[Frontiers in Bioscience, Landmark, 18, 106-119, January 1, 2013]

Platelet-derived growth factor signaling in mesenchymal cells

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1. ABSTRACT

Platelet-derived growth factors (PDGFs) and their receptors are major mitogens for many cell types of mensenchymal origin, including fibroblasts and vascular smooth muscle cells (VSMCs). Their role in enhancing migratory and proliferative responses and extracellular matrix synthesis in these cells, make them key regulators of critical biological functions and in tissue diseases including tissue remodeling, scarring and fibrosis. The activities of the PDGFs have been extensively characterized at the molecular and cellular level and in in vivo model systems. This, in turn, has lead to an increasing number of PDGF-based therapies designed to accelerate or combat defects in tissue repair. This review aims to summarize recent developments in the role of PDGF in key mesenchymal cell functions. Many of the current developments in this field have primarily focused on advancements in understanding cell differentiation, migration, proliferation and the development of emerging PDGF-based therapies and hence will be primary focus of this review.

2. INTRODUCTION - PDGF LIGAND-RECEPTOR SYSTEM

2.1. PDGF ligands

PDGFs are potent mitogens for cells of mesenchymal cell origin. PDGFs enhance mesenchymal cell migration and expression of factors that are pivotal in the deposition of extracellular matrix (ECM) proteins. They therefore play an important role in normal biological process such as cell survival, and differentiation and in normal wound healing (Figure 1). Dysregulated activity and function of PDGFs are also believed to be important determinants of human diseases including excessive dermal scarring, many forms of organ-based tissue fibrosis, vessel diseases such as atherosclerosis and pulmonary hypertension.

There are five different dimeric PDGF ligands: PDGF-AA, PDGF-BB, PDGF-CC, PDGF-DD, and PDGF-

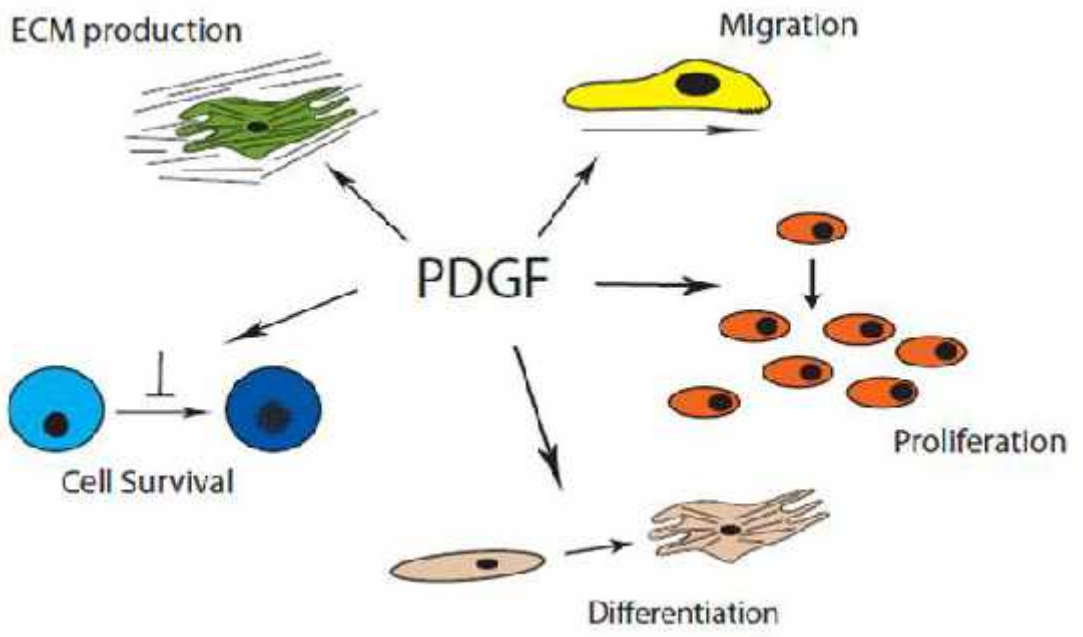

Figure 1. The roles of PDGF in mesenchymal cells. Some of the cellular functions that are influenced by the various isoforms of PDGF ligands and receptors include migration, proliferation, differentiation, cell survival and extracellular matrix (ECM) production. This occurs across various cell types including, fibroblasts, vascular smooth muscle cells, osteoblasts, osteoclasts and chrondrocytes, However, it has not been fully elucidated as to which PDGF ligand and receptor isoforms are responsible for each cellular function in the different cell types.

AB (1-5). PDGF-A and PDGF-B are synthesized as precursor polypeptides of 221 and 241 amino acids, respectively (reviewed by Heldin and Westermark (6)), whilst the more recently discovered PDGF-C and PDGF-D are 345 and 370 amino acids in length (4, 5). All PDGFs consist of a common growth factor domain plus other specific accessory/regulatory elements (Figure 1). The common growth factor domain is around 100 amino acids in length and has approximately 25% amino acid sequence identity, rising to 50% between PDGF-A and PDGF-B chains, and PDGF-C and PDGF-D chains. This indicates that the four PDGF genes arose from a common ancestor gene and have subsequently undergone a double gene duplication, as opposed to a single quadruplication (Figure 2) (7). The growth factor domain contains 8 cysteine residues that form a highly conserved cysteine knot motif, which is also found in the vascular endothelial growth factor (VEGF) family (8). The conserved cysteine residues contribute to both secondary structure via intramolecular disulphide bonds between cysteines 1-6, 3-7 and 5-8 and the formation of PDGF dimers via intermolecular disulphide bonds formed by cysteines 2 and 4 (6).

2.1.1. Biosynthesis

All four polypeptide chains, are inactive in their monomeric form, but assemble into biologically-active, disulfide-linked dimers in the endoplasmic reticulum (ER). All PDGFs form homodimers but PDGF-A and PDGF-B can also form a heterodimer. Both PDGF-A and PDGF-B pro-peptides undergo proteolytic processing of the amino-terminal region for activation (9). This is reported to occur in the Golgi by intracellular proteases such as furin (9) and have been reviewed elsewhere (7, 10). PDGF-B also contains a C-terminal region that is thought to be cleaved extracellularly (9). In contrast, PDGF-CC and PDGF-DD dimers are secreted as latent conditionally-inactive forms that contain an amino-terminal CUB (complement C1r/C1s, Uegf, Bmp1) domain which serves to sterically prevent binding of the ligand to its receptor (6). Activation of PDGF-CC and PDGF-DD in the extracellular space requires proteolytic cleavage of the CUB domain. In PDGF-CC this has been reported to be mediated by plasmin and tissue plasminogen activator (tPA) (10). CUB domains are often implicated in protein-protein and protein-carbohydrate interactions and may therefore have a role in regulating the distribution of PDGF-CC and PDGF-DD after being secreted into the extracellular space. Retention of PDGF-AA and PDGF-BB in the ECM is accomplished in part by a positively charged C-terminal "retention motif" that is determined by alternative splicing in PDGF-AA and proteolytic processing of PDGF-BB (11) (Figure 2). Retention of PDGFs in the ECM acts to regulate diffusion of the ligands into the tissue interstitium.

2.1.2. Expression

PDGFs are expressed in many different cell types. Each PDGF is expressed in a unique and partially overlapping manner. The syntheses of PDGFs are often increased in response to external stimuli, such as low oxygen tension, reactive oxygen species, and other soluble peptides and growth factors. They act in a primarily paracrine manor, but have also been reported to have

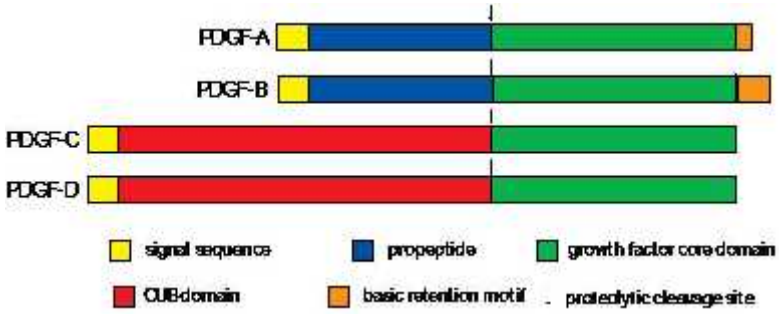

Figure 2. Domain organization of the mammalian PDGF ligand family. Mammalian PDGFs fall into two categories that are distinguished by the presence of either a basic retention motif (PDGF-A and PDGF-B) or a CUB domain (PDGF-C and PDGF-D). The differences in the structure of the unprocessed proteins between the two categories of PDGF ligand indicate that they each arose from a common ancestor, and have subsequently undergone a double gene duplication as opposed to a quadruplication. Numbers refer to the approximate number of residues in this the domain.

autocrine effects in tumors (6). The detailed expression pattern of individual ligands has been previously reviewed elsewhere and will not be covered in this review (6, 12). However there are some well established, general patterns: PDGF-BB is mainly expressed by vascular endothelial cells, megakaryocytes and neurons, PDGF-AA and PDGF-CC are expressed by epithelial cells, muscle, and neural progenitors. PDGF-DD is less well characterized, but has been reported to be secreted *in vitro* by fibroblasts and vascular smooth muscle cells (VSMCs) (11).

2.2. PDGF receptors

PDGF receptors (PDGFRs) belong to a family of tyrosine kinase receptors that also includes vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and epidermal growth factor (EGF) receptors (13). There are two different PDGFR chains – PDGFRalpha and PDGFRbeta. PDGFRalpha and PDGFRbeta receptors are transmembrane proteins of 170kd and 180kd, respectively. Each receptor contains five extracellular immunoglobulin-like domains, and an intracellularly tyrosine kinase domain which contains a specific inserted sequence that is not homologous to kinases, that is a characteristic of PDGFRs (14). Although, the two receptor isoforms are highly homologous, they are located on different chromosomes (4 and 5, respectively, in humans). Upon ligand binding PDGFR chains dimerise to form: PDFGRalphaalpha, PDGFRbetabeta homodimers or PDGFRalphabeta heterodimer. Each PDGF ligand binds each PDGFR with varying specificities as shown in Figure 3 (4-6, 15).

The PDGF ligand/receptor (L/R), axis is important in many key cellular processes including migration and proliferation. PDGFs are released during the inflammatory process and are potent activators of migration and proliferation in mesenchymal cells such as fibroblasts, VSMCs and osteoblasts, osteoclasts and chondrocytes.

3. PDGF SIGNALING IN MESENCHYMAL CELLS

3.1. Regulation of PDGF signaling

The PDGFs are key regulators of signal transduction in mesenchymal cells. As with other growth factor-receptor interactions, PDGF ligand-binding induces receptor dimerisation, activation of intrinsic receptor tyrosine kinase and phosphorylation of specific residues in the cytoplasmic region (16, 17). This results in selective recruitment of downstream intracellular effector molecules, such as phospholipases, Grb2, Ras GTPase-activating protein and PI3K and activation of downstream signaling cascades that elicit changes in gene transcription and changes in cell behavior. In the nucleus, PDGF signaling is known to activate various proto-oncogenes and immediate early response genes such as c-fos, JunB and c-myc (18). The specific signaling cascades ultimately result in changes in gene expression. PDGF can also modulate transcription factors. The most current research in this area has shown that PDGF-AA and PDGFRalpha are up-regulated in NFI-C$^{-/-}$ (nuclear factor I C) MEFs. NFI-C is a transcription factor that is thought to be implicated in TGF-beta signaling, ECM deposition and skin appendage pathologies. It is reported that NFI-C acts mainly by repressing genes expression in response to TGF-beta (19).

3.1.1. Modulation of PDGFRs

Much of the control of PDGF signaling is mediated via direct modulation of the signaling receptors. It has previously been shown that, similar to other growth factor receptors, PDGFRs are modulated via polyubiquitination, as a mechanism of negative regulation by mediating endocytosis, incorporation into lysosomes and degradation (20). It has also recently been shown that PDGFRbeta can be ubiquitated via the ubiquitin ligase Cbl. As Cbl binds at the same phospho-tyrosine as the downstream signaling mediator phospholipase C-gamma1 (PLCgamma1), it acts as a dual mechanism of negative regulation, by down-regulating the receptor, but also reducing downstream signaling by competing with one of its downstream effectors (21). However, the most well-established modulation of PDGFRs is the ligand-induced phosphorylation of the intracellular kinase domains (22). Previously autophosphorylation of the PDGFRbeta on Tyr579 and Tyr581 has been linked to signaling specifically via Src tyrosine kinases and Tyr1009, Try1021 has been linked to signaling via PLCgamma (23, 24). More recently it has been shown that a point mutation (D849N) in the C-terminal activation loop of PDGFRbeta causes

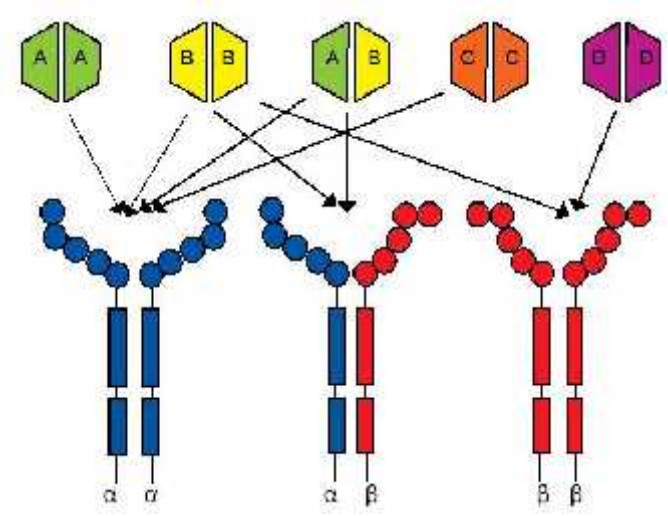

Figure 3. Diagram showing PDGFR-PDGF interactions *in vitro*. PDGF ligand dimers bind either PDGFRalpha or PDGFRbeta homodimers or the alpha/beta heterodimer. Arrows show proven ligand-receptor interactions. Each PDGF receptor has five extracellular immunoglobulin-like domains and two intracellular tyrosine kinase domains. PDGFR chains are shown in blue (PDGFRalpha) and red (PDGFRbeta). PDGF ligands are shown in green (PDGF-A), yellow (PDGF-B), orange (PDGF-C) and purple (PDGF-D).

elevated basal activation of the receptor, by lowering the threshold for kinase activation. This mutation also alters the pattern of tyrosine phosphorylation, deregulates recruitment of adaptor and signal transducers that enhance downstream signaling pathways, ligand-independent migration and proliferative response to PDGF-BB. In this case, a single modulation in PDGFRbeta results in a deregulation of the receptor and conveys an oncogenic predisposition to the cell, reinforcing the need for receptor modulation to be tightly controlled (25). This model has also been tested in *in vivo* liver and skin injury models. While the results correlate well with the altered kinetics previously reported, receptor activation in the injury model is not as high as the *in vitro* data would suggest, indicating that the activation of PDGFRbeta is more complex in injured tissues, highlighting the importance of studies using animal model systems (26). Some of the latest research on modulation of PDGFs has shown that PDGF signaling can also be modified through the addition of advanced glycation end-products (AGEs) to the PDGF ligands. Such modification, as found in hyperglycemia, results in decreased biological activity of the receptor and reduced signaling via pAKT (27).

3.1.2. Modulation of PDGF and PDGFR by integrins and other cell surface proteins

PDGFRs are also modulated by interactions with other receptors at the cell surface. PDGFR interactions with integrins are particularly fascinating, given their overlapping roles in mediating adhesion and motility. However, much of the previous work on this family of molecules has focused on examining the interactions between various integrins and PDGF ligands – for example alpha11beta1 integrin has been implicated in the attenuation of PDGF-BB-stimulated chemotaxis in mouse embryonic fibroblasts (MEFs) and PDGF-BB is important for regulating the recycling of alphavbeta3 back to the cell surface for the formation of focal complexes that are necessary for cell adhesion 28, 29). More recently, PDGFRs have also been shown to interact with integrins at the cell surface. The association between the PDGFRbeta and integrin receptors is enhanced by tissue trans-glutaminase (tTG), which acts as a bridge between the two receptors at the cell surface. Receptor clustering then results in enhanced PDGF binding, receptor activation, downstream signaling and receptor turnover. In this way tTG is thought to be essential for efficient PDGF-dependent proliferation and migration in fibroblasts (30). It has also recently been shown that the Na^+/H^+ exchanger (NHE1) is required for directional migration stimulated via PDGFRalpha in the primary cilium (31).

Modulation of all PDGF signaling also occurs via interactions of PDGFs with other signaling pathways and molecules at the cell surface. TGF-beta signaling is a good example of this. It has been previously shown that a low concentration TGF-beta induces autocrine expression of PDGF that stimulates cell proliferation, while a high concentration of TGF-beta reduces PDGF expression and cell growth (32). Similarly, a study by Jester *et al* reported that PDGF has a synergistic effect on TGF-beta-induced transformation of rabbit corneal keratocytes to myofibroblasts and subsequently proposed that PDGF is an important mediator of wound healing (33). However, the nature of the interaction between PDGF and TGF-beta is clearly dependent on the cell type and cellular context as TGF-beta and PDGF pathways have also, more recently, been suggested to antagonize each other in VSMCs (34).

The latest studies in this area have shown that PDGF works in synergy with other signaling molecules, particularly in the regulation of proliferation. For example,

PDGF has been shown to enhance the proliferative effect of lysophosphatidic acid on periodontal ligament fibroblasts and stimulate complete wound closure (35). Also, thrombospondin has been reported to be an important mediator of PDGF-DD-stimulated migration and proliferation of retinal pericytes (36). Conversely, Nincheri *et al* reported that PDGF can transactivate S1P receptor via sphingosine kinase SK-2 activation in mouse myoblasts. Activation of S1P negatively regulates PDGF-dependent proliferation, but positively regulates myoblast migration (via Erk) (37). This demonstrates that the regulation of PDGF-mediated cell function is strictly controlled, highly complex and yet to be fully elucidated

3.2. PDGF signal transduction pathways in migration

Signal transduction via PDGF has an essential role in both cellular adhesion and migration. Key regulators of growth factor signaling are kinase enzymes. Recent research in this area has shown that pharmacological inhibition of PI3K activity blocks PDGF-BB-induced migration in human mesangial cells (38). The dissolution of stress fibers and a change in focal adhesion dynamics associated with PI3K inhibition was only observed when PDGF-BB treatment was longer than 30min. This suggests that reorganization of the actin cytoskeleton (and hence migration) occurs in a biphasic manner - an initial PI3K-independent signal, followed by a 3-phosphoinositide-driven process (38). Whilst the relationship between these two effects is unclear; it is not thought to be mediated via PKC or Erk phosphorylation. This data provides further insight into the temporal regulation of PDGF-BB in mediating adhesion and migration in mesangial cells. Similarly, using Boyden chambers and scratch-wound assays to analyze migration in rat aortic smooth muscle cells, Lee *et al* identified spleen tyrosine kinase (Syk) as an upstream signal of the p38 MAPK pathway that contributes to PDGF-BB mediated migration (39). PDGF's multiple roles in migration and proliferation are also mediated by interactions with many different adaptor proteins. Most recently, two closely-related adaptors Nckalpha and Nckbeta have been shown to have non-compensatory roles in dermal fibroblast migration in response to PDGF-BB. While, Nckalpha is thought to mediate Cdc42 signaling of filapodium formation, Nckβ has been reported to mediate Rho-dependent induction of stress fibers. However, neither Nckalpha nor Nckbeta are thought to have a role in receptor activation, nor Akt, or Erk1/2 signaling (40). This is an example of how two closely related molecules, under the control of PDGF-BB signaling, mediate very different and specific cellular features to coordinate an aspect of cell behavior and illustrates the increasingly complex, but finely tuned, control of cell migration through PDGF-signaling.

3.3. PDGF signal transduction pathways in proliferation

PDGFs have a well-established role in eliciting a proliferative response in mesenchymal cells (41). Much of this proliferative response, as with other growth factor and tyrosine kinase receptors, is mediated via recognized mitogenic signaling pathways e.g. MAPK and PI3K-Akt (42, 43). Much of the current research focuses on elucidating the details of these pathways and which PDGF ligand/receptor combination is responsible for the specific effects, in different cell types. For example, it has recently been shown that PDGF-BB induces signaling via JAK2-STAT3 and is negatively regulated by SOCS3 in corneal fibroblasts (44). Such signaling is responsible for the regulation for PDGF-dependent cellular proliferation and migration. Also, ERK5 has been reported to be important for PDGF-BB-induced proliferation via Sac and downstream c-Jun in hepatic satellite cells. However, conversely depletion of ERK5 is associated with a decrease migration which is thought to be mediated by a redistribution of focal contacts and decreased pFAK and paxillin (45). Some of the most current research has further supported PDGF as having an important role in cell proliferation. It has been shown that stimulation of human fibroblasts with PDGF-AA rescues *in vitro*-induced cell senescence via stimulation/maintenance of the PI3K-Akt pathway. Therefore, PDGF is important in maintaining the ability of the cell to self renew and differentiate (46).

4. PDGFS IN FIBROBLASTS

4.1. Fibroblasts - Background

Fibroblasts are mesenchymal cells that have an essential role in connective tissues. Fibroblasts can originate from a variety of sources including: resident tissue populations, recruited fibrocytes of hematopoietic origin, pericytes, endothelial cells and epithelial cells. Fibroblasts secrete collagen proteins that are used to form a structural framework of tissues (17). Fibroblasts respond to changes in the environment during the inflammatory response and once activated, can proliferate, differentiate into myofibroblasts, add to and remodel ECM, contract wounds, and recruit other cell types. The control and regulation of fibroblast is therefore very important, especially in the context of tissue repair and wound healing (17).

Both PDGFRs are expressed in fibroblasts at low levels and are up-regulated in response to injury (47). However the relative expression of each receptor varies between tissues and environmental context, and is an area of on-going investigation. For example, in corneal myofibroblasts *in situ* the expression of PDGFRalpha is reported to increase and co-localize with α-smooth muscle actin (α-SMA) after injury (47). The expression of PDGFRbeta has yet to be investigated in this system.

4.2. Actions of PDGF in wound healing, tissue repair and fibrosis

Recent studies on PDGF have revealed some important insights into the role of PDGFs in fibroblasts. PDGFs are major chemotactic agents for fibroblasts during wound healing. Central to this process are integrins and PDGFs, and their subsequent role in mediating cell motility (48). Understanding the relationship between PDGF and integrin signaling is an area of increasing interest. In mouse fibroblasts it has recently been reported that beta1 integrin is required to maintain the stability of actin nucleating protein, neuronal Wiskott–Aldrich syndrome protein (N-WASP). N-WASP is required for PDGF internalization and chemotaxis. Therefore, depletion of beta1 integrin reduced the rate of

PDGF endocytosis and fibroblast chemotaxis. Furthermore, PDGF directly mobilizes and activates beta1 integrin within newly formed membrane ruffles which, in turn, controls assembly of Cdc42 and WASP-interacting protein (WIP) complexes with N-WASP that are required for both PDGF internalization and fibroblast chemotaxis (48). PDGF-BB-activated fibroblasts (fibroblasts adenovirally-transduced with PDGF-BB) have been shown to increase the rate of migration of mesenchymal stem cells, and induce differentiation of these cells into myofibroblasts - that are important for the remodeling phase of wound healing. This effect is thought to be mediated via fibroblast growth factor 2 (FGF2) as FGF2 was found to be elevated in the cell culture supernatant and differentiation to myofibroblasts was abrogated by FGF2 function blocking antibodies (49).

Fibroblasts are well established targets of PDGF, and have important roles in regulating ECM deposition and remodeling. The remodeling of ECM is key pathological feature of many inflammatory lung diseases such as fibrosis, chronic obstructive pulmonary disease (COPD), and asthma. Hypoxia has previously been associated with the pathogenesis of many of these diseases by increasing the secretion of pro-inflammatory cytokines and growth factors, such as TGF-beta and PDGF-BB (50-52). The effect of hypoxia on ECM production and cell proliferation is complex and varies between cell types and growth factor modulation (53). For example, it has recently been shown that stimulation of human lung fibroblasts with PDGF-BB, inhibits hypoxia-dependent matrix metalloproteinase (MMP) secretion, and increases the synthesis of soluble collagen. This is thought to be mediated via the Erk1/2 MAPK signaling pathway. While hypoxia may increase the secretion of PDGF-BB; PDGFBB may either inhibit or augment other hypoxia-dependent affects (53). It is clear that the role of PDGFs in ECM synthesis and remodeling are complex and requires much further study.

PDGF ligand/receptor axis has an essential role in regulating fibroblast function, particularly with regards to the precise control of, and balance between, migration and adhesion. Emerging knowledge in understanding the differences between populations of fibroblasts will be crucial in dissecting out the specific contributions of different ligand and receptor complexes to fibroblast function.

5. PDGFS IN VASCULAR SMOOTH MUSCLE CELLS (VSMC)

5.1. VSMC - Background

Phenotypic modulation of VSMCs from a quiescent, contractile type to synthetic phenotype is an important step in the development of several pathophysiological processes such as atherosclerosis, restenosis and vascular remodeling (54, 55). In VSMCs this is characterized by an increase in proliferation, migration and ECM protein production and a decrease in the expression of cytoskeletal and contractile proteins such as alphaSMA, calponin and myosin heavy chain (55). PDGF is one of the most potent inducers of VSMC synthetic phenotype (56).

5.2. PDGF signaling in VSMC in atherosclerosis

Previous studies on PDGFs in VSMC have primarily focused on understanding which of the ligands and specific cellular mechanisms regulate proliferation and migration, particularly in the context of developing therapies for atherosclerosis. Treating VSMCs with siRNA against PDGFRbeta has been shown to suppress intimal thickening (57). Similarly, the administration of antibodies to both PDGFRalpha and PDGFRbeta was reported to induce intimal atrophy in a baboon arterial graft model (58). These observations support the pathogenic role of PDGF and PDGFRs in proliferative vascular diseases and their potential as molecular targets for drug therapy to prevent myointimal hyperplasia.

Recently studies have used, PDGFRbeta, PDGFRalpha and PDGF-BB neutralizing antibodies to investigate the role of these receptors/ligands in a mouse reperfusion myocardial infarct model. Zymek *et al* showed that PDGFRbeta, PDGFRalpha and PDGF-BB mRNA was induced in re-perfused mouse infarcts. Inhibition, of PDGFRbeta, resulted in impaired pericyte support of the vessels, increased capillary density and impaired collagen deposition, while inhibition of PDGFRalpha had no effect on vascular maturation, but significantly attenuated collagen deposition in the infarcted myocardium. PDGF-BB and PDGFRbeta are crucial for neo-vessel stabilization and resolution of the inflammatory response (59).

PDGF-BB has received particular attention as it is mitogenic for cultured VSMCs through activation of either (PDGFRalpha or PDGFRbeta) and chemotactic through the activation of PDGFRbeta (60). It has been reported that polyclonal anti-PDGF antibodies block the migration of VSMC, but not their proliferation, indicating a discrete separation of signaling pathways mediating the two functions in VSMC (61).

5.3. PDGF in VSMA phenotypic modulation.

Some of the latest investigations in this area have focused on understanding the regulation of phenotypic modulation of VSMC from a contractile to a synthetic phenotype. It has been shown that PDGF-BB acts synergistically with FGF2 to induce cell proliferation and down-regulate aphaSMA and SM22alpha in VSMC. Furthermore, PDGF-BB induces tyrosine phosphorylation of FGFR1 via PDGFRbeta and that these, together with adaptor protein FRS2, form a multi-protein complex that is responsible for generating features of the synthetic phenotype. This is further confirmed as siRNA depletion of FRS2 inhibits PDGF-BB-induced down-regulation of alphaSMA and SM22alpha, but not PDGF-BB-induced cell proliferation or Erk activation (56).

More recently, another factor that has been shown to be important in regulating VSMCs is Micro RNAs (miRNA). miRNAs are a class of small non-coding RNAs that play a role in negative post-translation regulation of gene expression (62, 63). Recent studies have indicated that a single miRNA can affect a large number of protein coding genes, suggesting that modulation of a single miRNA can elicit multiple cellular events. miRNA-

221 has been identified as a factor that controls phenotypic modulation in response to PDGF-BB signaling. miRNA-221 is transcriptionally induced by PDGF-BB in primary VSMC, which in turn leads to down-regulation of c-Kit, resulting in inhibition of contractile genes and p25Kip1, which leads to induction of cell proliferation (64). Another miRNA involved in PDGF-induced phenotypic switching is miRNA24. Induction of miRNA24, by PDGF-BB, has been shown to down-regulate Tribbles-like protein 3 (Trb3) and subsequent Smad protein expression. This leads to a decrease in TGF-beta and BMP signaling, which is primarily responsible for maintaining the contractile phenotype in VSMC (34). These studies also provide evidence for antagonism between PDGFR and TGF-beta pathways.

6. PDGFS IN OSTEOBLASTS, OSTEOCLASTS AND CHONDROCYTES

6.1. Bone and cartilage - Background

Like many other growth factors, PDGF signaling is essential in the development and repair of bone and cartilage. In the formation and remodeling of bone, it is vital to regulate the balance between differentiation and proliferation of mesenchymally-derived osteoblasts and haematopoietically-derived osteoclasts. Unlike many mesenchymal cells *in vitro*, human oesteoblasts express relatively high levels of PDGFRalpha and are stimulated by PDGF-AA and PDGF-BB (65).

6.2. PDGFs in bone formation and remodeling

Current research into bone formation and remodeling has offered new and important insights into the role of PDGF over recent years. Previously PDGF-BB has been identified as factor that is secreted by osteoclasts which inhibits differentiation of osteoblasts *in vitro*, and hence bone formation (66). Conversely, PDGF-BB has also been reported to have a positive effect on osteogenic potential of MCSs (67). Furthermore, a recent review by Caplan and Correa, suggest that PDGF-BB is the key connector between cellular components and contributors of osteoblast differentiation (65). They argue that as i) bone formation/regeneration is a vascular driven process; ii) PDGF-BB has a well established role in pericyte recruitment/attachment to endothelial cells (68, 69); and, iii) that a high proportion of pericytes are MSCs with osteogenic potential as they exhibit similar markers *in situ* and *in vitro* and have similar multipotent differentiation ability, that it is clear to see the role of PDGF-BB and MSC in vascular driven bone formation (70-73). In this way, PDGF serves to stimulate perivascular proliferation of osteoblasts. Further investigations suggest that in angiogenesis, MSCs that reside in perivascular locations (near sheets of osteoblasts) have the ability to become pericytes and therefore able to stabilize new vessels (65, 74, 75). PDGFRbeta has also been shown to have an important function in MSCs in the bone marrow. Deletion of PDGFRbeta in MSC in the bone marrow results in decreased migratory and mitogenic responses, and increased osteogenic differentiation. PDGF-BB, primarily acting through PDGFRbeta, appears to be the major ligand responsible for this phenotype as its inhibition of osteogenic differentiation is abrogated by PDGFRbeta depletion. It has also been shown that PDGFRalpha deletion has a less significant effect on this phenotype compared to the effect observed in PDGFRbeta deletion (76).

It is clear that regulation of PDGF signaling in bone formation and remodeling is a complex process and has not yet been fully elucidated. This is, in part, attributed to the multiple cell types involved in the process and the very close, homeostatic relationship between them, which is tightly controlled by an array of hormones, growth factors and cytokines (65, 76). Furthermore, cells behave differently *in vitro* and *in vivo* and may have different clearance mechanisms for exogenous PDGF in these two systems. Therefore, PDGF-BB stimulation will produce very difference effects on the same cells, thus making direct comparison of much of the data difficult.

6.3. PDGF in cartilage formation

Recent studies on PDGF in chrondrocytes have revealed some important information as to their role in cartilage formation. PDGF signaling is thought to be important in regulating chondrocyte metabolism and therefore to be important in cartilage growth. PDGF-AB is has been reported to modulate the expression and synthesis of collagen (that conveys tensile strength) and proteoglycans, (that provide resistance to compressive forces) (77). Incubation of cartilage explant cultures with PDGF-AB has been shown to have very little effect on cartilage tissue composition (i.e. collagens and proteoglycans), but decreases tissue tensile integrity, thought to be the result of a loose and weak collagen network (77). This suggests that PDGF has an effect on collagen network formation in chondrocytes. This data is different from that observed by Schafer *et al*, who reported that PDGF-AB increase in proteoglycan synthesis in chondrocytes, However, this may be due to differences in cartilage tissue used and the concentration of PDGF-AB (77-78). The effects of other PDGFs are yet to be determined.

7. PDGFS AS THERAPEUTIC TOOLS IN WOUND HEALING AND TISSUE REPAIR

7.1. PDGF and therapeutics - background

As PDGFs have well established roles in cell proliferation and migration, they are thought to be involved in wound healing and tissue repair, and therefore make attractive therapeutic targets. Pre-existing drugs target PDGF and have been shown to have beneficial effects on wound healing. For example, a recent study by (79) reported that the anti-cancer drug Zeaxanthin, inhibited PDGF-BB-induced dermal fibroblast migration via inhibition of phospohorylation of PDGFRbeta, and subsequent MAPK activation. Treatment with Imatinib mesylate has been shown to improve repair of growth plate cartilage after injury (80). This is in contrast to its role in bone regeneration, and is attributed to Imatinib mesylate-mediated inhibition of PDGFR signaling, which attenuates expression of collagen type 2 (Col2a1) and osteocalcin and the subsequent abrogation of aberrant bone formation,

which can frequently form at the growth plate after injury (80).

7.2. PDGF as a target in complementary medicine

PDGF has also been shown to the key molecular mediator in complementary medicine. For example Crataegus extract WS 1442 (hawthorn extract) - used in the treatment of mild congestive heart failure - was shown to decrease migration and proliferation in VSMCs in a rat balloon catheter hyperplasia model. This effect is thought to be via inhibition of the PDGFRbeta kinase activity and downstream Erk signaling (81). In human aortic smooth muscle cells (HASMC), piceatannol, a metabolite of a polyphenol found in red wine, has been shown to inhibit PDGF-BB-induced cell migration and Akt phosphorylation via direct binding of piceatannol to PI3K, promoting its anti-atherosclerotic effects (82).

7.3. PDGF therapies in wound healing and tissue repair

Much of the current research involves optimization of PDGF delivery systems in wound healing models. A study by Grope *et al* showed that topical application of PDGF-AA and PDGF-BB to dermal wounds, improved wound healing by increasing the levels of phosphorylated mature PDGFR proteins to optimum levels at earlier time points in wound healing. An increase in phosphorylation of PDGFRs accelerated downstream signaling and hence promotes wound healing. This demonstrates that a simple topical application of PDGF can be an effective therapy (83).

7.3.1. Synthetic/artificial PDGF delivery systems

Exogenous matrices have recently proven to be useful tools in developing novel therapies for wound healing. For example, the use of collagen membranes loaded with collagen-binding human PDGF-BB was recently shown to improve wound healing in a dermal ulcer model (84). Also, modified dextran derivatives have been used as both a biopolymer surface to which growth factors may adhere but also to enhance the mitogenic effect of PDGF-BB (85). A modified heparin-binding delivery system of PDGF-BB and FGF2 in combination has recently been shown to increase proliferation and expression of ECM genes in tendon fibroblasts. This is thought to enhance remodeling and increase the strength of the tendon (84-86). A recent study which examined the effect of a fibrinogen/thrombin matrix impregnated with PDGF-BB on an *ex vivo* rabbit dermal fibroblast wound healing model showed an increased rate of wound healing (as measured by the amount of granulation tissue in biopsies taken at specific time-points) (87). An increasing number of studies have examined the role of exogenous PDGF as a therapeutic agent in osteogenic tissue repair. An ovine model of rotator cuff tears, demonstrated enhance healing and biomechanical strength when treated with an interposition graft consisting of recombinant PDGF-BB, combined with a collagen matrix (88). Similar observations have been reported in models of alveolar clef defects (89) and periodontal bone regeneration and healing (90-92). The use of surgical implants as a drug delivery system has also been studied. An extrudable ethylene-vinyl acetate co-polymer was used as a surgical implant to deliver a sustained release of PDGF and TGF-beta, and found to significantly improve cyclophosphamide-impaired dermal wound healing in rats (93).

7.3.2. Cell-based delivery systems

Currently, there is much research into developing modified cell-based therapies to improve wound healing. The two primary lines of investigation involve either impregnating ECM scaffolds with activated fibroblasts/keratinocytes or the use of cell-based gene transfer techniques in order to promote wound healing. Gene delivery of PDGF-BB induces sustained signal transduction in gingival fibroblasts compared with the recombinant protein. This may have important clinical applications in periodontal regenerative therapy (94). A recent study used fibroblasts and keratinocyes that were transduced with adenoviruses over-expressing VEGF and PDGF-BB, respectively, and applied to an artificial skin matrix, in a mouse model of dermal wounding. It was discovered that the two layer gene-modified artificial skin improved both vascularization and epidermalization of the skin compared to controls (95). Similarly, gene therapy has been used to improve bone healing and osteoregeneration. Bone repair was found to be accelerated in oral socket defects, through the addition of adenoviral PDGF-BB or hrPDGF-BB to a fibrillar collagen matrix, in a dose-dependent manner (96). Further addition of recombinant PDGF-BB in transplanted MSCs, was reported to inhibit apoptosis, thus enhancing their regenerative potential in a rat model of myocardial infarcts (97).

While it is difficult to directly compare each of the therapies and drug delivery systems, as they are performed in different experimental systems, the slow, sustained release drug delivery systems are likely to prove less invasive for patients and promote wound healing over a longer period of time, which will enable lower doses of the drug to be used, with potentially fewer adverse side-effects.

7.4. PDGF therapies in scarring and fibrosis

As PDGF has a well established role in ECM production, it is also true that dysregulation of PDGF signaling can result in excessive connective tissue production, resulting in scarring and fibrosis. This has been shown in recent mouse models where constitutively active PDGFRalpha signaling has been shown to disrupt connective tissue development and drive systemic fibrosis (98). Recent studies have shown that treatment of bleomycin-induced lung fibrosis was attenuated by treatment with tyrosine kinase inhibitors imatinib or nilotinib in mice. This study also reported a decrease in expression of PDGFRbeta associated with imatinib or nilotinib treatment, indicating that PDGFR is a valid target for anti-fibrotic therapies (99). Similarly, a mouse model of silica-induced pulmonary fibrosis showed significant decrease in fibrotic effects after imatinib treatment (100). Imatinib treatment has also been used in investigating the role of PDGFRB in, and treatment of, pulmonary hypertension (PH) (101). Anti-PDGFR approaches have been used in developing therapies to treat kidney fibrosis. The use of anti PDGFRalpha and PDGFRbeta antibodies

Table 1. Overview of the various PDGFs and PDGFR divided by cell type

Cell Type	PDGFs/PDGFRs in this review	Reference
Fibroblasts	PDGFRalpha	47
	PDGFRbeta	30
	PDGF-BB	28-29, 40, 48-53
VSMC	PDGFRalpha	58-60
	PDGFRbeta	56-60
	PDGF-BB	56, 59, 60, 64
Osteoblasts	PDGFRalpha	65
	PDGF-AA	65
	PDGF-BB	65-69
Osteoclasts	PDGF-BB	66
Chrondrocytes	PDGF-AB	77

reduced receptor activation and inflammatory infiltrates in a similar way to that observed with imatinib treatment, in a mouse kidney fibrosis model (102). PDGF signaling is clearly an important target in developing therapies for the treatment of fibrosis, although much of this work is ongoing.

8. PERSPECTIVES

It is clear that PDGF signaling is crucial in the regulation and repair of mesenchymal cells. In recent years there have been significant advances in understanding the complexities of both regulating PDGF signaling, and how PDGF regulates other molecules in specific tissue subsets (See Table 1 for a summary of the different PDGF receptor and ligands discussed, divided by cell type). This knowledge has led to an increase in the development of PDGF-based therapies, particular in the field of wound healing.

However, it is interesting to note that most of the studies that examine the effect of PDGF on cell behavior exclusively use the PDGF-BB ligand to stimulate PDGF signaling pathways. As the PDGF-BB ligand binds to all PDGFR isoforms, this approach is useful for examining broader PDGF effects; however, it is not suitable when investigating the effects of specific PDGF isoforms, as it is impossible to discern which receptor/ligand combination is responsible for the observed effects if all PDGFRs are artificially stimulated. An important next step in understanding the role of PDGF in tissue repair, and developing better therapies, is dissecting out the specific receptor ligand combination responsible for individual cellular responses.

To address these questions better *in vivo* models need to be developed. As PDGFR knockout is embryonic lethal, much of the research on these models has focused on their roles in development. *In vivo* models which are conditional, inducible, cell type specific deletions of individual PDGF receptor and ligand isoforms and ligand will enable a precise examination of contributions of specific cell types (such as fibroblasts or smooth muscle cells) to individual processes such as wound healing and tissue repair. In this way it will also be possible to investigate and establish the effects of redundancy and compensation between PDGF receptor and ligand isoforms.

Recent clinical trials involving imatinib have revealed some interesting insights to the treatment wound healing and tissue repair. Currently used in the treatment of gastrointestinal stromal tumors and chronic myelogenous leukemia, Imatinib specifically inhibits a number of tyrosine kinases such as c-abl and pro-fibrotic TGF-betaR and PDGFR. For this reason, it is thought to be useful in the treatment of fibrosis and diseases with a major fibrotic element such as systemic sclerosis (SSc). Whilst initial *in vitro* and *in vivo* appeared to positive, emerging data from phase I and II clinical trials into the used of imatinib in treating the fibrotic component of SSc reveal considerable variability in efficacy and failed to meet any of the primary end-points. The most recent double blind and open label trials have confirmed that the drug is poorly tolerated by SSc patients, even at sub-clinically significant doses, due to a high number of adverse effects. For this reason many of the studies are quite small and have poor retention rates. The variance in efficacy highlights the considerable difference in SSc disease subtypes and hence, the need for either a more multi-targeted approach to treatment, or more specifically targeted treatments to each well characterized subgroup. Current clinical trial with second phase drugs such as nilotinib and dasatinib may prove more successful, as will multicentre clinical trials whereby a greater number of patients can be compared and statistically significant conclusions may be made and changes in different SSc cohorts may be better observed.

9. ACKNOWLEDGEMENTS

The authors would like to thank Arthritis Research UK (Grant no 18420, 19427, 16379), The Rosetrees Trust and The Scleroderma Society for funding support.

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Key Words: PDGF, PDGFR, Fibroblast, Vascular smooth muscle cell, Chondrocyte, Osteoblast, Osteoclast, Review

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