Kindlin for the Fire: Targeting Proline Synthesis to Extinguish Matrix Production in Pulmonary Fibrosis

Pulmonary fibrosis is a scarring disease of the lung notable for epithelial injury followed by aberrant activation of lung fibroblasts, excessive matrix deposition, distortion of lung architecture, and abnormal gas exchange. Pulmonary fibrosis is commonly seen in patients with identified autoimmune disease or environmental exposures as well as in patients with no identified underlying cause (1). For patients with idiopathic pulmonary fibrosis (IPF), prognosis remains poor despite the emergence of antifibrotic medications, which have been shown to slow lung function decline. However, no existing treatment halts or reverses the progression of fibrosis once present. Targeting signaling pathways associated with collagen synthesis is an attractive avenue for the development of novel antifibrotic therapeutics, which is explored by Zhang and colleagues in this issue of the *Journal* (pp. 54–69). The authors hypothesized that targeting proline biosynthesis would reduce fibroblast activation and experimental pulmonary fibrosis. They addressed this question by testing whether kindlin-2 was critical for proline synthesis and subsequent lung fibroblast activation, collagen synthesis, and pulmonary fibrosis development (2).

As collagen is the primary component of extracellular matrix in pulmonary fibrosis, disruption of pathways that regulate collagen synthesis are of significant interest. In addition to transcriptional and translational regulation of collagen, several groups have focused on proline metabolism, as proline (and hydroxyproline) comprise 23% of collagen (3) and its abundance (or lack thereof) is critical for collagen synthesis. Several studies have described increased proline synthesis in pulmonary fibrosis (4). A recent publication made the connection that the translocation of kindlin-2 to the mitochondria and subsequent interaction with PYCR1 (pyrroline-5-carboxylate reductase 1) in lung cancer is critical for proline synthesis (5). PYCR1 is the enzyme that converts Δ^1-pyrroline-5-carboxylate into proline, the last step in proline synthesis from glutamate and ornithine. Targeting PYCR1 is sufficient to inhibit collagen synthesis in cancer-associated matrix–producing fibroblasts (6).

The kindlins (kindlin-1, kindlin-2, and kindlin-3) are a family of cytoplasmic focal adhesion proteins that bind to the cytoplasmic tail of β-integrin subunits, contain FERM domains, are critical in cytoskeletal reorganization in response to extracellular matrix (ECM), and function by increasing ligand–integrin binding affinity (7). The mediation of these protein interactions aids in assembly of ECM, maturation and localization of focal adhesions, cell spreading and migration, proliferation, differentiation, and cell survival. They are known to bind to ILK (8), β1-integrin, β3-integrin, and migfilin (9). Given their importance in many cellular processes, it is unsurprising that they also play an important role in many diseases. Loss of kindlin-1 leads to Kindler syndrome (10, 11), which is characterized by skin blistering and ulceration, photosensitivity, gingival atrophy and periodontal disease, reduced keratinocyte and epithelial adhesion, and increased apoptosis of keratinocytes. Loss of kindlin-3 leads to leukocyte adhesion deficiency type III, which is characterized by leukocyte adhesion deficits and deficient hematopoietic stem and progenitor cells (12). The loss of kindlin-2 is lethal in embryonic mice because of severe deficits in cellular adhesion to ECM and cytoskeletal organization (13, 14). Increased expression of kindlin-2 has been described in kidney fibrosis (15) through the activation of Erk1/2, Akt, and TGF-β signaling (16). During skin wound repair, fibroblasts within human scars express high concentrations of kindlin-2, which was shown to aid in the ability of fibroblasts to detect mechanical clues from the environment, driving their differentiation to myofibroblasts as mechanical stress increases during wound closure (17). Similarly, data presented in the current article reveal significant increases in kindlin-2 and its binding partner PYCR1 in αSMA^+ myofibroblasts in IPF tissue and in bleomycin-induced fibrosis in mice (2).

The connection of kindlin-2 to signaling pathways that are linked to progression of pulmonary fibrosis and its key role in directing normal wound repair in the skin make kindlin-2 an attractive target to inhibit the excessive repair process and matrix deposition occurring during pulmonary fibrosis. The authors show that TGFβ-1 treatment increased kindlin-2 and PYCR1 expression, kindlin-2 mitochondrial translocation, binding of kindlin-2 to PYCR1, and proline synthesis in fibroblasts. These effects were blocked by the depletion of kindlin-2 using lentiviral-mediated kindlin-2 shRNA. The authors also show that mice with intranasal adenoviral Cre-mediated depletion of kindlin-2 exhibit reduced bleomycin-induced proline synthesis and pulmonary fibrosis development. Furthermore, they found that treatment of lung fibroblasts with pirfenidone and nintedanib reduced kindlin-2 and PYCR1 expression and translocation to mitochondria and decreased proline synthesis.

Although this study provides compelling evidence for kindlin-2–dependent proline biosynthesis driving ECM production in pulmonary fibrosis, there are several questions that remain to be answered. First, although it appears that the critical cell type that requires kindlin-2 for the generation of pulmonary fibrosis is the fibroblast/myofibroblast, other cell types in the lung, including the epithelium and endothelium, interact with ECM, respond to TGFβ-1, and generate collagen and profibrotic growth factors in pulmonary fibrosis. Subsequent studies with cell-specific deletion of kindlin-2, for example, using *tcf21* to target lung fibroblasts (18), will be required to

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answer this question in a targeted manner. Second, further exploration of the mechanism behind kindlin-2 mitochondrial translocation and novel interaction with proteins outside of focal adhesion complexes, such as PYCR1, are still needed, as the mechanism by which kindlin-2 regulates PYCR1 remains unknown. The authors speculate that kindlin-2 prevents PYCR1 degradation through direct binding, but transcriptional regulation through an unknown mechanism is possible as well. Finally, the discovery that both pirfenidone and nintedanib disrupt kindlin-2–PYCR1 interactions and subsequent proline synthesis suggests that this pathway may be regulated by upstream profibrotic signaling, potentially through interaction with known receptor tyrosine kinases, including Src and EGFR (19, 20). As the phosphorylation of β3-integrin disrupts the ability of kindlin-2 to bind and coactivate it (21), therapeutics that alter the phosphorylation of integrins may also affect the other roles of kindlin-2.

The article by Zhang and colleagues highlights that understanding the mechanisms driving increased proline and collagen biosynthesis in the development and progression of pulmonary fibrosis is complex and relies on the interplay between ECM, metabolism, and fibroblast activation through the novel interaction between kindlin-2 and PYCR1. Although these pathways also appear to be affected by currently approved antifibrotic agents, the more precise targeting of kindlin-2 and proline biosynthesis may lead to improved therapies for pulmonary fibrosis. ■

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References

1. King TE Jr, Pardo A, Selman M. Idiopathic pulmonary fibrosis. *Lancet* 2011;378:1949–1961.
2. Zhang P, Wang J, Luo W, Yuan J, Cui C, Guo L, *et al.* Kindlin-2 acts as a key mediator of lung fibroblast activation and pulmonary fibrosis progression. *Am J Respir Cell Mol Biol* 2021;65:54–69.
3. Krane SM. The importance of proline residues in the structure, stability and susceptibility to proteolytic degradation of collagens. *Amino Acids* 2008;35:703–710.
4. Hamanaka RB, O'Leary EM, Witt LJ, Tian Y, Gökalp GA, Meliton AY, *et al.* Glutamine Metabolism Is Required for Collagen Protein Synthesis in Lung Fibroblasts. *Am J Respir Cell Mol Biol* 2019;61:597–606.
5. Guo L, Cui C, Zhang K, Wang J, Wang Y, Lu Y, *et al.* Kindlin-2 links mechano-environment to proline synthesis and tumor growth. *Nat Commun* 2019;10:845.
6. Kay EJ, Paterson K, Sumpton D, Stepanova E, Boldrini C, Hernandez-Fernaud JR, *et al.* Metabolic control of tumour extracellular matrix production in cancer-associated fibroblasts. bioRxiv; 2020 [accessed 2020 May 31]. Available from: https://doi.org/10.1101/2020.05.30.125237.
7. Larjava H, Plow EF, Wu C. Kindlins: essential regulators of integrin signalling and cell-matrix adhesion. *EMBO Rep* 2008;9:1203–1208.
8. Kadry YA, Huet-Calderwood C, Simon B, Calderwood DA. Kindlin-2 interacts with a highly conserved surface of ILK to regulate focal adhesion localization and cell spreading. *J Cell Sci* 2018;131: jcs221184.
9. Tu Y, Wu S, Shi X, Chen K, Wu C. Migfilin and Mig-2 link focal adhesions to filamin and the actin cytoskeleton and function in cell shape modulation. *Cell* 2003;113:37–47.
10. Jobard F, Bouadjar B, Caux F, Hadj-Rabia S, Has C, Matsuda F, *et al.* Identification of mutations in a new gene encoding a FERM family protein with a pleckstrin homology domain in Kindler syndrome. *Hum Mol Genet* 2003;12:925–935.
11. Siegel DH, Ashton GHS, Penagos HG, Lee JV, Feiler HS, Wilhelmsen KC, *et al.* Loss of kindlin-1, a human homolog of the Caenorhabditis elegans actin-extracellular-matrix linker protein UNC-112, causes Kindler syndrome. *Am J Hum Genet* 2003;73:174–187.
12. Kuijpers TW, van de Vijver E, Weterman MAJ, de Boer M, Tool ATJ, van den Berg TK, *et al.* LAD-1/variant syndrome is caused by mutations in FERMT3. *Blood* 2009;113:4740–4746.
13. Dowling JJ, Vreede AP, Kim S, Golden J, Feldman EL. Kindlin-2 is required for myocyte elongation and is essential for myogenesis. *BMC Cell Biol* 2008;9:36.
14. Dowling JJ, Gibbs E, Russell M, Goldman D, Minarcik J, Golden JA, *et al.* Kindlin-2 is an essential component of intercalated discs and is required for vertebrate cardiac structure and function. *Circ Res* 2008;102:423–431.
15. Bielesz B, Sirin Y, Si H, Niranjan T, Gruenwald A, Ahn S, *et al.* Epithelial Notch signaling regulates interstitial fibrosis development in the kidneys of mice and humans. *J Clin Invest* 2010;120:4040–4054.
16. Wei X, Xia Y, Li F, Tang Y, Nie J, Liu Y, *et al.* Kindlin-2 mediates activation of TGF-β/Smad signaling and renal fibrosis. *J Am Soc Nephrol* 2013;24:1387–1398.
17. He Y, Esser P, Schacht V, Bruckner-Tuderman L, Has C. Role of kindlin-2 in fibroblast functions: implications for wound healing. *J Invest Dermatol* 2011;131:245–256.
18. Park J, Ivey MJ, Deana Y, Riggsbee KL, Sörensen E, Schwabl V, *et al.* The Tcf21 lineage constitutes the lung lipofibroblast population. *Am J Physiol Lung Cell Mol Physiol* 2019;316:L872–L885.
19. Qu H, Tu Y, Guan JL, Xiao G, Wu C. Kindlin-2 tyrosine phosphorylation and interaction with Src serve as a regulatable switch in the integrin outside-in signaling circuit. *J Biol Chem* 2014;289:31001–31013.
20. Guo B, Gao J, Zhan J, Zhang H. Kindlin-2 interacts with and stabilizes EGFR and is required for EGF-induced breast cancer cell migration. *Cancer Lett* 2015;361:271–281.
21. Bledzka K, Bialkowska K, Nie H, Qin J, Byzova T, Wu C, *et al.* Tyrosine phosphorylation of integrin beta3 regulates kindlin-2 binding and integrin activation. *J Biol Chem* 2010;285:30370–30374.