EDITORIALS

A FOX-like Mechanism Regulating Lung Fibroblasts: Are We Getting There?

Excessive fibroblast proliferation, transdifferentiation (into myofibroblasts), and matrix (largely collagen) production are key features of interstitial lung diseases (ILDs) and reflect the response of the mesenchymal cell population to a given form of chronic lung injury, be it primarily chronic epithelial injury (as in case of idiopathic pulmonary fibrosis [IPF]) or chronic inflammation (as in the case of hypersensitivity pneumonia). Notwithstanding this important aspect of causality, the activated (myo)fibroblast population seems to develop mechanisms of self-perpetuation (1–3), the result of which is a decoupling of the fibroblast behavior from the original injurious process. To this end, the activated (myo) fibroblast turns from a victim into a culprit and actively maintains the process of progressive scarring of the lung, with all the devastating consequences for the affected patients.

From a clinical point of view, the concept of self-perpetuating fibroblast activation being decoupled from the original trigger reflects the clinical observation of patients with different forms of ILD showing a cancer-like (1) progressive fibrotic phenotype. According to more recent suggestions, patients showing this phenotype should be "lumped" together (4), as they seem to benefit from antifibrotic treatment modalities. In more detail, nintedanib has been proven effective in lung fibrosis in systemic sclerosis (5) and in progressive fibrotic lung disease other than IPF (6), and it has already been authorized for these indications. Likewise, pirfenidone has shown efficacy in slowing the decline of forced vital capacity in clinical trials undertaken in unclassifiable ILDs (7) or in some defined forms of progressive, fibrotic ILDs (chronic hypersensitivity pneumonia, collagen vascular disease ILD, asbestosis, and fibrotic nonspecific interstitial pneumonia) (8, 9). Still, a better understanding of the causative triggers for ILD development represents a top priority in ILD research and could be the basis for trigger-oriented, more causative treatment modalities, which—hopefully—could act in synergy with the existing and future more global and more efficient antifibrotic drugs.

What are the molecular pathways underlying the persistent and self-perpetuating activation of fibroblasts in lungs of patients with IPF?

In an article by Miyashita and colleagues in this issue of the *Journal* (pp. 831–842), the authors provide new answers to this pertinent question (10). An unsupervised, unbiased screening of the publicly available FANTOM5 (Functional ANnoTation Of the Mammalian Genome 5) database, including 45 different fibroblast lines, identified the FOX (forkhead box) transcription factor (TF) *FOXL1*, a marker of fibroblast identity, as being highly activated in lung fibroblasts. Further support was provided by analyzing the ENCODE (The Encyclopedia of DNA Elements) database and by their finding that the *FOXL1* gene was selectively hypomethylated in lung fibroblasts and associated with super enhancers. After a

knockdown of *FOXL1* in normal human lung fibroblasts, the authors identified several genes being differentially regulated, and these belonged to "inflammatory responses," "cell–cell signaling," and "regulation of cell growth" groups. Furthermore, by performing gene-set enrichment analysis, they identified commonalities between their siFOXL-downregulated signature and a previously published TAZ-regulated signature, suggestive of an interaction. Indeed, 12 common genes were identified to be differentially regulated in both signatures, and these included CTGF, GREM1 (gremlin 1), and FSTL1 (follistatin-like 1), which are BMP signaling antagonists. To further understand the interaction between FOXL1 and BMP signaling, the authors analyzed the expression of these factors by quantitative PCR in 17 different lung fibroblast lines and observed a positive correlation between FOXL1 expression and that of BMP2, BMP4, and GREM1. *In vitro*, BMP4 treatment of NHLF enhanced, whereas FOXL1 knockdown suppressed, cell proliferation as well as collagen gel contraction. Finally, the authors analyzed the regulation of FOXL1 in IPF lungs by analyzing the GSE2592 dataset for transcriptome levels by measuring the transcript levels of FOXL1 and GREMs/ FSTL1 in normal and IPF lung tissues and in isolated lung fibroblasts. Here, upregulation of FOXL1 correlated with enhanced GREM1 and FESTL1 expression and could be ascribed to the lung fibroblast.

Essential questions that arise from these mechanistic studies include:

1) Specificity of FOXL1 in mediating target gene regulation and fibroproliferative processes.

The FOXL1 binding-site analysis performed in the article is indeed not selective for FOXL1, as more than 20 FOX TFs are expressed in these particular cells that are all predicted to bind to the same consensus motif (11). Previous work demonstrated that the FOXO3 and FOXM1 TFs play a major role in driving lung fibrogenesis (12–14). Thus, it is important to investigate the cross-talk among the various FOX TFs (FOXO3, FOXM1, FOXL1, FOXF1, and others) (15) during IPF pathogenesis. It also raises the question of whether inhibition of any FOX TFs will result in attenuation of lung fibrosis, and, if so, which one among these should be targeted therapeutically.

2) FOXL1 association with super enhancers and its relevance to transcriptional control.

The analysis performed by the authors simply hints toward the gene regulation of *FOXL1/FOXC2* and *FOXF1/FENDRR* genes by distal super enhancers. Additional experiments are needed to show that FOXL1 largely exerts its transcriptional control via super enhancers that are marked by H3K27ac/H3K4 me3 and that FOXL1 is necessary for this chromatin mark at bound super enhancers and the activity of the

associated genes. These experiments will establish FOXL1-mediated transcriptional regulation at super enhancers and will provide an expanded set of target genes, resulting in a fundamental source to study FOXL1 function in normal and IPF settings.

3) Functional interaction between FOXL1 and YAP–TAZ signaling in the regulation of BMP and PDGF signaling molecules.

The authors determined that FOXL1 downstream target genes have an enrichment of YAP/TAZ (TEAD) consensus binding sites together with FOX consensus binding sites, and both FOX and TEAD consensus binding sites have been identified in the promoter regions of BMP and PDGF signaling pathway molecules. These findings suggest coregulation of FOXL1 target genes by YAP–TAZ signaling. This is compatible with a recent publication showing a functional interaction of YAP1 and another FOX family member, FOXO1, in cardiomyocytes (16). Although these studies provide some insight into the interaction of FOXL1 and YAP/TAZ, sophisticated and integrative epigenetic approaches such as chromatin IP assays and assessment of chromatin accessibility upon individual TF knockdown are warranted to confirm independent or codependent cooccupancy of FOXL1 and YAP/TAZ on FOXL1 target genes.

4) In vivo significance and development of novel therapeutic strategies focusing on FOXL1.

Although *in vitro* studies revealed a crucial role of FOXL1 in lung fibroblast proliferation and contraction, it is essential to perform fibroblast-specific loss-of-FOXL1-function experiments *in vivo* to implicate FOXL1 as a crucial player in the pathogenesis of IPF.

Taken together, our knowledge regarding the regulation of excessive fibroblast activation and extracellular matrix production steadily increases, including our understanding of the factors that are crucial for fibroblast identity. As a result, we all hope to see new innovative drugs that are able not only to interrupt the vicious cycle of perpetuation but also to completely stop further progression of fibrotic lung disease. ■

Soni Savai Pullamsetti, Ph.D.
University of Giessen and Marburg Lung Center
Justus-Liebig University Giessen
Giessen, Germany
and
Max Planck Institute for Heart and Lung Research
Bad Nauheim, Germany

Andreas Günther, M.D.
Center for Interstitial and Rare Lung Diseases
Justus-Liebig University Giessen
Giessen, Germany
Agaplesion Lung Clinic Waldhof-Elgershausen
Greifenstein, Germany
and
European Idiopathic Pulmonary Fibrosis Registry and Biobank
Gießen, Germany

ORCID ID: 0000-0003-0440-8831 (S.S.P.).

References

1. Vancheri C. Common pathways in idiopathic pulmonary fibrosis and cancer. *Eur Respir Rev* 2013;22:265–272.
2. Korfei M, Skwarna S, Henneke I, MacKenzie B, Klymenko O, Saito S, *et al*. Aberrant expression and activity of histone deacetylases in sporadic idiopathic pulmonary fibrosis. *Thorax* 2015;70: 1022–1032.
3. Rubio K, Singh I, Dobersch S, Sarvari P, Günther S, Cordero J, *et al*. Inactivation of nuclear histone deacetylases by EP300 disrupts the MiCEE complex in idiopathic pulmonary fibrosis. *Nat Commun* 2019; 10:2229.
4. Wells AU, Brown KK, Flaherty KR, Kolb M, Thannickal VJ; IPF Consensus Working Group. What's in a name? That which we call IPF, by any other name would act the same. *Eur Respir J* 2018;51: 1800692.
5. Miles WM, Chang MS, Heger JJ, Rinkenberger RL, Zipes DP, Prystowsky EN. Electrophysiologic and antiarrhythmic effects of oral encainide in patients with atrioventricular nodal reentry or nodoventricular reentry. *Am Heart J* 1987;114:26–33.
6. Flaherty KR, Wells AU, Cottin V, Devaraj A, Walsh SLF, Inoue Y, *et al*.; INBUILD Trial Investigators. Nintedanib in progressive fibrosing interstitial lung diseases. *N Engl J Med* 2019;381: 1718–1727.
7. Maher TM, Corte TJ, Fischer A, Kreuter M, Lederer DJ, Molina-Molina M, *et al*. Pirfenidone in patients with unclassifiable progressive fibrosing interstitial lung disease: a double-blind, randomised, placebo-controlled, phase 2 trial. *Lancet Respir Med* 2020;8: 147–157.
8. Behr J, Neuser P, Prasse A, Kreuter M, Rabe K, Schade-Brittinger C, *et al*. Exploring efficacy and safety of oral Pirfenidone for progressive, non-IPF lung fibrosis (RELIEF) - a randomized, double-blind, placebo-controlled, parallel group, multi-center, phase II trial. *BMC Pulm Med* 2017;17:122.
9. Guenther A, Prasse A, Kreuter M, Neuser P, Rabe K, Bonella F, *et al*. Late breaking abstract - exploring efficacy and safety of oral Pirfenidone for progressive, non-IPF lung fibrosis (RELIEF). *Eur Respir J* 2019; 54(suppl 63):RCT1879.
10. Miyashita N, Horie N, Suzuki HI, Saito M, Mikami Y, Okuda K, *et al*. FOXL1 regulates lung fibroblast function via multiple mechanisms. *Am J Respir Cell Mol Biol* 2020;63:831–842.
11. Golson ML, Kaestner KH. Fox transcription factors: from development to disease. *Development* 2016;143:4558–4570.
12. Al-Tamari HM, Dabral S, Schmall A, Sarvari P, Ruppert C, Paik J, *et al*. FoxO3 an important player in fibrogenesis and therapeutic target for idiopathic pulmonary fibrosis. *EMBO Mol Med* 2018;10: 276–293.
13. Nho RS, Hergert P, Kahm J, Jessurun J, Henke C. Pathological alteration of FoxO3a activity promotes idiopathic pulmonary fibrosis fibroblast proliferation on type I collagen matrix. *Am J Pathol* 2011; 179:2420–2430.
14. Penke LR, Speth JM, Dommeti VL, White ES, Bergin IL, Peters-Golden M. FOXM1 is a critical driver of lung fibroblast activation and fibrogenesis. *J Clin Invest* 2018;128:2389–2405.
15. Yao S, Fan LY-N, Lam EW-F. The FOXO3-FOXM1 axis: a key cancer drug target and a modulator of cancer drug resistance. *Semin Cancer Biol* 2018;50:77–89.
16. Morikawa Y, Zhang M, Heallen T, Leach J, Tao G, Xiao Y, *et al*. Actin cytoskeletal remodeling with protrusion formation is essential for heart regeneration in Hippo-deficient mice. *Sci Signal* 2015;8: ra41.