When things go wrong: exploring possible mechanisms driving the progressive fibrosis phenotype in interstitial lung diseases

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Shareable abstract (@ERSpublications)
ILDs of different aetiologies, which have the potential for improvement or stabilisation, may develop progressive pulmonary fibrosis (PF-ILD). Mechanisms underlying progression, including (epi)genetics, ageing and structural pattern, are proposed in this review. https://bit.ly/2NSJPXQ

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Abstract
Interstitial lung diseases (ILDs) comprise a large and heterogeneous group of disorders of known and unknown aetiology characterised by diffuse damage of the lung parenchyma. In recent years it has become evident that patients with different types of ILD are at risk of developing progressive pulmonary fibrosis, known as progressive fibrosing ILD (PF-ILD). This is a phenotype that behaves similar to idiopathic pulmonary fibrosis, the archetypical example of progressive fibrosis. PF-ILD is not a distinct clinical entity but describes a group of ILDs with similar clinical behaviour. This phenotype may occur in diseases displaying distinct aetiologies and different biopathology during their initiation and development. Importantly, these entities may have the potential for improvement or stabilisation prior to entering the progressive fibrosing phase. The crucial questions are: 1) why does a subset of patients develop a progressive and irreversible fibrotic phenotype even with appropriate treatment? and 2) what are the possible pathogenic mechanisms driving progression? Here, we provide a framework highlighting putative mechanisms underlying progression, including genetic susceptibility, ageing, epigenetics, structural fibrotic distortion, aberrant composition and stiffness of the extracellular matrix, and the emergence of distinct pro-fibrotic cell subsets. Understanding the cellular and molecular mechanisms behind PF-ILD will provide the basis for identifying risk factors and appropriate therapeutic strategies.

Introduction
In recent years it has become evident that in addition to idiopathic pulmonary fibrosis (IPF), the archetypical example of progressive pulmonary fibrosis, there are several other interstitial lung diseases (ILDs) that may develop a progressive fibrosing phenotype. This phenotype is known as progressive fibrosing ILD (PF-ILD), which importantly shows clinical and functional features of progression similar to IPF. In this context, PF-ILD is characterised by a progressive decline in lung function, increasing extent of fibrosis on high-resolution computed tomography (HRCT) and worsening of symptoms resulting in early mortality [1–4].

It is important to emphasise that PF-ILD is not a clinical entity but describes a clinical behaviour/phenotype that may occur in patients with ILD of several aetiologies. It is more frequently seen in hypersensitivity pneumonitis, autoimmune diseases such as rheumatoid arthritis and systemic sclerosis, idiopathic nonspecific interstitial pneumonia (NSIP), and unclassifiable ILD, whereas it seems to be uncommon in others such as lymphoid interstitial pneumonia or organising pneumonia [1–4]. The finding that some non-IPF ILDs may develop a progressive phenotype that behaves similar to IPF opened the possibility of using antifibrotic agents that are widely used in the management of IPF for non-IPF patients. Thus, both nintedanib and pirfenidone, which were developed primarily for IPF, have been recently...
explored in other ILDs with a progressive fibrotic phenotype. The positive impact of these drugs on non-IPF pulmonary fibrosis suggests that these diseases may share some common fibrogenic mechanisms [5–7].

Therefore, the crucial question to be addressed is why a subset of patients suffering from diseases that, in principle, have the potential for improvement or stabilisation develop a progressive and irreversible fibrotic phenotype even with appropriate (usually immunosuppressive) treatment.

In this review, we provide a framework discussing some mechanisms that are well established in IPF that could be involved in the pathogenesis of this aggressive behaviour in non-IPF ILD. For this purpose, we first briefly discuss the initiation and development of fibrosis in two inflammatory ILDs, *i.e.* hypersensitivity pneumonitis and rheumatoid arthritis (RA-ILD), that often may evolve to a progressive phenotype, and IPF, a typical progressive fibrosis with limited inflammation. We then explore the putative mechanisms that may contribute to the development of the progressive phenotype, focusing on the genetic architecture and transcriptomic signatures, environmental risk factors (primarily smoking), the role of the usual interstitial pneumonia (UIP) pattern, ageing-associated processes, the critical role of extracellular matrix (ECM) stiffness, and the emergence of active distinct pro-fibrotic subsets of epithelial cells, fibroblasts and macrophages.

### Initiation of an ILD and development of fibrosis

#### Inflammation-driven fibrosis

**Hypersensitivity pneumonitis**

Hypersensitivity pneumonitis is an immunologically mediated lung disease resulting from exposure to a wide variety of inhaled environmental antigens in a genetically predisposed individual. The conjunction of these (and other unknown) factors results in bronchioloalveolar inflammation characterised primarily by the infiltration of immune cells, mainly T-lymphocytes [8–10].

Then, persistent exposure to the offending antigen, ageing, cigarette smoking, development of autoimmune features, T-helper type 1 to type 2 switch and decrease of γδ T-cells, among others, contribute to the development of fibrosis. Immune and inflammatory cells release pro-fibrotic factors promoting fibroblast activation and the fibrotic response [8–10].

**Rheumatoid arthritis**

RA is a chronic autoimmune disease characterised primarily by synovial inflammation, but may affect many tissues including the lungs. Both genetic and environmental factors contribute to the development of autoimmunity, which is associated with the presence of pathogenic autoantigens [11, 12]. Clinically significant ILD occurs in ~10% of patients, but an additional one-third demonstrate subclinical ILD on chest HRCT [12]. RA presents a myriad of pulmonary manifestations aside from ILD, but RA-ILD shows some similarities with IPF [13].

The histopathological patterns are diverse, and (mainly) UIP and NSIP patterns are observed. In general, lungs show interstitial infiltration with lymphocytes and peribronchial/peribronchiolar lymphoid aggregates with a range of sizes and degrees of organisation [14, 15].

Similar to hypersensitivity pneumonitis, stimulated by the profound dysregulation of the immune system, T-lymphocytes and inflammatory cells release pro-fibrotic cytokines, chemokines and growth factors that promote fibroblast proliferation and differentiation to myofibroblasts, initiating a complex cross-talk between inflammatory and tissue-remodelling pathways [14, 15].

#### Epithelial-driven fibrosis

**Idiopathic pulmonary fibrosis**

A growing body of evidence supports the notion that IPF represents an epithelial-driven disorder that results from a complex interplay of genetic and environmental risk factors, ageing-associated processes, and a pro-fibrotic, partially stochastic, epigenetic drift [16, 17]. In IPF, lung epithelial cells undergo aberrant phenotypic and functional changes, and this “reprogramming” initiates a progressive and multistep process that involves fibroblast activation, ECM remodelling and, finally, the end-stage fibrosis [16].

Unlike inflammation-driven fibrosis, which starts as a chronic inflammatory process that does not resolve, IPF is from the outset a fibrotic disorder driven by the aberrant activation of the lung epithelium, but both biopathological processes trigger a similar fibrotic core signalling pathway (figure 1).
From pulmonary fibrosis to progressive pulmonary fibrosis

The molecular mechanisms underlying the persistence and progression of pulmonary fibrosis remain elusive.

In the following, we raise the hypothesis that some distinct cellular, molecular and structural mechanisms, and some potential interactions, are shared by IPF, per se PF-ILD, and by hypersensitivity pneumonitis and RA-ILD, contributing to the development of this aggressive phenotype (table 1).

Genetic architecture

An excess of rare variants in genes linked to familial and sporadic IPF has also been reported in RA-ILD. Thus, an increased frequency of mutations in telomere maintenance genes (TERT, PARN and RETL1) and SFTPC, involved in surfactant homeostasis, has been also observed in patients with this disease compared with controls [18, 19].

Similarly, mutations in telomere-associated genes and abnormal shortening of telomeres have been detected in families with different ILDs, in which some of the affected individuals had chronic hypersensitivity pneumonitis [20]. Moreover, a substantial proportion of patients diagnosed with sporadic hypersensitivity pneumonitis have rare, protein-altering variants in telomere-related genes, which are associated with short peripheral blood and lung telomere length [21, 22].

Taken together, these findings indicate that aberrant shortening of telomeres not only participates in the development of pulmonary fibrosis but the severity of telomere attrition also contributes to the PF-ILD phenotype in these two diseases. Supporting this notion, hypersensitivity pneumonitis patients with mutations in telomere-related genes and short peripheral blood telomere length display significantly...
reduced transplant-free survival [21]. In addition, patients with interstitial pneumonia with autoimmune features and leukocyte telomere length <10th percentile had a marked decline of lung function compared with those with leukocyte telomere length ≥10th percentile [23]. Likewise, patients with non-IPF showed the same mortality rate as patients with IPF when mutations in telomere-related genes and, consequently, severe telomere attrition were present [20].

The functional MUC5B rs35705950 promoter variant, which is a major genetic risk factor for IPF, was recently identified as a risk factor for RA with ILD, whereas it was not linked to RA without ILD [24]. MUC5B rs35705950 has also been associated with the extent of fibrosis, histopathological features of the UIP pattern and reduced survival in patients with chronic hypersensitivity pneumonitis [25]. These findings suggest that although phenotypically distinct and with different specific disease initiation factors, these diseases that may evolve to PF-ILD share some common genetic risk factors with IPF.

A question that persists is: is there a (yet) unknown “progressive fibrosis” gene variant(s) or are there some protective gene variants in those that do not evolve to a progressive phenotype?

**Transcriptomic signatures/gene and protein expression**

Independently of the aetiology, different progressive ILDs share some pro-fibrotic factors. For example, it was shown that concentrations of platelet-derived growth factor (PDGF)-AA, PDGF-BB, fibroblast growth factor (FGF)-2, vascular endothelial growth factor and macrophage colony-stimulating factor (M-CSF) were all increased in whole-lung homogenates from patients with PF-ILD of different aetiologies, including IPF, some connective tissue disease-associated ILD, sarcoidosis and exposure related-ILD, compared with healthy donor lungs [26]. It has also been recently reported that transcriptional signatures of IPF and chronic hypersensitivity pneumonitis share the upregulation of numerous genes and pathways related, among others, with collagen catabolism, collagen fibril organisation and epithelial development (this latter associated with reduced lung function in hypersensitivity pneumonitis) [27].

Taken together, these findings suggest that ILDs with a progressive fibrotic phenotype often share a similar core pro-fibrotic pathobiology with IPF (figure 1).

**Environmental linkage**

Long-term cigarette smoking is associated with IPF and RA-ILD, and although hypersensitivity pneumonitis is less frequent in smokers, progression to fibrosis is strongly linked to tobacco smoking [8–

### TABLE 1 Mechanisms and processes identified in patients with the progressive fibrosing interstitial lung disease phenotype

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Linking mechanism</th>
<th>Effect</th>
</tr>
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<tbody>
<tr>
<td>Genetic susceptibility</td>
<td>Common and rare variants (e.g. TERT, PARN and RETL1)</td>
<td>Aberrant shortening of telomeres</td>
</tr>
<tr>
<td>Transcriptional signature</td>
<td>Excessive expression of some growth factors (e.g. platelet-derived growth factor)</td>
<td>Fibroblast migration and activation</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>Numerous damaging molecules</td>
<td>Epithelial injury; epigenetic reprogramming</td>
</tr>
<tr>
<td>UIP pattern</td>
<td>Peculiar patchy dense fibrosis causing remodelling of lung architecture</td>
<td>Architectural distortion; induction of pro-fibrotic pathways (e.g. WNT signalling)</td>
</tr>
<tr>
<td>Epithelial senescence</td>
<td>Senescence-associated secretory phenotype</td>
<td>Induction of several pro-fibrotic pathways</td>
</tr>
<tr>
<td>Arrival of clusters of distinct highly active epithelial cells</td>
<td>KRT5-/KRT17 epithelial cells expressing collagen type I α1 chain, fibronectin 1 and other ECM components</td>
<td>Contribute to ECM accumulation</td>
</tr>
<tr>
<td>Fibroblast resistance to apoptosis</td>
<td>Reduction of pro-apoptotic mechanisms (p14ARF and Fas)</td>
<td>Persistence of myofibroblasts</td>
</tr>
<tr>
<td>Epigenetic reprogramming</td>
<td>Stochastic changes in DNA methylation; dysregulation of noncoding RNA</td>
<td>Cell senescence; ECM–fibroblast auto-loop with permanent activation</td>
</tr>
<tr>
<td>ECM stiffness</td>
<td>Lysyl oxidase-induced increase of cross-linking</td>
<td>Increased expression of transforming growth factor-β and others resulting in a pro-fibrotic loop</td>
</tr>
<tr>
<td>Nonresolved chronic inflammation</td>
<td>Persistent activation of immune and inflammatory cells</td>
<td>Unremitting fibroblast migration/proliferation/activation</td>
</tr>
<tr>
<td>Arrival of clusters of highly active fibroblasts</td>
<td>HAS1fibroblasts; CTHRC1 fibroblasts</td>
<td>Critical for ECM accumulation</td>
</tr>
<tr>
<td>Arrival of clusters pro-fibrotic macrophages</td>
<td>M-CSF/M-CSF receptor loop</td>
<td>Drive permanent fibroblast activation</td>
</tr>
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</table>

UIP: usual interstitial pneumonia; ECM: extracellular matrix; M-CSF: macrophage colony-stimulating factor.
Nicotine and other components of tobacco smoke provoke epithelial and endothelial cell damage, and enhance transforming growth factor (TGF)-β activity, thus contributing to fibrotic activity [28]. Chronic exposure to cigarette smoke also induces numerous alterations in DNA methylation, influencing gene transcription and, as a consequence, affecting many biological processes that may enhance a fibrotic response [29, 30]. For example, chronic exposure to cigarette smoke silences E-cadherin by hypermethylation, inducing epithelial to mesenchymal transition [31]. However, whether the smoking risk factor enhances the development of fibrosis and/or the progressive phenotype is unclear.

The UIP pattern

The UIP pattern is characteristic of IPF, but it is also observed in RA-ILD and hypersensitivity pneumonitis, and in these diseases is often associated with progression. Moreover, CT honeycombing has been shown to be prevalent in several ILDs and identifies a progressive fibrotic phenotype associated with increased mortality rates [32]. Notably, in RA-ILD the UIP pattern is more frequent in older males with a history of smoking and confers a poorer prognosis, paralleling IPF [15].

The reason why the UIP pattern confers a progressive phenotype compared with other fibrotic patterns in ILD is unclear. It may be related to its destructive characteristic; honeycombing and traction bronchiectasis/bronchiolectasis provoke strong architectural distortion and are associated with rapid functional decline and early mortality [33]. Likewise, combined pulmonary fibrosis and emphysema (CPFE) occurs in a subgroup of ILD patients of different aetiologies, primarily in smokers that develop a UIP pattern, and worsens the prognosis, accelerating progression [34, 35].

However, the UIP pattern may also trigger or sustain some distinct pathobiology pathways. Of note, regardless of the underlying aetiology (IPF or autoimmune diseases), UIP exhibits epithelial senescence in remodelled areas as well as high expression of all markers of autophagy [36]. Moreover, a recent study that included 169 IPF patients and 57 non-IPF patients from different aetiologies, all of them with the UIP pattern, demonstrated that molecular markers of telomere dysfunction and epithelial senescence are expressed in both groups. Diagnosis of non-IPF UIP was supported by the presence of lymphocytic infiltration, noncaseating granulomas, airway-centred inflammation or small airways disease [37].

Likewise, high expression of WNT5a has been reported in extracellular vesicles of IPF lung fluids and increased plasma levels of WNT5a (presumably from lung origin) were recently also found in patients with RA-ILD, primarily in patients with the UIP pattern compared with those with the NSIP and other ILD patterns [38, 39]. Finally, exaggerated enzymatic activity, mainly associated with matrix metalloproteinases (MMPs), may play a role in the biopathology of CPFE and may contribute to progression [40].

The ageing connection

Ageing is a driving force associated with IPF, and strongly contributes to the development and disease progression of other ILDs such as chronic hypersensitivity pneumonitis and RA-ILD through several interrelated mechanisms [8, 15]. Ageing is a complex biological process characterised by numerous alterations, including genomic instability, cellular senescence, telomere attrition, stem cell exhaustion, loss of protein homeostasis, epigenetic reprogramming, deregulated nutrient sensing, altered intercellular communication and mitochondrial dysfunction [41]. As already mentioned, at least two of these interrelated hallmarks are shared by IPF, hypersensitivity pneumonitis and RA-ILD, i.e. abnormal shortening of telomeres and cell senescence. Telomeres are specialised nucleoprotein complexes that cap the ends of chromosomes; when they reach a critically short length it may trigger apoptosis or more often cellular senescence, limiting the proliferative capacity of the cells.

Cellular senescence is characterised by a state of stable cell cycle arrest associated with the secretion of a set of cytokines, chemokines, MMPs and growth factors, known as the senescence-associated secretory phenotype (SASP), which influences the micro-environment where they are located [42].

Exaggerated epithelial cell senescence has been documented in IPF and its persistence likely contributes to progression through the secretion of multiple pro-fibrotic mediators.

Moreover, it has been recently demonstrated that senescence of type 2 alveolar epithelial cell (AECs) is sufficient to initiate progressive lung fibrosis that closely resembles pathological remodelling seen in IPF lungs [43]. Of note, transient senescence is important for key functional processes such as the transition of type 2 to type 1 AECs [44]. By contrast, persistent epithelial senescence results, through the SASP, in pathological responses including fibrosis. As already mentioned, epithelial senescence has been also
noticed in cystic remodelled areas of UIP lungs of different aetiologies, suggesting that a similar pro-fibrotic process may be operating [36, 37].

Likewise, it has been suggested that loss of plasticity (e.g. the failure to dedifferentiate) and resistance to apoptosis occur in senescent IPF myofibroblasts [45]. More importantly, resistance to apoptosis appears to be a common feature of fibrotic diseases [46–48]. However, whether this contributes to the development of non-IPF PF-ILD is uncertain.

Interestingly, it has been recently demonstrated that, through WNT signalling, epithelial senescent cells induce the myofibroblast expression of Nanog, a marker of stem cells which may play a critical role in myofibroblast activation as well as sustaining self-renewal and the anti-apoptosis phenotype [49]. This finding supports the notion of an age-associated abnormal recapitulation of developmental pathways resulting in antagonistic pleiotropy that may enhance the fibrotic response [50].

In general terms, these findings suggest that the development of epithelial senescence, likely associated with the abnormal shortening of telomeres, and fibroblast resistance to apoptosis may be two mechanisms implicated in the progressive fibrosis phenotype of ILD independent of aetiology.

Other mechanisms associated with ageing may also be operating in this process. For example, mitochondrial dysfunction, which has been revealed in IPF, has long been assumed to be related to the progression of fibrosis in many end-stage disorders [51, 52]. Mitochondrial dysfunction can lead to the release of mitochondrial DNA and permanently increased oxidative stress, which eventually exacerbates the fibrotic process.

Another important link with ageing is the partially stochastic age-associated epigenetic drift that leads to unpredictable differences in the methylome among individuals of similar age [53]. Therefore, we can at least hypothetically suggest that in some older patients with ILD, some pro-fibrotic genes may be demethylated and/or some antifibrotic genes may be hypermethylated at random, resulting in the persistence of fibrosis programmes. Likewise, persistent dysregulation of noncoding RNA may play a role in progression as exemplified by miRNA-29 (discussed in the following section) and several microRNAs that help to drive cell senescence, such as the miRNA-34 family [54].

Accumulation of stiff and disorganised ECM is a central factor in promoting disease progression

Lung tissue stiffening creates a micro-environment where changes in matrix mechanosensing may drive fibrotic disease progression. The composition and cross-linking of structural components, especially fibrillar collagens and elastin, seems to be strongly implicated in ECM stiffness. Lysyl oxidase (LOX) family members play a key role in ECM cross-linking, and at least two of them (LOXL1 and LOXL2) gene and protein levels are increased in the lungs of IPF patients [55]. Although data in other fibrotic lung diseases are scarce, increased LOX expression and collagen cross-linking might also account for stiffening in other fibrotic ILDs such as scleroderma [56, 57]. Likewise, tenascin C, which interacts with a variety of ECM molecules, may drive persistence of organ fibrosis and is markedly increased in fibrotic lungs, including in IPF, hypersensitivity pneumonitis, scleroderma/ILD and others [58–61].

The stiffened ECM stimulates myofibroblast activity through positive feedback loops, including the mechanically driven release and activation of matrix-bound latent TGF-β, which in turns activate several transcriptional pathways stimulating the expression of pro-fibrotic genes, including TGF-β1 itself, resulting in a progressive pro-fibrotic loop [62–65]. Supporting this notion, studies performed in healthy and IPF-derived lung fibroblasts seeded on healthy or IPF-derived matrices indicated that the dominant effect is matrix dependent and not cell autonomous [66]. Thus, the IPF matrix repressed miR-29 expression, which caused an increased translation of matrix proteins when fibroblasts were seeded on IPF-derived matrices, also indicating that some epigenetic reprogramming may contribute to unremitting progression [66]. These findings demonstrate that a positive feedback loop created by a stiff matrix develops a vicious cycle that ultimately perpetuates fibrosis.

Although studies on this field in other ILDs are scarce, it is highly possible that the same process may occur once the fibrosis reaches a point of no return. Actually, ECM mechanical stiffness and its dynamic change contribute to progressive fibrosis in many tissues such as the heart, liver and kidneys [67–69].

The linkage between lung epithelium, alveolar regeneration, ECM stiffness and progression

Along the same line of thought, it has been shown that impaired alveolar regeneration increases mechanical tension resulting in PF-ILD. Thus, loss of Cdc42 gene function in type 2 AECs in mice causes
periphery-to-centre progressive lung fibrosis, where sustained increased mechanical tension spatially activates a TGF-β signalling loop in type 2 AECs that is more evident in epithelial cells at the lung periphery [70]. In other words, there was a direct functional link between progressive lung fibrosis and elevated mechanical tension caused by impaired alveolar regeneration.

Single-cell RNA sequencing (scRNA-Seq) provides deep insights at the cellular level to identify the transcriptome of individual cells and allows inference of context-dependent phenotypes of individual cells to reveal the cellular diversity of complex tissues. In the case of pulmonary fibrosis, recent studies have dissociated cells and then analysed them to investigate their transcriptional signatures and behaviour, and have demonstrated that subsets of pro-fibrotic epithelial cells, fibroblasts and macrophages emerge during progression.

In the case of lung epithelium, a recent study using scRNA-Seq in transplant lungs showed that abnormal highly active epithelial cells are present not only in IPF but also in other ILDs such as chronic hypersensitivity pneumonitis and ILD associated with autoimmune disorders, and revealed a cluster of epithelial cells almost exclusively contained in fibrotic lungs which were characterised by increased expression of genes previously reported to be associated with pulmonary fibrosis [71].

Moreover, another scRNA-Seq study, examining lungs from IPF, hypersensitivity pneumonitis, NSIP, sarcoidosis and unclassifiable ILD, identified numerous distinct epithelial and mesenchymal cells genes involved in the pathological remodelling tissue in pulmonary fibrosis. The study revealed a previously undescribed population of KRT5⁺/KRT17⁺ epithelial cells that expressed collagen type I α1 chain (COL1A1), fibronectin 1 (FN1) and other pathological ECM components. Importantly, this population is conserved across a subset of histopathological patterns of pulmonary fibrosis, providing direct evidence of an epithelial role in collagen/ECM production in fibrotic lungs [72]. Of note, these cells lacked α-smooth muscle actin, PDGF receptor α, fibroblast-specific protein 1/S100A4 and other canonical markers of fibroblasts.

Taken together, these studies indicate that aberrantly activated lung epithelial cells are critical not only in IPF pathogenesis and progression but also in other fibrotic lung diseases.

An active fibroblast programme orchestrates not only initiation but also the irreversible progression of fibrosis

It is conceivable that some normal wound-healing regulatory mechanisms may operate in fibrotic processes that revert or stop and stabilise. At least theoretically, the resolution of an ongoing fibrotic response would be possible via the de-differentiation of myofibroblasts or loss by cell death signalling. In progressive fibrosis, these mechanisms are disrupted, and myofibroblasts persist and become resistant to apoptosis, remaining persistently activated [16, 17, 45, 46]. Among others, epigenetic mechanisms and resistance to apoptosis induction by the death receptor Fas have been shown to play a key role [73, 74]. Persistent myofibroblasts may continue to release large amounts of ECM components such as collagens and fibronectin, and thus contribute to progressive maladaptive remodelling. Data obtained by scRNA-Seq underscore the concept that a diversity of fibroblast phenotypes is found in fibrotic lungs. Interestingly, a specific enrichment in ACTA²⁺ myofibroblast, a PLIN2⁺ lipofibroblast-like group and a previously undescribed HAS1ha fibroblast population was identified in fibrotic lungs of different aetiologies [72]. Likewise, a comparison through scRNA-Seq between normal and fibrotic lungs identified a unique population of fibroblasts that expressed the highest levels of components of the ECM, which was marked by high levels of expression of Chhrc1 in IPF and lung fibrosis associated with scleroderma [75]. These cells were found within clusters of collagen-producing cells in fibroblastic foci and showed high migratory capacity. Moreover, these cells colonise the fibrosing lung in mice after intratracheal transfer, indicating a relevant role in driving pulmonary fibrosis [75].

Macrophages

Mechanical cues and matrix stiffness also regulate the function of macrophages. Macrophages represent a complex and heterogeneous population of cells that may be present in several states of activation as well as transitioning among diverse states at any given moment. Alterations in timely activation and inactivation of macrophages in appropriate sites in the lung probably contribute to progressive fibrosis [76].

In this context, a distinct population of pro-fibrotic alveolar macrophages was identified exclusively in patients with pulmonary fibrosis independently of aetiology [71]. Moreover, heterogeneity within macrophages was observed in most of the fibrotic lungs, with distinct clusters of cells enriched for different pro-fibrotic genes (e.g. CHI3L1 or SPP1). Recently, Joshi et al. [77] showed the recruitment of monocyte-derived alveolar macrophages to spatially restricted fibrotic niches and identified putative
mechanisms of intercellular communication within the fibrotic niche, including epithelial cells, macrophages and fibroblasts. Moreover, the findings of Joshi et al. [77] suggest that macrophages might maintain their population via an autocrine M-CSF/M-CSF receptor loop, continually driving fibroblast proliferation even in the absence of active injury.

Finally, some putative independent mechanisms (not shared by IPF and non-IPF) may participate in the development of progressive pulmonary fibrosis in ILDs of different aetiologies. Thus, for example, the innate immune response, vascular endothelium and microbiome changes (among others) may trigger diverse mechanisms in different ILDs. Nonresolved inflammation provoked by continuous extrinsic (hypersensitivity pneumonitis) or intrinsic (autoimmunity) injury/inflammation may evolve to PF-ILD through the permanent secretion of pro-fibrotic molecules. Likewise, widespread endothelial cell dysregulation is critical in the fibroproliferative response observed in scleroderma [78], while impaired diversity in the lung microbiome seems to be associated with progression in IPF [79].

**Concluding remarks**

In this review we have attempted to explore whether some mechanisms that are well established for IPF could also be involved in the development of the progressive fibrosis phenotype in other ILDs.

In response to (known or unknown) injury, sophisticated and complex mechanisms are engaged to revert the lesions and regenerate the damaged lung. Effective regeneration of the alveolar epithelium requires bidirectional communication between epithelial and mesenchymal cells. Thus, for example, a single cell signalling pathway initiated by activation of the signal transducer and activator of transcription STAT3 induces the expression of brain-derived neurotrophic factor, which through the receptor TrkB induces the mesenchymal expression of FGF-7 and supports epithelial regeneration [80]. If this and other regulatory mechanisms are disrupted, damage persists which could result in a fibrotic response. In some cases, however, a persistent fibrotic remodelling programme characterised by the progressive cellular and molecular feed-forward loops is switched on. These points of no return may (co)exist in diverse ILDs, contributing to the detrimental orchestration of the PF-ILD phenotype (figure 2). We acknowledge that our

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**FIGURE 2** Proposed integral model for the development of progressive fibrosing interstitial lung disease (ILD). TGF: transforming growth factor; PDGF: platelet-derived growth factor; UIP: usual interstitial pneumonia; ECM: extracellular matrix. The combination of genetic, environmental and host factors in the lungs triggers an immunoinflammatory reaction or the hyperactivation of resident cells such as epithelial or endothelial cells. The disease may eventually be reverted under treatment if regulatory pathways operate appropriately. More often, these lung disorders evolve to fibrosis through several mechanisms that result in the expansion and activation of fibroblasts and excessive deposition of ECM. The fibrotic response may be stopped and stabilised either spontaneously through endogenous regulatory mechanisms or with treatment. However, in the presence of several self-progression and activation loops, a fibrosis progressive phenotype is acquired with the chaotic accumulation of ECM and end-stage lung remodelling.
perspective has limitations, mostly due to the scarcity of studies dealing specifically with the mechanisms involved in the point of no return in non-IPF ILD. Therefore, our proposal is based mainly on the pathogenic mechanisms known to participate in IPF, the prototype of PF-ILD, theoretically assuming that if they occur in other types of fibrotic ILD they may also participate in the development of this progressive behaviour. However, there are many knowledge gaps and issues to resolve, including the reasons why some ILDs have a marked susceptibility for progression while others do not. We also need to better identify and separate the pathobiological processes that trigger the fibrotic response post-injury from those that perpetuate and irreversibly sustain the fibrotic process. To understand PF-ILD certainly raises many challenges since the pathophysiology and even the natural history have not yet been widely studied. Further in-depth studies using multilevel system biological approaches in combination with longitudinal analysis will allow us to understand the complex network underlying PF-ILD and identify appropriate antifibrotic targets.

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