

Role of PDGF in fibrotic diseases and systemic sclerosis

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PDGF functions as a primary mitogen and chemoattractant for cells of mesenchymal origin. Members of the PDGF family play an important role during embryonic development and contribute to the maintenance of connective tissue in adults. Deregulation of PDGF signalling has been linked to atherosclerosis, pulmonary hypertension and organ fibrosis. Elevated expression of PDGF and its receptors has been found in scleroderma skin and lung tissues. There is evidence for a TGF- β and IL-1 α -dependent autocrine PDGF-A/PDGFR α signalling loop in scleroderma skin and lung fibroblasts, suggesting that a cross-talk between TGF- β and PDGF pathways may regulate chronic fibrosis in scleroderma.

KEY WORDS: Platelet-derived growth factor, Platelet-derived growth factor receptor, Transforming growth factor- β , Fibrosis, Pulmonary arterial hypertension, Scleroderma, Myofibroblast.

PDGF ligands and receptors

PDGFs are the primary mitogens for the cells of mesenchymal and neuroectodermal origin. PDGF, which was first described in the 1970s as a serum factor that stimulates proliferation of smooth muscle cells, is now one of the best characterized growth factor-receptor systems. The PDGF family is composed of four different polypeptide chains, the traditional PDGF-A and PDGF-B, and more recently discovered PDGF-C and PDGF-D. The biologically active PDGF protein forms disulphide-bonded dimers, including four homodimers PDGF-AA, PDGF-BB, PDGF-CC and PDGF-DD, and one heterodimer, PDGF-AB. PDGF-A and PDGF-B are processed intracellularly and secreted in their active forms, while PDGF-C and PDGF-D are secreted as latent factors requiring proteolytic activation [1].

PDGFs exert their biological activities by activating two structurally related tyrosine kinase receptors, PDGFR α and PDGFR β . Ligand-induced receptor homo- or heterodimerization leads to autophosphorylation of specific tyrosine residues within the cytoplasmic domain. PDGF-A activates PDGFR α , exclusively, while PDGF-B is capable of activating PDGFR α , PDGFR $\alpha\beta$ and PDGFR β . PDGF-AB and PDGF-C activate PDGFR α and PDGFR $\alpha\beta$, whereas PDGF-D preferentially activates PDGFR β [1]. Receptor activation leads to induction of several signalling pathways resulting in cellular proliferation, chemotaxis and actin reorganization. PDGF α and - β receptors are structurally similar and activate overlapping signal transduction pathways including phosphatidylinositol 3 kinase (PI3K), Ras-MAPK, Src family kinases and phospholipase C γ (PLC γ) resulting in overlapping biological properties *in vitro*.

Genetic analysis of PDGF system

Physiological functions of PDGFs and their receptors *in vivo* have been elucidated through extensive genetic studies in mice [2]. Knockout studies have demonstrated that PDGFs are essential during mouse embryonic development. Interestingly, however, PDGF-A and PDGF-B and their respective receptors have distinct functions *in vivo*. PDGF-B and the PDGFR β are primarily required for the development of the vasculature. Specifically, PDGF-B/PDGFR β regulate recruitment of mural cell progenitors during vessel remodelling and maturation, but are

not involved in the initial stages of formation of the vascular network. Studies show that endothelial cells secrete PDGF-B, which acts at close range in a paracrine manner to promote proliferation and spreading of mural cells, which express the PDGFR β . Pericyte coverage of blood vessels in the central nervous system, skin, lungs and heart is particularly dependent on the PDGF-B/PDGFR β signalling.

PDGF-A and PDGFR α have a broader role in embryogenesis and function at the sites of epithelial–mesenchymal interactions during organogenesis. PDGF-A/PDGFR α is required for the development of the central nervous system, lung, gonads, gut and kidneys. Absence of PDGF-A signalling during lung development leads to a severe emphysema-like phenotype due to the failure of alveolar septum formation. The expression pattern of the PDGF-A/PDGFR α suggests that during development PDGF-A is expressed by the epithelium, while PDGFR α is expressed on the adjacent mesenchymal cells, which constitute the progenitors of the alveolar smooth muscle cells. PDGF-A signalling appears to be required for the proper positioning and differentiation of these cells and a subsequent deposition of elastin. On the other hand, overexpression of PDGF-A in the lung epithelium leads to excessive expansion of the lung mesenchyme and compression of distal respiratory airways. PDGF-A/PDGFR α is also required for the formation of mesenchymal dermis. In the PDGFR α -null mice there is a general absence of mesenchymal cells including dermal fibroblasts and the mesenchymal cells located in the hair follicle. PDGF-A secreted by the epidermal cells supports proliferation and the maintenance of the dermal mesenchymal cells. PDGF-C knockout shows similarities to mice lacking PDGF-A during early embryonic development [3]. PDGF-D knockout has not yet been reported.

PDGFs and human disease

Excessive activity of PDGF has been associated with several human disorders, including atherosclerosis, balloon injury-induced restenosis, pulmonary hypertension, organ fibrosis and tumorigenesis [4]. PDGF-A and PDGF-B, as well as PDGF receptors are almost undetectable in normal vessels, but are highly expressed in the diseased vessels. The main sources of PDGF are the lesional macrophages. A causal role for PDGF in atherosclerosis has been supported by several animal *in vivo* studies. Blockade of PDGF using pharmacological inhibitors or specific antibodies resulted in diminished smooth muscle cell (SMC) accumulation following injury [5]. These studies have also established that blockade of PDGF-B/PDGFR β is the most beneficial, suggesting a lesser role for the PDGFR α signalling in this disease.

Pulmonary arterial hypertension (PAH) is characterized by the pathological changes in the pulmonary circulation leading to

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sustained elevation of pulmonary arterial pressure and death. Recent animal studies, as well as analyses of human biopsies from patients with PAH have suggested that overexpression of PDGF receptors may contribute to SMC dysregulation in PAH. This notion was supported by a study that demonstrated reversal of PAH in two animal models by administration of STI587 (imatinib mesylate) [6]. The authors of this study have shown that STI587 specifically targeted PDGF signalling resulting in lower levels of PDGF-B and diminished phosphorylation levels of PDGFR β .

Numerous studies have implicated PDGF working in concert with TGF- β in development of organ fibrosis [7]. Fibrosis results from the excessive deposition of extracellular matrix primarily by the activated mesenchymal cells termed myofibroblasts. PDGF plays a key role in expansion of myofibroblasts by stimulating their proliferation, migration and survival. Elevated levels of PDGF have been consistently demonstrated in the fibrotic lesions of various organs. In pulmonary fibrosis, PDGF-B is produced by the alveolar macrophages, while myofibroblasts are the main source of PDGF-A. Myofibroblasts also express elevated levels of PDGFR α , suggesting that both the paracrine and autocrine modes of signalling operate in these cells. Interestingly, in a bleomycin model of fibrosis PDGF-C and PDGF-D were up-regulated together with increase in levels of PDGFR α , while the levels of PDGF-A and PDGF-B remained unchanged.

PDGF is also the most prominent mitogen contributing to fibrosis of the liver [7]. Hepatic fibrogenesis involves activation and proliferation of hepatic stellate cells that acquire a myofibroblastic phenotype and ultimately are responsible for the excessive production of extracellular matrix proteins. PDGF-B and PDGFR β are the key players in development of hepatic fibrosis. Recent reports have also linked PDGF-C and PDGF-D to development of hepatic fibrosis, steatosis and hepatocellular carcinoma. PDGF is one of the best characterized growth factor systems in renal disease with all four PDGF isoforms being implicated in various renal pathologies [8]. These studies point to a common mechanism of organ fibrosis with PDGF playing a central role in expanding the population of collagen-producing cells and TGF- β -stimulating matrix production.

The role of PDGF in scleroderma

PDGF may also play an important role in the pathogenesis of scleroderma. PDGF is almost undetectable in healthy skin or lung. Immunohistochemical studies have revealed increased presence of PDGF and PDGF receptors in scleroderma skin biopsies. Expression of PDGF-B was detected in endothelial cell lining of small capillaries and in the infiltrating cells [9, 10]. PDGF-A was prominently expressed in small capillaries, around the hair follicles and in selective stromal cells [11]. Likewise, elevated levels of PDGF-A and PDGF-B were found in bronchoalveolar lavage (BAL) fluid obtained from scleroderma patients [12]. An interesting observation was made regarding TGF- β regulation of PDGFR α in scleroderma fibroblasts. Unlike normal fibroblasts, which are unaffected by TGF- β treatment or show decreased PDGFR α expression in response to TGF- β , scleroderma fibroblasts respond to TGF- β with up-regulation of PDGFR α [11]. As a result TGF- β treatment renders scleroderma fibroblasts more responsive to the subsequent mitogenic stimulation with PDGF. This unique characteristic is present in both skin fibroblasts and fibroblasts obtained from scleroderma BAL fluid [12]. Relevant to this finding, the studies of Kawaguchi *et al.* [13] have shown that scleroderma fibroblasts express elevated levels of endogenous IL-1 α , which in turn stimulates production of PDGF-A and IL-6 [13]. These findings imply the existence of an autocrine PDGF-A/PDGFR α loop operating in scleroderma fibroblasts (Fig. 1). The functional significance of the activation of PDGF signalling in scleroderma fibroblasts has not been fully evaluated, but it may contribute to the enhanced proliferation and migratory and contractile potential of cultured scleroderma fibroblasts.

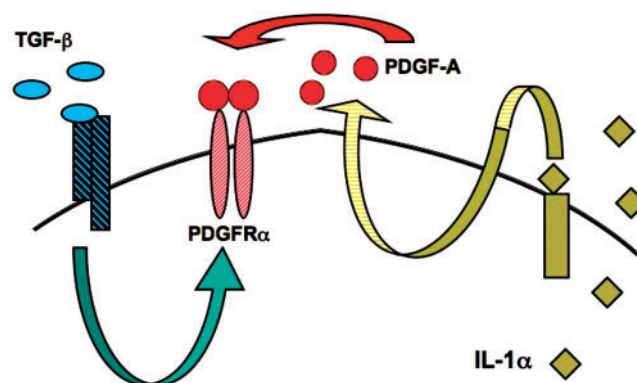


FIG. 1. Aberrant activation of PDGF-A/PDGFR α signalling in scleroderma fibroblasts. In scleroderma fibroblasts, constitutive PDGF-A production is driven by the endogenous IL-1 α , whereas PDGFR α expression is up-regulated in response to TGF- β or thrombin stimulation.

Recent studies have also suggested that sera from patients with scleroderma contain pathological autoantibodies directed against PDGF receptors [14]. These autoantibodies were capable of stimulating reactive oxygen species and subsequent activation of ERK1/2. These intriguing studies, if confirmed, may provide additional support for the persistent activation of PDGF signalling and its contribution to scleroderma fibrosis. This new concept, however, awaits additional independent confirmation.

The studies of the mechanism of scleroderma fibrosis point to the principal role of lesional fibroblasts, which are responsible for the uncontrolled matrix deposition. Cultured scleroderma fibroblasts continue to exhibit many abnormal characteristics when compared with healthy fibroblasts, including elevated matrix synthesis, resistance to Fas-induced apoptosis, a high proportion of cells with myofibroblast characteristics, and as discussed earlier, enhanced proliferation [15]. This abnormal behaviour has been attributed to the alterations of the components of the TGF- β signalling pathway resulting in autocrine TGF- β signalling. As discussed earlier, there is also a positive cross-talk between the TGF- β and PDGF signalling through up-regulation of PDGFR α , which occurs in scleroderma, but not in healthy fibroblasts. It may be of interest that in dermal fibroblasts, PDGF has been shown to stimulate TGF- β receptors, suggesting a possibility of the reciprocal mode of regulation, as well.

The combined sustained PDGF and TGF- β signalling in scleroderma lesions may enable the expansion of a subpopulation of cells with increased synthetic capacity and contribute to the perpetual fibrotic response. Furthermore, interruption of this perpetual signalling should be beneficial to scleroderma patients suggesting that both TGF- β and PDGF are attractive targets for therapy in scleroderma.

Rheumatology key messages

- Activation of the PDGF/PDFGR signalling pathway has been linked to a number of proliferative and fibrotic disorders including PAH and SSc.
- In SSc fibroblasts, PDGF-A synthesis is driven by enhanced endogenous IL-1 α , while PDGFR α expression is up-regulated in response to TGF- β thereby creating an autocrine PDGF-A/PDGFR α loop.
- In dermal fibroblasts, PDGF has been shown to stimulate TGF- β receptors, suggesting an additional reciprocal mode of regulation.
- Both TGF- β and PDGF are therefore attractive targets for therapy in SSc.

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