Review

Pathogenesis of combined pulmonary fibrosis and emphysema

Common pathogenetic pathways

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INTRODUCTION

Emphysema is defined on pathology grounds as an abnormal permanent enlargement of air spaces distal to the terminal bronchioles, accompanied by the destruction of alveolar walls and without obvious fibrosis[1]. Idiopathic Pulmonary Fibrosis (IPF) has a distinct appearance on HRCT defined as the UIP and possible UIP pattern[2]. Although initially considered as two separate disorders (as it is clearly depicted in the definition of emphysema), emphysema and fibrosis have been found to co-exist. The widespread use of HRCT given its high accuracy for the diagnosis of emphysema and IPF helped in recognizing the co-existence of these two diseases. The association of IPF and emphysema was initially described by Wiggins et al in 1990[3]. The term Combined Pulmonary Fibrosis and Emphysema (CPFE) was first proposed by Cottin et al[4], who retrospectively described a homogenous group of 61 patients with both emphysema of the upper zones and diffuse parenchymal lung disease with fibrosis of the lower zones of the lungs on chest computed tomography. The patients were all smokers, male (but one), presenting with dyspnea on exertion, relatively preserved lung volumes and a disproportionate reduction in DLco. Although in the pivotal study by Cottin, patients with connective tissue disease were excluded, the CPFE syndrome has been described in the latter group[5]. Interestingly, in a retrospective study by Tzouvelekis et al[6], a significant increased number of CPFE patients exhibited elevated serum ANA with or without positive p-ANCA titers compared to patients with IPF without emphysema. Furthermore, patients with CPFE and positive autoimmune markers (mainly ANA) exhibited improved survival compared to patients with a negative autoimmune profile. This improvement in survival was also correlated with the presence of a massive infiltration of clusters of CD20+ B cells forming lymphoid follicles[6].

Whether the co-existence of emphysema and fibrosis is pure coincidence, due to the presence of a common predisposing factor (i.e. smoking) or it is related to shared pathogenetic mechanisms, is not yet clarified. However there are indications supporting the latter theory. Further studies are needed in order to reach a more robust conclusion.

DEFINITION

CPFE is defined by the co-existence of emphysema and pulmonary fibrosis based on HRCT. This definition is broad as it includes patients with any amount of emphysema and any type of fibrosis.

This broad definition has some limitations. The absence of a specific threshold for emphysema leads to the possible inclusion of patients with limited emphysema of no clinical significance. This can underscore the actual impact of emphysema in the natural course of CPFE. Several groups have proposed diagnostic criteria for CPFE that include a threshold of 10% emphysema visible on HRCT.

The inclusion of multiple types of ILD in the definition, leads to a heterogeneous cohort. This makes it impossible to draw comparative conclusions regarding the natural course of CPFE against a specific ILD. For example, it is well established that IPF has a better prognosis compared to iNSIP and also collagen tissue related interstitial lung diseases (CTD-ILD). Hence, the inclusion of iNSIP or CTD-ILD patients in the CPFE population can produce a bias in favor of CPFE when compared to IPF. When studying clinically meaningful endpoints (rate of FVC decline, rate of DLco decline, hospitalizations, acute exacerbations of ILD), the type of fibrotic pattern should be defined. Given the fact that IPF is the commonest ILD, special interest should be given to the syndrome emphysema-IPF, as it can have serious implications in the rate of FVC decline and thus in the evaluation of outcome and impact of therapeutic approaches.

PATHOGENESIS

The relationship between smoking and emphysema is well established. Also, smoking is known to be related with certain interstitial lung diseases as respiratory bronchiolitis interstitial lung disease (RB-ILD), desquamative interstitial pneumonia (DIP), pulmonary Langerhans' cell histiocytosis (PLCH) and IPF[7,8]. It is noteworthy that in the latest classification of the Idiopathic Interstitial Pneumonias smoking is used as a classification element.[9] Furthermore, in recent years there is an increasing amount of literature regarding a new form of smoking related interstitial disease linking smoking with fibrosis[10-15]. It comprises of distinctly circumscribed fibrotic lesions that radiate from a respiratory bronchiole to the pleura. The interstitial fibrosis is mixed with emphysematous changes. On HRCT these lesions correspond to localized areas of reticulation concentrated about regions of upper and mid zone emphysema[15]. Several names have been proposed for this distinct interstitial lung disease as respiratory bronchiolitis–interstitial lung disease with fibrosis[10], smoking-related interstitial fibrosis (SRIF)[11,12] airspace enlargement with fibrosis[13,14] and respiratory bronchiolitis with fibrosis (RBF)[15]. Although in the majority of cases RBF doesn't seem to have functional consequences, it provides an additional link between emphysema and fibrosis.

Based on the above, it is not surprising that there is an extremely strong association between smoking and CPFE. Almost all CPFE patients (98%)[16] are current or former smokers. Below we will describe the pathogenesis of CPFE focusing on those aspects that support common pathogenetic routes in favor of a mere co-existence due to a common etiological factor. Smoking is a common predisposing factor for both conditions. However, it seems that in patients with CPFE there are diverse susceptible genetic backgrounds that result in the development of both emphysema and fibrosis.

1. Senescence-Telomeres

A characteristic example of such genetic background is related to accelerated senescence via telomere length shortening. A telomere is a region of repetitive nucleotide sequences at each end of a chromatid. Telomeres shorten with each cell division. Thus, they act as disposable buffers at the ends of chromosomes. When telomeres reach a critical threshold (Hayflick limit), they are sensed as double stranded DNA break and trigger a DNA damage response that provokes cellular senescence and apoptosis[17]. Telomerase is a specialized polymerase that renews telomeres. It has two core components: TERT, the telomerase reverse transcriptase, and TERC, the telomerase RNA that provides the template for telomere repeat addition[18,19].

Interestingly, telomerase mutations leading to short telomeres have been implicated in the pathogenesis of emphysema[20] and IPF[21]. Recently it was shown that telomerase mutations may be associated with a more severe phenotype of emphysema with a frequent occurrence of pneumothorax, especially in women[22]. Mutations in TERT and TERC are risk factors for pulmonary fibrosis underlying the inheritance in 8–15% of familial cases[23,24]. Mutations in the essential telomerase genes are also found in 1–3% of sporadic IPF cases[25,26]. Furthermore, IPF patients have shorter telomeres than age-matched controls, even when no mutation in telomerase is detected[25,26]. Based on the above it is expected that telomerase mutations have been proposed as a possible common link for the development of CPFE[27,28].

Telomerase mutations have important clinical implications. Since they are not restricted to the lungs the term "telomere syndrome" has been proposed[28]. This contradicts the classic view that IPF has no extra-pulmonary manifestations. When IPF is associated with telomerase mutations, it is in fact part of a telomere-mediated syndrome[29]. Other manifestations of this syndrome can be present as well.

Indeed, IPF patients are at increased risk for the development of diseases that have been associated with short telomeres as liver cirrhosis[25], diabetes[30,31] and bone marrow failure. Bone marrow failure can manifest as red blood cells macrocytosis, single lineage cytopenias, aplastic anemia, myelodysplastic syndrome, or acute myeloid leukemia[23,32-34]. The syndrome complex of IPF and bone marrow failure is specific for the presence of germline defects in telomerase[35]. Thus, in IPF patients, a high degree of suspicion for the presence of hematologic abnormalities is needed.

The presence of hematologic abnormalities can be extremely deleterious regarding outcome, when IPF patients need to receive marrow suppressive drugs in the context of chemotherapy or immunosuppressive drugs after lung transplantation. IPF patients with telomerase mutations that were subjected to lung transplantation, showed an increased rate of hematological complications and renal failure requiring renal replacement therapy[36].

2. SFTP

Another susceptible genetic background involves mutations in genes encoding surfactant proteins C and A2 (SFTPC and SFTPA2). Mutations in SFTPC and SFTPA2 have been implicated in the development of pulmonary fibrosis. They exert their fibrogenic effect by increasing the levels of endoplasmic reticulum stress and activating the unfolded protein response (UPR)[37-39]. Cottin et al reported a case of CPFE in a never smoker, 32 year old female with a dominant I73T mutation in the SFTPC gene. Her infant (bearing the same mutation) was also diagnosed with interstitial lung disease[40]. Other genes involved in surfactant metabolism, such as ATP-binding cassette A3 (ABCA3) are also associated with pulmonary fibrosis[41-43]. Epaud et al reported a case of CPFE in a nonsmoker, 41 year old male with mutations in the ABCA3 gene[44].

3. MMP-9 and TGF-β

Matrix-metalloproteinase (MMP)-9 has been implicated in the pathogenesis of emphysema and lung fibrosis. Sputum MMP-9 concentrations in COPD patients are directly associated with the extent of emphysema measured by CT and MMP-9 expression levels are inversely associated with DLco[45]. Regarding fibrosis, it is important that MMP-9 proteolytically cleaves latent TGF-β, leading to its activation[46].TGF-β is known to play a pivotal role in the development of IPF as it induces fibroblast chemotaxis, proliferation and transdifferentiation into myofibroblasts, and it largely promotes the production and secretion of extracellular matrix compounds, mainly collagen[47]. Furthermore, it seems that TGF-β plays an important regulatory role in the transcription of MMP-9 via the Smad3 signalling pathway. TGF-β ligand activates TGF-β receptor/Smad3 signalling, which in turn collaboratively activates SIRT1 transcription with c-Jun. Subsequently, SIRT1 can deacetylate NF-κB at lysine 30, as well as histones adjacent to the transcription factors AP-1, NF-κB, and Pea3 binding sites of the MMP9 promoter. The final result is the suppression of MMP-9 transcription (Figure 1). When Smad3 is missing, this regulatory pathway is neutralized so that MMP9 is epigenetically activated[48,49]. These developmental epigenetic mechanisms by which Smad3 regulates MMP9 transcription may be important in modulating both emphysema and pulmonary fibrosis. This could explain why both pathologies can appear within the same lung specimen[49,50].

4. TNF-α, PDGF-β

Lunndbland et al demonstrated that in transgenic mice, TNF-α overexpression caused pathologic changes consistent with both emphysema and pulmonary fibrosis[51]. Hoyle et al studied transgenic mice that expressed the platelet-derived growth factor-B (PDGF-β) gene from the lung-specific surfactant protein C (SPC) promoter. Results of these studies indicated that overexpression of PDGF-β induced distinct abnormalities in the developing and adult lung that led to a complex phenotype encompassing aspects of both emphysema and fibrosis[52].

5. Neutrophil elastase

In bleomycin treated mice Lucatelli et al demonstrated that emphysematous and fibrotic lesions can be significantly attenuated by using a protease inhibitor active against neutrophil elastase. Also, in a strain of mice that developed both emphysema and fibrosis after chronic cigarette-smoke exposure, the presence of neutrophil elastase in alveolar structures was associated with a positive immunohistochemical reaction for both TGF-β and TGF-α. This led to the suggestion that neutrophil

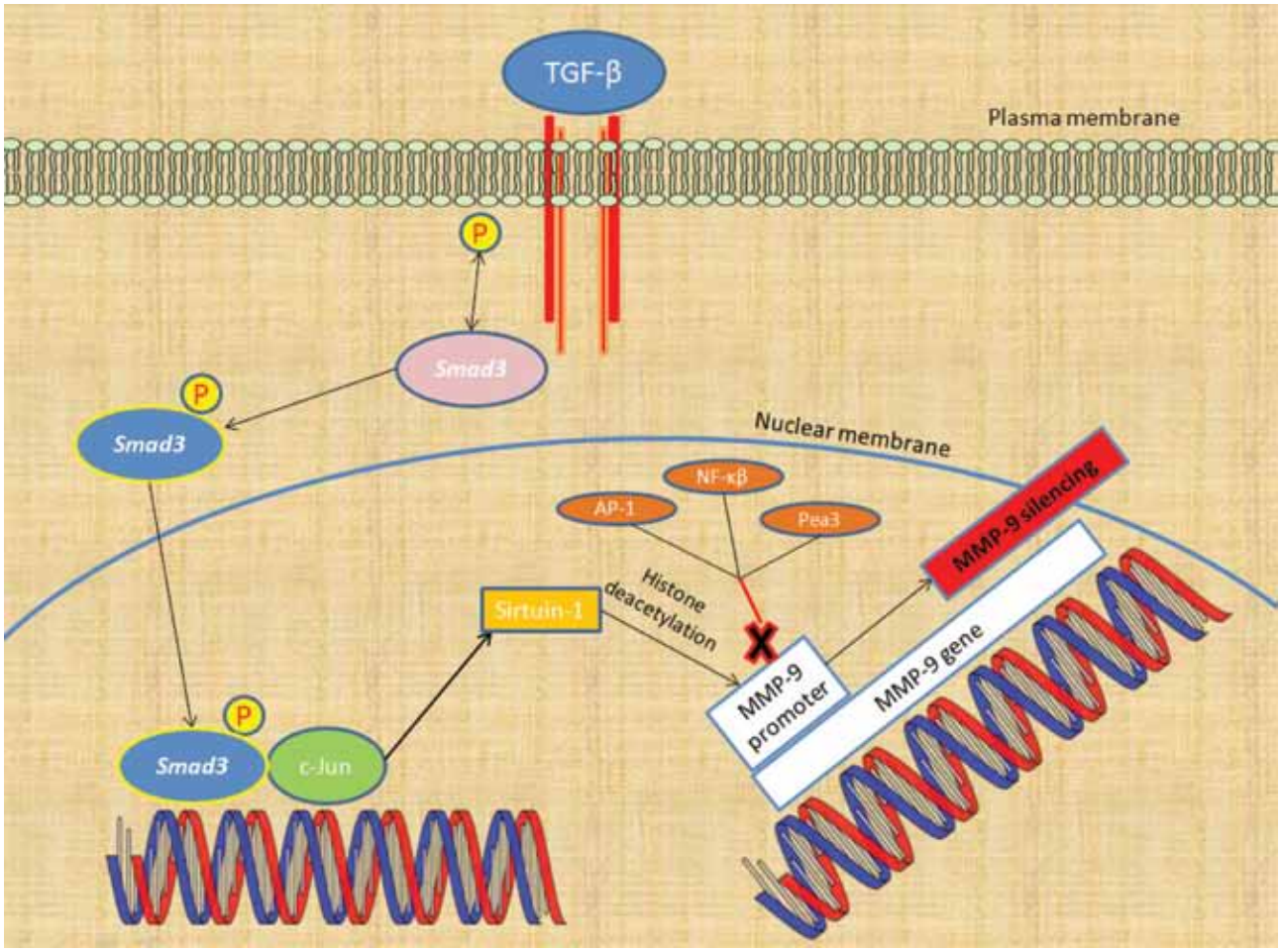

FIGURE 1. Regulation of MMP-9 transcription by TGF-β via Smad3. TGF-β ligand activates TGF-β receptor/Smad3 signalling, which in turn collaboratively activates SIRT1 transcription with c-Jun. Subsequently, sirtuin 1 deacetylates histones adjacent to the binding sites of the MMP9 promoter regarding transcription factors AP-1, NF-κB, and Pea3. The final result is the suppression of MMP-9 transcription.

elastase could be the missing link between emphysema and fibrosis[53]. It is possible that neutrophil elastase is involved in the release of the active TGF-β from its initial inactive complex. This complex comprises from TGF-β, latent associated peptide (LAP) and latent TGF-β binding protein (LTBP)[54].

6. CXCL5 and CXCL8

CXCL5 and CXCL8 (epithelial neutrophil activating peptide 78, and interleukin 8) have been found elevated in patients with IPF[55,56]. CXCL8 is known to be implicated in the pathogenesis of emphysema[57]. Tasaka et al[58] examined cytokine levels and differential cell counts in bronchoalveolar lavage (BAL) from patients with IPF. The concentrations of CXCL5 and CXCL8 were significantly greater in patients with concomitant emphysema. CXCL8 concentrations were significantly correlated with the absolute numbers of neutrophils in BAL fluid and with the extent of emphysema on HRCT and inversely correlated with FVC.

7. Interleukin 13

Overexpression of interleukin 13 (IL-13) in a murine model resulted in the development of lung fibrosis, and airspace enlargement (emphysema)[59]. Recently, it was shown that inhibition of IL-13 via overexpression of IL-13Rα2 resulted in inhibition of bleomycin induced pulmonary fibrosis[60]. This is in line with previous observations where blocking IL-13 with tralokinumab in a humanized mouse model of IPF inhibited lung fibrosis and reduced

epithelial apoptosis in the lung[61].

8. Caveolae

Caveolae are 50 to 100 nm flask-shaped invaginations of the plasma membrane enriched in cholesterol and glycosphingolipids. Caveolin-1, the structural protein component of caveolar membranes, acts as a scaffolding protein to concentrate and functionally regulate signaling molecules. Oxidative stress upregulates Caveolin-1 protein expression. Caveolin-1 plays an important role in the development of emphysema through activation of the ATM-p53-p21 pathway. The development of pulmonary emphysema was significantly inhibited in caveolin-1 null mice compared to wild type mice after 6 months of exposure to cigarette smoking[62]. Regarding IPF, caveolin-1 expression was found reduced in lung tissues and in primary pulmonary fibroblasts from idiopathic pulmonary fibrosis patients, compared with controls[63]. It is very interesting that restoration of caveolin-1 function in skin fibroblasts from patients with scleroderma reverses their pro-fibrotic phenotype while skin from caveolin-1 null mice exhibits many characteristics found in the skin of scleroderma patients[64].

9. T-regulatory cells (Treg)

Treg (CD4+CD25+FOXP3+) play a key role in immune regulation. They generally suppress or downregulate induction and proliferation of effector T cells[65]. In IPF there is a global impairment of Treg[66]. This impairment is not only numerical but also functional. Specifically, Treg from IPF patients exhibited a defective antiproliferative activity and a defective suppression of Th1 and Th2 cytokine secretion. This functional impairment was strongly correlated with a decrease in FVC, DLco and TLC[66]. In lung tissue from emphysema patients[67] and also in the small airways of COPD patients, an impairment of Treg has also been observed[68]. In COPD patients, Hou et al[69] dissected Treg into three distinct subpopulations based on the intensity of FOXP3 expression and the secretion of proinflammatory cytokines: denominated resting, active and cytokine secreting Treg. In accordance to the previous studies there was an imbalance between different Treg subsets in peripheral blood and BAL. Specifically, there was a downregulation of suppressive Treg and an increased proportion of the cytokine-secreting non-Treg subpopulation in patients with COPD when compared to smokers with normal pulmonary function tests[69]. The impairment of Treg in emphysema and COPD is correlated with a decrease in FEV1[68,69]. Thus, Treg impairment provides an intriguing common pathway between IPF and emphysema and a potential therapeutic target[70].

SYNOPSIS

CPFE vividly illustrates the importance of thinking

TABLE 1. Common pathogenetic mechanisms in emphysema and pulmonary fibrosis

Category	Mechanism	Comments
Susceptible genetic background	Telomerase mutations (TERT, TERC)	8–15% of familial cases and 1–3% of sporadic IPF cases
	Mutations in SFTPC, SFTPA2, ABCA3	Case reports
Cytokines	CXCL5	Increased in BALF from CPFE patients
	CXCL8 (Interleukin 8)	Increased in BALF from CPFE patients
	Interleukin 13	Possible therapeutic target
	TNF-α	Overexpression in a murine model resulted in combined emphysema/lung fibrosis
Growth Factors	TGF-β	Pivotal cytokine in pulmonary fibrosis Regulates MMP-9 transcription via Smad3
	PDGF-β	Overexpression in a murine model resulted in combined emphysema/lung fibrosis
Proteases	MMP-9	Cleavage of latent TGF-β releasing its active form
	Neutrophil elastase	Cleavage of latent TGF-β releasing its active form
Adaptive immunity	Tregs	Impaired (numerically and functionally) in IPF and emphysema patients
Cell anatomy	Caveolae	Oxidative stress acts as a first hit

beyond the obvious. A dogmatic dichotomous categorization between emphysema on one hand and fibrosis on the other has limitations. The recognition and further study of this syndrome can have serious implications. On the level of basic science the exploration of shared pathogenetic mechanisms can offer new therapeutic targets. As described this is an area of intensive ongoing research with very interesting findings (telomere length shortening, mutations in SFTPC and SFTPA2, IL-8, IL-13, TNF-α, MMP-9, Treg, Table 1) that could translate to feasible therapeutic targets. From a clinical trial perspective it is important to clarify if and how the inclusion of CPFE patients in the IPF trials can impact the results. CPFE patients tend to exhibit a delay in the reduction of FVC and DLco. This can underestimate the efficacy of an investigational drug. Further research is needed in order to seek for a better end point for CPFE. Also, it is important to accurately define CPFE as well as the comparator populations in further studies in order to produce comparable results across clinical trials. Finally, from a clinical point early detection of pulmonary hypertension is of utmost importance as it is associated with an ominous prognosis and can have impact on crucial management decisions as early referral for lung transplantation[71-73]. A high degree of vigilance is required regarding the early diagnosis of lung cancer in CPFE patients as well[74,75]. Finally, stem cell therapy is a promising approach for COPD[76] and IPF[77,78]. Conducting clinical trials of stem cell therapy in CPFE is an intriguing project that could shed further light in the areas of pathogenesis and treatment.

REFERENCES

1. Snider GL. Chronic obstructive pulmonary disease: a definition and implications of structural determinants of airflow obstruction for epidemiology. Am Rev Respir Dis 1989;140:S3-8.
2. Raghu G, Collard HR, Egan JJ, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med 2011;183:788-824
3. Wiggins J, Strickland B, Turner-Warwick M. Combined cryptogenic fibrosing alveolitis and emphysema: the value of high resolution computed tomography in assessment. Respir Med 1990;84:365-9.
4. Cottin V, Nunes H, Brillet P, et al. Combined pulmonary fibrosis and emphysema: a distinct underrecognised entity. Eur Respir J 2005;26:586–93.
5. Cottin V, Cordier JF. Combined pulmonary fibrosis and emphysema in connective tissue disease. Curr Opin Pulm Med 2012;18:418-27.
6. Tzouvelekis A, Zacharis G, Oikonomou A, et al. Increased incidence of autoimmune markers in patients with combined pulmonary fibrosis and emphysema. BMC Pulm Med 2013;13:31.
7. Flaherty KR, Fell C, Aubry MC, et al. Smoking-related idiopathic interstitial pneumonia. Eur Respir J 2014; 44:594–602.
8. Vassallo R, Ryu JH. Smoking-related interstitial lung diseases. Clin Chest Med 2012; 33:165–78.
9. Travis WD, Costabel U, Hansell DM, et al. An Official American Thoracic Society/EuropeanRespiratory Society Statement: Update of the International Multidisciplinary Classification of the Idiopathic Interstitial Pneumonias. Am J Respir Crit Care Med 2013;188:733–48.
10. Yousem SA. Respiratory bronchiolitis-associated interstitial lung disease with fibrosis is a lesion distinct fromfibrotic nonspecific interstitial pneumonia: a proposal. Mod Pathol 2006;19:1474–9.
11. Katzenstein AL, Mukhopadhyay S, Zanardi C, Dexter E. Clinically occult interstitial lfibrosis in smokers: classification and significance of a surprisingly commonfinding in lobectomy specimens. Hum Pathol 2010;41:316–25.
12. Katzenstein AL. Smoking-related interstitial fibrosis (SRIF): pathologic findings and distinction from other chronicfibrosing lung diseases. J Clin Pathol 2013;66:882–7.
13. Kawabata Y, Hoshi E, Murai K, et al. Smoking related changes in the background lung of specimens resected for lung cancer: a semiquantitative study with correlation to postoperative course. Histopathology 2008;53:707–14.
14. Yamada T, Nakanishi Y, Homma T, et al. Airspace enlargement with fibrosis shows characteristic histology and immunohistology different from usual interstitial pneumonia, nonspecific interstitial pneumonia and centrilobular emphysema. Pathol Int 2013;63:206–13.
15. Reddy TL, Mayo J, Churg A. Respiratory Bronchiolitis with Fibrosis High-Resolution Computed Tomography Findings and Correlation with Pathology. Ann Am Thorac Soc 2013; 10:590-601.
16. Jankowich MD, Rounds SI. Combined pulmonary fibrosis and emphysema syndrome: a review. Chest 2012; 141:222–31.
17. Armanios M. Telomeres and age-related disease: how telomere biology informs clinical paradigms. J Clin Invest 2013;123:996–1002.
18. Greider CW, Blackburn EH. A telomeric sequence in the RNA of Tetrahymena telomerase required for telomere repeat synthesis. Nature 1989;337(6205):331–7.
19. Lingner J, Hughes TR, Shevchenko A, Mann M, Lundblad V, Cech TR. Reverse transcriptase motifs in the catalytic subunit of telomerase. Science 1997;276(5312):561–7.
20. Alder JK, Guo N, Kembou F, et al. Telomere Length is a Determinant of Emphysema Susceptibility. Am J Respir Crit Care Med 2011; 184:904–12.
21. Armanios M. Telomerase and idiopathic pulmonary fibrosis. Mutat Res 2012;730(1-2):52-8.
22. Stanley SE, Chen JJ, Podlevsky JD, et al. Telomerase mutations in smokers with severe emphysema. J Clin Invest 2015;125:563-70.
23. Armanios MY, Chen JJ, Cogan JD, et al. Telomerase mutations in families withidiopathic pulmonary fibrosis. N Engl J Med 2007;356:1317–26.
24. Tsakiri KD, Cronkhite JT, Kuan PJ, et al. Adult-onset pulmonary

fibrosis caused by mutations in telomerase. Proc Natl Acad Sci U S A 2007; 104:7552–7.
25. Alder JK, Chen JJ, Lancaster L, et al. Short telomeres are a risk factor for idiopathic pulmonary fibrosis. Proc Natl Acad Sci U S A 2008;105:13051–6.
26. Cronkhite JT, Xing C, Raghu G, et al. Telomere shortening in familial and sporadic pulmonary fibrosis. Am J Respir Crit Care Med 2008; 178:729–37.
27. Nunes H, Monnet I, Kannengiesser C, et al. Is telomeropathy the explanation for combined pulmonary fibrosis and emphysema syndrome?: Report of a family with TERT mutation. Am J Respir Crit Care Med 2014;189:753-4.
28. Armanios M. Reply: telomerase makes connections between pulmonary fibrosis and emphysema. Am J Respir Crit Care Med 2014; 189:754-5.
29. Armanios M. Syndromes of telomere shortening. Annu Rev Genomics Hum Genet 2009; 10:45–61.
30. Gribbin J, Hubbard R, Smith C. Role of diabetes mellitus and gastro-oesophageal reflux in the aetiology of idiopathic pulmonary fibrosis. Respir Med 2009; 103:927–31.
31. Garcia-Sancho Figueroa MC, Carrillo G, Perez-Padilla R, et al. Risk factors for idiopathic pulmonary fibrosis in a Mexican population. A case-control study. Respir Med 2010; 104:305–9.
32. Armanios M, Chen JL, Chang YP, et al. Haploinsufficiency of telomerase reverse transcriptase leads to anticipation in autosomal dominant dyskeratosis congenita. Proc Natl Acad Sci U S A 2005;102:15960–4.
33. Diaz de Leon A, Cronkhite JT, Katzenstein AL, et al. Telomere lengths, pulmonary fibrosis and telomerase (TERT) mutations. PLoS One 2010; 5 e10680.
34. Kirwan M, Vulliamy T, Marrone A, et al. Defining the pathogenic role of telomerase mutations in myelodysplastic syndrome and acute myeloid leukemia. Hum Mutat 2009; 30:1567–73.
35. Parry EM, Alder JK, Qi X, Chen JJ, Armanios M. Syndrome complex of bone marrow failure and pulmonary fibrosis predicts germline defects in telomerase. Blood 2011; 117:5607–11.
36. Silhan LL, Shah PD, Chambers DC, et al. Lung transplantation in telomerase mutation carriers with pulmonary fibrosis. Eur Respir 2014;44:178-87.
37. Amin RS, Wert SE, Baughman RP, et al. Surfactant protein deficiency in familial interstitial lung disease. J Pediatr 2001; 139:85-92.
38. Thomas AQ, Lane K, Phillips J, 3rd, et al. Heterozygosity for a surfactant protein C gene mutation associated with usual interstitial pneumonitis and cellular nonspecific interstitial pneumonitis in one kindred. Am J Respir Crit Care Med 2002;165:1322-8.
39. Tanjore H, Blackwell TS, Lawson WE. Emerging evidence for endoplasmic reticulum stress in the pathogenesis of idiopathic pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 2012;302:L721-L729.
40. Cottin V, Reix P, Khouatra C, Thivolet-Béjui F, Feldmann D, Cordier JF. Combined pulmonary fibrosis and emphysema syndrome associated with familial SFTPCmutation. Thorax 2011; 66:918–9.
41. Bullard JE, Wert SE, Whitsett JA, Dean M, Nogee LM. ABCA3 mutations associated with pediatric interstitial lung disease. Am J Respir Crit Care Med 2005; 172:1026-31.
42. Crossno PF, Polosukhin VV, Blackwell TS, et al. Identification of early interstitial lung disease in an individual with genetic variations in ABCA3 and SFTPC. Chest 2010; 137:969-73.
43. Young LR, Nogee LM, Barnett B, Panos RJ, Colby TV, Deutsch GH. Usual interstitial pneumonia in an adolescent with ABCA3 mutations. Chest 2008;134:192-5.
44. Epaud R, Delestrain C, Louha M, Simon S, Fanen P, Tazi A. Combined pulmonary fibrosis and emphysema syndrome associated with ABCA3 mutations. Eur Respir 2014;43:638-41.
45. Chaudhuri R, Mcsharry C, Spears M, et al. Sputum matrix metalloproteinase-9 is associated with the degree of emphysema on computed tomography in COPD. Translational Respiratory Medicine 2013;1:11.
46. Yu Q, Stamenkovic I. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-β and promotes tumor invasion and angiogenesis. Genes Dev 2000; 14:163-176.
47. Fernandez IE, Eickelberg O. The impact of TGF-β on lung fibrosis: from targeting to biomarkers. Proc Am Thorac Soc 2012;9:111-6.
48. Xu B, Chen H, Xu W, et al. Molecular mechanisms of MMP9 overexpression and its role in emphysema pathogenesis of Smad3-deficient mice. Am J Physiol Lung Cell Mol Physiol 2012;303:L89-96.
49. Warburton D, Shi W, Xu B. TGF-β-Smad3 signaling in emphysema and pulmonary fibrosis: an epigenetic aberration of normal development? Am J Physiol Lung Cell Mol Physiol 2013;304:L83-5.
50. Gauldie J, Kolb M, Ask K, et al. Smad3 signaling involved in pulmonary fibrosis and emphysema. Proc Am Thorac Soc 2006;3:696-702.
51. Lundblad LK, Thompson-Figueroa J, Leclair T, et al. Tumor necrosis factor-α overexpression in lung disease: a single cause behind a complex phenotype. Am J Respir Crit Care Med 2005;171:1363–70.
52. Hoyle GW, Li J, Finkelstein JB, et al. Emphysematous lesions, inflammation, and fibrosis in the lungs of transgenic mice overexpressing platelet-derived growth factor. Am J Pathol 1999; 154:1763–75.
53. Lucattelli M, Bartalesi B, Cavarra E, et al. Is neutrophil elastase the missing link between emphysema and fibrosis? Evidence from two mouse models. Respir Res 2005;6:83.
54. Annes JP, Munger JS, Rifkin DB. Making sense of latent TGFbeta activation. J Cell Sci 2003;116:217–224.
55. Keane MP, Arenberg DA, Lynch JP, III, et al. The CXC chemokines, IL-8 and IP-10, regulate angiogenic activity in idiopathic pulmonary fibrosis. J Immunol 1997;159:1437–43.
56. Keane MP, Belperio JA, Burdick MD, et al. ENA-78 is an important angiogenic factor in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2001;164:2239–42.
57. Mukaida N. Pathophysiological roles of interleukin-8/CXCL8 in pulmonary diseases. Am J Physiol Lung Cell Mol Physiol 2003; 284:L566–773.
58. Tasaka S, Mizoguchi K, Funatsu Y, et al. Cytokine profile of bronchoalveolar lavage fluid in patients with combined pulmonary fibrosis and emphysema. Respirology 2012;17:814-20.
59. Fulkerson PC, Fischetti CA, Hassman LM, et al. Persistent ef-

fects induced by IL-13 in the lung. Am J Respir Cell Mol Biol 2006; 35:337–46.
60. Lumsden RV, Worrell JC, Boylan D, et al. Modulation of pulmonary fibrosis by IL-13Rα2. Am J Physiol Lung Cell Mol Physiol 2015;308:L710-8.
61. Murray LA, Zhang H, Oak SR, et al. Targeting interleukin-13 with tralokinumab attenuates lung fibrosis and epithelial damage in a humanized SCID idiopathic pulmonary fibrosis model. Am J Respir Cell Mol Biol 2014;50:985-94.
62. Volonte D, Kahkonen B, Shapiro S, et al. Caveolin-1 expression is required for the development of pulmonary emphysema through activation of the ATM-p53-p21 pathway. J Biol Chem 2009;284:5462-6.
63. Wang XM, Zhang Y, Kim HP, et al. Caveolin-1: a critical regulator of lung fibrosis in idiopathic pulmonary fibrosis. J Exp Med 2006; 203:2895–906.
64. Castello-Cros R, Whitaker-Menezes D, Molchansky A, et al. Scleroderma-like properties of skin from caveolin-1-deficient mice: implications for new treatment strategies in patients with fibrosis and systemic sclerosis. Cell Cycle 2011;10:2140-50.
65. Tzouvelekis A, Bouros E, Bouros D. The Immunology of pulmonary fibrosis: The role of Th1/Th2/Th17/Treg cells. Pneumon 2010;1:17-20.
66. Kotsianidis I, Nakou E, Bouchliou I, et al.Global impairment of CD4+CD25+FOXP3+ regulatory T cells in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2009;179:1121-30.
67. Lee SH, Goswami S, Grudo A, et al. Antielastin autoimmunity in tobacco smoking–induced emphysema. Nat Med 2007;13:567–9.
68. Isajevs S, Taivans I, Strazda G, et al. Decreased FOXP3 expression in small airways of smokers with COPD. Eur Respir J 2009;33:61–7.
69. Hou J, Sun Y, Hao Y, et al. Imbalance between subpopulations of regulatory T cells in COPD. Thorax 2013;68:1131–9.
70. Tzouvelekis A, Bouros D. COPD and IPF: it›s all about regulation and balance. Thorax 2014;69:490.
71. Corte TJ, Wort SJ, Gatzoulis MA, et al. Pulmonary vascular resistance predicts early mortality in patients with diffuse fibrotic lung disease and suspected pulmonary hypertension. Thorax 2009;64:883–8.
72. Hamada K, Nagai S, Tanaka S, et al. Significance of pulmonary arterial pressure and diffusion capacity of the lung as prognosticator in patients with idiopathic pulmonary fibrosis. Chest 2007;131:650–6.
73. Lettieri CJ, Nathan SD, Barnett SD, et al. Prevalence and outcomes of pulmonary arterial hypertension in advanced idiopathic pulmonary fibrosis. Chest 2006;129:746–52.
74. Kwak N, Park CM, Lee J, et al. Lung cancer risk among patients with combined pulmonary fibrosis and emphysema. Respir Med 2014;108:524e30.
75. Usui K, Tanai C, Tanaka Y, Noda H, Ishihara T. The prevalence of pulmonary fibrosis combined with emphysema in patients with lung cancer. Respirology 2011;16:326-31.
76. Tzouvelekis A, Laurent G, Bouros D. Stem cell therapy in chronic obstructive pulmonary disease. Seeking the Prometheus effect. Curr Drug Targets 2013;14:246-52.
77. Tzouvelekis A, Antoniadis A, Bouros D. Stem cell therapy in pulmonary fibrosis. Curr Opin Pulm Med 2011;17:368-73.
78. Tzouvelekis A, Paspaliaris V, Koliakos G, et al. A prospective, non-randomized, no placebo-controlled, phase Ib clinical trial to study the safety of the adipose derived stromal cells-stromal vascular fraction in idiopathic pulmonary fibrosis. J Transl Med 2013;11:171.