Idiopathic pulmonary fibrosis

Zhe Hui Hoo, Moira K B Whyte

Department of Infection and Immunity, Academic Unit of Respiratory Medicine, University of Sheffield, Sheffield, UK

Correspondence to
Professor Moira Whyte, Department of Infection and Immunity, Academic Unit of Respiratory Medicine, University of Sheffield, Sheffield S10 2RX, UK; m.k.whyte@sheffield.ac.uk

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ABSTRACT

Idiopathic pulmonary fibrosis is a progressive lung disease that carries a poor prognosis and for which there are no effective therapies. Although the excessive deposition of extracellular matrix, combined with evidence of recurrent injury to the alveolar epithelium, are well-described there is a pressing need to understand these processes better at a molecular level and thus to identify potential therapeutic targets in this intractable disease. This review considers some recent advances published in *Thorax* and elsewhere that have improved our understanding of the pathophysiology of idiopathic pulmonary fibrosis, using data both from human cells and tissue and from animal models of pulmonary fibrosis. The studies particularly address the fate of the alveolar epithelial cell and mechanisms of fibrogenesis, and identify mechanistic pathways shared with co-existing conditions such as lung cancer and pulmonary hypertension. The concepts of physiological biomarkers of disease progression and prognosis are also discussed.

Idiopathic pulmonary fibrosis (IPF) is a progressive fibrotic lung condition that is uncommon but not rare. In the UK, the incidence of IPF is 4.6 per 100 000 person-years and has increased over recent years.[1] The pathophysiology of IPF remains poorly understood, but is an area of very active research that should yield effective treatments for this condition, which currently has a poorer prognosis than many malignancies. The increased research activity in this area is reflected in the publication of 15 IPF-related studies in *Thorax* since 2008, addressing aspects of disease pathophysiology and prognostic factors.

THE PATHOPHYSIOLOGY OF IPF

Usual interstitial pneumonia is the histological hallmark of IPF. Injury to the alveolar epithelium followed by aberrant repair is a central pathogenic mechanism in IPF,[2] and this dysregulated repair is characterised by 'fibroblast foci', aggregates of activated myofibroblasts[3 4] whose extent correlates with poorer prognosis.[5] Myofibroblasts are highly synthetic cells and important effector cells in IPF.[6] The origins of fibroblast foci remain unknown, but they may arise from epithelial–mesenchymal transition (EMT), from division and differentiation of resident lung fibroblasts or from recruitment of circulating fibrogenic stem cells to the lung.[6 7] Airway smooth muscle cells (ASMCs) are also found in the interstitium of fibrotic lungs[8] and may be recruited by coagulation signalling.[9] ASMCs can be induced to secrete chemokines, cytokines and growth factors, and to perpetuate inflammation.[10] The role of inflammation in IPF is controversial—early concepts of IPF pathophysiology proposed that inflammation was central to disease initiation[11 12]; more recently inflammation has been viewed as of little significance.[2] Recent studies, for example correlating pulmonary inflammation and poorer prognosis[13] or providing evidence of adaptive immune responses in the IPF lung,[14] have suggested that immune mechanisms play a role in disease progression and potentially in disease initiation.

Epithelial response to injury

Death of alveolar epithelial cells

Alterations in the phenotype of the alveolar epithelium are a central feature in IPF. The pulmonary epithelial cell has a number of potential fates following injury: recovery and repair, caspase-dependent death by apoptosis or phenotypic differentiation into a fibroblastic cell (EMT). Inhibition of epithelial cell apoptosis prevents development of fibrosis in the mouse bleomycin model,[15] and two recent papers have identified a mediator of epithelial cell death that merits further investigation in IPF. Oikonomou and colleagues found that gelsolin, a gene overexpressed in the lungs of bleomycin-treated mice,[16] was also upregulated in patients with IPF or fibrotic non-specific interstitial pneumonia (NSIP), but not other forms of interstitial lung disease (ILD).[17] Mice deficient in gelsolin were protected from bleomycin-induced inflammation and fibrosis, and bone marrow transplant experiments demonstrated that gelsolin expression in lung tissue cells (as opposed to leucocytes) was required for development of lung fibrosis. At a molecular level, gelsolin regulates cellular cytoskeletal dynamics, and its cleavage by active caspase-3 leads to cellular collapse during apoptotic cell death. The authors propose that actin-modifying or gelsolin-targeting drugs could 'rescue' epithelial cells in IPF, assuming gelsolin cleavage is not too far downstream in apoptotic signalling pathways for long-term cell viability.[18] Richter *et al* examined the effects of endostatin, a product of collagen XVIII cleavage, on lung epithelial cells.[19] Endostatin levels were elevated in bronchoalveolar lavage (BAL) and plasma from patients with IPF compared with controls. Endostatin induced apoptotic death and impaired proliferation of pulmonary epithelial cells in vitro. These findings, together with the known antiangiogenic effects of endostatin, suggest a generalised deleterious effect of endostatin on wound repair. Further studies await the development of effective inhibitors of endostatin activity.

Epithelial regeneration

Recurrent epithelial cell death induces a regenerative response, activating signalling pathways more

traditionally associated with lung development, such as the Wnt/β-catenin pathway.[20] Bronchiolisation of the distal airways occurs in IPF, with alveolar structures replaced by enlarged distal airspaces or cysts, covered by epithelial cells with characteristics of proximal airway epithelial cells and often mucus producing. Plantier *et al*[21] examined pathways governing mucus cell differentiation in lung tissue from patients with IPF and other chronic pulmonary disorders. They found that mucus cells lining the IPF distal airways expressed the mucin gene MUC5B, typically associated with submucosal glands, but not MUC5AC, although both are major components of airway mucus. Neuregulin1α, which drives mucus cell differentiation in vitro, was ectopically expressed in lungs from patients with IPF and may signal in a paracrine manner to cause bronchiolisation of the alveolar epithelium. Altered transcription factor expression was also observed, suggesting abnormal epithelial programming in IPF. Fascinatingly, a genome-wide linkage study in IPF has subsequently identified a common polymorphism in the MUC5B gene promoter, greatly over-represented in IPF patient populations, that is associated with markedly increased production of MUC5B.[22]

Epithelial–mesenchymal transition

Mechanisms of EMT have also received attention in *Thorax*. Calabrese and colleagues[23] assessed the role of squamous cell carcinoma antigen (SCCA) as a marker of, and a potential contributing factor to, epithelial instability in IPF. SCCA is a serine protease inhibitor with pleiotropic biological activities, including roles in cell migration, adhesion and proliferation. SCCA was overexpressed in the lungs of patients with IPF and specifically in metaplastic squamous and bronchiolar epithelial cells that also stain strongly for the fibrogenic cytokine, transforming growth factor β (TGFβ). The authors highlight the potential of SCCA as a marker of poor prognosis but, more interestingly, given that IPF populations have a greatly increased risk of developing squamous carcinoma of the bronchus,[24 25] speculate that SCCA expression could identify cells with potential for malignant transformation.

Fibrinolytic activity is suppressed in IPF, resulting in fibrin deposition in the interstitium and alveolar spaces.[26] Fibrinolysis is mediated by production of urokinase-type plasminogen activator, which is inactivated by plasminogen activator inhibitor-1 (PAI-1). Studies by Senoo and colleagues[27] showed that PAI-1 is predominantly expressed in the epithelial lining of the honeycomb lung, hyperplastic type 2 pneumocytes and alveolar macrophages in IPF. They showed that inactivation of PAI-1 using small interfering RNA (siRNA) limits the development of bleomycin-induced pulmonary fibrosis in mice. Delayed administration of PAI-1-siRNA during the 'fibrotic phase' of the bleomycin model also limited injury, implying that PAI-1-siRNA has direct antifibrotic effects. In addition, cell culture experiments showed that PAI-1-siRNA could inhibit TGFβ-mediated EMT in lung epithelial cell lines. Notwithstanding the potential challenges of siRNA delivery in humans, this pathway is clearly worthy of further investigation.

An interesting study by Jayachandran and colleagues[28] evaluated the role of zinc finger (SNAI) transcription factors in TGFβ-induced EMT. SNAIs regulate EMT during embryonic development and in disease,[29] and SNAI expression is induced by TGFβ.[30] Jayachandran and colleagues showed that TGFβ1 induces EMT and increases SNAI1 and SNAI2 protein expression in mouse and human epithelial cells. Overexpression of SNAIs could induce EMT in human epithelial cells, even in the absence of TGFβ. Conversely, siRNA silencing of SNAIs attenuated cell migration, a key requirement for EMT, and reduced EMT in those cells in response to TGFβ. Increased levels of SNAIs could be detected both in the bleomycin mouse model and in patients with IPF.

Mechanisms of fibrogenesis

Borie and colleagues[31] studied the expression of receptors for somatostatin (growth hormone release-inhibiting hormone, GH-RIH) in bleomycin mouse models and the effects of pasireotide, a long half-life somatostatin analogue. The authors had previously shown that human lung fibroblast binding of a somatostatin agonist, octreotide, was increased in cells derived from patients with IPF and correlated with the severity of fibrosis.[32] All five known somatostatin receptors (sst1–sst5) were detected in the lungs of bleomycin-naïve mice; four were modulated by bleomycin treatment. The sst2 receptor was expressed in both inflammatory cells and resident lung cells, and upregulated in fibrotic areas of IPF lungs. Sst2 also plays a prominent role in the biological effects of somatostatin analogues.[33] In mice given intratracheal bleomycin, immediate subcutaneous administration of pasireotide reduced inflammatory cell influx into the airways, improved lung injury scores, reduced lung collagen and inhibited increases in TGFβ and connective tissue growth factor. Delayed administration of pasireotide improved the majority of these parameters, but to a lesser degree. This study provides encouraging evidence for an antifibrotic action of pasireotide, although the reduced efficacy of delayed treatment may indicate a reduced effect in established fibrosis.

In similar experimental systems, Königshoff and colleagues[34] evaluated the expression of 5-hydroxytryptamine (serotonin, 5-HT) receptors in IPF and experimental pulmonary fibrosis. Serotonin is best known as a neurotransmitter but is mostly found outside the central nervous system.[35] Serotonin is synthesised from tryptophan, stored in platelets (which release it with the membrane-bound serotonin transporter, 5-HTT) and metabolised to 5-hydroxyindoleacetic acid (5-HIAA) in the liver. Serotonin has multiple actions in different physiological systems (including regulation of cell migration, proliferation, cytokine production and vasoregulation) that are mediated by seven different 5-HT receptor subtypes (5-HTR_1 to 5-HTR_7). This study found that 5-HTT was downregulated and several 5-HTRs upregulated in IPF and NSIP lungs compared with controls. 5-HTR_{2A} upregulation appeared specific to IPF and, with 5-HTR_{2B}, localised to key cell types involved in disease pathogenesis. Similarly, 5-HTR_{2A} and 5-HTR_{2B} were upregulated and 5-HTT downregulated in bleomycin-instilled mice. Moreover, intraperitoneal administration of a $5\text{-HTR}_{2A/B}$ antagonist, terguride, had beneficial effects upon lung compliance, collagen content and fibrosis. Terguride treatment of human lung fibroblasts also significantly reduced collagen production in response to TGFβ and WNT3a. These data are in agreement with a previous study[36] and support the concept that 5-HT signalling is altered in IPF and that $\text{HTR}_{2A/B}$ antagonism may have potential antifibrotic effects. Interestingly, serotonin is also a potent vasoconstrictor and may contribute to the pathogenesis of pulmonary hypertension (PH),[37] which is common among patients with IPF.[38] The decreased levels of 5-HTT among patients with IPF may increase serotonin levels, and a clinical trial of terguride in IPF with or without PH merits consideration.

Oxidant–antioxidant imbalances in the lower respiratory tract may have a role in the development of IPF,[39] with multiple strands of evidence of increased oxidant stress.[40 41] Reactive oxygen species (ROS) have widespread effects on pulmonary cells and growth factors (including TGFβ), and can promote

a profibrogenic environment.[39] The phagocyte NADPH oxidase is a membrane-bound enzyme complex that catalyses ROS production, while a family of non-phagocytic NADPH oxidases are designated as NOX.[42] NOX4 is expressed in pulmonary artery smooth muscle cells and may contribute to the pathogenesis of PH.[43] A previous study of cardiac fibrosis showed that NOX4 may mediate TGFβ-induced fibroblast differentiation into myofibroblasts.[44] On this background, Amara and colleagues[45] investigated NOX expression in human lung fibroblasts and their roles in mediating TGFβ-induced fibroblast differentiation into myofibroblasts. They found that IPF fibroblasts have increased NOX4 expression, increased intracellular ROS production and increased myofibroblast differentiation. A significant correlation between expression of the myofibroblast marker α-smooth muscle actin (α-SMA) and NOX4 is suggestive of a role for NOX4 in myofibroblast differentiation. TGFβ treatment of lung fibroblasts increased NOX4 and α-SMA expression, and ROS production. These effects were more marked with IPF fibroblasts perhaps due to autocrine effects of raised TGFβ levels in IPF. TGFβ-mediated increases in α-SMA mRNA were inhibited by pretreatment of fibroblasts with *N*-acetylcysteine, or by siRNA silencing of NOX4, indicating that oxidant–antioxidant imbalance may influence TGFβ signalling. The efficacy of *N*-acetylcysteine as a single agent for IPF treatment is being addressed by the PANTHER-IPF trial (clinicaltrials.gov/ NCT00650091), following previous evidence of beneficial effects in the IFIGENIA trial.[46]

The last paper in this section, from Knobloch *et al*,[47] pertains to the roles of ASMCs and inflammation in the pathogenesis of IPF. Endothelin-1 (ET-1) is both a potent vasoconstrictor and a proinflammatory cytokine.[48] ET binds to two G protein-coupled receptors (ET_AR and ET_BR) that are widely expressed in the lung. The authors showed that ET-1, signalling via ET_AR, could induce its own transcription and that of granulocyte–macrophage colony-stimulating factor (GM-CSF) in human ASMCs, while tumour necrosis factor α (TNFα) induced transcription of both ET-1 and GM-CSF, revealing a complex network of interactions between these molecules. ET-1 activation of the downstream ERK/p38MAPK signalling pathways was inhibited by bosentan (a dual endothelin receptor antagonist). By inhibiting the TNFα/ET-1/GM-CSF network, bosentan demonstrated anti-inflammatory properties and the authors speculated that it would be a useful treatment in early IPF. However, the BUILD-3 trial of bosentan failed to achieve its primary end point of 'reduced morbidity/mortality' in IPF, perhaps because these cytokine networks are more relevant to early disease. ET-1 is also a known common mediator in IPF and PH.[49] All three papers in this section, therefore, investigate mediators that have been implicated in both PH and IPF, reflecting increasing recognition of shared pathophysiological mechanisms as well as clinical co-existence of these two conditions.

One further pathophysiological insight came from Richter *et al*'s investigation of bacterial colonisation of the lower airways in patients with Wegener granulomatosis or IPF.[50] It was found that 36% of patients with IPF had lower airway bacteria detected in BAL, some of whom remained culture positive 3 months later. Positive bacteriology was associated with elevated levels of the anti-inflammatory cytokine, interleukin 1 receptor antagonist (IL-1ra). It is unclear whether the presence of bacteria was related to radiological changes such as traction bronchiectasis and whether bacterial colonisation is a cause or a consequence of structural lung damage. Nonetheless, recent evidence shows that neutrophilic inflammation is associated with poorer prognosis in IPF and may be induced by infectious as well as non-infectious stimuli in the lung.[13] In the light of these data, and the availability of new molecular microbiomic techniques, it may be timely to revisit the roles of inflammation and infection in disease progression in IPF.

PROGNOSTIC INDICATORS IN IPF

IPF has a variable natural history; some patients die within a year of diagnosis while others live >10 years.[51] We currently lack prospectively validated, robust biomarkers of disease progression to advise patients or inform decisions such as referral for and timing of lung transplantation.[52] Forced vital capacity (FVC; >10%) and transfer factor of the lung for carbon monoxide (TL_{CO}; >15%) decline within 6 months of diagnosis are specific markers of poor short-term prognosis in patients with IPF[53–55] but are not particularly sensitive[56] and provide prognostic information only after 6 months of a median 2–3 years' life expectancy have elapsed. Several trials have shown the usefulness of quantitative scoring of high resolution CT (HRCT) scans in predicting prognosis at baseline,[57 58] but HRCT is susceptible to interpretive variability, even among expert radiologists.[58] There remains a need for a simple, practical and accurate prognostic indicator in IPF.

Corte *et al*[59] evaluated an inert gas rebreathing Innocor device for non-invasive measurement of pulmonary blood flow (PBF) in patients with ILD. The authors found strong correlation and, importantly, reasonable agreement between $PBF_{INNOCOR}$ and the 'gold standard' Fick calculation of cardiac output. The sample size was small (15 patients with IPF) and, while CO has prognostic significance in pulmonary arterial hypertension, its role in ILD is less certain. It would be useful to conduct a prospective trial in patients with IPF to correlate $PBF_{INNOCOR}$ with mean pulmonary artery pressure, pulmonary vascular resistance, lung function decline and mortality.

Gas exchange is impaired in IPF and worsens with exercise.[60] The 6 min walk test (6MWT) is the most common and best validated form of exercise testing for IPF. Both Sa_{O_2} desaturation to <88% during exercise[61] and shorter walk distance[62] at baseline can predict prognosis. However, only the walk distance is highly reproducible,[63] allowing its serial use to monitor disease progression. Flaherty and colleagues found that a 6-month decrease in walk distance of >200 feet is associated with increased mortality in IPF[61] and a change in 6MWT walk distance (6MWD) is increasingly used as an end point in clinical trials. However, the figure of 200 feet (~61 m) was chosen a priori and only a few patients will exhibit a 6MWD decline of that magnitude. It is, therefore, uncertain what distance constitutes the minimum important difference (MID) that is clinically meaningful. Swigris *et al*[64] attempted to answer this question by calculating changes in 6MWD among patients with IPF over a 12-month period and determining the MID for 6MWD by retrospective analysis of data from the BUILD-1 trial.[65] The authors used anchor-based (with Saint George's Respiratory Questionnaire (SGRQ) score and FVC selected as the two 'anchor categories') and distribution-based methods to generate 10 MID estimates. The range was 10.8–58.5 m, with a mean of 28 m. This mean corresponds closely to the estimated MID (29–34 m) from another published study.[66] However, it should be noted that patients with severe and mild disease were not represented, so the estimated MID may not be applicable to those groups. The MID for SGRQ scores used in the anchor-based method was derived from the same patient population so further validation of 6MWD MID in other patients with IPF is required.

Quantifying perception of quality of life of patients with IPF is important since IPF has a devastating effect on functional status as well as life expectancy. Improvements in health-related quality of life (HR-QOL) represent a tangible benefit for patients, and several clinical trials have used HR-QOL as a secondary end point. An ideal instrument to measure HR-QOL should be valid, reliable, responsive and interpretable.[67] Condition-specific instruments, tailored to patients with the disease of interest, are considered more sensitive and better targeted than generic instruments in detecting effects of that disease on patients' lives.[67] Yorke and colleagues[68] developed an IPF-specific version of the SGRQ, again using retrospective analysis of data from the BUILD-1 trial and statistically based methods used to develop the chronic obstructive pulmonary disease (COPD)-specific SGRQ. They derived the first IPF-specific HR-QOL score, the SGRQ-I, that can be used to track changes in patients' perception of HR-QOL over time. Prospective studies in other groups of patients with IPF will be needed to validate the SGRQ-I.

CLINICAL TRIALS IN IPF

Between 2008 and 2010, there was only one clinical trial pertaining to IPF published in *Thorax*, the first randomised controlled trial of exercise training in ILD.[69] Exercise training improves exercise capacity and HR-QOL in patients with COPD[70] but its role in patients with ILD is less clear. This study compared a twice-weekly exercise training programme for 8 weeks versus once-weekly telephone contact for support and general health advice in 57 patients with ILD (34 with IPF) and included patients with a wide range of disease severity. The primary end point was 6MWD. Drop-out rates were lower than in many pharmacological trials, suggesting that exercise training is well tolerated. At 9 weeks there was an improvement in 6MWD in the exercise compared with the control group, with a mean increase of 35 m, and improvements in symptom scores. The improvements in exercise capacity and symptoms were not sustained at 6 months. This could be due to disease progression negating the earlier benefits of exercise training or reflect the need for a formal maintenance exercise programme to sustain improvement. Although the improvements are only modest, and larger studies with longer follow-up and inclusion of maintenance exercise are warranted, exercise training should be considered for patients with IPF who have dyspnoea and diminished functional capacity and no contraindications.

CONCLUSIONS

The papers on IPF pathogenesis have not only highlighted the complexity of this disease but also suggested common mechanistic pathways for conditions (such as lung cancer and PH) that may co-exist with IPF. Impressively, many studies combined the use of patient tissue with interventional studies in the bleomycin mouse model and, despite the recognised limitations of the model for study of IPF, showed good congruity of experimental results. These studies identify some encouraging therapeutic possibilities. Further papers have increased our understanding of the natural history of the disease and identified useful end points for clinical trials. The clinical trial discussed showed the potential benefit of pulmonary rehabilitation among patients with IPF. Future trials should robustly assess improvements in the HR-QOL of patients with IPF, in addition to effects of treatments on lung function and, most importantly, on the prognosis of this condition.

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