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Epithelial stem cell exhaustion in the pathogenesis of idiopathic pulmonary fibrosis

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Abstract. New paradigms have been recently proposed in the pathogenesis of idiopathic pulmonary fibrosis (IPF), evidencing that in IPF the cumulative action of an accelerated parenchymal senescence determined by either telomere dysfunction or genetic defects, together with the concurrent noxious activity of tobacco smoking, are able to severely compromise the regenerative potential of parenchymal epithelial stem cells, triggering a cascade of molecular signals and events (scarring, bronchiolar proliferation, abnormal remodelling) eventually leading to severe and irreversible functional impairment. New pathogenic schemes focus on the complex molecular mechanisms driving in a vicious circle the different signalling pathways (e.g. Wnt/ -catenin, TGF-beta, caveolin-1, etc.) potentially involved in epithelial-mesenchymal transition and irreversible lung remodelling. *(Sarcoidosis Vasc Diffuse Lung Dis 2010; 27: 7-18)*

Key words: IPF, epithelial stem cell, telomere dysfunction, senescence, Wnt-signalling-pathway

Introduction

Idiopathic pulmonary fibrosis (IPF) is a severe multifactorial pulmonary syndrome functionally characterised by a progressive restrictive pattern with low diffusion capacity for carbon monoxide and low total capacity. IPF is the most common and severe form of idiopathic interstitial pneumonia, and its median survival is 3-4 years (1-3). Effective treatments are lacking for IPF, and this can be a consequence, at least in part, of the limited understanding of its pathogenesis, despite the overwhelming plethora of studies and theories proposed so far. In addition, although many clinical and pathological evidences have been provided favoring IPF as a clinical entity, a general consensus has not been definitely reached on its distinction from other idiopathic interstitial pneumonias, and conflicting opinions remain regarding the utility of distinguishing IPF from other diseases characterized by the usual interstitial pneumonia (UIP) pattern (4-7).

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Evolving views in the pathogenesis of IPF

In recent years evolving opinions regarding the pathogenesis of IPF have raised stirring discussion, and different models have been proposed. The "inflammatory theory" of IPF/UIP has been challenged, and new hypotheses assuming that abnormal epithelial-mesenchymal interactions and aberrant wound healing are in fact the crucial events in its pathogenesis have been proposed (8-11). It is now clear and widely accepted that the central pathogenic mechanism in IPF is the progressive loss of alveolar-capil-

lary barrier integrity determined by increased injury and death of alveolar epithelial cells, followed by a deranged regeneration of parenchymal tissue. According to this notion, parenchymal fibrosis and tissue remodelling are triggered by the abnormal activation of reparative processes leading to fibroblast and myofibroblast recruitment and activation. A large number of human and experimental studies and models support this pathogenic scenario for IPF (12-22). Accordingly, patchy alveolar damage and regeneration can be observed in most UIP samples, focal pneumocyte-type-II hyperplasia and cytologic atypia are common, and causal relationship between type II alveolar epithelial cell injury and interstitial collagen accumulation can be experimentally demonstrated in pulmonary fibrosis (20). Nevertheless, a number of issues remain open including the causes of alveolar loss in IPF, as well as the mechanisms linking pneumocyte apoptosis with the striking subversion of pulmonary structure leading to progressive and irreversible scarring and remodelling.

What causes alveolar loss and apoptosis of alveolar epithelial cells in IPF?

Accelerated senescence of epithelial precursors. The lesson from familial IPF.

A major issue regards the specific cause (or causes) of the epithelial damage occurring in IPF. The search for an etiology of IPF has been so far frustrated, although a large number of hypothetic factors have been proposed (viral infections, toxic substances, gastro-esophageal reflux, etc.) (23). In other interstitial lung diseases (e.g. nonspecific interstitial pneumonia, organising pneumonia, or hypersensitivity pneumonitis), alveolar epithelial cell damage is considered among the major pathogenic mechanisms as it is in IPF, but the underlying mechanisms are known or partially known, and identification of the cause (e.g. chronic exposure to antigens, drugs, or autoimmunity-related damage) may represent a relevant step in the diagnostic work-up. In addition, in many cases of interstitial pneumonitis other than IPF the alveolar loss can be variably reversed by therapy, and complete restoration of normal parenchyma is possible in a proportion of cases, whereas the alveolar damage in IPF is progressive and irreversible.

Recently, genetic studies have provided new precious information about the mechanisms that can lead to alveolar damage and their failure to regenerate. There is in fact accumulating evidence supporting that in both familial and sporadic IPF cases, abnormalities affecting the telomere length are common (24-28). Efficient telomere length control is central to the survival of stem cells (29), and a defective regeneration potential and senescence of parenchymal epithelium can occur in IPF when this mechanism is impaired (30, 31). Type-II pneumocytes in fact, behave as precursor cells and express telomerase after alveolar damage (32). Taking into account these accumulating evidences, the cause of abnormal alveolar re-epithelialization occurring in IPF should be ascribed to the precocious hexaustion of the renewal potential of epithelial stem cells. The concurrent action of environmental factors, such as the exposure to toxic substances, and especially tobacco smoking and pollution, can be necessary to the full development of the pathogenic process. Telomere stricture and endoplasmic reticulum stress are in fact relevant injurious effects of tobacco smoking on pulmonary parenchyma (33, 34). Accordingly, IPF is typically a disease of advanced age, and there is increased risk for IPF in male smokers and in people exposed to other toxic substances (35-38). All these data taken together suggest that IPF can be included within the category of diseases of lung "premature aging" with similarities with diseases characterised by telomere stricture and stem cell dysfunction (31, 39-42). This latter category of progeroid diseases, mostly represented by dyskeratosis congenita, are characterised by stem cell failure due to mutations affecting the telomerase complex, forming a spectrum of variants presenting with bone marrow failure, liver fibrosis, and also pulmonary fibrosis (40, 43, 44). Pulmonary disease is described in up to 20% of patients with dyskeratosis congenita (45). Nevertheless, in a proportion of familial and sporadic IPF cases short telomeres are present in leukocytes, without evidence of abnormalities affecting the telomerase complex. The mechanisms accounting for defective telomere maintenance are complex and only partly understood, but the concurrence of variability in the genetic background, including polymorphisms in telomere maintenance, and in environmental exposures are likely candidates to explain this heterogeneity (40, 46, 47). Promising new approaches for

deciphering this complexity include the identification and evaluation of signalling hubs and networks of human longevity and age-related diseases (48).

It is possible to argue that other genetic abnormalities characterize a proportion of familial IPF, mostly affecting the production of surfactant proteins (mainly surfactant protein C, surfactant protein A2, and the phospholipid transporter ABCA-3) (49-54). These abnormalities are able to impair the regenerative potential of pneumocyte precursors (type-II pneumocytes), to disrupt the cellular protein trafficking, and/or to cause endoplasmic reticulum stress and apoptosis, thus determining in alveolar precursors a functional defect comparable to the one induced by telomere shortening and accelerated senescence (51, 55). Similar pathogenic mechanisms can operate in Hermanski-Pudlak, another genetic pulmonary syndrome pathologically characterized by a UIP-like pattern, and where lysosomal defects can trigger alveolar apoptosis and pulmonary fibrosis (56-58).

In conclusion, in different forms of familial and sporadic pulmonary fibrosis a common pathogenic scheme can be proposed, where different underling genetic defects and/or predisposing conditions affecting the "stemness" of epithelial precursors, acting in concert with relevant concurrent environmental toxic exposures, can be able to induce breakdown of epithelial regeneration leading to chronic and irreversible alveolar loss. Obviously, these "intrinsic" defects can not be reversed as in other interstitial pneumonias. Further studies are needed to better define the reasons why this breakdown is commonly triggered at susceptible locations (peripheral lower lung sections as commonly observed in IPF).

Mechanisms of fibroblast and myofibroblasts recruitment and activation

If we accept this general scheme, a further issue is represented by mechanisms triggering the progressive and irreversible parenchymal fibrosis occurring in IPF. In all injured tissues, the damage of an epithelial layer is followed by a concurrent stimulation of epithelial and mesenchymal precursors, aimed to repair the wound. This repair process follows a series of stereotypical events, tightly regulated by molecular signalling finely tuning cell proliferation and differentiation of both the epithelial and mesenchymal components. When this renewal occurs in a pathologic environment, e.g. at sites where epithelial stem cells are exhausted and/or impaired in their functions as occurring in IPF, excess of molecular pro-fibrotic signals are generated by damaged basement membrane and activated type-II pneumocytes, leading to over-production of reparative signals (growth factors, cytokines, chemokines), and abnormal recruitment of fibroblasts and myofibroblasts. Scarring and remodelling in this pathogenic scenario can be consequences of the establishment of a vicious circle of injury and abnormal repair, leading to perturbation of the ordered cross-talk between epithelial and mesenchymal cells (59-60). Interestingly, the molecular pathways aberrantly activated in IPF seem to be the same involved in epithelial-mesenchymal communications occurring in lung development and differentiation (21-60).

In IPF, a overwhelming number of data have been produced concerning abnormal features of fibroblasts and myofibroblasts compared to normal. The numerical increase of fibroblast and myofibroblasts in IPF has been ascribed to a variety of concurrent mechanisms such as increased local proliferation and/or resistance to apoptotic signals, recruitment of fibrocytes from the circulation, and also epithelial mesenchymal transition (EMT) (10, 61, 62). Many studies have revealed molecular abnormalities characterising IPF fibroblasts/ myofibroblasts, focussing on the paradoxical imbalance in cell death between IPF pneumocytes, characterised by increased apoptosis, and IPF fibroblasts/myofibroblasts, characterized by decreased apoptosis. This imbalance has been attributed to abnormal expression of a variety of molecules, but the precise role of these abnormalities is far from clear. Epigenetic suppression of Thy-1/CD90, a GPI-ancored protein involved in the regulation of fibroblast proliferation and migration, characterises IPF myofibroblasts, and this decrease has been considered as part of their pro-fibrotic phenotype (63, 64). Decreased production of caveolin-1, a critical regulator of lung fibrosis, has been evidenced in IPF, and this abnormality has been proposed as relevant in its pathogenesis (65, 66). Interestingly, caveolin-1 participates in a complex molecular network including TGFβ and telomerase, thus linking molecular signalling and genetic predisposition in the pathogenesis of lung fibrosis (67-68). Caveolin-1 is a key player in promoting fibroblast senescence (69-70), and its down-modulation in IPF could contribute to their increased survival

and activation. Nevertheless, perturbation of caveolin-1 functions can not be considered as specific, since abnormal levels of this molecule are not restricted to IPF (71, 72). It is not easy to substantiate whether all these features are intrinsically related to specific abnormalities of IPF myofibroblasts, or merely result from abnormal stimulation of "normal" fibroblasts by "abnormal" environmental signalling.

In IPF/UIP the fibrotic reaction is distinctive, since discrete collections of myofibroblasts, generally known as fibroblast foci (FF), are scattered at the borders between normal and dense scar tissue. FF are considered a key element for defining the UIP pattern, since they give the appearance of temporal heterogeneity and represent the leading edge of ongoing lung injury and abnormal repair. FF occur in the majority of UIP samples, and their frequency has been related to disease severity and prognosis (73). The FF in UIP are similar to those observed in organizing pneumonia (the so-called Masson's body), but have different location (interstitial/intramural versus intra-alveolar), and biological features such as the absence of blood vessels and inflammatory cells (74).

A number of studies have been focused on the molecular characterization of myofibroblasts forming the FF in UIP samples, and abnormalities and differences have been in fact described (e.g. increased proportion of myofibroblasts expressing the proliferation marker Ki67+, elevated expression of TIMP-2, a member of tissue inhibitor of metalloproteinases), justifying the increased proliferation and/or decreased apoptotic clearance of these cells (75).

Abnormal nuclear accumulation of β-catenin has been demonstrated in FF of UIP, at variance with samples of organizing pneumonia (76). This finding can be considered as evidence of activation of the Wnt-pathway in IPF (see below in more detail), and suggests that transactivation of anti-apoptotic and/or pro-proliferative β-catenin target genes is implicated in the abnormal persistence of FF (76, 77). As above described, myofibroblasts in IPF can accumulate with several mechanisms, including local recruitment and differentiation from fibroblasts, migration of fibrocytes from the periphery (78), and EMT (79). The transition between fibroblasts and alpha-smooth-muscle (α-SMA) expressing myofibroblasts is regulated by a complex array of molecular signals, including TGF-β1, tumour necrosis factor alpha, and also telomerase activity (80). Activation of the Wnt- and/or TGFβ pathways can synergistically contribute to mechanisms triggering EMT, and can also induce an abnormal survival phenotype in myofibroblasts, increasing their resistance to apoptosis and invasive behaviour (62, 81, 82).

Interestingly, β-catenin stabilisation (e.g by gene mutation) can lead to abnormal Wnt activation and eventual development of aggressive myofibroblastic lesions (83, 84). Although β-catenin mutations are not demonstrable in IPF, abnormal Wnt activation can be responsible of the expansion of mesenchymal stem cells and the invasive behaviour of myofibroblasts in involved areas (76, 60, 85).

Functional abnormalities of IPF fibroblasts can be in turn involved in the abnormal regeneration of pneumocytes and the progressive alveolar loss observed in this disease, as elegantly shown in a recent study demonstrating that the basal HGF secretion by IPF fibroblasts is decreased by 50% when compared with control fibroblasts (86).

Abnormal activation of the Wnt-pathway in IPF

It is possible to speculate that the abnormal genetic background occurring in IPF can affect differently the diverse tissue components forming the pulmonary parenchyma, because the precursor cells are different, use different molecular mechanisms, and likely heterogeneously respond to the renewal impairment. Following this hypothesis, we proposed that a severe perturbation of the molecular signals interconnecting pneumocytes, mesenchymal cells and proximal lung epithelial precursors can drive a divergent effect on the proximal versus distal pulmonary components leading to the abnormal remodelling of the pulmonary structure (21). A key element in this pathogenic model is represented by the abnormal activation of the canonical Wnt-signalling pathway (76). The Wnt-signaling pathway plays diverse key roles in development and morphogenesis, in stem cell renewal and differentiation, and in tissue maintenance (87, 88). Beta-catenin signalling in fact determines a vast array of fundamental functions including cell proliferation, migration, apoptosis, and also epithelial mesenchymal transition (EMT) (62, 89, 90). EMT is a physiological mechanism involved in embryogenesis, but it is also relevant in patholog-

ical conditions such as metastatic spread and fibrosis (79). Several human and experimental studies have confirmed that the Wnt-pathway is abnormally activated in IPF, and this notion is included in recent pathogenic models for IPF (60, 76, 91-95). In line with the relevance of Wnt-signalling in IPF, a number of molecules that are abnormally expressed in human or experimental pulmonary fibrosis are among the downstream target genes of beta-catenin. These include the metallo-proteinase matrilysin/MMP7, cyclin-D1, tenascin, osteopontin, and different factors regulating epithelial cell survival, cell migration, and EMT (c-Myc, laminin-5 gamma-2 chain, WISP1, snail, twist, slug)(82, 96-101) (figure 1). Alveolar type II pneumocytes after injury show in fact many features of precursor cells with increased levels of telomerase, and also activation of EMT, as revealed by the expression of the transcription factors snail, slug, and twist, as well as decreased levels of E-cadherin (figure 1) (21, 32, 102). Telomerase

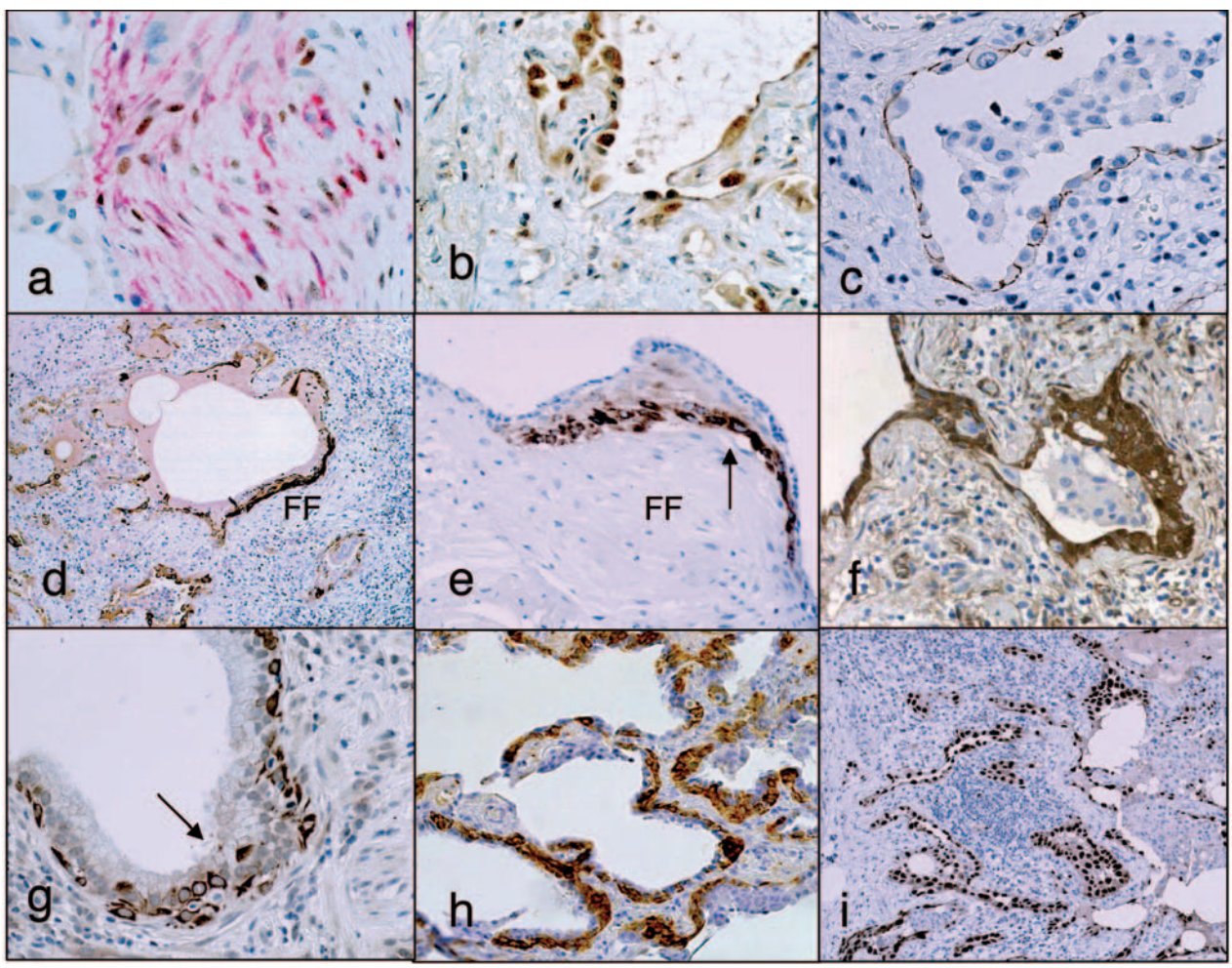

Fig. 1. *Evidences of epithelial mesenchymal transition in IPF*. A fibroblast focus located in lung parenchyma is shown in **a**, where α-SMA expressing myofibroblasts (red) co-express the EMT-related transcription factor twist (brown). Reactive type-II pneumocytes express nuclear slug (**b**), and decreased E-cadherin (**c**) in affected alveoli.
Abnormal remodeling in IPF. An enlarged bronchiole, with some features of microscopic honeycombing is shown in **d**, where laminin-5 γ2 expression is evident in activated type II pneumocytes in neighboring damaged alveoli, and also in a basal cell cluster overlying fibroblast foci (FF) (**d, e**). Abnormal nuclear and cytoplasmic expression of beta-catenin is observed in both alveolar and bronchiolar epithelial cells (**f**), together with increased expression of matrylisin/MMP7 in basal cells (arrow - **g**). Bronchiolar hyperplasia and abnormal colonization are evidenced by the basal-cell marker CK5 (**h**). Increased expression of cyclin-D1 (a Wnt-pathway target gene product) is evident in hyperplastic bronchiolar structures and affected alveolar epithelial cells (**i**)

seems to be crucial for maintaining the stem cell properties of type II pneumocytes in smokers, since telomere shortening can be increased by oxidative stress, and lung alveolar integrity is compromised in telomerase-null mice (103, 104).

Interestingly, there is a substantial connectivity between Wnt and other signalling pathways orchestrating the physiological tissue renewal and repair, including bone morphogenetic protein (BMP) and transforming growth factor beta (TGF-β), and deregulation of this network may trigger a vicious circle amplifying abnormal tissue remodelling in IPF (105, 106). Gremlin, an inhibitor of BMP, is hyper-expressed in IPF, and induces lung fibrosis in the mouse lung via decreased BMP- and increased TGF-β signalling. (107, 108). The TGF-β pathway is able to trigger EMT and fibrosis, and its pathogenic role in IPF has been suggested in many studies, as also the involvement of its downstream effectors SNAI and transgelin (81, 109-112). The possible role of WNT5A and non-canonical Wnt-signalling has been also hypothesized (113).

In different systems, the classical role of the Notch signalling, acting in concert with Wnt, is the maintenance of self-renewal potential of epithelial stem cells by regulating cell differentiation (114-117). Notch is crucial for myofibroblast differentiation and alveogenesis, and Notch- and Wnt-signalling can cooperate in triggering pulmonary fibrosis (118-119). The abnormal expression of c-FLIP, an inducible endogenous inhibitor of Fas-mediated apoptosis, in the lung tissues of patients with IPF/UIP compared with non-diseased control subjects also suggests a role for the NF-kB pathway (120).

Taking all these data into account, a general pathogenic model can be traced for IPF where aberrant activation of the WNT-signalling and the other involved pathways are chronically and aberrantly triggered by signals derived from senescent alveolar epithelial cell precursors, attempting in vain to repair injured epithelium (figure 2).

Lung remodelling, bronchiolar proliferation and honeycombing

In IPF the UIP pattern is characterized by heterogeneously distributed alterations of the pulmonary parenchyma, usually described with the generic term "remodelling". A number of abnormalities are encompassed under this term, including dense fibrosis, structural distortion, and abnormal proliferative lesions affecting the small airways, (hyperplasia, metaplasia, bronchiolization and honeycombing) (76, 96, 121). How can be reconciled these morphological appearances with the pathogenic model above described? We proposed a scheme where the aberrant activation of Wnt/β-catenin signalling, and other involved pathways, is able to trigger a divergent epithelial regeneration at bronchiolo-alveolar junctions, with loss of alveolar epithelium on one side, and abnormal proliferation of bronchiolar epithelium on the other, concurrent with mesenchymal proliferation and scarring (76). In fact, we can expect that epithelial precursors (type II pneumocytes in the alveoli and basal cells in bronchioles) provide distinct responses to abnormal molecular proliferative and apoptotic signals, since these precursors utilise distinct molecular strategies and mechanisms for their survival and differentiation. In our view, the variegated appearance, from early lesions to extensive fibrosis and remodelling characterizing IPF/UIP lesions, is produced by the progressive interference on physiological tissue repair mechanisms due to abnormal β-catenin activation, starting from foci of ongoing injury and repair processes, as following a gradient of Wnt-signal concentration. Deregulated expression of Wnt target genes could exert divergent effects on different airway components (namely bronchiolar and alveolar), eventually leading to alveolar loss on one hand, and bronchiolar proliferation on the other. In lung development Wnt-signalling can differentially stimulate the expansion of distal and proximal lung components, and Inhibition of Wnt/β-catenin signalling by tissue-specific deletion of β-catenin results in disruption of distal airway development and expansion of proximal airways (122). In this model, senescent alveolar cells in IPF could be particularly vulnerable to pro-apoptotic signaling (as evidenced by increased expression of p53, $p21^{waf1}$, and also transactivating isoforms of p63 (121, 123-126). On the other hand, bronchiolar basal cells could be more protected from apoptosis by the constitutive expression of truncated dominant-negative ΔN-p63 isoforms exerting potent anti-apoptotic signals (128-130). The β-catenin/TCF complex is the master switch that controls proliferation versus differentiation, and c-myc

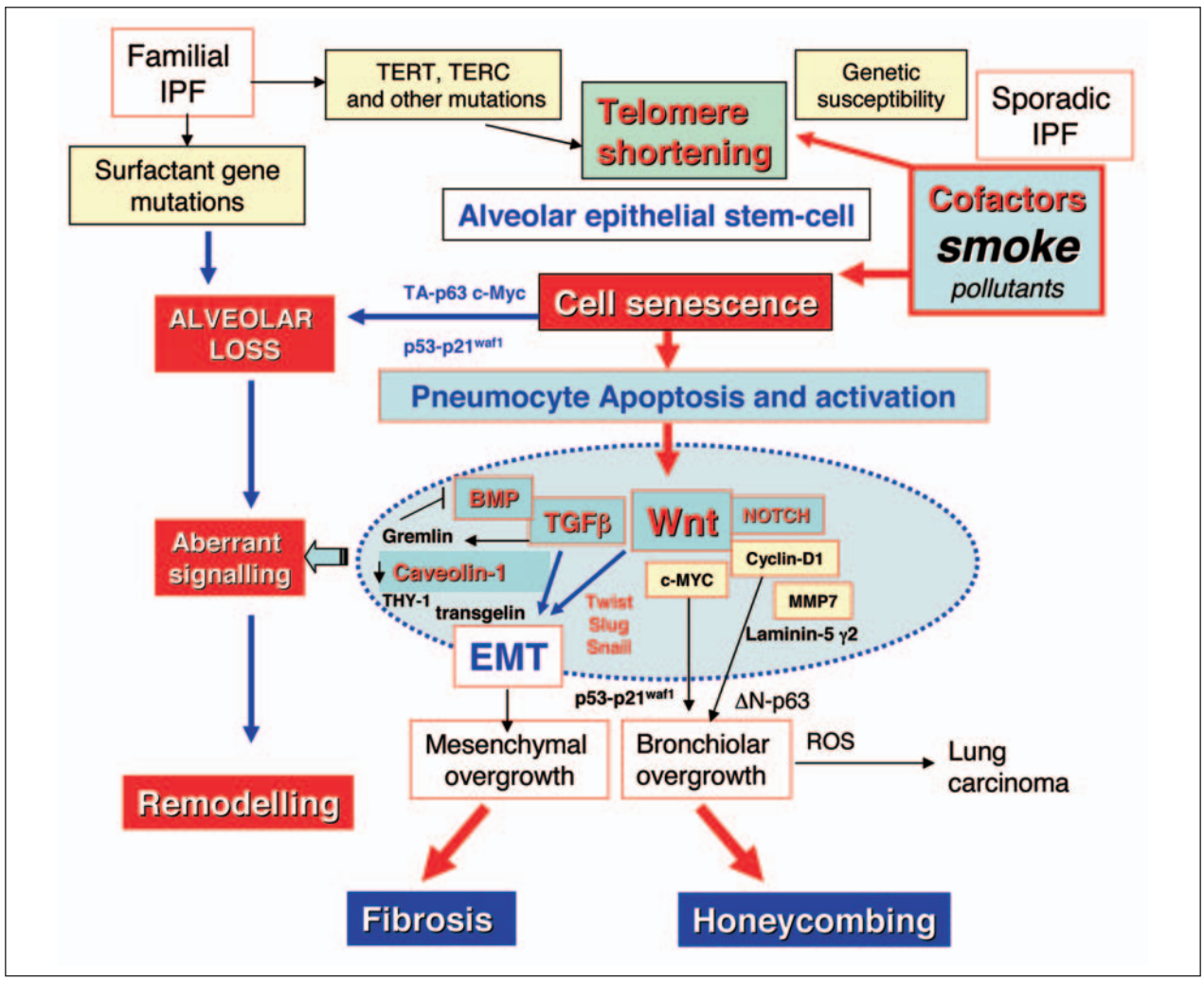

Fig. 2. General scheme describing the different steps of IPF pathogenesis as proposed in this review.

is one of its most relevant mediator (131-133). Accordingly, in different systems and cell types, the activation of the Wnt-pathway is able to either trigger or inhibit survival and death by modulating the availability of cyclin-D1 and c-myc, two proteins that play roles in both cellular proliferation and apoptosis (132, 133). The specific role of c-Myc, in amplifying divergence between distal and proximal lung tissue components warrants further investigation, since at sites where abnormal levels of β-catenin, and hence c-myc, are expressed, migrating bronchiolar cells could behave as super-competitors (131, 133-135).

The epithelial cells overlying fibroblast foci of UIP have been described as "cuboidal" epithelial cells of undetermined nature. These epithelial cells, that likely represent target cells in the injury-repair sequence occurring in IPF, have been recognized as either alveolar or bronchiolar in different studies. At immunophenotypic analysis the majority of FF are covered by bronchiolar epithelium (21, 96, 121), and honeycomb cysts have frequently myofibroblast collections resembling FF in their wall (figure 1). In most cases honeycomb cysts at histology appear as dilated bronchiolar structures, frequently filled by mucus and muciphages (136), and show features of hyperplasia and bronchiolization (so-called "microscopic" honeycombing). This latter type of honeycomb cyst are in most instances peripheral, and appear early in the evolution of the disease. Bronchio-

lar lesions in affected areas are frequent, and encompass basal cell hyperplasia, bronchiolization, squamous metaplasia and atypia (21, 121). In a large proportion of cases of IPF/UIP, the bronchiolar changes are prominent, and sometimes can mimic an epithelial neoplasm. Interestingly, the molecular abnormalities occurring in bronchiolar proliferative lesions are all centered on basal cells, mostly exhibiting activation of the wnt-β-catenin pathway, as evidenced by the expression of nuclear β-catenin, cyclin-D1 and MMP-7/matrilysin (76). This observation is relevant since basal cells are stem cells involved in the renewal of airway epithelium (129), and in physiologic conditions they do not express significant levels of these molecules (76). In a recent study, we demonstrated that bronchiolar basal cells overlying fibroblast foci aberrantly express molecules involved in cell motility and invasiveness, including laminin-5-γ2-chain (LAM5γ2), the calcium binding protein fascin, and phosphorylated heat-shock-protein-27 (HSP27) (96). We hypothesized that this phenotype is likely related to the alveolar colonization observed in IPF (bronchiolization), and can significantly contribute to lung structure distortion and remodelling. HSP27 in fact can mediate resistance against cell death induced by stress and differentiation, preventing apoptosis by blocking interactions of Daxx with Ask-1 and Fas (137). The trimeric protein laminin-5 (α3, β3, γ2-chain) is an integral part of the basal lamina of stratified epithelia where it plays a crucial role in the organization of the basal stem-cell niche by providing epithelial-mesenchymal connections by interacting with integrin α6β4 (138). These interactions are critical for regulating cell migration, an event required in different processes, such as wound healing, embryogenesis and metastatic dissemination (139,140). The γ2 chain of laminin-5 (LAM5γ2) is induced by β-catenin, and acts as a soluble cell motility factor in a variety of conditions after its cleaving by metalloproteinases (141-142). The enhanced expression of LAM5γ2 is considered one of the best marker of invasiveness in different carcinomas (142,143). Within the FF the LAM5γ2 positive cells appear as linear clusters of cytokeratin-5 expressing basal cells, wedged between luminal epithelial cells and myofibroblasts, forming peculiar negative-positive-negative three-layered lesions (that we named "sandwich" fibroblast-foci, figure 1)(96). According to this study, the "sandwich-foci" appear as specific for IPF/UIP, since similar lesions are extremely rare in other interstitial lung diseases. Myofibroblasts are able to produce reactive oxygen species (ROS), and can then contribute to the induction of cell death in alveolar epithelium (144). In basal stem cells of sandwich foci, on the other hand, the coincidental expression of survival signals (provided by ΔN-p63 and Wnt-activation) and the genotoxic action of ROS locally produced by myofibroblasts might be involved in carcinoma development in IPF (145-147).

Conclusions

These evolving concepts open new options to better understand the pathogenesis of IPF, and also new perspectives for alternative treatment options, including drugs specifically addressing cell senescence. The possible action of drugs inhibiting and/or regulating the Wnt-pathway could positively interfere with EMT, but also with the complex mechanisms involved in the divergent behaviour of different epithelial components as described in this paper (148, 149). The central role of telomere-shortening in epithelial stem cells exhaustion is emphasised, and future efforts should be focused on their pharmacological protection and/or specific replacement (150-154).

Acknowledgements

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References

1. 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: 646-64.
2. Poletti V, Kitaichi M. Facts and controversies in the classification of idiopathic interstitial pneumonias. Sarcoidosis Vasc Diffuse Lung Dis 2000;17:229-38.
3. Fernández Pérez ER, Daniels CE, Schroeder DR, et al. Incidence, prevalence, and clinical course of idiopathic pulmonary fibrosis: a population-based study. Chest 2010; 137: 129-37.
4. King TE Jr. Clinical advances in the diagnosis and therapy of the interstitial lung diseases. Am J Respir Crit Care Med 2005; 172: 268-79.
5. du Bois R, King TE Jr. Challenges in pulmonary fibrosis: the NSIP/UIP debate. Thorax 2007; 62: 1008-12.

6. Meltzer EB, Noble PW. Idiopathic pulmonary fibrosis. Orphanet J Rare Dis 2008; 3: 8.
7. Myers JL, Katzenstein AL. Beyond a consensus classification for idiopathic interstitial pneumonias: progress and controversies. Histopathology 2009; 54: 90-103.
8. Selman M, Pardo A. Idiopathic pulmonary fibrosis: misunderstandings between epithelial cells and fibroblasts? Sarcoidosis Vasc Diffuse Lung Dis 2004; 21: 165-72.
9. Selman M, King TE.Jr, Pardo A. Idiopathic pulmonary fibrosis: prevailing and evolving hypotheses about its pathogenesis and implications for therapy. Ann Intern Med 2001; 134: 136-51.
10. Strieter RM, Mehrad B. New mechanisms of pulmonary fibrosis. Chest 2009; 136: 1364-70.
11. Strieter RM. What differentiates normal lung repair and fibrosis? Inflammation, resolution of repair, and fibrosis. Proc Am Thorac Soc 2008; 5: 305-10.
12. Williams K, Malarkey D, Cohn L, Patrick D, Dye J, Toews G. Identification of spontaneous feline idiopathic pulmonary fibrosis: morphology and ultrastructural evidence for a type II pneumocyte defect. Chest 2004; 125: 2278-88.
13. Barbas-Filho JV, Ferreira MA, Sesso A, Kairalla RA, Carvalho CR, Capelozzi VL. Evidence of type II pneumocyte apoptosis in the pathogenesis of idiopathic pulmonary fibrosis (IFP)/usual interstitial pneumonia (UIP). J Clin Pathol 2001; 54: 132-8.
14. Gauldie J, Kolb M, Sime PJ. A new direction in the pathogenesis of idiopathic pulmonary fibrosis? Respir Res 2002; 3: 1.
15. Noble PW, Homer RJ. Back to the future: historical perspective on the pathogenesis of idiopathic pulmonary fibrosis. Am J Respir Cell Mol Biol 2005; 33: 113-20.
16. Geiser T. Idiopathic pulmonary fibrosis - a disorder of alveolar wound repair? Swiss Med Wkly 2003; 133: 405-11.
17. Korfei M, Ruppert C, Mahavadi P, et al. Epithelial endoplasmic reticulum stress and apoptosis in sporadic idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2008; 178: 838-46.
18. Lawson WE, Grant SW, Ambrosini V, et al. Genetic mutations in surfactant protein C are a rare cause of sporadic cases of IPF. Thorax 2004; 59: 977-80.
19. Plataki M, Koutsopoulos AV, Darivianaki K, Delides G, Siafakas NM, Bouros D. Expression of apoptotic and antiapoptotic markers in epithelial cells in idiopathic pulmonary fibrosis. Chest 2005; 127: 266-74.
20. Sisson TH, Mendez M, Choi K, et al. Targeted injury of type II alveolar epithelial cells induces pulmonary fibrosis. Am J Respir Crit Care Med 2010; 181: 254-63.
21. Chilosi M, Murer B, Poletti V. Usual Interstitial Pneumonia. In: Molecular Pathology of lung diseases. Zander S., Popper HH, Jagirdar J, Haque AK, Cagle PT and Barrios R Eds. Springer, 2008, pp. 607-15.
22. Gharaee-Kermani M, Gyetko MR, Hu B, Phan SH. New insights into the pathogenesis and treatment of idiopathic pulmonary fibrosis: a potential role for stem cells in the lung parenchyma and implications for therapy. Pharm Res 2007; 24: 819-41.
23. Kottmann RM, Hogan CM, Phipps RP, Sime PJ. Determinants of initiation and progression of idiopathic pulmonary fibrosis. Respirology 2009; 14: 917-33.
24. Armanios MY, Chen JJ, Cogan JD, et al. Telomerase mutations in families with idiopathic pulmonary fibrosis. N Engl J Med 2007; 356: 1317-2.
25. Tsakiri KD, Cronkhite JT, Kuan PJ, et al. Adult-onset pulmonary fibrosis caused by mutations in telomerase. Proc Natl Acad Sci USA 2007; 104: 7552-7.
26. Mushiroda T, Wattanapokayakit S, Takahashi A, et al. Pirfenidone Clinical Study Group. A genome-wide association study identifies an association of a common variant in TERT with susceptibility to idiopathic pulmonary fibrosis. J Med Genet 2008; 45: 654-6.
27. Alder JK, Chen JJ, Lancaster L, et al. Short telomeres are a risk factor for idiopathic pulmonary fibrosis. Proc Natl Acad Sci USA 2008; 105: 13051-6.
28. 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.
29. Hao LY, Armanios M, Strong MA, et al. Short telomeres, even in the presence of telomerase, limit tissue renewal capacity. Cell 2005; 123: 1121-31.
30. Armanios M, Alder JK, Parry EM, Karim B, Strong MA, Greider CW. Short telomeres are sufficient to cause the degenerative defects associated with aging. Am J Hum Genet 2009; 85: 823-32.
31. Thannickal VJ, Loyd JE. Idiopathic pulmonary fibrosis: a disorder of lung regeneration? Am J Respir Crit Care Med 2008; 178: 663-5.
32. Driscoll B, Buckley S, Bui KC, Anderson KD, Warburton D. Telomerase in alveolar epithelial development and repair. Am J Physiol Lung Cell Mol Physiol 2000; 279: L1191-8.
33. Jorgensen E, Stinson A, Shan L, Yang J, Gietl D, Albino AP. Cigarette smoke induces endoplasmic reticulum stress and the unfolded protein response in normal and malignant human lung cells. BMC Cancer 2008; 8: 229.
34. Babizhayev MA, Savel'yeva EL, Moskvina SN, Yegorov YE. Telomere length is a biomarker of cumulative oxidative stress, biologic age, and an independent predictor of survival and therapeutic treatment requirement associated with smoking behavior. Am J Ther 2010 Mar 11.
35. Baumgartner KB, Samet JM, Stidley CA, Colby TV, Waldron JA. Cigarette smoking: a risk factor for idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 1997; 155: 242-8.
36. Baumgartner KB, Samet JM, Coultas DB, et al. Occupational and environmental risk factors for idiopathic pulmonary fibrosis: a multicenter case-control study. Collaborating Centers. Am J Epidemiol 2000; 152: 307-15.
37. Raghu G, Weycker D, Edelsberg J, Bradford WZ, Oster G. Incidence and prevalence of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2006; 174: 810-6.
38. Borie R, Crestani B, Bichat H. Prevalence of telomere shortening in familial and sporadic pulmonary fibrosis is increased in men. Am J Respir Crit Care Med 2009; 179: 1073.
39. Armanios M. Syndromes of telomere shortening. Annu Rev Genomics Hum Genet 2009; 10: 45-61.
40. Ly H. Genetic and environmental factors influencing human diseases with telomere dysfunction. Int J Clin Exp Med 2009; 2: 114-30.
41. Calado RT, Young NS. Telomere diseases. N Engl J Med 2009; 361: 2353-65.
42. Vulliamy T, Beswick R, Kirwan M, et al. Mutations in the telomerase component NHP2 cause the premature ageing syndrome dyskeratosis congenita. Proc Natl Acad Sci USA 2008; 105: 8073-8.
43. Walne AJ, Marrone A, Dokal I. Dyskeratosis congenita: a disorder of defective telomere maintenance? Int J Hematol 2005; 82: 184-9.
44. Kirwan M, Dokal I. Dyskeratosis congenita, stem cells and telomeres. Biochim Biophys Acta 2009; 1792: 371-9.
45. Marrone A, Walne A, Dokal I. Dyskeratosis congenita: telomerase, telomeres and anticipation. Curr Opin Genet Dev 2005; 15: 249-57.
46. Choi JE, Kang HG, Jang JS, et al. Polymorphisms in telomere maintenance genes and risk of lung cancer. Cancer Epidemiol Biomarkers Prev 2009; 18: 2773-81.
47. Herrera E, Samper E, Martín-Caballero J, Flores JM, Lee HW, Blasco MA. Disease states associated with telomerase deficiency appear earlier in mice with short telomeres. EMBO J 1999; 18: 2950-60.
48. Wolfson M, Budovsky A, Tacutu R, Fraifeld V. The signaling hubs at the crossroad of longevity and age-related disease networks. Int J Biochem Cell Biol 2009; 41: 516-20.
49. Nogee LM, Dunbar AE 3rd, Wert SE, Askin F, Hamvas A, Whitsett JA. A mutation in the surfactant protein C gene associated with familial interstitial lung disease. N Engl J Med 2001; 344: 573-9.

50. Whitsett JA. Genetic basis of familial interstitial lung disease: misfolding or function of surfactant protein C? Am J Respir Crit Care Med 2002; 165: 1201-2.
51. Wang Y, Kuan PJ, Xing C, Cronkhite JT, Torres F, Rosenblatt RL, et al. Genetic defects in surfactant protein A2 are associated with pulmonary fibrosis and lung cancer. Am J Hum Genet 2009; 84: 52-9.
52. Chibbar R, Shih F, Baga M, Torlakovic E, Ramlall K, Skomro R, et al. Nonspecific interstitial pneumonia and usual interstitial pneumonia with mutation in surfactant protein C in familial pulmonary fibrosis. Mod Pathol 2004; 17: 973-80.
53. 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.
54. Bullard JE, Nogee LM. Heterozygosity for ABCA3 mutations modifies the severity of lung disease associated with a surfactant protein C gene (SFTPC) mutation. Pediatr Res. 2007; 62: 176-9.
55. Lawson WE, Crossno PF, Polosukhin VV, et al. Endoplasmic reticulum stress in alveolar epithelial cells is prominent in IPF: association with altered surfactant protein processing and herpesvirus infection. Am J Physiol Lung Cell Mol Physiol 2008; 294: L1119-26.
56. Young LR, Pasula R, Gulleman PM, Deutsch GH, McCormack FX. Susceptibility of Hermansky-Pudlak mice to bleomycin-induced type II cell apoptosis and fibrosis. Am J Respir Cell Mol Biol. 2007; 37: 67-74.
57. Suzuki T, Li W, Zhang Q, et al. Hermansky-Pudlak syndrome is caused by mutations in HPS4, the human homolog of the mouse light-ear gene. Nat Genet 2002; 30: 321-4.
58. Wei ML. Hermansky-Pudlak syndrome: a disease of protein trafficking and organelle function. Pigment Cell Res. 2006; 19: 19-42.
59. Morishima Y, Nomura A, Uchida Y, et al. Triggering the induction of myofibroblast and fibrogenesis by airway epithelial shedding. Am J Respir Cell Mol Biol 2001; 24: 1-11.
60. Selman M, Pardo A, Kaminski N. Idiopathic pulmonary fibrosis: aberrant recapitulation of developmental programs? PLoS Med 2008; 5(3): e62.
61. Strieter RM, Keeley EC, Hughes MA, Burdick MD, Mehrad B. The role of circulating mesenchymal progenitor cells (fibrocytes) in the pathogenesis of pulmonary fibrosis. J Leukoc Biol 2009; 86: 1111-8.
62. Willis BC, duBois RM, Borok Z. Epithelial origin of myofibroblasts during fibrosis in the lung. Proc Am Thorac Soc 2006; 3: 377-82.
63. Sanders YY, Pardo A, Selman M, et al.. Thy-1 promoter hypermethylation: a novel epigenetic pathogenic mechanism in pulmonary fibrosis. Am J Respir Cell Mol Biol 2008; 39: 610-8.
64. Hagood JS, Prabhakaran P, Kumbla P, et al.. Loss of fibroblast Thy-1 expression correlates with lung fibrogenesis. Am J Pathol 2005; 167: 365-79.
65. 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.
66. Le Saux O, Teeters K, Miyasato S, et al. The role of caveolin-1 in pulmonary matrix remodeling and mechanical properties. Am J Physiol Lung Cell Mol Physiol 2008; 295: L1007-17.
67. Igarashi J, Shoji K, Hashimoto T, et al. Transforming growth factor-beta1 downregulates caveolin-1 expression and enhances sphingosine 1-phosphate signaling in cultured vascular endothelial cells. Am J Physiol Cell Physiol 2009; 297: C1263-74.
68. Verma S, Slutsky AS. Idiopathic pulmonary fibrosis - new insights. N Engl J Med. 2007 29; 356: 1370-2.
69. Park WY, JS Park K.A, Cho DI, et al. Up-regulation of caveolin attenuates epidermal growth factor signaling in senescent cells. J Biol Chem 2000; 275: 20847-52.
70. Linge A, Weinhold K, Bläsche R, Kasper M, Barth K. Downregulation of caveolin-1 affects bleomycin-induced growth arrest and cellular senescence in A549 cells. Int J Biochem Cell Biol 2007; 39: 1964-74.
71. Del Galdo F, Lisanti MP, Jimenez SA. Caveolin-1, transforming growth factor-beta receptor internalization, and the pathogenesis of systemic sclerosis. Curr Opin Rheumatol. 2008; 20: 713-9.
72. Le Saux CJ, Teeters K, Miyasato SK, et al. Down-regulation of caveolin-1, an inhibitor of transforming growth factor-beta signaling, in acute allergen-induced airway remodeling. J Biol Chem 2008; 283: 5760-8.
73. Nicholson AG, Fulford LG, Colby TV, du Bois RM, Hansell DM, Wells AU. The relationship between individual histologic features and disease progression in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2002; 166: 173-7.
74. Lappi-Blanco E, Kaarteenaho-Wiik R, Soini Y, Risteli J, Paakko P. Intraluminal fibromyxoid lesions in bronchiolitis obliterans organizing pneumonia are highly capillarized. Hum Pathol 1999; 30: 1192-6.
75. Selman M, Ruiz V, Cabrera S, Segura L, Ramirez R, Barrios R, Pardo A. TIMP-1, -2, -3, and -4 in idiopathic pulmonary fibrosis. A prevailing nondegradative lung microenvironment? Am J Physiol Lung Cell Mol Physiol 2000; 279: L562-L574.
76. Chilosi M, Poletti V, Zamò A, et al. Aberrant Wnt/beta-catenin pathway activation in idiopathic pulmonary fibrosis. Am J Pathol 2003; 162: 1495-502.
77. Neth P, Ciccarella M, Egea V, Hoelters J, Jochum M, Ries C. Wnt signalling regulates the invasion capacity of human mesenchymal stem cells. Stem Cells 2006; 24: 1892-903.
78. Andersson-Sjöland A, de Alba CG, Nihlberg K, et al. Fibrocytes are a potential source of lung fibroblasts in idiopathic pulmonary fibrosis. Int J Biochem Cell Biol 2008; 40: 2129-40.
79. Guarino M, Tosoni A, Nebuloni M. Direct contribution of epithelium to organ fibrosis: epithelial-mesenchymal transition. Hum Pathol 2009; 40: 1365-76.
80. Liu T, Hu B, Chung MJ, Ullenbruch M, Jin H, Phan SH. Telomerase regulation of myofibroblast differentiation. Am J Respir Cell Mol Biol 2006; 34: 625-33.
81. Willis BC, Liebler JM, Luby-Phelps K, et al. Induction of epithelial-mesenchymal transition in alveolar epithelial cells by transforming growth factor-beta1: potential role in idiopathic pulmonary fibrosis. Am J Pathol 2005; 166: 1321-32.
82. Pozharskaya V, Torres-González E, Rojas M, et al. Twist: a regulator of epithelial-mesenchymal transition in lung fibrosis. PLoS One. 2009; 4: e7559.
83. Neth P, Ries C, Karow M, Egea V, Ilmer M, Jochum M. The Wnt signal transduction pathway in stem cells and cancer cells: influence on cellular invasion. Stem Cell Rev 2007; 3: 18-29.
84. Cheon SS, Cheah AY, Turley S, et al. beta-Catenin stabilization dysregulates mesenchymal cell proliferation, motility, and invasiveness and causes aggressive fibromatosis and hyperplastic cutaneous wounds. Proc Natl Acad Sci USA 2002; 99: 6973-8.
85. Klapholz-Brown Z, Walmsley GG, Nusse YM, Nusse R, Brown PO. Transcriptional program induced by Wnt protein in human fibroblasts suggests mechanisms for cell cooperativity in defining tissue microenvironments. PLoS One 2007; 2: e945.
86. Marchand-Adam S, Marchal J, Cohen M, et al. Defect of hepatocyte growth factor secretion by fibroblasts in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2003; 168: 1156-61.
87. Cadigan KM, Peifer M. Wnt signaling from development to disease: insights from model systems. Cold Spring Harb Perspect Biol 2009; 1: a002881.
88. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol 2004; 20: 781-810.
89. Goss AM, Morrisey EE. Wnt signaling and specification of the respiratory endoderm. Cell Cycle 2010; 9: 10-1.
90. Zhang Y, Goss AM, Cohen ED, et al. A Gata6-Wnt pathway required for epithelial stem cell development and airway regeneration. Nat Genet 2008; 40: 862-70.

91. Morrisey EE. Wnt signaling and pulmonary fibrosis. Am J Pathol 2003; 162: 1393-7.
92. Königshoff M, Balsara N, Pfaff EM, et al. Functional Wnt signaling is increased in idiopathic pulmonary fibrosis. PloS One 2008; 3: e2142.
93. Königshoff M, Kramer M, Balsara N, et al. WNT1-inducible signalling protein-1 mediates pulmonary fibrosis in mice and is upregulated in humans with idiopathic pulmonary fibrosis. J Clin Invest 2009; 119: 772-87.
94. Kuhn C, Mason RJ. Immunolocalization of SPARC, tenascin, and thrombospondin in pulmonary fibrosis. Am J Pathol 1995; 147: 1759-69.
95. Borok Z. Role for alpha3 integrin in EMT and pulmonary fibrosis. J Clin Invest 2009; 119: 7-10.
96. Chilosi M, Zamò A, Doglioni C, et al. Migratory marker expression in fibroblast foci of idiopathic pulmonary fibrosis. Respir Res 2006; 7: 95.
97. Tanjore H, Xu XC, Polosukhin VV, et al. Contribution of epithelial-derived fibroblasts to bleomycin-induced lung fibrosis. Am J Respir Crit Care Med 2009; 180: 657-65.
98. Cohen ED, Ihida-Stansbury K, Lu MM, Panettieri RA, Jones PL, Morrisey EE. Wnt signaling regulates smooth muscle precursor development in the mouse lung via a tenascin C/PDGFR pathway. J Clin Invest 2009; 119: 2538-49.
99. Pardo A, Gibson K, Cisneros J, et al. Up-regulation and profibrotic role of osteopontin in human idiopathic pulmonary fibrosis. PLoS Med. 2005; 2: e251.
100. Rohde F, Rimkus C, Friederichs J, et al. Expression of osteopontin, a target gene of de-regulated Wnt signaling, predicts survival in colon cancer. Int J Cancer 2007; 121: 1717-23.
101. Zuo F, Kaminski N, Eugui E, et al. Gene expression analysis reveals matrilysin as a key regulator of pulmonary fibrosis in mice and humans. Proc Natl Acad Sci USA 2002; 99: 6292-7.
102. Reddy R, Buckley S, Doerken M, et al. Isolation of a putative progenitor subpopulation of alveolar epithelial type 2 cells. Am J Physiol Lung Cell Mol Physiol 2004; 286: L658-67.
103. von Zglinicki T. Role of oxidative stress in telomere length regulation and replicative senescence. Ann N Y Acad Sci 2000; 908: 99-110.
104. Lee J, Reddy R, Barsky L, Scholes J, Chen H, Shi W, Driscoll B. Lung alveolar integrity is compromised by telomere shortening in telomerase-null mice. Am J Physiol Lung Cell Mol Physiol 2009; 296: L57-70.
105. Eblaghie MC, Reedy M, Oliver T, Mishina Y, Hogan BL. Evidence that autocrine signaling through Bmpr1a regulates the proliferation, survival and morphogenetic behavior of distal lung epithelial cells. Dev Biol 2006; 291: 67-82.
106. Weaver M, Yingling JM, Dunn NR, Bellusci S, Hogan BL. Bmp signaling regulates proximal-distal differentiation of endoderm in mouse lung development. Development 1999; 126: 4005-15.
107. Koli K, Myllärniemi M, Vuorinen K, et al. Bone morphogenetic protein-4 inhibitor gremlin is overexpressed in idiopathic pulmonary fibrosis. Am J Pathol 2006; 169: 61-71.
108. Myllärniemi M, Lindholm P, Ryynänen MJ, et al. Gremlin-mediated decrease in bone morphogenetic protein signaling promotes pulmonary fibrosis. Am J Respir Crit Care Med 2008; 177: 321-9.
109. Kim KK, Kugler MC, Wolters PJ, 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: 13180-5.
110. Jayachandran A, Königshoff M, Yu H, et al. SNAI transcription factors mediate epithelial-mesenchymal transition in lung fibrosis. Thorax 2009; 64: 1053-61.
111. 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.
112. Yu H, Königshoff M, Jayachandran A, et al. Transgelin is a direct target of TGF-beta/Smad3-dependent epithelial cell migration in lung fibrosis. FASEB J 2008; 22: 1778-89.
113. Vuga LJ, Ben-Yehudah A, Kovkarova-Naumovski E, et al. WNT5A is a regulator of fibroblast proliferation and resistance to apoptosis. Am J Respir Cell Mol Biol 2009; 41: 583-9.
114. Zhu X, Zhang J, Tollkuhn J, et al. Sustained Notch signaling in progenitors is required for sequential emergence of distinct cell lineages during organogenesis. Genes Dev 2006; 20: 2739-53.
115. Lowry WE, Blanpain C, Nowak JA, Guasch G, Lewis L, Fuchs E. Defining the impact of beta-catenin/Tcf transactivation on epithelial stem cells. Genes Dev 2005; 19: 1596-611.
116. Katoh M, Katoh M. Notch ligand, JAG1, is evolutionarily conserved target of canonical WNT signaling pathway in progenitor cells. Int J Mol Med 2006; 17: 681-85.
117. Shimizu T, Kagawa T, Inoue T, et al. Stabilized beta-catenin functions through TCF/LEF proteins and the Notch/RBP-Jkappa complex to promote proliferation and suppress differentiation of neural precursor cells. Mol Cell Biol 2008; 28: 7427-41.
118. Xu K, Nieuwenhuis E, Cohen BL, et al. Lunatic Fringe-mediated Notch signaling is required for lung alveogenesis. Am J Physiol Lung Cell Mol Physiol 2010; 298: L45-56.
119. Liu T, Hu B, Choi YY, et al. Notch1 signaling in FIZZ1 induction of myofibroblast differentiation. Am J Pathol 2009; 174: 1745-55.
120. Cha SI, Groshong SD, Frankel SK, et al. Compartmentalized expression of c-FLIP in lung tissues of patients with idiopathic pulmonary fibrosis. Am J Respir Cell Mol Biol 2010; 42: 140-8.
121. Chilosi M, Poletti V, Murer B, et al. Abnormal re-epithelialization and lung remodeling in idiopathic pulmonary fibrosis: the role of deltaN-p63. Lab Invest. 2002; 82: 1335-45.
122. Shu W, Guttentag S, Wang Z, et al. Wnt/beta-catenin signaling acts upstream of N-myc, BMP4, and FGF signaling to regulate proximal-distal patterning in the lung. Dev Biol 2005; 283: 226-39.
123. Kuwano K, Kunitake R, Kawasaki M, et al. P21Waf1/Cip1/Sdi1 and p53 expression in association with DNA strand breaks in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 1996; 154: 477-83.
124. Senoo M, Pinto F, Crum CP, McKeon F. p63 Is essential for the proliferative potential of stem cells in stratified epithelia. Cell 2007; 129: 523-36.
125. Lena AM, Shalom-Feuerstein R, Rivetti di Val Cervo P, et al. miR-203 represses 'stemness' by repressing DeltaNp63. Cell Death Differ 2008; 15: 1187-95.
126. Blanpain C, Fuchs E. p63: revving up epithelial stem-cell potential. Nat Cell Biol 2007; 9: 731-3.
127. Chilosi M, Doglioni C. Constitutive p63 expression in airway basal cells. A molecular target in diffuse lung diseases. Sarcoidosis Vasc Diffuse Lung Dis 2001; 18: 23-26.
128. Daniely Y, Liao G, Dixon D, et al. Critical role of p63 in the development of a normal esophageal and tracheobronchial epithelium. Am J Physiol Cell Physiol 2004; 287: C171-81.
129. Hong KU, Reynolds SD, Watkins S, Fuchs E, Stripp BR. Basal cells are a multipotent progenitor capable of renewing the bronchial epithelium. Am J Pathol 2004; 164: 577-88.
130. Massion PP, Taflan PM, Jamshedur Rahman SM, et al. Significance of p63 amplification and overexpression in lung cancer development and prognosis. Cancer Res 2003; 63: 7113-21.
131. Packham G, Porter CW, Cleveland JL. c-Myc induces apoptosis and cell cycle progression by separable, yet overlapping, pathways. Oncogene 1996; 13: 461-9.
132. He TC, Sparks CAB, Rago C, H. Hermeking H, et al. Identification of c-MYC as a target of the APC pathway. Science 1998; 281: 1509-12.
133. Donaldson TD, Duronio RJ. Cancer cell biology: Myc wins the competition. Curr Biol 2004; 14: R425-7.

134. Moreno E, Basler K. dMyc transforms cells into super-competitors. Cell 2004; 117: 117-29.
135. de la Cova C, Abril M, Bellosta P, Gallant P, Johnston LA. Drosophila myc regulates organ size by inducing cell competition. Cell 2004; 117: 107-16.
136. Reghellin D, Poletti V, Tomassetti S, et al. Cathepsin-K is a sensitive immunohistochemical marker for detection of micro-granulomas in hypersensitivity pneumonitis. Sarcoidosis Vasc Diffuse Lung Dis 2010; 27: .
137. Charette SJ, Landry J. The interaction of HSP27 with Daxx identifies a potential regulatory role of HSP27 in Fas-induced apoptosis. Ann N Y Acad Sci 2000; 926: 126-31.
138. Niessen CM, Hogervorst F, Jaspars LH, et al. The alpha 6 beta 4 integrin is a receptor for both laminin and kalinin. Exp Cell Res 1994; 211: 360-7.
139. Hintermann E, Quaranta V. Epithelial cell motility on laminin-5: regulation by matrix assembly, proteolysis, integrins and erbB receptors. Matrix Biol 2004; 23: 75-85.
140. Coraux C, Meneguzzi G, Rousselle P, Puchelle E, Gaillard D. Distribution of laminin 5, integrin receptors, and branching morphogenesis during human fetal lung development. Dev Dyn 2002; 225: 176-85.
141. Kariya Y, Miyazaki K. The basement membrane protein laminin-5 acts as a soluble cell motility factor. Exp Cell Res 2004; 297: 508-20.
142. Hlubek F, Jung A, Kotzor N, Kirchner T, Brabletz T. Expression of the invasion factor laminin gamma2 in colorectal carcinomas is regulated by beta-catenin. Cancer Res 2001; 61: 8089-93.
143. Pyke C, Romer J, Kallunki P, et al. The gamma 2 chain of kalinin/laminin 5 is preferentially expressed in invading malignant cells in human cancers. Am J Pathol 1994; 145: 782-91.
144. Waghray M, Cui Z, Horowitz JC, et al. Hydrogen peroxide is a diffusible paracrine signal for the induction of epithelial cell death by activated myofibroblasts. FASEB J 2005; 19: 854-6.
145. Aubry MC, Myers JL, Douglas WW, et al. Primary pulmonary carcinoma in patients with idiopathic pulmonary fibrosis. Mayo Clin Proc. 2002; 77: 763-70.
146. García-Sancho Figueroa MC, Carrillo G, Pérez-Padilla R, et al. Risk factors for idiopathic pulmonary fibrosis in a Mexican population. A case-control study. Respir Med 2010; 104: 305-9.
147. 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: 1115-9.
148. Hu J, Dong A, Fernandez-Ruiz V, et al. Blockade of Wnt signaling inhibits angiogenesis and tumor growth in hepatocellular carcinoma. Cancer Res 2009; 69: 6951-9.
149. Tang Y, Simoneau AR, Liao WX, et al. WIF1, a Wnt pathway inhibitor, regulates SKP2 and c-myc expression leading to G1 arrest and growth inhibition of human invasive urinary bladder cancer cells. Mol Cancer Ther 2009; 8: 458-68.
150. Serrano-Mollar A, Nacher M, Gay-Jordi G, Closa D, Xaubet A, Bulbena O. Intratracheal transplantation of alveolar type II cells reverses bleomycin-induced lung fibrosis. Am J Respir Crit Care Med 2007; 176: 1261-8.
151. Agarwal S, Loh YH, McLoughlin EM, et al. Telomere elongation in induced pluripotent stem cells from dyskeratosis congenita patients. Nature 2010 Feb 17.
152. Scotton CJ, Chambers RC. Molecular targets in pulmonary fibrosis: the myofibroblast in focus. Chest 2007; 132: 1311-21.
153. Fauce SR, Jamieson BD, Chin AC, et al. Telomerase-based pharmacologic enhancement of antiviral function of human CD8+ T lymphocytes. J Immunol. 2008; 181: 7400-6.
154. Effros RB. Telomerase induction in T cells: a cure for aging and disease? Exp Gerontol 2007; 42:416-20.