

REVIEW ARTICLE

IPF: new insight on pathogenesis and treatment

S. Harari & A. Caminati

Unità Operativa di Pneumologia e Terapia Semi-Intensiva Respiratoria – Servizio di Fisiopatologia Respiratoria ed Emodinamica Polmonare
Ospedale San Giuseppe, Milan, Italy

To cite this article: Harari S, Caminati A. IPF: new insight on pathogenesis and treatment. *Allergy* 2010; DOI: 10.1111/j.1398-9995.2009.02305.x.

Keywords

idiopathic interstitial pneumonia; idiopathic pulmonary fibrosis; pathogenesis; therapy; usual interstitial pneumonia.

Correspondence

Dr. Sergio Harari, Unità Operativa di
Pneumologia, Ospedale San Giuseppe, Via
San Vittore, 12, 20123 Milano, Italy.
Tel.: +39 (02) 85994580
Fax: +39 (02) 85994400
E-mail: sharari@ilpolmone.it

Accepted for publication 21 November 2009

DOI:10.1111/j.1398-9995.2009.02305.x

Edited by: Marc Humbert

Abstract

Recent years have seen a robust influx of exciting new observations regarding the mechanisms that regulate the initiation and progression of pulmonary fibrosis but the pathogenesis remains poorly understood. The search for an alternative hypothesis to unremitting, chronic inflammation as the primary explanation for the pathophysiology of idiopathic pulmonary fibrosis (IPF) derives, in part, from the lack of therapeutic efficacy of high-dose immunosuppressive therapy in patients with IPF. The inflammatory hypothesis of IPF has since been challenged by the epithelial injury hypothesis, in which fibrosis is believed to result from epithelial injury, activation, and/or apoptosis with abnormal wound healing. This hypothesis suggests that recurrent unknown injury to distal pulmonary parenchyma causes repeated epithelial injury and apoptosis. The resultant loss of alveolar epithelium exposes the underlying basement membrane to oxidative damage and degradation. Emerging concepts suggest that IPF is the result of epithelial–mesenchymal interaction. The initiation of this fibrotic response may depend upon genetic factors and environmental triggers; the role of Th1 or Th2 cell-derived cytokines may also be important. This process appears to be unique to usual interstitial pneumonia/IPF. It is clear that IPF is a heterogeneous disease with variations in pathology, high-resolution computed tomography findings, and patterns of progression. Idiopathic pulmonary fibrosis is a complex disorder, and no unifying hypothesis has been identified at present that explains all the abnormalities.

Clinical evaluation and diagnostic approach to IPF

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive parenchymal lung disease with a median survival of less than 3–5 years following diagnosis, although the clinical course can be highly variable (1, 2). Idiopathic pulmonary fibrosis is the most common of the idiopathic interstitial pneumonias (IIPs) with a prevalence of 13–20 per 100 000 people in the general population (3, 4). Predictors of a worse outcome include progressive dyspnea, oxygen desaturation, and distance walk during the 6-min walking test (5, 6), worsening pulmonary function and gas-exchange (7–10), the presence and extent of honeycombing on high-resolution computed tomography (HRCT) (11, 12), and the presence of pulmonary hypertension (PH) (13).

The diagnosis of IPF is based on clinical, radiographic, and histopathologic evaluations (4). Common clinical features consist of progressive dyspnea, dry cough, and the presence of basilar ‘velcro-like’ rales on examination. Digital clubbing and clinical signs of cor-pulmonale may be present.

Secondary causes of pulmonary fibrosis such as collagen-vascular disease, chronic hypersensitivity pneumonitis, adverse drug reactions, granulomatous diseases, and pneumoconiosis must be excluded. High-resolution computed tomography has taken a prominent role in the diagnosis of IPF and can help distinguish IPF from other IIPs (14). A patchy pattern of peripheral, sub-pleural and predominantly lower lobe reticular opacities combined with honeycombing, traction bronchiectasis, and the absence of significant ground-glass together constitute the classic radiographic features of IPF (Fig. 1). The presence of these features on HRCT in the context of an appropriate clinical presentation may abrogate the need for a surgical lung biopsy.

In the absence of typical clinical and radiographic features, a surgical biopsy is recommended for the definitive diagnosis of IPF. Diagnostic accuracy may be improved if biopsies are obtained from multiple lobes, as it has shown that several distinct histopathologic patterns may coexist in the same patient, and the presence of usual interstitial pneumonia (UIP) on any biopsy confer a worse prognosis (15). Usual

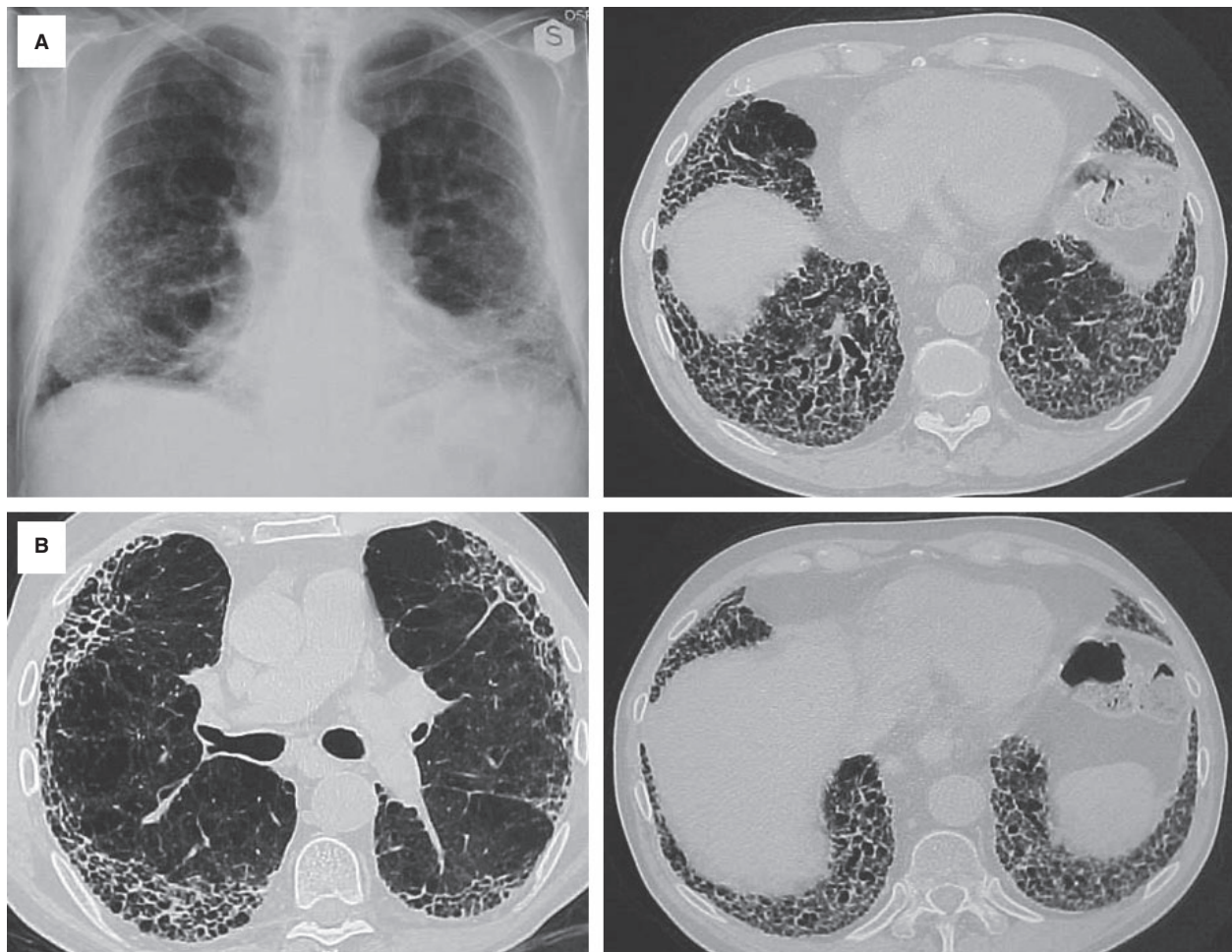


Figure 1 A 73-year-old man, current smokers, with clinical-radiological diagnosis of end-stage idiopathic pulmonary fibrosis. (A) Chest X-ray shows reticular opacities, reduced lung volume. High-resolution computed tomography (HRCT) at three levels shows

intra-lobular interstitial thickening, diffuse honeycombing, predominantly in the lower lungs, architectural distortion, and traction bronchiectasis; ground-glass is absent. Honeycomb cysts typically share walls on HRCT and occur in several layers in the sub-pleural lung.

interstitial pneumonia is the pathological hallmark of IPF. Histopathologic features of UIP include patchy areas of fibrosis in association with areas of normal lung architecture, the so-called temporal heterogeneity of UIP (Fig. 2). Mild inflammatory cell infiltration may be present in UIP, but is not a prominent feature. Fibroblastic foci, consisting of aggregates of myofibroblasts underlying 'injured', reparative epithelium, are key histologic features of IPF (16) (Fig. 3). The presence and extent of fibroblastic foci, while not pathognomonic, are of prognostic value in IPF as these lesions correlates with a worse prognosis (17).

Patients with IPF may experience a rapidly progressive decline in lung function from onset or a slowly progressive decline course of illness, during which the development of acute exacerbations (AEs) can lead to rapid respiratory failure and early death (18). Prognosis is extremely poor, and there is no known curative therapy for IPF. Immunosuppressive treatment regimens have been used in the treatment of

IPF; however, there is little evidence to support their efficacy and safety. The combination of the unique pathologic features of UIP on biopsy, the progressive clinical course and resistance to anti-inflammatory therapy constitute the cardinal manifestations of what is now termed IPF/UIP and have led to recent suggestions that new therapies should be directed at regulating fibroblast functions rather than targeting the inflammatory response (19). There has been growing interest in the diagnosis and prevention of AEs in patients with IPF (20). It has been shown that AEs are quite prevalent among patients with IPF that they occur at any stage of the illness and that they are frequently fatal events (19, 20).

Pathogenesis of IPF – fibroblastic foci

The etiopathogenesis of IPF remains enigmatic. Despite considerable recent investigation of UIP, neither the initial cause nor the reasons for progression are known. The precise

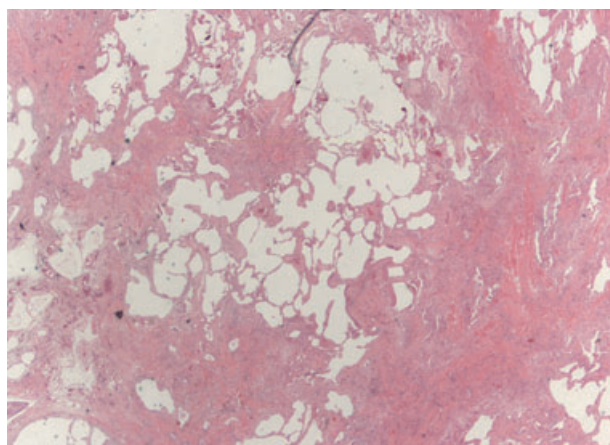


Figure 2 Low-power examination of a surgical lung biopsy demonstrating the patchy nature of fibrosis in idiopathic pulmonary fibrosis. Fibrotic zones of dense collagen with a typically sub-pleural and paraseptal distribution, a sharp demarcation between normal lung and abnormal honeycomb lung, and little nonspecific chronic inflammation are the histological hallmark of usual interstitial pneumonia.

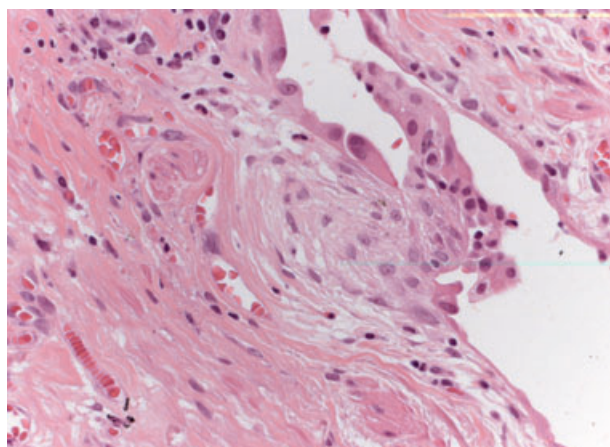


Figure 3 On higher magnification, a fibroblastic foci, a cardinal feature of usual interstitial pneumonia (UIP), is evident. These appear as areas of loose fibroblastic proliferation, either re-epithelializing or de-epithelializing that are highly interconnected on sequential sectioning. It is a reactive rather than a malignant process. The fibroblastic foci are of lower vascularity than the intra-alveolar foci of fibroblastic proliferation seen in organising pneumonia and inflammatory cells are typically seen in areas of established fibrosis rather than the fibroblastic areas. These findings, amongst others, have led to the view that, although inflammation still has a role in pathogenesis, there may be significant epithelial–mesenchymal interaction in UIP as a response to repetitive alveolar epithelial injury and an altered alveolar microenvironment.

factors that initiate and maintain the inflammatory and fibrotic responses observed in IPF are unknown. However, some risk factors are associated with IPF, including smoking, infections, environmental pollutants, chronic aspiration, and drugs

(4). A question arose about the importance of fibroblastic foci in IPF. Although IPF is a disease of unknown etiology, the current hypothesis regarding its development conceptualizes ongoing multiple, small, focal, and isolated episodes of epithelial injury followed by a pathologic fibrotic-repair mechanism (21). It is probable that the disease results from a series of nonspecific insults to both the epithelial barrier and the pulmonary parenchyma (21, 22). The clinical features of IPF – insidious onset, progression over years, infrequent but abrupt episodes of deterioration (23), and failure to respond to immunosuppressive therapy – have offered indirect evidence in support of this hypothesis. More substantial support comes from the histopathologic features of the disease. Usual interstitial pneumonia is characterized by a temporally heterogeneous appearance with areas of normal appearing lung intermixed with older collagenized fibrosis, microscopic honeycombing, and small focal areas of younger, myxoid-appearing matrix with aggregates of actively proliferating and collagen-producing myofibroblasts termed ‘fibroblast foci’ (24, 25). These foci are often identified in a transition zone between the more normal uninvolved lung and the abnormal fibrotic lung. They have been interpreted to represent an organizing phase of focal acute lung injury (24, 26, 27) and believed to recapitulate the events that typify the healing skin wound. Three-dimensional reconstruction indicated that foci are the leading edge of a continuous fibrotic reticulum that extends from the pleural surface into the lung parenchyma. In addition, clonality analysis revealed that the foci are polyclonal, rather than monoclonal (28). This supports the longstanding impression that UIP is not a malignant but a reactive process. These findings argue against the underlying biological cause of UIP being the result of multiple, small, focal, and isolated episodes of epithelial injury followed by a pathologic fibrotic-repair mechanism. This concept, of a ‘wave’ of fibrosis progressing through the lung, alters our underlying assumptions regarding the initiation and progression of IPF and suggests new hypotheses for the initiation and progression of fibrosing lung disease. It is increasingly apparent that mesenchymal cells in fibroblastic foci in IPF exhibit a variety of abnormalities compared to normal lung fibroblasts or fibroblasts from other lung diseases. The origin of pathological fibroblast foci within the IPF lesion remains puzzling. Possibilities include differentiation of resident fibroblasts, recruitment of circulating fibroblast precursors, and transdifferentiation of epithelial cells into pathological fibroblast phenotypes (29–31) (Fig. 4).

Epithelial cell activation

A marked disruption in the integrity of the alveolar epithelium with presence of several altered phenotypes is a distinctive feature in IPF lung. Morphologic phenotypes may include: (i) hyperplastic type 2 pneumocytes, (ii) reactive large and elongated epithelial cells (putative transitional cells among type 2 and type 1 pneumocytes), (iii) flattened and attenuated epithelial cells usually overlying the fibroblastic foci, (iv) bronchiolar-type epithelium lining the enlarged airspaces of honeycomb lesions, and (v) squamous

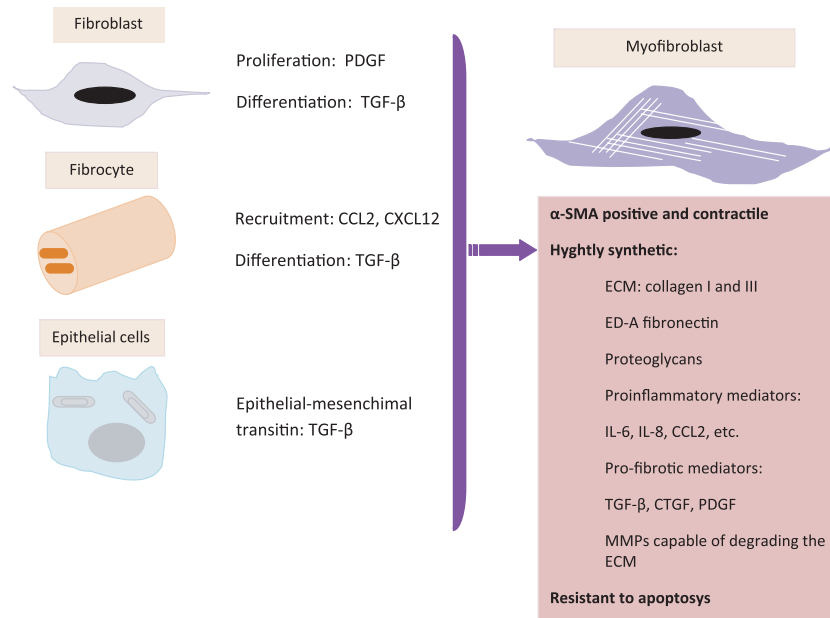


Figure 4 The primary effector cell in idiopathic pulmonary fibrosis is the myofibroblast; a cell that is highly synthetic, exhibits a

contractile phenotype, and is characterized by the presence of α -smooth muscle actin stress fibres (adapted from reference 108).

metaplasia. Importantly, lung carcinomas develop in a high percentage of patients with IPF, and it may be difficult to differentiate prominent reactive epithelial proliferations from well-differentiated adenocarcinomas (32) (Fig. 5). The reasons for all these epithelial changes are unknown, but some of them seem to be related to the initial insult, whereas others appear to represent a reactive response to lung remodeling. Regarding the former, although the etiology of IPF is

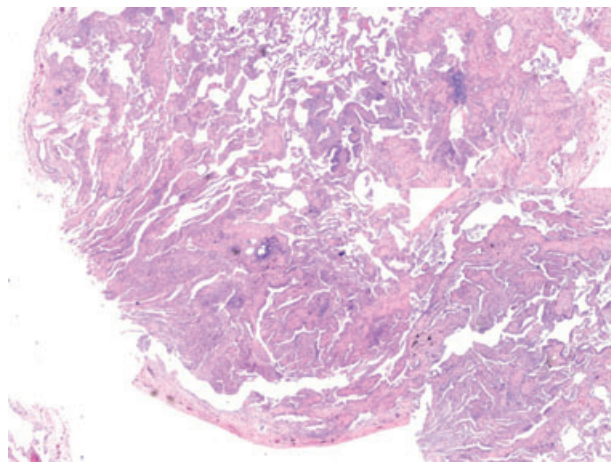


Figure 5 Patchy fibrosis with remodeling of the lung architecture is present in this surgical lung biopsy. In addition to a usual interstitial pneumonia pattern, this case shows atypical epithelial proliferation. It can be especially difficult to separate prominent reactive epithelial proliferations from well-differentiated adenocarcinomas.

unknown and probably multifactorial in a susceptible host, the putative injuring candidates are more likely associated with an initial alveolar epithelial damage. Thus, environmental exposure to different dusts, gastro-esophageal reflux, and viral infections has been associated with the development of IPF, suggesting that diverse epithelial injuries may trigger the disease in different individuals (33). Re-establishing an intact epithelium following injury is a key component of normal wound healing. This may require orchestrated epithelial cell responses that include proliferation and expansion of progenitor/stem cells, migration, and differentiation. The homeostatic function of an intact epithelium may be necessary to suppress mesenchymal cell activation. Idiopathic pulmonary fibrosis more likely results from an aberrant activation of alveolar epithelial cells (AECs) after injury that provoke the migration, proliferation, and activation of mesenchymal cells with the formation of fibroblastic/myofibroblastic foci, leading to the exaggerated accumulation of extracellular matrix (ECM) with the irreversible destruction of the lung parenchyma (21, 34). The precise process by which AECs become 'activated' and communicate with fibroblasts leading to unremitting fibrosis in IPF remains poorly understood. Nonetheless, various theories have been proposed to explain this process, including the production of fibroblasts chemotactic factors by activated AECs and the release of growth factors involved in re-epithelialization. These growth factors include transforming growth factor- β (TGF- β), platelet-derived growth factor (PDGF), insulin-like growth factor (ILGF)-1, and endothelin (ET)-1, among others. Thus, when the distal epithelium in the lung becomes injured and the basement membrane loses its integrity, it endeavours to re-epithelialize the surface and, as a result, growth factor is produced that can

potentially recruit fibroblasts or myofibroblasts. Thus, instead of healing by repair, myofibroblasts proliferation and ECM deposition continues unabated (35) (Fig. 6). In IPF, there is loss of epithelial type I cells and a marked proliferation of epithelial type II cells; however, these cells generally do not appear to re-epithelialize the alveolar space because the basement membranes of patients with IPF remain abnormal, demonstrating microscopic alterations including duplication and fragmentation (36). These basement membrane abnormalities also permit the migration of mesenchymal cells from interstitium to the alveolar regions of the injured lung. These changes suggest that a chronic injury to the alveolar epithelium may result in perpetual nature of intra-alveolar exudates, fibrosis, and remodeling. Dysregulated interactions between epithelial and mesenchymal cells within the context of a damaged basement membrane and the surrounding ECM are increasingly recognized as a key control point in the pathogenesis of fibrotic disorders. Morphologic changes suggestive of epithelial cell injury and apoptosis are early features in the histopathology of IPF (16, 37). Several studies have described phenotypic alterations in type II AECs including proliferation (38), bronchiolarization (39), apoptosis, and regenerative hyperplasia (40). Underlying areas of atypical

and apoptotic epithelial cells are fibroblastic foci containing activated contractile myofibroblasts that secrete abundant ECM proteins (16). These myofibroblasts are closely associated with areas of epithelial cell death and regeneration. The molecular mechanisms that determine the persistent nature of IPF are poorly understood. While aberrant activation of developmental pathways that are usually suppressed in adult tissues is often noticed in cancer, it is only rarely observed in nonmalignant diseases. Microarray analysis identified an IPF-specific gene expression signature characterized by the up-regulation of genes indicative of an active tissue remodeling program, including ECM and a large number of myofibroblast/smooth muscle cell-associated and epithelial cell-related genes (34). Idiopathic pulmonary fibrosis lungs are significantly enriched with genes associated with lung development (41). The up-regulated development-relevant genes included several members of transcription factor families. While transcription factors active in morphogenesis and differentiation of the embryonic lung may be transiently expressed during adult lung repair, they are only rarely expressed in the normal lung. In fact, their sustained expression is often thought of as a marker for malignant transformation. In IPF, there is increasing evidence supporting the

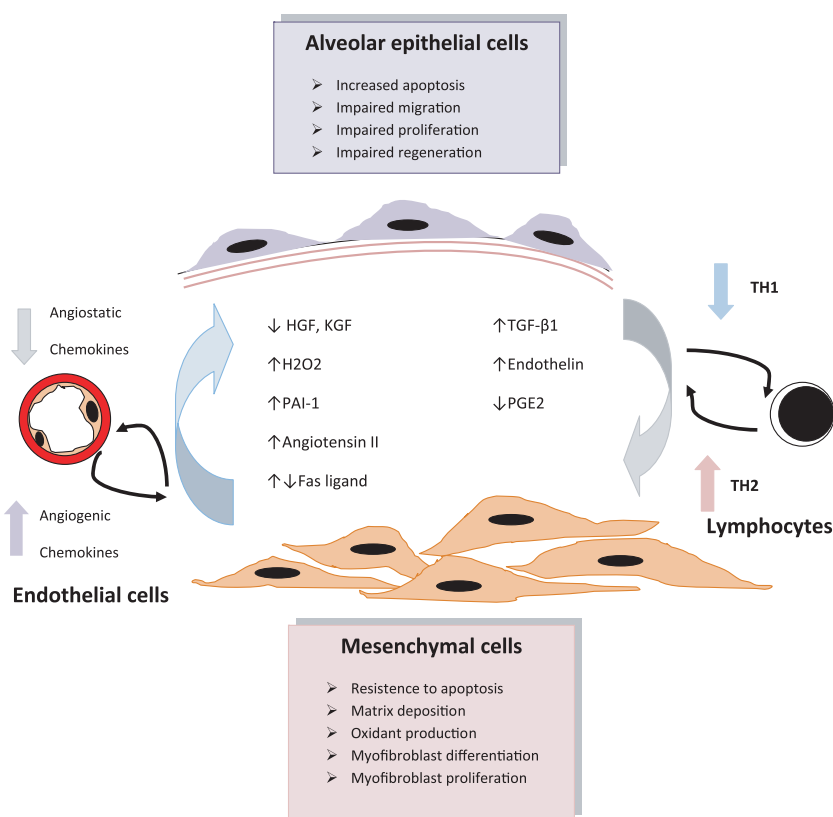


Figure 6 The pathogenesis of idiopathic pulmonary fibrosis idiopathic pulmonary fibrosis idiopathic pulmonary fibrosis is complex, and three major hypothesis or paradigms for the pathogenesis of idiopathic pulmonary fibrosis

have emerged: dysregulated epithelial-mesenchymal interactions, aberrant angiogenesis, and the Th1/Th2 cytokine imbalance (adapted from reference 61).

idea that AECs are the primary source of cytokines and growth factors involved in fibroblast migration and proliferation, and myofibroblast differentiation. Thus, various studies performed by *in situ* hybridization and immunohistochemistry have demonstrated that in this disease, AECs are the main site of synthesis of PDGF, TGF- β , and tumor necrosis factor- α (TNF- α), all of them central pieces for the development of pulmonary fibrosis (33, 42–44). Likewise, ET-1, a multifunctional peptide able to induce mesenchymal cell mitosis is strongly up-regulated in type 2 pneumocytes primarily in those located close to fibroblastic foci (45). Connective tissue growth factor (CTGF), a chemotactic and mitogenic factor for fibroblasts, is also up-regulated in type 2 AECs and fibroblasts in IPF lungs (46).

Epithelial cell plasticity

Epithelial cells are motile and can migrate away from their nearest neighbors (47). However, under normal conditions, they do not detach and move away from the epithelial layer. This arrangement can be disturbed by a process known as epithelial–mesenchymal transition (EMT). In the process of EMT, epithelial cells lose many of their epithelial characteristics and obtain properties that are distinctive of mesenchymal cells (48). They become migratory, down-regulate the expression of cell adhesion molecules, primarily E-cadherin, lose their apical-basal polarity, and express mesenchymal molecules such as fibronectin and N-cadherin (49, 50). Epithelial–mesenchymal transition is a key process in embryogenesis. An EMT-like process has been reported in cancer progression and metastasis and in fibrotic disorders (51–54). Recently,

EMT was also observed in lung fibrosis and is another possible explanation for AEC activation: numerous cells co-expressing epithelial and mesenchymal markers (thyroid transcription factor-1/ α -smooth muscle actin or surfactant protein C/N-cadherin) within the expanded interstitium in IPF lungs (54, 55). Bone morphogenetic proteins (BMPs) and TGF- β belong to a superfamily of multifunctional cytokines that includes different isoforms with highly specific functions including wound healing, ECM remodeling, and the control of epithelial–mesenchymal interactions during embryogenesis (56). Importantly, BMPs antagonize the effects of TGF- β regarding EMT and induce the inverse process of mesenchymal-to-epithelial transition (48). Gremlin, the main BMP antagonist, is increased in IPF lung (57). Transforming growth factor- β induces gremlin expression in association with EMT in lung epithelial cells (57). Taken together, these data suggest that increased TGF- β expression and active BMP inhibition by gremlin create an EMT-favoring environment in IPF lungs (Fig. 7). The occurrence of EMT in the lung represents a dramatic shift in cellular phenotype and requires reversal of early embryonic programs. In the lung, the formation of the various cell types lining the proximal and distal airways occurs through the differentiation of the epithelial precursor cells (58). Thus, EMT in IPF probably represents a dramatic reprogramming of epithelial cells (59).

Cell apoptosis

There is growing circumstantial evidence to suggest that in IPF the alveolar epithelium is prone to undergoing programmed cell death, although the mechanism for inducing

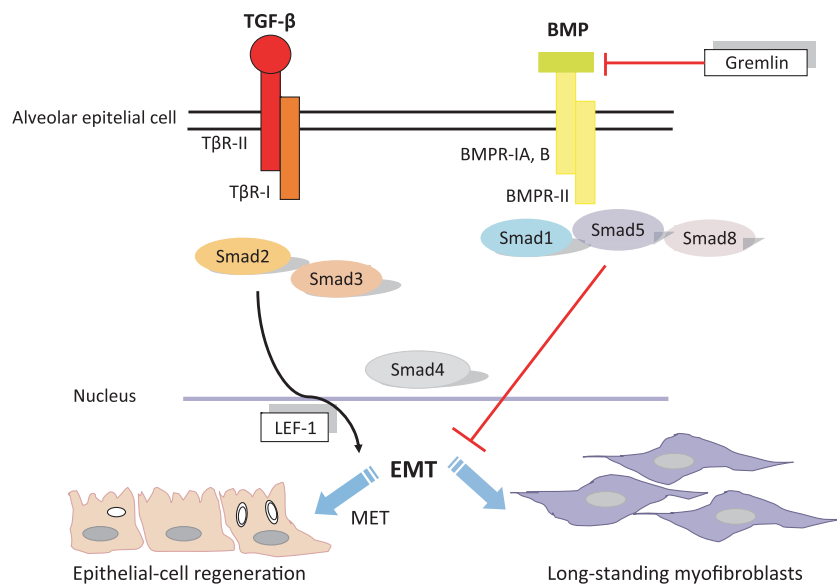


Figure 7 Schematic overview of the epithelial–mesenchymal transition as modulated by transforming growth factor- β (TGF- β) and BMPs. In idiopathic pulmonary fibrosis lungs, TGF- β 3, gremlin, and lymphoid enhancer factor-1 (LEF-1) are up-regulated, while BMP-2 is down-regulated. Dysregulation of TGF- β /BMP pathway induces a fibrotic phenotype. Epithelial–mesenchymal transition (EMT), so

important in development, is more likely to represent one possible response of the alveolar epithelial cell facing sustained injury. Increased expression of BMPs may not only antagonize TGF- β -induced EMT, enhancing re-epithelialization, but also may cause the opposite process, mesenchymal-to-epithelial transition (MET) (adapted from reference 56).

epithelial apoptosis is, as yet, unknown. Epithelial apoptosis is accompanied by damage to the basement membrane leading to the release of growth factors and chemokines. It appears that some of the products of epithelial cell injury may stimulate fibroblasts to produce ECM components, most notably collagen, that can lead to dysregulated repair of the epithelial/endothelial barrier (35). The specific mediator(s) responsible for epithelial cell apoptosis in pulmonary fibrosis remain unclear, and several different mechanisms, including TGF- β 1, oxidative stress, Fas activation, and angiotensin II have been proposed. In epithelial cells, TGF- β 1 typically functions as a tumor suppressor through growth-inhibition and induction of apoptosis (60, 61). Oxidative stress resulting from the release of reactive oxygen species (ROS) by activated phagocytic cells has also been proposed as a mechanism for epithelial cell injury in IPF (62). Although ROS are well recognized to induce cell injury, they are also important regulators of intra- and inter-cellular signaling (63). Transforming growth factor- β 1 is a strong inducer of the sustained production of extracellular oxidants in human lung fibroblasts (64), and extracellular hydrogen peroxide (H_2O_2) generated by IPF myofibroblasts in response to TGF- β 1 has been shown to mediate paracrine induction of epithelial cell apoptosis/death (64). Up-regulation of the Fas signaling pathway has been demonstrated in human IPF (65), and a number of studies in animal models have explored the role of Fas-Fas ligand cascade in epithelial cell apoptosis, sometimes with conflicting results (66, 67). Angiotensin II has also been proposed as a soluble mediator of epithelial cell apoptosis in the pathogenesis of IPF. Inhibition of angiotensin II or the angiotensin receptor has been shown to block Fas-mediated epithelial cell apoptosis (68, 69). Moreover, IPF-derived fibroblasts can induce paracrine epithelial cell apoptosis through generation of angiotensin II (70). In animal models, inhibition of angiotensin II activation and blockade of its receptor abrogates bleomycin-induced fibrosis (71, 72). Telomeres are noncoding DNA sequences at the end of eukaryotic chromosomes that maintain chromosomal integrity and prevent replication of defective genes (73). When normal cells reach a critical telomere length, they exit the cell cycle and undergo senescence. The putative relationship between telomerase and human disease is demonstrated in two opposite situations. On one side of the spectrum, most human cancers are characterized by the expression of telomerase, which helps to maintain telomere length and enhance indefinite cell proliferation (74). On the other side of the spectrum, in pulmonary fibrosis, telomerase activity seems to be diminished, with consequent premature telomere shortening. Two recent reports demonstrated that mutations in the genes encoding telomerase components are detected in patients with familial IPF and occasionally in patients with sporadic IPF (75). According to these studies, pulmonary fibrosis may be at least partially related to a telomerase deficiency and short dysfunctional telomeres, which after DNA damage leads to cell death or cell cycle arrest. Interestingly, cigarette smoking, a strong environmental risk factor for IPF, causes telomere shortening in a dose-dependent manner (76). As AECs play a critical role in the pathogenesis

of IPF, and telomerase expression is generally restricted to cells with the capacity to proliferate, it has been speculated that fibrotic response in patients with short telomeres is provoked by a loss of alveolar cells (75), which may be enhanced by exposure to cigarette smoke. Stresses within the endoplasmic reticulum (ER), the site of protein synthesis within the cell, can lead to protein misfolding and aggregation. To prevent misfolded protein aggregation, the ER activates the unfolded protein response (UPR). This signal transduction cascade allows for: (i) an increase in capacity for protein folding; (ii) a reduction in newly translated proteins entering the ER; and (iii) an increase in the breakdown of misfolded proteins. Ultimately, the UPR signals cell apoptosis (35).

Mesenchymal cell phenotypes

Underlying areas of epithelial injury in lungs of patients with IPF are fibroblastic foci composed of aggregates of activated fibroblasts/myofibroblasts, which are the key effector cells in fibrogenesis (16, 61). Myofibroblasts are contractile and highly activated, secretory cells with characteristics intermediate between fibroblasts and smooth muscle cells (77). Following injury, mesenchymal cells infiltrate wounds, differentiate into myofibroblasts, and secrete ECM proteins that help to constitute provisional matrix, which serves as the 'scaffold' for normal tissue repair (77). Contraction of the provisional matrix approximates epithelial cells margins to facilitate re-epithelialization while collagen secretion stabilizes the contracted matrix. Interestingly, recent studies indicate that lung mesenchymal cells following injury may derive from several sources including resident interstitial fibroblasts (29), circulating fibrocytes (30, 78), bone marrow-derived fibroblast precursors (79), and lung epithelial cells via EMT (31, 55). For normal healing to occur, wound myofibroblasts must undergo apoptosis (80); failure of apoptosis leads to myofibroblasts accumulation, exuberant ECM production, persistent tissue contraction, and pathologic scar formation (81). Transforming growth factor- β 1 is a critical mediator of the myofibroblast phenotype and has been implicated in the pathogenesis of fibrotic disease in virtually every organ system studied (82–84). In contrast to its effects on epithelial cells, TGF- β 1 promotes an anti-apoptotic phenotype in fibroblasts (85, 86). Accumulating evidence supports the emergence of an 'apoptosis paradox' in IPF – apoptosis susceptibility in epithelial cells and apoptosis resistance in myofibroblasts. Another contrasting feature is the susceptibility of epithelial cells to ROS-induced injury and apoptosis whereas myofibroblasts appear to be relatively resistant to similar levels of oxidative stress.

Multiple other soluble and insoluble substances in the cellular microenvironment have been shown to promote a 'pro-fibrotic' mesenchymal cell phenotype. Soluble factors that induce myofibroblast differentiation include ET-1 (87), thrombin (88), and thrombospondin-1 (89). Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) is a phosphatidylinositol phosphate phosphatase that is frequently deleted or inactivated in human cancers (90). Phosphatase and tensin homologue deleted on chromosome

10 is critical in development (91, 92). A critical role for PTEN in the orchestrated death and removal of myofibroblasts has been suggested. Myofibroblasts from IPF fibroblast foci undergo decreased apoptosis compared to myofibroblasts from fibromyxoid lesions of cryptogenic organizing pneumonia, a potentially reversible lung disorder (93, 94). Impaired apoptosis may be at least partially associated with the decreased expression of PTEN observed in IPF lungs (95). Additionally, as PTEN suppresses fibroblast differentiation to myofibroblasts (95), its reduced expression in IPF fibroblastic foci may have a dual deleterious effect, inducing myofibroblast differentiation and increasing survival.

Caveolin-1 has been proposed as a protective regulator of pulmonary fibrosis (96, 97). Caveolin-1 limits TGF- β 1-induced production of ECM and restores alveolar epithelial-repair processes. It is the predominant structural protein of caveolae, which are flask-shaped invaginations of the plasma membrane and are abundantly present in many terminally differentiated cells (98). These plasmalemmal microdomains serve many fundamental biologic processes, including vesicular and cholesterol trafficking and transcytosis. They represent a regulatory hub controlling the signaling activity of many molecules, including nitric oxide. Wang et al. (96) used several strategies to test the hypothesis that caveolin-1 is an endogenous inhibitor of pulmonary fibrosis. First, they observed that expression of the molecule was markedly reduced in lung tissue from patients with IPF and that this reduction was predominant in AECs. Furthermore, they found that fibroblasts, the key cellular element of fibrosis, had low levels of caveolin-1 expression in patients with IPF. The authors also observed an inverse relationship between caveolin-1 and TGF- β 1 signaling. Transforming grow factor- β 1 attenuated the expression of caveolin-1 in human pulmonary fibroblasts, but overexpression of caveolin-1 suppressed TGF- β 1-induced production of ECM protein by fibroblasts. Thus, decreased caveolin-1 expression may unleash TGF- β 1 signaling in epithelial cells and fibroblasts. Transforming grow factor- β 1 negatively regulates telomerase activity (99), the key regulator of telomere length, cell division, and senescence. Genetic mutations affecting telomerase may also increase susceptibility to IPF (75). Assessing caveolin-1 expression in patients with mutant telomerase may uncover a link between molecular signaling and a genetic predisposition to IPF. Caveolin-1 is a novel and essential regulator of fibroblast proliferation, and these recent observations suggest that it is a logical target for therapeutic intervention to reduce the development and progression of IPF. Treatment approaches that augment caveolin-1 bioavailability may help to restore normal alveolar epithelial repair and regeneration (97).

Pulmonary hypertension in IPF

Pulmonary hypertension may be an important risk factor for increased mortality in patients with IPF (2, 61). Early studies showed that patients with IPF had elevated mean pulmonary artery pressures compared to patients with non-IPF interstitial lung diseases; pulmonary pressures increased with exercise to a greater extent in patients with IPF than in

non-IPF patients (100). When present at rest, PH is, in general, of mild to moderate severity (100) but may also sometimes be severe (101). Chronic alveolar hypoxia with subsequent vascular remodeling is probably involved in pathogenesis of PH in this setting, but the most prominent mechanism is thought to be reduction in pulmonary vascular bed because of extension of fibrosis (102). In particular, vascular changes are reported as frequent in scarred areas. They include intimal proliferation and medial thickening of muscular pulmonary arteries, muscularization of the arterioles, and intimal proliferation of pulmonary veins (32, 103). An occlusive venopathy was observed in 65% of cases in a recent study (104). Diffusion capacity is strongly correlated with PH, being inversely related (105).

In patients with IPF, early studies demonstrated presence of pulmonary-systemic vascular anastomoses (106). Abnormalities in vascular remodeling are an important component of IPF pathology. A more recent study showed increased levels of proangiogenic chemokines and cytokines in plasma of patients with IPF compared to normal volunteers (107). An imbalance of angiogenic and angiostatic chemokines contributes to pulmonary fibrosis pathogenesis and intervention targeted at this imbalance may be beneficial in IPF treatment. There is good evidence that in patients with pulmonary fibrosis, the balance is greatly shifted in favor of procoagulant activity and also antifibrinolytic activity (108).

Progression of disease

Idiopathic pulmonary fibrosis is unresponsive to current therapy, and most patients die within 3–5 years after diagnosis (21). However, it is increasingly apparent that patients with IPF exhibit distinct patterns of disease progression (18). Most of them show a long duration of symptoms before diagnosis and then experience a slowly progressive clinical course ('slow' progressors) (18). Often, an acute clinical deterioration ('AE' of IPF) precedes the terminal phase of the illness in this subgroup (18, 109). Acute exacerbations of IPF is defined as a clinical syndrome characterized by sudden worsening of dyspnea, newly developing diffuse radiographic opacities (Fig. 8), worsening hypoxemia, and absence of infectious pneumonia, heart failure, or sepsis (20). This process is characterized by diffuse alveolar damage on the background of the otherwise typical UIP pattern of injury found in IPF. Quite distinct from these observations, some patients with IPF display a more rapidly progressive clinical course with a shorter duration of symptoms before diagnosis and progression to death ('rapid' progressors). However, a systematic characterization of these distinct disease progression phenotypes has not been performed (18). A recent study represents the first identification of a distinct clinical phenotype of IPF – one that differs in clinical course and transcriptional profile, despite having similar lung function, chest imaging, and histology findings (110). Up-regulation of several functional pathways became apparent in the lungs from rapid progressor patients. These pathways seem to reflect diverse molecular mechanisms mostly operating in alveolar epithelial and mesenchymal cells and include genes involved in cell motility, myofibroblast differentiation, oxidative stress,

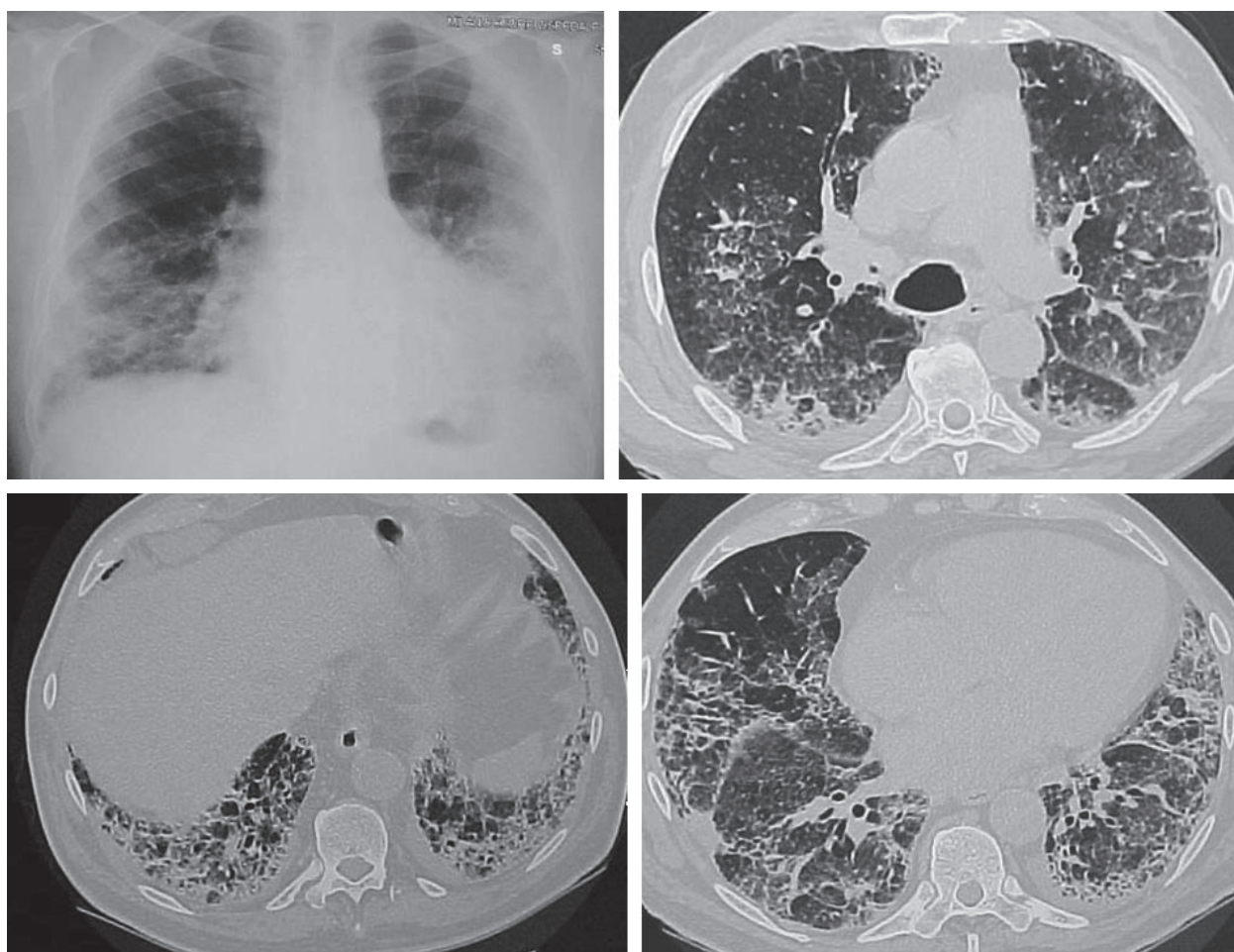


Figure 8 Ground-glass opacity in a 63-year-old man with acute exacerbation of idiopathic pulmonary fibrosis. The high-resolution computed tomography findings consist of extensive multifocal,

diffuse ground-glass opacification superimposed on a background of irregular linear opacities and sub-pleural peripheral honeycombing.

coagulation, and development. These findings suggest that during the development of this complex disease, genetic modifiers (and environmental factors, i.e. smoking) may play an important role in determining the eventual clinical phenotype and that some modifiers genes are inducing a more aggressive IPF phenotype. Taken together with reports of the impact of AEs of IPF on morbidity and mortality, these results further highlight the variability in the progression and outcome of IPF. These findings may explain, in part, the difficulty in obtaining significant and reproducible results in studies of therapeutic interventions in patients with IPF (111) and support prospective studies to identify highly reproducible biomarkers for disease progression and outcome (110).

Acute exacerbation of IPF

The etiology of AE of IPF is unknown. Perhaps, AE of IPF represents a distinct, pathobiological manifestation of the primary disease process, characterized by idiopathic lung

injury. Second, AEs of IPF may represent clinically occult but biologically distinct conditions that go undiagnosed (e.g. viral infection, aspiration). Acute exacerbations of IPF may be the sequelae of an acute direct stress to the lung, with a subsequent acceleration of the already abnormal fibroproliferative process intrinsic to IPF (20, 21). Loss of AEC integrity and injury may play an important role in AEs, leading to the extrusion of fibrin onto the alveolar surface and remodeling (20). Acute exacerbations is morphologically characterized by bronchoalveolar lavage (BAL) neutrophilia and histopathology showing diffuse alveolar damage. Both of these observations suggest that loss of AEC integrity and injury may play an important role (109, 112, 113). Environmental factors more likely interact with a genetic variability in epithelial cell function, which may explain why only a subset of patients with IPF appears to develop AEs (114). Patients with rapidly progressive IPF show increases in active matrix metalloproteinases (MMPs)-9 in BAL (115). Excessive MMP-9 may severely disrupt the structural and functional

integrity of the alveolar-capillary basement membrane (116) and may activate latent TGF- β , a profibrotic cytokine (117). Thus, increases in MMP-9 and TGF- β activation may promote the development of AE in IPF. Disordered coagulation and fibrinolysis may be important components of AEs of IPF (118). Recently, the presence of heterozygous mutations in the telomerase reverse transcriptase (hTERT) and/or RNA component (hTR) genes encoding telomerase components have been reported in several families with IPF and in one patient with sporadic IPF (75, 119). These mutations lead to shortened telomeres that may limit the regenerative capacity of AECs and contribute to the pathobiology of AE (20). Initially described as a primary response gene at the initial stage of fibroblast proliferation, ST2 gene products are expressed during the T-helper type 2 inflammatory response (120), which is the predominant immune response seen in patients with IPF. Elevated serum-soluble ST2 protein has been noted in patients with AEs compared with those with stable disease and healthy control subjects (121). ST2 elevation during an AE-IPF suggests that the T-helper type 2 inflammatory response may be an important part of the pathogenesis in this setting (122). Acute exacerbations of IPF have become increasingly important as a target for therapy (112). For example, one clinical study (123) comparing pirfenidone with placebo was prematurely terminated by the Data Safety Monitoring Board when a higher number of AEs was noted in the placebo group (placebo group, 5 of 35 patients; pirfenidone group, 0 of 35 patients). Kubo et al. (118), in studying the utility of anticoagulation as a possible treatment for IPF, noted that the major cause of clinical deterioration was AE. In a study of cyclosporine-treated patients vs those treated without cyclosporine, Homma et al. (124) suggested a better prognosis with regard to the AE of IPF in the cyclosporine-treated group. These studies indicate that AEs may be a potential therapeutic target in IPF (125).

Relationship between IPF and other IIPs

The relationship between IPF and other IIPs, such as non-specific interstitial pneumonia (NSIP) and desquamative interstitial pneumonia (DIP) are not well defined. Recent studies have identified UIP, NSIP, and even DIP in biopsy specimens from the same patient, suggesting that host responses may produce different histopathologic tissue reactions to the same stimulus/injury or that these histopathologic patterns represent a continuum that varies temporally and spatially within the same lung (126). Although fibroblastic foci are the histologic hallmark of UIP, they can occasionally be found in other IIPs and by themselves are not pathognomonic for IPF. Fibroblastic foci observed in these different conditions might reflect different disease processes. It is noteworthy that a classic UIP pattern, including honeycombing, is sometimes observed with hypersensitivity pneumonitis and in others diseases (connective tissue diseases, asbestosis and sarcoidosis). Connective tissue disease, hypersensitivity pneumonitis, drug-induced lung disease, resolved acute respiratory distress syndrome and some cases of cryptogenic organising pneumonia may all produce a his-

tological picture of NSIP (127, 128). Overall, the prognosis for patients with NSIP on biopsy is better than that for patients with UIP (median survival 33 months vs 56 months) (129). However, histology is not the sole determinant of disease outcome. Latsi et al. (129) have shown that mortality during the first 2 years following diagnosis is primarily linked to severity of pulmonary function impairment and not to histological diagnosis. Furthermore, patients with UIP on biopsy but an indeterminate or NSIP appearance on HRCT have a better prognosis than those with UIP and a typical IPF appearance on HRCT (130). Another recent observation is that disease progression in IPF, rather than always being gradual and progressive, frequently occurs through a series of rapid stepwise deteriorations (131). These apparent AEs of IPF are characterized on biopsy by the appearance of diffuse alveolar damage, the histological lesion of acute interstitial pneumonia (131). This coexistence of histological patterns and disease behavior challenges classical thinking concerning the separate and unrelated nature of these conditions (Fig. 9) (126). Familial IPF may manifest as either UIP or NSIP in different members of the same affected family. A recent study of gene expression profiling in UIP and NSIP of either familial or nonfamilial origin found only minor gene expression changes between sporadic UIP and sporadic NSIP cases but much greater variation between familial and nonfamilial cases (132). In an earlier study, Selman et al. (34) demonstrated significant gene expression profile differences between UIP and hypersensitivity pneumonitis, but found cases of NSIP with a profile indistinguishable from that of UIP. These data provide compelling evidence for the idea that a single disease entity may be responsible for causing a spectrum of histological abnormality (126).

New therapies

Idiopathic pulmonary fibrosis is among the most therapeutically challenging lung disorders. Conventional approaches to IPF treatment have been largely empirical and were based on the belief that IPF was a chronic inflammatory disease. More recently, following the emergence of a better understanding of underlying disease mechanisms, treatment approaches have been based on targeting the molecular events believed to perpetuate and sustain the fibrotic process in IPF. Patients should be told that there are no studies that prove that any medication is efficacious for IPF treatment (19). The most effective approaches are obvious; patients should stop smoking, because cigarette smoking increases the risk of superimposed inflammatory bronchial disease and neoplasia and aggravates UIP. Influenza and pneumonia vaccine should be administered. Oxygen saturation should be maintained at a level above 90% through the use of oxygen therapy. Treatment of PH with newer agents may be of benefit (133). Although in some cases improvement has been reported with corticosteroids and immunosuppressive therapies, a Cochrane Review found no data that support the use of immunosuppressive medications (134). Lung transplantation is the last resort. The search for an effective agent to treat IPF

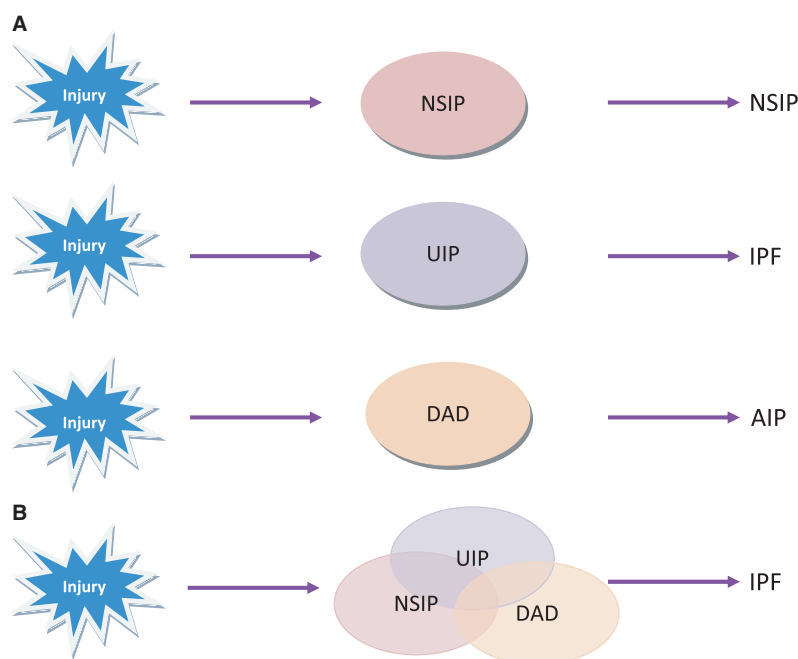


Figure 9 (A) The paradigm suggested by the current classification system is one of separate injuries giving rise to separate histological lesions and hence separate diseases. (B) A more recent hypothesis proposes that the same injury may result in one or a

combination of histological lesions that, when sharing a common clinical phenotype, represent the single disease idiopathic pulmonary fibrosis (adapted from reference 126).

continues. Many clinical trials of new therapies for IPF are ongoing and planned. Elevated levels of $\text{TNF-}\alpha$, a cytokine with inflammatory and fibrogenic properties, have been detected in the lungs in patients with IPF. Etanercept is a recombinant soluble human TNF receptor, which binds to TNF and neutralizes its activity *in vitro* (135). The negative result of the etanercept study (a well-designed randomized controlled trial of $\text{TNF-}\alpha$ antagonist therapy) is unequivocal because the trial used a placebo-controlled design, which is ethically acceptable when no known effective therapy exists (136, 137). Even though there was a trend suggesting slower disease progression for patients on etanercept vs placebo, no positive conclusions about its benefit or efficacy should be made based on this study (137). Apart from mortality, the appropriate primary endpoints for IPF trials are arguable and undecided. The use of surrogate markers for mortality in clinical trials is attractive because it allows investigators to design trials of shorter duration, thus lowering costs and improving patient retention. There has been much interest in identifying physiologic variables that may be used as surrogates for mortality in IPF (7, 8, 129). This study used two measures of pulmonary function (forced vital capacity and diffusing capacity) and a measure of gas exchange, rather than mortality, as primary endpoints. Other IPF trials have used similar surrogate measures as primary outcomes (111, 123, 138). All have failed to show the efficacy of the treatment arm. These negative results may be because surrogate markers of mortality are insensitive to the effects of the experimental treatments. Another possibility is that the

treatment outcomes used in murine model studies for IPF (prevention or resolution of scar tissue) vary greatly from those used in human trials (prevention of disease progression or decreased mortality). When investigators of the negative trial, who explored treatment of IPF with interferon-gamma 1b, examined the efficacy of the study endpoints (all surrogate markers for mortality), they concluded that mortality was the best endpoint for therapeutic studies in IPF (23). Although the study of IPF requires large numbers of subjects, the power of such studies to find differences in mortality is critical for effective drug development, and mortality should be the primary endpoint for phase 3 clinical trials in IPF. Validated outcome measures that represent either improvement or progression of diseases are need (137).

Endothelin-1, a potent, endogenous vasoconstrictor, is implicated in the pathophysiology of PH through proliferation of smooth muscle cells, inflammation, fibrosis, and endothelial dysfunction. In addition, ET-1 is a profibrotic molecule that can modulate matrix production and turnover, resulting in increased collagen synthesis and decreased interstitial collagenase production (139). In BUILD-1 study, was hypothesized that bosentan, a dual ET receptor antagonist, may influence the course of IPF through its anti-inflammatory and antifibrotic effects. In this study, bosentan treatment in patients with IPF did not show superiority over placebo on change in 6-min walking distance (6-MWD) from baseline up to month 12, the primary endpoint. However, bosentan was well tolerated and the observed clinically relevant delay in time to disease progression or death warrants further

investigation (138). A more pronounced effect was observed in patients with surgical lung biopsy-proven IPF. In conclusion, the results of the BUILD-1 study are sufficiently encouraging to be evaluated further in a larger trial. Selection of a primary endpoint of accepted clinical relevance is of paramount importance in any new trial and has already been addressed in the design of the ongoing, larger BUILD-3 morbidity/mortality study in patients with IPF (138). A phase 3 randomized, double-blind, placebo-controlled study to evaluate the safety and effectiveness of ambrisentan, an ET receptor antagonist that is selective for the type A ET receptor, in early IPF is ongoing. Recently macitentan, a nonselective ET A and B receptor antagonist, is being studied in a multicenter phase 2 study in patients with IPF.

Pirfenidone is a novel anti-inflammatory, antifibrotic compound that has generated significant interest as a potential therapeutic agent. Patients treated with pirfenidone had decreased loss of vital capacity when compared with placebo at 9 months (123). This improved preservation of pulmonary function, while statistically significant, is small in absolute terms and has questionable clinical significance. However, the pirfenidone-treatment group had no AEs, while 14% of those treated with placebo had an AE (123). Some experts have questioned the design of this initial investigation, but these findings should pave the way for larger, long-term trials that can further define the role of pirfenidone in the treatment of IPF, and results of a large phase 3 clinical trial (CAPACITY) are ongoing.

Anticoagulation has also been investigated as a therapeutic modality in patients with pulmonary fibrosis. Efficacy potentially occurs through the prevention of *in situ* thrombosis, the attenuation of the prothrombotic stimulus occur-

ring after alveolar disruption, or the prevention of fibrin deposition in the alveolar space after acute lung injury (118).

Another potential therapy for IPF is N-acetylcysteine (NAC), which is a molecular precursor to the naturally occurring antioxidant glutathione. Glutathione is known to be depleted in the lungs of patients with IPF (140). Theoretically, oral NAC should replete glutathione stores and restore natural oxidant/antioxidant balance to prevent oxidative injury that precedes fibroproliferation (62). A large, randomized, double-blinded study of 182 patients with IPF to compare the efficacy of a regimen that included prednisone, azathioprine, and NAC to a regimen of only prednisone and azathioprine was conducted (141). A statistically significant difference in lung function was found to favor treatment with prednisone/azathioprine/NAC. However, the study is flawed because of a high rate of drop-out among the study subjects. Drop-out was addressed in statistical analysis by deriving and using imputed data for missing data points. Both groups in the study experienced decline of lung function over the 12-month study period; the difference was that one group declined more. The regimen of prednisone/azathioprine/NAC was shown to be possibly superior to prednisone/azathioprine alone. However, the efficacy of prednisone/azathioprine/NAC has not been compared to placebo and has never been shown to improve or stabilize patients with IPF. The PANTHER study for patients in the early stage of the disease will compare prednisone/azathioprine/NAC against placebo. Roughly, 400 randomized patients will be treated for 60 weeks. Patients will be divided into three categories: those who take NAC only, those who take a combination of all three drugs, and those who take the placebo.

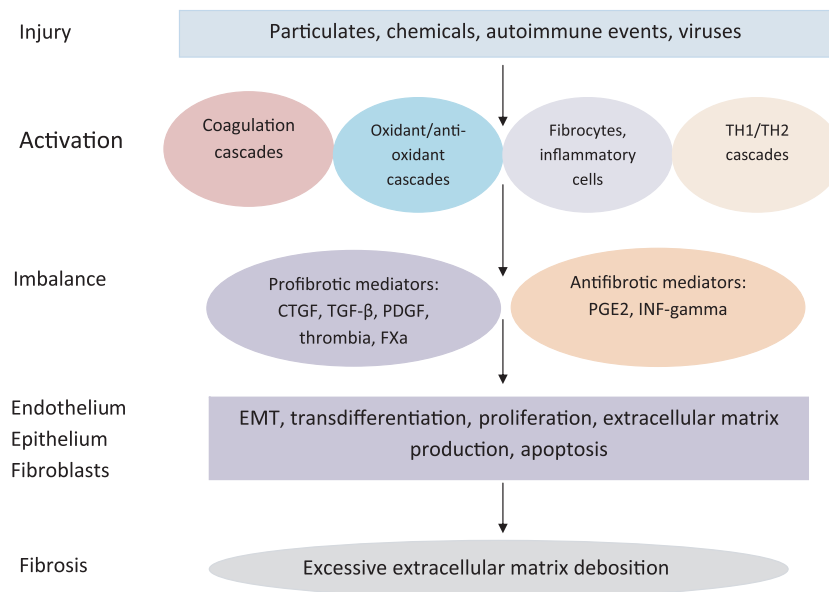


Figure 10 A new model for the pathogenesis of idiopathic pulmonary fibrosis: injury activates multiple inflammatory, cell signaling, and repair pathways. Activation of these cascades causes an imbalance in pro- and antifibrotic mediators. In turn, these

mediators activate multiple cell types, causing changes in cellular functioning and cell-cell interactions that ultimately result in progressive fibrosis (adapted from reference 126).

The interferon-gamma-1b INSPIRE trial was terminated recently because of lack of efficacy at a predefined interim analysis.

Many patients with IPF also have secondary PH. Sildenafil is a phosphodiesterase-5 inhibitor that dilates pulmonary vasculature. A recently published case series found an average increase in 6-MWD of 49 m in approximately half of the patients with IPF treated with sildenafil (142). Future-controlled studies may confirm that sildenafil represents an important treatment for improving quality of life in a subset of patients with IPF. The STEP trial will evaluate the effectiveness of sildenafil on patients with late-stage IPF.

Imatinib mesylate inhibits activation of the PDGF receptor significantly; a phase 2, randomized, double-blinded, placebo-controlled study of the safety and clinical effect of imatinib administered orally has been concluded. The efficacy of the biological agent has been encouraging (143), but susceptibility to infections has been a major concern. BIBF 1120 is a small molecule inhibitor targeting the receptor kinases of PDGF, basic fibroblast growth factor and vascular endothelial growth factor, which have known roles in the pathogenesis of pulmonary fibrosis; a phase 2 clinical trial to evaluate the efficacy and safety of this molecule is ongoing.

Understanding the mechanism of recruitment of stem cells to sites of injury, and their involvement in tissue repair, regeneration, and remodeling may offer a novel therapeutic target for developing more effective treatments against this fatal disorder.

Conclusions

The process of evolution has created considerable pleiotropism, as well as much redundancy, in cell signaling and wound healing pathways. It is possible that IPF develops as a consequence of abnormalities occurring in multiple biological pathways that affect inflammation and wound repair. Therefore, IPF represents a syndrome rather than one specific disease and that it is the end stage of a fibrosing process.

A consequence of evolutionary adaptation is that major abnormalities in single key molecules can be compensated for by the recruitment of otherwise redundant mechanisms. It is often only when errors occur in multiple pathways that protective mechanisms are overcome and disease develops. The

idea of multiple hits has best been elucidated in malignancy, where it has been demonstrated that somatic mutations in a sequence of key oncogenes are required before malignancy develops (144). It is now clear that both individual susceptibility to IPF and the pattern of disease behavior and progression are linked to patient genotype (145). A number of investigators have undertaken an extensive search for fibrosis genes. A range of polymorphisms have been related to susceptibility to IPF and to disease severity and progression (145). Furthermore, tentative evidence has been published suggesting that somatic gene mutation plays a role in the development of IPF (146). Following injury, a multiple pathway mechanism is at play in the pathogenesis of IPF (Fig. 10). A range of molecular abnormalities that can result in fibrosis are now well characterized. The clotting cascade, antioxidant pathways, apoptosis, inflammatory cytokines, angiogenesis and vascular remodeling, growth factors, surfactant, and matrix regulatory factors have all been implicated in pulmonary fibrosis (93, 147). Furthermore, these cascades initiate changes in the behavior and morphology of multiple cell types, including epithelial cells, fibroblasts, endothelial cells, resident and migratory inflammatory cells, and, as has recently been described, circulating fibroblast progenitor cells (fibrocytes) (126). Evidence for the relative roles of these key pathways and the interactions between different cell types in the pathogenesis of fibrosis in humans is gradually emerging (126). As is the case in other complex diseases, it believes that the balance of abnormalities in each of these key pathways may vary between affected individuals. This would explain the range of clinical, radiological, and pathological phenotypes observed in IPF. Current thinking in IPF has tended to focus on individual mediators of fibrosis or single pathways linked to fibrogenesis. The limitations of this model have been reflected in disappointing trials of therapy targeting individual disease pathways. Ongoing adherence to this approach threatens to affect the development of future therapies and therapeutic strategies. Adopting a multiple-pathway model for understanding the pathogenesis of IPF would facilitate a future research approach better suited to the understanding of this complex disease. Furthermore, if the multiple-pathway hypothesis is correct, it seems more likely that the most effective approach to treatment would be to target multiple fibrosis pathways simultaneously. This approach to treatment is analogous to that used in the treatment of many cancers and idiopathic PH.

References

1. Schwartz DA, Helmers RA, Galvin JR, Van Fossen DS, Frees KL, Dayton CS et al. Determinants of survival in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 1994;**149**:450–454.
2. King TE Jr, Tootz JA, Schwarz MI, Brown KR, Cherniack RM. Predicting survival in idiopathic pulmonary fibrosis: scoring system and survival model. *Am J Respir Crit Care Med* 2001;**164**:1171–1181.
3. Coultas DB, Zumwalt RE, Black WC, Sobonya RE. The epidemiology of interstitial lung diseases. *Am J Respir Crit Care Med* 1994;**150**:967–972.
4. American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). *Am J Respir Crit Care Med* 2000;**161**:646–664.
5. Lama VN, Flaherty KR, Toews GB, Colby TV, Travis WD, Long Q et al. Prognostic value of desaturation during a 6-minute walk test in idiopathic interstitial pneumonia. *Am J Respir Crit Care Med* 2003;**168**:1084–1090.
6. Caminati A, Bianchi A, Cassandro R, Mirenda MR, Harari S. Walking distance on 6-MWT is a prognostic factor in idiopathic pulmonary fibrosis. *Respir Med* 2009;**103**:117–123.

7. Flaherty KR, Mumford JA, Murray S, Kazerooni EA, Gross BH, Colby TV et al. Prognostic implications of physiologic and radiographic changes in idiopathic interstitial pneumonia. *Am J Respir Crit Care Med* 2003;**168**:543–548.
8. Collard HR, King TE Jr, Bartelson BB, Vourlekis JS, Schwarz MI, Brown KK. Changes in clinical and physiologic variables predict survival in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2003;**168**:538–542.
9. Harari S, Caminati A. Idiopathic pulmonary fibrosis. *Allergy* 2005;**60**:421–435.
10. Martinez FJ, Flaherty K. Pulmonary function testing in idiopathic interstitial pneumonias. *Proc Am Thorac Soc* 2006;**3**:315–321.
11. Gay SE, Kazerooni EA, Toews GB, Lynch JP, Gross BH, Cascade PN et al. Idiopathic pulmonary fibrosis: predicting response to therapy and survival. *Am J Respir Crit Care Med* 1998;**157**:1063–1072.
12. Caminati A, Harari S. Which prognostic indicator should we use for the clinical practice in the initial evaluation and follow-up of IIP: should we stand on PFT, HRCT or... whatever? *Sarcoidosis Vasc Diffuse Lung Dis* 2005;**22**:S24–S30.
13. Nadrous HF, Pellikka PA, Krowka MJ, Swanson KL, Chaowalit N, Decker PA et al. Pulmonary hypertension in patients with idiopathic pulmonary fibrosis. *Chest* 2005;**128**:2393–2399.
14. Hunninghake GW, Lynch DA, Galvin JR, Gross BH, Müller N, Schwartz DA et al. Radiologic findings are strongly associated with a pathologic diagnosis of usual interstitial pneumonia. *Chest* 2003;**124**:1215–1223.
15. Flaherty KR, Travis WD, Colby TV, Toews GB, Kazerooni EA, Gross BH et al. Histopathologic variability in usual and nonspecific interstitial pneumonias. *Am J Respir Crit Care Med* 2001;**164**:1722–1727.
16. Katzenstein AL, Myers JL. Idiopathic pulmonary fibrosis: clinical relevance of pathologic classification. *Am J Respir Crit Care Med* 1998;**157**:1301–1315.
17. King TE Jr, Schwarz MI, Brown K, Tooze JA, Colby TV, Waldron JA et al. Idiopathic pulmonary fibrosis: relationship between histopathologic features and mortality. *Am J Respir Crit Care Med* 2001;**164**:1025–1032.
18. Martinez FJ, Safrin S, Weycker D, Starko KM, Bradford WZ, King TE et al. The clinical course of patients with idiopathic pulmonary fibrosis. *Ann Intern Med* 2005;**142**:963–996.
19. King TE Jr, du Bois RM. Elucidating the causes and examining the latest clinical findings in pulmonary fibrosis. *Eur Respir Rev* 2008;**17**:105–107.
20. Collard HR, Moore BB, Flaherty KR, Brown KK, Kaner RJ, King TE et al. Acute exacerbations of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2007;**176**:636–643.
21. Selman M, King TE, Pardo A. Idiopathic pulmonary fibrosis: prevailing and evolving hypotheses about its pathogenesis and implications for therapy. *Ann Intern Med* 2001;**134**:136–151.
22. Gross TG, Hunninghake GW. Idiopathic pulmonary fibrosis. *N Engl J Med* 2001;**345**:517–525.
23. King TE Jr, Safrin S, Starko KM, Brown KK, Noble PW, Raghu G et al. Analyses of efficacy end points in a controlled trial of interferon-gamma 1b for idiopathic pulmonary fibrosis. *Chest* 2005;**127**:171–177.
24. Myers JL, Katzenstein AL. Epithelial necrosis and alveolar collapse in the pathogenesis of usual interstitial pneumonia. *Chest* 1988;**94**:1309–1311.
25. Daniil ZD, Gilchrist FC, Nicholson AG, Hansell DM, Harris J, Colby TV et al. A histologic pattern of nonspecific interstitial pneumonia is associated with a better prognosis than usual interstitial pneumonia in patients with cryptogenic fibrosing alveolitis. *Am J Respir Crit Care Med* 1999;**160**:899–905.
26. Fukuda Y, Basset F, Ferrans VJ, Yamanaka M. Significance of early intra-alveolar fibrotic lesions and integrin expression in lung biopsy specimens from patients with idiopathic pulmonary fibrosis. *Hum Pathol* 1995;**26**:53–61.
27. Kuhn C, McDonald JA. The roles of the myofibroblast in idiopathic pulmonary fibrosis: ultrastructural and immunohistochemical features of sites of active extracellular matrix synthesis. *Am J Pathol* 1991;**138**:1257–1265.
28. Cool CD, Groshong SD, Rai PR, Henson PM, Stewart JS, Brown KK. Fibroblast foci are not discrete sites of lung injury or repair. The fibroblast reticulum. *Am J Respir Crit Care Med* 2006;**174**:654–658.
29. Phan SH. The myofibroblast in pulmonary fibrosis. *Chest* 2002;**122**:286S–289S.
30. Moore BB, Kolodick JE, Thannickal VJ, Cooke K, Moore TA, Hogaboam C et al. CCR2-mediated recruitment of fibrocytes to the alveolar space after fibrotic injury. *Am J Pathol* 2005;**166**:675–684.
31. Kasai H, Allen JT, Mason RM, Kamimura T, Zhang Z. TGF-beta1 induces human alveolar epithelial to mesenchymal cell transition (EMT). *Respir Res* 2005;**6**:56.
32. Travis WD, Colby TV, Koss MN, Rosado-Christenson ML, Müller NL, King TE Jr. Idiopathic interstitial pneumonia and other diffuse parenchymal lung disease. In: Donald West King, editor. Non-neoplastic disorders of the lower respiratory tract. Washington: American Registry of Pathology and the Armed Forces Institute of Pathology, 2002:59–72.
33. Selman M, Pardo A. The epithelial/fibroblastic pathway in the pathogenesis of idiopathic pulmonary fibrosis. *Am J Respir Cell Mol Biol* 2003;**29**:93S–97S.
34. Selman M, Pardo A, Barrera L, Estrada A, Watson SR, Wilson K et al. Gene expression profiles distinguish idiopathic pulmonary fibrosis from hypersensitivity pneumonitis. *Am J Respir Crit Care Med* 2006;**173**:188–198.
35. Noble PW. Epithelial fibroblast triggering and interactions in pulmonary fibrosis. *Eur Respir Rev* 2008;**17**:123–129.
36. Basset F, Ferrans VJ, Soler P, Takemura T, Fukuda Y, Crystal RG. Intraluminal fibrosis in interstitial lung disorders. *Am J Pathol* 1986;**122**:443–461.
37. Chilosi M, Poletti V, Murer B, Lestani M, Cancellieri A, Montagna L et al. Abnormal re-epithelialization and lung remodeling in idiopathic pulmonary fibrosis: the role of deltaN-p63. *Lab Invest* 2002;**82**:1335–1345.
38. Katzenstein AL. Pathogenesis of “fibrosis” in interstitial pneumonia: an electron microscopic study. *Hum Pathol* 1985;**16**:1015–1024.
39. Kawanami O, Ferrans VJ, Crystal RG. Structure of alveolar epithelial cells in patients with fibrotic lung disorders. *Lab Invest* 1982;**46**:39–53.
40. Corrin B, Dewar A, Rodriguez-Roisin R, Turner-Warwick M. Fine structural changes in cryptogenic fibrosing alveolitis and asbestosis. *J Pathol* 1985;**147**:107–119.
41. Studer SM, Kaminski N. Towards systems biology of human pulmonary fibrosis. *Proc Am Thorac Soc* 2007;**4**:85–91.
42. Antoniadis HN, Bravo MA, Avila RE, Galanopoulos T, Neville-Golden J, Maxwell M et al. Platelet-derived growth factor in idiopathic pulmonary fibrosis. *J Clin Invest* 1990;**86**:1055–1064.
43. Nash JR, McLaughlin PJ, Butcher D, Corrin B. Expression of tumour necrosis factor- α in cryptogenic fibrosing alveolitis. *Histopathology* 1993;**22**:343–347.
44. Khalil N, O'Connor RN, Unruh HW, Warren PW, Flanders KC, Kemp A et al. Increased production and immunohistochemical localization of transforming growth factor- β in idiopathic pulmonary fibrosis. *Am J Respir Cell Mol Biol* 1991;**5**:155–162.
45. Giaid A, Michel RP, Steward DJ, Sheppard M, Corrin B, Hamid Q. Expression of endothelin-1 in lung of patients with cryptogenic fibrosing alveolitis. *Lancet* 1993;**341**:1550–1554.
46. Pan LH, Yamauchi K, Uzuki M, Nakanishi T, Takigawa M, Inoue H et al. Type II alveolar epithelial cells and interstitial fibro-

- blasts express connective tissue growth factor in IPF. *Eur Respir J* 2001;**17**:1220–1227.
47. Schock F, Perrimon N. Molecular mechanisms of epithelial morphogenesis. *Annu Rev Cell Dev Biol* 2002;**18**:463–493.
 48. Thiery JP, Sleeman JP. Complex networks orchestrate epithelial–mesenchymal transitions. *Nat Rev Mol Cell Biol* 2006;**7**:131–142.
 49. Moustakas A, Heldin CH. Signaling networks guiding epithelial–mesenchymal transitions during embryogenesis and cancer progression. *Cancer Sci* 2007;**98**:1512–1520.
 50. Zavadil J, Bottinger EP. TGF- β and epithelial-to-mesenchymal transitions. *Oncogene* 2005;**24**:5764–5774.
 51. Huber MA, Kraut N, Beug H. Molecular requirements for epithelial–mesenchymal transition during tumor progression. *Curr Opin Cell Biol* 2005;**17**:548–558.
 52. Christiansen JJ, Rajasekaran AK. Reassessing epithelial to mesenchymal transition as a prerequisite for carcinoma invasion and metastasis. *Cancer Res* 2006;**66**:8319–8326.
 53. Willis BC, Borok Z. TGF- β -induced EMT: mechanisms and implications for fibrotic lung disease. *Am J Physiol Lung Cell Mol Physiol* 2007;**293**:L525–L534.
 54. Willis BC, Liebler JM, Luby-Phelps K, Nicholson AG, Crandall ED, du Bois RM et al. Induction of epithelial–mesenchymal transition in alveolar epithelial cells by transforming growth factor- β 1: potential role in idiopathic pulmonary fibrosis. *Am J Pathol* 2005;**166**:1321–1332.
 55. Kim KK, Kugler MC, Wolters PJ, Robillard L, Galvez MG, Brumwell AN et al. Alveolar epithelial cell mesenchymal transition develops in vivo during pulmonary fibrosis and is regulated by the extracellular matrix. *Proc Natl Acad Sci USA* 2006;**103**:13180–13185.
 56. Selman M, Pardo A, Kaminski N. Idiopathic pulmonary fibrosis: aberrant recapitulation of developmental programs? *PLoS Med* www.plosmedicine.org 2008;**5**:e62.
 57. Koli K, Myllärniemi M, Vuorinen K, Salmenkivi K, Ryyänänen MJ, Kinnula VL et al. Bone morphogenetic protein-4 inhibitor gremlin is overexpressed in idiopathic pulmonary fibrosis. *Am J Pathol* 2006;**169**:61–71.
 58. Warburton D, Schwarz M, Tefft D, Flores-Delgado G, Anderson KD, Cardoso WV. The molecular basis of lung morphogenesis. *Mech Dev* 2000;**92**:55–81.
 59. Selman M, Pardo A. Role of epithelial cells in idiopathic pulmonary fibrosis: from innocent targets to serial killers. *Proc Am Thorac Soc* 2006;**3**:364–372.
 60. Globe GC, Schiemann WP, Lodish HF. Role of transforming growth factor β in human disease. *N Engl J Med* 2000;**342**:1350–1358.
 61. Horowitz JC, Thannickal VJ. Idiopathic pulmonary fibrosis: new concepts in pathogenesis and implications for drug therapy. *Treat Respir Med* 2006;**5**:325–342.
 62. Cantin AM, North SL, Fells GA, Hubbard RC, Crystal RG. Oxidant-mediated epithelial cell injury in idiopathic pulmonary fibrosis. *J Clin Invest* 1987;**79**:1665–1673.
 63. Thannickal VJ, Fanburg BL. Reactive oxygen species in cell signaling. *Am J Physiol Lung Cell Mol Physiol* 2000;**279**:L1005–L1028.
 64. Thannickal VJ, Aldweib KD, Fanburg BL. Tyrosine phosphorylation regulates H₂O₂ production in lung fibroblasts stimulated by transforming growth factor β 1. *J Biol Chem* 1998;**273**:23611–23615.
 65. Maeyama T, Kuwano K, Kawasaki M, Kunitake R, Hagimoto N, Matsuba T et al. Upregulation of Fas-signalling molecules in lung epithelial cells from patients with idiopathic pulmonary fibrosis. *Eur Respir J* 2001;**17**:180–189.
 66. Kuwano K, Hagimoto N, Kawasaki M, Yatomi T, Nakamura N, Nagata S et al. Essential roles of the Fas–Fas ligand pathway in the development of pulmonary fibrosis. *J Clin Invest* 1999;**104**:13–19.
 67. Aoshiba K, Yasui S, Tamaoki J, Nagai A. The Fas/Fas-ligand system is not required for bleomycin-induced pulmonary fibrosis in mice. *Am J Respir Crit Care Med* 2000;**162**:695–700.
 68. Wang R, Zagariya A, Ang E, Ibarra-Sunga O, Uhal BD. Fas-induced apoptosis of alveolar epithelial cells require ANG II generation and receptor interaction. *Am J Physiol* 1999;**277**:L1245–L1250.
 69. Papp M, Li X, Zhuang J, Wang R, Uhal BD. Angiotensin receptor subtype AT₁ (1) mediates alveolar epithelial cell apoptosis in response to ANG II. *Am J Physiol Lung Cell Mol Physiol* 2002;**282**:L713–L718.
 70. Uhal BD, Gidea C, Bargout R, Bifero A, Ibarra-Sunga O, Papp M et al. Captopril inhibits apoptosis in human lung epithelial cells: a potential antifibrotic mechanism. *Am J Physiol* 1998;**275**:L1013–L1017.
 71. Li X, Rayford H, Uhal BD. Essential roles for angiotensin receptor AT₁a in bleomycin-induced apoptosis and lung fibrosis in mice. *Am J Pathol* 2003;**163**:2523–2530.
 72. Otsuka M, Takahashi H, Shiratori M, Chiba H, Abe S. Reduction of bleomycin induced lung fibrosis by candesartan cilexetil, an angiotensin II type 1 receptor antagonist. *Thorax* 2004;**59**:31–38.
 73. Phatak P, Burger AM. Telomerase and its potential for therapeutic intervention. *Br J Pharmacol* 2007;**152**:1003–1011.
 74. Holt SF, Shay JW, Wright WF. Refining the telomere-telomerase hypothesis of aging and cancer. *Nat Biotechnol* 1996;**14**:1734–1741.
 75. Armanios MY, Chen JJ, Cogan JD, Alder JK, Ingersoll RG, Markin C et al. Telomerase mutations in families with idiopathic pulmonary fibrosis. *N Engl J Med* 2007;**356**:1317–1326.
 76. Morlà M, Busquets X, Pons J, Saulea J, MacNee W, Agustí AG. Telomere shortening in smokers with and without COPD. *Eur Respir J* 2006;**27**:525–528.
 77. Gabbiani G. The myofibroblast in wound healing and fibrocontractive diseases. *J Pathol* 2003;**200**:500–503.
 78. Phillips RJ, Burdick MD, Hong K, Lutz MA, Murray LA, Xue YY et al. Circulating fibrocytes traffic to the lungs in response to CXCL 12 and mediate fibrosis. *J Clin Invest* 2004;**114**:438–446.
 79. Hashimoto N, Jin H, Liu T, Chensue SW, Phan SH. Bone marrow-derived progenitor cells in pulmonary fibrosis. *J Clin Invest* 2004;**113**:243–252.
 80. Desmouliere A, Redard M, Darby I, Gabbiani G. Apoptosis mediates the decrease in cellularity during the transition between granulation tissue and scar. *Am J Pathol* 1995;**146**:56–66.
 81. Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nat Rev Mol Cell Biol* 2002;**3**:349–363.
 82. Thannickal VJ, Toews GB, White ES, Lynch JP, Martinez FJ. Mechanisms of pulmonary fibrosis. *Annu Rev Med* 2004;**55**:395–417.
 83. Border WA, Noble NA. Transforming growth factor β in tissue fibrosis. *N Engl J Med* 1994;**331**:1286–1292.
 84. Chapman HA. Disorders of lung matrix remodeling. *J Clin Invest* 2004;**113**:148–157.
 85. Horowitz JC, Lee DY, Waghay M, Keshamouni VG, Thomas PE, Zhang H et al. Activation of the pro-survival phosphatidylinositol 3-kinase/AKT pathway by transforming growth factor- β 1 in mesenchymal cells is mediated by p38 MAPK-dependent induction of an autocrine growth factor. *J Biol Chem* 2004;**279**:1359–1367.
 86. Zhang HY, Phan SH. Inhibition of myofibroblast apoptosis by transforming growth factor β 1. *Am J Respir Cell Mol Biol* 1999;**21**:658–665.
 87. Sun G, Stacey MA, Bellini A, Marini M, Mattoli S. Endothelin-1 induces bronchial myofibroblast differentiation. *Peptides* 1997;**18**:1449–1451.
 88. Bogatkevich GS, Tourkina E, Silver RM, Ludwicka-Bradley A. Thrombin differentiates normal lung fibroblasts to a myofibroblast phenotype via the proteolytically

- activated receptor-1 and a protein kinase C-dependent pathway. *J Biol Chem* 2001;**276**:45184–45192.
89. Morishima Y, Nomura A, Uchida T, Noguchi Y, Sakamoto T, Ishii Y et al. Triggering the induction of myofibroblast and fibrogenesis by airway epithelial shedding. *Am J Respir Cell Mol Biol* 2001;**24**:1–11.
 90. Gericke A, Munson M, Ross AH. Regulation of the PTEN phosphatase. *Gene* 2006;**374**:1–9.
 91. Di Cristofaro A, Pesce B, Cordon-Cardo C, Pandolfi PP. PTEN is essential for embryonic development and tumour suppression. *Nat Genet* 1998;**19**:348–355.
 92. Stiles B, Groszer M, Wang S, Jiao J, Wu H. PTEN less means more. *Dev Biol* 2004;**273**:175–184.
 93. Thannickal VJ, Horowitz JC. Evolving concepts of apoptosis in idiopathic pulmonary fibrosis. *Proc Am Thorac Soc* 2006;**3**:350–356.
 94. Lappi-Blanco E, Soini Y, Paakko P. Apoptotic activity is increase in the newly formed fibromyxoid connective tissue in bronchiolitis obliterans organizing pneumonia. *Lung* 1999;**177**:367–376.
 95. White ES, Atrasz RG, Hu B, Phan SH, Stambolic V, Mak TW et al. Negative regulation of myofibroblast differentiation by PTEN (Phosphatase and Tensin Homolog Deleted on Chromosome 10). *Am J Respir Crit Care Med* 2006;**173**:112–121.
 96. Wang XM, Zhang Y, Kim HP, Zhou Z, Feghali-Bostwick CA, Liu F et al. Caveolin-1: a critical regulator of lung fibrosis in idiopathic pulmonary fibrosis. *J Exp Med* 2006;**203**:2895–2906.
 97. Verma S, Slutsky AS. Idiopathic pulmonary fibrosis – new insights. *N Engl J Med* 2007;**356**:1370–1372.
 98. Schwencke C, Braun-Dullaeus RC, Wunderlich C, Strasser RH. Caveolae and caveolin in transmembrane signaling: implications for human disease. *Cardiovasc Res* 2006;**70**:42–49.
 99. Li H, Xu D, Li J, Berndt MC, Liu JP. Transforming growth factor beta suppresses human telomerase reverse transcriptase (hTERT) by Smad3 interactions with c-Myc and the hTERT gene. *J Biol Chem* 2006;**281**:25588–25600.
 100. Weitzenblum E, Ehrhart M, Rasaholainahary J, Hirth C. Pulmonary hemodynamics in idiopathic pulmonary fibrosis and other interstitial pulmonary diseases. *Respiration* 1983;**44**:118–127.
 101. Leuchte HH, Neurohr C, Baumgartner R, Holzapfel M, Giehl W, Vogeser M et al. Brain natriuretic peptide and exercise capacity in lung fibrosis and pulmonary hypertension. *Am J Respir Crit Care Med* 2004;**170**:360–365.
 102. Weitzenblum E, Chaouat A. Pulmonary hypertension due to chronic hypoxic lung disease. In: Peacock AJ, Rubin LJ, editors. Pulmonary circulation: diseases and their treatment. London: Arnold, 2004:374–386.
 103. Katzenstein AL. Idiopathic interstitial pneumonia. In: Katzenstein AL, editor. Katzenstein and Askin's surgical pathology of non-neoplastic lung disease. Philadelphia: WB Saunders Co, 1997:48–80.
 104. Colombat M, Mal E, Groussard O, Capron F, Thabut G, Jebrak G et al. Pulmonary vascular lesions in end-stage idiopathic pulmonary fibrosis: histopathologic study on lung explant specimens and correlations with pulmonary hemodynamics. *Human Pathol* 2007;**38**:60–65.
 105. Lettieri CJ, Nathan SD, Barnett SD, Ahmad S, Shorr AF. Prevalence and outcomes of pulmonary arterial hypertension in advanced idiopathic pulmonary fibrosis. *Chest* 2006;**129**:746–752.
 106. Turner-Warwick M. Precapillary systemic-pulmonary anastomoses. *Thorax* 1963;**18**:225–237.
 107. Simler NR, Brenchley PE, Horrocks AW, Greaves SM, Hasleton PS, Egan JJ. Angiogenic cytokines in patients with idiopathic interstitial pneumonia. *Thorax* 2004;**59**:581–585.
 108. Chambers RC. Abnormal wound healing responses in pulmonary fibrosis: focus on coagulation signalling. *Eur Respir Rev* 2008;**17**:130–137.
 109. Ambrosini V, Cancellieri A, Chilosi M, Zompatori M, Trisolini R, Saragoni L et al. Acute exacerbation of idiopathic pulmonary fibrosis: report of a series. *Eur Respir J* 2003;**22**:821–826.
 110. Selman M, Carrillo G, Estrada A, Mejia M, Becerril C, Cisneros J et al. Accelerated variant of idiopathic pulmonary fibrosis: clinical behavior and gene expression pattern. *PLoS ONE* 2007;**2**:e482.
 111. Raghu G, Brown KK, Bradford WZ, Starko K, Noble PW, Schwartz DA et al. Idiopathic pulmonary fibrosis study group. A placebo-controlled trial of interferon gamma-1b in patients with idiopathic pulmonary fibrosis. *N Eng J Med* 2004;**350**:125–133.
 112. Parambil JG, Myers JL, Ryu JH. Histopathologic features and outcome of patients with acute exacerbation of idiopathic pulmonary fibrosis undergoing surgical lung biopsy. *Chest* 2005;**128**:3310–3315.
 113. Rice AJ, Wells AU, Bouros D, du Bois RM, Hansell DM, Polychronopoulos V et al. Terminal diffuse alveolar damage in relation to interstitial pneumonias: an autopsy study. *Am J Clin Pathol* 2003;**119**:709–714.
 114. Schwartz DA. The importance of gene-environment interactions and exposure assessment in understanding human diseases. *J Expo Sci Environ Epidemiol* 2006;**16**:474–476.
 115. Suga M, Iyonaga K, Okamoto T, Gushima Y, Miyakawa H, Akaike T et al. Characteristic elevation of matrix metalloproteinase activity in idiopathic interstitial pneumonias. *Am J Respir Crit Care Med* 2000;**162**:1949–1956.
 116. Atkinson JJ, Senior RM. Matrix metalloproteinase-9 in lung remodeling. *Am J Respir Cell Mol Biol* 2003;**28**:12–24.
 117. Yu Q, Stamenkovic I. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. *Genes Dev* 2000;**14**:163–176.
 118. Kubo H, Nakayama K, Yanai M, Suzuki T, Yamaya M, Watanabe M et al. Anticoagulant therapy for idiopathic pulmonary fibrosis. *Chest* 2005;**128**:1475–1482.
 119. Tsakiri K, Cronkhite J, Kuan P, Xing C, Raghu G, Weissler JC et al. Adult-onset pulmonary fibrosis caused by mutations in telomerase. *Proc Natl Acad Sci USA* 2007;**104**:7552–7557.
 120. Xu D, Chan WL, Leung BP, Hunter D, Schulz K, Carter RW et al. Selective expression of a stable cell surface molecule on type 2 but not type 1 helper T cells. *J Exp Med* 1998;**187**:787–794.
 121. Tajima S, Oshikawa K, Tominaga S, Sugiyama Y. The increase in serum soluble ST2 protein upon acute exacerbation of idiopathic pulmonary fibrosis. *Chest* 2003;**124**:1206–1214.
 122. Hyzy R, Huang S, Myers J, Flaherty K, Martinez F. Acute exacerbation of idiopathic pulmonary fibrosis. *Chest* 2007;**132**:1652–1658.
 123. Azuma A, Nukiwa T, Tsuboi E, Suga M, Abe S, Nakata K et al. Double-blind, placebo-controlled trial of pirfenidone in patients with idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2005;**171**:1040–1047.
 124. Homma S, Sakamoto S, Kawabata M, Kishi K, Tsuboi E, Motoi N et al. Cyclosporin treatment in steroid-resistant and acutely exacerbated interstitial pneumonia. *Intern Med* 2005;**44**:1144–1150.
 125. Noth I, Martinez FJ. Recent advances in idiopathic pulmonary fibrosis. *Chest* 2007;**132**:637–650.
 126. Maher TM, Wells AU, Laurent GJ. Idiopathic pulmonary fibrosis: multiple causes and multiple mechanisms? *Eur Respir J* 2007;**30**:835–839.
 127. American Thoracic Society, European Respiratory Society. American Thoracic Society/European Respiratory Society international multidisciplinary consensus classification of the idiopathic interstitial

- pneumonias. *Am J Respir Crit Care Med* 2002;**165**:277–304.
128. Nicholson AG, Wells AU. Nonspecific interstitial pneumonia – nobody said it's perfect. *Am J Respir Crit Care Med* 2001;**164**:1553–1554.
 129. Latsi PI, du Bois RM, Nicholson AG, Colby TV, Bisirtzoglou D, Nikolakopoulou A et al. Fibrotic idiopathic interstitial pneumonia: the prognostic value of longitudinal functional trends. *Am J Respir Crit Care Med* 2003;**168**:531–537.
 130. Flaherty KR, Thwaite EL, Kazerooni EA, Gross BH, Toews GB, Colby TV et al. Radiological versus histological diagnosis in UIP and NSIP: survival implications. *Thorax* 2003;**58**:143–148.
 131. Kim DS, Park JH, Park BK, Lee JS, Nicholson AG, Colby T. Acute exacerbation of idiopathic pulmonary fibrosis: frequency and clinical features. *Eur Respir J* 2006;**27**:143–150.
 132. Yang IV, Burch LH, Steele MP, Savov JD, Hollingsworth JW, McElvania-Tekippe E et al. Gene expression profiling of familial and sporadic interstitial pneumonia. *Am J Respir Crit Care Med* 2007;**175**:45–54.
 133. Teirstein AS. The elusive goal of therapy for usual interstitial pneumonia. *N Eng J Med* 2004;**350**:181–183.
 134. Davies HR, Richeldi L, Walters EH. Immunomodulatory agents for idiopathic pulmonary fibrosis. *Cochrane Database Syst Rev* 2003;**3**:CD003134.
 135. Mohler KM, Torrance DS, Smith CA, Goodwin RG, Stremmer KE, Fung VP et al. Soluble tumor necrosis factor (TNF) receptors are effective therapeutic agents in lethal endotoxemia and function simultaneously as both TNF carriers and TNF antagonists. *J Immunol* 1993;**151**:1548–1561.
 136. Raghu G, Brown KK, Cottin V, du Bois RM, Lasky JA et al. Treatment of idiopathic pulmonary fibrosis with etanercept. An exploratory, placebo-controlled trial. *Am J Respir Crit Care Med* 2008;**178**:948–955.
 137. Jackson RM, Fell CD. Etanercept for idiopathic pulmonary fibrosis. Lessons on clinical trial design. *Am J Respir Crit Care Med* 2008;**178**:889–893.
 138. King TE Jr, Behr J, Brown KK, du Bois RM, Lancaster L, de Andrade JA et al. BUILD-1: a randomized placebo-controlled trial of bosentan in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2008;**177**:75–81.
 139. Shi-Wen X, Denton CP, Dashwood ME, Holmes AM, Bou-Gharios G, Pearson JD et al. Fibroblast matrix gene expression and connective tissue remodeling: role of endothelin-1. *J Invest Dermatol* 2001;**116**:417–425.
 140. Cantin AM, Hubbard RC, Crystal RG. Glutathione deficiency in the epithelial lining fluid of the lower respiratory tract in idiopathic pulmonary fibrosis. *Am Rev Respir Dis* 1989;**139**:370–372.
 141. Demedts M, Behr J, Buhl R, Costabel U, Dekhuijzen R, Jansen HM et al. High-dose acetylcysteine in idiopathic pulmonary fibrosis. *N Engl J Med* 2005;**353**:2229–2242.
 142. Collard HR, Anstrom KJ, Schwarz MI, Zisman DA. Sildenafil improves walk distance in idiopathic pulmonary fibrosis. *Chest* 2007;**131**:897–899.
 143. Bouros D, Antoniou KM. Current and future therapeutic approaches in idiopathic pulmonary fibrosis. *Eur Respir J* 2005;**26**:693–702.
 144. Benito M, Diaz-Rubio E. Molecular biology in colorectal cancer. *Clin Transl Oncol* 2006;**8**:391–398.
 145. Lawson WE, Lloyd JE. The genetic approach in pulmonary fibrosis: can it provide clues to this complex disease? *Proc Am Thorac Soc* 2006;**3**:345–349.
 146. Vassilakis DA, Sourvinos G, Spandidos DA, Siafakas NM, Bouros D. Frequent genetic alterations at the microsatellite level in cytologic sputum samples of patients with idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2000;**162**:1115–1119.
 147. Chambers RC. Role of coagulation cascade proteases in lung repair and fibrosis. *Eur Respir J* 2003;**22**:33S–35S.