotic lung parenchyma and at the sites of active fibrosis, the fibroblastic foci, which consist of proliferative fibroblast-like cells and myofibroblasts. Gremlin was highly expressed in primary pulmonary fibroblast lung cell lines from IPF/UIP patients, whereas no corresponding over-expression was found in the skin fibroblasts of scleroderma patients [7]. These findings suggested that elevated gremlin expression might be a useful indicator of IPF/UIP and lung fibrosis rather than fibrosis in general. Gremlin over-expression may contribute to inadequate BMP-4 signalling and impaired epithelial repair as well as sustained TGFβ signalling in the fibrotic lung [7].

The current study was undertaken to investigate whether gremlin expression levels could be used to differentiate IPF/UIP from NSIP. Gremlin and BMP-4 expression levels at the early stages of IPF/UIP and NSIP were determined and correlated with lung function parameters and α-smooth muscle actin (α-SMA) expression.

Patients and methods

Gremlin, BMP-4 and α-SMA localization was detected by immunohistochemistry and quantitated morphometrically from 36 patients. Gremlin and BMP-4 mRNA levels were measured by real-time polymerase chain reaction (RT–PCR) from snap-frozen tissue specimens collected from 15 patients.

Patients

Open-lung or thoracoscopic biopsies from patients with IPF/UIP (n = 24) or NSIP (n = 12) were used for immunohistochemical localization of gremlin, BMP-4 and α-SMA (Table 1).

For quantitative PCR analysis, lung biopsies or explanted lung obtained during lung transplantation were acquired from patients from Helsinki and Tampere University Central hospitals. All patients received written information and gave their permission to use the samples. The Ethics Committees of the Helsinki and Tampere University Central hospitals approved the study, and the use of the clinical material has been registered at www.hus.fi/clinicaltrials. IPF/UIP lung biopsies were either diagnostic biopsies (IPF/UIP early, n = 6) or obtained from lung explants during lung transplantation (IPF/UIP advanced, n = 4). All NSIP biopsies (n = 6) were taken at the time of diagnosis, via either open lung surgery or thoracoscopy. Control biopsies (n = 7) were obtained from healthy lung derived from operations in which benign tumours were removed or from unused donor lung tissue obtained during lung transplantation (Table 2).

Immunohistochemistry

Paraffin-embedded tissue sections from normal/healthy and IPF lung biopsies were deparaffinized in xylene and rehydrated in graded alcohol. Antigens were retrieved by heating the sections in citrate buffer (pH 6.0). Endogenous peroxidase activity was neutralized with 0.3% hydrogen peroxide. For immunostaining, Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA, USA) or Zymed ABC Histostain-Plus kit (Zymed, San Francisco, CA, USA) was used according to the manufacturer’s protocol. The primary antibodies used were goat polyclonal for human gremlin (sc-18 274, Santa Cruz Biotechnology, Santa Cruz, CA, USA), mouse monoclonal for human BMP-4 (MAB1049, Chemicon, Hampshire, UK) and mouse monoclonal for human α-SMA (MS-113-P0, Neo Markers, Fremont, CA, USA). Detection was performed with biotinylated anti-goat secondary antibody (Vector Laboratories), horseradish peroxidase

<table>
<thead>
<tr>
<th>Parameter</th>
<th>UIP early</th>
<th>UIP advanced</th>
<th>NSIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>19</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Age (years)</td>
<td>65.3 ± 2.67</td>
<td>51.4 ± 5.18</td>
<td>60.1 ± 4.50</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>10/9</td>
<td>4/1</td>
<td>9/3</td>
</tr>
<tr>
<td>Smoking/non-smoking</td>
<td>1/18</td>
<td>0/5</td>
<td>3/9</td>
</tr>
<tr>
<td>FEV1 (%)</td>
<td>71.9 ± 4.30</td>
<td>48.8 ± 10.7</td>
<td>78.9 ± 4.30</td>
</tr>
<tr>
<td>FVC (%)</td>
<td>67.0 ± 4.05</td>
<td>36.6 ± 3.14</td>
<td>81.3 ± 4.75</td>
</tr>
<tr>
<td>DLCO</td>
<td>45.3 ± 4.44</td>
<td>19.4 ± 6.96</td>
<td>57.9 ± 4.47</td>
</tr>
<tr>
<td>DLCO-VA</td>
<td>69.2 ± 4.37</td>
<td>43.2 ± 6.35</td>
<td>69.7 ± 4.08</td>
</tr>
</tbody>
</table>

Table 1. Patient characteristics for immunohistochemical studies

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>UIP early</th>
<th>UIP advanced</th>
<th>NSIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>7</td>
<td>6</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Age (years)</td>
<td>68.3 ± 5.19</td>
<td>62.0 ± 4.44</td>
<td>53.3 ± 5.59</td>
<td>59.5 ± 4.45</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>4/3</td>
<td>3/3</td>
<td>4/0</td>
<td>5/1</td>
</tr>
<tr>
<td>Smoking/non-smoking</td>
<td>—</td>
<td>0/6</td>
<td>0/4</td>
<td>1/5</td>
</tr>
<tr>
<td>FEV1 (%)</td>
<td>69.2 ± 4.98</td>
<td>52.0 ± 13.2</td>
<td>66.3 ± 12.0</td>
<td></td>
</tr>
<tr>
<td>FVC (%)</td>
<td>71.3 ± 4.34</td>
<td>38.0 ± 3.63</td>
<td>68.4 ± 8.35</td>
<td></td>
</tr>
<tr>
<td>DLCO</td>
<td>55.0 ± 3.15</td>
<td>22.3 ± 8.19</td>
<td>54.0 ± 7.46</td>
<td></td>
</tr>
<tr>
<td>DLCO-VA</td>
<td>80.5 ± 4.92</td>
<td>48.3 ± 4.97</td>
<td>79.0 ± 7.90</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Patient characteristics for real-time PCR studies

Patient characteristics (RT–PCR samples) data are presented as mean ± SEM. FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; DLCO, diffusion capacity of carbon monoxide; DLCO-VA, DLCO corrected for alveolar volume.
complex, and 3-amino, 9 ethyl-carbazole (AEC) chromogen (Zymed). The sections were counterstained with Mayer’s haematoxylin and mounted on glass slides. Control sections were treated with goat IgG (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) to determine the specificity of the staining.

Double staining was performed with α-SMA (Neo Markers) and gremlin (Santa Cruz Biotechnology) antibodies. Vectastain Elite ABC Mouse IgG and Goat IgG kits (Vector Laboratories) were used according to the manufacturer’s protocols. A blocking step was performed prior to the addition of the secondary antibody with an avidin/biotin blocking kit according to the manufacturer’s instructions (Vector Laboratories). α-SMA was detected with DAB substrate kit for peroxidase (Vector Laboratories), producing a black reaction product. Gremlin was visualized with Vector® NovaRed™ substrate kit (Vector Laboratories), producing a red reaction product. The sections were counterstained briefly with Mayer’s haematoxylin and mounted in non-aqueous mounting media (Vector Laboratories).

Morphometry
Morphometrical analysis of gremlin and α-SMA immunoreactivity was performed using Image-Pro Plus 6.1. (Media Cybernetics, Inc, Silver Spring, MD, USA) software. Three randomly chosen images from the lung parenchyma of each stained section were taken using an Olympus U-CMAD3 camera (Olympus Corporation, Japan) and QuickPHOTO CAMERA 2.1 software (Promicra, Czech Republic). If the area in the microscope field did not contain >50% tissue, the slide was moved vertically until a representative area was seen. The areas of positive and negative staining were calculated using Image-Pro Plus 6.1. BMP-4 staining was quantitated by counting individual positive or negative cells (300 cells/section).

Real-time PCR
Total RNA was extracted from snap-frozen lung biopsies by mechanical homogenization and Phase Lock Gel Heavy kit, according to the manufacturer’s (Eppendorf, Hamburg, Germany) instructions. Reverse transcription was carried out with Random hexamer primers (Invitrogen, Carlsbad, CA, USA) and Superscript II reverse transcriptase (Life Technologies, Gaithersburg, MD, USA), using 1.0 µg total RNA, according to the manufacturer’s instructions. The cDNAs were amplified using TaqMan Assays-on-Demand gene expression products (Applied Biosystems, Foster City, CA, USA) and GeneAmp 7500 Sequence Detector thermal cycler (Applied Biosystems). Control amplifications directly from RNA were taken using an Olympus U-CMAD3 camera (Olympus. There was more α-SMA+ fibroblastic foci (Figure 2). In contrast, in NSIP sections gremlin immunoreactivity predominated in the alveolar epithelium, especially at the areas of epithelial activation/atypia, although in some specimens intense parenchymal immunoreactivity was also detectable. In control biopsies (n = 5) taken from normal/healthy lung, only faint gremlin immunoreactivity was seen in alveolar macrophages and in cells lining the alveolar wall. BMP-4-positive (BMP-4+) cells were observed only around the alveolar wall (Figure 1). Nearly all samples had BMP-4+ cells, although a few IPF/UIP (n = 4) and NSIP (n = 3) biopsies were completely negative.

α-SMA and gremlin double-staining immunohistochemistry was performed to demonstrate the difference in gremlin localization between IPF/UIP and NSIP samples. In IPF/UIP sections, gremlin was seen mainly in the lung parenchyma around α-SMA+ fibroblastic foci (Figure 2). In contrast, in NSIP sections gremlin immunoreactivity predominated in the alveolar epithelium. There was more α-SMA+ staining in IPF/UIP biopsies than in NSIP biopsies (Figure 2, p < 0.0001), and the level of gremlin immunoreactivity correlated with α-SMA immunoreactivity (r = 0.37, p = 0.034, n = 33, Figure 2).

Correlation of gremlin and BMP-4 immunoreactivity to lung function parameters
When gremlin immunoreactivity in IPF/UIP and NSIP biopsies was compared using morphometric quantitation of the positively stained areas, a significantly higher proportion of positive area was observed in the IPF/UIP specimens (Figure 3A). The IPF/UIP

Statistics
Data were analysed using SPSS 12.0.1 for Windows (SPSS, Chicago, IL, USA). Spearmann’s rank correlation analysis for non-parametric data was used to correlate gremlin and BMP-4 mRNA expression levels and immunoreactivity with clinical parameters. Inter-group differences were analysed using the Mann–Whitney U-test and Student’s t-test. A p value of <0.05 was considered to be statistically significant.
Figure 1. Localization of gremlin and BMP-4 immunoreactivity in representative sections of UIP, NSIP and normal control lungs. In the normal lung, faint gremlin-positivity is seen in epithelial areas (Ctr, first and second columns from the left). In an early UIP lesion, positive staining is observed in the mildly thickened parenchymal lesion (UIP, first column from the left). In more advanced lesions (UIP, second and third columns), intense immunoreactivity is observed in the parenchyma but not the epithelial area. In fibrotic NSIP, the atypical epithelial layer is positive for gremlin (NSIP, first and second columns). Cellular type NSIP (NSIP, third column) shows immunoreactivity of the alveolar lining, but not in the lung parenchyma. BMP-4⁺ cells are observed only at the alveolar epithelial lining. In the normal lung, positive cells in alveolar branching points are observed. In UIP, occasional epithelial cells stain positive for BMP-4, whereas in a representative NSIP section almost all cells are positive for BMP-4.

Figure 2. Double-staining immunohistochemistry of representative sections of advanced lesions of UIP and NSIP; 8–10 digital images from the same spot with different focusing planes of a ×1000-magnified field were used to create an image stack. Red colour, gremlin; black, α-SMA; and blue, haematoxylin counterstain. Isotype control is a serial section from the UIP biopsy. In UIP, the alveolar epithelium is negative for gremlin, but positive staining (red) is observed in an α-SMA-positive (α-SMA⁺) fibroblast focus (black). In NSIP, gremlin immunoreactivity is observed predominantly at the alveolar epithelium. Gremlin and α-SMA⁺ staining was quantitated morphometrically. The proportions of gremlin and α-SMA⁺ tissue areas are shown.

The group was divided further into early (patients who underwent a diagnostic biopsy, n = 19) and advanced (patients who underwent transplantation, n = 5) stages of the disease. The proportion of gremlin-positive (gremlin⁺) area was highest in the advanced IPF/UIP (6.065 ± 0.619, p = 0.001 for IPF/UIP adv. versus NSIP), high in early IPF/UIP (4.821 ± 0.676, p = 0.005 for IPF/UIP early versus NSIP) and lowest in NSIP (1.775 ± 0.657). The area of gremlin⁺ staining correlated negatively with the forced expiratory vital capacity (FVC; Figure 3B).

The numbers of BMP-4⁺ cells were calculated from the lung biopsies. The proportion of BMP-4⁺ cells seemed to be higher in NSIP than in IPF/UIP,
Gremlin in interstitial pneumonia subtypes

Figure 3. Morphometric analysis of immunoreactivity for gremlin (A) and BMP-4 antibodies (C) in 24 IPF/UIP and 12 NSIP biopsies. Three representative areas consisting of the parenchymal portion of lung tissue were analysed from all stained sections. (B) Correlation between gremlin immunoreactivity and FVC ($n = 34$) although the difference was not statistically significant (Figure 3C). There was no correlation between the lung function parameters and the proportions of BMP-4$^+$ cells.

Gremlin and BMP-4 mRNA expression in IPF/UIP and NSIP

The mean levels of gremlin mRNA in patients with IPF/UIP (early $n = 6$, advanced $n = 4$), NSIP ($n = 6$) or no pulmonary disease ($n = 7$) are shown in Figure 4A. Both advanced IPF/UIP and NSIP biopsies exhibited higher levels of gremlin mRNA ($p = 0.008$ and $p = 0.007$, respectively) than the control lung. In addition, gremlin levels were higher in patients with end-stage fibrosis than in patients with early disease ($p = 0.01$; Figure 4A). There was no significant difference in gremlin mRNA expression between the early IPF/UIP biopsies and the NSIP biopsies (Figure 4A). The mRNA expression levels of BMP-4 were elevated in NSIP ($n = 6$), low in IPF/UIP and lowest in the advanced IPF/UIP ($p = 0.006$; Figure 4B) when compared to normal/healthy controls.

Correlation between gremlin and BMP-4 mRNA levels and lung function parameters

We have found an up to 35-fold elevation of gremlin mRNA levels in a small set of IPF/UIP patients who underwent lung transplantation [7]. In the present study we assessed whether gremlin and BMP-4 mRNA levels would correlate with the disease severity in a larger group of patients. A negative correlation ($r = -0.69$, $p = 0.007$, $n = 15$) between gremlin mRNA levels and specific diffusion capacity corrected for alveolar volume (DLCO/VA) was indeed observed when IPF/UIP and NSIP patients were analysed (Figure 5A). Even if the NSIP patients were excluded from the dataset, gremlin mRNA correlated negatively with DLCO/VA ($r = -0.766$, $p = 0.01$, $n = 10$, not shown). A positive correlation ($r = 0.801$, $p < 0.0001$, $n = 16$) was observed between BMP-4 mRNA and FVC (Figure 5B). A similar positive correlation was found between BMP-4 mRNA and diffusion...
Figure 5. Correlation between relative gremlin mRNA levels and diffusion capacity, corrected for alveolar volume (DLCO/VA) \((n=15)\) (A) and relative BMP-4 mRNA and forced expiratory vital capacity \((n=16)\) (B).

capacity \((\text{DLCO}: r = 0.601, p = 0.01, n = 16)\) and BMP-4 and DLCO/VA \((r = 0.594, p = 0.02, n = 16)\).

### Discussion

Idiopathic pulmonary fibrosis is a progressive lung disease characterized by alveolar epithelial damage and an inflammatory response that leads to fibroblast proliferation, excessive extracellular matrix (ECM) deposition, and ultimately to the loss of normal lung architecture. Gremlin mRNA has been shown to be highly up-regulated in the human IPF/UIP lung [7]. The present study was designed to explore whether gremlin or BMP-4 localization or mRNA expression in IPF/UIP or NSIP could be used as a differential diagnostic marker in determining the idiopathic interstitial pneumonia subtype. The correlation between gremlin and BMP-4 expression with the extent of lung damage was assessed by comparison to lung function parameters and \(\alpha\)-SMA immunoreactivity. There was a distinct difference in the localization of gremlin. In IPF/UIP-type interstitial pneumonia, gremlin was predominantly in the lung interstitium, whereas in NSIP biopsies gremlin immunoreactivity was mostly in the cells that lined the alveolar epithelium. The levels of gremlin mRNA were comparable between IPF/UIP and NSIP at diagnosis and were lower than those observed in advanced IPF/UIP. Importantly, gremlin mRNA levels correlated with disease severity in IPF/UIP and NSIP patients. This suggests that gremlin is an indicator of the stage of pulmonary fibrosis in these patients, independent of the diagnosis. On the other hand, BMP-4 levels were down-regulated in the most advanced stages of the disease and correlated positively with lung function parameters.

TGF\(\beta\) is a factor that enhances the progression of fibrosis by inducing myofibroblast differentiation [12,13], ECM accumulation [14] and epithelial–mesenchymal transition [15] in the lungs of IPF patients. BMP-4 is a member of the TGF\(\beta\) family of growth-regulatory cytokines. It is an important signalling molecule involved in lung morphogenesis, but BMP-4 is also expressed in the adult lung. It has also been identified as a negative regulator of intracellular TGF\(\beta\) signalling [16]. BMP-4 signalling, in turn, is negatively regulated by three proteins, noggin, chordin and gremlin [17]. In addition to lung fibrosis, gremlin up-regulation has been detected in liver [18] and renal [19] fibrosis. Experimentally induced mouse kidney fibrosis can be reversed by administration of recombinant human BMP-7, pointing to a potential therapeutic role for gremlin inhibition [20,21]. It also suggests that BMPs may have a fibroprotective role in vivo, as shown recently in liver [22] and cardiac [23] fibrosis.

The initial observation of gremlin over-expression was made with a small group of IPF/UIP patients who had undergone lung transplantation [7]. In the present study, gremlin expression was assessed in samples of the early stages of the disease and in other forms of idiopathic interstitial pneumonia, namely NSIP. The localization of gremlin immunoreactivity was different between IPF/UIP and NSIP, also during the earlier stages of the disease. In IPF/UIP gremlin localized to the lung interstitium, whereas in NSIP, gremlin was concentrated in the normal-looking or hypertrophied alveolar epithelium. Analysis of the very early stages of the diseased lung was not possible, since the diagnosis of idiopathic interstitial pneumonia is typically delayed and a significant loss of normal pulmonary function was found even in those early-diagnosed patients.

Recent studies have shown that the transcriptional profiles of IPF/UIP and NSIP are unexpectedly

\(J\ Pathol\ (2008)\ DOI: \text{10.1002/path}\)

Copyright \(\text{© 2007 Pathological Society of Great Britain and Ireland. Published by John Wiley & Sons, Ltd.}\)
similar [24,25]. Although a significantly different transcriptional profile has been observed between sporadic and familial idiopathic interstitial pneumonias, only minor gene expression changes have been detected between the histopathological disease subtypes of UIP and NSIP [26]. Accordingly, we found no differences between gremlin mRNA levels of early IPF/UIP and NSIP biopsies that were taken at the time of diagnosis.

Our observations revealed certain differences between IPF/UIP and NSIP in the staining patterns and mRNA expression levels of gremlin. It seems that the epithelial layer in IPF/UIP is devoid of gremlin expression, whereas in NSIP biopsies the activated epithelial layer was often positive. However, significant overlap was observed, as occasional NSIP samples showed similar parenchymal gremlin immunoreactivity as the IPF/UIP samples. This finding depicts the fact that the histopathological differential diagnosis of IPF/UIP and NSIP is difficult [5,6,27]. Even if gremlin staining could be helpful in evaluating some difficult cases, more sophisticated methods will be needed in order to confirm whether gremlin could be used as a differential diagnostic marker between IPF/UIP and problematic cases of NSIP. The current finding, that gremlin expression levels correlate negatively with BMP-positively with lung function tests, suggests that gremlin may be a marker of an advanced stage pulmonary fibrosis. Future studies will determine whether this finding can evolve to diagnostic or therapeutic applications.

Acknowledgements

We are grateful to the patients who consented to participate in our study. We thank Tiina Marjomaa and Anitra Ahonen for excellent technical assistance. This work was supported by the Academy of Finland, the Finnish Cancer Foundation, the Finnish Cultural Foundation, the Sigrid Juselius Foundation, Biocentrum Helsinki, Helsinki University Hospital Fund, the University of Helsinki, the Finnish Antituberculosis Association Foundation, the Finnish Medical Foundation, the Jalmari and Rauha Ahokas Foundation and the Yrjo Jahnsson Foundation.

References


