Journal of Pathology
J Pathol 2017; **241:** 6–9
Published online 29 November 2016 in Wiley Online Library (wileyonlinelibrary.com) DOI: 10.1002/path.4825

INVITED COMMENTARY

Fibroblast growth factors and pulmonary fibrosis: it's more complex than it sounds†

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†Invited commentary for Shimbori *et al.* Fibroblast growth factor-1 attenuates TGF-β1-induced lung fibrosis. *J Pathol* 2016; **240:** 197–210.

Abstract

Lung fibrosis results from the cumulative effect of dysfunctional wound repair involving multiple cell types, including fibroblasts, epithelial cells, and macrophages responding to an array of soluble and matrix-mediated stimuli. Recent studies have shown that a tyrosine kinase inhibitor that targets FGF, VEGF, and PDGF receptors can slow the rate of decline in pulmonary function in patients with idiopathic pulmonary fibrosis. However, each of these growth factor families is comprised of multiple ligands and receptors with pleiotropic activities on different cell types such that their broad inhibition might have both pro-fibrotic and anti-fibrotic effects, limiting the potential therapeutic efficacy. Continued investigation and delineation of specific roles of individual proteins and receptors on different cell types hold promise for targeting specific pathways with precision and optimizing the potential efficacy of future approaches to lung fibrosis therapy.

Keywords: fibrosis; precision medicine; EMT; fibroblast growth factor; nintedanib; idiopathic pulmonary fibrosis

Received 23 September 2016; Revised 7 October 2016; Accepted 10 October 2016

Conflict of interest statement: Dr Horowitz has received research funding from Boehringer Ingelheim International, which manufactures nintedanib. Drs Sisson and Kim have no conflicts of interest to disclose.

Lung fibrosis results from dysfunctional wound repair involving aberrant responses of multiple cell types, including fibroblasts, epithelial cells, and macrophages, to an array of soluble and matrix-derived stimuli [1]. Within the last few years, the demonstration that pharmacological intervention can alter the natural history of declining lung function in idiopathic pulmonary fibrosis (IPF) enhanced optimism and enthusiasm among physicians, scientists, and patients previously frustrated by more than a decade of well-executed, but negative, clinical trials [2]. However, a lack of detailed understanding of the specific mechanisms by which the clinically efficacious drugs, pirfenidone and nintedanib, exert their anti-fibrotic effects highlights the ongoing need for a deeper mechanistic understanding of disease pathogenesis and the development of therapeutic options with increased specificity. The molecular target of pirfenidone has not been identified, and nintedanib is a 'triple kinase inhibitor' that broadly targets the fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF) receptor tyrosine kinases as well as intracellular kinases from the Src family [3,4]. Pre-clinical data support a role for each of these in lung fibrosis, but their relative contributions to disease development and progression have not been determined. It is not clear if the anti-fibrotic effect of nintedanib requires inhibition of each of these kinases or if similar efficacy can be achieved with specific targeting of an individual kinase. The cellular targets by which nintedanib exerts its beneficial effects have also not been clearly defined. Importantly, the FGF, PDGF, and VEGF families each consist of several distinct ligands and receptors with diverse expression patterns and an array of activities on cellular function [5–7]. Of these, the FGF family is the most diverse, with more than 20 ligands that signal through four distinct receptors (FGFR1–4) [5]. Nintedanib potently inhibits FGFR1–3 and has similar potency for VEGFR1–3 and PDGFRα and β [4]. It is feasible to hypothesize that such broad inhibition of multiple kinases with pleiotropic functions that vary by cell type could have unintended effects that limit the potential clinical benefits.

The FGF family was characterized (and named) by the ability of its members to promote fibroblast proliferation. However, it is now recognized that FGF family members influence the phenotype of multiple cell types. Indeed, members of the FGF family can also stimulate

epithelial cell proliferation. For example, FGF-7 (also referred to as keratinocyte growth factor) stimulates proliferation in lung epithelial cells and has been studied as a potential therapy for lung injury [8]. In this manner, within the FGF family some members can be considered 'pro-fibrotic' through their mitogenic activity on fibroblasts, while others have 'anti-fibrotic' effects by promoting epithelial cell regeneration. Furthermore, individual FGF family members may exert pro- and anti-fibrotic effects, depending on the responding cell and the context of other signalling molecules.

In a recent issue of *The Journal of Pathology* [9], Shimbori *et al* examined the effect of fibroblast growth factor 1 (FGF-1) on alveolar epithelial cells and fibroblasts *in vitro* and lung fibrosis *in vivo* using a well-established murine model of lung fibrosis induced by TGF-beta (TGFβ) overexpression [10]. This study demonstrates that FGF-1 is anti-fibrotic. Specifically, the *in vitro* experiments show that FGF-1 antagonizes TGFβ-induced epithelial–mesenchymal transition (EMT) by promoting the caveolin-1-dependent proteosomal degradation of the type 1 TGFβ1 receptor (TGFβR1), thereby reducing TGFβ-mediated signalling. In fibroblasts, FGF-1 similarly inhibits TGFβ1-mediated signalling and myofibroblast differentiation through suppression of TGFβR1 transcription and through enhanced proteosomal degradation of the receptor. These results are consistent with prior reports demonstrating the requirement of TGFβ signalling in both alveolar epithelial cells and fibroblasts for the development of fibrosis [11,12]. Importantly, the *in vivo* studies in this paper also support an anti-fibrotic role for FGF-1, as both preventive and therapeutic overexpression of FGF-1 diminished TGFβ1-induced lung fibrosis. Together, these findings demonstrate that despite its name, FGF-1 inhibits TGFβ1-mediated cellular phenotypes that are thought to be integral to fibrogenesis and that these *in vitro* actions of FGF-1 are associated with *in vivo* limitation of fibrosis. Extrapolating from these findings, one would predict that inhibition of FGF-1 might actually exacerbate lung fibrosis. The current study similarly shows increased FGF-1 levels in the serum of IPF patients and increased FGF-1 expression in the alveolar epithelium, but not myofibroblasts in IPF lungs, which the authors speculate represents a failed endogenous attempt to limit the extent of lung fibrosis. Consistent with this supposition, others have shown that FGF-1 and several FGF receptors are expressed at increased levels in IPF lungs and that treating IPF-derived lung fibroblasts with FGF-1 decreased collagen production and increased apoptosis [13].

A complex role for the FGFs in fibrosis is not limited to FGF-1, and the 'anti-fibrotic' activities of several family members have been reported (Figure 1). One study reported that FGF-9 and FGF-18 were strongly expressed by epithelial cells and myofibroblasts in IPF lung tissue, while mesenchymal cells within the fibroblastic foci expressed several FGF receptors [14], suggesting that these ligand–receptor interactions

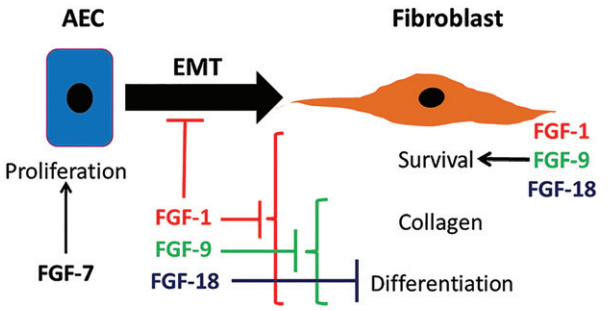

Figure 1. Pleiotropic effects of different fibroblast growth factors. FGF-1 (red) prevents TGFβ1-induced alveolar epithelial cell (AEC) transition to a mesenchymal cell phenotype (EMT), while also preventing TGFβ1-mediated myofibroblast differentiation and elaboration of collagen. FGF-9 (green) inhibits collagen production by fibroblasts, while both FGF-9 and FGF-18 (blue) prevent myofibroblast differentiation. FGF-1 has been reported to inhibit fibroblast survival, but has also been reported to inhibit apoptosis (as have FGF-9 and FGF-18). FGF-7 (also known as keratinocyte growth factor) promotes the proliferation of alveolar epithelial cells.

contribute to epithelial–mesenchymal crosstalk. The reported effects of FGF family members on fibroblast survival and apoptosis have been variable. In a recent report, FGF-1 (as well as FGF-9 and FGF-18) decreased the apoptosis susceptibility of normal fibroblasts but had no impact on fibroblasts isolated from patients with IPF [14]. In contrast, a prior study by Ramos *et al* reported that FGF-1 treatment was sufficient to induce fibroblast apoptosis [15]. FGF-9 and FGF-18 also increased fibroblast migration but prevented myofibroblast differentiation. Additionally, FGF-1 and FGF-9, but not FGF-18, decreased basal and TGFβ1-mediated collagen expression and myofibroblast differentiation in fibroblasts [14]. Collectively, these studies demonstrate the heterogeneity and contextual nature of cellular responses to soluble stimuli within the FGF family, illustrate the limitation in defining a family of growth factors as either 'pro-' or 'anti-fibrotic', and emphasize the importance of understanding how growth factors modulate different behaviours within a cell population and in different types of cells.

The findings in this study highlight several of the challenges and opportunities in basic and translational lung fibrosis research. For one, the effects of ligand–receptor interactions and signalling pathways need to be appreciated in multiple cell types, as inhibiting a pathway for beneficial effects on one cell type may have deleterious effects in other cells. Evidence for this is provided by previous studies of imatinib mesylate, a multi-tyrosine kinase inhibitor targeting Bcr/c-Abl and PDGFR, which was viewed as a promising potential therapeutic agent due to its inhibition of fibroblast proliferation, differentiation, and ECM production [16,17], and the prevention of bleomycin-induced fibrosis in mice [16]. However, this tyrosine kinase inhibitor was also found to induce apoptosis and impair differentiation in epithelial cells, and failed to demonstrate an *in vivo* effect on fibrosis when it was introduced during the late-inflammatory

phase of the bleomycin model [17]. It was speculated that the adverse effects of the drug on the epithelium masked any beneficial anti-fibrotic effects on myofibroblasts. Ultimately, a clinical trial of imatinib for IPF demonstrated no benefit [18].

In the current study, FGF-1 effectively inhibited EMT. Although it is clear that alveolar epithelial cells (AECs) have a critical role in the pathogenesis of fibrosis, the mechanisms by which the alveolar epithelium contributes to the perpetuation of the wound-repair process in fibrosis have not been fully defined. We have shown that TGFβ1-treated AECs undergo a phenotypic transition and elaborate soluble mediators that, in turn, promote fibroblast activation [19,20]. While 'epithelial–mesenchymal transition' has become a controversial term, it is clear that AECs have phenotypic plasticity in the context of injury and repair responses [21]. This controversy regarding full or partial EMT has distracted from important studies on the regulation and precise functional contribution of AEC plasticity during fibrosis. As noted above, AEC responses to TGFβ are critical for fibrosis and TGFβ is a major regulator of AEC plasticity. Importantly, TGFβ signalling can be modified through crosstalk with other signalling pathways such as FGF-1, as demonstrated in this report.

The variable responses of different cell types to soluble growth factors and cytokines and the dynamic evolution of lung injury, repair, and fibrosis highlight the need to use *in vivo* models in which the comprehensive effects of altered cell signalling can be assessed. Much has been written about the strengths and weakness of different murine models, which will not be recapitulated here [1,22], but it is important to recognize that there are no perfect murine models of IPF. While developing better models is a goal in the field, it remains to be seen if any model will fully recapitulate IPF [1,23]. Accordingly, each murine model of lung fibrosis represents a tool that can be used to address specific aspects of the pathogenesis of fibrosis. Through integration of findings from several models, we hope to gain valuable insight into fundamental mechanisms of lung repair and assess the feasibility of specific therapeutic approaches. Transgenic models that allow us to control the cell specificity and timing of interventions can complement the broader manipulation of a pathway achieved with adenoviral delivery to the lungs, systemic administration of a drug, or germline knockouts, and contribute to an increasing mechanistic understanding.

It is said that perfection is the enemy of the good. However, good must not become the enemy of better. There is a growing appreciation that diseases involving every organ and tissue in humans are attributable to fibrosis, and the likelihood is that shared underlying mechanisms contribute to the pathogenesis across organ systems such that novel and targeted anti-fibrotic therapies have the potential to have a significant impact on human health. The success of recent clinical trials in IPF provides the motivation for us to redouble our efforts to develop a better understanding of the basic mechanisms that underlie fibrosis. Such advancement should embrace mechanistic heterogeneity and define different endotypes that can be targeted with more selective therapies [24,25].

Acknowledgements

Funding was provided by NIH/NHLBI grants HL105489 (JCH), HL078871 (THS), and HL108904 (KKK).

Author contribution statement

JCH, KKK, and THS all contributed to the planning, writing, and editing of this manuscript.

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