New tale for an old fox in IPF?

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IDIOPATHIC PULMONARY FIBROSIS (IPF) is a fatal progressive disease, with a poorly understood etiology and pathogenesis. Current estimates propose that IPF affects about 500,000 people in the USA and Europe with an increasing incidence. Histologically IPF is characterized by alveolar epithelial cell injury and activation, the formation of fibroblastic foci, and excessive matrix deposition in the lung parenchyma, leading to decreased lung function and ultimately respiratory failure. Most patients present with established disease with an average survival of less than 3 years from diagnosis (reviewed in Ref. 5). The insidious nature of the disease, combined with an incomplete understanding of pathogenesis, accounts for the paucity of currently available therapeutic interventions.

Hypotheses for the initiation and progression of pulmonary fibrosis propose an unknown alveolar epithelial injury initiating dysregulated epithelial-mesenchymal interactions, which sustain a vicious cycle of aberrant tissue repair and injury. It is likely that multiple pathways, including abnormal matrix regulation, defective epithelial reconstitution, uncontrolled coagulation, abnormal neovascularization, and inadequate antioxidant pathways, contribute to this defective cross talk and thereby drive relentless disease progression (5).

MMPs as Markers of Tissue Repair in IPF

Microarray studies have been invaluable in informing our understanding of IPF at a molecular level as well as in identifying potential disease biomarkers (8, 18–20, 24). The gene expression signature in IPF is dominated by genes involved in development, extracellular matrix turnover, cellular growth, and differentiation consistent with active tissue remodeling and repair processes (19), exemplified by the matrix metalloproteinases (MMPs), which are coordinately upregulated in the lungs of IPF patients (24).

In addition to promoting matrix turnover, the MMP superfamily, which comprises over 20 proteinases, influences a range of processes including cellular interaction and adhesion, cytokine and chemokine activation, and modulation of microbrial peptides (4, 14). In IPF, MMP-1 and MMP-7 (matrilysin) have been widely shown to be increased in the plasma, lung tissue, and bronchoalveolar lavage fluid from IPF patients, with plasma levels of these proteinases differentiating IPF from sarcoidosis and chronic obstructive pulmonary disease. In particular, plasma MMP-7 levels were found to correlate with increasing disease severity in subclinical disease in IPF patients (18).

MMP-7 is constitutively expressed by airway epithelial cells and alveolar type II cells following injury (6, 12, 17) and promotes epithelial cell motility, reepithelialization, and wound closure by mediating E-cadherin and syndecan-1 shedding (2, 11, 12). MMP-7 therefore plays a key role in promoting normal repair following epithelial injury (reviewed in Refs. 4, 14). In the context of pulmonary fibrosis, however, this MMP may play a key role in aberrant epithelial repair mechanisms, including bronchiolization of the distal lung (3, 13, 21). In animal models of lung injury and fibrosis, MMP-7-deficient mice lack the ability to reepithelialize tracheal epithelium following mechanical wounding (6); conversely, these mice are partially protected from bleomycin-induced pulmonary fibrosis (24). These studies support the notion that increased MMP-7 levels in IPF are suggestive of ongoing aberrant epithelial injury and repair. However, little is known about both the nature of the injury and the mechanisms responsible for inducing or maintaining increased MMP-7 levels in IPF.

FOXA2 as a Novel Transcription Factor for MMP-7

Having identified an MMP-7 signature in IPF, understanding its relevance to pathology is imperative; how and why is MMP-7 upregulated in IPF?

A study by Richards and coworkers, published in a recent issue of the American Journal of Physiology Lung Cellular and Molecular Physiology (16), attempts to address these questions, suggesting a novel mechanism underlying elevated MMP-7 expression in IPF.

The authors provide compelling evidence for the involvement of the forkhead ortholog box (FOX) transcription factor, FOXA2 (16), in the upregulation of MMP-7 expression in the IPF epithelium. The FOX family transcription factors are critical during lung development. Together with other transcription factors, including Sry-related HMG box (SOX)2, thyroid transcription factor (TTF-1), forkhead ortholog box (FOX)A2, and Sam pointed domain Ets-like factor (SPDEF), they interact to form a system of cues for epithelial differentiation and cell fate during embryogenesis (reviewed in Ref. 22). In particular, increased expression of TTF-1 and FOXA2 accompanies differentiation of the alveolar epithelium and negatively associates with expression of the goblet cell marker, SPDEF (10, 15). Following lung injury, the coordinated cell-specific reactivation of these developmental transcription factors directs the epithelial differentiation repair response (reviewed in Ref. 22) and it is notable that global expression analysis has revealed that several developmental genes, including FOX, SOX, and Wnt/β-catenin-related genes, are enriched in IPF (reviewed in Ref. 20).

Richards and coworkers investigated the relationship between two single-nucleotide polymorphisms (SNPs) in the MMP-7 promoter-rs11568818 (G to A transition at position -181) and rs11568819 (C to T transition at position -153) and elevated levels of plasma MMP-7 in IPF. Both SNPs have previously been associated with decreased arterial dimensions in hypercholesteremia patients, as well as the progression of both colorectal and breast cancer (1, 7, 9). Within the IPF patient cohort, plasma MMP-7 was significantly increased in individuals homozygous for the rs11568818A variant (rs11568818AA) or heterozygous for rs11568818T (rs11568818CT). The influence of
these SNPs was underlined by the copy number-dependent effect of the GC haplotype, associating with reduced plasma levels of MMP-7.

Transition G to A at position −181 (rs11568818) creates a binding site for FOXA2 in the MMP-7 promoter, rendering FOXA2 a novel modulator of MMP-7 expression in individuals expressing this allele (Fig. 1). The functionality of the SNP in binding FOXA2 was elegantly confirmed by transient expression of both haplotype and allele specific luciferase reporter constructs, showing the rs11568818A allele was critical in mediating increased binding of the transcription factor. Colocalization of FOXA2 and MMP-7 in the alveolar epithelium of IPF patients emphasized the potential functional significance of these findings, although it was unclear whether the patients sampled for immunohistochemistry were of rs11568818AA genotype, information that could further strengthen the functional linkage between novel FOXA2 binding and increased MMP-7. Although the rs11568819CT genotype was also shown to be associated with increased plasma MMP-7 in IPF patients, no differential nuclear protein complex formation was observed by using probes specific for either allelic variant of this SNP.

The description of this novel regulatory mechanism represents an important step in understanding the biology of this important biomarker in IPF.

Unanswered Questions

This work raises several questions. First, it is noteworthy that no association was observed between the MMP-7 promoter variants and predisposition to develop IPF. The polymorphisms only apparently affect MMP-7 levels in individuals with disease. This implies that mechanisms affecting MMP-7 expression are modifying rather than directly causal factors in IPF (16). It is also unclear whether increased MMP-7 levels in plasma correlate with disease severity; however, previous studies would suggest this to be the case (18). Both MMP-1 and MMP-7 are significantly upregulated in IPF plasma and strongly distinguish IPF from hypersensitivity pneumonitis (19). Subsequent analyses showed that plasma MMP-7 levels distinguish IPF from a control population and from patients with COPD or sarcoidosis and potentially correlate with progression.

Fig. 1. rs11568818A in the matrix metalloproteinase 7 (MMP-7) promoter permits novel forkhead box A2 (FOXA2) binding. Schematic illustrating the permissive effect of rs11568818 (transition G to A at position −181) on FOXA2 binding and the postulated downstream effect on MMP-7 expression. Anti-sense sequences for both G and A variants of the single-nucleotide polymorphism are shown, along with the consensus core sequence for FOX transcription factor binding (R = A/G, K = T/G, Y = T/C).

That said, MMP-7 levels alone, although elevated in IPF, are potentially less robust than MMP-1 in distinguishing IPF from hypersensitivity pneumonitis (18). Moreover, MMP-7 has also been reported to be elevated in the lungs of a small number of patients with cryptogenic organizing pneumonia (8). The observation that MMP-7 is increased across divergent interstitial lung diseases suggests that it may form part of an aberrant control response, rather than being causal. Richards et al.’s (16) study finding that polymorphisms affecting MMP-7 expression modify the disease rather than predispose adds support to this notion.

Second, although this study elegantly delineates the role of rs11568818A in enabling FOXA2-mediated regulation of the MMP-7 gene, it is noteworthy that only 32% of IPF cases exhibit the AA genotype, leaving wide scope for the prosecution of additional mechanisms involved in modulating MMP-7 expression in IPF. In this regard, although the T allele of rs11568819 neither mediated increased FOXA2 binding to the MMP-7 promoter nor showed preferential affinity for nuclear proteins, the CT genotype nonetheless showed significant association with increased plasma MMP-7; rs11568819CT accounted for 8% of IPF cases. How exactly this SNP functionally affects MMP-7 levels will doubtless form the basis of future investigations. Outside of the promoter region, epigenetic and posttranscriptional regulation could also prove to be critical in modulating MMP-7 expression.

Finally, despite there being increased FOXA2 expression in IPF compared with normal lung, the upstream events influencing FOXA2 expression or activation in the IPF epithelium remain unresolved. Previous studies by this group have shown that the major profibrotic mediator, TGF-β, downregulates FOXA2 expression (23), suggesting that additional pathways are responsible for upregulating FOXA2 in IPF. Wnt/β-catenin signaling was a likely TGF-β-independent pathway candidate pathway, prior to the current study. Wnt/β-catenin signaling plays a key role in epithelial differentiation, and Wnt/β-catenin signature genes are enriched in IPF (20). Importantly, both FOXA2 and MMP-7 are known downstream targets of Wnt signaling. Although Richards and coworkers found that FOXA2 and MMP-7 colocalize to the IPF epithelium, they could not identify significant association with nuclear β-catenin. Collectively, these data suggest that it is unlikely that either TGF-β or the Wnt/β-catenin signaling activate the FOXA2/MMP-7 axis and reveal the need to identify key pathways which influence this axis.

Conclusion

Epithelial-derived MMP-7 is emerging as an important biomarker for lung injury and repair, showing significant association with IPF severity and clinical course. How MMP-7 contributes to the pathogenesis of IPF and mechanisms involved in control of its expression are not fully understood. The identification of novel SNPs in the MMP-7 promoter, and description of their functional significance is an important step forward in understanding the mechanisms that underlie MMP-7 expression in IPF. Whether similar SNP-mediated control mechanisms exist in the context of other important IPF biomarkers such as MMP-1 remains unknown but could provide further avenue for fruitful future investigation.
REFERENCES


