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Involvement of PDGF in Fibrosis and Scleroderma: Recent Insights from Animal Models and Potential Therapeutic Opportunities

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Abstract

Fibrosis is the principal characteristic of the autoimmune disease known as scleroderma or systemic sclerosis (SSc). Studies published within the last three years suggest central involvement of platelet-derived growth factors (PDGFs) in SSc-associated fibrosis. PDGFs may also be involved in SSc-associated autoimmunity and vasculopathy. The PDGF signaling pathway is well understood and PDGF receptors are expressed on collagen-secreting fibroblasts and on mesenchymal stem and/or progenitor cells that may affect SSc in profound and unexpected ways. Although much work remains before we fully understand how PDGFs are involved in SSc, there is much interest in using PDGF inhibitors as a therapeutic approach to SSc.

Keywords

PDGF; Scleroderma; Fibrosis; Mouse model; Animal models; Pericyte; Imatinib; Neutralizing antibody; Therapeutic

Introduction

Scleroderma, or systemic sclerosis (SSc), is an autoimmune disease with skin and other organ fibrosis as its principal manifestation. SSc-associated fibrosis has the potential to involve the gastrointestinal tract, skeletal muscle, kidneys, lungs, and heart. Additional characteristics of SSc include loss of adipose tissue, loss of capillaries and small lymphatic vessels, and chronic inflammation with autoantibody production [1–3]. Despite better management of its complications, SSc is an incurable disease with median survival of 11 years [4]. The initial causes of SSc are unknown, and there are many proposals of how the characteristics of this disease might be integrated in SSc pathogenesis. A major impediment to developing effective SSc therapy has been a poor understanding of the biological processes involved in fibrosis. It is widely accepted that resident dermal fibroblasts and/or myofibroblasts promote SSc skin fibrosis by overproducing collagen-rich extracellular matrix (ECM) [5]. However, the origin of these ECM-producing fibroblasts in SSc has yet to be conclusively demonstrated [6]. Many different cell types have been proposed as the origin

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of ECM-producing fibroblasts in fibrotic disease, including endothelial cells, mural cells, bone marrow-derived cells, and mesenchymal stem and/or progenitor cells. It is well established that profibrotic pathways can be activated by cytokines, for example transforming growth factor (TGF)- β and platelet-derived growth factor (PDGF), which are likely to be crucially involved in the pathogenesis of SSc. In this review, we first provide an overview of PDGF and PDGF receptors (PDGFRs). We then discuss what is known about the involvement of PDGF in SSc, including recent animal models that suggest crucial involvement of PDGFR α in organ fibrosis. Finally, our review will focus on possible therapeutic opportunities to inhibit PDGF signaling in SSc.

PDGF and Scleroderma

PDGF was identified almost forty years ago as a growth factor in serum that stimulated proliferation of fibroblasts and vascular smooth muscle cells [7, 8]. Increased PDGF signaling has been associated with major human diseases including cancer, atherosclerosis, and fibrosis [9]. Fibrotic skin lesions in SSc patients express higher levels of PDGF and PDGF receptors [10–12]. Significantly increased PDGFR β expression on pericytes within the dermal microvasculature is observed for patients with early SSc [13]. Moreover, elevated levels of PDGF have been detected in bronchoalveolar lavage of SSc patients [14, 15]. Many investigators believe TGF β is the master regulator of fibrosis because it stimulates collagen and alpha smooth muscle actin (α SMA) expression [6]. PDGF was initially judged most likely to have indirect involvement in SSc by regulating fibroblast proliferation [16]. However, more recent work using genetic mouse models has revealed that either PDGF or TGF β signaling can be sufficient to induce SSc-like fibrosis [17, 18]. Thus, the potential for PDGF signaling to be crucially involved in SSc must be considered.

PDGF is secreted by platelets, monocytes, vascular endothelial cells, and other cell types as a disulfide-linked dimer of two PDGF peptides, giving rise to multiple isoforms including PDGF-AA, BB, AB, CC, and DD [9]. Mesenchyme-derived cells, for example fibroblasts, smooth muscle cells, and pericytes, are all responsive to PDGF. There are two PDGFR isoforms, PDGFR α (CD140a) and PDGFR β (CD140b), each capable of homo or heterodimerization in the presence of PDGF. PDGF-AA, BB, AB, and CC bind to PDGFR $\alpha\alpha$ homodimers whereas PDGF-BB and DD bind PDGFR $\beta\beta$ homodimers. All PDGF isoforms except PDGF-AA can activate PDGFR $\alpha\beta$ heterodimers, but the biological importance of heterodimers has been difficult to test in vivo. PDGF binding induces PDGF receptor tyrosine kinase activity, which in turn activates multiple downstream signaling pathways including Ras/MAPK, PI3K/AKT, PLC γ /PKC, Src family kinases, and STATs [19]. Inflammatory cells produce cytokines that up-regulate PDGFR expression on fibroblasts and other mesenchymal cells (reviewed in Ref. [20]). In a pioneering study, Baroni et al. described an alternative mechanism for activation of PDGFRs in SSc by circulating autoantibodies that bind PDGFRs and activate downstream signaling [21]. Two subsequent studies used different screening procedures and patient groups and failed to identify stimulatory PDGFR autoantibodies [22, 23]. Therefore, while cognate PDGF ligands are certainly overexpressed in SSc, the existence of stimulatory autoantibodies has yet to be confirmed.

PDGF signaling is required for many aspects of embryogenesis and an understanding of these may give insight into how PDGF signaling mediates fibrosis. In general, PDGF regulates the proliferation, migration, survival, and differentiation of mesenchymal progenitor cells that give rise to diverse types of stromal cells in the adult organism [9, 24]. For example, mice lacking PDGF-A develop thinner dermis, suggesting that epidermal-expressed PDGF-A is important for proliferation of mesenchymal progenitors that become dermal fibroblasts [25]. Moreover, PDGFR α ^{-/-} mutant embryos and PDGF-A^{-/-}, PDGF-C^{-/-} double-mutant embryos develop more severe deficiency of dermal mesenchyme [26, 27]. PDGF-A, PDGF-C, and PDGFR α are also required for formation of interstitial connective tissue in the kidney cortex, intestine, and lung [26–30]. Epicardial-specific knockout of PDGFR α results in specific loss of cardiac fibroblasts as a result of defective epithelial-to-mesenchymal transition [31]. Embryos lacking PDGF-B or PDGFR β have normal connective tissue and dermis but are deficient in pericytes in the vasculature of the skin, skeletal muscle, intestine, lung, and heart [32]. Thus, from a developmental perspective, there is ample reason to suspect that activation of PDGFR α and PDGFR β could promote diseases involving connective tissue fibrosis and dysregulated microcirculation.

Exciting new findings indicate that PDGFRs are expressed on adult stem or progenitor cells located in perivascular niches in most mouse organs, including those in which fibrotic pathology occurs in SSc. Both PDGFR α and PDGFR β are expressed on mouse adipocyte progenitor cells that resemble pericytes and reside close to the vasculature [33, 34]. In skeletal muscle, PDGFR α marks perivascular cells located between muscle fibers that have the potential to generate fibrotic scar tissue or ectopic adipose tissue in response to different types of injury [35, 36]. PDGFR α , with the protease ADAM12, marks progenitor cells that are a major source of activated fibroblasts and/or myofibroblasts in response to acute injury of the muscle or dermis [37•]. In each of these studies PDGFR expression served as a useful marker to identify progenitor cells in adult tissue, but the functional involvement of PDGF signaling is not yet identified. Nevertheless, it is interesting to speculate that PDGF may affect a disease such as SSc by inducing profibrotic changes in rare adult stem or progenitor cells rather than (or in addition to) inducing ECM production in resident tissue fibroblasts.

In addition to directly promoting fibrosis, PDGF signaling could also be involved in the vasculopathy and immune dysfunction seen in SSc. As mentioned above, PDGFR β is upregulated on microvascular pericytes in early-stage SSc skin biopsies [13]. This leads to the hypothesis (which is currently untested) that increased PDGFR β activity and pericyte activation in SSc causes capillary occlusion and regression. Moreover, it has recently been observed that lymphatic vessel counts are severely reduced in SSc skin biopsies [38]. New research suggests that impaired lymphatic drainage in mouse lung causes or exacerbates pulmonary fibrosis. In this study, PDGF-B/PDGFR β signaling mediated lymphatic dysfunction by recruiting ectopic pericyte and SMC coverage of lymphatic vessels [39•]. Finally, it was recently shown that PDGFR β is expressed on perivascular progenitor cells that give rise to follicular dendritic cells (FDCs) in secondary and ectopic lymphoid tissue [40•], suggesting the possibility that PDGFR β could indirectly modulate lymphocytic inflammation and autoimmunity by regulating FDC numbers or differentiation.

Mouse Models

Transgenic overexpression of different PDGF isoforms in mice has been shown to induce tissue-specific fibrosis in the heart and liver [41–43]. These approaches rely on tissue-specific promoters to force secretion of PDGF from differentiated cardiomyocytes and hepatocytes.

To investigate the profibrotic function of PDGFR α and PDGFR β signaling in a more physiological context, we generated knockin mouse models with single amino acid mutations that enable hyperresponsive PDGFR α or PDGFR β signaling [17, 44•]. Briefly, point mutations in the juxtamembrane or kinase domains of PDGFRs can destabilize intramolecular contacts that normally suppress receptor kinase activity in the absence of ligand. We used this information to design PDGFR α or PDGFR β cDNAs encoding hyperresponsive receptors and targeted the mutant cDNAs to the appropriate *Pdgfra* and *Pdgfrb* genes. Importantly, PDGF signaling activity under these conditions remains physiological in most respects because the mutant receptors are expressed from the endogenous *Pdgfr* promoters and are still responsive to exogenous PDGF ligand. Moreover, the targeting events included a lox-Stop-lox cassette inserted upstream of the cDNA to enable conditional activation of the mutations using a variety of Cre-mediated approaches.

Our studies using a globally-active Cre to generate PDGFR α ^{V561D} (juxtamembrane domain mutant) and PDGFR α ^{D842V} (kinase domain mutant) embryos indicated that increased PDGFR α signaling during development promotes connective tissue hyperplasia [17]. As a consequence, PDGFR α ^{D842V} neonates died at birth with hyperplasia of mesenchymal cells in the lung. Surviving PDGFR α ^{V561D} pups developed lethal hyperplasia of connective tissue in the gastrointestinal and renal systems by three weeks of age. These phenotypes support the hypothesis that increased PDGFR α signaling promotes fibrotic disease, for example SSc. We also used a tamoxifen-inducible Cre approach to determine whether increased PDGFR α signaling in adult tissues would generate fibrosis and which organs would be affected. By this method, mosaic Cre recombination and increased PDGFR α phosphorylation were achieved in cells, and widespread organ fibrosis was observed 6–8 months after tamoxifen treatment. Severe fibrosis of the gastrointestinal tract had a significant effect on the lifespan of PDGFR α ^{D842V} mutants, leading to a median survival of less than one year. Adult PDGFR α ^{D842V} mice also developed cutaneous, musculoskeletal, renal, pulmonary, and cardiac fibrosis. Of note, widespread fibrosis occurred without ectopic expression of α SMA, the prototypical marker of myofibroblasts and a direct target of TGF β signaling. PDGF is known to repress smooth muscle genes including α SMA [45]. Therefore, an important question is whether the fibrosis seen in PDGFR α ^{D842V} mice involves TGF β signaling. Activation of the canonical TGF β signaling pathway involves phosphorylation of several SMAD proteins including SMAD2. Phospho-SMAD2 was not detected in fibrotic lung or intestine from PDGFR α ^{D842V} mice, suggesting that the TGF β signaling pathway is not overactivated in this mouse model ([17]; Iwayama, unpublished observation).

The next question was whether PDGFR β signaling would induce a similar fibrosis phenotype. Our studies using PDGFR β ^{V536A} (juxtamembrane domain mutant) and

PDGFR β ^{D849V} (kinase domain mutant) mice indicate that widespread fibrosis does not occur with increased PDGFR β signaling ([44•], unpublished observations). Instead, microarray analysis and histological studies revealed that juvenile mutant mice develop a complex inflammatory condition [44•]. Pericytes, which express PDGFR β in the brain, skin, and adipose tissue, were found to overexpress a gene expression profile normally associated with interferon signaling and dendritic cell maturation. Some of the most highly overexpressed genes were chemokines, including CXCL10 and CCL2, which have previously been implicated in SSc inflammation [46]. At first this autoinflammatory phenotype was a surprise because pro-inflammatory signaling had not been attributed to pericytes. However, a new biological framework for considering pericytes and inflammation was provided by the recent finding that follicular dendritic cells are derived from ubiquitous PDGFR β + pericytes [40••]. Therefore, the PDGFR β -induced inflammatory gene program seen in PDGFR β ^{V536A} and PDGFR β ^{D849V} mice could be deployed during the pathogenesis of SSc and other inflammatory diseases. Future studies will determine whether this occurs.

The clearly distinguishable phenotypes of these mouse models strongly suggest that PDGFR α and PDGFR β have different involvement in SSc. Of the two receptors, PDGFR α has the greater involvement in fibrosis. Lineage tracing and transplantation studies are in progress in our laboratory to definitively test whether PDGFR α directly induces stem and/or progenitor cells to exit the perivascular niche and differentiate into ECM-producing fibroblasts. On the other hand, PDGFR β may not readily promote fibrosis but potentially participates in other aspects of SSc, for example autoimmunity and vasculopathy (Fig. 1).

How could increased signaling through PDGFR α and PDGFR β have such distinct consequences? The two receptors are structurally very similar, yet biologically significant differences in signaling have been revealed by in-vivo studies. For example, PI3K is the primary effector of PDGFR α during development [47], but PI3K operates in an additive fashion with other effectors of PDGFR β signaling [48]. From this one could conclude that PDGFR α or PDGFR β activation in the same cell type could have distinct consequences. It is also possible that differences in cell-type expression explain the distinct consequences of increased PDGFR α or PDGFR β signaling. Fibroblast and pericyte-like progenitor cell populations in vivo may be more heterogeneous than expected and specialized subpopulations may preferentially express one isoform. Thus, PDGFR α may identify subpopulations that mediate fibrosis and PDGFR β may be expressed on a different subpopulation. Remarkably, we are still in the early stages of defining mesenchymal cell types in vivo.

Possible Therapeutic Opportunities

Clinical trials have been conducted on potential drugs to alter the course of fibrosis in SSc, but so far a treatment with a major therapeutic effect remains elusive. There has recently been interest in Imatinib (Gleevec, Novartis), which is a small molecule tyrosine kinase inhibitor (TKI) that blocks PDGFR isoforms and the related receptor c-Kit and the non-receptor tyrosine kinase c-Abl. Because c-Abl is an important mediator of TGF β signaling, Imatinib would seem to be a highly promising candidate with potential for dual inhibition of two major profibrotic signals. Moreover, Imatinib is already used to treat chronic

myelogenous leukemia (CML), gastrointestinal stromal tumors (GISTs), and several myeloproliferative diseases driven by mutant tyrosine kinases. Initial reports showed that Imatinib inhibited ECM production by SSc fibroblasts and prevented SSc-like skin fibrosis in bleomycin-treated mice [49]. A second study showed that Imatinib could stop and even reverse fibrosis in the TSK-1 genetic mouse model and in mice pretreated with bleomycin for a prolonged time before therapy [50]. These exciting findings encouraged early clinical studies. However, as of 2012, three small clinical trials have been reported and two of these indicated that imatinib is poorly tolerated in patients with SSc, with a large number of adverse effects including edema and fatigue [51, 52]. A third study reported acceptable levels of adverse events and some improvement in pulmonary and cutaneous manifestations of SSc [53]. Therefore, the efficacy of Imatinib as a strategy for SSc treatment is unresolved and several studies conclude that Imatinib is poorly tolerated in SSc. Second-generation TKIs that include PDGFRs among their molecular targets, for example Dasatinib, Nilotinib, and Sunitinib, have also been the topic of pre-clinical study in mouse models of SSc [50, 54]. However, all of these TKIs, similarly to Imatinib, have multiple molecular targets and should be expected to have more side effects than drugs with few targets.

A different approach would be to use monoclonal antibodies to neutralize PDGF signaling and treat SSc. This approach could be highly specific and target a specific PDGF or PDGFR isoform. This approach has been effective in mouse models of cardiac fibrosis, in which a specific neutralizing PDGFR α antibody has been shown to attenuate atrial fibrosis in pressure-overloaded mouse hearts [55]. In a model of healing myocardial infarcts and subsequent fibrosis, blockade of either PDGFR α or PDGFR β reduced collagen deposition in the healing infarct, with PDGFR β blockade additionally impairing the maturation of infarct vasculature [56]. Proof-of-concept studies using PDGFR-neutralizing antibodies in models of SSc have not yet been reported. As detailed above, PDGFR α and PDGFR β clearly have different involvement in the development and in the adult physiology, including fibrosis. Therefore, the isoform-specificity of PDGFR-blocking antibodies may prove to be of major importance in treating SSc without the adverse side effects of current TKIs. This approach certainly merits more basic and clinical attention.

Conclusions

Scleroderma is a terrible disease and effective treatments are still being sought. Patients may some day benefit from Imatinib, second generation TKIs, or even monoclonal antibody therapy. However, a more complete understanding of the mechanisms of fibrosis will lead to new therapeutic approaches capable of limiting or reversing this disease. It is believed that PDGF contributes to fibrotic diseases primarily by stimulating fibroblasts, but this growth factor also has the potential to regulate inflammation and angiogenesis via pericytes. The discovery that PDGF receptors are expressed on diverse types of mesenchymal stem and/or progenitor cells in adult tissues raises the provocative possibility that PDGF could promote SSc by changing the behavior of cells that give rise to fibroblasts. Whereas numerous functional, genomic, and proteomic studies have compared fibroblasts from normal and SSc tissue, there has been little research on stem and/or progenitor cells from SSc patients [57]. We believe that this different approach, combined with functional studies in animal models

of fibrosis, will reveal unknown mechanisms of SSc pathogenesis and eventually lead to new therapy.

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Fig. 1.

Different involvement of PDGFR α and PDGFR β in SSc pathogenesis. Injury and inflammation initiate secretion of PDGF isoforms that bind and activate their cognate PDGFRs. Some autoantibodies might also induce PDGFR signaling in SSc. Genetic mouse models of hyperresponsive PDGFR signaling reveal distinct consequences for each receptor. This leads to the prediction that the PDGFR α signaling pathway predominates in SSc fibrosis; PDGFR β signaling, which is not sufficient to promote fibrosis, is likely to promote other hallmarks of SSc