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How Do We Know What We Are Missing? Loss of Signaling through CD148 Drives Fibroblast Activation in Pulmonary Fibrosis

Examination of signaling pathways associated with idiopathic pulmonary fibrosis (IPF) has proven to be complex, and easily identifiable targets with therapeutic efficacy still are lacking (1). Despite the increasing clinical trials dedicated to IPF and other fibrosing interstitial lung diseases and the approval of two antifibrotic therapies, nintedanib and pirfenidone, there is still no curative treatment (2). IPF remains a persistent disease characterized by damaged epithelium, fibroblast/myofibroblast accumulation and activation, excessive extracellular matrix (ECM) deposition, and progressive scarring (3). As our understanding of the pathogenesis has evolved and we recognize fibrosis as a process of aberrant wound healing (4), it is clear that identifying signaling pathways in each cellular population compared with changes seen in whole lung tissue will allow for increased specificity during novel therapeutic development (5). Dissecting out the role of overexpressed genes and pathways in cellular lineages is critical, as the upregulation may be an attempt at a normal repair process with the continued activation driving a fibrotic response. However, absent or downregulated pathways may also end up acting in a profibrotic manner by failing to provide signals of inhibition. This leads us to a critical question: how do we distinguish, find, and activate repair pathways that are missing?

In this issue of the *Journal*, Tsoyi and colleagues (pp. 312–325) examine one such antifibrotic signaling pathway specifically in fibroblasts: the receptor-like protein PTPRJ/CD148 (tyrosine phosphatase-eta) and its ligand syndecan-2 (6). An examination of α-smooth muscle actin–positive myofibroblasts within IPF tissue demonstrated decreased CD148 expression. Therefore, they hypothesized that decreased signaling through this antifibrotic pathway allows for hyperactivated PI3K/Akt/mTOR signaling, reduced autophagy, increased p62 accumulation, and subsequent NF-κB activation (6).

CD148 is highly expressed in the lungs and many other tissues as well as numerous cell lineages, including fibroblasts, endothelial cells, and leukocytes (7–9). It dephosphorylates and regulates proteins involved in fibrosis, including PDGF, EGF, and VEGF (10, 11), by inhibiting growth factor signals and proliferation, and its activation is antifibrotic in a mouse model of radiation-induced fibrosis (9). Interestingly, CD148 is significantly upregulated in synovial monocytes/macrophages in a mouse model of collagen-induced arthritis and in patients with rheumatoid arthritis (12).

In 2011, Whiteford and colleagues identified syndecan-2 as a novel ligand for CD148 (13). Syndecan-2 is one of four syndecans that comprise a family of heparan sulfate (HS) proteoglycans. Each syndecan contains a specific extracellular ectodomain, a conserved transmembrane domain, and a short cytoplasmic domain. Because of the interaction of HS-glycosaminoglycan chains with matrix proteins, cytokines, growth factors, and their receptors, syndecans are important signaling molecules in cancer, angiogenesis, and wound repair (14, 15). Soluble syndecans have been implicated in wound healing processes and are often generated in response to stress and pathogenesis. Syndecan-1 is increased in dermal fluid of mice during wound repair, and syndecan-1–deficient mice have delayed skin wound healing because of defective proliferation and migration of keratinocytes and epithelial cells (16, 17). Syndecan-4 is also significantly increased during dermal repair in mice and humans. It facilitates fibroblast adhesion through fibronectin, integrin interactions, and focal adhesion formation by binding to connective tissue growth factor. Syndecan-4 also interacts with tenascin-C (an ECM protein) during wound closure, and its expression closely regulates matrix contraction, fibronectin response, and fibroblast morphology (18). Syndecan-2 expression is increased during pulmonary fibrosis, is upregulated in the presence of TGF-β, can directly bind and regulate TGF-β through its ectodomain, and is expressed in macrophages and endothelial and mesenchymal cells (19–22). Syndecan-2 has been shown to support fibroblast proliferation, spreading, and attachment through activation of MMPs (23), and its shedding can inhibit angiogenesis through binding to its receptor CD148 on endothelial cells (13, 24–26). Syndecan-2 overproduction by macrophages acts in an antifibrotic manner via inhibition of TGF-β signaling in epithelial cells, thereby promoting the internalization of TGFβR1 and reducing cellular apoptosis. It also reduced fibroblast to myofibroblast differentiation and decreased ECM production (9, 20). Administration of recombinant human syndecan-2 was able to attenuate bleomycin-induced fibrosis (20).

In the current article, the authors extend their previous findings and examine the mechanistic role of CD148–syndecan-2 signaling in fibroblasts during pulmonary fibrosis (6). For the first time, they demonstrate that although syndecan-2 has clear antifibrotic functions, it is unable to exert them because of decreased levels of CD148 in IPF

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fibroblasts/myofibroblasts. Single-cell analysis of normal and IPF lungs supports this, with decreased expression in IPF myofibroblasts compared with controls (27, 28). They also show that targeted genetic loss of CD148 under the inducible control of the Col1a2 promoter in fibroblasts increased fibrosis development after bleomycin. An examination of the mechanism indicated that loss of signaling through CD148 reduced autophagy, enhanced apoptosis resistance, and activated the PI3K/Akt/mTOR pathways together with p62 accumulation and NF-κB activation, ultimately enhancing the profibrotic phenotype of fibroblasts (6).

Another strength of the manuscript lies in the development of a novel CD148-activating peptide sequence (SDC2-pep) derived from the ectodomain of syndecan-2. When given therapeutically during the development of bleomycin-induced pulmonary fibrosis, SCD2-pep inhibited pulmonary fibrosis development and activation of profibrotic genes. Ex vivo analysis in IPF precision-cut lung slices also resulted in inhibited ECM gene expression, cell contractility, reduced p62 expression, and increased sensitivity to FasL-induced apoptosis. Further testing to determine off-target and CD148-independent effects of the SCD2-pep will be required to validate the therapeutic potential of this target. In addition, the use of a persistent fibrotic model (29) will also be needed to determine whether the activation of CD148 during established disease initiates resolution and alters PI3K/Akt/mTOR-dependent increases in autophagy, p62 accumulation, and Nf-κB signaling. Finally, because CD148 is also suggested to be downregulated in the alveolar epithelial type I and II cells, additional experiments examining the role of CD148 activation in these cells and the regulation of tight junctions during epithelial response to injury will also be needed.

The article by Tsoyi and colleagues highlights that further understanding and exploration into the role of PTPs in fibrotic disease is warranted, as there is significant heterogeneity in their functions based on expression and cell type. In addition to their importance in fibrosis, PTP activation of signaling pathways, including PI3K/AKt, has been implicated in many cancers in regulating cell proliferation, apoptosis, and differentiation of mesenchymal cells. However, the signaling pathway of CD148–syndecan-2 is a novel interaction that could be further explored by the development of therapies that induce its upregulation to take advantage of its natural ligand syndecan-2, which is overexpressed in fibrotic lungs. Understanding the factors involved in driving the development of fibrosis becomes critical as we continue to define the natural antifibrotic pathways that are missing in progressive fibrotic disease. ■

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All Roads Lead to Rome? Resident or Interspecies Chimera-derived Pulmonary Endothelial Progenitors for Cell-based Therapy

Aberrant development or extensive damage to the lung endothelium are involved in diseases such as bronchopulmonary dysplasia (1), pulmonary arterial hypertension (2), or even coronavirus disease (COVID-19) (3). Coordinated regenerative responses of the lung endothelium and epithelium are necessary to restore lung function and architecture after various types of injury (4). Cell-based therapeutic approaches for pulmonary vascular diseases are currently at various stages of development, including clinical trials (5, 6). These approaches are propelled by basic studies that aim to elucidate endothelial cell heterogeneity and mechanisms of epithelial–endothelial cross-talk in lung development and disease (7, 8).

Endothelial cell heterogeneity across tissues has recently been described and analyzed in depth with the introduction of single-cell RNA-sequencing (9). The lung vascular field has also benefited from this tool that has allowed for the identification of capillary subpopulations in the mouse lung. The main source of endothelial heterogeneity arises from capillaries (7–10), an observation that is also conserved across organs (9) and that has been depicted in the human lung (11).

Pulmonary endothelial cells, specifically, include two distinct capillary types: general capillaries labeled by *cKit* (cKit tyrosine kinase, 85% of the endothelium) and Car4 endothelial cells (15%), named after their marker gene carbonic anhydrase 4 (7, 10). The morphology, location, and direct association of the Car4 cell to the alveolar type 1 cell (7) indicate a possible role in gas exchange, which has led to the name "aerocytes" (10); still, there is no functional data to support that role thus far. Car4 endothelial cells first appear at Embryonic Day 19, specified by VEGFA (vascular endothelial growth factor A) secreted by alveolar type 1 cells. However, earlier in lung development at Embryonic Day 17, there is only one type of capillary cell type that can be identified by the expression of *cKit*, a progenitor population that gives rise to the mature *cKit*$^+$ general capillaries and to the Car4 population (7).

In this issue of the *Journal*, Wang and colleagues (pp. 326–338) have identified additional heterogeneity within this cKIT$^+$ progenitor population consisting of *Foxf1* (forkhead box F1)-positive or -negative cells by means of single-cell RNA-sequencing (12). FOXF1$^+$cKIT$^+$ cells, also present in the human lung, decrease in the adult and are enriched in genes related to angiogenesis and endothelial cell proliferation. Underlining their progenitor potential, these cells were able to rescue neonatal angiogenesis in a mouse model of alveolar capillary dysplasia with misalignment of pulmonary veins (ACDMPV), a rare disease related to mutations in the *Foxf1* gene. Moreover, their presence improved alveologenesis, highlighting the developmental association and coordinated growth of the endothelium and epithelium.

In a similar vein to their previous work on lung epithelial reconstitution via intraspecies (mouse–mouse) blastocyst complementation (13), the authors then produced mouse endothelial cells via interspecies (mouse–rat) complementation (i.e., mouse embryonic stem cell injection into rat blastocysts). Mouse-derived cells were found incorporated in most tissues, including mouse endothelial cells in the rat pulmonary vasculature. Flow-cytometry–purified mouse FOXF1$^+$cKIT$^+$ lung endothelial progenitors from chimeric animals were able to improve neonatal angiogenesis and alveolarization in their ACDMPV mouse model after adoptive transfer. These cells were transcriptionally similar to endogenous FOXF1$^+$cKIT$^+$ progenitors, overall indicating that interspecies chimeras are, in principle, a viable way of producing functional endothelial progenitors via *in vivo* differentiation of pluripotent stem cells.

The presence of "transitional cells" in the vasculature—cells that coexpress markers of distinct phenotypes simultaneously—raises interesting questions about identity and potential (9). In the lung, we can find this phenomenon as part of the anatomical transition along the vascular tree, exemplified by cells that express *Esm1* (endothelial cell specific molecule 1), as well as macrovasculature and capillary genes, which are located in the transition zone from arteries to capillaries (7). Nevertheless, this transition could also be the result of cell fate change. Mainly, these cKIT$^+$ progenitors, which then subdivide into FOXF1-expressing cells to finally give rise to mature capillaries, point to the dynamic nature of the lung during development and how little is known about the mechanisms for endothelial heterogeneity and its role in regeneration (7). Whether FOXF1$^+$cKIT$^+$ progenitors play a part in endothelial repair during disease, what cell types they are able to give rise

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