Stiffness of the Extracellular Matrix: A Regulator of Prostaglandins in Pulmonary Fibrosis?

Aberrant deposition of extracellular matrix (ECM) proteins is a hallmark of idiopathic pulmonary fibrosis (IPF) (1). Activated lung fibroblasts, characterized by either α-SMA (α-smooth muscle actin) or PDGFRα (platelet-derived growth factor receptor α), are the main source of ECM production (2). Deposition and crosslinking of ECM proteins results in ECM stiffening. Indeed, the biomechanical properties of the lung change dramatically in IPF, and Young's modulus, as a measure of stiffness, ranges from areas with normal values between 0.5 and 10 kPa (resembling normal lung tissue values) to extremes between 50 and 100 kPa in highly fibrotic areas (3, 4).

Until recently, it was believed that matrix stiffening is a result of end-stage fibrotic remodeling processes. However, increasing evidence suggests that changes in ECM stiffening can actively contribute, propagate, and even initiate the disease (5). Indeed, increased matrix stiffness alone is sufficient to induce fibroblast activation and collagen secretion (6), and, in turn, soft matrix can reverse fibroblast activation (7). Therefore, targeting the ECM and its biomechanics has risen as a new promising concept for future IPF therapies. For a long time, no treatment option was available for IPF, and many potential candidates failed clinical trials. Only recently, two antifibrotic drugs, nintedanib and pirfenidone, were shown to successfully slow the disease progression (8). However, additional reverse-remodeling and antifibrotic approaches are still needed to not only slow down but also to halt disease progression. Thus, targeting matrix stiffening holds great promise.

This issue of the *Journal* contains a study by Berhan and colleagues (pp. 819–830) showing how matrix stiffness interferes with multiple steps of prostaglandin (PG) E_2 production in human lung fibroblasts (9). Investigation of prostaglandins in that context is indeed promising, as many members of that family show antifibrotic activity, and several highly specific receptor agonists/antagonists have been developed with the potential for clinical use (NCT00296556). All PGs are derived from the precursor PGH_2 *via* the arachidonic and COX (cyclooxygenase) pathway. Terminal synthetic enzymes then convert PGH_2 to bioactive individual prostaglandins, namely, PGI_2 (prostacyclin), $PGF_{2\alpha}$, PGD_2, and PGE_2. Among other members of the family, PGE_2 has a strong antifibrotic and antiinflammatory action, which is mostly mediated by its EP2 and EP4 (E-type prostanoid receptors 2 and 4) (10).

However, multiple studies indicate that the physiologic local antifibrotic action of PGE_2 is limited in pulmonary fibrosis, as PGE_2 concentrations in the BAL are lower in patients with IPF (11). The reasons for the decreased PGE_2 concentrations are not yet fully understood and are likely multifactorial. Therefore, much effort has been put into investigation of the key enzymes for PG production, COX-1 and COX-2. Here, the gathered data remained inconclusive, ranging from decreased mRNA and protein concentrations in lung parenchyma (12) and isolated IPF lung fibroblasts (13) to increased concentrations in fibrotic foci (14), therefore not yet fully explaining the decreased PGE_2 concentrations in IPF. This leaves room for a more detailed investigation into PGE_2 production and degradation. Indeed, increased expression of the PGE_2-degrading enzyme 15-PGDH is found in several areas in the fibrotic lung (15), and increased matrix stiffness further limits PGE_2 production and secretion (16). However, so far, investigation of the terminal enzyme of PGE_2 production, PTGES (prostaglandin E synthase), has been missing, until Berhan and colleagues were first to detect decreased concentrations of PTGES in IPF lungs.

In a plethora of *in vitro* experiments using human lung fibroblasts, they extensively and comprehensively investigated how all players involved in PGE_2 production respond to soft and stiff environments. They undertook sophisticated *in vitro* setups to mimic healthy (soft) and diseased (stiff) environment conditions for lung fibroblasts by using two-dimensional (2D) plastic, soft matrix (cytosoft and hydrogel), and three-dimensional (3D) soft spheroid culture, all together investigating stiffness ranges from ∼3 GPa to 0.4 kPa. For the first time, the authors identified decreased concentrations of the terminal enzyme of PGE_2 production (PTGES) in patients with IPF. They provide a molecular mechanism behind their finding by showing that a stiff matrix reduced PTGES expression, whereas soft 3D culture conditions induced and rescued its expression and activity in non-IPF fibroblasts and, by additional arachidonic acid supplementation, in IPF fibroblasts. The authors confirmed the stiffness-driven suppression of COX-2 expression and PGE_2 secretion (16) and closed the cycle by the additional investigation of PGE_2 receptors, identifying lower expression of the antifibrotic EP4 receptor in stiff settings compared with soft settings (Figure 1).

Although the authors convincingly show that the PGE_2 synthesis pathway is sensitive to biophysical changes in its microenvironment in non-IPF fibroblasts, there are still pieces of the puzzle that are needed for a full and comprehensive picture because PGE_2 concentrations were not fully recovered in IPF fibroblasts. In addition, the maintenance of lung fibroblasts on soft and stiff 2D matrix over several passages, indeed, robustly affected mRNA and protein concentrations of members of the PGE_2 pathway. In experiments over a shorter time period, however, changes on mRNA concentrations were not always recapitulated in protein concentrations, and often the most prominent effects on enzymes involved in PGE_2 synthesis were observed in soft 3D spheroid culture compared with stiff 2D conditions. This robust, quick, and profound effect of soft 3D culture is an intriguing finding by Berhan and colleagues, as it further highlights the complexity and sensitivity of cellular reactions in the eicosanoid

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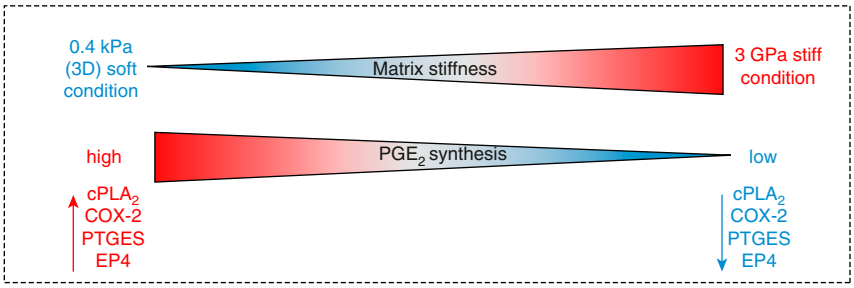

Figure 1. PGE_2 (prostaglandin E_2) synthesis of human lung fibroblasts is dependent on the biomechanical properties of its surrounding extracellular matrix. Two-dimensional and three-dimensional soft matrix conditions increased the concentrations of PGE_2, which was accompanied by increased expression of $cPLA_2$ (cytosolic phospholipase A2), COX (cyclooxygenase) isoforms, and the terminal enzyme, PTGES (PGE_2 synthase). 3D = three-dimensional; EP4 = E-type prostanoid receptor 4.

pathways and the demand and necessity for 3D experimental setups. Therefore, the authors could have unraveled an additional regulatory checkpoint of PGE_2 synthesis with further potential for future investigation. In future experiments, important open questions need to be answered, such as whether biomechanical changes in soft and stiff 3D experimental settings can still affect the PGE_2 pathway. To answer that, decellularized lung tissue from patients with IPF and control subjects or fibrotic and nonfibrotic IPF lung sections could serve as a scaffold for recellularization to investigate PG production. In addition, local prostanoid concentrations in fibrotic and nonfibrotic areas should be examined to identify potential shifts in profibrotic and antifibrotic PGs and their dependence on matrix stiffness.

Together, the authors have comprehensively identified multiple points in the regulation of PGE_2 synthesis depending on the biomechanical environment. They provide further explanation of the decreased concentrations of PGE_2 in patients with IPF and highlight the potential for targeting the PGE_2 pathway in pulmonary fibrosis. ■

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