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REVIEW

Tissue remodelling in pulmonary fibrosis

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Abstract Many lung diseases result in fibrotic remodelling. Fibrotic lung disorders can be divided into diseases with known and unknown aetiology. Among those with unknown aetiology, idiopathic pulmonary fibrosis (IPF) is a common diagnosis. Because of its progressive character leading to a rapid decline in lung function, it is a fatal disease with poor prognosis and limited therapeutic options. Thus, IPF has motivated many studies in the last few decades in order to increase our mechanistic understanding of the pathogenesis of the disease. The current concept suggests an ongoing injury of the alveolar epithelium, an impaired regeneration capacity, alveolar collapse and, finally, a fibroproliferative response. The origin of lung injury remains elusive but a diversity of factors, which will be discussed in this article, has been shown to be associated with IPF. Alveolar epithelial type II (AE2) cells play a key role in lung fibrosis and their crucial role for epithelial regeneration, stabilisation of alveoli and interaction with fibroblasts, all known to be responsible for collagen deposition, will be illustrated. Whereas mechanisms of collagen deposition and fibroproliferation are the focus of many studies in the field, the awareness of other mechanisms in this disease is currently limited to biochemical and imaging studies including quantitative assessments of lung structure in IPF and animal models assigning alveolar collapse and collapse induration crucial roles for the degradation of the lung resulting in de-aeration and loss of surface area. Dysfunctional AE2 cells, instable alveoli and mechanical stress trigger remodelling that consists of collapsed alveoli absorbed by fibrotic tissue (i.e., collapse induration).

Keywords Idiopathic pulmonary fibrosis · Mechanical stress · Alveolar collapse · Alveolar epithelial type 2 cells · Collapse induration

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Functional microarchitecture of healthy lung

Lung parenchyma is composed of alveolar and ductal airspaces and inter-alveolar septal walls containing a capillary network. To accommodate its central role in gas exchange, the parenchyma of the mammalian lung provides a huge surface area within a limited volume, together with a very thin barrier between the air and blood in order to minimise diffusion distances for oxygen (Gehr et al. 1978; Weibel et al. 1993). The lung is stabilised by an economically designed fibre network, which in particular consists in the so-called axial, septal and peripheral elastic and collagen fibre system (Wilson and Bachofen 1982). The axial network of elastic fibres takes its origin in the walls of the conducting airways and forms the stress-bearing component of the alveolar entrance rings. This axial network is connected to the peripheral fibre system by the septal system so that it is fixed to the subpleural connective tissue (Mercer and Crapo 1990;

Weibel 2009; Wilson and Bachofen 1982). Hence, elastic fibres and collagen fibrils are concentrated at the edges of the septal walls and, although they possess a central role for stabilisation of lung parenchyma and airways, they contribute less than 10 % to the volumes of septal walls in the human lung, thereby minimising interference with gas exchange capacity (Mercer and Crapo 1990). In the healthy lung, this stabilising system of connective tissue allows the volume to change during respiration with minimal effort and without interfering with the crucial gas exchanging function of the parenchyma.

The pulmonary surfactant system contributes to pulmonary mechanics and stabilises alveoli at lower lung volumes (Schürch et al. 2001). High surface tension leads to a reduction in alveolar surface area by causing the collapsibility of airspaces (Schiller et al. 2001). This is counteracted by the intra-alveolar surfactant through the reduction of surface tension. The intra-alveolar surfactant consists of 90 % lipids (mostly phospholipids) and 10 % proteins. The alveolar epithelial type II cells (AE2 cells) synthesize the components of the surfactant in the Golgi apparatus and rough endoplasmic reticulum and then move these components via transporters and multi-vesicular bodies into specialised organelles called the lamellar bodies (LB). With regard to the relevance of dysfunction in surfactant function and homeostasis in lung diseases, the reader is referred to the review article by Lopez-Rodriguez et al. in this special issue of Cell and Tissue Research.

Alterations in either the interstitial connective tissue components or the surfactant system or both of them will directly affect lung mechanical properties and diffusion capacity and are typical features of lung diseases resulting from pro-fibrotic pulmonary remodelling. Pulmonary fibrosis is not merely an excessive accumulation of extracellular matrix (ECM) components within the pulmonary interstitium and/or intra-alveolar space produced by highly activated fibroblasts and myofibroblasts. In particular, in the case of idiopathic pulmonary fibrosis (IPF), pulmonary fibrosis can furthermore be considered as a disease resulting in severely disorganised connective tissue and the loss of ventilated lung parenchyma following alveolar collapse (i.e., volume loss; Leslie 2012; Myers and Katzenstein 1988) resulting in the stiffening of lung parenchyma. In this review, we focus on IPF, which, in view of its fatal and progressive nature, still represents a severe health-care problem in respiratory medicine. Taking relevant findings from animal models of pulmonary fibrosis used in translational research into account, we will discuss aspects in the pathogenesis of pulmonary fibrosis including the roles of AE2 cells and their interaction with fibroblasts and myofibroblasts. In addition, we will discuss alveolar instability and mechanical stress as a potential trigger of pro-fibrotic remodelling and the relevance of a mechanism called collapse induration for the degradation of lung function.

Effects of IPF on lung structure and function

The histopathological pattern found in patients with IPF is the usual interstitial pneumonia (UIP) pattern (ATS and ERS 2002). UIP is characterised by temporal and spatial heterogeneity. Temporal heterogeneity refers to the fact that, within the same lung, regions can be found representing the various stages from the initiation of fibrotic remodelling to end-stage lung fibrosis. Spatial heterogeneity reflects the patchy distribution of the various pathological alterations in the lung; this means that completely normal-appearing lung parenchyma with maintained acinar architecture can be found in the close neighbourhood of affected and even severely affected areas. Typical light microscopic findings in UIP are fibroblast foci, thickening of inter-alveolar septal walls, microscopic honeycombing with bronchiolarisation of distal airspaces and hypertrophy and hyperplasia of alveolar epithelial cells (Katzenstein et al. 2008). These pathological alterations are predominantly located in basal and subpleural regions of the lung. Hence, an apical to basal gradient of pathological findings including the formation of honey comb cysts and the volume loss in high-resolution chest computed tomography (HRCT) is an important feature for the diagnosis of IPF (Raghu et al. 2011). The existence of fibroblast foci is a crucial characteristic for the diagnosis of a UIP pattern at the histopathological level. Fibroblast foci are considered to be sites of ongoing lung injury with fibroproliferation within the alveolar and interstitial space and the presence of fibroblasts and myofibroblasts, with both of them being responsible for the deposition of ECM components including immature collagen (Cool et al. 2006; Katzenstein et al. 2008; Kuhn et al. 1989; Kuhn and McDonald 1991). The clinical meaning of fibroblast foci as locations of disease activity has been emphasised in several observational clinical studies. The number of profiles of fibroblast foci and the volume fraction of fibroblast foci in light microscopic sections from surgical lung biopsies from IPF patients have been found to correlate with the impairment in lung function (degree of restrictive ventilator failure), disease progression and prognosis of the patients in terms of mortality (Enomoto et al. 2006; Harada et al. 2013; Nicholson et al. 2002). However, in two-dimensional light microscopic sections, fibroblast foci appear to be isolated "islands". Using serial sections from patients with the diagnosis of IPF and a three-dimensional reconstruction of lung parenchyma including fibroblast foci, Cool and co-workers demonstrated that fibroblast foci are not isolated. Instead, fibroblast foci form a three-dimensional fibroblast reticulum meaning that islands of fibroblast foci are interconnected by connective tissue bridges (Cool et al. 2006). Moreover, the finding that interconnected fibroblast foci form a three-dimensional network has direct implications for lung mechanical properties of the IPF lung. IPF is a disease that is supposed to have a subclinical phase meaning that a period

of unknown duration ranges from the initiation of the disease to its first symptoms, the establishment of the diagnosis and finally respiratory failure attributable to progression (Ley et al. 2011). The degradation of lung function characterised by the loss of forced vital capacity with time is a typical and clinically highly relevant IPF feature, which is often used as an endpoint in clinical studies (King et al. 2014; Noble et al. 2011; Richeldi et al. 2014) and predictive of the prognosis (Collard et al. 2003). In this regard, Bates and co-workers linked the progression of lung parenchymal pathology with functional pulmonary degradation by using a computational modelling approach based on the concept of percolation (Bates et al. 2007). In this model, an elastic network of springs corresponded to lung parenchyma and defined the lung mechanical properties. Progressive pulmonary fibrosis at a structural basis was modelled by progressive and random stiffening of individual springs. As long as the fibrotic springs were not interconnected, the modelling predicted only minor effects of progressing lung pathology on lung mechanical impairment. However, as soon as a network of stiffened springs appeared with progressive disease attributable coalescence, meaning that a threshold was reached, the overall stiffness of the lung suddenly markedly increased (Bates et al. 2007). This modelling emphasises that not only the total amount or volume of pathological lesions (e.g., fibroblast foci) within the lung increased but also the three-dimensional organisation of such lesions, which determines lung mechanical properties (Bates et al. 2007) underscoring the pathophysiological relevance of the three-dimensional architecture of the fibroblast foci (Cool et al. 2006). Moreover, the modelling could, at least in part, explain the time course of the disease with a subclinical phase followed by a phase of rapid deterioration of symptoms and lung function.

Another description with regard to the fibroblast foci in UIP has included not only acute lung injury but also alveolar collapse, so that fibroblast foci have been considered as sites of alveolar epithelial cell necrosis followed by collapse of distal airspaces and fibroproliferation (Kuhn and McDonald 1991; Myers and Katzenstein 1988). In the context of UIP, ultrastructural evaluation of fibroblast foci showed remnants of the alveolar epithelial basal lamina deep within the connective tissue (Kuhn and McDonald 1991; Myers and Katzenstein 1988), whereas in cases of acute interstitial pneumonia, denuded basal lamina with alveolar collapse and apposition of alveolar septal walls were typical findings (Katzenstein 1985). Moreover, hyperplastic alveolar epithelial cells overgrew such apposing alveolar septal walls. Hence, Katzenstein concluded that not only interstitial fibrosis but also alveolar collapse, plus the incorporation of intra-alveolar exudates into the septal wall are responsible for the thickening of the septal walls found in interstitial lung diseases such as IPF or acute interstitial pneumonia, a disease which is characterised by a much more rapid and acute clinical presentation and time course (Katzenstein 1985). Of note, Kuhn and co-workers were also able to demonstrate that epithelial cells covering fibroblast foci quite often have no basal lamina and are in direct contact with underlying collagen fibrils (Kuhn and McDonald 1991), a finding that we have reproduced in our own material from explanted IPF lungs (Fig. 1). Using design-based stereology, Coxson and co-workers underscored the relevance of the collapse of distal airspaces in IPF (Coxson et al. 1997). By means of computed tomography and surgical lung biopsies up to the ultrastructural level taken from IPF patients at the time point of diagnosis for quantitative morphology, the authors demonstrated a dramatic decline of alveolar surface area per lung from 100 m^2 in healthy controls to 30 m^2 in IPF patients. Of interest, this study did not find an overall increase in the total amount of tissue per lung. These data do not support the concept of an excessive proliferation of connective tissue as the main factor for lung functional degradation, which is in agreement with earlier findings by using hydroxyproline levels for the quantification of fibrosis

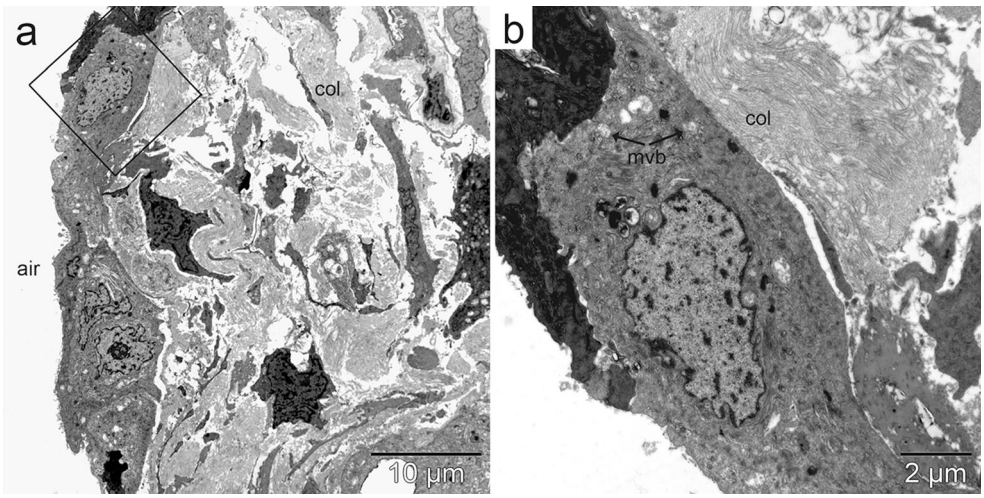

Fig. 1 Severe fibrosis in idiopathic pulmonary fibrosis (IPF). Hyperplastic epithelium covers severely remodelled tissue. No basal lamina is detectable and epithelial cells are directly located on collagen fibrils (*col*). Note the multi-vesicular bodies (*mvb*). The *boxed area* in **a** is shown at higher magnification in **b**

in IPF (Fulmer et al. 1980). Nevertheless, within the parenchymatous tissue of the lung, an increase in the volume fraction of collagen fibrils was observed (Coxson et al. 1997). Taking the ultrastructural findings from Myers and Katzenstein into consideration (Myers and Katzenstein 1988), the quantitative structural data provide evidence for the relevance of the instability of the distal airspaces, such as permanent alveolar collapse in IPF (Galvin et al. 2010; Leslie 2012; Todd et al. 2015), giving at the same time, an explanation how the lung loses lung capacity with only a limited increase in components of connective tissue elements (Coxson et al. 1997; Fulmer et al. 1980; Selman et al. 1986). Using micro-computed tomography (μCT) of explanted IPF lungs, Mai and co-workers recently performed a correlative morphological study and closed the gap with regard to the resolution between light microscopy and HRCT. In areas with minor alterations at the HRCT level, evidence of alveolar collapse could be found adjacent to the consolidations, which most likely represented fibroblast foci. From these observations, the authors concluded that alveolar collapse occurs at an initial stage predating the profibrotic remodelling of IPF (Mai et al. 2016). Similar observations were reported in animal models of lung injury and fibrosis including the bleomycin model (Lutz et al. 2015), the amiodarone model (Birkelbach et al. 2015; Mahavadi et al. 2014) and the transforming growth factor (TGF) β1 model (Lopez-Rodriguez et al. 2016a) and after paraquat challenge of monkey lungs (Fukuda et al. 1985). Thus, the important effects of IPF on lung structure are the formation of a fibroblast reticulum in concert with alveolar collapse, with both of these structural alterations impacting lung mechanical properties.

Aetiological aspects of IPF

Known triggers for the development of lung fibrosis include drugs (bleomycin, amiodarone), radiation and chronic inflammatory diseases such as connective tissue diseases or those diseases following inhalation of organic (e.g., farmer's lung) or inorganic (e.g., asbestosis) particles. Some of these entities of interstitial lung disease triggered predominantly by inflammatory stimuli respond to anti-inflammatory therapies. However, in the case of IPF, the aetiology of the disease remains elusive and anti-inflammatory drugs have turned out to be harmful for these patients (Raghu et al. 2012). In other words, IPF is the result of ongoing lung injury with impaired regeneration but the factors that cause the lung injury and the aberrant repair are unclear. As such, current concepts suggest the merging of a diversity of potentially harmful hits on the lung including those genetic, environmental and behavioural factors thought to be necessary for the development of IPF (Fig. 2; Brownell et al. 2016; Mulugeta et al. 2015). Several conditions and activities including smoking, farming and rearing livestock and wood-dust and stone/sand inhalation have been identified as being associated with a higher risk for the development of IPF, although a clear cause-effect relationship has not been established (Ekström et al. 2014; Taskar and Coultas 2006). Case–controlled genome-wide association

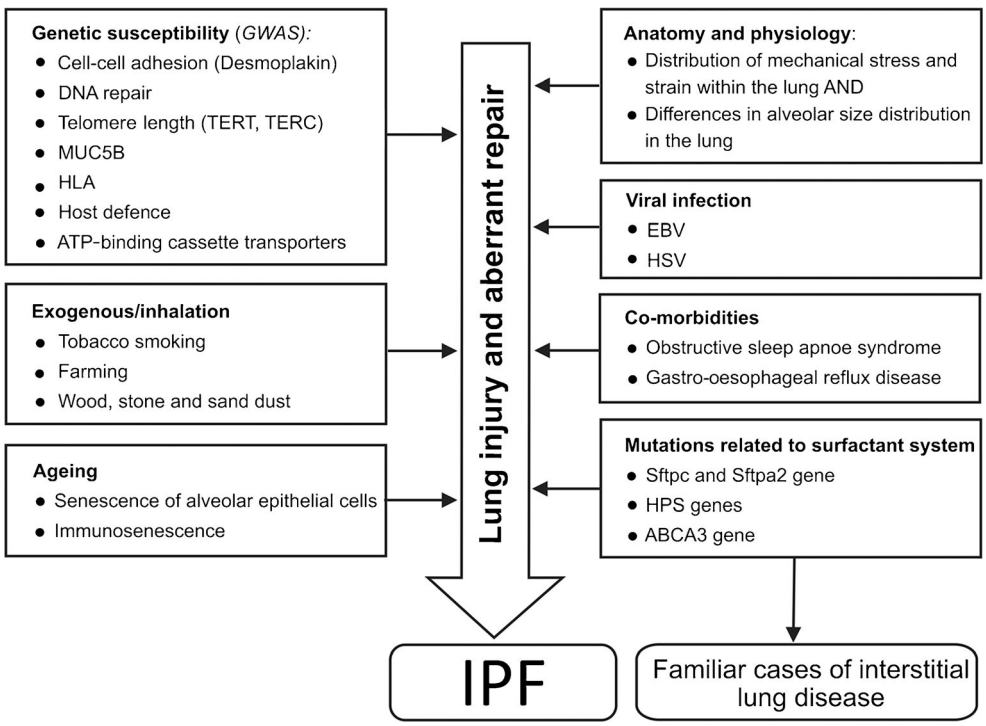

Fig. 2 Aetiological factors in lung fibrosis. Several factors including genetics, environmental exposure, aging, mechanical stress and co-morbidities have been shown to be associated with fibrotic lung diseases but for most factors, a causal relationship could not be demonstrated. Nevertheless, current concepts suggest a multiple hit model, meaning that the disease results from the interplay of a diversity of different factors

studies of patients with interstitial lung diseases have been performed to find genes and single nucleotide polymorphisms (SNPs) that might be linked to an increased susceptibility for lung fibrosis (Fingerlin et al. 2013, 2016). These studies have demonstrated a set of genes related to cell-cell adhesion, DNA repair, host defence and HLA to be associated with idiopathic interstitial lung diseases among which IPF is the most common representative. A strong association could be found between the DSP gene and idiopathic interstitial pneumonias. DSP encodes for desmoplakin, a component of the desmosome, which provides epithelial cells with resistance towards mechanical stress (Fingerlin et al. 2013). Hence, SNPs of the DSP gene might be linked with an increased susceptibility of alveolar epithelial cells to be injured as a result of mechanical challenge. Moreover, genes or SNPs related to ATP-binding cassette transporters, which are found in AE2 cells and involved in surfactant homeostasis, have been associated with an increased risk for idiopathic interstitial pneumonias (Fingerlin et al. 2013; Postle et al. 2011). Another very strong association has been established and also confirmed, in several studies, between a SNP of the MUC5B gene (rs35705950) and pulmonary fibrosis. In cases of homozygous alleles of rs35705950, a 20-fold increase in the risk of pulmonary fibrosis has been seen (Fingerlin et al. 2013; Seibold et al. 2013). Moreover, the rs35705950 allele has been linked to a dramatic up-regulation of MUC5B expression by epithelial cells of distal conducting airways, both in healthy controls and in IPF patients (Nakano et al. 2016; Seibold et al. 2011). Several prospective cohorts of the population, including the cohort of the Framingham Heart study, were recently further evaluated by computed tomography of the chest and by lung functional data and followed for several years (Putman et al. 2014, 2016). These studies demonstrated that interstitial lung abnormalities are not rare findings in chest computed tomography of the lung and were observed in 7 % of subjects, although lung functional parameters were not impaired in these subjects and the diagnosis of an interstitial lung disease was not established (Hunninghake et al. 2013). However, a clear association of the interstitial lung abnormalities with MUC5B SNP rs35705950, increased mortality and increased loss of lung function was noted at follow-up (Araki et al. 2016; Hunninghake et al. 2013; Putman et al. 2016). The mechanism of action of MUC5B SNP rs35705950 is not yet clear but some hypotheses include impaired host defence mechanisms or increased mechanical stress imposed on lung parenchyma because of the interaction of MUC5B protein with surfactant leading to impaired surface tension lowering function, in cases in which MUC5B reaches the alveolar portion, e.g., caused by the bronchiolarisation of the distal airspaces (Kolb et al. 2016; van Moorsel et al. 2015). SNPs of TOLLIP, a gene regulating the innate immune response via Toll-like receptors and transforming growth factor-β1 (TGF-β1) signalling have also been shown to be associated with IPF but with the same prognosis and a response upon treatment with N-acetylcystein, an anti-oxidative drug (Noth et al. 2013; Oldham et al. 2015). Hence, TOLLIP determines susceptibility for IPF but also modulates disease progression. Other genes that have been linked not only to IPF but also to familiar cases of pulmonary fibrosis, which can manifest with an UIP pattern at histopathological evaluation, include those that regulate the telomere length (TERT and TERC; Armanios et al. 2007; Fingerlin et al. 2013) and a shortening of telomere length has been associated with IPF. Hence, ageing such as the senescence of alveolar epithelial cells or the immunosenescence or exhaustion of stem cell resources necessary for regeneration are also factors that seem to play important roles with respect to the susceptibility for the development of fibrotic lung diseases including IPF (Araya et al. 2013; Chilosi et al. 2013). Aging as such is very likely to increase susceptibility of the alveolar epithelium with respect to harmful factors. Viral triggers such as an infection of the alveolar epithelium with Epstein-Barr virus (EBV), human herpes virus (HHV) or herpes simplex virus (HSV) have been discussed as a potential etiological factor for lung injury, as both the protein and DNA of the viruses have been traced in the lung tissue of IPF patients (Lok et al. 2001; Molyneaux and Maher 2013). Compared with the general population, gastroesophageal reflux (disease) is much more frequent and a common finding in patients with IPF (e.g., 10–19 % vs. 87–94 %), indicating that acid aspiration might play an etiological role in pathogenesis (Ghebre and Raghu 2016; Raghu et al. 2006). However, from a lung mechanical point of view, the higher prevalence of gastroesophageal reflux in IPF might also be a secondary effect resulting from increased lung stiffness and an increased intra-thoracic pressure swing that might lead to an insufficient sphincter function at the oesophageal-gastral junction (Ghebre and Raghu 2016). Further comorbidities that are more often found in IPF than in the general population are sleep-related breathing disorders including obstructive sleep apnoe syndrome (OSAS) or alveolar hypoventilation (Milioli et al. 2016). Whether untreated OSAS contributes to disease progression is not clear but the combination of OSAS and IPF has been shown to be associated with a poorer prognosis (Milioli et al. 2016). In principle high trans-thoracal pressure gradients acting on lung parenchyma during phases of obstruction of the upper airways might contribute to ongoing or repetitive lung injury by means of mechanical stress.

Pathogenesis of IPF

In recent years, IPF has in general been accepted to result from repetitive or ongoing injury of the alveolar epithelium with aberrant pro-fibrotic repair including clot formation and the generation of a provisional matrix, activation of fibroblasts

and myofibroblasts and interstitial and intra-alveolar scar formation (Fig. 3; Geiser 2003). This concept with regard to the pathogenesis of IPF was described more than 30 years ago and ever since has been confirmed not only in several animal models but also in familiar forms of interstitial lung diseases resulting from mutations of the surfactant protein C gene, which is linked to the chronic endogenous injury of AE2 cells (Mulugeta et al. 2015; Uhal and Nguyen 2013). Repetitive minor injuries of the alveolar epithelium in concert with an impaired regeneration capacity results in aberrant wound repair with the activation of fibroblasts and the deposition of collagen fibrils (Geiser 2003). This concept is based on animal experiments performed by Haschek and Witschi dating back to the 1970s (Haschek et al. 1981; Haschek and Witschi 1979). Butylated hydroxytoluene was intraperitoneally injected into the mouse and induced acute injury of the

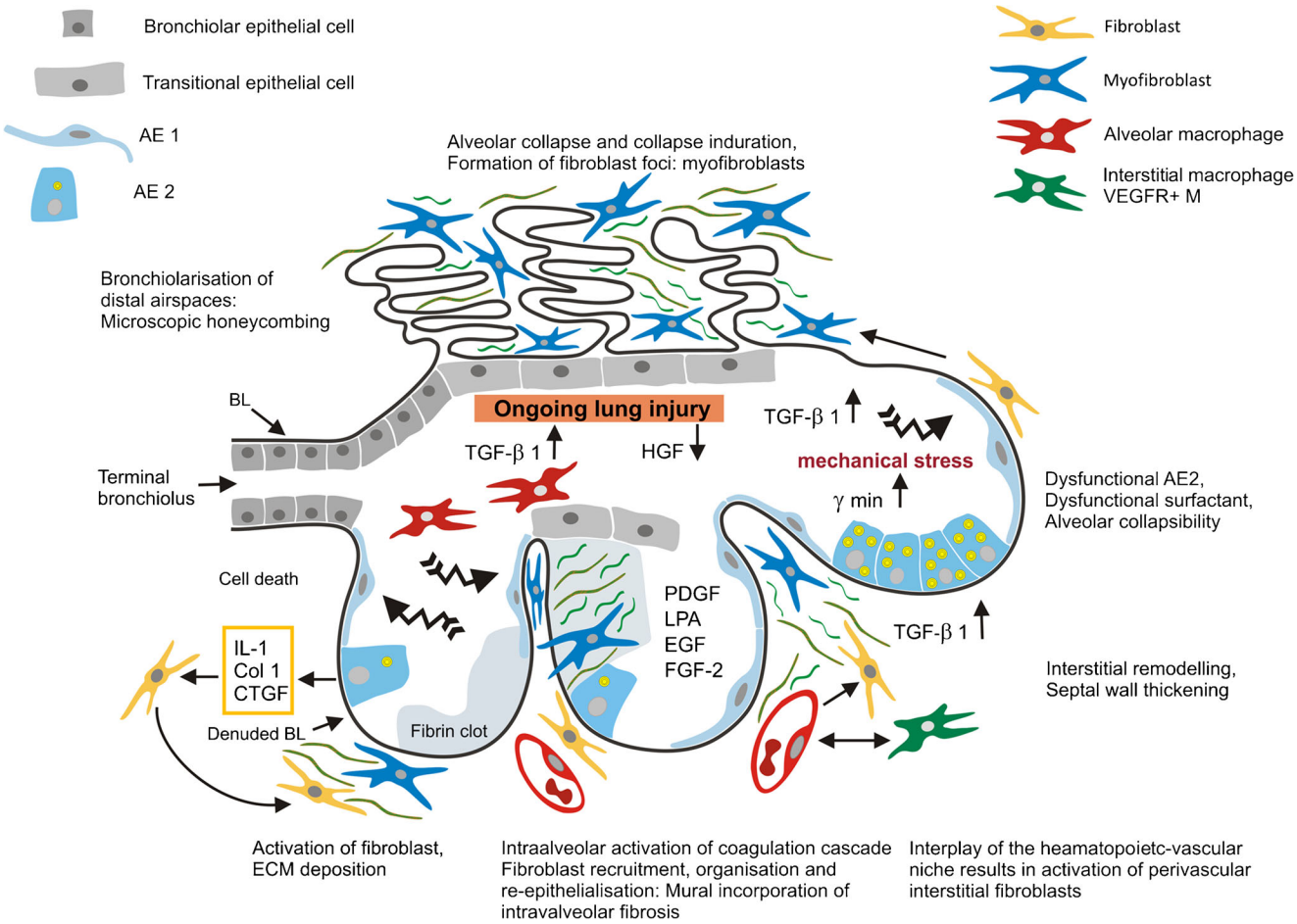

Fig. 3 Summarised pathophysiological concept of pulmonary fibrosis. Ongoing injurious events operating on the alveolar epithelium are considered key in IPF, although the injurious triggers are still not defined. The alveolar milieu is characterised by an imbalance of profibrotic (e.g., activated TGF-β1) and antifibrotic (e.g., HGF) factors. The Alveolar epithelial type II (*AE2*) cells, which are responsible for regeneration, fail to repopulate the denuded epithelial basal lamina (*BL*) resulting in activation in fibroblasts. Factors related to alveolar epithelial cells, such as direct cell-cell contacts with interstitial cells or the production of paracrine factors (Il-1, Col 1, CTGF), are involved in this process. After injury, the coagulation cascade is activated resulting in the formation of intra-alveolar clots, which are invaded and remodelled by fibroblasts and myofiboblasts. Involved mediators include PDGF, LPA, EGF, or FGF-2. Intra-alveolar fibrotic tissue is repopulated by epithelial cells and engulfed in the interstitial compartment. Not only alveolar epithelial cells but also endothelial cells and interstitial macrophages expressing VEGF-receptor have been shown to be involved in the regulation of alveolar epithelial cell and fibroblast function (so-called haematopoietic vascular niche). AE2 cell dysfunction also results in impaired surfactant function with instability of distal airspaces resulting in alveolar collapsibility and subsequently mechanical stress, alveolar epithelial injury and TGF-β activation predominantly in areas of the lung, which are, from a physiological and anatomical point of view, underprivileged: these are areas in which volume changes (strain) during breathing are maximal within the lung and the alveoli are the smallest. Finally, collapsed alveoli cannot be reopened. The entrance is overgrown by hyperplastic transitory epithelial cells and these collapsed alveoli are engulfed in fibrotic tissue, a process referred to as "collapse induration". As a result, massive de-aeration, volume loss and loss of alveolar epithelial surface area occur, characteristics that from an imaging perspective are key in IPF (*Il-1* interleukin-1, *Col 1* collagen type 1, *CTGF* connective-tissue-derived growth factor, *ECM* extracellular matrix, *HGF* hepatocyte growth factor, *PDGF* platelet-derived growth factor, *LPA* lysophosphatidic acid, *EGF* epithelial growth factor, *FGF-2* fibroblast growth factor-2, *TGF-β1* activated transforming growth factor-β1, *VEGFR+ M* vascular endothelial growth factor receptor positive macrophages, γ_{min} minimum surface tension)

alveolar epithelial type I (AE1) cells, while leaving AE2 cells intact according to ultrastructural criteria (Adamson et al. 1977, 1990). This injury was repaired without relevant fibrotic remodelling as long as the AE2 cell function was not affected: the AE2 cells proliferated and differentiated into AE1 cells. However, exposure of these mice lungs to, for example, 70 % oxygen in the inspiratory air during the days following butylated hydroxytoluene exposure resulted in a failure of AE2 cells to regenerate the loss of AE1. Instead, an increase in collagen deposition and hydroxyproline per lung was found (Haschek and Witschi 1979). In IPF lungs, an injury of AE1 and AE2 cells can be seen at the ultrastructural level resulting in detachment of cells from the basal lamina and, finally, in denudation of the alveolar epithelial basal lamina (Fig. 4). The lung injury is accompanied by an inflammatory response not only in animal models but also in human acute or chronic lung injury. Reducing the influx of neutrophils into the bleomycin-injured rat lung by antibody-induced depletion resulted in an increase in lung fibrosis, meaning that at least the neutrophil-related and injury-associated inflammatory response fulfils a protective role in this context (Thrall et al. 1981). Findings from interleukin 10 (IL-10)-deficient mice support the concept that acute-lung-injury-related inflammation and fibrotic remodelling do not correlate with each other and can be regarded to be dissociated from each other (Huaux et al. 1998). The silicate animal model of lung injury and fibrosis in wildtype and IL-10-knockout mice revealed a decreased inflammatory response in wildtype animals related to anti-inflammatory functions of IL-10. However, the fibrotic response was more pronounced in the wildtype than in the IL-10-knockout mice. Hence, IL-10 is anti-inflammatory on the one hand but is pro-fibrotic on the other (Huaux et al. 1998). Until the beginning of the last decade, human IPF was considered to result predominantly from uncontrolled inflammation. Hence, diverse clinical trials involved various forms and combinations of immunosuppressive drugs, none of which showed any efficiency in IPF. Instead, the PANTHER trial testing the efficiency and safety of a combination of steroids, azathioprine and N-acetylcystein was terminated ahead of schedule, since immunosuppressive treatment was associated with an increased mortality and hospitalisation rate (Raghu et al. 2012). These data defeat the hypothesis that at least those inflammatory factors that can be suppressed by steroids and azathioprine are involved in the pathogenesis of IPF. Instead, an explanation based on dysregulated wound repair attributable to dysfunction of the alveolar epithelium, or more specifically, dysfunction of the AE2 cells, moved closer to the centre of the pathogenic concept of fibrosing lung diseases (Selman and Pardo 2006). AE2 cells are responsible for surfactant metabolism, which is essential for keeping alveoli open, dry and clean (Ochs 2010) and for the regeneration of alveolar epithelium, giving credence for their role as "defenders of the alveolus" (Fehrenbach 2001).

AE2 cell in focus: ER stress, autophagy and apoptosis

Consistent with a central role for the alveolar epithelium in the pathogenesis of pulmonary fibrosis, the apoptosis of AE2 cells is a prominent finding in the histopathology of IPF (Barbas-Filho et al. 2001; Kuwano et al. 1996; Myers and Katzenstein 1988; Uhal et al. 1998). Experimentally, the targeted injury of AE2 cells with subsequent cell death has been shown to be sufficient to induce fibrotic remodelling in mice lungs (Sisson et al. 2010). Apoptosis of AE2 cells leads to a denudation of the basal lamina and might reflect the initial damaging event in the development of this disease (Horowitz and Thannickal 2006), although the involved molecular mechanisms are incompletely understood.

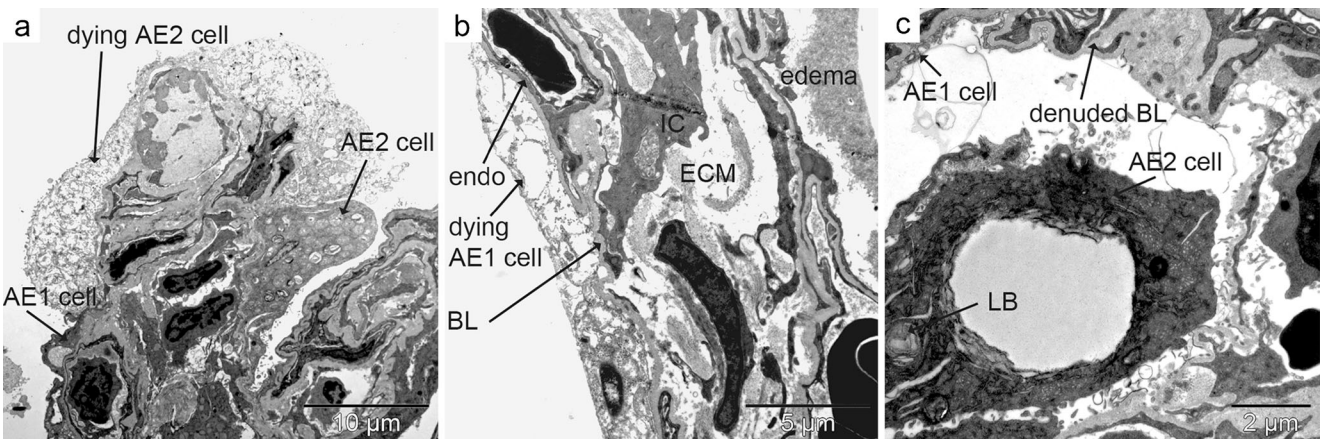

Fig. 4 Injury to alveolar epithelial cells in IPF. Ultrastructural evidence of AE2 and AE1 cell injury in samples from explanted IPF lungs analysed within the frame of a previous study (Lutz et al. 2015). Tissue taken from a macroscopic non-fibrotic-appearing area of the lung. **a** Swollen (oncotic) AE2 cell in the direct neighbourhood of a normal-appearing AE2 cell. **b** Swollen (oncotic) AE1 cell covering an alveolar septum (*BL* basal lamina, *ECM* extracellular matrix, *endo* endothelial cell). On the other side of this septum, alveolar oedema (*edema*) is visible. **c** Completely denuded basal lamina (*BL*) and a hyperplastic lamellar body (*LB*) containing an AE2 cell detached from the BL

Recently, several studies have demonstrated evidence for the pivotal role of cellular stress, in particular endoplasmic reticulum (ER) stress, as the underlying cause for epithelial apoptosis in IPF and other forms of pulmonary fibrosis (for a review, see Korfei et al. 2016).

Initial insights into the role of ER stress in the pathogenesis of lung fibrosis came from the observations in familial interstitial pneumonias. Mutations in the surfactant protein C gene (SFTPC) are associated with familial forms of lung fibrosis in both adults and paediatric patients (Lawson et al. 2008; Mulugeta et al. 2005; Nogee et al. 2001; Thomas et al. 2002). Meanwhile, over 60 different mutations in the SFTPC gene have been described (Mulugeta et al. 2015), many of them being located within the pre-protein BRICHOS domain, an approximately 100-amino-acid region in the COOH-terminal area. This region bears homology to a number of proteins linked to familial neurodegenerative disease and amyloid formation (Sánchez-Pulido et al. 2002). The mutations result in the misfolding of the pro-protein, impaired proteolytic processing and trafficking through the regulated secretory pathway with subsequent intracellular aggregate formation and accumulation (Mulugeta et al. 2007; Thomas et al. 2002; Wang et al. 2003). As a consequence of an accumulation of misfolded proteins, the activation of ER stress pathways (PERK, ATF6α and IRE1) and the unfolded protein response (UPR), the induction of the pro-apoptotic transcription factor CHOP and, finally, the activation of caspase-3 have been observed (Korfei et al. 2016; Mulugeta et al. 2005, 2007; Wang et al. 2003).

In addition to mutations in SFTPC, mutations in the genes encoding surfactant protein A (SPA, SFTPA2; Maitra et al. 2010) and ABCA3 (Bullard et al. 2005; Wambach et al. 2014; Weichert et al. 2011; Young et al. 2008), an ATP-binding cassette transporter involved in intracellular surfactant transport, have been discovered in patients with various forms of lung fibrosis. Overexpression of these mutant proteins in alveolar epithelial cells in vitro also results in the significant induction of ER stress. The finding of pro-apoptotic ER stress in AE2 cells, however, is not restricted to familial forms but is also prominent in patients with sporadic IPF (and other idiopathic interstitial pneumonias) in the absence of any gene mutation (Korfei et al. 2008, 2011, 2013; Lawson et al. 2008). A significant activation of ER stress pathways, including ATF6-upregulation and -cleavage, the activation of the IRE1α/XBP1-pathway and significant increases in the expression of ATF4 and CHOP have been demonstrated in AE2 of sporadic IPF patients but not in AE2 of organ donors.

Another example of a link between defective AE2 homeostasis and ER stress in the development of pulmonary fibrosis comes from lysosomal storage diseases. Patients with Hermansky-Pudlak syndrome (HPS), who develop a form of lung fibrosis that is indistinguishable from the typical UIP histology found in IPF, show an impaired lamellar body genesis and disturbed surfactant processing with intracellular accumulation of surfactant proteins and lipids (Brantly et al. 2000; Nakatani et al. 2000). A severe ER stress response can develop as a consequence of the defective trafficking, targeting and secretion of proteins and lipids and the accumulation of unprocessed proteins and lipids in distal lysosomes, vesicles, Golgi and (finally) also in the proximal ER. Indeed, hyperplastic AE2 cells of patients with HPS1 exhibit pro-apoptotic ER stress, as shown by immunohistochemistry for the ER stress markers ATF4 and CHOP and the apoptosis-marker cleaved capase-3 (Mahavadi et al. 2010). In corresponding animal models (Hps1/Hps2 double-mutant mice), lysosomal stress has been demonstrated by the increased expression of cathepsin D and of its apoptotic pro-form and seems to precede the induction of severe ER stress (Mahavadi et al. 2010). In agreement with these findings, defective surfactant storage attributable to the inhibition of trafficking and transport mechanisms in vesicles of the lysosomal compartment with the subsequent induction of autophagy, lysosomal and ER stress has been found in amiodarone-induced lung fibrosis in mice (Birkelbach et al. 2015; Mahavadi et al. 2014, 2015).

Additionally, AE2 apoptosis might be the cellular consequence of genomic instability, DNA damage and aberrant DNA repair. In this regard, several cases of familial interstitial pneumonias, mostly IPF, with mutations in the telomerase genes, namely telomerase-reverse transcriptase (TERT) and telomerase RNA component (TERC; Armanios et al. 2007; Tsakiri et al. 2007) and other telomere-associated genes (e.g., dyskerin/DKC1 and shelterin-complex protein TINF2; Fukuhara et al. 2013; Kropski et al. 2014) have been reported. These mutations have been demonstrated to be associated with telomere shortening, which is a potential cause for the triggering of the activation of a persistent DNA damage response and the induction of a replicative senescence or apoptosis in affected cells. Moreover, shortened telomeres have been observed directly in the AE2 of familial IPF patients and have even been commonly detected in AE2 of sporadic IPF cases, in the absence of any gene mutations in the telomerase complex (Alder et al. 2008).

Taken together, all these independent observations suggest that the ER-stress-induced apoptosis of AE2 represents a common pathomechanistic principle in IPF and other forms of lung fibrosis. Various cellular stress mechanisms, including defective protein folding and processing, DNA damage stress and genomic instability or lysosomal stress can induce or aggravate ER stress and thereby contribute to the loss of AE2. If the cellular stress is not resolved, an otherwise protective ER stress response on normal cells can be converted into maladaptive severe ER stress and subsequent apoptosis. Second hits might further trigger the maladaptive switch (Xu et al. 2005). Oxidative stress (e.g., as caused by cigarette smoking) is known to disrupt protein folding and is the leading cause of

telomere shortening (Jones 2006) and viral/bacterial infections might critically overwhelm protein synthesis in the ER and induce or aggravate ER stress (Endo et al. 2005). In agreement with such reasoning, an imbalanced alveolar oxidant-antioxidant status in the lung attributable to an increased production of oxidants and the depletion of antioxidants has been observed in the fibrotic lung (Kinnula and Myllärniemi 2008) and respiratory infections are a common phenomenon that frequently antecede the clinical appearance of the disease and also seem to accelerate the clinical course (Molyneaux and Maher 2013).

AE2 cells, fibroblasts and their interaction in fibrotic remodelling: lessons learned from animal models

Upon injury of the alveolar epithelium resulting in denudation of the epithelial basal lamina, AE2 cells start to proliferate to fill the gap and to repopulate the denuded basal lamina. Finally, AE2 cells differentiate into AE1 cells (Adamson and Bowden 1974). If the AE2 cells fail to repopulate the denuded basal lamina, interstitial fibroblasts are activated and start to produce excessive amounts of ECM components (Adamson et al. 1988, 1990). These observations were originally made in animal models of lung injury and fibrosis but the local association of denuded basal lamina and interstitial collagen deposition can also be found in IPF samples quite commonly: at the ultrastructural level, excessive amounts of connective tissue components can be observed in areas of denuded basal lamina in human IPF explants (Fig. 5). Moreover, the deposition of collagen in the interstitial tissue is correlated with the ultrastructural changes in AE2 cells in IPF and also in the amiodarone mouse animal model of lung injury and fibrosis (Birkelbach et al. 2015; Kawanami et al. 1982).

Hence, dysfunctional AE2 cells, which, on the one hand, fail to regenerate the alveolar epithelium and, on the other hand, show hypertrophy, hyperplasia and severe ultrastructural abnormalities related to the intracellular surfactant pool, appear to be of relevance for the interstitial deposition of connective tissue components. Based on observations from the bleomycin-induced lung injury and fibrosis model, Adamson and co-workers (1990) suggested a reciprocal epithelial-fibroblast control system: epithelial cell damage with delayed repair promotes the growth of fibroblasts and direct cell-cell contacts between AE2 cells and fibroblasts or fibrillary collagen seem to be involved in regulating AE2 cell growth and differentiation (Adamson et al. 1990). Thus, treatment strategies with the goal of stimulating migration, proliferation and differentiation of AE2 cells have been tested in animal models of lung injury and fibrosis. In order to enhance the regeneration capacity of AE2 cells, hepatocyte growth factor (HGF) has been successfully used either by unspecific overexpression of the HGF gene by alveolar epithelial cells or by specific expression in AE2 cells under the control of the surfactant protein C promotor or by bone-marrow-derived stem cells passed intratracheally into the bleomycin injured lung (Gazdhar et al. 2007, 2013a, 2013b). In this regard, a relative deficiency of HGF in the alveolar space has also been reported in IPF patients providing evidence of an imbalance of factors promoting alveolar epithelial regeneration (e.g., HGF; Marchand-Adam et al. 2006; Mason et al. 1994; Phin et al. 2010), on the one hand and factors that inhibit regeneration directing fibrotic repair mechanisms, such as TGF-β1, on the other hand. TGF-β1, a key player for fibrotic remodelling, has major effects on AE2 cells in vivo and the increased expression of an active variant of TGF-β1 with increased secretion into the alveolar space by using an adenoviral vector for gene-transfer results in a loss of polarisation of AE2 cells

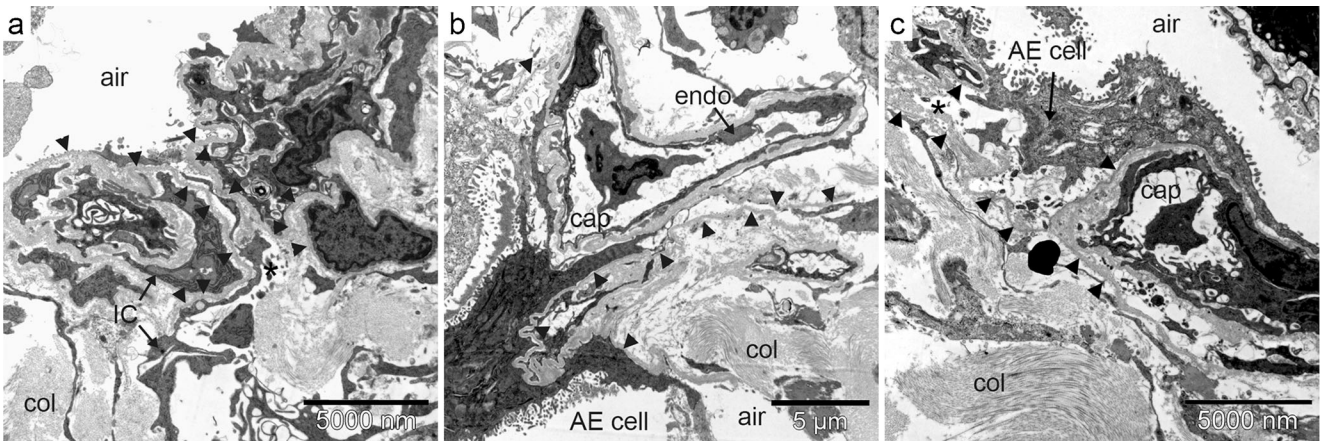

Fig. 5 Alveolar collapse and collapse induration in less severe fibrotic areas. Areas of the lung from an IPF explant with thickened septal walls. Denuded basal lamina (*arrowheads*), indicating the course of the original alveolar epithelium, can be followed deep into the interstitial tissue. The former lumen (*asterisk* in **a**) is partly filled with interstitial cells, most probably fibroblasts and extracellular matrix. In **b**, **c**, the presumed former entrances of the alveoli are overgrown by alveolar epithelial cells that partly show characteristics of AE2 cells (*air* airspace, *IC* interstitial cell, *col* collagen fibrils, *endo* endothelial cell, *cap* capillary lumen)

characterised by a decrease in the surface area of the apical membrane and an increase in the surface area of the basolateral membrane (Lopez-Rodriguez et al. 2016a). These changes correlate with lung mechanical properties such as decreased compliance indicating the functional relevance of ultrastructural alterations of AE2 cells (Lopez-Rodriguez et al. 2016a). Dysfunctional AE2 cells and dysregulated crosstalk between alveolar epithelial cells and interstitial fibroblasts have been suggested to be of great importance for the progression of fibrotic remodelling (Selman and Pardo 2006). Markers of apoptosis and electron microscopy have revealed an increased proportion of apoptotic and necrotic AE1 and AE2 cells with shortened telomere length not only in fibroblast foci but also in normal-appearing alveoli (Uhal et al. 1998; Waisberg et al. 2010). In vivo experiments on animal models of lung injury and fibrosis have brought to light many signalling factors and pathways that might also orchestrate the pathogenesis of IPF and lead to both a pro-apoptotic milieu for alveolar epithelial cells and a pro-fibrotic milieu in which fibroblasts appeared to be more resistant for apoptosis. Lysophosphatidic acid (LPA), for example, which is a bioactive lipid, plays an important role during alveolarisation in the mouse lung via the LPA_1-receptor by mediating the migration of peripheral myofibroblasts and the synthesis of elastin, with both of these factors being highly relevant for early and late alveolarisation in young mice (Funke et al. 2016). However, good evidence is available for LPA acting as a factor enhancing pro-apoptotic signalling in AE2 cells and, at the same time, protecting fibroblasts from apoptosis (Funke et al. 2012). In IPF patients, the levels of LPA are increased in the bronchoalveolar lavage and LPA_1-receptor knockout mice are protected from bleomycin-induced fibrosis providing evidence that this pathway is also involved in human IPF (Tager et al. 2008).

Nevertheless, the biomechanical properties of the matrix in which the fibroblasts and myofibroblasts are embedded have also been shown to be of importance regarding the prevention of apoptosis and the maintenance of a pro-fibrotic phenotype via mechanotransduction (Kulkarni et al. 2016; Liu et al. 2010, 2015; Saito and Nagase 2015; Zhou et al. 2013). Matrix stiffness (by analogy to TGF-β1 stimulation) as such induces a remodelling of the actin cytoskeleton in fibroblasts and triggers the actin-myosin-related contractile system, which in turn, via nuclear translocation of transcription factor MKL1 (megakaryoblastic leukaemia 1), leads to the differentiation of fibroblasts to myofibroblasts and the increased production of collagen and anti-apoptotic mediators (e.g., BCL-2; Zhou et al. 2013). This results in a feed-forward loop, since the increasing stiffness of the matrix in scared lung tissue and fibroblast foci promotes, at least in part, further myofibroblast contraction, differentiation and survival via the GTPase Rho A/Rho-associated kinase (ROCK) and also an integrin-mediated release of active TGF-β1 from extracellular sources (Hinz 2012). Another aspect of this feed-forward loop is a progressive shrinkage of tissue caused by the contraction of the myofibroblasts. Of interest is the finding that mechanical stress in concert with TGF-β1 signalling is important for the differentiation of myofibroblasts from fibroblasts (Hinz 2012). Whereas the mechanical properties of the normal lung parenchyma, e.g., low stiffness and a normal shear modulus of tissue, inhibit fibroblast activation, increasing stiffness, for example, via the down-regulation of cyclooxygenase (COX) 2 and prostaglandin E_2 (Liu et al. 2010) or increased signalling of the Hippo-pathway, leads to fibroblast stimulation and pro-fibrotic remodelling (Liu et al. 2015; Saito and Nagase 2015).

Further mechanisms that are involved in the misdirected epithelial-mesenchymal cross-talk and that are similar to pathways activated during organogenesis of the lung have been revealed, such as the Wnt/β-catenin signalling pathway (Königshoff and Eickelberg 2010) resulting in an elevated expression of Wnt target genes (Königshoff et al. 2008). In AE2 cells, the activated Wnt/β-catenin pathway, e.g., via Wnt3a, induces the production of pro-inflammatory IL-1β and IL-6 after bleomycin challenge of mouse lung (Aumiller et al. 2013). Prolonged overexpression of IL-1β by epithelial cells for 7–10 days by using adenoviral-mediated gene-transfer has been linked to fibrotic remodelling, most likely via excessive production of other pro-fibrotic factors including TGF-β1 and platelet-derived growth factor (PDGF; Kolb et al. 2001). Moreover, the Wnt/β-catenin signalling cascade promotes the proliferation of AE2 cells followed by hyperplasia and the epithelial-mesenchymal transition and mediates the deposition of ECM by fibroblasts via Wnt1-induceable signalling protein-1 (WISP1; Königshoff et al. 2009). However, other factors produced by challenged AE2 cells also mediate fibroblast activation. For example, selective deletion of connective-tissue-derived growth factor (CTGF) or collagen I in alveolar epithelial cells results in the attenuation of bleomycin-induced lung fibrosis in mice (Yang et al. 2013, 2014). AE2 cells are also known to be involved, in collaboration with interstitial cells, in the turnover of components of the ECM (Leuenberger et al. 2012). Yang and co-workers (2013) showed that murine AE2 cells after injury produce excessive collagen type 1, which is involved in the resolution of inflammation but also, via the collagen receptor discoidin domain receptor (DDR2), capable of directly activating fibroblasts leading to excessive production of matrix proteins. These and other mechanisms might explain the co-localisation of denuded basal laminae, the hyperplasia and hypertrophy of AE2 cells and the deposition of ECM in animal models and IPF samples (Birkelbach et al. 2015; Knudsen et al. 2015).

Other important issues are the injury-induced exudates and activation of the coagulation cascade within the alveolar space resulting in the formation of a provisory matrix necessary for wound healing (Ruppert et al. 2008). The latter is then invaded

by fibroblasts and myofibroblasts, which, because of their contractile properties, cause tissue shrinkage and the organised intra-alveolar connective tissue is re-epithelialised and incorporated into the septal walls (Basset et al. 1986; Kuhn et al. 1989; Kuhn and McDonald 1991). Hence, previous studies in animal models have demonstrated decreased fibrosis after bleomycin-induced injury as a result of the inhibition of components of coagulation cascade (e.g., thrombin; Howell et al. 2001). However, clinical trials involving the use of anticoagulation with Warfarin have not shown a benefit for IPF patients (Noth et al. 2012). Nevertheless, the invasion of fibroblasts into the provisory matrix located in the alveolar space means that fibroblasts have to overcome the basal lamina and other matrix components, e.g., by the activation of proteases involved in the degradation of matrix proteins, allowing the fibroblasts to generate gaps through which they can invade (Li et al. 2011). In this regard, Li and co-workers assigned fibroblast-produced hyaluronan and its receptor CD44 a critical role for promoting an invasive fibroblast phenotype in the context of bleomycin-induced fibrosis and human IPF (Li et al. 2011; Pardo et al. 2016). In addition, Ahluwalia and co-workers (2016) identified several factors found in the broncho-alveolar lavage fluid taken 7 days after bleomycin challenge and from IPF patients; these factors can mediate the development of an invasive phenotype of fibroblasts enabling them to traverse through a matrigel ex vivo (Ahluwalia et al. 2016) and include PDGF-BB, LPA, epithelial growth factor (EGF) and fibroblast growth factor 2 (FGF-2). The knockdown of their corresponding receptors might consequently attenuate the invasive phenotype of the fibroblasts (Ahluwalia et al. 2016). Diverse cell types might be involved in producing these factors but, in principle, AE2 cells are capable of such production and have been shown to be a major source and therefore an important regulator of fibroblast/myofibroblast function (Selman and Pardo 2014).

However, the mechanisms involved in regulating AE2 cell functions are not entirely clear, with other cell types possibly also being involved. In this context, further key players interacting with alveolar epithelial cells and fibroblasts have been identified in the so-called pulmonary capillary vascular niche consisting of endothelial cells and perivascular macrophages directing the response upon injury either towards regeneration or fibrosis of the lung (Cao et al. 2016). Cao and co-workers compared the behaviour of the pulmonary vascular niche cells following single and repetitive injuries of the blood-gas barrier in the lung with bleomycin or hydrochloric acid. Of note, in this study, a single bleomycin instillation was associated with a complete recovery of oxygenation and hydroxyproline content at day 35 but this was not the case after repetitive bleomycin instillations mimicking the time course and current pathophysiological concept of IPF in a realistic manner (Cao et al. 2016; Degryse et al. 2010). Mechanistic studies performed by Cao and co-workers revealed that, after a single injury to the alveolar epithelium, pulmonary capillary endothelial cells promote, via chemokine receptor CXCR7, a regeneration of alveolar epithelial cells including their proliferation and repopulation of denuded basal lamina. Chronic injury by repetitive instillation of bleomycin or hydrochloric acid (e.g., 6 times), however, results in the downregulation of the chemokine receptor CXCR7 with hampered proliferation/function of AE2 cells, on the one hand and the recruitment of vascular endothelial growth factor receptor 1 (VEGFR1)-positive perivascular macrophages, on the other hand. These macrophages via the pulmonary capillary endothelial cells and Wnt/β-catenin pathway activate stromal fibroblasts, thereby generating fibrotic repair mechanisms (Cao et al. 2016). The presented data are in agreement with findings that pulmonary resident stromal fibroblasts are an important source of effector cells with regard to fibrotic remodelling in the lung, whereas other sources, such as epithelial-mesenchymal transition or differentiation from pericytes, appear not to be dominant (Rock et al. 2011).

Mechanical stress, alveolar collapse and collapse induration

A major feature of IPF is the distribution of the lesions in the lung; lesions emerge in basal and subpleural regions and ascend to upper apical regions of the lung with disease progression. Hence, the apical to basal gradient of pathological lesions, such as honeycombing and the volume loss as revealed by HRCT are critical for diagnosis (Raghu et al. 2011). From a functional point of view, the lesions predominantly occur in regions of the lung in which volume changes during respiration are maximal and in which the smallest alveoli are located (Leslie 2012). In other words, the local strain operating on lung parenchyma in these regions is the largest within the lung. Taking these functional-anatomical aspects into consideration, Carloni and co-workers (2013) used a mathematical model with the goal of simulating the distribution of mechanical stresses within the lung parenchyma. This modelling predicted the highest mechanical stresses in the basal and subpleural regions of the lung coinciding with the typical distribution pattern of pathological alterations seen by HRCT (Carloni et al. 2013). The relevance of mechanical stresses for disease progression is well known in acute lung injury in which mechanical ventilation with associated atelectrauma (e.g., recruitment and derecruitment of distal airspaces during each respiratory cycle) and volutrauma (dynamic stress attributable to overdistension of the open lung) contribute to disease progression (Bilek et al. 2003; Mead et al. 1970; Nieman et al. 2015; Slutsky and Ranieri 2013). Mechanical stress in the context of acute lung injury and ventilator-induced lung injury has been shown to activate TGF-β1 and a pro-fibrotic

response, with mechanisms such as epithelial-mesenchymal transition being described to occur (Cabrera-Benítez et al. 2012; Heise et al. 2011). However, in spontaneously breathing animals suffering from high inspiratory pressure gradients operating on the lung parenchyma and induced either by increased resistance of a partially ligated trachea or by high surface tension, mechanical stress has also been shown to be sufficient to induce acute lung injury in vivo (Ikegami et al. 2005; Mascheroni et al. 1988; Toumpanakis et al. 2010). These findings suggest that mechanical stress in spontaneously breathing IPF patients might also play an important role in the pathogenesis of pulmonary remodelling. The relevance of mechanical stress for the generation of a pro-fibrotic milieu within the lung parenchyma was recently highlighted by an elegant *ex vivo* experimental setup (Froese et al. 2016). Lung tissue strips with defined dimensions from an animal model of lung fibrosis and surgical lung biopsies of IPF patients were subjected to cyclic mechanical stresses by using forces in a range that are also likely to occur in the IPF lung during normal breathing. As a result, the fibrotic lung tissue but not the normal controls, demonstrated an increased release of active TGF-β1 and the activation of the Smad2/3 pathway following phosphorylation; this increase was correlated with stiffness of the lung tissue as measured by Young's module, a measure indicating the force per area (Pascal) for a defined strain. Moreover, use of either inhibitors of the mechanosensitive Rho/kinase pathway (so-called ROCK inhibitors) or of αv integrin but not of matrix metalloproteinases, led to an attenuation of the release of active TGF-β1 from endogenous pools, indicating that mechanotransduction is involved in generating an pro-fibrotic milieu in a fibrotic lung upon mechanical stress (Froese et al. 2016). Hence, TGF-β1 release is a function of lung stiffness and matrix mechanical properties: latent TGF-β1 complex is bound to collagen fibrils and, via αv integrin, is connected to the actin-myosin filaments of contractile myofibroblasts so that a stiff matrix, in concert with myofibroblasts, are capable to release active TGF-β1 by means of mechanotransduction upon stretch (Hinz and Suki 2016). These data provide evidence for mechanical stress during spontaneous breathing as being an additional aggravating factor in fibrotic remodelling in diseased lungs but not in healthy lungs supporting the concept that several hits in concert are involved in the pathogenies of IPF. The relevance of mechanical stress as a pro-fibrotic stimulus and as a regulator of a variety of processes including cytoskeletal structure, cell adhesion and ECM has been pointed out recently. In the context of acute lung injury and mechanical ventilation and independently of the release of active TGF-β1 from extracellular stores, the NOX1 – Midkine – Notch2 signalling pathway has been shown to contribute to fibrotic remodelling in response to stretch and mechanical stress (Zhang et al. 2015). In this regard, studies of the bleomycin model have suggested that, after reaching a critical mass of stiff tissue, the remodelling might be perpetuated because of a positive forward loop of mechanical stress and pro-fibrotic signalling (Froese et al. 2016; Liu et al. 2010). The idea that mechanical stress is involved in the pathogenesis of IPF is definitely reasonable bearing in mind the distribution of the pathology within the lung, the damage occurring predominantly in areas in which mechanical stresses are the highest (Carloni et al. 2013). However, the question remains: what is the initial event increasing lung stiffness and thereby inducing such a positive feed-forward mechanism of progressive fibrosis? In the rodent model of TGF-β1 in which an adenoviral vector for intra-tracheal instillation and gene-transfer of active porcine TGF-β1 is used, pro-fibrotic remodelling is known predominantly to occur after the expression of porcine TGF-β1 has declined and the endogenous active TGF-β1 is increased (Ask et al. 2008; Knippenberg et al. 2015; Lopez-Rodriguez et al. 2016a; Sime et al. 1997). Moreover, during the expression of porcine TGF-β1 by epithelial cells, which are the main target of the intratracheal instillation of the adenoviral vector and the resulting increased levels within alveolar space, a high proportion of alveoli collapse, because of high surface tension and surfactant dysfunction can be found with no signs of fibrosis (Lopez-Rodriguez et al. 2016a). At least at the organ scale, alveolar collapse is known to be linked to an increase in lung stiffness as measured by invasive pulmonary function tests (Lutz et al. 2015; Smith et al. 2013). Based on the Bachofen-Wilson model of acinar micromechanics, alveolar collapse, for example, caused by high surface tension, leads to a considerable stretch of the fibre network consisting of elastin and collagen fibrils (Wilson and Bachofen 1982) and such a stretch of collagen fibrils has been suggested to be involved in the release of active TGF-β1 by means of mechano-transduction, in the fibrotic lung (Hinz and Suki 2016). Hence, we can speculate that alveolar-collapse-related tissue stiffness and the stretching of ECM components might be sufficient to generate a pro-fibrotic milieu in the lung. In IPF samples, alveolar collapse has recently been found in areas with only a little fibrotic remodelling and is suggested to be a trigger for disease progression including fibroproliferation (Mai et al. 2016).

The establishment of structure-function relationships in animal models of lung injury and fibrosis demonstrates that the degradation of lung function happens before the fibroproliferative process starts; this is particularly true in the bleomycin model in which the number of open alveoli is the structural parameter best correlated with static compliance during an observational period of 14 days (Lutz et al. 2015). Hence, alveolar collapse is a structural mechanism increasing lung stiffness and, based on a modelling approach published by Mead et al. in 1970, alveolar collapse in its function as a stress concentrator might be the reason for potentially harmful mechanical stress in the lung (Mead et al. 1970) and therefore for the release of endogenously

active TGF-β1, as seems to be the case in the animal model of the adenoviral-mediated transfer of active porcine TGF-β1. In human IPF, mechanical stress probably starts to be of relevance in the pathogenesis very early during disease development. Recently, inspiratory "Velcro crackles" heard during auscultation of the basal parts of the lung have been pointed out to be early findings in IPF patients predating the occurrence of visible pathological alterations in conventional chest X-ray evaluations (Cottin and Cordier 2012). Based on a modelling approach, inspiratory Velcro crackles are suggested to originate from the energy-rich explosive reopening of distal airspaces (Vyshedskiy et al. 2009) and this in turn can impose potentially harmful stresses on lung parenchyma by recruitment/derecruitment of distal airspaces, on the one hand and heterogeneous ventilation, on the other hand (Bilek et al. 2003; Mead et al. 1970). The instability of distal airspaces, however, implies high surface tensions and surfactant dysfunction in IPF lungs, an issue that has indeed been shown to be the case in IPF (Günther et al. 1999). In essence, the IPF lung is exposed to high surface tension and alveolar instability. However, high surface tension leads not only to alveolar instability but also to a process referred to as hydrodynamic stress at the air-liquid interface at which alveolar fluid oscillates during the respiratory cycle resulting in shear stress at the apical membrane of AE2 cells (Hobi et al. 2012; Ravasio et al. 2011). In vitro experiments have linked such hydrodynamic stress to severe dysfunctions of AE2 cells, which finally are subject to apoptosis (Hobi et al. 2012). Therefore, an initial dysfunction of AE2 cells, for example, because of aging, genetic susceptibility or smoking, with increased surface tension might result in the most severe mechanical stress in the basal and subpleural regions characterised by the recruitment/derecruitment of distal airspaces, heterogeneous ventilation and hydrodynamic stress aggravating injury by means of inducing AE2 cell apoptosis and the release of active TGF-β1, all culminating in a vicious circle with disease progression over time (Fig. 6). Of note, the primary effect

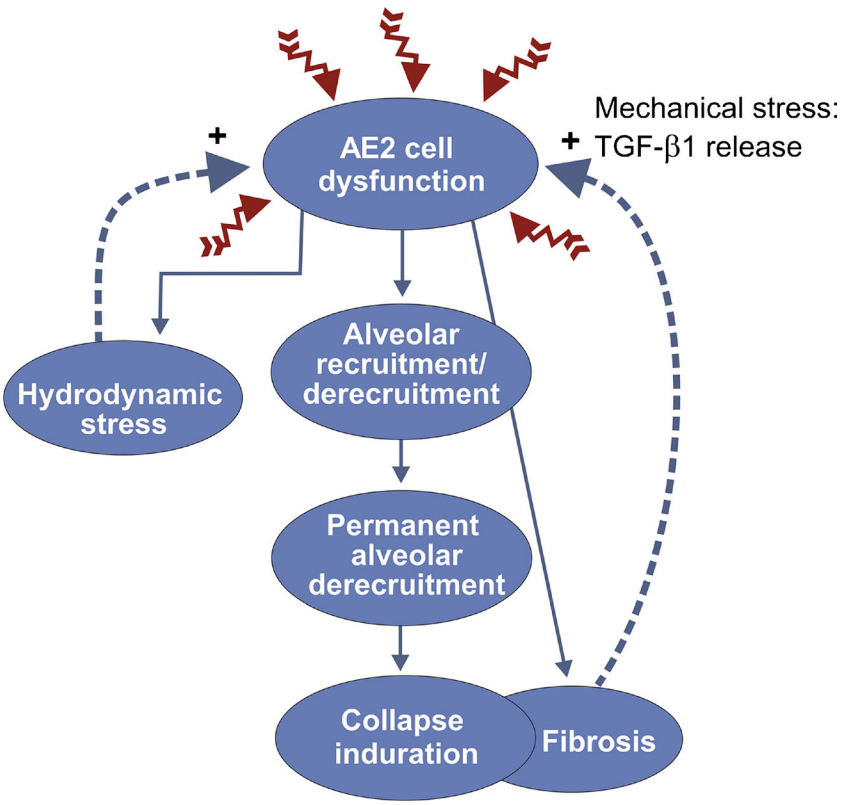

Fig. 6 Mechanical stress and disease progression; effects on AE2 cells. A model of the way that AE2 cell dysfunction and mechanical stress can result in a vicious circle of disease progression. Repetitive injury of AE2 cells of unknown origin results in fibrotic remodelling and surfactant dysfunction. Surfactant dysfunction leads to alveolar instability and oscillation of the hypophase during breathing. Oscillation of the hypophase in the presence of high surface tension has been shown to result in hydrodynamic stress and further AE2 cell dysfunction in vitro. Collapse induration and pulmonary fibrosis result in heterogeneous ventilation, stress concentrators and a stiff matrix. These factors in concert cause the release of TGF-β1 as a response to cyclic stretch. TGF-β1 leads not only to fibrotic remodelling but also to the down-regulation of surfactant proteins and therefore aggravates AE2 cell dysfunction and disease progression. Mechanical stress might explain the occurrence of IPF predominantly in basal and subpleural areas, since here the volume changes during breathing are maximal. Here too the alveoli are the smallest and thus, according to the law of Laplace, are prone to high-surface-tension-induced collapse in the presence of surfactant dysfunction

of active TGF-β1 on lung structure, if produced after gene-transfer by using an adenoviral vector for intratracheal instillation, is alveolar collapse attributable to surfactant dysfunction and not to the activation of fibroblasts and the excessive production of collagen fibrils within septal walls; these effects can be found with some delay, meaning that mechanical stress predates fibrotic remodelling (Lopez-Rodriguez et al. 2016a). This reasoning of the interaction of alveolar instability and lung injury in the context of the chronic disease IPF leads to the pathogenetic concept of collapse induration, which has probably been underestimated in the past (Burkhardt 1989; Crouch 1990; Gibson and Pride 1977; Leslie 2012; Todd et al. 2015). Moreover, previous findings in animal models provide a link between dysfunctional surfactant with high surface tension and the spontaneous development of lung fibrosis (Mahavadi et al. 2010; Zhang et al. 2012). Using the bleomycin model of acute lung injury and fibrosis, we have recently shown that surfactant dysfunction and alveolar collapse at low lung volumes represent a very early event occurring as soon as 1 day after challenge in the absence of oedema formation at the light microscopic level: approximately one third of alveoli are subject to alveolar derecruitment below airway opening pressures of 10 cmH_2O (Knudsen et al. 2016; Lutz et al. 2015). Although, during the early stage, alveoli can be recruited with increasing end-expiratory positive airway pressure (PEEP), this is no longer possible at later timepoints. Hence, alveolar micromechanics in progressive disease are characterised by initial intratidal alveolar recruitment/derecruitment, permanent derecruitment and, finally, collapse induration. Collapse induration is characterised by collapsed alveoli with denuded epithelial basal lamina being piled up and embedded in interstitial fibrotic tissue; the former alveolar entrances are then overgrown by hyperplastic alveolar epithelial cells (Burkhardt 1989; Lazenby et al. 1990; Lutz et al. 2015). Similar findings have also been observed in samples characterised by a UIP or acute interstitial pneumonia (AIP) pattern (Katzenstein 1985; Kuhn and McDonald 1991; Myers and Katzenstein 1988); an example from an explanted IPF lung is given in Fig. 5. The term "collapse induration" was introduced into the English literature by Burkhardt (1989). It refers to the German term "Kollapsinduration", which had been used in pathology textbooks for a long time (e.g., Ziegler 1881).

Many drugs addressing the fibrotic response by, for example, attenuating the activation of fibroblasts/myofibroblasts and the deposition of ECM have been demonstrated to be efficient in animal models of lung injury of fibrosis but most of these treatments have failed to prove efficacious in clinical trials of IPF (Ahluwalia et al. 2014). As an exception, pirfenidone and nintedanib have recently been shown to decelerate the decline in lung function in IPF patients (King et al. 2014; Raghu et al. 2016; Richeldi et al. 2014), although the degradation of lung function with time remains very high compared with that of healthy controls evaluated in a former study (Fletcher and Peto 1977). Whereas the mechanism of action for nintendanib is known, as it is an unselective tyrosine kinase inhibitor and develops anti-fibrotic properties via the receptors of PDGF, VEGF and FGF (Ahluwalia et al. 2016; Wollin et al. 2014), that for pirfenidone is less clear. Animal studies have shown an associated decrease in pro-fibrotic factors including active TGF-β1 and others (Myllärniemi and Kaarteenaho 2015); however, an upregulation of biophysically active surfactant proteins resulting in a reduction in surface tension has also been demonstrated, indicating beneficial effects of this drug on AE2 cell function (Lopez-Rodriguez et al. 2016b). With regard to the effects of nintenanib on AE2 dysfunction, little is known so far.

In an attempt to regenerate AE2 cells and their function as defenders of the alveoli, cell-based therapies might be an attractive approach in the future. After acute lung injury in mice, alveolar epithelial cells and endothelial cells have been demonstrated to be regenerated from bone-marrow-derived progenitor cells (Yamada et al. 2004). In the bleomycin model, systemic treatment with mesenchymal stem cells has been shown to be an efficient strategy to prevent lung injury and fibrotic remodelling in mice and experimental evidence has been provided that these cells also differentiate to some extent into AE2 cells (Ortiz et al. 2003).

Concluding remarks

In summary, IPF is a disease with complex effects on pulmonary structure and function. Whereas the role of the injury of alveolar epithelial cells as a trigger for interstitial and intra-alveolar fibrotic remodelling including the activation of fibroblasts and myofibroblasts is well established, the relevance of alveolar collapse and collapse induration for the degradation of lung function might have been underestimated so far. Increasing evidence supports the role of mechanical stress as an important trigger for progressive remodelling processes in IPF and animal models. Hypothetically, alveolar collapse might act as an additional stress concentrator resulting in harmful mechanical stresses in the lung contributing to fibrosis in the context of collapse induration. Therefore, treatments aimed at a reduction of surface tension and the stabilisation of distal airspaces might represent important additional therapeutic strategies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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