RNA Sequencing Uncovers Antifibrotic Genes during Lung Fibrosis Resolution

Progressive scarring of the lungs is a lethal outcome of idiopathic pulmonary fibrosis (IPF) (1). The U.S. Food and Drug Administration–approved drugs nintedanib and pirfenidone have been shown to reduce the rate of progression of IPF; however, they cannot halt or reverse lung fibrosis (2). The mechanisms of action of these drugs remain poorly understood, but they likely target pathways involved in lung fibrosis initiation and development (3) rather than mechanisms promoting fibrosis resolution. Thus, further understanding of the biological and molecular mechanisms promoting resolution of persistent lung fibrosis is desperately needed to develop novel drugs that may reverse lung fibrosis, ultimately inducing regeneration of the damaged lung tissue. In this issue of the *Journal*, Tan and colleagues (pp. 453–464) describe studies using RNA-sequencing (RNA-seq) analysis to identify endogenous antifibrotic genes that promote resolution of lung fibrosis *in vivo* in mouse models of lung fibrosis (4).

Lung fibrosis has traditionally been considered an irreversible process. Although the lungs lack the dramatic regenerative capacity of the liver (5), lung fibrosis has been shown to spontaneously resolve in human lung diseases, including acute respiratory distress syndrome, severe acute respiratory syndrome, and coronavirus disease (COVID-19) (6–9). Similarly, lung fibrosis resolves in most murine lung injury models induced by LPS, bleomycin, asbestos fibers, or FITC (5, 10, 11), especially in commonly used young C57BL/6 mice. Taking advantage of these self-resolving models of lung fibrosis in young mice, Tan and colleagues applied RNA transcriptomics to identify genes and mechanisms associated with fibrosis resolution in the bleomycin-induced lung fibrosis model. They first investigated changes in gene expression of primary lung fibroblasts isolated from healthy lungs compared with fibroblasts isolated from lungs of bleomycin-challenged mice at Day 14 (peak of fibrosis) and Day 30 (early-stage fibrosis resolution). Notably, the authors used the Col1a1-GFP reporter mouse to facilitate fluorescence-activated cell sorting of collagen-producing cells in this model before RNA-seq analysis (12). They identified 3,018 candidate genes whose expression was increased/decreased during the peak of lung fibrosis

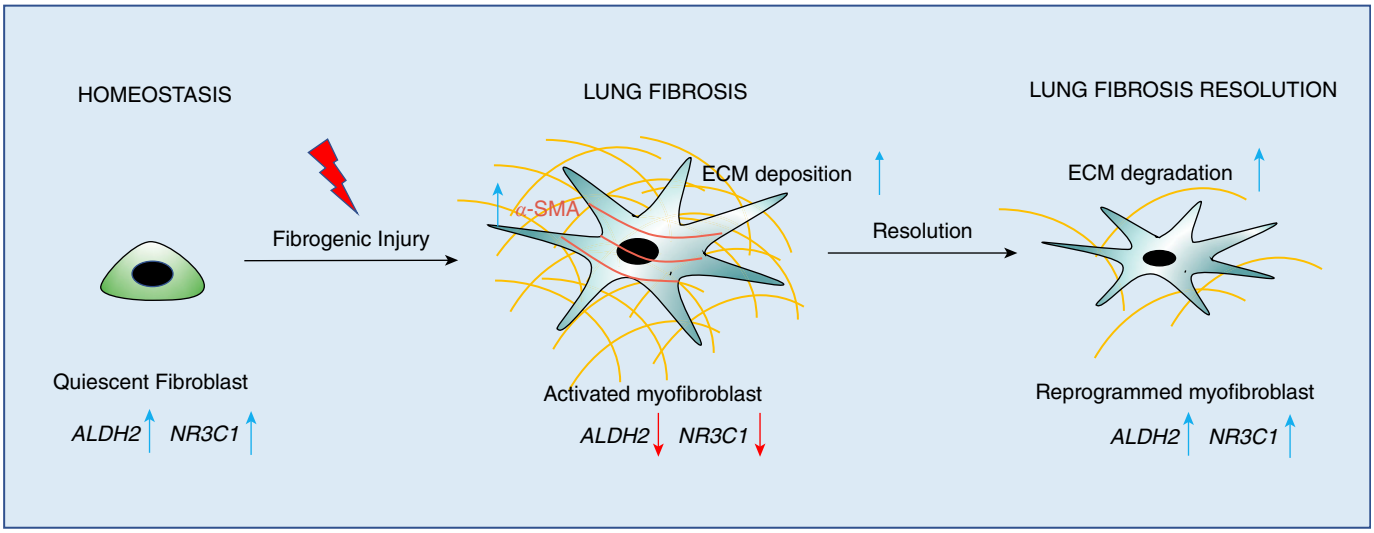

Figure 1. Lung fibrosis resolution is associated with upregulation of antifibrotic genes in fibroblasts. Activated α-*SMA*$^{+}$ myofibroblasts display exaggerated ECM production during lung fibrogenesis. In the current study, Tan and colleagues (4) use RNA-sequencing analysis to investigate endogenous antifibrotic genes that promote resolution of fibrosis using the self-resolving murine model of bleomycin-induced lung fibrosis. They identify two antifibrotic genes, *ALDH2* and *NR3C1*, whose expression is reduced at the peak of lung fibrosis but is restored during fibrosis resolution, providing novel mechanistic insights for lung fibrosis resolution. Blue arrows indicate upregulation/increase levels of these genes. Red arrows indicate downregulation/decrease levels of these genes. α-SMA = α-smooth muscle actin; *ALDH2* = aldehyde dehydrogenase 2 family member; ECM = extracellular matrix; *NR3C1* = nuclear receptor subfamily 3 group C member 1.

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at Day 14 and normalized to baseline by Day 30 during the resolution of fibrosis.

Pathway analysis identified two genes, *ALDH2* and *NR3C1*, whose expression was decreased at the peak of lung fibrosis but restored during fibrosis resolution (Figure 1). The *ALDH2* gene encodes a metabolic enzyme in the oxidative pathway of alcohol metabolism, suggesting metabolic reprogramming of lung fibroblasts during fibrosis resolution. The *NR3C1* gene encodes the GR (glucocorticoid receptor), which controls cell metabolism and immune responses. To validate downregulation of these two antifibrotic genes in human fibrotic disease, the authors mined publicly available, single-cell RNA-seq data sets to verify that *ALDH2* and *NR3C1* transcripts were also decreased in IPF as compared with normal lungs (12). They further confirmed that protein expression levels of ALDH2 and NR3C1 were lower in human IPF fibroblasts compared with healthy lung fibroblasts. *In vitro*, forced overexpression of *ALDH2* and *NR3C1* by a CRISPR activation approach in primary IPF lung fibroblasts showed that increased expression of both genes led to reduced expression of profibrotic genes, including *COL1a1* and *ACTA2*. Furthermore, *ALDH2* and *NR3C1* overexpression inhibited TGF-β1–induced profibrotic effects on IPF lung fibroblasts, providing potential mechanistic insight into the role of *ALDH2* and *NR3C1* in promoting fibrosis resolution by limiting fibroblast profibrotic activities.

The persistence of activated myofibroblasts is known to be a hallmark of progressive organ fibrosis (13). The study by Tan and colleagues suggests that upregulation of the antifibrotic genes *ALDH2* and *NR3C1* promotes lung fibrosis resolution by impairing TGF-β1–induced myofibroblast activation and extracellular matrix synthesis; however, the full spectrum of mechanisms by which *ALDH2* and *NR3C1* modulate the biology of activated myofibroblasts and promote lung fibrosis resolution *in vivo* deserve further investigation. Potential mechanisms related to fibrosis regression include myofibroblast deactivation, reprogramming from scar-forming to scar-resolving cells, and induction of senescent myofibroblast apoptosis (13–15). Thus, further molecular studies are needed to understand how *ALDH2* and *NR3C1* modulate not only the TGF-β1 pathway but also signaling pathways involved in myofibroblast fate and persistence. More importantly, the current study suggests that therapeutically enhancing *ALDH2* and *NR3C1* expression may serve as a novel strategy for resolving established lung fibrosis by restoring the normal tissue repair program, a hypothesis that could be tested on aged mice, which show non–self-resolving pulmonary fibrosis. Taken together, the results of the current study open the door to harnessing endogenous repair mechanisms to treat established lung fibrosis. ■

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References

1. Richeldi L, Collard HR, Jones MG. Idiopathic pulmonary fibrosis. *Lancet* 2017;389:1941–1952.
2. Maher TM, Strek ME. Antifibrotic therapy for idiopathic pulmonary fibrosis: time to treat. *Respir Res* 2019;20:205.
3. Knüppel L, Ishikawa Y, Aichler M, Heinzelmann K, Hatz R, Behr J, *et al*. A novel antifibrotic mechanism of nintedanib and pirfenidone: inhibition of collagen fibril assembly. *Am J Respir Cell Mol Biol* 2017; 57:77–90.
4. Tan Q, Link PA, Meridew JA, Pham TX, Caporarello N, Ligresti G, *et al*. Spontaneous lung fibrosis resolution reveals novel antifibrotic regulators. *Am J Respir Cell Mol Biol* 2021;64:453–464.
5. Glasser SW, Hagood JS, Wong S, Taype CA, Madala SK, Hardie WD. Mechanisms of lung fibrosis resolution. *Am J Pathol* 2016;186: 1066–1077.
6. Xie L, Liu Y, Fan B, Xiao Y, Tian Q, Chen L, *et al*. Dynamic changes of serum SARS-coronavirus IgG, pulmonary function and radiography in patients recovering from SARS after hospital discharge. *Respir Res* 2005;6:5.
7. Burnham EL, Hyzy RC, Paine R III, Coley C II, Kelly AM, Quint LE, *et al*. Chest CT features are associated with poorer quality of life in acute lung injury survivors. *Crit Care Med* 2013;41:445–456.
8. Burnham EL, Janssen WJ, Riches DW, Moss M, Downey GP. The fibroproliferative response in acute respiratory distress syndrome: mechanisms and clinical significance. *Eur Respir J* 2014;43: 276–285.
9. Pan F, Ye T, Sun P, Gui S, Liang B, Li L, *et al*. Time course of lung changes on chest CT during recovery from coronavirus disease 2019 (COVID-19). *Radiology* 2020;295:715–721.
10. Moore BB, Hogaboam CM. Murine models of pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2008;294:L152–L160.
11. Yang H-Z, Wang J-P, Mi S, Liu HZ, Cui B, Yan HM, *et al*. TLR4 activity is required in the resolution of pulmonary inflammation and fibrosis after acute and chronic lung injury. *Am J Pathol* 2012;180: 275–292.
12. Tsukui T, Sun K-H, Wetter JB, Wilson-Kanamori JR, Hazelwood LA, Henderson NC, *et al*. Collagen-producing lung cell atlas identifies multiple subsets with distinct localization and relevance to fibrosis. *Nat Commun* 2020;11:1920.
13. Hinz B, Lagares D. Evasion of apoptosis by myofibroblasts: a hallmark of fibrotic diseases. *Nat Rev Rheumatol* 2020;16:11–31.
14. Jun J-I, Lau LF. Resolution of organ fibrosis. *J Clin Invest* 2018;128: 97–107.
15. Lagares D, Santos A, Grasberger PE, Liu F, Probst CK, Rahimi RA, *et al*. Targeted apoptosis of myofibroblasts with the BH3 mimetic ABT-263 reverses established fibrosis. *Sci Transl Med* 2017;9:eaal3765.