

Fibroblast Growth Factors and Pulmonary Fibrosis: It's more complex than it sounds[†]

Running Title: Pleiotropic actions of FGFs in lung fibrosis

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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 holds promise for targeting specific pathways with precision and optimizing the potential efficacy of future approaches to lung fibrosis therapy.

Key Words: Fibrosis, Precision medicine, EMT, fibroblast growth factor, nintedanib, idiopathic pulmonary fibrosis

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 pharmacologic 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 are each comprised 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 twenty ligands that signal through four distinct receptors (FGFR1-4) [5]. Nintedanib potently inhibits FGFR1-3, and has similar potency for VEGFR1-3 and PDGFR α 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 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 signaling molecules.

In this issue of The Journal of Pathology [9], Shimbori and colleagues investigate 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 (TGF β) overexpression [10]. This study demonstrates that FGF-1 is anti-fibrotic. Specifically, the in vitro experiments show that FGF-1 antagonizes TGF β induced epithelial-mesenchymal transition (EMT) by promoting the caveolin-1 dependent proteosomal degradation of the type 1 TGF β 1 receptor (TGF β R1), thereby reducing TGF β -mediated signaling. In fibroblasts, FGF-1 similarly inhibits TGF β 1 mediated signaling and myofibroblast differentiation through suppression of TGF β R1 transcription and through enhanced proteosomal degradation of the

receptor. These results are consistent with prior reports demonstrating the requirement of TGF β signaling 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 TGF β 1 induced lung fibrosis. Together, these findings demonstrate that despite its name, FGF-1 inhibits TGF β 1 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 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 -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 and colleagues reported that FGF-1 treatment was sufficient to induce fibroblast apoptosis [15]. FGF-9 and -18 also increased fibroblast migration but prevented myofibroblast differentiation. Additionally, FGF-1 and -9, but not FGF-18, decreased basal and TGF β 1 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 behaviors 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 signaling 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, that 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 TGF β 1 treated alveolar epithelial cells 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 TGF β are critical for fibrosis and TGF β is a major regulator of AEC plasticity. Importantly TGF β signaling can be modified through crosstalk with other signaling 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 signaling 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 re-double 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].

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References

1. Blackwell TS, Tager AM, Borok Z, *et al.* Future Directions in Idiopathic Pulmonary Fibrosis Research. An Nhlbi Workshop Report. *Am J Respir Crit Care Med* 2014; **189**: 214-222.
2. Raghu G, Rochwerf B, Zhang Y, *et al.* An Official Ats/Ers/Jrs/Alat Clinical Practice Guideline: Treatment of Idiopathic Pulmonary Fibrosis. An Update of the 2011 Clinical Practice Guideline. *Am J Respir Crit Care Med* 2015; **192**: e3-19.
3. Richeldi L, du Bois RM, Raghu G, *et al.* Efficacy and Safety of Nintedanib in Idiopathic Pulmonary Fibrosis. *N Engl J Med* 2014; **370**: 2071-2082.
4. Hilberg F, Roth GJ, Krssak M, *et al.* Bmf 1120: Triple Angiokinase Inhibitor with Sustained Receptor Blockade and Good Antitumor Efficacy. *Cancer Res* 2008; **68**: 4774-4782.
5. Gong SG. Isoforms of Receptors of Fibroblast Growth Factors. *J Cell Physiol* 2014; **229**: 1887-1895.
6. Neufeld G, Cohen T, Gengrinovitch S, *et al.* Vascular Endothelial Growth Factor (Vegf) and Its Receptors. *FASEB J* 1999; **13**: 9-22.
7. Ostendorf T, Eitner F, Floege J. The Pdgf Family in Renal Fibrosis. *Pediatr Nephrol* 2012; **27**: 1041-1050.
8. Ware LB. Keratinocyte Growth Factor as an Epithelial Protective Agent: Where Do We Stand? *Int J Radiat Oncol Biol Phys* 2004; **60**: 1345-1346.
9. Shimbori CB, P. Xia, J. Gauldie, J. Ask, K. Ramos, C. Becerril, C. Pardo, A. Selman, M. Kolb, M. Fibroblast Growth Factor-1 Attenuates Tgf-B1-Induced Lung Fibrosis. *The Journal of Pathology* (In Press).
10. Bonniaud P, Kolb M, Galt T, *et al.* Smad3 Null Mice Develop Airspace Enlargement and Are Resistant to Tgf-Beta-Mediated Pulmonary Fibrosis. *J Immunol* 2004; **173**: 2099-2108.
11. Hoyles RK, Derrett-Smith EC, Khan K, *et al.* An Essential Role for Resident Fibroblasts in Experimental Lung Fibrosis Is Defined by Lineage-Specific Deletion of High-Affinity Type Ii Transforming Growth Factor Beta Receptor. *Am J Respir Crit Care Med* 2011; **183**: 249-261.
12. Li M, Krishnaveni MS, Li C, *et al.* Epithelium-Specific Deletion of Tgf-Beta Receptor Type Ii Protects Mice from Bleomycin-Induced Pulmonary Fibrosis. *J Clin Invest* 2011; **121**: 277-287.
13. MacKenzie B, Korfei M, Henneke I, *et al.* Increased Fgf1-Fgfr Expression in Idiopathic Pulmonary Fibrosis. *Respir Res* 2015; **16**: 83.
14. Joannes A, Brayer S, Besnard V, *et al.* Fgf9 and Fgf18 in Idiopathic Pulmonary Fibrosis Promote Survival and Migration and Inhibit Myofibroblast Differentiation of Human Lung Fibroblasts in Vitro. *Am J Physiol Lung Cell Mol Physiol* 2016; **310**: L615-629.
15. Ramos C, Montano M, Becerril C, *et al.* Acidic Fibroblast Growth Factor Decreases Alpha-Smooth Muscle Actin Expression and Induces Apoptosis in Human Normal Lung Fibroblasts. *Am J Physiol Lung Cell Mol Physiol* 2006; **291**: L871-879.
16. Daniels CE, Wilkes MC, Edens M, *et al.* Imatinib Mesylate Inhibits the Profibrogenic Activity of Tgf-Beta and Prevents Bleomycin-Mediated Lung Fibrosis. *J Clin Invest* 2004; **114**: 1308-1316.
17. Vittal R, Zhang H, Han MK, *et al.* Effects of the Protein Kinase Inhibitor, Imatinib Mesylate, on Epithelial/Mesenchymal Phenotypes: Implications for Treatment of Fibrotic Diseases. *J Pharmacol Exp Ther* 2007; **321**: 35-44.

18. Daniels CE, Lasky JA, Limper AH, *et al.* Imatinib Treatment for Idiopathic Pulmonary Fibrosis: Randomized Placebo-Controlled Trial Results. *Am J Respir Crit Care Med* 2010; **181**: 604-610.
19. Yang J, Velikoff M, Canalis E, *et al.* Activated Alveolar Epithelial Cells Initiate Fibrosis through Autocrine and Paracrine Secretion of Connective Tissue Growth Factor. *Am J Physiol Lung Cell Mol Physiol* 2014; **306**: L786-796.
20. Yang J, Wheeler SE, Velikoff M, *et al.* Activated Alveolar Epithelial Cells Initiate Fibrosis through Secretion of Mesenchymal Proteins. *Am J Pathol* 2013; **183**: 1559-1570.
21. Chapman HA. Epithelial Responses to Lung Injury: Role of the Extracellular Matrix. *Proc Am Thorac Soc* 2012; **9**: 89-95.
22. Moore BB, Hogaboam CM. Murine Models of Pulmonary Fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2008; **294**: L152-160.
23. White ES, Borok Z, Brown KK, *et al.* An American Thoracic Society Official Research Statement: Future Directions in Lung Fibrosis Research. *Am J Respir Crit Care Med* 2016; **193**: 792-800.
24. Brownell R, Kaminski N, Woodruff PG, *et al.* Precision Medicine: The New Frontier in Idiopathic Pulmonary Fibrosis. *Am J Respir Crit Care Med* 2016; **193**: 1213-1218.
25. Horowitz JC, Osterholzer JJ, Marazioti A, *et al.* "Scar-Cinoma": Viewing the Fibrotic Lung Mesenchymal Cell in the Context of Cancer Biology. *Eur Respir J* 2016; **47**: 1842-1854.

Figure Legend:

Figure 1: Pleiotropic effects of different fibroblast growth factors. FGF-1 (red) prevents TGF- β 1 induced alveolar epithelial cell (AEC) transition to a mesenchymal cell phenotype (EMT) while also preventing TGF- β 1 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 has FGF-9 and FGF-18). FGF-7 (also known as keratinocyte growth factor), promotes proliferation of alveolar epithelial cells.

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