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Mini Review

Roles of eicosanoids in pulmonary fibrosis

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Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive disease characterized by fibroblast proliferation and excess deposition of extracellular matrix proteins in the lungs. As anti-inflammatory therapies such as steroids or immunosuppressive agents do not improve disease progression, targets to block fibrogenesis per se are needed. Mice lacking cytosolic phospholipase A_2, a key enzyme triggering the production of arachidonic acid metabolites such as eicosanoids including prostanoids and leukotrienes, did not develop bleomycin-induced pulmonary fibrosis, indicating that arachidonic acid metabolites play an important role in the pathogenesis of pulmonary fibrosis. Previous reports indicated that leukotrienes have fibrogenic effects, and prostaglandins (PGs) E_2 and I_2 have antifibrogenic effects. We have reported that, using mice lacking each prostaglandin receptor, $PGF_{2\alpha}$ and its receptor FP signaling facilitated bleomycin-induced pulmonary fibrosis through increased fibroblast proliferation and collagen deposition, independently of transforming growth factor (TGF)-β signaling. Furthermore, to evaluate the clinical significance of $PGF_{2\alpha}$ in IPF, using the liquid chromatography-tandem mass spectrometry, the levels of 15-keto-dihydro $PGF_{2\alpha}$ as well as $PGF_{2\alpha}$ increased in the bronchoalveolar lavage fluid of patients with IPF to a greater extent than in patients with sarcoidosis. Thus, we have suggested $PGF_{2\alpha}$-FP signaling as a therapeutic target for IPF. Eicosanoids may be promising targets for novel treatments and management of IPF, in addition to TGF-β.

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Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive disease characterized by fibroblast proliferation and excess deposition of collagen and other extracellular matrix (ECM) proteins in the lungs. It is usually lethal and is of unknown etiology. There are limited therapeutic options. As the currently used anti-inflammatory and immunosuppressive therapies do not improve disease progression, therapies that block fibrogenesis per se are needed. Modeling pulmonary fibrosis with bleomycin has been most fre-

quently used as an animal model of IPF. Although previous experiments have indicated that transforming growth factor (TGF)-β plays the most significant role in fibrogenesis in the lungs as well as other organs including the liver, kidney, skin, arteries and central nervous system[1], antagonizing this pathway in the bleomycin animal model did not completely prevent fibrosis[2], suggesting the presence of other mediators contributing to fibrogenesis. It has been proposed that the pathogenesis of IPF is not based on individual mediators of fibrosis or single pathways, but that multiple causes and multiple pathways are involved[3]. This approach may clarify the complex pathogenesis of IPF and provide a novel effective treatment approach targeting multiple fibrosis pathways simultaneously.

Mice lacking cytosolic phospholipase A_2 (cPLA$_2$), a key enzyme triggering the production of arachidonic acid metabolites such as eicosanoids including prostanoids and leukotrienes (LTs), showed significantly suppressed lung fibrosis in the bleomycin model[4], indicating that arachidonic acid metabolites play an important role in the pathogenesis of pulmonary fibrosis. We briefly review the roles of eicosanoids in pulmonary fibrosis, and would like to introduce our basic and clinical studies focusing on a novel profibrotic pathway.

Various eicosanoids are involved in pulmonary fibrosis

Eicosanoids are produced through the activation of cPLA$_2$, which cleaves phospholipids in response to stimuli and releases arachidonic acid. It is metabolized by cyclooxygenase (COX) to produce prostanoids and by 5-lipoxygenase (5-LO) to produce leukotrienes. Prostanoids include prostaglandin (PG) D_2, PGE_2, $PGF_{2\alpha}$, PGI_2 and thromboxane (TX) A_2 acting at their cognate receptors which are designated as DP, EP1 — EP4, FP, IP and TP, respectively[5]. Leukotrienes are synthesized through an intermediate product of LTA_4, which is converted to LTB_4 or cycteinyl leukotrienes (LTC_4, LTD_4, LTE_4). Prostanoids and leukotrienes are released outside of cells, and act at the sites due to their short half-life. Using liquid chromatography-tandem mass spectrometry, we systematically measured the amount of arachidonic acid metabolites in the lung, and found that various eicosanoids are produced in the bleomycin-induced pulmonary fibrosis model[6]. In addition, as loss of cPLA$_2$ suppressed fibrotic process[4], it may play an important role in the pathogenesis of fibrogenesis.

In experiments[7-9] using mice deficient in 5-LO or CysLT2 receptor (a low affinity receptor for cyteinyl leukotrienes), or treated with a LTB_4 receptor antagonist, bleomycin-induced inflammation and its subsequent fibrosis were reduced, suggesting that leukotrienes may exert a proinflammatory action and then contribute to fibrosis. Thus, leukotriene receptor antagonists and Zileuton, a 5-LO inhibitor, which are currently used or studied in asthma, have been investigated for use in pulmonary fibrosis in animal models and humans[10].

On the other hand, regarding prostanoids, in experiments using COX-2-deficient mice, pulmonary fibrosis worsened or did not change compared with that in wild-type (WT) mice[11-13]. Conversely, using indomethacin (COX inhibitor) or COX-2 inhibitor, bleomycin-induced pulmonary fibrosis was reduced[14,15]. These inconsistent results indicate that different prostanoids may play opposing roles in the pathogenesis of pulmonary fibrosis. Among them, the antifibrotic effects of PGE_2 have been most investigated[16]. PGE_2 reduces fibroblast activity through diminished fibroblast proliferation, migration and collagen production, mainly through EP2 receptor-mediated elevations in cyclic adenosine monophosphate (cAMP). EP2 levels are diminished in fibroblasts after bleomycin treatment, which resulted in blunted cAMP responses and a reduced ability of PGE_2 to inhibit proliferation and collagen production in fibroblasts[17]. This seems to be due to hypermethylation of the PGE receptor 2 gene promoter in fibroblasts from IPF patients or fibrotic mice, or deficiency in enzyme PTEN, which upregulates EP2 expression, in the fibrotic foci[18]. Thus, signaling via inhibitory EP2 receptor diminished, and then fibroblasts in patients with IPF are refractory to PGE_2 inhibitory signaling.

Similarly, PGI receptor, IP-deficient mice showed exaggerated fibrosis[11], and, conversely, iloprost, a PGI_2 analogue, abrogated bleomycin-induced pulmonary fibrosis[19]. Thus, PGE_2-EP2 and PGI_2-IP signaling have protective roles in fibrogenesis; however, prostanoids promoting fibrosis remain to be elucidated.

FP signaling promotes fibrosis without affecting inflammation

To reveal the roles of prostanoids in pulmonary fibrosis, we subjected mice deficient in DP, EP1-3, FP, IP and TP and administered by a selective EP4 antagonist to bleomycin-

induced pulmonary fibrosis, and found out that only mice deficient in FP ($FP^{-/-}$) showed a reduced fibrotic response as assessed by hydroxyproline contents, histology and lung function, compared with WT mice[6]. Furthermore, to analyze whether attenuated fibrosis is related to reduced inflammation or not, we studied the inflammatory response by examining the number of and composition of cells in bronchoalveolar lavage fluid (BALF), and found that it did not differ between the $FP^{-/-}$ and WT mice on Day 7 when inflammatory response peaked after bleomycin administration. Thus, FP signaling may directly promote fibrosis without affecting peak inflammatory responses.

We then performed gene expression analysis of the lungs of $FP^{-/-}$ and WT mice on Days 0, 7 and 14 after bleomycin administration[6]. Based on cluster analysis, the lung of $FP^{-/-}$ mice revealed induction of inflammation-related genes similarly to WT mice, but the induction of fibrosis-related genes was suppressed, indicating that $PGF_{2\alpha}$-FP functions not in inflammation but in fibrosis, where it facilitates fibrogenesis by enhancing the expression of fibrosis-related genes.

FP signaling promotes fibrosis independently of TGF-β pathway

Furthermore, to analyze the effect of $PGF_{2\alpha}$-FP signaling on the TGF-β pathway, firstly, using $FP^{-/-}$ and WT mice after bleomycin treatment, we measured the content of active TGF-β1 levels in BALF, and then assessed Smad2 phosphorylation, a crucial intracellular step in TGF-β signal transduction, in the lung homogenate. There were no significant differences in the increased active TGF-β1 levels and Smad2 phosphorylation between $FP^{-/-}$ and WT mice after bleomycin treatment. Additionally, we administered TGF-β receptor I kinase inhibitor, SD-208, to $FP^{-/-}$ and WT mice, and then found that FP deficiency and inhibition of TGF-β signaling additively decreased bleomycin-induced fibrosis. Thus, $PGF_{2\alpha}$-FP signaling and TGF-β signaling work independently of each other in the development of fibrosis in mice.

Fibroblasts are the key cells producing collagen and ECM proteins in fibrogenesis. $PGF_{2\alpha}$ increased collagen production of lung fibroblasts via FP through stimulating fibroblast proliferation and the activity of the collagen α2(I) promoter, additively to TGF-β[6]. In addition, collagen production induced by $PGF_{2\alpha}$ was inhibited by treatment with not only AL8810 (FP antagonist)[20] but also Y-27632[21], a specific Rho kinase inhibitor, in IMR cells, a human lung fibroblast cell line. This indicates that $PGF_{2\alpha}$ mobilize signaling to induce collagen production through the Rho kinase pathway differently from TGF-β1 whose main intracellular signaling is through Smad (Fig.1).

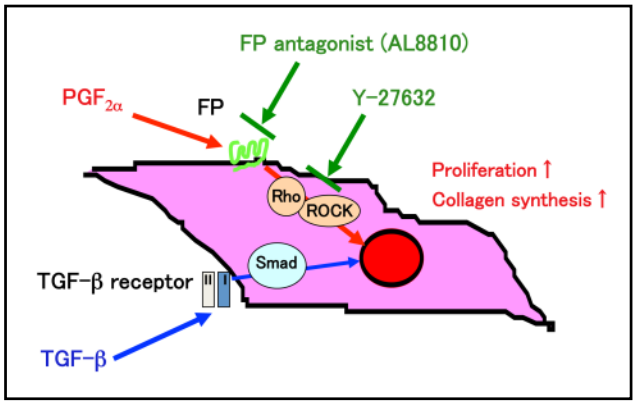

Fig.1 Possible mechanism of $PGF_{2\alpha}$-FP signaling inducing pulmonary fibrosis

Possible mechanism of $PGF_{2\alpha}$-FP signaling inducing fibroblast proliferation and collagen synthesis through Rho kinase-ROCK pathway in fibroblasts, differently from TGF-β signaling mainly through Smads.

$PGF_{2\alpha}$ and other lipid mediators may be a therapeutic target for fibrosis

We demonstrated that $PGF_{2\alpha}$-FP signaling mediates pulmonary fibrosis additive to that induced by TGF-β *in vivo* and *in vitro*. While the median survival of IPF patients of 3-5 years is short, developing drugs targeted against fibrogenesis is urgent. Although therapy against TGF-β signaling would be most promising, other therapeutic targets are needed and blocking $PGF_{2\alpha}$-FP signaling, for example, by an FP antagonist may be one possible alternate approach for treating pulmonary fibrosis. Recently, similar effects of $PGF_{2\alpha}$ on collagen synthesis through protein kinase C and Rho kinase independently of TGF-β1 were observed in cardiac fibroblasts, indicating $PGF_{2\alpha}$-FP signaling as a new therapeutic target for myocardial fibrosis[22]. Thus, more attention may be paid to this pathway for treating fibrosis in various organs.

$cPLA_2$ yields eicosanoids as well as lysophospholipids. Among lysophospholipids, lysophosphatidic acid (LPA) and one of its receptors, LPA1, are implicated in the pathogenesis of pulmonary fibrosis[23], and an LPA1 antagonist is now being investigated in clinical trials of IPF or systemic sclerosis skin fibrosis[10].

Plasma $PGF_{2\alpha}$ metabolite may be a promising candidate for a biomarker of IPF

The clinical course of IPF is highly variable, and there are a considerable number of patients who show rapid disease progression (rapid decliners). Therefore, sensitive biomarkers that can predict future outcomes of patients with IPF are needed in clinical practice. Endogenous $PGF_{2\alpha}$ is swiftly degraded in various organs to 13,14-dihydro-15-keto $PGF_{2\alpha}$ (15-keto-dihydro $PGF_{2\alpha}$), a stable metabolite of $PGF_{2\alpha}$, which has a longer half-life in the circulation and has been used as a reliable indicator of in vivo $PGF_{2\alpha}$ biosynthesis[24]. We found that, using the aforesaid liquid chromatography-tandem mass spectrometry, the levels of 15-keto-dihydro $PGF_{2\alpha}$ as well as $PGF_{2\alpha}$ increased in the BALF of patients with IPF to a greater extent than in patients with sarcoidosis[6]. We further measured plasma concentrations of this metabolite in patients with IPF. We then found that the plasma concentration of 15-keto-dihydro $PGF_{2\alpha}$ in IPF patients was higher than that in controls, and was significantly associated with mortality (unpublished data). Thus, plasma 15-keto-dihydro $PGF_{2\alpha}$ may have a prognostic value in IPF and be useful in clinical management of patients, although further study would be needed. Although the source of $PGF_{2\alpha}$ and its metabolite remains speculative, activated macrophages, type II alveolar epithelial cells, metaplastic epithelium, and contractile interstitial cells (a suspected precursor of myofibroblasts) may produce $PGF_{2\alpha}$[25-27], causing increased levels of $PGF_{2\alpha}$ and its metabolite in BALF in IPF patients, which probably also leak into the circulation. In addition, pulmonary arterial endothelium and/or smooth muscles may upregulate the COX-2 pathway through hypoxic stimulus and/or development of pulmonary hypertension seen in advanced IPF[28], which increases the plasma levels.

Conclusion

Although many drug targets have been proposed through an animal model of IPF and many clinical trials have been performed for novel treatments in patients with IPF, effective therapies have not yet been established and the prognosis of IPF remains poor. However, recently, the understanding of IPF has progressed, and pirfenidone, a novel drug for IPF, has been developed. Studies on novel signaling pathways and effective clinical trials have to be continued. Eicosanoids including PGs (PGE_2, $PGF_{2\alpha}$, PGI_2) and LTs may be one area of promising targets for novel treatments and management, different from the classical models of IPF.

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Conflict of interests

Authors declare no conflict of interest.

References

1) Border WA, Noble NA: Transforming growth factor β in tissue fibrosis. N Engl J Med. 1994; 331: 1286-1292.
2) Wang Y, Hyde DM, Gotwals PJ, Koteliansky VE, Ryan ST, Giri SN: Reduction of bleomycin induced lung fibrosis by transforming growth factor β soluble receptor in hamsters. Thorax. 1994; 54: 805-812.
3) Maher TM, Wells AU, Laurent GJ: Idiopathic pulmonary fibrosis: multiple causes and multiple mechanisms? Eur Respir J. 2007; 30: 835-839.
4) Nagase T, Uozumi N, Ishii S, Kita Y, Yamamoto H, Ohga E, Ouchi Y, Shimizu T: A pivotal role of cytosolic phospholipase A_2 in bleomycin-induced pulmonary fibrosis. Nat Med. 2002; 8: 480-484.
5) Narumiya S: Physiology and pathophysiology of prostanoid receptors. Proc Jpn Acad Ser B. 2007; 83: 296-319.
6) Oga T, Matsuoka T, Yao C, Nonommura K, Kitaoka S, Sakata D, Kita Y, Tanizawa K, Taguchi Y, Chin K, Mishima M, Shimizu T, Narumiya S: Prostaglandin $F_{2\alpha}$ receptor signaling facilitates bleomycin-induced pulmonary fibrosis independently of transforming growth factor-β. Nat Med. 2009; 15: 1426-1430.
7) Peters-Golden M, Bailie M, Marshall T, Wilke C, Phan SH, Toews GB, Moore BB: Protection from pulmonary fibrosis in leukotriene-deficient mice. Am J Respir Crit Care Med. 2002; 165: 229-235.
8) Beller TC, Maekawa A, Friend DS, Austen KF, Kanaoka

Y: Targeted gene disruption reveals the role of the cysteinyl leukotriene 2 receptor in increased vascular permeability and in bleomycin-induced pulmonary fibrosis in mice. J Biol Chem. 2004; 279: 46129-46134.
9) Izumo T, Kondo M, Nagai A: Effects of a leukotriene B_4 receptor antagonist on bleomycin-induced pulmonary fibrosis. Eur Respir J. 2009; 34: 1444-1451.
10) Castelino FV: Lipids and eicosanoids in fibrosis: emerging targets for therapy. Curr Opin Rheumatol. 2012; 24: 649-655.
11) Lovgren AK, Jania LA, Hartney JM, Parsons KK, Audoly LP, Fitzgerald GA, Tilley SL, Koller BH: COX-2-derived prostacyclin protects against bleomycin-induced pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol. 2006; 291: L144-L156.
12) Bonner JC, Rice AB, Ingram JL, Moomaw CR, Nyska A, Bradbury A, Sessoms AR, Chulada PC, Morgan DL, Zelden DC: Susceptibility of cyclooxygenase-2-deficient mice to pulmonary fibrogenesis. Am J Pathol. 2002; 161: 459-470.
13) Card JW, Voltz JW, Carey MA, Bradbury JA, Degraff LM, Lih FB, Bonner JC, Morgan DL, Flake GP, Zelden DC: Cyclooxygenase-2 deficiency exacerbates bleomycin-induced lung dysfunction but not fibrosis. Am J Respir Cell Mol Biol. 2007; 37: 300-308.
14) Mall G, Zimmermann P, Siemens I, Burkhardt A, Otto HF: Prevention of bleomycin-induced fibrosing alveolitis with indomethacin: stereological studies on rat lungs. Virchows Arch A Pathol Anat Histopathol. 1991; 419: 339-347.
15) Arafa HM, Abdel-Wahab MH, El-Shafeey MF, Badary OA, Hamada FM: Anti-fibrotic effect of meloxicam in a murine lung fibrosis model. Eur J Pharmacol. 2007; 564: 181-189.
16) Bozyk PD, Moore BB: Prostaglandin E_2 and the pathogenesis of pulmonary fibrosis. Am J Respir Cell Mol Biol. 2011; 45: 445-452.
17) Moore BB, Ballinger MN, White ES, Green ME, Herrygers AB, Wilke CA, Toews GB, Peters-Golden M: Bleomycin-induced E prostanoid receptor changes alter fibroblast responses to prostaglandin E_2. J Immunol. 2005; 174: 5644-5649.
18) White ES, Atrasz RG, Hu B, Phan SH, Stambolic V, Mak TW, Hogaboam CM, Flaherty KR, Martinez FJ, Kontos CD, Toews GB: Negative regulation of myofibroblast differentiation by PTEN (Phosphatase and Tensin Homolog Deleted on chromosome 10). Am J Respir Crit Care Med. 2006; 173: 112-121.
19) Zhu Y, Liu Y, Zhou W, Xiang R, Jiang L, Huang K, Xiao Y, Guo Z, Gao J: A prostacyclin analogue, iloprost, protects from bleomycin-induced pulmonary fibrosis in mice. Respir Res. 2010; 11: 34.
20) Griffin BW, Klimko P, Crider JY, Sharif NA: AL-8810: a novel prostaglandin $F_{2\alpha}$ analog with selective antagonist effects at the prostaglandin $F_{2\alpha}$ (FP) receptor. J Pharmacol Exp Ther. 1999; 290: 1278-1284.
21) Ishizaki T, Uehata M, Tamechika I, Keel J, Nonomura K, Maekawa M, Narumiya S: Pharmacological properties of Y-27632, a specific inhibitor of rho-associated kinases. Mol Pharmacol. 2000; 57: 976-983.
22) Ding WY, Ti Y, Wang J, Wang ZH, Xie GL, Shang YY, Tang MX, Zhang Y, Zhang W, Zhong M: Prostaglandin $F_{2\alpha}$ facilitates collagen synthesis in cardiac fibroblasts via an F-prostanoid receptor/protein kinase C/Rho kinase pathway independent of transforming growth factor β1. Int J Biochem Cell Biol. 2012; 44: 1031-1039.
23) Tager AM, LaCamera P, Shea BS, Campanella GS, Selman M, Zhao Z, Polosukhin V, Wain J, Karimi-Shah BA, Kim ND, Hart WK, Pardo A, Blackwell TS, Xu Y, Chun J, Luster AD: The lysophosphatidic acid receptor LPA1 links pulmonary fibrosis to lung injury by mediating fibroblast recruitment and vascular leak. Nat Med. 2008; 14: 45-54.
24) Basu S: Novel cyclooxygenase-catalyzed bioactive prostaglandin $F_{2\alpha}$ from physiology to new principles in inflammation. Med Res Rev. 2007; 27: 435-468.
25) Taylor L, Polgar P, McAteer JA, Douglas WH: Prostaglandin production by type II alveolar epithelial cells. Biochim Biophys Acta. 1979; 572: 502-509.
26) Lappi-Blanco E, Kaarteenaho-Wiik R, Maasilta PK, Anttila S, Paakko P, Wolff HJ: COX-2 is widely expressed in metaplastic epithelium in pulmonary fibrous disorders. Am J Clin Pathol. 2006; 126: 717-724.
27) Fukui M, Fujimoto T, Watanabe K, Endo K, Kuno K: Prostaglandin F synthase is localized to contractile interstitial cells in bovine lung. J Histochem Cytochem. 1996; 44: 251-257.
28) Archer SL, Weir EK, Wilkins MR: Basic science of pulmonary arterial hypertension for clinicians: new concepts and experimental therapies. Circulation. 2010; 121: 2045-2066.