SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2016; 33; 41-50

MFAP4: a candidate biomarker for hepatic and pulmonary fibrosis?

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Abstract. *Background:* Several comparable mechanisms have been identified for hepatic and pulmonary fibrosis. The human microfibrillar associated glycoprotein 4 (MFAP4), produced by activated myofibroblasts, is a ubiquitous protein playing a potential role in extracellular matrix (ECM) turnover and was recently identified as biomarker for hepatic fibrosis in hepatitis C patients. The current study aimed to evaluate serum levels of MFAP4 in patients with pulmonary fibrosis in order to test its potential as biomarker in clinical practice. A further aim was to determine whether MFAP4 deficiency in mice affects the formation of pulmonary fibrosis in the bleomycin model of lung fibrosis. *Methods:* 91 patients with idiopathic pulmonary fibrosis (IPF), 23 with hypersensitivity pneumonitis (HP) and 31 healthy subjects were studied. In the mouse model, C57BL/6 *Mfap4+/+* and *Mfap4-/-* mice between 6-8 weeks of age were studied. Serum levels of MFAP4 were measured by ELISA in patients and in mice. Surfactant protein D (SP-D) and LDH were measured as comparison biomarkers in patients with pulmonary fibrosis. Morphometric assessment and the Sircol kit were used to determine the amount of collagen in the lung tissue in the mouse model. *Results:* Serum levels of MFAP4 were not elevated in lung fibrosis – neither in the patients with IPF or HP nor in the animal model. Furthermore no significant correlations with pulmonary function tests of IPF patients could be found for MFAP4. MFAP4 levels were increased in BAL of bleomycin treated mice with pulmonary fibrosis. *Conclusions:* MFAP4 is not elevated in sera of patients with pulmonary fibrosis or bleomycin treated mice with pulmonary fibrosis. This may be due to different pathogenic mechanisms of liver and lung fibrogenesis. MFAP4 seems to be useful as serum biomarker for hepatic but not for lung fibrosis. *(Sarcoidosis Vasc Diffuse Lung Dis 2016; 33: 41-50)*

Key words: biomarkers for pulmonary fibrosis, idiopathic pulmonary fibrosis (IPF), extracellular matrix, fibrogenesis, mouse model of pulmonary fibrosis

Received: 22 March 2015
Accepted after revision: 3 August 2015
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List of abbreviations

BAL	Bronchoalveolar Lavage
CTGF	Connective tissue growth factor
dNTPs	Nucleotides (deoxyNucleosideTriPhosphate)
ECM	Extracellular matrix
ELISA	Enzyme-Linked Immunosorbent Assay
EMT	Epithelial-to-mesenchymal transition
FDA	US Food and Drug Administration

FReD	Fibrinogen Related Domain
HE	Haematoxylin-eosin dye
HP	Hypersensitivity pneumonitis
HRCT	High resolution computer tomography
HSC	Hepatic stellate cell
ILD	Interstitial Lung Disease
IPF	Idiopathic pulmonary fibrosis
KO	Knock-out
MAGP-36	36-kDa Microfibrillar-Associated GlycoProtein
MFAP4	Microfibrillar Associated Protein 4
OPD	O-phenylenediamine
PaO2	Arterial pO2
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PFT	Pulmonary function tests
RIPA	RadioImmunoPrecipitation Assay
ROS	Reactive Oxygen SpeciesSP-D
SEM	Standard Error of the Mean
SD	Standard Deviation
sMFAP4	Systemic Microfibrillar Associated Protein 4
SMS	Smith-Magenis Syndrome
SP-D	Surfactant protein D
TBS	Tris Buffered Saline
TGFβ-1	Transforming growth factor β-1
TLCO	Transfer factor of the lung for carbon monoxide
TNFα	Tumor necrosis factor-alpha
UIP	Usual interstitial pneumonia
WT	Wild-type

Introduction

Fibrosis is a common late disease pattern in chronic disorders affecting parenchymal organs. The development of fibrosis is promoted by chronic infections, metabolic and toxic injuries or idiopathic inflammatory diseases (1, 2). Patients with advanced organ fibrosis have a poor prognosis and often require organ transplantation (3). The impairment of cellular homeostasis by chronic injuries leads to a damage and subsequently to a loss of parenchymal cells (4-7). The injury of endothelial cells in parenchymal organs results in an increased permeability (8), influx of inflammatory cells and cell death. This is paralleled by a release of pro-fibrogenic and inflammatory cytokines such as transforming growth factor TGFβ-1, connective tissue growth factor (CTGF) and tumor necrosis factor (TNFα) (4, 5, 9, 10). TGF beta-1 is essential for recruitment and activation of fibrogenic cells (11) which may take their origin from resident fibrocytes, from bone marrow-derived fibroblasts/fibrocytes or from epithelial-to-mesenchymal transition (EMT) (5,12).

Following a TGF beta 1 stimulation, resident lung fibroblasts proliferate and differentiate into collagen-synthesizing myofibroblasts (12-14). Similarly, chronic liver injury leads to the activation of hepatic stellate cells (HSC) which transform into myofibroblasts (4). It has been hypothesized that parenchymal cells in lung and liver take their origin from a common sheet during embryogenesis (15).

The human microfibrillar associated glycoprotein 4 (MFAP4) is a ubiquitous protein playing a potential role in extracellular matrix (ECM) turnover during fibrogenesis (16). MFAP4 was recently identified as a potential biomarker for hepatic fibrosis in patients suffering from hepatitis C (17). This protein contains fibrinogen-like domains and an arg-gly-asp sequence in the N-terminus that serves as the ligand motif for the cell receptor integrin (18). The bovine form displays a calcium-dependent binding to collagen structures of lung surfactant proteins (19). Furthermore, MFAP4 interacts in a calcium-dependent manner with different collectins in the lung and might fix them in the ECM during inflammation (19). Besides vascular smooth muscle cells it is unknown which cells are the principal source of MFAP4 which is expressed throughout the body and significantly more in the lungs than in other organs (35, 38). Pulmonary fibrosis is a component of many interstitial lung diseases (ILDs), the etiology of which may or may not be known.

In a large cohort of patients suffering from hepatitis C, serum MFAP4 showed high diagnostic accuracy for detection of liver cirrhosis and fibrosis (17). Whether MFAP4 has a similar potential as biomarker in pulmonary fibrosis has not been investigated. Therefore, the aim of the current study was to assess whether MFAP4 serum levels are elevated in patients affected by idiopathic pulmonary fibrosis (IPF), a chronic progressive fibrotic lung disease, or by chronic hypersensitivity pneumonitis (HP), a disease with fibrotic and inflammatory components, and to evaluate the potential role of serum MFAP4 as a biomarker for pulmonary fibrosis in clinical practice.

A further aim of the current study was to determine whether MFAP4 deficiency in mice has any

effect on the formation of pulmonary fibrosis following bleomycin challenge as a model for human lung fibrosis.

Our hypothesis is that MFAP4 might have a role in development, structure and remodeling of the lungs, and therefore, as being involved in the formation of fibrosis in the lungs, that increased systemic levels might indicate the presence of lung disease.

Methods

Patients

We retrospectively studied consecutive patients with a diagnosis of IPF or chronic HP admitted to the Ruhrlandklinik (Essen, Germany) between 2008 and 2010. 91 IPF patients, 22 patients with chronic HP, and 31 healthy controls (HC) were studied. The diagnosis of IPF was established according to the ATS/ERS guidelines 2011.

HP was diagnosed according to the following criteria: (1) a history of exposure to organic antigens, (2) clinical signs and symptoms consistent with HP (3), radiologic features and/or functional abnormalities characteristic of interstitial lung disease (4), evidence of serum precipitins against one or more organic antigens, and (5) BAL fluid with increased lymphocytes.

The study was approved by the local IRB (06-3170). All patients and healthy volunteers gave written informed consents and permission to use their samples.

Sampling and laboratory measurements

Blood samples were collected by vein-puncture. Serum was separated by centrifugation at 1500 g for 15 minutes. Aliquots of serum were stored at -80°C until use. The time from collection to frozen storage was no longer than 60 minutes.

Serum MFAP4 was measured by enzyme-linked immunosorbent assay (ELISA) as described before (19). This assay also measures murine serum MFAP4. Quality controls made from culture supernatant diluted to 625 mU/ml and 150 mU/ml, respectively, were included on each plate. All sera were tested in duplicates diluted 1:100 and if out of range retested in appropriate dilution.

Serum SP-D was measured with a commercially available ELISA kit (Yamasa, Chiba, Japan) according to the manufacturer's instruction (21). Serum LDH was measured routinely (upper limit of normal in our laboratory: 225 U/l).

Pulmonary Function Tests

Measurements included vital capacity (VC), FEV1, TLC, DLCO and DLCO/VA and arterial blood gas analysis.

Animal model – bleomycin treated MF4P4+/+and MFAP4-/- mice

C57Bl/6 mice deficient in MFAP4 were bred in-house (Schlosser et al., 2014, unpublished data). Lights in the animal facility were switched on from 6.00 to 18.00 hours and the temperature was maintained at 20-22°C with a relative humidity of 40-60%. The animals were fed *ad libitum* with a standard rodent chow. Water was constantly available. The study protocol was approved by The Animal Experiment Inspectorate of Denmark.

C57Bl/6N *Mfap4+/+*and *Mfap4-/-*littermate mice between 6-8 weeks of age were used for the experiments. The mice received either 2,5 mg/kg bleomycin (Bleomycin sulfate from Streptomyces verticillus, SIGMA-Aldrich) orsaline byoropharyngeal administration and using 4% isoflurane (IsoFlo Vet, Orion Pharma) mixed with 0,4 L/min $O_2$for anesthesia. The mice were terminated after 22 days and the left lung was inflated with 4% formaldehyde at a constant 25 cm H_20 pressure. Obtained sections were used for immunohistochemical staining for MFAP4 or Masson's Trichrome stain for collagen. The right lung was used for measurement of collagen content. Some mice were terminated7 days after administration and BAL fluid was obtained by injecting and drawing out 1 mL of saline from the lungs a total of three times.

Morphometric assessment of collagen content in the lung

Using the CAST software (Olympus), the operator added up the total number of points out of 70x25 software selected points that had touched a collagen stained area, and a percentage of the total number of points that were within the tissue could be calculated.

Lung tissue collagen content

Lung tissue was homogenized using 500mg homogenization beads (24 homogenizer, Precellys) in 0,5 ml RIPA-bufferand 10 µL protease inhibitor cocktail (Protease Inhibitor Cocktail with mammalian cell and tissue extracts, DMSO solution, SIGMA). The lungs were homogenized at 6200 rpm in2 x 20 sec. with a 20 sec break in between. Following the tubes were centrifuged at 10,000 rpm for 10 min. The Sircol kit Sircol (Biocolor) was following used according to the manufacturers' instructions.

BAL cell differential count

The BAL fluid was centrifuged at 500xg for 5 minutes and the pellet was resuspended in 1 ml PBS. A total cell count was made using tryphan blue dye exclusion method. 100.000 cells were subsequently used for differential count using the cytospin method. A differential cell stain was made by first fixating in methanol-1, drying, dipping in eosin solution and then dipping in methylene blue solution.

Statistical analysis

Data were expressed as mean ± standard deviation. Student's T-tests, ANOVA (in combination with analysis of covariance) for comparison of two or multiple groups respectively, were used for normally distributed variables (log concentrations of MFAP4, SP-D and LDH were used) followed by Tukey's HSD post-hoc test. Comparison of non-normally distributed variables between two groups was done with the Mann-Whitney U test. Spearman correlation analysis was used to test whether parameters of pulmonary function or blood gas analysis correlate with serum levels of MFAP4, SP-D and LDH.

Statistica 10 software package (StatSoft, Tulsa, OK, USA) was used for the above mentioned analyses. OriginPro 8.5G (OriginLab, Northampton, MA, USA) was used for receiver operating characteristic curve (ROC) analysis to test the power of biomarkers to detect fibrosis.

Comparison of categorical variables between the three groups was done by the Fisher's exact probability test using R version 2.14.1 (The R Foundation for Statistical Computing). Differences were considered statistically significant when the p value was < 0.05.

Results

Patients' characteristics

Demographics and patients' characteristics are summarized in the Table 1. The groups were not well matched for age and gender, so we analyzed their influence as a confounder and included them as covariates in the statistical analyses if necessary.

Table 1. Demographics and patients´characteristics

	IPF	HP	HC	p
Patients(n)	91	22	31	
Gender (M/F)	65/26	11/11	9/22	< 0.001
Age, years (mean ± SD)	68± 10	54± 14	40 ± 10	< 0.001
Smoking habits(n=41/10/0)				0.86
Current smokers	3	0	n.a.	
Ex-smokers	25	6	n.a.	
Never-smokers	13	4	n.a.	
Pulmonary function at diagnosis				
FEV1, %pred. (n=81/20/0)	69±18	57±16	n.a.	0.004
FVC, % pred. (n=82/22/0)	68±18	62±20	n.a.	0.2
TLC, % pred. (n=77/19/0)	63±16	64±16.5	n.a.	0.9
DLCO, % pred. (n=66/21/0)	47±15	45±14	n.a.	0.7
Blood gas analysis at diagnosis				
PaO2, mmHg (n=61/22/0)	70±14	71±14	n.a.	0.7
PaCO2, mmHg (n=61/22/0)	40±9	39.5±4	n.a.	0.7
(A-a)DO2, mmHg (n=37/22/0)	28±13	31±10	n.a.	0.3

n.a.= not available

Serum levels of biomarkers

MFAP4 serum levels were 13.4±5 U/ml in healthy controls (HC), 14.5±6.5 U/ml in IPF patients, and 12.4±5 U/ml in HP patients. There was no difference between the groups (overall p= 0.09 ANOVA including gender) (Figure 1).

SP-D serum levels were 19±18 ng/ml in HC (n=26), 83±68 ng/ml in IPF patients (n=84) and 175±190 ng/ml in HP patients (n=20) (overall ANOVA p < 0.000001, Tukey HSD Test p=0.000022 (HC, HP), p=0.000022 (HC, IPF), p=0.09 (HP, IPF) (Figure 2).

LDH serum levels were 192±28 U/l in HC (n=31), 288±105 U/l in IPF patients (n=83) and 256±77U/l in HP patients (n=19). There was a significant difference in LDH serum levels between controls and both the IPF and HP patients but not between IPF and HP patients (overall ANOVA p<0.000001, Tukey HSD Test p=0.0089 (HC, HP), p=0.000022 (HC, IPF), p=0.18 (HP, IPF)) (Figure 3).

ROC analysis

MFAP4, Serum SP-D and LDH were tested for their value to discriminate IPF patients from HC. (Figure 4). Both SP-D and LDH showed a discriminative power, whereas MFAP4 did not (AUC of 0.53). For SP-D we estimated an AUC of 0.91 ($p=1.9 * 10^{-10}$); using the cut off at 33 ng/ml, SP-D reached a sensitivity of 81% and a specificity of 85%

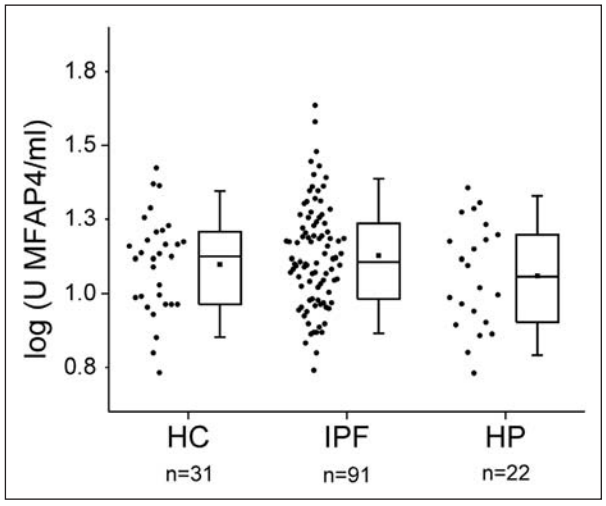

Fig. 1. Scatter plot and box plot of MFAP4 serum concentrations in healthy controls (HC) and patients suffering from IPF and HP. The small black circle and the horizontal line mark the mean and the median of MFAP4 serum concentrations respectively. The large boxes constitue 50% of the measurments, whereas the error bars mark the 1.5 fold standard deviation.After log transformation, values are normally distributed.No significant difference could be established between the three analysed groups

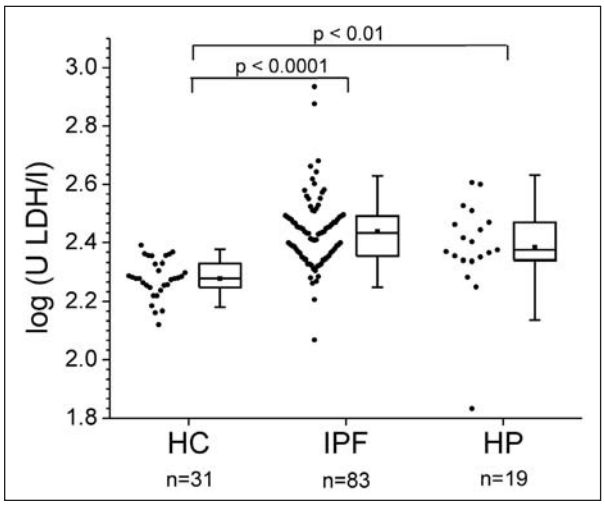

Fig. 2. Scatter plot and box plot of LDH serum concentrations in healthy controls (HC) and patients with IPF and HP. The small black circle and the horizontal line mark the mean values and the median of LDH serum concentrations respectively. The large boxes constitue 50% of the measurments, whereas the error bars mark the 1.5 fold standard deviation. After log transformation, values are normally distributed

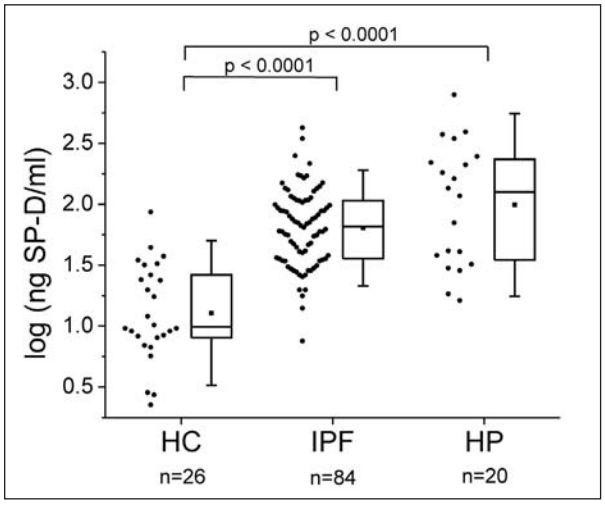

Fig. 3. Scatter plot and box plot of SP-D serum concentrations in healthy controls (HC) and patients with IPF and HP. The small black circle and the horizontal line mark the mean values and the median of SP-D serum concentrations respectively. The large boxes constitue 50% of the measurments, whereas the error bars mark the 1.5 fold standard deviation. After log transformation, values are normally distributed

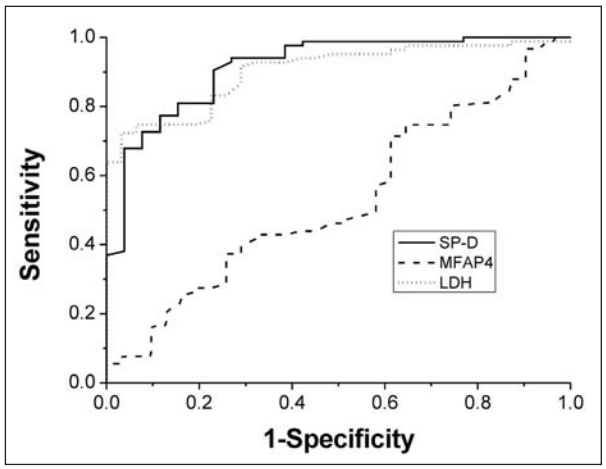

Fig. 4. Receiver operating characteristic curve based on SP-D (n = 84/26), MPAP4 (n = 91/31) and LDH (n = 81/31) serum levels in patients suffering from IPF and healthy controls. MFAP4 showed no diagnostic value for IPF detection in contrast to SP-D exhibiting 81% sensitivity at a specificity of 84.6% and LDH reaching 72.3% sensitivity at 96.8% specificity

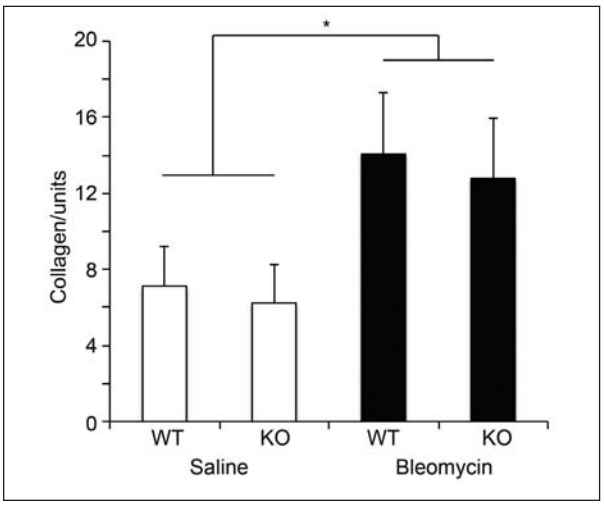

Fig. 5. The collagen content of the right lung measured using the Sircol assay twentyseven days after oropharyngeal administration of bleomycin or control treatment. (N = 9-13) WT = Mfap4+/+, KO = Mfap4-/--/-. Data are mean+SD.

for discriminating IPF patients from healthy controls. The AUC for LDH was 0.90 ($p=5 * 10^{-11}$); at the cut off at 233U/l, LDH reached a sensitivity of 72% at specificity of 97%.

Correlations of serum biomarkers with PFT and blood gas analyses

We didn't found any correlation between MFAP4 serum levels and PFTs (TLC, FEV1, IVC, KCO, DLCO) or blood gas analysis parameters ($PaCO_2$, PaO_2, O_2 saturation, $AaDO_2$) as well as correlations between MFAP4 levels and SP-D or LDH.

Bleomycin-induced pulmonary fibrosis in MFAP4+/+ and MFAP4-/- mice

ANOVA analysis determined that there was no significant association between the *Mfpa4*-genotype and the newly formed collagen content in the lung, p=0.7, as measured by the Sircol kit. As expected, there was a significant association between treatment and collagen content in the lung, p=0.01. Post hoc T-test analysis demonstrated that the bleomycin treatment significantly induced an increase in the measured amount of newly formed collagen in the lung by 2 fold, p<0.005 (Figure 5).

Masson's trichrome staining was additionally used to quantify the relative increase in the total collagen content in bleomycin-induced fibrosis (Figure 6), which was significantly associated to bleomycin treatment, p<0.005.Post hoc T-tests demonstrated that total collagen was significantly induced by 2.29 fold in mice treated with bleomycin, p<0.005.

In the lung of saline treated *Mfap4-/-* mice the collagen-staining was concentrated around blood

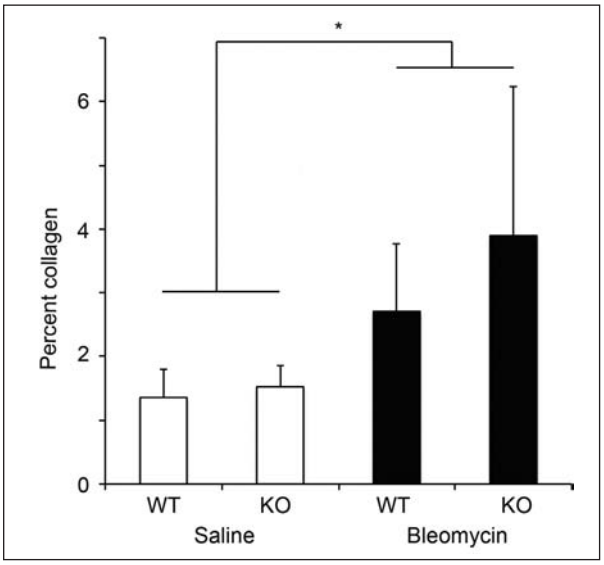

Fig. 6. Relative quantification of collagen in the left lung twentytwodays after oropharyngeal administration ofbleomycin by morphometric analysis of Masson's trichrome stained sections (N = 9-13).WT = *Mfap4+/+*, KO = *Mfap4-/-*.Data are mean+SD

vessels and bronchi, and it was not seen in the parenchyma (Figure 7A).The observed focal fibrotic lesions were generally subpleural and extended to varying degree into the lung parenchyma (Figure 7B). There was no apparent difference between the *Mfap4*-genotypes (Figures 7C+D). MFAP4 was predominantly located to the elastic fibers of blood vessels and to the basal membrane beneath epithelial cells of bronchioles as previously described.

Measurement of murine serum and BAL MFAP4

Anova analysis determined that serum MFAP4 was not associated to the bleomycin treatment, p = 0.41. An apparent decrease in serum MFAP4 was seen during the experimental period; however this observation was not repeated between two independent experiments.

Corresponding measures of sMFAP4 and BAL MFAP4 were obtained from seven mice seven days after administration of bleomycin. T-student test determined that MFAP4 in BAL was significantly associated to treatment, p = 0.0012, with a 63 fold induction (Figure 8). Like in the previous experiments, there was no induction of systemicMFAP4 by the bleomycin treatment and Pearsons correlation analysis of sMFAP4 and MFAP4 in BAL thus showed no association between the two measurements, r =-0.0136, p=0.98.

Pulmonary influx of inflammatory cells

There was no detectable influence of MFAP4 deficiency on the total cell count, or differential cell count (p =0.15 and p=0.77 respectively) in the utilized model (data not shown). The BAL fluids ob-

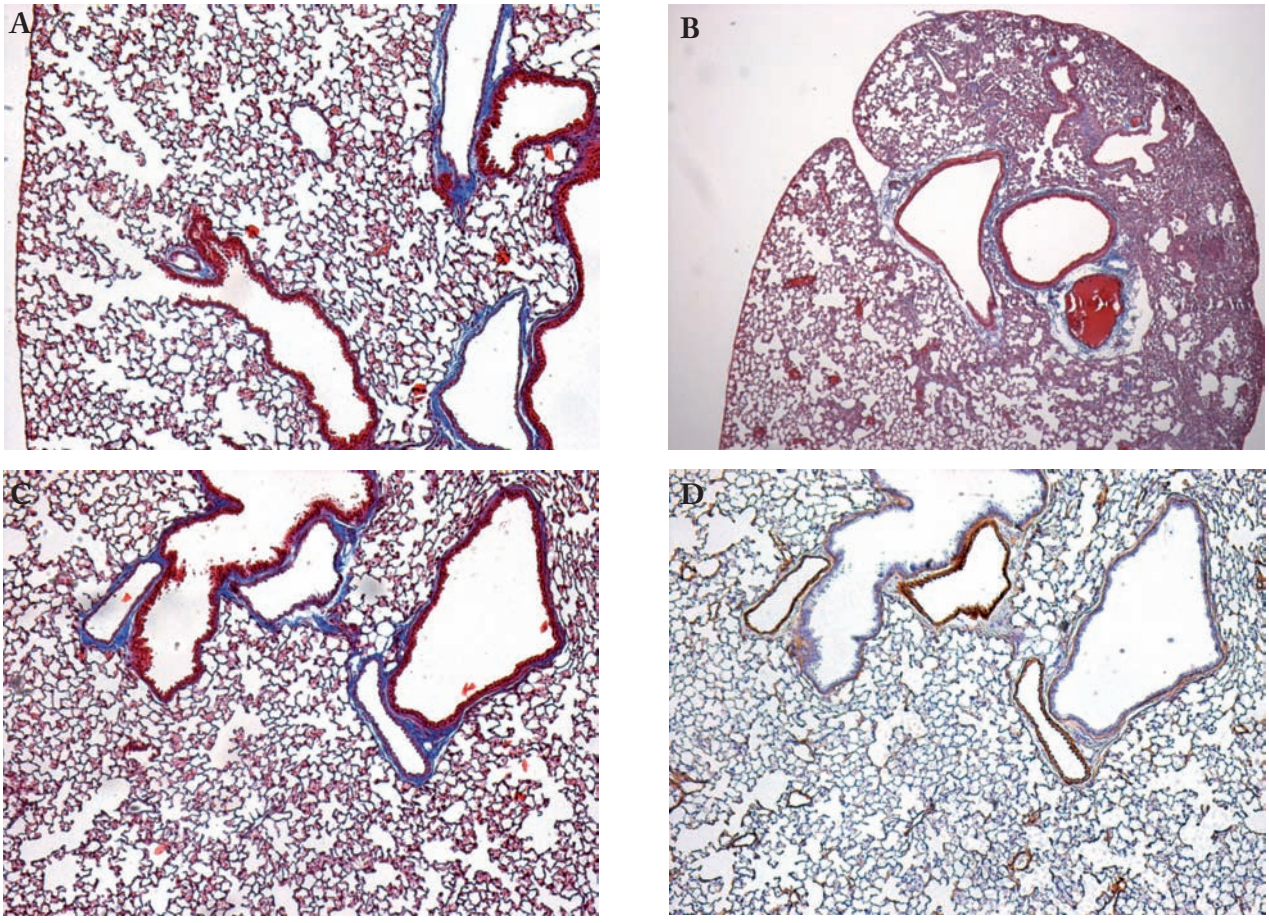

Fig. 7 A-D. Lung histology of mice treated with oropharyngealadministration of bleomycin. Masson's trichrome staining (A and C), Monoclonal antiMFAP4 antibody (HG-HYB71-4) (B and D). (A-B)*Mfap4+/+* treated with bleomycin. (C-D)*Mfap4-/-* treated with bleomycin. Original magnification X

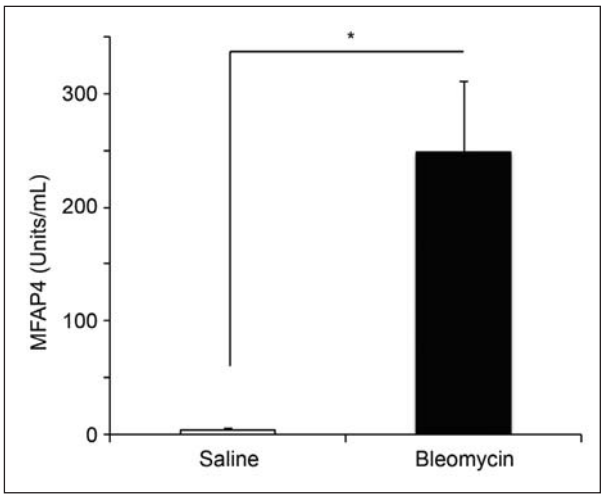

Fig. 8. The MFAP4 in BAL fluid of *Mfap4+/+*mice 7 days after administration treated with oral bleomycin (N = 3-4).WT = Wildtype. Data are mean+SD.

tained from mice receiving saline were dominated by macrophages (more than 90%) irrespective of the genotype.

Discussion

The human microfibrillar associated glycoprotein 4 (MFAP4) is a ubiquitous protein playing a potential role in extracellular matrix (ECM) turnover and was recently identified as a biomarker for hepatic fibrosis in hepatitis C patients (17).

The current study showed that MFAP4 was not elevated in patients with pulmonary fibrosis in comparison to healthy controls. Furthermore, no differences were seen between IPF and HP patients. The two known biomarkers for pulmonary fibrosis, serum SP-D and LDH, both had a discriminative power between patients with pulmonary fibrosis and healthy controls. No correlations were found between serum MFAP4 and pulmonary function tests or blood gas analyses in IPF patients.

In the bleomyin mouse model of lung fibrosis, data from wild type and MFAP4 -/- mice showed twenty-two days after oropharyngeal bleomycin administration that MFAP4 deficiency does not have a major influence on the progression of bleomycin induced pulmonary fibrosis. There was no significant difference in the amount of lung collagen between WT mice and MFAP4 deficient mice. Moreover, the BAL fluid cell differential count was not different between WT and MFAP4 -/- mice, seven days after oropharyngeal bleomycin administration.

Pulmonary fibrosis is a very complex disease, which involves many parts of the immune system, as well as local tissue and fibrocytes, and has a variable etiology. We can only conclude that MFAP4 is not a major player in the formation of bleomycin-induced pulmonary fibrosis in the mouse model. The apparent lack of involvement in the development of pulmonary fibrosis in the mouse model is in line with the clinical data showing that this biomarker is not useful in IPF and HP patients.

Data on the source of MFAP4 and its kinetic are poor which consequently complicates the interpretation of the serum MFAP4 levels in IPF / HP patients and in the animal model. A small experiment including seven WT mice showed that direct administration of bleomycin led to a highly significant, 63 fold induction in BAL MFAP4 when compared to controls, seven days after administration. This could indicate that MFAP4 production is upregulated in the lungs during the early inflammatory phase of the bleomycin model of lung injury but not in the late fibrotic phase. It is possible that MFAP4 leaks into the alveolar space following acute lung inflammation and injury, either from the blood through the tissue, or as a consequence of increased local MFAP4 production, or possibly both.

IPF presents peculiar aspects in the fibrogenesis. First, abnormal wound healing but not inflammation appears to play a pivotal role in the pathogenesis of IPF (29, 30). In the liver, inflammation represents the first step leading to fibrotic liver: Parenchymal cell injury and the subsequent inflammatory reaction induce transdifferentiation of vitamin A–storing hepatic stellate cells into myofibroblasts, which synthesize collagen, matrix proteins, and a broad range of inflammatory and anti-inflammatory cytokines, chemokines, and growth factors. Second, in contrast to hepatocytes, pneumocytes can directly differentiate into myofibroblasts via EMT (12). Pneumocyte-derived myofibroblasts represent the major population (60-80%) of collagen producing cells in the fibrotic lung (27). In the fibrotic liver, resident macrophages (Kupffer cells) are the main source of TGFβ-1 (23-26) and induce collagen production by hepatic stellate cells as they differentiate into activated myofibroblasts. Hepatic stellate cells-derived

myofibroblasts represent 80% of all collagen-producing cells 20% of hepatic myofibroblasts are recruited from bone marrow cells (26). In addition, bone marrow derived cells, circulating mesenchymal precursor cells and fibrocytes migrate to the damaged liver and correspond to 5 to 7% and 4 to 6%, respectively, of all collagen producing cells (26). It can only be speculated that the different proportion of bone marrow derived myofibroblasts between lung and liver explain a difference in the pattern of extracellular matrix proteins.

With regard to the spill-over mechanisms of MFAP4 into the blood, it is known that the augmented permeability of the endothelial barrier is a common phenomenon in hepatic and lung fibrosis, above all in the early stages (31). MFAP4 is a peptide with a mass between 36 and 250 kDa according to the reduced or unreduced state, respectively, and co-localize with surfactant-Protein A (SP-A) in the interalveolar septum, mostly in chronic inflamed lung tissue (31). It is possible that the low presence of inflammation in lung fibrosis and the binding to surfactant proteins in the fibrotic alveolar septum are the reason why MFAP4 is not released into the blood. Another explanation could be that MFAP4 is immobilized by binding to the elastic fibers in the alveolo-arterial membrane. Further investigations are needed to elucidate this.

There are several limitations of this study. Regarding the animal model with *Mfap4*+/+ and *Mfap4*-/- deficient mice, blood samples were taken between 9 am and 15 pm. There has not been any research into the murine variation of sMFAP4, and circadian variation of sMFAP4 could be a source of variation.

The studied patients groups could not be well matched per age and gender, due to the fact that HP patients are usually younger than IPF patients. This may affect the levels of biomarkers in serum.

Concluding, even if similarities exist between liver and lung fibrosis, the pattern of biomarkers associated with fibrosis secreted by hepatic and pulmonary myofibroblasts seems to be different. MFAP4 seems to be a useful serum biomarker for hepatic but not for pulmonary fibrosis, and has no apparent effect in a mouse model of fibrosis.

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