

## REVIEW

# Targeting platelet-derived growth factor as a therapeutic approach in pulmonary fibrosis

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**Abstract :** Idiopathic pulmonary fibrosis (IPF) is a progressive and lethal lung disease characterized by the proliferation of fibroblasts and deposition of extracellular matrix. Since the prognosis of IPF is still poor, novel therapeutic modalities are strongly required. For this reason, to find molecular target for therapy of IPF is of much importance. The recent understanding of pathogenesis in IPF indicates the critical role of alveolar epithelial type II cells (AECII) and fibroblasts. Although the detailed mechanisms involved in IPF is still unclear, various profibrotic mediators which are produced by the injured AECII are thought to play a role in the progression of pulmonary fibrosis via stimulating fibroblasts. Among them, platelet-derived growth factor (PDGF) is one of critical growth factors by stimulating the proliferation and migration of fibroblasts. In this review, we discuss the role of PDGF in pulmonary fibrosis and the possibility as a therapeutic target for IPF. *J. Med. Invest.* 60 : 175-183, August, 2013

**Keywords :** *idiopathic pulmonary fibrosis, platelet-derived growth factor, tyrosine kinase, fibroblast*

## INTRODUCTION

Idiopathic pulmonary fibrosis (IPF)/usual interstitial pneumonia (UIP) is a progressive and lethal lung disease characterized by the proliferation of fibroblasts and deposition of extracellular matrix (ECM) including fibrillar collagens, fibronectin, elastic fibers and proteoglycans (1, 2). While corticosteroids and other immunosuppressants have been used for the treatment of patients with IPF, the response rate to these agents was low, and the five-year survival rate of IPF is less than 50% (3, 4). Recent guidelines for IPF describe that no effective pharmacological therapy has been developed so far

(5). For this reason, novel therapeutic modalities are still of strong interest.

However, the molecular pathogenesis involved in pulmonary fibrosis is not fully investigated. In addition, from the therapeutic point of view, we do not have the right answer to the question which is best target to treat pulmonary fibrosis.

More recently, Nintedanib (BIBF1120) which is the multikinase inhibitor of three receptors for platelet-derived growth factor (PDGF), fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) have been shown the inhibitory activity for the progression of IPF in TOMORROW phase II clinical trial (6). Based on this trial, PDGF is believed to be one of promising targets for therapy of IPF. In this review, we consider the update molecular pathogenesis in IPF, and discuss the possibility of targeting PDGF as a therapeutic approach of IPF.

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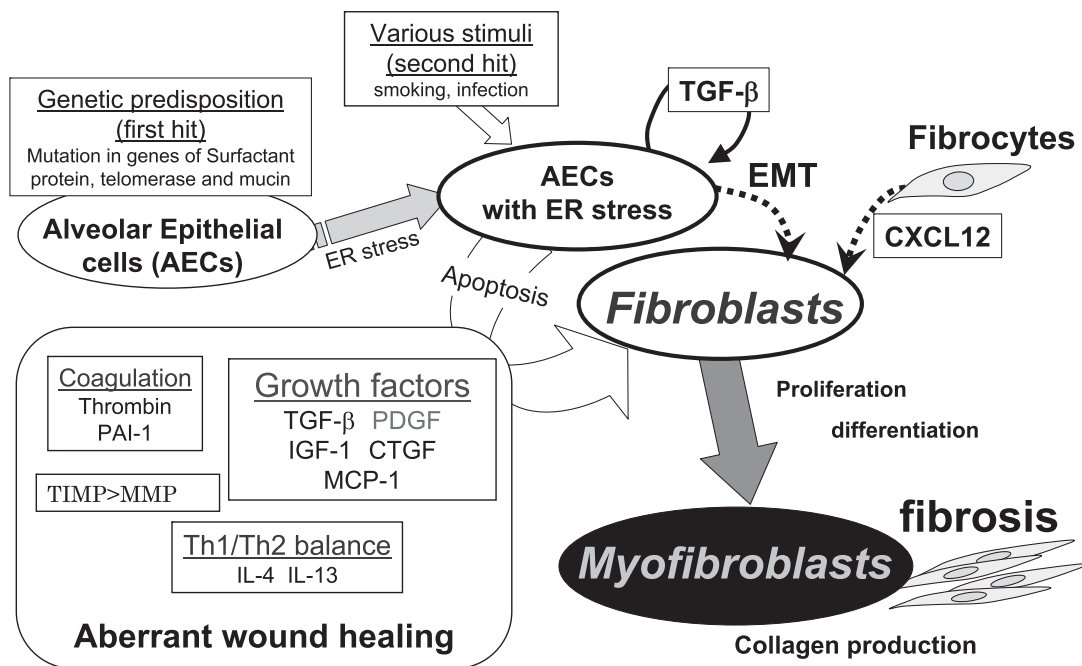
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MOLECULAR PATHOGENESIS IN IPF

The molecular mechanisms involved in the progression of IPF are still not fully understood, whereas the alveolar epithelial type II cells (AECII) and fibroblasts are thought to be main players (Figure 1). As the first event, AECII injury is caused by endoplasmic reticulum (ER) stress, lysosomal stress, and mitochondrial and DNA damage (7, 8). AECII are known to have genetic predisposition like gene mutations of surfactant protein C or A2, telomerase and MUC5B in subjects with familial pulmonary fibrosis, which lead to be susceptible for the second stimuli such as cigarette smoking or viral infection. After the second stimuli, the injured AECs have the tendency to be apoptotic. The link of injured AECII and fibrosis is not fully understood, but it was reported that those cells could produce the fibrotic mediators. Among profibrotic mediators, transforming growth factor (TGF)- $\beta$  and PDGF are thought to play a critical role in the fibrogenesis of the lungs.

PDGF AND PDGF RECEPTORS

PDGF is a homo- or heterodimeric molecule with a molecular weight of 30 kDa (7). PDGF genes are consisted of four different genes including PDGF-A, -B, -C and -D, which are located on chromosomes 7, 22, 4 and 11, respectively (9, 10). There are two types of PDGF receptors (PDGFRs),  $\alpha$  and  $\beta$ , which have a molecular weight of 170-180 kDa, and composed of homo- or heterodimers. The possible PDGF-PDGFR interactions are multiple and complex in *in vitro* study as shown in figure 2 (11). However, the *in vivo* study showed the binding of PDGF-AA or -CC to PDGFR- $\alpha$ , and PDGF-BB to PDGFR- $\beta$  (11). PDGF dimers can bind their receptors and induce the dimerization of PDGFR, which allow their autophosphorylation of tyrosine residues in *trans* between two receptors (9). After the autophosphorylation, activated PDGFR can coupled with various signal transduction molecules such as Ras-MAPK through Grb2 and Shc adaptor proteins, and phosphatidyl inositol-3 kinase and phospholipase C- $\gamma$ . PDGFR interacts with integrins and induced cell migration through activation focal adhesion kinase (FAK).



**Figure 1** Molecular pathogenesis of IPF Alveolar epithelia cells (AECs) are known to have genetic predisposition like gene mutations of surfactant protein C or A2, telomerase and MUC5B in subjects with familial pulmonary fibrosis, which induce endoplasmic reticulum (ER) stress and lead to be susceptible for the second stimuli such as cigarette smoking or viral infection. After the second stimuli, the injured AECs have the tendency to be apoptotic and produce the profibrotic mediators. Although the link of injured AECs and fibrosis is not fully understood, among profibrotic mediators, transforming growth factor (TGF)- $\beta$  and PDGF are thought to play a critical role in the fibrogenesis of the lungs, which induce the proliferation and differentiation to myofibroblasts of fibroblasts.

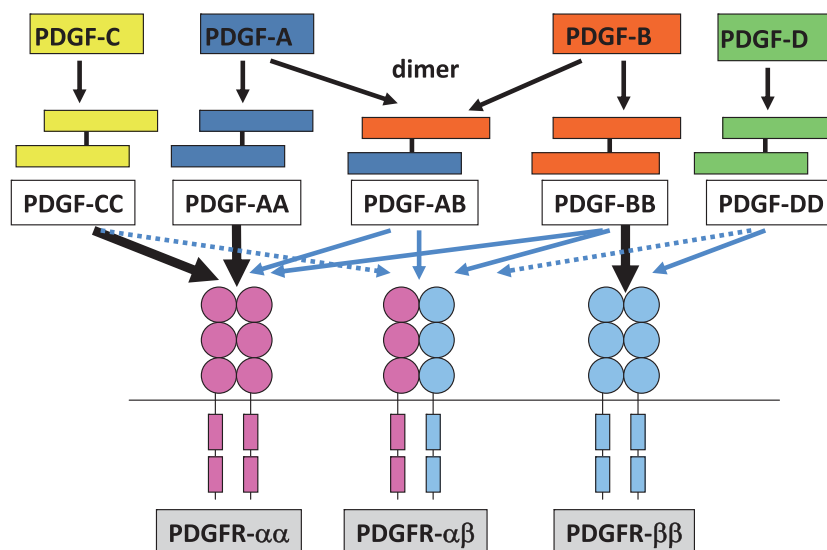


Figure 2 PDGF-PDGFR system

The four PDGF proteins make a homo- or hetero-dimers and bind to PDGF receptors. The black bold arrow indicates the bindings which are confirmed *in vivo*. The blue arrows indicate the binding in cell culture. The hatched arrows indicate weak interactions or conflicting results.

## EXPRESSION OF PDGFs AND PDGFRs

PDGFs are expressed in many types of cells including fibroblasts, vascular endothelial cells, macrophages as well as platelets/megakaryocytes (9). It is known that the expression of PDGFs is up-regulated by various inflammatory cytokines and growth factors including TGF- $\beta$  and PDGF themselves. PDGFRs are also expressed in various cells although the classical targets of PDGF are fibroblasts and smooth muscle cells. The expression of PDGFRs is also induced by various stimuli. In contrast to PDGFs, expression of PDGFR in some cells is limited to PDGFR- $\alpha$  or PDGFR- $\beta$ , but not both.

## BIOLOGICAL ACTIVITIES THROUGH PDGF/PDGFR

PDGF is known to be a major mitogen for mesenchymal cells. PDGF is a strongest stimulus of proliferation of fibroblasts (9). In addition, binding of PDGF to their receptors induces  $Ca^{2+}$  influx and rearrangement of the cytoskeleton involving changes in the arrangement of actin stress fibers. In terms of these responses, PDGF also stimulates the migration of various cells.

## LUNG DEVELOPMENT AND PDGFs/PDGFRs

The knock-out mice of the PDGF-A gene showed the homozygous lethal with two different restriction points, one prenatally and one postnatally (12). Postnatally surviving PDGF-A-deficient mice develop lung emphysema secondary due to the loss of alveolar myofibroblasts which has PDGFR- $\alpha$ . On the other hand, the PDGFR- $\alpha$  null mice showed the cranial malformations and deficiency of myotome formation (13). Mice deficient for PDGF-B revealed renal, cardiovascular and hematological abnormalities (14). Similarly, PDGFR- $\beta$  mutant mice exhibited abnormal kidney development and hematological disorders, but cardiovascular abnormality was not detected (15). In summary, PDGF-A/PDGFR- $\alpha$  pathway plays a role in the secondary septation process, since PDGFR- $\alpha$ -expressing cells locate in the alveolar entry ring and have characteristics of myofibroblasts (16).

## PDGF AND PULMONARY FIBROSIS

It is known that PDGF is one of the growth factors which play a role in the pathogenesis of pulmonary fibrosis (9, 11). In the animal models, bleomycin-induced pulmonary fibrosis has been used for the analysis of molecular pathogenesis in mice or rats.

Maeda *et al.* reported that expression of the PDGF-A gene increased in bleomycin-induced pulmonary fibrosis models in mice using semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) (17). Walsh *et al.* have examined the bronchoalveolar lavage fluid of rats treated with a bleomycin, and could find the 38-40 kDa and 29 kDa peptides, which were detected with anti-PDGF-BB and anti-PDGF-AA antibodies, respectively, showed the growth-promoting activity of lung fibroblasts (18). The growth-promoting activity was neutralized 64% by anti-PDGF-BB antibody and 15% by anti-PDGF-AA antibody. In contrast, Zhuo *et al.* showed that PDGF-C gene, but not PDGF-A, B and D, was induced in the lungs of mice treated with bleomycin by Northern blot analysis (19). Shimizu *et al.* reported that both PDGF-A and -B was induced and elevated in bleomycin-treated lungs of mice at the levels of both mRNA and protein (20). Adoptive transfer of an adenovirus expressing the PDGF-B gene into the lung induced severe fibrosis in mice (21). From these reports, the expression of PDGF isoforms could be enhanced in fibrogenesis of the lungs, but the details are required to be further analyzed.

On the other hand, enhanced expression of PDGF in the epithelial cells and alveolar macrophages in lungs of patients with IPF has been reported (22, 23). However, the mechanisms involved in the enhanced expression and action of PDGF in the fibrotic lung are poorly understood. Recently, Gochuico *et al.* has examined the growth factors in alveolar lining fluids of patients with rheumatoid arthritis complicated with pulmonary fibrosis, and reported that PDGF-AB and -BB, but not TGF- $\beta$  and PDGF-AA, was associated with the progressive stage of pulmonary fibrosis (24), indicating the importance of PDGF-B in the fibrogenesis of the lungs.

#### ANTIFIBROTIC EFFECTS OF TARGETING PDGF/PDGFR SIGNALING PATHWAY IN ANIMAL MODELS

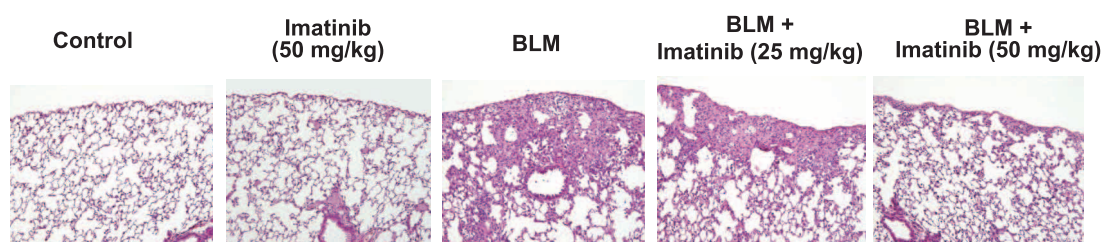
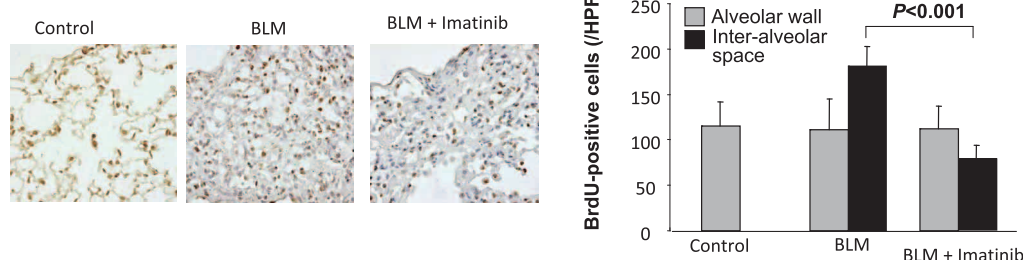
Several evidences described above suggest the targeting PDGF/PDGFR signaling pathway might lead to show the therapeutic effects against pulmonary fibrosis. This hypothesis has been investigated using animal models of pulmonary fibrosis with specific inhibitors for PDGFR.

Rice *et al.* firstly reported that AG1296, the inhibitor for the tyrosine kinase of PDGFR, prevented

pulmonary fibrosis induced by vanadium pentoxide ( $V_2O_5$ ) in rats (25). Since AG1296 is a compound which has been used for *in vitro* experiments, molecular targeted drugs which have been developed for treatment of patients with malignancy should be investigated their antifibrotic effects. Among them, imatinib mesylate (previously called STI571, Gleevec in the United States and Glivec in Europe) is a potent and specific tyrosine kinase inhibitor against c-abl, bcr-abl and c-kit. Imatinib has been demonstrated to be highly active in chronic myeloid leukemia (CML) and gastrointestinal stromal tumors (GIST) (26-29). The reported data regarding the specificity of imatinib for various tyrosine kinases shows that imatinib also specifically inhibits PDGFR tyrosine kinase (30). Therefore, we and others have tried to demonstrate antifibrotic effects of imatinib in various pulmonary fibrosis models. As shown in figure 3, imatinib strongly inhibited the fibrogenesis in the lungs treated with bleomycin via inhibiting the growth of mesenchymal cells *in vivo* in mice (31). Daniels *et al.* reported that imatinib could inhibit the activity of TGF- $\beta$  via inhibiting c-Abl kinase in addition to the blocking of PDGFR signaling (32). They also demonstrated that imatinib prevented bleomycin-induced pulmonary fibrosis in mice. Furthermore, Abdollahi *et al.* demonstrated the antifibrotic effects of imatinib in murine radiation-induced lung fibrosis (33). In their experiments, they used three kinds of small molecule inhibitors, imatinib, SU9518 and SU11657, which can inhibit the common receptor PDGFR. Therefore antifibrotic effects of three inhibitors were strongly suggested to be mediated by blocking PDGFR. In addition, Yoshida *et al.* reported that the *in vivo* gene transfer of an extracellular domain of PDGFR- $\beta$  reduced bleomycin-induced pulmonary fibrosis (34). These results suggested that PDGFR is one of therapeutic target for pulmonary fibrosis.

Imatinib has also been reported to prevent fibrogenesis in the liver and kidneys (35-38). These results in addition to inhibitory effects of myelofibrosis (39, 40) suggest that imatinib might serve as an antifibrotic drug for various fibrotic diseases in humans.

Recently, the antifibrotic effects of other inhibitors, which could show higher activity compared to imatinib, have been reported. For example, BIBF1000 which inhibited PDGFR, VEGFR and FGFR was reported to reduce pulmonary fibrosis induced by a bleomycin in rats (41). Nilotinib was also reported to show higher antifibrotic activity as compared to imatinib (42).

**A: H&E staining****B: BrdU staining**

**Figure 3** Antifibrotic effects of imatinib in pulmonary fibrosis induced by bleomycin in mice. Treatment with imatinib inhibited pulmonary fibrosis induced by bleomycin in a dose-dependent manner (A). The BrdU staining showed the reduced number of proliferating cells in inter-alveolar space (B). Adapted from reference 31.

## ANTIFIBROTIC EFFECTS OF TARGETING PDGF/PDGFR SIGNALING PATHWAY IN HUMAN

Based on the preclinical studies, the application of imatinib for treatment of patients with pulmonary fibrosis has been expected.

In the earlier experiences in clinical use of imatinib, several case reports showed the favorable activity of imatinib in patients with connective tissue diseases including systemic sclerosis (SSc) or mixed connective tissue disease (MCTD) (43-45). Chung *et al.* reported two cases with SSc treated with imatinib. Both patients showed the improvement of skin tightening after the use of 200 mg/day of imatinib for three months (43). In addition, a forced vital capacity (FVC) in one patient who has lung involvement of SSc was improved from 48% of predicted to 52%. Sfikakis *et al.* reported the severe case of SSc who have treated with 400 mg/day imatinib for six months (44). This patient showed the improvement of FVC from 68.0% to 88.3% predicted. Distler *et al.* also reported the improvement of pulmonary function and HRCT findings in patients with MCTD (45). The recent phase IIa, single-arm, open-label clinical trial with imatinib against SSc showed the efficacy in modified Rodnan skin score and FVC (46), whereas the increased adverse events were

observed, and some trials were prematurely terminated for safety reasons (47).

On the other hand, the clinical trial of IPF with single dose of imatinib has been conducted and performed in USA (48). Although serious adverse events were not more common in the imatinib group, treatment with imatinib did not affect lung function such as FVC and the diffusing capacity of the lung (DLco) as well as survival of IPF patients. However, this trial has several problems in the design and results which was pointed out in the discussion of this paper and the editorial by Thannickal VJ and Roman J (49). First, this study was conducted by the anticipation of 50% incidence of achieving the primary endpoint of disease progression or death over 96 weeks of treatment, whereas the incidence in placebo group was 31.6%, indicating the underpower of this study to detect the differences between groups. Second, theoretically, PDGFR are expressed not only in fibroblasts, but also in epithelial or other cells. To inhibit PDGFR signaling pathway can block the proliferation and migration of fibroblasts, but the blocking of PDGFR may affect the function of epithelial cells to prevent the fibrogenesis. In fact, Vuorinen *et al.* reported that PDGFR- $\beta$  are preferentially expressed in lung parenchyma, but PDGFR- $\alpha$  are strongly expressed in epithelium in addition to lung parenchyma in the lungs of IPF patient (50).

Third, the pharmacological consideration of imatinib was also pointed out. We reported the resistance mechanism of imatinib mediated by  $\alpha$ 1-acid glycoprotein (AGP), which can bind to imatinib and block the effects of imatinib (51). AGP was elevated in serum of both bleomycin-treated mice and patients with IPF. Therefore, the patients enrolled in clinical trial might be better to be selected by the level of AGP. The more extensive and fine preclinical study could be required to focus the molecular target for therapeutic approach of IPF.

However, the growth factors are still in central mediators to promote pulmonary fibrosis. Recently, promising clinical trials targeting the signaling of growth factors have been reported. BIBF1120, which is derivative of BIBF1000, was reported to inhibit the progression and acute exacerbation of IPF in phase II TOMORROW study (6).

## FUTURE PERSPECTIVES

The international phase III clinical trial using BIBF1120 for IPF has been in progress. BIBF1120 can inhibit VEGFR and FGFR in addition to PDGFR. The role of VEGFR and FGFR in the progression of pulmonary fibrosis is still unclear. Therefore, PDGFR still play a central role as a therapeutic target in the progression of IPF. The establishment of molecular targeting therapy against growth factors including PDGF would be expected in clinic.

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