Out of Tune: Fibroblasts Turn Fibrotic When They Lack a FENDRR

Idiopathic pulmonary fibrosis (IPF) is a progressive and incurable disease. Patients have a mean life expectancy of 3–5 years after diagnosis (1). IPF is characterized by widespread remodeling of lung tissue, with excessive deposits of collagen and fibronectin in the extracellular matrix (ECM) and pockets of fibrotic foci. Lung function and gas exchange are significantly impaired by the loss of alveolar space and increased tissue stiffness. In fibrotic disease, unchecked signaling by TGF-β (transforming growth factor-β) and other profibrotic cytokines pushes fibroblasts to become contractile myofibroblasts expressing ACTA2, large amounts of collagen, and other ECM components needed to drive tissue remodeling and disease (2, 3). Therefore, to identify novel strategies to combat IPF, it is vital to understand the molecular mechanisms involved in fibroblast-to-myofibroblast differentiation and excessive ECM production. In this issue of the *Journal*, Huang and colleagues (pp. 440–453) report on novel roles for the long noncoding RNA (lncRNA) FENDRR (fetal-lethal noncoding developmental regulatory RNA) in controlling the fibrotic phenotype of lung fibroblasts (4).

lncRNAs are ncRNAs that are typically longer than 200 nt and are transcribed from DNA that was once thought to be "junk" (5). These RNAs are larger than small ncRNAs such as microRNAs, Piwi-interacting RNAs, siRNAs, and small nucleolar RNAs, which are typically ∼20–180 nt in length. Current estimates suggest that ∼20,000 distinct lncRNAs are present in humans (6). Despite their relative abundance, their role in mammalian development and disease is still poorly understood. For example, mice lacking FENDRR exhibit embryonic lethality because FENDRR is essential for proper heart and body wall development (7). However, it is also highly expressed in the adult lung. Using deposited RNA-sequencing data from IPF and control samples, Huang and colleagues discovered that FENDRR expression was reduced in samples from patients with IPF and in an animal model of lung fibrosis. Adenoviral delivery of FENDRR attenuated bleomycin-induced lung fibrosis in mice, suggesting that FENDRR has therapeutic potential. So how does FENDRR block fibrosis?

Earlier studies found that FENDRR resides in the nucleus and alters chromatin-associated histone modifications in the fetal mesenchyme (7). In contrast, Huang and colleagues found that FENDRR is largely cytoplasmic in adult lung fibroblasts, where it can influence TGF-β–induced collagen and ACTA2 levels. Furthermore, a FENDRR RNA-protein pulldown experiment showed that it complexed with IRP1 (iron-regulatory protein 1). IRP1, which additionally functions as aconitase in the citric acid cycle, plays a key role in controlling intracellular iron homeostasis. When iron is low, IRP1 binds iron-response elements located within mRNAs encoding proteins that are involved in iron import and storage to promote iron uptake (8). Interestingly, overexpression of FENDRR reduced intracellular iron levels and aconitase activity, suggesting an ability to block IRP1 function. FENDRR also contains six conserved miR-214 binding sites, which could serve to bind or "sponge" up miR-214, preventing it from binding to target mRNAs involved in fibrosis. In fact, blocking miR-214 reduces renal fibrosis (9). Huang and colleagues found that FENDRR could indeed block miR-214 from repressing a miR-214 reporter gene in HEK293FT cells, suggesting that FENDRR might limit miR-214 function in lung fibroblasts. Taken together, the findings of Huang and colleagues suggest that FENDRR is an antifibrotic lncRNA that can block both IRP1 and miR-214 functions to limit the fibrotic phenotype of lung fibroblasts (Figure 1).

Although this study uncovers a novel role for FENDRR in attenuating fibrotic signaling in lung fibroblasts, there are several unanswered questions that require attention. The authors found that cytoplasmic FENDRR influences the fibrotic phenotype of fibroblasts, yet FENDRR was originally identified in the nucleus of fetal mesenchymal cells. The significance of this discrepancy remains to be determined. Hypothetically, nuclear forms of FENDRR seen in the fetus influence fibroblast differentiation, whereas the cytoplasmic forms seen in adults influence the fibroblast phenotype. Because FENDRR modifies TGF-β signaling, this may help explain why TGF-β promotes alveolar simplification in neonates and fibrosis in adults. Does FENDRR expression increase under all inflammatory states or just those that promote fibrosis? Such information would help us understand how inflammation can be both fibrotic and nonfibrotic. In the current study, Huang and colleagues overexpressed FENDRR in the mouse lung using adenovirus as the carrier. Given that adenovirus targets epithelial cells, it will be important to determine whether overexpression of FENDRR affected epithelial injury and survival. A previous report found that FENDRR could also reduce non–small-cell lung cancer cell growth by sponging miR-761 (10). Although fibroblast expression of FENDRR may be beneficial, it will be important to determine whether targeting FENDRR to epithelial cells inhibits proliferation, because that could be detrimental to restoring lung function. Another interesting question is whether or not FENDRR could be delivered as a paracrine or autocrine signal to suppress fibrotic signaling. Fibroblasts can produce antifibrotic extracellular vesicles (EVs) that could be delivered to neighboring cells and prevent myofibroblast formation and ECM production (11). Although it was found that the antifibrotic lipid mediator PGE_2 was in the cargo of the EVs, it would be interesting to determine whether FENDRR could be delivered as well. Indeed, lncRNAs have been found in fibroblast-derived EVs and other membrane-bound carriers such as exosomes (12, 13). Other studies have identified various factors, including RNAs such as microRNAs and lncRNAs, that are differentially expressed in PF and IPF fibroblasts (14, 15). How these ncRNAs coordinate the fibrotic phenotype of fibroblasts

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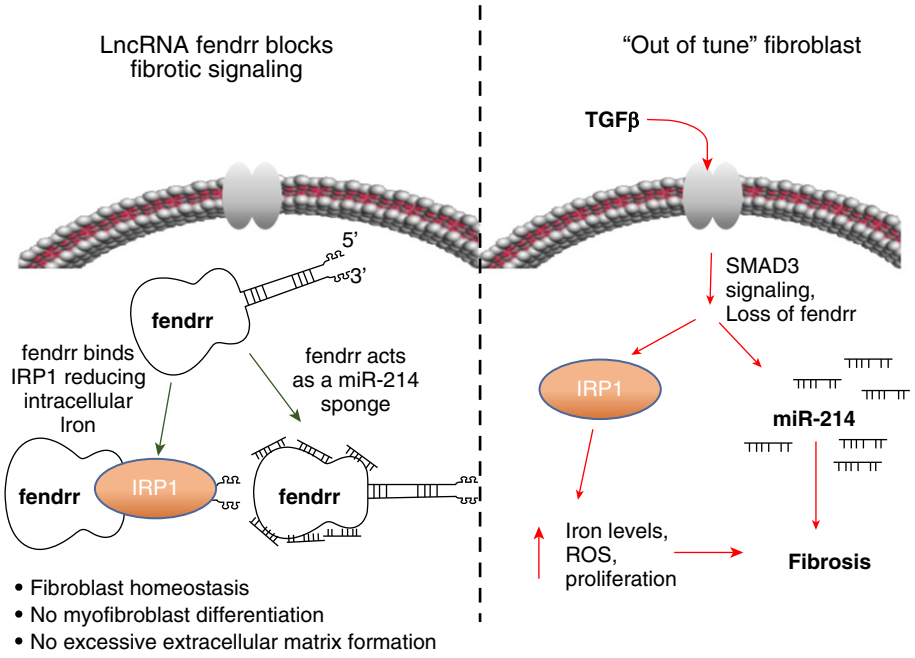

Figure 1. The long noncoding RNA (lncRNA) FENDRR (fetal-lethal noncoding developmental regulatory RNA) blocks IRP1 (iron-regulatory protein 1) and miR-214 functions to prevent fibrotic signaling in lung fibroblasts. FENDRR is an antifibrotic lncRNA that can block both IRP1 and miR-214 functions to limit fibrotic signaling in the cytoplasm of lung fibroblasts. FENDRR can sequester IRP1, preventing increases in intracellular iron that could lead to increased production of reactive oxygen species (ROS) and to fibroblast activation and proliferation, which drive fibrotic signaling. FENDRR also contains six binding sites for the profibrotic microRNA miR-214. Thus, FENDRR can also act as a miR-214 "sponge" and block its fibrotic activity. In idiopathic pulmonary fibrosis, FENDRR levels are decreased, which may be caused by elevated TGF-β (transforming growth factor-β)-induced SMAD3 signaling. When FENDRR levels are low, fibroblasts become "out of tune," turning profibrotic with higher levels of intracellular iron and miR-214, which promote fibrotic signaling. miR-214 = microRNA 214.

with FENDRR is unexplored. Although the ability of the lncRNA FENDRR to limit fibrotic signaling is very interesting, more studies are needed to further characterize the role of FENDRR and other lncRNAs both in lung fibroblasts and *in vivo* so that we can understand why fibroblasts are "out of tune" in IPF. ■

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References

1. Kolahian S, Fernandez IE, Eickelberg O, Hartl D. Immune mechanisms in pulmonary fibrosis. *Am J Respir Cell Mol Biol* 2016;55:309–322.
2. Sime PJ, Xing Z, Graham FL, Csaky KG, Gauldie J. Adenovector-mediated gene transfer of active transforming growth factor-beta1 induces prolonged severe fibrosis in rat lung. *J Clin Invest* 1997;100:768–776.
3. Khalil N, O'Connor RN, Unruh HW, Warren PW, Flanders KC, Kemp A, *et al*. Increased production and immunohistochemical localization of transforming growth factor-beta in idiopathic pulmonary fibrosis. *Am J Respir Cell Mol Biol* 1991;5:155–162.
4. Huang C, Liang Y, Zeng X, Yang X, Xu D, Guo X, *et al*. Long Noncoding RNA FENDRR exhibits antifibrotic activity in pulmonary fibrosis. *Am J Respir Cell Mol Biol* 2020;62:440–453.
5. Kapranov P, Cheng J, Dike S, Nix DA, Duttagupta R, Willingham AT, *et al*. RNA maps reveal new RNA classes and a possible function for pervasive transcription. *Science* 2007;316:1484–1488.
6. Hon CC, Ramilowski JA, Harshbarger J, Bertin N, Rackham OJ, Gough J, *et al*. An atlas of human long non-coding RNAs with accurate 5′ ends. *Nature* 2017;543:199–204.
7. Grote P, Wittler L, Hendrix D, Koch F, Währisch S, Beisaw A, *et al*. The tissue-specific lncRNA Fendrr is an essential regulator of heart and body wall development in the mouse. *Dev Cell* 2013;24:206–214.
8. Wang J, Pantopoulos K. Regulation of cellular iron metabolism. *Biochem J* 2011;434:365–381.
9. Denby L, Ramdas V, Lu R, Conway BR, Grant JS, Dickinson B, *et al*. MicroRNA-214 antagonism protects against renal fibrosis. *J Am Soc Nephrol* 2014;25:65–80.
10. Zhang MY, Zhang ZL, Cui HX, Wang RK, Fu L. Long non-coding RNA FENDRR inhibits NSCLC cell growth and aggressiveness by sponging miR-761. *Eur Rev Med Pharmacol Sci* 2018;22:8324–8332.
11. Anderson DD, Woeller CF, Chiang EP, Shane B, Stover PJ. Serine hydroxymethyltransferase anchors de novo thymidylate synthesis pathway to nuclear lamina for DNA synthesis. *J Biol Chem* 2012;287:7051–7062.
12. Yang X, Li Y, Zou L, Zhu Z. Role of exosomes in crosstalk between cancer-associated fibroblasts and cancer cells. *Front Oncol* 2019;9:356.
13. Kenneweg F, Bang C, Xiao K, Boulanger CM, Loyer X, Mazlan S, *et al*. Long noncoding RNA-enriched vesicles secreted by hypoxic cardiomyocytes drive cardiac fibrosis. *Mol Ther Nucleic Acids* 2019;18:363–374.
14. Salamo O, Mortaz E, Mirsaeidi M. Noncoding RNAs: new players in pulmonary medicine and sarcoidosis. *Am J Respir Cell Mol Biol* 2018;58:147–156.
15. Hadjicharalambous MR, Roux BT, Feghali-Bostwick CA, Murray LA, Clarke DL, Lindsay MA. Long non-coding RNAs are central regulators of the IL-1β-induced inflammatory response in normal and idiopathic pulmonary lung fibroblasts. *Front Immunol* 2018;9:2906.