

IPF: time for the (ciliary) beat generation?

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Idiopathic pulmonary fibrosis (IPF), the most common of the idiopathic interstitial pneumonias remains a disabling, progressive lung disease with extremely poor prognosis, in which no pharmacological intervention significantly alters outcome.¹ A relatively poor understanding of the complex pathophysiology of IPF continues to hinder the identification of effective therapies, and of patients most likely to benefit from existing treatments.

Usual interstitial pneumonia is the histological hallmark of IPF, characterised by temporospatial heterogeneity in which normal lung is interspersed with areas of subpleural interstitial fibrosis, loss of normal alveolar architecture and the presence of fibroblastic foci.² Histological sections may also show ‘bronchiolisation’ of the distal airway with alveolar structures replaced by enlarged airspaces (forming characteristic honeycomb cysts) lined by epithelial cells more akin to proximal airway epithelium, often ciliated and mucus producing.^{3–4} The potential importance of mucus production in the pathogenesis of IPF has been highlighted by a landmark study demonstrating that a common variant within the putative promoter region of *MUC5B* (an airway mucin gene) is strongly associated with familial interstitial pneumonia and IPF.⁵ Moreover, subjects with IPF had significantly higher expression of *MUC5B* protein levels compared with controls, and this was localised to areas of lung fibrosis. It has been hypothesised that excessive *MUC5B* may impair the host response to alveolar injury through excess mucus plugging and impaired clearance of inhaled substances and micro-organisms. Alternative theories propose that *MUC5B* may interfere directly with alveolar repair mechanisms, either through disruption of interactions between type II alveolar epithelial cells and extracellular matrix, or by interfering with properties of surfactant and promoting alveolar collapse.

While there has been a proliferation of interest surrounding mucin production in IPF, the importance of cilia has received much less attention. The fascinating gene expression study from Yang *et al*⁶ in this edition of *Thorax* seems set to change this. The authors performed comparative RNA microarray analysis on lung tissue from 119 patients with well-characterised IPF and from patients with brain death whose lungs were considered unusable for lung transplantation. Genes differentially expressed in IPF were interrogated using hierarchical clustering, yielding two subgroups of IPF differentiated by distinct expression profiles. Intriguingly, the strongest signal distinguishing the two cohorts of patients with IPF appeared to come from cilia-associated genes and their structural components (DNAH6, DNAH7, DNAI1 and RPGRI1L) as well as *MUC5B*. The authors broadly replicated their findings in lung tissue from an independent cohort with IPF. Interestingly, the two IPF cohorts were indistinguishable clinically, but the ‘cilia-high’ group had more histological honeycombing in tissue taken from the area immediately adjacent to that used for microarray analysis.

This meticulous study adds important new knowledge in IPF. Consistent findings in two independent cohorts of well-characterised patients, relevant tissue, strong genetics expertise and decent sample sizes represent obvious strengths of the study, and increase the likelihood that the observation is ‘real’. Furthermore, the biological plausibility of the findings is enticing, because ciliated ‘bronchiolised’ epithelium is seen in honeycomb cysts.⁷ While the motile functions of cilia are well described, there has been a recent explosion in understanding of the role of the primary cilium in ‘sensing’ the cellular microenvironment, and in cell motility. Interestingly, ciliary chemosensing appears to be mediated via molecules already implicated in the pathogenesis of IPF such as Sonic-Hedgehog, platelet-derived growth factor receptor and canonical Wnt.⁸ Furthermore, primary cilia coordinate a series of signalling pathways critical to fibroblast migration in response to wound healing. In particular platelet-derived growth factor receptor α is compartmentalised to the primary cilium to activate pathways that regulate reorganisation of the cytoskeleton.⁹ Cigna *et al*¹⁰ recently

demonstrated that hedgehog pathways were reactivated in the IPF lung and have significant crosstalk with transforming growth factor β signalling in lung fibroblasts. Wnt and β -catenin are located on the membrane and basal body of the primary cilium—canonical Wnt signalling inactivates glycogen synthase kinase 3 β , thus stabilising β -catenin, which enters the nucleus to activate Wnt target genes (including fibronectin, matrix metalloproteinase 7, cyclins, axins and vascular endothelial growth factor). It has been suggested that upregulation of Wnt signalling may inhibit the ability of type II alveolar epithelial cells to differentiate into type I alveolar epithelial cells in response to injury (thus disrupting repair mechanisms).¹¹

As with all clinical IPF studies (however meticulous), some aspects of the paper by Yang *et al* must be interpreted cautiously. The cohort with upregulated cilia-associated genes had far more histological honeycomb cysts, and the implication may simply be that more ‘bronchiolised’, and therefore ciliated, tissue was available to sample (though the authors provide a fair argument against this bias playing a significant part). Also, the patients with IPF all had lung biopsies, and were younger than the average patient with IPF. In addition, the majority of the control group is likely to have suffered significant trauma, all will have been mechanically ventilated, and the implication is that there was sufficient inflammatory change to preclude use of these lungs for transplantation—these factors may have skewed the panel of genes selected to distinguish IPF cohorts. Finally, the specific cell type(s) producing the upregulation in ciliary genes remains unknown.

Equally, as with all fresh, important clinical research, the study stimulates more questions than it answers. One key question that remains to be answered is whether the two IPF cohorts differed in terms of outcomes such as mortality, loss of lung function or response to treatment? Also, could the study findings simply reflect duration of disease? Certainly one cohort of IPF had surprisingly little microscopic honeycombing—perhaps this could reflect sampling bias, or perhaps the natural history of the disease would be for these patients to go on and develop histological microcysts? Are the transcriptional changes actually associated with changes in cellular function? Perhaps most importantly, is upregulation of cilia-associated genes an essential component of disease pathogenesis, a compensatory and appropriate defence mechanism, or a functionally unimportant reaction to more important pathogenetic processes?

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Despite these considerations, the potential importance of this study is considerable. Certainly it highlights the ability of good genetic studies to generate new hypotheses and new avenues for exploration. In this particular case it throws attention onto the bronchiolisation process. Considerable focus has centred on epithelial-to-mesenchymal transition in recent years, but perhaps we have ignored the potential of stressed alveolar epithelium to transform into an entirely different epithelial configuration(s). The tantalising possibility, of course, is that secretion of mucin and upregulation of cilia-associated genes may be intrinsically linked. The authors of the present study are ideally placed to begin to address this issue, and their recent finding that specific polymorphisms in the *MUC5B* promoter region may be associated with better prognosis in IPF¹² highlights the complex interplay between biological factors and pathogenesis.

Of course, we remain frustratingly distant from the day when we can perform something like a transbronchial biopsy and have its transcriptome reliably inform us how best to manage patients with IPF. Nevertheless, a real momentum seems to be building in terms of high quality clinical studies identifying potentially important processes in the biology of IPF. The important next step is obviously to determine whether these can be manipulated to therapeutic advantage. In this regard, we currently await results

from the final part of the PANTHER-IPF trial,¹³ assessing whether the mucolytic, antioxidant N-acetylcysteine has beneficial effects in IPF. Meantime, the elegant study from Yang *et al* suggests that perhaps the ciliary beat is the beat to follow. Perhaps IPF researchers are ready to answer the question Jack Kerouac himself posed in *On The Road*—‘You boys going to get somewhere, or just going?’

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