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Considerations for Targeting β-Catenin Signaling in Fibrosis

Idiopathic pulmonary fibrosis (IPF) is a progressive and fatal chronic lung disease, which exhibits a median survival between 2 and 4 years after diagnosis. Although our understanding of the pathogenesis of IPF has significantly improved over the last years, we still lack effective therapies for IPF (1). Unbiased screening approaches analyzing IPF lung tissue, as well as experimental animal models thereof, suggest

that developmental signaling pathways (e.g., transforming growth factor β [TGF-β], Wnts, and growth factor receptor signaling) are dysregulated during IPF; in fact, the gene expression profile in IPF lungs looks similar to that of a developing lung (2). A working hypothesis in the field reasons that epithelial injuries trigger fibroblast-dependent repair processes that patients with IPF cannot resolve normally, which leads to excessive fibroproliferation and matrix deposition at the expense of normal tissue remodeling. Thus, although dysregulation of developmental signaling pathways may not be the underlying cause of fibrosis, there is much interest in assessing their contribution to fibrogenesis, given the development of small molecule therapeutics that are becoming available to target these pathways.

The Wnt/β-catenin (β-cat) signaling pathway is one such pathway found dysregulated in microarrays from patients with lung fibrosis (2). Wnts are secreted ligands that promote nuclear accumulation of β-cat, which mediates the T-cell factor (TCF)-dependent activation of genes important for cell fate decisions and behaviors required for tissue homeostasis. Several animal studies using Wnt/β-cat/TCF reporter animals provided evidence that activation of β-cat signaling is an early event in the lung epithelium during the development of experimental fibrosis (3, 4). Although the cell-specific gene targets of β-cat signaling in the lung are still emerging, there has been considerable interest in understanding the contribution of β-cat signaling to lung fibrogenesis nonetheless. Four recently published studies have provided evidence that inhibitors of β-cat signaling (5–7) or a target of β-cat signaling (3) can attenuate fibrosis in the bleomycin model. Collectively, these data indicate that too much β-cat signaling is somehow bad for fibrosis. However, in this issue of the *Journal*, Tanjore and colleagues (pp. 630–639) show that depletion of β-cat protein from surfactant protein C–expressing alveolar epithelial type 2 (AT2) cells using a doxycycline-regulatable Cre-recombinase approach did not protect from, but rather *promoted*, fibrosis in the bleomycin model (8). Evidence suggests that worsened fibrosis may be due to a reduced capacity for epithelial "healing," as alveolar epithelial cells (AECs) lacking β-cat manifest increased apoptosis and closed an epithelial sheet wound more slowly than β-cat–expressing cells.

Evidence for a reparative role of β-cat in lung is further supported by a recent study showing that epithelial β-cat signaling increases during leukocyte transmigration, and its inhibition limits AEC proliferation and delays acquisition of transepithelial resistance (9). In addition, activation of β-cat signaling by lithium chloride improves parenchymal lung architecture in an experimental emphysema model, suggesting that enhancing β-cat signaling may promote lung repair (10). Given that epithelial cells in IPF appear injured and hyperplastic, despite evidence of β-cat activation (11), it is possible that the cellular defect in IPF represents a failure to respond adequately to β-cat–dependent survival and repair signals. In this regard, telomerase loss-of-function mutations are associated with IPF (12), and telomerase reverse transcriptase has been recently shown to be a target of β-catenin signaling (13), raising the possibility that a failure to up-regulate certain epithelial-specific β-cat-responsive genes may contribute to the AT2 cell dysregulation thought to drive disease.

It is also likely that dynamic changes in the microenvironment and extracellular matrix composition further modify the AEC repair capacity. In this respect, Tanjore and colleagues observed active β-catenin signaling in epithelial (and other) cell populations throughout all stages of lung fibrosis in the bleomycin model, from initiation to progression and resolution. Better understanding of the factors that promote the resolution of fibrosis in this model may lead to novel therapeutic targets for patients with IPF, given that active β-cat signaling is observed in end-stage IPF with no signs of resolving disease.

Evidence that epithelial-specific removal of β-cat worsens fibrosis must also be considered in light of studies showing that β-cat is required for the efficient transmission of TGF-β signals. For example, TGF-β1–mediated induction of α-smooth muscle actin in AECs depends on a β-cat/CREB-binding protein/Smad3 DNA-binding complex (14). In addition, TGF-β1 can promote the formation of a particular β-cat phospho-form that interacts with phospho-Smad2 and up-regulates factors that can drive epithelial-to-mesenchymal transition (EMT) apparently independent of TCFs (6). These studies might have predicted that the AT2 cell–specific β-cat knockout (KO) used by Tanjore and colleagues would block EMT/profibrotic TGF-β signaling, thereby inhibiting fibrogenesis. Absent this outcome, it is important to further delineate the impact of TGF-β1/β-cat signaling and EMT on repair process in IPF.

One way to reconcile the opposing outcomes observed in Tanjore and colleagues' study compared with the β-cat signaling inhibitor approaches that preceded it (5–7) is to consider the manner in which β-cat signaling is inhibited. In contrast to these latter studies, which target β-cat's signaling function, the study by Tanjore and colleagues removed the β-cat gene completely. Because most of the cellular pool of β-cat is associated with cadherin-type intercellular adhesion receptors, it stands to reason that removing β-cat may negatively impact *both* adhesion and β-cat signaling. Thus, perturbed AEC adhesion may be the feature that is worsening fibrosis in this model. However, currently available data challenges this view, as the original β-cat KO mouse showed defects in anterior–posterior axis formation (i.e., Wnt signaling), with no obvious perturbation in adhesion (15). It appears that plakoglobin, typically associated with desmosomes, gets co-opted by cadherins in the absence of β-cat, effectively rescuing basic cell–cell adhesion (16). These data offer a rationale for why loss of β-cat in surfactant protein C–expressing cells in Tanjore and colleagues' study was not sufficient to impact lung mechanics (i.e., compliance or airway resistance) or promote frank epithelial barrier defects, as inferred from increased protein leak into the bronchoalveolar lavage or immune cell recruitment. Thus, although we cannot be certain that plakoglobin rescues all aspects of cadherin/β-cat–based adhesion, precedence suggests that β-cat–depleted AECs can have morphologically normal cell–cell adhesions. It is also worth noting that inhibition of β-cat signaling but not adhesive function in AT2 cells using the β-cat transcriptional inhibitor, inhibitor of β-catenin and TCF (ICAT), phenocopies the β-cat loss-of-function approach with regard to promoting apoptosis and antagonizing repair in a scratch wound assay (4), suggesting that the primary defect in the study by Tanjore and coworkers is due to a loss of β-cat signaling.

An alternative explanation for why genetic depletion of β-cat in AT2 cells worsens fibrosis compared with the various pathway inhibitor approaches discussed above may lie simply in the degree to which β-cat signaling is inhibited across these studies. Systemic inhibitor strategies did not likely achieve the same level of β-cat signaling inhibition in AECs as the targeted loss of β-cat performed in the study by Tanjore and colleagues. We speculate, therefore, that β-cat–mediated AEC repair mechanisms were likely outside the therapeutic window in these previous studies, allowing the inhibitor to target an as-yet-unidentified cell type where excessive β-cat signaling drives fibrosis. Because a high rate of Wnt pathway mutations are observed in fibroproliferative desmoid tumors, and forced activation of β-cat signaling in fibroblasts can enhance proliferation, migration, and matrix deposition, it is possible that sustained activation of β-catenin signaling in fibroblasts ultimately drives fibrosis (17, 18), although cell-specific β-cat KO approaches will be required to support this model.

Altogether, the study by Tanjore and colleagues supports a model where, upon epithelial injury, activation of β-cat signaling in AT2 cells reflects an attempt to repair and regenerate (18). β-cat signaling promotes AT2 cell survival, proliferation, and ability to

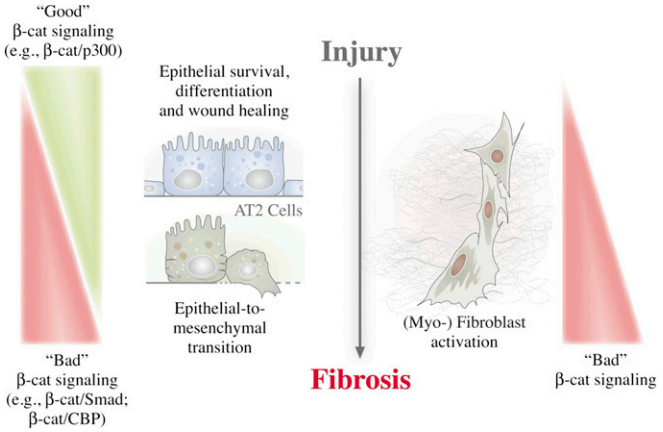

Figure 1. Model for β-cat signaling during lung injury, repair, and fibrosis. *See* text and Reference 8 for details. AT2 = alveolar epithelial type 2; β-cat = β-catenin; CBP and p300 = transcriptional coactivator histone acetyltransferases; Smad = TGFβ nuclear effector protein.

migrate and close a wound, playing an important protective role during the early stages of repair. These data raise the possibility that the contributing role for β-cat signaling in fibrosis will follow the "Goldilocks" model of cell signaling: *too little* β-cat signaling in AT2 cells will promote epithelial cell death and potentially exacerbate a lung injury/fibrosis phenotype; *too much* β-cat signaling in a presently unidentified cell type will enhance the fibrotic phenotype through promoting fibroproliferation, migration, and activation. Moreover, the fate of "good" versus "bad" β-cat signaling in lung epithelial cells is most probably dictated by β-cat binding to other cofactors (Figure 1). This model predicts that β-cat inhibitor strategies will need to interfere with specific β-cat interactions and provide sufficient control of dosing, which may be critical for providing the "*just right*" amount of β-cat signaling necessary for alveolar repair, without promoting fibroproliferation associated with fibrosis.

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