Prostaglandin E_2 and the Pathogenesis of Pulmonary Fibrosis

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Prostaglandin (PG)E_2 is a bioactive eicosanoid that regulates many biologically important processes in part due to its ability to signal through four distinct G-protein–coupled receptors with differential signaling activity and unique expression patterns in different cell types. Although PGE_2 has been linked to malignancy in many organs, it is believed to play a beneficial role in the setting of fibrotic lung disease. This is in part due to the ability of PGE_2 to limit many of the pathobiologic features of lung fibroblasts and myofibroblasts, including the ability of PGE_2 to limit fibroblast proliferation, migration, collagen secretion, and, as originally reported in the *Journal* by us in 2003, the ability to limit transforming growth factor (TGF)-β–induced myofibroblast differentiation. In the setting of lung fibrosis, PGE_2 production and signaling is often diminished. In the last 8 years, significant advances have been made to better understand the dysregulation of PGE_2 production and signaling in the setting of lung fibrosis. We also have a clearer picture of how PGE_2 inhibits myofibroblast differentiation and the receptor signaling pathways that can influence fibroblast proliferation. This review highlights these recent advances and offers new insights into the potential ways that PGE_2 and its downstream signals can be regulated for therapeutic benefit in a disease that has no validated treatment options.

Keywords: PGE_2; myofibroblasts; collagen; lung; epigenetics

CLINICAL RELEVANCE

This review highlights the current understanding of how prostaglandin E_2 can limit lung fibrosis and reviews research findings to explain why production and signaling of this eicosanoid is impaired in lung fibrosis. It also reviews how E prostanoid receptor use can influence the response of prostaglandin E_2 on fibroblasts.

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive interstitial lung disease characterized by alveolar epithelial cell injury, fibroblast accumulation, and differentiation to myofibroblasts (1). The end result is contraction of alveolar architecture and the relentless deposition of extracellular matrices including collagen 1, collagen 3, and fibronectin (2, 3). The pathogenesis of the disease is poorly understood, but studies in patients with IPF have indicated that this disease is characterized by a loss in production of prostaglandins, including prostaglandin (PG)E_2 (4–6). PGE_2 signaling has many inhibitory actions on lung cells that could potentially suppress fibrogenesis, including the ability of PGE_2 to limit lung myofibroblast differentiation (7). This observation, which is being highlighted in this issue, was first reported by us in 2003.

More recent work in animal models and human tissues has expanded our understanding of the regulation and role of prostaglandins in fibrotic lung disease. These advances include the identification of epigenetic changes that explain the inhibition of prostaglandin production in fibrotic lung tissue (8, 9) and studies that have elucidated PGE_2 signaling defects in fibrotic fibroblasts (10–12). We also have a better understanding of how epithelial–mesenchymal crosstalk is regulated by PGE_2 (13–15) and the signaling pathways that allow PGE_2 to limit myofibroblast differentiation and migration (16, 17) and the circumstances in which PGE_2 can promote fibroblast proliferation to serve a profibrotic role (18–20). This review summarizes the current state of our understanding regarding the role(s) that PGE_2 signaling plays in modulating lung fibrosis.

PULMONARY FIBROSIS: CLINICAL AND PATHOLOGICAL FEATURES

The term "pulmonary fibrosis" is used clinically to describe several forms of diffuse interstitial lung diseases classified as idiopathic interstitial pneumonias (IIP) with a common hallmark of fibrosis. Histopathological assessment can further differentiate usual interstitial pneumonia (UIP) from other forms of IIP (21). Idiopathic pulmonary fibrosis (IPF), the clinical correlate to histological UIP in the absence of an identifiable cause, is the most common and most progressive form of IIP (22), while being least responsive to therapy. Although IPF is considered rare, precise epidemiological data are lacking primarily due to methodologies that lack histological confirmation. However, one study examining narrow criteria by diagnostic coding estimated prevalence at approximately 14 cases per 100,000, with increasing prevalence associated with aging (23).

Typical symptoms include progressive breathlessness and nonproductive cough, with pulmonary function testing classically demonstrating reduced lung volumes and impaired gas exchange (21). High-resolution computer tomography typically shows lower lobe–predominant disease with septal thickening, traction bronchiectasis, and often the presence of honeycombing, which represents cystic fibrotic airspaces (24). Such findings in the proper clinical context are strongly related to the likely finding of UIP by surgical lung biopsy (24). Histologically, there is the presence of patchwork fibrosis containing significant collagen deposition and patchy inflammation in a nonuniform distribution (25). Securing a histological diagnosis of UIP requires the identification of "fibroblastic foci," which are focal clusters of fibroblasts and myofibroblasts within young connective tissues thought to represent sites of ongoing lung injury. The presence of these fibroblastic foci is inversely correlated

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with disease survival and response to therapy (26). Shortened survival is associated with extent of fibroblastic foci rather than degree of cellularity or alveolar wall fibrosis, suggesting that mortality in IPF arises from abnormal fibrobroblast and myofibroblast accumulation rather than a predominantly inflammatory process (26).

IPF carries an overall poor prognosis. A histological diagnosis of UIP is the most important factor determining survival in patients with IIP (27). Median survival from time of diagnosis has been reported as 2.5 to 3.5 years (28), with most patients dying from disease progression to respiratory insufficiency. Subsequent studies have revealed that there can be a variable clinical course, ranging from subclinical and slowly progressive disease to rapid acute decompensation and death (29, 30). The search for biomarkers to predict disease progression is ongoing. Treatment options generally center around immunosuppressive regimens including prednisone and azathioprine, but there is no clear clinical benefit for these therapies in most patients and use of these drugs is being reevaluated in an ongoing NIH-sponsored clinical trial.

FIBROBLAST/MYOFIBROBLAST FUNCTION IN PULMONARY FIBROSIS

Fibroblasts are largely responsible for synthesizing the extracellular matrix components seen in fibrotic lesions. The matrix proteins in pulmonary fibrosis are mostly composed of collagens type 1 and 3 and fibronectin (3). Many growth and differentiation factors can affect the ability of fibroblasts to proliferate and produce matrix proteins. The best studied molecule in this regard is transforming growth factor (TGF)-β. TGF-β added to purified fibroblasts can have proliferative or differentiative effects (7, 31, 32). TGF-β induces expression of α-smooth muscle actin (α-SMA) within fibroblasts. α-SMA expression is associated with the formation of stress fibers in nonmuscle cells. This fibroblast with contractile properties is designated as a myofibroblast (33). Myofibroblasts are thought to be instrumental for the lung contraction, alveolar collapse, and matrix deposition (3, 33, 34) seen in pulmonary fibrosis. As such, strategies that limit myofibroblast differentiation may be beneficial for the treatment of lung fibrosis. As discussed below, PGE_2 is a potent inhibitor of myofibroblast differentiation (7).

PROSTAGLANDIN BIOSYNTHESIS AND REGULATION

All cells are capable of arachidonic acid (AA) release and its metabolism to bioactive eicosanoids (35). Free AA is liberated from the sn2 position of membrane phospholipids via the actions of phospholipase A2. Once liberated, the free AA can be metabolized via either of two major pathways (36). The 5-lipoxygenase pathway gives rise to leukotrienes and is found primarily in leukocytes (37), although alveolar epithelial cells can produce low levels of cysteinyl leukotrienes (38). In contrast, the cyclooxygenase pathway yields prostanoid products, including prostaglandins, thromboxane, and prostacyclin (PGI_2). This pathway is active in bone-marrow–derived cells and in structural cells (36, 39, 40). The initial step in this pathway involves the conversion of AA to PGH_2 via cyclooxygenase (COX)-1 or COX-2 enzymes. Conventional dogma suggests that the COX-1 enzyme is responsible for constitutive production of PGH_2, whereas the expression of the COX-2 enzyme is inducible and transient via a number of inflammatory stimuli. However, bronchial and alveolar epithelial cells express COX-2 constitutively (39, 41, 42). Formation of specific prostanoid end-products from PGH_2 is mediated by cell-specific distal prostaglandin synthase enzymes (e.g., PGE synthases), which are present in constitutive and inducible isoforms (43). The COX enzymes are the targets for nonsteroidal antiinflammatory drugs, including aspirin and indomethacin (44). Biological changes that result in the shunting of AA preferentially to the lipoxygenase or the cyclooxygenase pathway can have profound effects on homeostasis and disease.

COX-2 is considered the rate-limiting enzyme for the production of prostaglandins, and numerous inflammatory and injury signals are known to up-regulate COX-2 expression, including lipopolysaccharide, IL-1β, TGF-β, hepatocyte growth factor (HGF), and plasmin (8, 13, 45). Transcriptional up-regulation of COX-2 is mediated via activation of transcription factor binding to the 5′ UTR of the COX-2 gene, which contains DNA binding sites for NFkB, AP-1, and cAMP response elements (CREs) (46). In addition, inflammatory and injury signals regulate COX-2 via regulation of histone acetylation and chromosome accessibility (8). In contrast, transcriptional silencing of the COX-2 gene is associated with hypermethylation of the CpG islands in the 5′UTR (47). Because COX-2 expression must be tightly controlled, additional posttranscriptional and posttranslational mechanisms have been described that limit COX-2 activity. One such mechanism is mRNA stability, which is influenced by the complex array of AU-rich domains within the 3′UTR of COX-2. Various transacting factors can bind to these elements to regulate stability (reviewed in Ref. 48). Additional regulatory mechanisms for COX-2 expression involve miRNA inhibition of translation via interactions with the 3′UTR of COX-2 (48), differential utilization of polyadenylation sites (48), proteosomal protein degradation pathways, and suicide inactivation of COX catalytic activity (reviewed in Ref. (49). Expression of the distal synthetic enzyme microsomal PGE_2 synthase-1 has also been shown to be regulated via mRNA stability (50). Many diseases, including chronic inflammation, fever, arthritis, and certain cancers, have been linked to aberrant expression of COX-2 and enhanced prostanoid synthesis (49, 51, 52). In some cases, these pathologies are linked to the inhibitory actions of prostaglandins on innate and adaptive immune function (53–55). In contrast, mounting evidence (discussed below) suggests that decreased expression of prostaglandins relative to leukotrienes is predictive of, and contributes to, fibrotic lung disease (4, 6, 8, 14, 56, 57).

PROSTAGLANDIN RECEPTORS

There are four E prostanoid (EP) receptors, designated EP1, EP2, EP3, and EP4. In many cases, the differential effects of prostaglandins in different cell types and tissues are mediated through differential activation of 7-transmembrane–spanning EP receptors (58). The functions of the EP receptors are dictated by the intracellular signaling machinery coupled to each receptor (reviewed in Ref. 58). Stimulation of EP2 and EP4 increases cyclic adenosine monophosphate (cAMP) levels within the receptor-bearing cell and can signal smooth muscle cell relaxation. Signaling via EP1 increases intracellular Ca^{2+} and induces smooth muscle cell contraction. The EP3 receptor decreases cAMP and inhibits smooth muscle relaxation (58–61). Regarding lung fibroblasts, the antifibrotic actions of PGE_2 have been shown to be mediated via stimulation of EP2 and EP4, resulting in the activation of cAMP, and via downstream effects on protein kinase (PK)A or exchange protein activated by cAMP (7, 11, 62). Inhibition of fibroblast collagen expression involves activation of PKA (62, 63), whereas inhibition of proliferation requires exchange protein activated by cAMP (62).

Similar to COX-2, expression of the EP receptors, especially EP2, is also tightly controlled. EP2 is frequently silenced in neuroblastoma cell lines, and the DNA methylation pattern in

a portion of the CpG islands is correlated inversely with EP2 expression (64). Additionally, aberrant methylation of EP2 is noted more frequently in advanced neuroblastoma cancers (64). Similarly, a loss of EP2 expression is noted in many non–small cell lung carcinomas, and expression can be restored after treatment with a demethylating agent (65). Because PGE_2 can promote tumorigenesis in lung cancer, aberrant methylation, which limited EP2 expression, was correlated with better patient outcome. As discussed below, a similar epigenetic reduction of EP2 expression in patients with lung fibrosis (where PGE_2 signaling is beneficial) can have the opposite effect and worsen patient outcomes.

PGE_2 MEDIATES HOMEOSTASIS IN THE LUNG

Homeostasis within the healthy lung microenvironment is dependent on alveolar epithelial cell (AEC)–mesenchymal cell crosstalk (2, 66). The evidence for this concept is: (*1*) in normal lung sections, foot processes from AECs can be seen extending through the basement membrane and making contact with the mesenchymal cells; (*2*) *in vitro*, AECs from normal animals inhibit fibroblast proliferation; and (*3*) fibrotic injury results in loss or damage to AECs. The ability of epithelial cells to limit fibroblast proliferation is critically dependent on the ability of the AECs to produce prostaglandins (41). The actions of prostaglandins (specifically PGE_2 and PGI_2) inhibit fibroblast function. Specifically, prostaglandins decrease fibroblast chemotaxis (67), decrease fibroblast proliferation (11, 62, 63, 68–70), decrease fibroblast growth factor receptor expression (71), decrease fibroblast collagen synthesis (63, 72, 73), inhibit myofibroblast differentiation (7, 16), and increase collagen degradation (74). The pathways by which PGE_2 limits myofibroblast differentiation are discussed in more detail below.

Prostaglandins also play important roles in the regulation of apoptosis within the lung, and the fact that patients with IPF exhibit increased apoptosis of AECs but diminished apoptosis of fibroblasts has been termed the "apoptosis paradox." Recent studies have demonstrated that reduced expression of COX-2 and PGE_2 in fibroblasts from patients with IPF is one factor that promotes fibroblast survival in the fibrotic lung (14). In normal human lung fibroblasts, PGE_2 increases apoptosis and potentiates apoptotic signals delivered by Fas ligand. The ability of PGE_2 to promote normal fibroblast apoptosis requires signaling via EP2/EP4 and a reduction in activity of the prosurvival molecule protein kinase B (Akt) (75). In contrast, fibroblasts from patients with IPF are resistant to the proapoptotic effects of PGE_2 (75). Figure 1 diagrams the homeostatic and antifibrotic actions of PGE_2 signaling in lung epithelial cells and fibroblasts.

MYOFIBROBLAST DIFFERENTIATION AND INHIBITION BY PGE_2

In response to TGF-β, fibroblasts undergo Smad-dependent signaling and develop characteristic changes known as stress fibers caused by the reorganization of α-SMA into filamentous bundles (7, 16, 76, 77). In addition, TGF-β signaling induces the reorganization of the actin cytoskeleton and the formation of adhesive signaling complexes known as focal adhesions. Focal adhesions are populated by focal adhesion kinase, F-actin, paxillin, vinculin, and αvβ3 integrins (78, 79). The formation of these adhesive contacts is critical for myofibroblast differentiation. When cells are treated with TGF-β in suspension culture, no myofibroblast differentiation occurs (77). The formation of focal adhesions involves activation of one or more members of the small Ras GTPase family (Rho A, Rac, or CDC42) (80, 81).

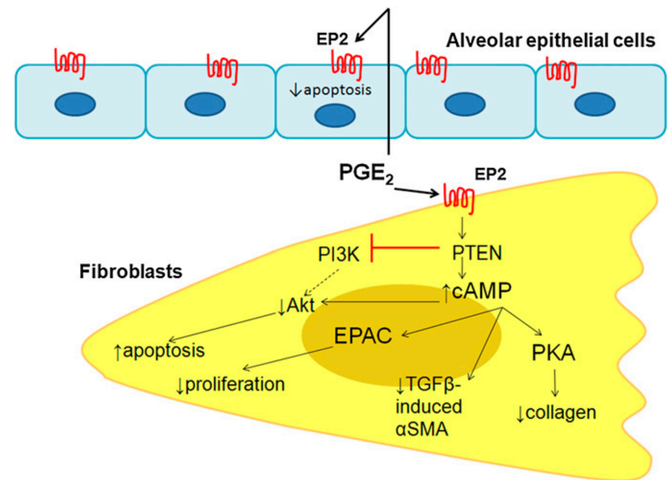

Figure 1. The homeostatic signaling of prostaglandin (PG)E_2 in alveolar epithelial cells (AECs) and fibroblasts. Diagram showing that in the normal lung fibroblast, PGE_2 functions to limit proliferation, collagen secretion, and myofibroblast differentiation. With respect to apoptosis, PGE_2 can induce apoptosis in fibroblasts while protecting lung AECs.

Our original study published in 2003 demonstrated that PGE_2 signaling led to increased cAMP via the EP2 receptor, which, in turn, could inhibit TGF-β–induced myofibroblast differentiation and limit collagen secretion (7). Our study showed that PGE_2 did not function by interfering with TGF-β–induced Smad phosphorylation or translocation to the nucleus. Rather, PGE_2 altered cytoskeletal architecture and disrupted the formation of focal adhesions (16) (Figure 2). Additionally, PGE_2 signaling through EP2 activates phosphatase and tensin homolog deleted on chromosome 10 (PTEN), and this results in diminished fibroblast proliferation (82). TGF-β is only able to induce myofibroblast differentiation in the absence of PTEN (76); thus, PGE_2 activation of PTEN is another mechanism by which this prostanoid limits myofibroblast differentiation. Strategies that amplify the inhibitory cAMP signals generated by PGE_2 are also beneficial in limiting myofibroblast differentiation. Phosphodiesterase 4 (PDE4) inhibitors can prevent the breakdown of cAMP, and, as such, potentiate the effects of PGE_2 in limiting myofibroblast differentiation (83, 84) and can limit collagen gel contraction and chemotaxis (85). More recently, knockdown of PDE4B and PDE4D subtypes were shown to limit TGF-β–induced myofibroblast differentiation on their own (86). In summary, these data suggest that PGE_2 signaling should be beneficial in limiting fibrosis; thus, investigators were eager to understand why production of PGE_2 was diminished in IPF lungs (4–6). Furthermore, the observation that fibroblasts from fibrotic murine and human lungs were often refractory to the inhibitory effects of PGE_2 (10–12) stimulated additional studies to understand this disease-related heterogeneity.

EICOSANOID DERRANGMENTS IN IPF

Derangement of eicosanoid synthesis can be seen in human and animal lung fibrosis studies. Leukotriene levels have been reported to be greater in bronchoalveolar lavage fluid and lung homogenates from patients with IPF than from healthy volunteers (87, 88). Alveolar macrophages are the main source of leukotriene synthesis and are responsible for the increased production of leukotriene C_4 and leukotriene B_4 noted in IPF

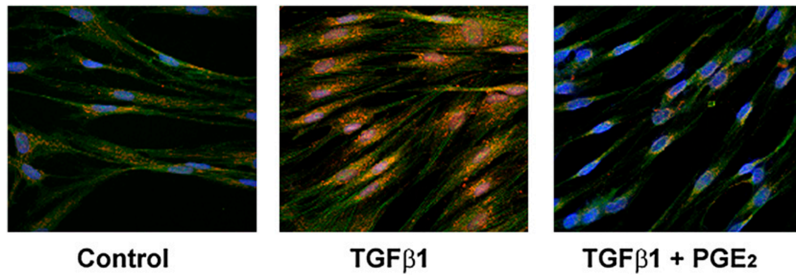

Figure 2. Transforming growth factor (TGF)-β1 induces the formation of focal adhesions, but cotreatment with PGE_2 prevents focal adhesion formation. IMR90 lung fibroblast cells were serum starved before treatment with serum-free media alone (control) or TGF-β1 alone (2 ng/ml) or in combination with 10 nM PGE_2 for 24 hours. Cells were fixed and stained with FITC-phalloidin (*green*) to visualize F-actin, Cy3–anti-paxillin (*red*), and DAPI (*blue*) for the nuclei and analyzed by laser-scanning confocal microscopy. Areas of coimmunofluorescence with FITC and paxillin are focal adhesions (*orange/yellow*). The colocalization of F-actin and paxillin was confirmed by Z-stack analysis.

lung homogenates (57). Animal models are also characterized by increased leukotriene production after lung injury. Mice genetically deficient in leukotriene production (5-lipoxygenase knock-out mice) are protected from bleomycin-induced pulmonary fibrosis (89). Because cysteinyl leukotrienes are known to induce proliferation and collagen synthesis in mesenchymal cells (68, 90, 91), increases in metabolism of AA via the 5-lipoxygenase pathway can enhance fibrogenesis.

Conversely, reduced PGE_2 levels have been reported in bronchoalveolar lavage fluid and alveolar macrophage–conditioned media from patients with IPF (4, 56). These observations are consistent with findings of reduced COX-2 expression in patients with IPF (8, 14, 92). Fibroblasts from patients with IPF are unable to up-regulate the COX-2 enzyme in response to stimuli and as such are deficient in PGE_2 production (5, 6, 93). Reduced PGE_2 synthesis has also been reported for fibroblasts isolated from rat lungs after bleomycin-induced pulmonary fibrosis (94). Animal models characterized by reduced PGE_2 synthesis in the lung via administration of indomethacin (95) or the gene-deletion of COX-2 (96) manifest worse bleomycin-induced fibrosis. Thus, in the injured lung, the functional loss of prostaglandins has severe consequences for fibroproliferation.

MECHANISMS FOR PGE_2 DEFICIENCY IN LUNG FIBROSIS

The mechanisms responsible for PGE_2 loss in the fibrotic lung are varied and include effects of soluble mediators and epigenetics. Profibrotic injury to epithelial cells often results in the release of chemokines, including CCL2 (97). In the presence of CCL2, PGE_2 production by lung AECs is diminished, and fibroproliferation increases (15). This is one mechanism that explains why mice defective in CCR2 (the receptor for CCL2) are protected from fibrotic injury (97, 98). Another well known consequence of fibrotic injury is the leakage of plasma from damaged vasculature and the activation of the coagulation cascade. However, in the fibrotic lung, the extravascular fibrin is not effectively cleared, and epithelial repair is blocked due to a marked increase in the expression of plasminogen activation inhibitor (PAI)-1 relative to urokinase-type plasminogen activator (uPA) (99–101). PAI-1 blocks uPA and thus prevents the generation of plasmin and inhibits the proteolytic activation and release of the epithelial repair molecule HGF (102, 103). We recently showed that plasmin up-regulates COX-2 and stimulates PGE_2 production in AECs, fibroblasts, and fibrocytes via HGF (13). Thus, one consequence of the increased PAI-1 levels noted in fibrotic lungs is diminished production of PGE_2. Because PGE_2 has also been shown to promote HGF activation (104), this positive antifibrotic feedback loop is likely missing in IPF.

More recently, epigenetic changes have been identified that contribute to diminished expression of COX-2 and thus PGE_2 in fibrotic lungs. Using fibroblasts isolated from normal or fibrotic lungs, Coward and colleagues demonstrated that COX-2 mRNA levels were reduced in fibrotic fibroblasts, but mRNA degrada-

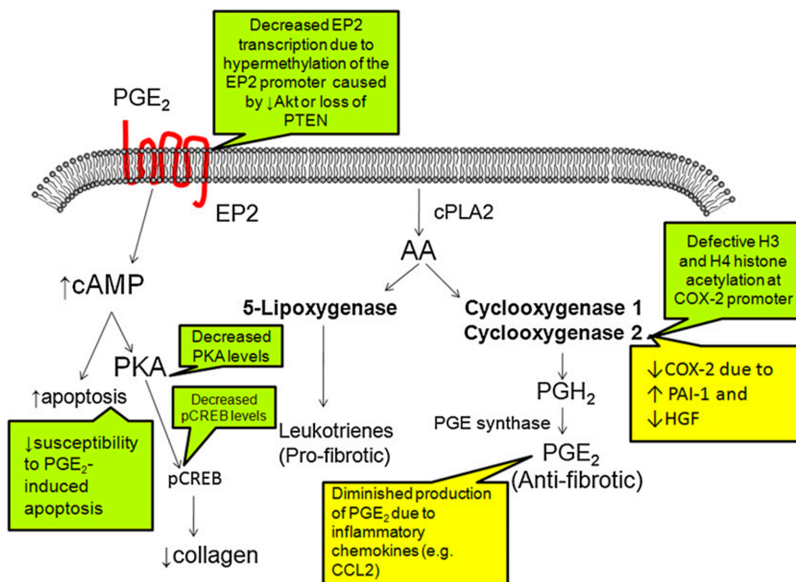

Figure 3. Alternations in PGE_2 production and signaling that have been noted in fibrotic fibroblasts. Schematic showing the epigenetic and inflammatory signals that contribute to the dysregulation of PGE_2 production and signaling in the fibrotic lung. *Green boxes* represent changes due to epigenetic alterations. *Yellow boxes* denote changes due to inflammatory signals.

tion rates were similar (8). Further investigation demonstrated that although some fibroblasts from IPF lungs contained appropriate levels of transcription factors that drive COX-2 expression (including NFkB p65, CEBPb, and CREB-1), these factors were prevented from binding to the COX-2 promoter in the native chromatin configuration. This inhibition of transcription factor binding was correlated with defective histone H3 and H4 acetylation due to reduced recruitment of histone acetyltransferases and increased recruitment of histone deactylease containing co-repressor complexes (8). In other fibrotic fibroblasts, the phosphorylation of CREB (a downstream effector of cAMP activation of PKA) was shown to be defective (105). Taken together, these epigenetic changes noted in fibroblasts from patients with IPF prevent COX-2 gene transcription in IPF and contribute to diminished production of PGE_2. Figure 3 highlights alterations in the PGE_2 production and signaling pathways that have been noted during lung fibrosis.

MECHANISMS FOR DIMINISHED PGE_2 EFFECTS IN LUNG FIBROSIS

Not only is PGE_2 production altered in the fibrotic lung, but PGE_2 signaling can also be impaired. Transcriptional or epigenetic decreases in EP2 or EP4 expression can limit the inhibitory signaling of PGE_2 in fibroblasts. We have previously shown that EP2 levels are diminished in fibroblasts isolated from mice on Day 14 after bleomycin or fluorescein isothiocyanate treatment (11). This loss of EP2 expression resulted in blunted cAMP responses and a reduced ability of PGE_2 to inhibit proliferation and collagen secretion in these cells. Fibroblasts from patients with IPF have also been shown to be refractory to PGE_2 signaling and identified mechanisms include decreased expression of EP2 as well as diminished expression of PKA (a downstream target of cAMP and EP2 signaling) (10). More recently, hypermethylation of the PGE receptor 2 gene (*PTGER2*) promoter has been identified as a mechanism for reduced EP2 expression in fibroblasts from patients with IPF and fibrotic mice (9). An increase in Akt signal transduction is believed to be one mechanism that drives the hypermethylation status of the *PTGER2* promoter (9). Additionally, action of the enzyme PTEN is known to upregulate EP2 expression (12) and IPF fibroblasts; particularly those in the fibrotic foci are known to be PTEN deficient (76). Each of these mechanisms would result in diminished signaling via the inhibitory EP2 receptor, and, as such, these findings explain in part why fibroblasts from patients with IPF are largely refractory to PGE_2 inhibitory signaling. Understanding the differences that exist in the cell types that comprise the normal and fibrotic lung is important for the design of future therapeutics.

PROLIFERATIVE EFFECTS OF PGE_2 SIGNALING VIA EP1 AND EP3

Although we have detailed the antifibrotic actions of PGE_2 signaling via EP2 and EP4 and noted that lung fibrosis is often associated with reduced production of PGE_2 or defective EP2 signaling, in some instances, PGE_2 can promote fibroblast proliferation. When this occurs, it is via EP1 or EP3 signaling. Studies looking at the fibroproliferative response after acute lung injury (ALI) identified a dose-dependent effect of PGE_2 on fibroblast proliferation. At midrange concentrations (10^{-9} to 10^{-7} M), PGE_2 enhanced proliferation of lung fibroblasts via EP3 stimulation, whereas at extremely low ($< 10^{-10}$ M) or high concentrations ($> 10^{-6}$ M), PGE_2 suppressed lung fibroblast proliferation via EP2 stimulation (20). This study found that the range of PGE_2 concentrations noted in edema fluid from patients with ALI were in the concentration range to stimulate fibroblast proliferation, thus implicating EP3 stimulation in the fibroproliferative consequences of ALI (20). EP1 stimulation has also been reported to promote fibroblast calcium mobilization and increased proliferation of NIH 3T3 fibroblasts (19). Additionally, in recent studies using neonatal rat ventricular fibroblasts that expressed all four EP receptor subtypes, PGE_2 stimulation increased the number of cells in S phase and increased expression of cyclin D3. These same effects were mimicked with the EP1/EP3 agonist sulprostone (18) and implicate EP1/EP3 stimulation in cardiac fibrosis. Thus, PGE_2 is capable of inhibiting fibroproliferation via EP2/EP4 or promoting proliferation via EP1/EP3 stimulation depending on the particular context of the fibroblasts.

CONCLUSIONS

Homeostatic balance within the lung requires appropriate crosstalk between alveolar epithelial cells, fibroblasts, and inflammatory cells (*see* Figure 1). Production of PGE_2 by alveolar epithelial cells is believed to be an important factor for limiting fibroproliferation and promoting appropriate alveolar epithelial repair. In the normal lung, PGE_2 signaling via EP2 receptor–mediated elevations in cAMP can induce fibroblast apoptosis and, as we showed, limit myofibroblast transformation, proliferation, and collagen secretion. However, in the fibrotic lung, various perturbations alter the homeostatic balance. PGE_2 production is limited via inflammatory mediators and epigenetic silencing of the COX-2 promoter. Furthermore, fibrotic fibroblasts lose EP2 receptor expression and may lose expression of the downstream effectors PKA and phospho-CREB. It is also interesting that fibrosis is a male-predominant disease, and at least one study has suggested that male gender is associated with reduced EP2 and EP4 levels and reduced PGE_2 production in splenic macrophages after trauma (106). If these gender differences are true in response to lung injury as well, it may in part explain the gender differences that are noted in IPF.

FUTURE DIRECTIONS AND CLINICAL IMPLICATIONS

It is clear that PGE_2 production plays an important role in determining homeostasis in the normal lung. As such, it is tempting to suggest that strategies aimed at delivering PGE_2 to the lung might be beneficial in treating patients with IPF. However, there are several caveats to this type of therapy. First, the therapy would only be effective in patients in whom EP2 signaling was intact. Determining this may require surgical or transbronchial biopsies, and PGE_2 responsiveness in isolated fibroblasts must be determined on an individual patient basis. Second, the half-life of PGE_2 is quite short, so new innovations would be needed to deliver this lipid as a therapeutic. One possibility includes creating derivatives with similar EP2 binding and signaling capacities. Alternatively, EP2 agonists may be more stable. Another important aspect to consider is that PGE_2 has differential effects depending on the cell type and the EP receptor profile it encounters. Thus, intravenous administration may not be effective due to deleterious effects on the vascular system. It is also possible that systemic administration of PGE_2 could promote diseases that are often associated with pathological overexpression of PGE_2, such as colon cancer, persistent inflammation, and arthritis (49, 51, 52). This means that inhalation therapy may be the best option for patients with lung fibrosis, but at present nebulization of PGE_2 or EP2 agonists is not practical. Alternative therapeutic strategies could be to prevent the breakdown of cAMP to maximize its inhibitory signaling capacity in fibrotic lungs. This may be achieved by

administration of PDE4 inhibitors. Again, this therapy would only be effective in patients in whom modest PGE_2 production and cAMP generation was intact in the lung, and this type of therapy may predispose to other diseases and malignancy. Methylation of the promoters for COX-2 and EP2 has been demonstrated to limit PGE_2 expression in some patients. Thus, it is possible that inhibitors of methyl transferases may be effective in reversing the methylation status of these genes and increasing PGE_2 production and signaling. The potential down sides to this therapy involve the off-target effects that these agents could have on other genes. Global demethylation may not be beneficial.

It may be possible to target signals downstream of EP2, such as cAMP elevation or PKA activation. Particularly if these therapies could be delivered in a cell-specific manner, they may be effective in inhibiting myofibroblast differentiation and activation even in patients with EP2 receptor defects. This targeted therapy may also avoid the potential proliferative effects of PGE_2 binding to EP1 or EP3 receptors in some organs. Finally, it is possible that cell-directed therapies could be evolved to treat patients with IPF. Fibrotic regions of the lung may be quite difficult to treat by inhalational therapies due to limited airflow in these regions. It is possible that cells such as mesenchymal stem cells or potentially fibrocytes could be engineered to deliver abundant PGE_2, EP2 agonists, or adenyl cyclase stimulators. In this way, the therapeutic cell type may be able to use chemokine receptors and inflammatory signals to colocalize to areas of fibrosis and as such may offer a more targeted delivery of an antifibrotic therapy.

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