# Fibrogenic Effects of Heparin-Binding Epidermal Growth Factor-like Growth Factor: Myeloid or Epithelial Origin?

Idiopathic pulmonary fibrosis (IPF) is a condition with a poor prognosis and for which therapeutic options are still limited to drugs that slow the progression of disease. Arresting and reversing the condition remains an elusive goal. Many molecular mechanisms have been explored through the use of animal models of lung fibrosis. Administration of bleomycin and adenoviral vector-mediated overexpression of transforming growth factor-β (TGF-β) are among those frequently reported. These studies have addressed the regulation of collagen synthesis by fibroblasts and their phenotype shift to myofibroblasts in fibrogenesis, whereas alveolar epithelial cells and macrophages are candidate cells upstream in fibrogenesis.

In this issue of the *Journal,* Hult and colleagues (pp. 641–653) report on their exploration of a novel pathway regulating pulmonary fibrosis involving the epidermal growth factor receptor (EGFR) ligand, heparin-binding epidermal growth factor-like growth factor (HB-EGF), in the bleomycin murine model (1). The rationale for addressing this molecule was derived from the observation that HB-EGF and EGFR were prognostic markers in the blood of patients with IPF, in which elevated circulating concentrations of these molecules were associated with poorer survival. Bleomycin increased the expression of HB-EGF in lung tissue and BAL fluid cells. Two of the lung cell types of interest, namely, airway epithelial cells and monocyte-derived macrophages, express HB-EGF. Ostensibly, to explore the role of HB-EGF in macrophages, the authors used *Lyz2*$^{Cre}$/*HB-EGF*$^{flox/flox}$ mice to delete HB-EGF. The Lyz2 gene is expressed selectively, although not exclusively, in myeloid lineage cells, providing a tool to examine the effects of the deletion of HB-EGF in the monocyte-derived macrophages. Deletion of HB-EGF from monocytes/macrophages resulted in the improvement of several outcomes in bleomycin-treated mice. Hydroxyproline concentrations in the lung tissues, collagens 1 and 3, and fibronectin expression were reduced in *Lyz2*$^{Cre}$/*HB-EGF*$^{flox/flox}$ mice 21 days after bleomycin administration. Histology showed improvement, and the pressure required to inflate the respiratory system during tidal breathing on volume-controlled mechanical ventilation was reduced, suggesting improved lung mechanics. By Day 7 after bleomycin administration, interstitial macrophages increased less than in wild-type mice, and monocytes/macrophages did not increase in the knockout (KO) mice. BAL fluid amounts of the CC-chemokine ligand (CCL)2, the chemoattractant that mediates the major portion of monocyte/macrophage recruitment to the lungs, were lower in KO mice, presumably explaining altered macrophage numbers.

Macrophage involvement in fibrosis has been widely reported (2, 3). Single-cell sequencing of lung cells from patients with pulmonary fibrosis has revealed complex macrophage subsets, some of which express fibrogenic cytokines (3). Similarly, single-cell RNA sequencing of macrophages from mice in which fibrosis was initiated by intratracheal instillation of silica revealed an expansion of several subsets of interstitial macrophages (4). The deletion of the subset of macrophages expressing complement protein-1q reduced fibrosis, although it increased neutrophilic inflammation (4). Recruitment of macrophages is mediated in part by CCL2 interacting with the CCR2 receptor on monocytes. CCL2 has been shown to be elevated in the BAL fluid and blood of patients with pulmonary fibrosis (5). However, a phase II clinical trial of carlumab, a monoclonal antibody that binds human CCL2, failed to retard the progression of IPF in patients with documented progressive disease (6). Perhaps this therapy had too broad a reach, possibly targeting several macrophage subsets with opposing actions.

Despite the observations suggesting a role for HB-EGF synthesis by macrophages in bleomycin-induced fibrosis (1), the LysM gene is also expressed in nonmyeloid cells such as alveolar type II (ATII) cells (7). Thus, depletion of HB-EGF from airway epithelial cells provided an alternate explanation for the findings. A previous study of the effects of depletion of cells expressing the LysM gene demonstrated acute lung injury resulting from ATII cell apoptosis, and in this context, macrophages proved to be protective (7). Many studies have implicated dysfunction of alveolar epithelial type II cells in pulmonary fibrosis, with evidence of increased apoptosis (reviewed in [8]). Consistent with the finding of expression of the LysM gene in ATII cells, the airway epithelial cells also showed reduced expression of HB-EGF in the *Lyz2*$^{Cre}$/*HB-EGF*$^{flox/flox}$ mice, raising questions as to whether the deficiency in monocytes/macrophages or epithelial cells was responsible for the reduced fibrogenic response to bleomycin. Experiments with chimeric mice favored the airway epithelial cells as the responsible cell type because the transplantation of wild-type bone marrow to KO mice to restore the HB-EGF–sufficient monocytes/macrophages did not augment the bleomycin-induced fibrosis.

Studies in mice have implicated the EGFR in lung fibrosis, and the role of the EGFR and its ligands in pulmonary fibrosis has been extensively reviewed (9). Overexpression of transforming growth factor-α leads to pulmonary fibrosis (10), and amphiregulin mediates the effects of TGF-β on fibroblast proliferation (11). Gefitinib, an EGFR tyrosine kinase inhibitor, attenuates bleomycin and silica-induced fibrosis in mice (12, 13). Although Hult and colleagues are the first to report on HB-EGF involvement in bleomycin-induced lung fibrosis, the work falls short of providing the mechanism by which HB-EGF is profibrotic in this context. Administration of recombinant HB-EGF to fibroblast cultures augmented their migration in a wound assay but did not promote their differentiation to myofibroblasts; moreover, the expression of collagens 1 and 3 and fibronectin was not altered. Thus, the effects of HB-EGF are not mediated by direct actions on fibroblasts. Whether HB-EGF mediates TGF-β effects on fibroblasts was not tested.



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There is no model that faithfully reproduces the features of IPF. However, the bleomycin murine model has been used to better understand the factors controlling myofibroblast activation in the lungs and the deposition of matrix proteins. It has provided significant insights into the acute phase of matrix deposition. A shortcoming of the model that deviates from the human condition is that it is a form of lung injury characterized by an increase in collagen synthesis that regresses with time. Whether the current findings will lead to an opportunity for translation to IPF in humans is not clear; patients treated with gefitinib for non–small-cell lung cancer are at increased risk of interstitial lung disease (14), and inhibition of the EGFR does not appear to have been implicated in the antifibrotic effects of the current tyrosine kinase inhibitors, nintedanib or pirfenidone. However, the thorough studies presented by Hult and colleagues provide further insight into the complex interplay of inflammatory/repair pathways in pulmonary fibrosis. ■

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James G. Martin, M.D., D.Sc.
Ilan Azuelos, M.D.
*McGill University Health Center Research Institute*
*McGill University*
*Montreal, Quebec, Canada*

and

*Department of Medicine*
*McGill University*
*Montreal, Quebec, Canada*

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