"Yap"-ing about the Antifibrotic Benefits of Prostacyclin

Prostaglandins were first discovered in 1935 (1) and this paved the way for some of our earliest understanding of how cells communicate and signal in an autocrine or paracrine manner. Prostaglandins are derived from the lipid arachidonic acid, which is present in all cellular membranes, and their synthesis is highly regulated by the actions of cyclooxygenase 1 and 2 and distinct prostaglandin synthases, which are highly conserved in all animal species. Prostaglandins are often best known for being responsible for the cardinal manifestations of inflammation, including "rubor" (redness), calor (heat), tumor (swelling), and dolor (pain) (2), which account for the pharmacologic efficacy of nonsteroidal cyclooxygenase inhibitors (3). However, their broad synthesis, variety of chemical structures, and diverse signaling through an array of different G-protein–coupled receptors allow prostaglandins to exert a myriad of diverse effects. The 1982 Nobel Prize in Physiology and Medicine was bestowed upon Sune K. Bergström, Bengt I. Samuelsson, and John R. Vane for their discovery of prostaglandins and the seminal importance of these molecules in biology.

Although prostaglandins were discovered more than three-quarters of a century ago, new and important findings continue to emerge due to their diverse functions and actions. Prostaglandin E_2 (PGE_2), which is synthesized by cyclooxygenase and PGE synthase, is one of the most ubiquitous and highly synthesized prostaglandins in the lung. Many years ago, it was discovered that PGE_2 had potent antifibrotic properties. PGE_2 inhibits all aspects of fibroblast function, including matrix production, fibroblast survival (4) and proliferation (5), and myofibroblast differentiation (6). PGE_2 is one of the few molecules that can even reverse myofibroblast differentiation (7), triggering established, differentiated myofibroblasts to downregulate expression of the contractile protein, smooth muscle actin. PGE_2 exerts these actions by signaling through the E prostanoid 2 (EP2) receptor, a $G\alpha_s$-coupled receptor that generates cAMP as a second messenger (5). The downstream targets of cAMP, including protein kinase A and exchange protein activated by cAMP, have been shown to be responsible for the antifibrotic actions of PGE_2 (8). Although PGE_2 promotes fibroblast apoptosis, it also paradoxically promotes epithelial cell survival and proliferation, further supporting the notion that PGE_2 may be beneficial against pulmonary fibrosis (PF) (9). For all of these reasons and because PGE_2 synthesis is diminished in the idiopathic PF (IPF) lung (10, 11), treatment with exogenous PGE_2 or its mimetics provides an attractive option to treat PF. Unfortunately, several limitations temper enthusiasm for this approach. Most prominently, fibroblasts from patients with IPF have been shown to be resistant to the antifibrotic actions of PGE_2, in part through a decrease in EP2 receptor expression and downstream PGE_2 signaling (12). PGE_2 is also difficult to administer because it degrades quickly. Inhalational delivery of PGE_2 has been considered as a route of therapy, but this approach has been hampered by its ability to cause cough (13).

Enter prostacyclin (PGI_2). PGI_2, which is also synthesized from arachidonic acid by cyclooxygenases and PGI synthase, is produced in the lung predominantly by vascular endothelial cells and it plays a critical role in vascular smooth muscle relaxation. Accordingly, both PGI_2 (14) and its analogs are used as therapy for pulmonary hypertension. Lung fibroblasts, however, also express receptors for PGI_2, and because the I prostanoid (IP) receptor also signals through a $G\alpha_s$-coupled G protein, activation of this receptor should have many of the same biological effects as PGE_2 does in signaling through EP2.

In this issue of the *Journal*, Zmajkovicova and colleagues (pp. 578–591) report that levels of the IP receptor are not downregulated in IPF fibroblasts (15). In their study, they show that activation of IP receptor signaling by the IP receptor agonist ACT-333679 inhibits fibroblast proliferation, fibroblast matrix production, fibrosis-associated cytokine expression, and myofibroblast differentiation. Like PGE_2, PGI_2 can also reverse the differentiation of established myofibroblasts. Unbiased analyses demonstrated that mechanistically, elevations in cAMP led to the inhibition of YAP and TAZ, two transcription factors that are critical for the myofibroblast phenotype, especially in situations where mechanotransduction and tissue stiffness lead to the activation of these cells (16). Although the promise of PGI_2 as an antifibrotic in PF has been suggested in the past (17), the article by Zajkovicova and colleagues provides a comprehensive analysis of all of the diverse functions PGI_2 is capable of inhibiting in fibroblasts, and links its actions to YAP/TAZ transcription factor activity. One of the most intriguing aspects of this study is that these findings were achieved through the use of ACT-333679, an IP receptor agonist that is the active metabolite of the commercially available orally administered drug selexipag, which is approved for use in pulmonary arterial hypertension (18).

How optimistic should we be about IP receptor agonists as therapy for IPF? Obviously, studies in animal models would first need to be done to confirm efficacy *in vivo*. The demonstration that ACT-333679 was effective at attenuating fibrosis markers in precision-cut lung slices and in primary IPF lung fibroblasts is a good first step toward extending *in vitro* findings to disease-relevant cells and tissues. Zmajkovicova and colleagues also do an excellent job of demonstrating the pharmacologic efficacy (and relative superiority) of ACT-333679 compared to other PGI_2 analogs and to the Food and Drug Administration-approved antifibrotic agents, nintedanib and pirfenidone. Finally, as opposed to inhibitors of other pathways that have the promise of inhibiting fibroblasts but might also be deleterious to epithelial cells, prostanoids and their signaling pathways have the advantage of inhibiting all functions of fibroblasts and reversing myofibroblast

differentiation while also enhancing epithelial cell proliferation, which is critical for lung repair and regeneration.

Patients with interstitial lung disease often have concomitant pulmonary hypertension, and several studies have examined the efficacy of various medications used to treat pulmonary hypertension in these patients (19). However, such studies are generally limited to oral compounds and focus on endpoints specific to pulmonary hypertension and the consequences of pulmonary hypertensive disease (20). With the recent Food and Drug Administration approval and availability of the orally active, selective IP receptor agonist selexipag, the time is right to consider whether this drug might help treat not just pulmonary hypertension but also the scarring present in the lungs of patients with IPF. Taking a page out of the pulmonary arterial hypertension therapy playbook, combining that treatment with a phosphodiesterase inhibitor (or a cAMP/cGMP stimulant such as riociguat) may further enhance the activity of selexipag for treatment of IPF. However, it is important to note that PGI_2 analogs, including selexipag, show variable efficacy among patients with pulmonary hypertension, and even vasodilator studies cannot always predict who will or will not respond to the treatment. IPF is no less heterogeneous in terms of genetics, molecular pathophysiology, and clinical prognosis. It is conceivable, then, that IPF fibroblasts, as with their resistance to PGE_2 (12), may also demonstrate variable responsiveness to ACT-333679 and that selexipag may demonstrate variable efficacy among different patients. Biochemical tests or cell-based biomarkers may be necessary to determine which patients could benefit from the drug. Now, more than 80 years after the initial discovery and description of prostaglandins, and despite the above caveats, these molecules and signaling pathways continue to remain an attractive area for the development of new treatments against this deadly disease. ■

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