PDGF Receptors as Targets in Tumor Treatment

Arne Östman* **and Carl-Henrik Heldin†**

**Department of Pathology-Oncology, Cancer Center Karolinska, Karolinska Institutet, R8:03, SE-171 76 Stockholm, Sweden;*
†Ludwig Institute for Cancer Research, Uppsala University, SE-751 24 Uppsala, Sweden

Signaling through platelet-derived growth factor (PDGF) receptors contributes to multiple tumor-associated processes. The recent introduction of clinically useful PDGF inhibitors have the last years validated PDGF receptors in malignant and stromal cells as relevant cancer drug targets. Mutational activation of PDGF receptor signaling in malignant cells has been described in some rare tumor types such as dermatofibrosarcoma protuberans, a subset of GISTs, and some hematologic malignancies. Furthermore, expression of PDGF receptors on pericytes is a common characteristic of solid tumors. The clinical efficacy of novel multikinase inhibitors, such as sunitinib and sorafenib, most likely involves targeting of PDGF receptor-dependent pericytes. Preclinical studies suggest that targeting of stromal PDGF receptors might also constitute a novel strategy to enhance tumor drug uptake. Finally, recent studies have implied both pro- and antimetastatic effects of PDGF receptors on malignant and stromal cells. The studies on the roles of PDGF receptors in cancer signaling are thus presently in a dynamic phase where collaborations between oncologists, pathologists, and tumor biologists are predicted to be highly productive.

Advances in CANCER RESEARCH
0065-230X/07 $35.00
DOI: 10.1016/S0065-230X(06)97011-0

I. MOLECULAR BIOLOGY OF PDGF

Members of the platelet-derived growth factor (PDGF) family stimulate the proliferation, survival, and motility of connective tissue cells and certain other cell types (Heldin and Westermark, 1999). PDGF isoforms have important roles during the embryonal development, particularly to promote the development of various mesenchymal cell types in different organs (Betsholtz, 2004). In the adult, PDGF stimulates normal wound healing (Robson *et al.*, 1992) and regulates the interstitial fluid pressure (IFP) of tissues (Rodt *et al.*, 1996).

Overactivity of PDGF has been linked to tumorigenesis, as well as to the development of other diseases involving excessive cell proliferation, such as atherosclerosis and various fibrotic conditions (Östman and Heldin, 2001). In the case of cancer, PDGF receptor activation can drive tumor growth directly by autocrine PDGF stimulation or activating mutations in PDGF receptors (Fig. 1). However, PDGF produced by cancer cells, which themselves do not respond to PDGF, can also act in a paracrine manner on nontumor cells, such as cells in tumor blood vessels and stromal fibroblasts, which may also be important for tumor growth and homeostasis (Fig. 1) (Pietras *et al.*, 2003a).

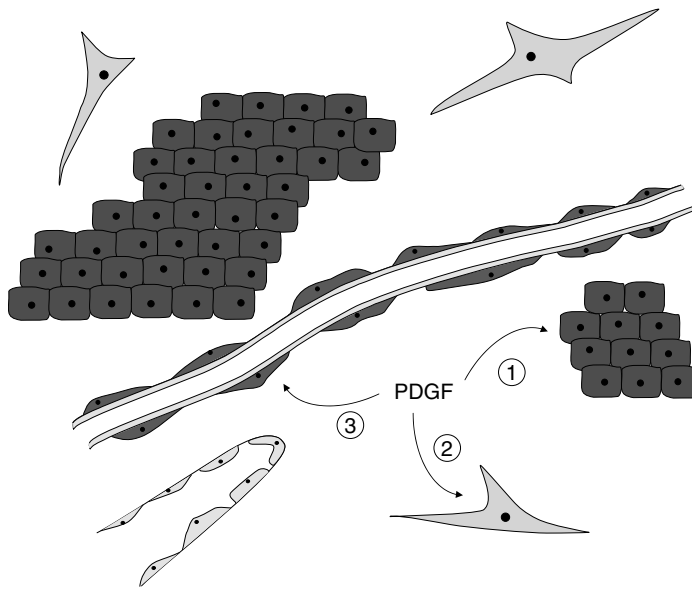

Fig. 1 Effects of PDGF in tumors. PDGF can, in an autocrine or paracrine manner, stimulate the growth and survival of certain types of tumor cells (1). PDGF can also stimulate stromal fibroblasts (2), and cells of blood vessels, particularly pericytes (3). For references, see the text.

The purpose of the present communication is to review the development of PDGF antagonists, and their use in preclinical animal models and patients for the treatment of tumors characterized by autocrine or paracrine PDGF stimulation.

A. PDGF Isoforms and PDGF Receptors

The PDGF family consists of five isoforms that are homodimers of A-, B-, C-, and D-polypeptide chains, that is PDGF-AA, -BB, -CC, and -DD, and a heterodimer PDGF-AB (Heldin *et al.*, 2002). The A- and B-chains are synthesized as inactive precursors, but are cleaved during secretion from the producer cell, and are thus present extracellularly in active forms. In contrast, the C- and D-chains are secreted as inactive forms containing N-terminal CUB domains, which have to be removed before these isoforms can bind to receptors (Li and Eriksson, 2003). It has been shown that PDGF-CC and -DD are activated by tissue plasminogen activator and urokinase plasminogen activator, respectively (Fredriksson *et al.*, 2004; Ustach and Kim, 2005).

The PDGF isoforms exert their cellular effects by binding to structurally similar α- and β-tyrosine kinase PDGF receptors. Each receptor contains five extracellular Ig-like domains to which ligands bind, and an intracellular tyrosine kinase domain which has a characteristic inserted sequence of about 100-amino acid residues without similarity to kinase domains. The receptors are activated by ligand-induced receptor dimerization. Since the A-, B-, and C-chains of PDGF bind to the α-receptors, whereas the B- and D-chains bind to the β-receptor, different types of receptor dimers are formed depending on which PDGF isoform that binds, and on which receptor isoforms the target cell expresses.

Ligand-induced receptor dimerization allows autophosphorylation in *trans* between the receptor subunits in the dimer. Phosphorylation of a tyrosine residue in the activation loop of the kinase and of specific tyrosine residues in other regions of the cytoplasmic parts of the receptors leads to an increase in the catalytic activity of the kinase. Moreover, the phosphorylated tyrosines provide docking sites for downstream signaling molecules containing SH2 domains.

B. Signaling via PDGF Receptors

The PDGF α- and β-receptor homo- and heterodimers induce similar, but not identical, cellular effects. Whereas all dimeric receptor complexes mediate potent mitogenic effects, only $\beta\beta$ homodimers and $\alpha\beta$ heterodimers mediate chemotaxis of smooth muscle cells and fibroblasts; activation of $\alpha\alpha$

homodimeric receptors, in fact, inhibits chemotaxis. Moreover, whereas all receptor dimers mediate rearrangements of the actin filament system of cells, only $\beta\beta$- and $\alpha\beta$-receptor dimers stimulate the formation of circular actin structures of the dorsal surface of the cell (Eriksson *et al.*, 1992).

The cellular effects of PDGF isoforms are mediated by activation of several signal transduction pathways at the respective receptor complexes. Many of these signaling pathways are initiated by the docking of SH2-domain-containing molecules at specific autophosphorylated residues in the receptors. In total, at least 11 and 12 autophosphorylation sites have been identified in the PDGF α- and β-receptors, respectively (Heldin *et al.*, 1998). They bind, in a specific manner, about 10 different types of SH2-domain-containing molecules, including tyrosine kinases of the Src family, phosphatidylinositol-3′-kinase (PI3K), phospholipase C-γ1 (PLC-γ1), the Grb2/Sos1 complex that activates Ras and the Erk MAP kinase pathway, GTPase-activating protein for Ras (RasGAP), the tyrosine phosphatase SHP-2, and transcription factors of the STAT family. In addition, the activated receptors bind several SH2-domain-containing adaptor molecules, including Nck, Shc, and Crk, that do not have any enzymatic activity and whose function is to form bridges between the receptors and other signaling molecules.

Accumulating observations have shown that PI3K and PLC-γ are particularly important for PDGF-induced actin reorganization and chemotaxis, whereas the Erk MAP kinase pathway and Src are particularly important for stimulation of cell proliferation. However, there is an extensive cross-talk between the different signaling pathways, and the ultimate effect of activation of different signaling pathways may differ between different cell types.

An interesting aspect of signaling via PDGF receptors, and other receptors, is that modulatory signals are often induced in parallel to stimulatory signals. Thus, activation of Ras occurs by docking to the PDGF receptors of the Grb2/Sos1 complex. However, the PDGF β-receptor, but not the α-receptor, also binds RasGAP which counteracts the Sos1-induced Ras activation. Since Grb2/Sos1 and RasGAP bind to different phosphotyrosines, the efficiency in Ras activation is determined by the stoichiometry of phosphorylation of these residues. Interestingly, the phosphorylation of Tyr771 in the PDGF β-receptor, which binds RasGAP, is higher in a $\beta\beta$-receptor homodimer than in an $\alpha\beta$-receptor heterodimer, leading to different efficiencies in Ras activation by the different dimeric receptor complexes (Ekman *et al.*, 2002). Moreover, activation of PI3K by PDGF receptors is modulated by the docking to the receptor, via the PDZ-domain-containing protein Na^+/H^+ exchanger isoform 3 regulatory factor (NHERF), of the phosphoinositide phosphatase PTEN which dephosphorylates the PI3K product (Takahashi *et al.*, 2006). In addition, the tyrosine phosphatase SHP-2 binds to α- and

β-receptors and counteracts the phosphorylation induced by the receptors, and thus modulates the strength of the signaling from the receptors. Importantly, different tyrosine phosphatases can selectively dephosphorylate different autophosphorylation sites in the PDGF receptors, and thereby modulate signaling (Klinghoffer and Kazlauskas, 1995; Kovalenko *et al.*, 2000; Persson *et al.*, 2004). In fact, efficient PDGF signaling is dependent on transient and reversible inhibition of tyrosine phosphatases, which is obtained by PDGF-induced production of H_2O_2 which oxidizes and thereby inactivates phosphatases (Bae *et al.*, 2000; Sundaresan *et al.*, 1995). Interestingly, peroxiredoxin type II, a cellular peroxidase that eliminates H_2O_2, was shown to associate with the PDGF receptor and to suppress tyrosine phosphatase inactivation, thereby suppressing PDGF receptor activation (Choi *et al.*, 2005).

Signaling via PDGF receptors has also been shown to be modulated by certain membrane proteins. Thus, binding of urokinase to its receptor (UPAR) induces an association with and activation of the PDGF β-receptor in a PDGF-independent manner (Kiyan *et al.*, 2005). Moreover, the PDGF β-receptor forms a complex with the hyaluronan receptor CD44; binding of hyaluronan to CD44 suppresses PDGF β-receptor activation most likely by recruiting a tyrosine phosphatase to the receptor (Li *et al.*, 2006).

II. PHYSIOLOGICAL ROLES OF PDGF

During the embryonal development, PDGF isoforms are often expressed by epithelial cells in various organs, and PDGF receptors on neighboring mesenchymal cells, suggesting paracrine roles for the PDGF isoforms in the development of different types of mesenchymal cell types in different organs. Detailed insights into the physiological roles of PDGF isoforms have come from the knockout of genes for PDGF isoforms and receptors in mice (Betsholtz, 2004).

Inactivation of the gene for PDGF-B (Leveen *et al.*, 1994) or the β-receptor (Soriano, 1994) gives similar phenotypes. On one hand, mesangial cells of kidneys do not develop, resulting in poor filtration in the glomeruli. On the other hand, there is a defect in the development of smooth muscle cells of the blood vessel walls, resulting in bleedings at the time of birth, which is the cause of death of these animals. The fact that the phenotypes of B-chain and β-receptor knockout mice are so similar suggests that during embryonal development, PDGF-BB is a more important ligand for this receptor than PDGF-DD.

Mice in which the PDGF A-chain gene has been knocked out die at about 3 weeks of age because of lung emphysema (Boström *et al.*, 1996). The defect involves poor development of lung alveoli because of lack of spreading of

alveolar smooth muscle cell progenitors. There is also an abnormal mucosal lining in the gastrointestinal tract with fewer villus clusters in A-chain knockout mice (Karlsson *et al.*, 2000).

Knockout of the PDGF C-chain gene causes perinatal death due to feeding and respiratory difficulties (Ding *et al.*, 2004). When both the A- and C-chain genes were inactivated a severe phenotype was observed, including cleft palate, subepidermal blistering, deficiency of renal cortex mesenchyme, spina bifida, and skeletal and vascular defects (Ding *et al.*, 2004). Interestingly, these defects phenocopies the loss of the PDGF α-receptor (Soriano, 1997), consistent with the notion that the α-receptor *in vivo* is stimulated by both PDGF-AA and PDGF-CC.

The different roles of the α- and β-receptors during embryogenesis can largely be explained by their different expression patterns. However, differences in their signaling capacities also contributes, as shown in an *in vivo* experiment in which the intracellular parts of the receptors were exchanged (Klinghoffer *et al.*, 2001). Interestingly, the intracellular α-receptor part failed to mediate the β-receptor's effect on vascular development.

In the adult, PDGF has been shown to promote wound healing (Robson *et al.*, 1992). Topical application of PDGF-BB in the wounded area increases the amount of granulation tissue through stimulation of mitogenicity and chemotaxis of fibroblasts and smooth muscle cells, and chemotaxis of neutrophiles and macrophages, as well as through stimulation of the production of various matrix molecules.

The importance of PDGF for the connective tissue is also reflected by the fact that it regulates the IFP of tissues (Rodt *et al.*, 1996). The mechanism probably involves the ability of PDGF to enhance the formation of contacts between connective tissue components and stromal fibroblasts, and to stimulate contractility of these cells. Signaling via PI3K was found to be particularly important for this effect (Heuchel *et al.*, 1999).

III. ROLES OF PDGF RECEPTORS IN TUMORS

A. PDGF Stimulation of Malignant Cells

1. MALIGNANCIES ASSOCIATED WITH MUTATIONAL ACTIVATION OF PDGF SIGNALING

In general, mutational activation of PDGF receptor signaling is rare in solid tumors and hematologic malignancies. However, some rare diseases are associated with mutations in PDGF ligands and receptors (Table I).

Dermatofibrosarcoma protuberans (*DFSP*) and the juvenile *giant cell fibroblastoma* (*GCF*) are rare skin tumors of intermediate malignancy

Table I Tumors with Known Genetic Alterations of PDGF or PDGF Receptor Genes

Tumor	Genetic alteration	Estimated frequency of genetic alteration in the disease (%)	Activating mechanism	Imatinib sensitivity	Clinical effect of imatinib treatment
Dermatofibrosarcoma protuberans (DFSP)	Translocation of the PDGFB gene to the COL1A1 gene	100	Autocrine PDGF-BB stimulation	Yes	Yes
Gastrointestinal stromal tumors (GIST)	Mutations in the PDGFRA gene	10	Activating missense mutations unleashing the receptor kinase activity	One-third of cases sensitive; two-third of cases insensitive	Yes
Chronic myelomonocytic leukemia (CMML)	Fusion of the PDGFRB gene with the TEL gene	<30	Constitutive dimerization and activation of the intracellular receptor domain	Yes	Yes
Other Bcr-Abl negative chronic myelocytic leukemia	Fusion of the PDGFRB gene with the genes for HIP-1, Rab-5, CEV14, or H4/D10S170	<10	Constitutive dimerization and activation of the intracellular receptor domain	Yes	Yes
Idiopathic hypereosinophilic syndrome (IHES)	Fusion of the PDGFRA gene with genes for FIP1L1, K1F5B, or CDKRAP2	10–30	Constitutive dimerization and activation of the intracellular receptor domain	Yes	Yes
Glioblastoma	Amplification of the PDGFRA gene	5–25	Production of abnormally large amount of receptor protein	Yes	Yes

associated with translocation affecting the PDGF B-chain gene (McArthur, 2006). DFSP most commonly occurs in the middle age, and incidence has been estimated at 0.8 cases per 1 million persons per year. Standard treatment is surgery. However, this is sometimes problematic due to a difficult anatomical location of the tumor, and is also associated with a risk of local recurrence.

DFSP has since long been known to be associated with a specific chromosome 17/22 translocation. Studies during the late nineties by Dumanski *et al.* demonstrated that this translocation led to the generation of fusion genes encoding novel collagen1A1 (COL1A1)-PDGF B-chain proteins (Simon *et al.*, 1997). Fusion genes displayed a consistent structure where N-terminal parts of COL1A1 of variable length were fused to the N-terminal prosequence of PDGF B-chain.

Functional characterization of the protein products of this translocation showed that the fusion proteins were processed to functional PDGF-BB that on expression in fibroblasts exerted transforming activity that could be neutralized by PDGF antagonists (Greco *et al.*, 1998; Shimizu *et al.*, 1999; Sjöblom *et al.*, 2001). Furthermore, primary DFSP cultures were also shown to be sensitive to inhibition of PDGF receptor signaling.

Gastrointestinal stromal tumors (*GISTs*) are tumors derived from the interstitial Cajal cells of the GI-tract. The most common location is the stomach. A population-based study indicated an incidence of about 15 cases per million people and year (Nilsson *et al.*, 2005). The most common genetic alteration of this disease is activating c-kit mutations which occur in about 85% of the cases (Corless *et al.*, 2004).

However, a significant fraction of GIST patients also present with activating mutations of the PDGF α-receptor (Heinrich *et al.*, 2003). A study of 1015 patients identified PDGF α-receptor mutations in 69 patients indicating that approximately one-third of GIST patients without c-kit mutations harbor PDGF α-receptor mutations (Corless *et al.*, 2005). Similar frequencies of PDGF α-receptor mutations in GISTs have also been reported in other studies with fewer cases (Sihto *et al.*, 2004).

PDGF α-receptor mutations occur in exons 11, 12, 14, and 18, and have been shown to be associated with activation of the receptor (Corless *et al.*, 2005). The most common variants are exon 18 mutations involving D842, which is located in the activation loop of the kinase and account for about two-third of all PDGF α-receptor mutations. Analysis of the mutants with regard to sensitivity to the PDGF receptor kinase inhibitor imatinib indicates that the large majority of D842 mutant receptors are not blocked by imatinib. However, many other PDGF α-receptor variants are blocked by imatinib. Together these studies indicate that about one-third of GIST patients with PDGF α-receptor mutations could benefit from imatinib treatment.

An involvement of mutationally activated PDGF receptors in hematologic malignancies was first suggested by the description of a translocation involving the PDGF β-receptor in *chronic myelomonocytic leukemia* (*CMML*) (Golub *et al.*, 1994). The chromosome 5/12 translocation of CMML creates a fusion gene encoding, a protein composed of part of the TEL transcription factor fused to the kinase domain of the PDGF β-receptor. This fusion protein displays constitutive kinase activity, dependent on dimerization mediated by the TEL part of the protein (Carroll *et al.*, 1996; Jousset *et al.*, 1997; Sjöblom *et al.*, 1999). Numerous studies in tissue culture and animal models have subsequently confirmed the ability of this fusion protein to induce hematologic malignancies, in a manner which can be blocked by imatinib or other PDGF receptor kinase inhibitors (Tomasson *et al.*, 1999, 2001).

Subsequent to the original identification of the TEL-PDGF β-receptor fusion protein in CMML, numerous other fusion proteins containing the kinase domain of PDGF β-receptor has been described in BCR-ABL negative chronic myeloid leukemias including HIP-1, Rab-5, CEV14, H4/D10S170 (Jones and Cross, 2004). A common theme among these alterations is that the fusion partner of the kinase mediates protein dimerization which leads to activation of the kinase domain. Loss of regulatory elements in the juxtamembrane part of the receptor could also contribute to activation of the kinase activity of the receptor. In addition to these findings, there are also reports of rare translocations involving the PDGF α-receptor in myeloid disorders.

Idiopathic hypereosinophilic syndrome (*IHES*) is characterized by persistent hypereosinophilia and is frequently associated with symptoms caused by organ damage of, for example, the skin, the cardiovascular system, and the lungs. An involvement of PDGF α-receptor signaling in this disease has been established by the demonstration of the identification of at least three different PDGF α-receptor fusion proteins expressed in this disease (Cools *et al.*, 2003; Jones and Cross, 2004). The best characterized of these aberrant proteins is the FIP1L1-PDGF α-receptor fusion caused by an interstitial deletion on chromosome 4. The FIP1L1-PDGF α-receptor protein is lacking the regulatory extracellular domain of the receptor and retains the kinase domain. Transforming activity of this protein is dependent on the FIP1L1-domain and can be blocked by PDGF receptor kinase inhibitors. In a larger study of 89 patients with moderate to severe eosinophilia, the FIP1L1-PDGF α-receptor translocation was found in 11 patients (Pardanani *et al.*, 2004). Most of these were found in patients with systemic mast cell disease associated with eosinophilia.

Other imatinib-sensitive PDGF α-receptor fusion proteins have been described in IHES (Score *et al.*, 2006; Walz *et al.*, 2006).

The presence of PDGF receptor mutations in tumors has also been analyzed in systematic sequencing studies. So far, these studies have not provided any evidence that activating mutations occur in any larger fractions of common solid tumors.

2. PDGF STIMULATION OF GLIOBLASTOMA, SARCOMAS, AND LEYDIG TUMOR CELLS

PDGF ligands and receptors are coexpressed in human glioblastoma tissue. Amplification of PDGF α-receptor also occurs in 5-25% of glioblastomas (Fleming *et al.*, 1992; Hermanson *et al.*, 1992). These findings suggest an involvement of autocrine PDGF signaling in glioblastoma growth. This notion is also supported by experiments in which glioblastoma-like tumors could be induced by experimental induction of autocrine PDGF receptor signaling in different animal models (Dai *et al.*, 2001; Hesselager *et al.*, 2003; Uhrbom *et al.*, 2000). Furthermore, growth of glioblastoma cells has been shown to be sensitive to treatment with PDGF antagonists (Kilic *et al.*, 2000; Vassbotn *et al.*, 1994). In a study, a subset of glioblastoma cultures was identified that appeared to be particularly dependent on PDGF receptor signaling (Hägerstrand *et al.*, 2006). Key characteristics of this subset included high expression of PDGF receptors and the chemokine CXCL12/SDF1.

The potent effects of PDGF on fibroblasts have prompted analyses of a possible involvement of autocrine PDGF receptor signaling in sarcomas other than DFSP. Immunohistochemical studies have confirmed coexpression of ligands and receptors in human tumor tissue; however, the functional significance remains unclear. Among subgroups of sarcomas, particular attention has been drawn to Ewing family tumors (Zwerner and May, 2001, 2002). PDGF ligands are targets of specific transcription factors associated with this disease type, and cell lines derived from these tumors are growth inhibited by PDGF antagonists.

Clinical activity of imatinib was also reported in patients with advanced aggressive fibromatosis (AF) (Heinrich *et al.*, 2006). This study could not definitely identify the imatinib-target mediating this effect. However, PDGF β-receptors were expressed on tumor cells, and responders were also characterized by elevated levels of serum PDGF-BB, which together suggest that clinical effects involved inhibition of PDGF receptor signaling in tumor cells.

The growth of the Leydig cells of the testis is dependent on epithelial production of PDGF-A acting on PDGF α-receptors expressed on Leydig cells (Gnessi *et al.*, 2000). An involvement of PDGF receptor signaling also in the growth of Leydig tumor cells has been suggested based on the findings

that imatinib blocks the *in vitro* and *in vivo* growth of these tumors cells (Basciani *et al*., 2005).

3. PDGF SIGNALING IN MALIGNANT CELLS OF EPITHELIAL TUMORS

As discussed above, the physiological roles of PDGF receptors mostly, if not exclusively, occur in mesenchymal and glial cells. Furthermore, there are until now no strong indications that mutational activation of PDGF receptors is a common feature of epithelial tumors. There are nevertheless a number of studies which have implicated PDGF receptor signaling also in the growth of the malignant cells of various epithelial tumors such as ovarian, prostate, and renal cell cancer (reviewed in Östman, 2004). The suggestions that PDGF can be involved in the growth of the malignant cells of these tumors are mostly derived from studies which have demonstrated PDGF receptor expression in human tumor tissue samples, combined with observations of growth inhibitory effects of PDGF antagonists on established tumor cell lines (Matei *et al*., 2004; Ustach *et al*., 2004). The major caveats of these studies thus include the use of antibodies with incompletely characterized specificity, as well as the reliance on established cell lines which might not be relevant for human disease.

It is highly warranted that these intriguing findings, with major clinical implications, are consolidated. A highly prioritized topic for such studies would be continued validation of the expression of activated PDGF receptors in human tumor tissue.

B. Tumor Angiogenesis and PDGF Receptor Signaling

Angiogenic activity of PDGF ligands was first described already in the early nineties, using the chick chorioallantoic membrane angiogenesis assay (Risau *et al*., 1992). These findings have subsequently been confirmed in other *in vivo* models of angiogenesis such as the cornea angiogenesis assay and analyses of revascularization of ischemic tissues (Cao *et al*., 2002; Li *et al*., 2005). As discussed earlier, analyses of the phenotypes of knockout mice have established critical roles for PDGF in vessel maturation through pericyte recruitment. Studies, relying on sophisticated mouse genetics have demonstrated that proper PDGF receptor-dependent pericyte recruitment relies on endothelial production of PDGF-B, and also that local disposition of this growth factor, mediated by a C-terminal retention-sequence, is critical for proper vessel development (Enge *et al*., 2002; Lindblom *et al*., 2003).

Whether PDGF has direct effects on endothelial cells remains unclear. Most normal tissues appear to lack PDGF receptor expression on mature endothelial cells. However, a role for PDGF during endothelial cell differentiation has been suggested based on studies of endothelial precursor cells (Rolny *et al.*, 2006).

1. PDGF AND TUMOR PERICYTES

PDGF β-receptor expression on human tumor pericytes was first described in colorectal cancers (Sundberg *et al.*, 1993). Subsequent studies, including ongoing analyses on tumor tissue arrays, indicate that most common solid tumors have vessels with PDGF β-receptor expressing pericytes (Paulsson, Sjöblom, unpublished observation).

The functional significance of PDGF recruitment of pericytes has been evaluated in different animal tumor models in which PDGF stimulation of pericytes have been manipulated (Abramsson *et al.*, 2003; Furuhashi *et al.*, 2004; Guo *et al.*, 2003). These studies include analyses of the effects of PDGF overproduction by tumor cells, or depletion of PDGF production by endothelial cells. Together these studies have clearly established that PDGF stimulation of pericytes is critical for their recruitment to tumor vessels. Concerning the effect of pericyte coverage of vessels on tumor growth, one study identified a direct tumor growth stimulatory effect of enhanced PDGF-dependent pericyte recruitment (Furuhashi *et al.*, 2004). Detailed characterization of these faster-growing pericyte-rich tumors, including MRI-based analyses, revealed that increased pericyte coverage also was associated with improved perfusion, and a shift toward the use of smaller-caliber capillaries (Robinson, unpublished observation).

The progenitors of PDGF-responsive tumor pericytes remain incompletely characterized but are likely to include local precursor cells. Additionally, bone-marrow-derived precursor cells, undergoing a local differentiation process defined by variable marker expression, might also contribute to the pool of tumor pericytes (Song *et al.*, 2005).

The importance of PDGF-dependent pericytes for tumor angiogenesis has also been confirmed in studies where the effects of PDGF antagonists have been analyzed (Bergers *et al.*, 2003; Erber *et al.*, 2004). A pioneering study by Bergers *et al.* (2003) showed that an enhanced antiangiogenic effect could be achieved by combining VEGF- and PDGF-antagonists, and thereby obtaining simultaneously antiendothelial and antipericyte effects. Later studies in other tumor models, and other models of angiogenesis, have confirmed this interesting concept. This approach has been further developed by inclusion also of drugs directed predominantly against the epithelial compartment of tumors (Pietras and Hanahan, 2005).

2. PDGF AND TUMOR HEM ANGIOGENIC ENDOTHELIAL CELLS

As discussed above, the analyses of the phenotypes of mice lacking PDGF ligands or receptors have not yet provided firm evidence for a direct role of PDGF on endothelial cells. However, it is well known that endothelial cells in tumors differ from endothelial cells in normal tissues, thus leaving open the possibility that PDGF may act on endothelial cells of tumors.

A series of studies from the laboratory of Fidler have provided evidence for significant functions of PDGF receptors on endothelial cells in various models (Apte *et al*., 2004; Hwang *et al*., 2003; Kim *et al*., 2006; Lev *et al*., 2005). An intriguing finding from their studies is the indication that PDGF receptor expression is up-regulated in endothelial cells of metastatic tumors. This has been demonstrated in mouse models of bone metastases of prostate and breast cancer. Therapeutic effects of PDGF antagonists were also obtained in these models which were, at least partially, ascribed to inhibition of PDGF receptors on endothelial cells. The same group has also described the expression of ligand-activated receptors in models of intraperitoneal ovarian cancer and orthotopically grown pancreatic carcinoma.

Concerning PDGF receptor expression on endothelial cells of human tumors, the information is still scarce. Continued studies in this area would obviously benefit from improvement of the methodology for tissue analyses so that expression of PDGF receptors in pericytes and juxtaposed endothelial cells could reliably be distinguished.

C. PDGF and Recruitment of Tumor Fibroblasts

PDGF receptor expression on tumor fibroblasts is a common feature of human tumors. A recent survey, using an NCI-derived human tumor tissue array, indicated variable frequencies of PDGF receptor expression in fibroblasts of different tumors, as exemplified by 45% of positive samples among the colon cancers, whereas less than 20% of melanomas were positive. In general, PDGF β-receptor expression appeared to be more common. Highest PDGF α-receptor expression was seen in colon tumors, where 41% of the cases expressed PDGF α-receptors.

Studies of animal models have provided indications that PDGF stimulation of fibroblasts is critical for tumor recruitment of stroma. Transfection of melanoma cells with PDGF B-chain cDNA increased the ability of these cells to form subcutaneous tumors in a manner involving enhanced fibroblast recruitment (Forsberg *et al*., 1993). Similar observations have been made in models of skin cancer (Skobe and Fusenig, 1998). Also, the desmoplasia of breast cancer has been implied to involve PDGF-dependent expansion

of the tumor stroma, as suggested by the dramatic reduction in desmoplasia observed after inhibition of PDGF production in a breast cancer model (Shao *et al*., 2000).

Besides contributing to the recruitment and expansion of tumor fibroblasts, PDGF stimulation has also been shown, in animal models, to be important for the secretion of VEGF by these cells (Dong *et al*., 2004). These findings thus suggest that inhibition of PDGF receptor signaling in fibroblasts might not only reduce the volume of the stromal compartment but also indirectly exert antiangiogenic effects.

D. Regulation of Tumor Drug Uptake and IFP by PDGF Receptors

Increased IFP is a common feature of solid tumors (Jain, 2001). The underlying mechanisms remain incompletely understood. However, components of tumor pathophysiology that have been linked to this phenotype include the absence of lymphatic vessels, the leakiness of immature tumor vessels, and the presence of "activated" fibroblasts (Heldin *et al*., 2004). The increased IFP of tumors has been postulated to exert a negative influence of transcapillary drug-transport.

An involvement of PDGF receptor signaling in the increased IFP on tumors was originally suggested by demonstrations that IFP in normal loose connective tissue could be increased by PDGF-BB stimulation of fibroblasts (Rodt *et al*., 1996). Similar activities have been demonstrated for PDGF-DD (Uutela *et al*., 2004).

Studies using three different subcutaneous tumor models have now demonstrated that inhibition of PDGF receptors on fibroblasts, and possibly pericytes, also leads to a transient and reversible reduction in tumor IFP (Baranowska-Kortylewicz *et al*., 2005; Pietras *et al*., 2001, 2002, 2003b). Interestingly, these effects are associated with an improved tumor drug uptake and a concomitantly enhanced therapeutic effect of agents directed against the tumor epithelial cells. This effect was first demonstrated with conventional chemotherapeutical drugs such as Taxol and 5-FU (Pietras *et al*., 2002). Treatment with the PDGF receptor tyrosine kinase inhibitor imatinib of animals with LS174T subcutaneous tumors was shown to increase tumor uptake also of a tumor-targeting antibody through mechanisms possibly involving a reduction in tumor IFP (Baranowska-Kortylewicz *et al*., 2005). In one of these studies, the effect on drug uptake in normal organs was also analyzed and revealed that systemic treatment with PDGF inhibitors did not appear to increase drug uptake in normal tissue (Pietras *et al*., 2003b). Together these findings thus suggest inhibition of

stromal PDGF receptors as a general mechanism to improve tumor drug uptake.

Many aspects of these intriguing observations still remain unclear. As the experiments have been performed, it is not possible to strictly evaluate the relative importance of inhibition of PDGF receptors on pericytes and on fibroblasts for the observed effects. Furthermore, it can formally not be excluded that the effects on IFP and tumor drug uptake are temporally, but not causally, linked. Finally, the molecular mechanisms involved in PDGF receptor-mediated control of IFP and tumor drug uptake should be better clarified. Studies in this area indicate that the effect on IFP require PDGF receptor activation of PI3K (Heuchel *et al.*, 1999). Furthermore, characterization of the IFP regulation in normal loose connective tissue suggests that PDGF receptor-dependent interactions between integrins and extracellular matrix are involved in the fibroblast-mediated control of IFP (Liden *et al.*, 2006).

E. Implications of Roles for PDGF Receptor Signaling in Metastasis

Metastasis is a complex multistep process. In addition to the possible roles of PDGF receptor in the angiogenesis of bone metastasis discussed above, other specific aspects of metastasis has been proposed to be PDGF dependent. These include epithelial-mesenchymal transition (EMT) of malignant epithelial cells, lymphangiogenesis, alteration in tumor vasculature that is permissive for extravasation of tumor cells, and, finally, local interactions between the metastatic microenvironment and the malignant cells (Fig. 2).

One established model of EMT is TGFβ-stimulated hepatocytes expressing activated Ha-ras. Recent characterization of this model demonstrated that induction of autocrine PDGF α-receptor signaling occurred in association with EMT induced by TGFβ. A functional importance of the PDGF receptor signaling was inferred based on the observations that PDGF receptor blockade reduced TGFβ-induced *in vitro* migration as well as the *in vivo* tumor formation of these cells (Jechlinger *et al.*, 2006). Similar observations were also obtained in TGFβ-dependent models of EMT in breast cancer in which experimental metastasis was blocked by interference with PDGF receptor signaling (Gotzmann *et al.*, 2006).

Lymphangiogenesis is required for lymphatic spread of cancers. This process is generally believed to be highly dependent on VEGFR-3 expressed on lymphatic endothelial cells (Alitalo *et al.*, 2005). A study also suggested a role for PDGF receptor signaling in lymphangiogenesis (Cao *et al.*, 2004). The key finding of this study was that overproduction of PDGF by

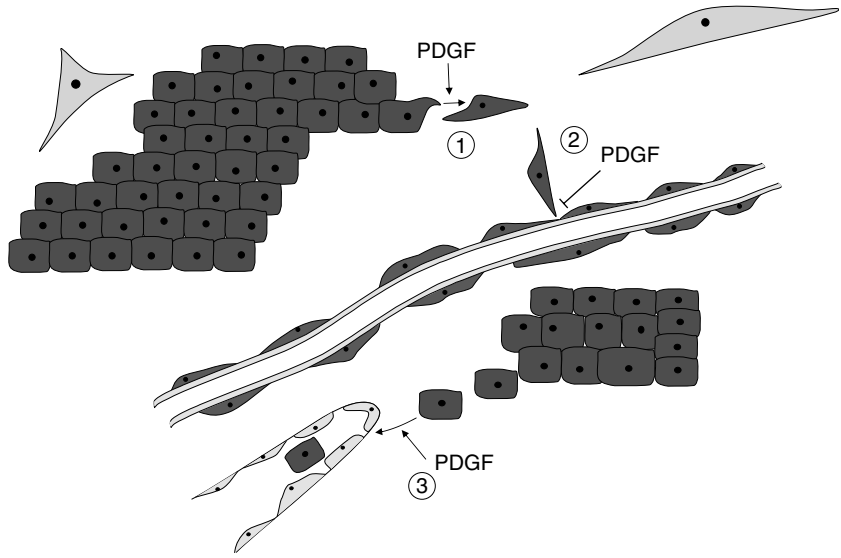

Fig. 2 Involvement of PDGF in tumor metastasis. PDGF may promote migration and invasiveness of certain tumor cells (1), and may also promote the spread of tumor cells via lymphatic vessels (3). On the other hand, PDGF may also promote pericyte coverage of blood vessels, and thereby prevent tumor cell uptake and spread via the blood stream (2). For references, see the text.

murine fibrosarcoma cells increased tumor lymphangiogenesis, possibly via induction of VEGF-C or -D production.

Concerning pericytes and metastasis, it was observed in the RIP-Tag model of insulinomas that PDGF-dependent pericyte-coverage appeared to represent a barrier for metastatic spread (Xian *et al.*, 2006). Breeding of RIP-Tag mice with Pdgfb (ret/ret) mice, in which endothelial retention of PDGF-BB is perturbed, led to the formation of primary tumors with reduced pericyte coverage and a concomitant increase in metastases in distant organs and local lymph nodes. The notion of a metastasis-protective effect of pericytes was independently supported by analyses of tumors in N-CAM-deficient derivatives of RIP-Tag mice, which were also characterized by a reduction in pericyte coverage and increased formation of metastases. On the other hand, analyses of animal models of renal cell cancer metastasis have suggested that PDGF stimulation promotes metastasis through mechanisms including stimulation of pericytes (Xu *et al.*, 2005).

Finally, a role for PDGF receptor signaling in the establishment of bone metastases of prostate and breast cancer has been suggested (Dolloff *et al.*, 2005; Yi *et al.*, 2002). In prostate cancer, PDGF α-receptor responses have been demonstrated specifically in highly metastatic variants. Since both

osteoclasts and osteoblasts produce PDGF-AA, these cells might be involved in the recruitment of the metastatic cells.

Thus, using different animal models, both pro- and antimetastatic effects of PDGF receptor signaling, have been implied. It will be important to elucidate to what extent these observations are relevant for human tumors.

IV. CLINICAL STUDIES

A. PDGF Antagonists

Isoform-specific neutralizing polyclonal and monoclonal antibodies against PDGF ligands and receptors have been generated and successfully used to define PDGF-dependent processes in animal disease models (Östman, 2004). Most of these studies have analyzed the importance of PDGF receptor signaling in atherosclerosis and restenosis, but significant effects have also been obtained in tumor models.

As candidate drugs for clinical application, humanized antibodies are most attractive. PDGF α-receptor-neutralizing activity of one such antibody was characterized (Loizos *et al.*, 2005). Clinical cancer studies with neutralizing PDGF receptor antibodies are still scarce and the only published example is a report where a PEG-conjugated Fab-fragment targeting the PDGF β-receptor was used to analyze effects on tumor perfusion (Jayson *et al.*, 2004).

Recombinant soluble receptors constitute a generic class of growth factor antagonists. PDGF-antagonistic effects of such proteins have also been demonstrated. Fusion proteins where PDGF receptor sequence have been fused to either the conserved part of the immunoglobulin heavy chain or GST have been described (Heidaran *et al.*, 1995; Leppänen *et al.*, 2000). These compounds both displayed inhibitory effects at nanomolar concentrations; the high potency most likely is dependent on the dimeric nature of these receptor constructs. These types of antagonists have not yet been developed for clinical applications.

The first selective LMW inhibitor of the PDGF receptor tyrosine kinase was described in 1994 (Kovalenko *et al.*, 1994). Since then a series of compounds have been introduced, some of which are now in various phases of clinical development (some examples are detailed below). All these compounds inhibit both PDGF α- and β-receptors, and they block to different extents also other tyrosine kinases at the concentration required for PDGF receptor inhibition. Thus, these compounds differ from each other not only in potency, that is concentration required for PDGF receptor inhibition, but also in selectivity profile.

Imatinib is the most established PDGF receptor inhibitor, with demonstrated clinical efficiency in PDGF-dependent malignancies (Capdeville *et al.*, 2002; Östman, 2004). Two multikinase inhibitors, sunitinib and sorafenib, with anti-PDGF receptor activity have also now been approved following results in phase III trials (Gollob *et al.*, 2006; Motzer *et al.*, 2006).

Finally, some novel highly potent PDGF receptor inhibitors, such as CP-673451 (Roberts *et al.*, 2005), with a more specific target profile than sorafenib and sunitinib have been introduced and are now entering clinical trials.

B. Clinical Effects Ascribed to PDGF Receptor Inhibition

1. PDGF RECEPTOR TARGETING IN MALIGNANCIES ASSOCIATED WITH MUTATIONAL ACTIVATION OF PDGF SIGNALING

In the case of DFSP, wide surgical excisions are still the standard treatment. However, a series of reports have also demonstrated clinical activity of the PDGF receptor inhibitor imatinib in this disease. In a study of 10 patients, all 8 individuals with locally advanced disease became free of disease through imatinib-induced complete responses or surgery following partial responses (McArthur *et al.*, 2005). Activity was also observed in patients with metastatic disease. It should also be noted that DFSP-like tumors lacking the t(17/22) translocations appear to be nonresponsive to imatinib.

As discussed above, 30–50% of GIST patients lacking c-kit mutations instead display mutational activation of PDGF α-receptors (Corless *et al.*, 2005). Biochemical characterization of these variants, including the most common D842V mutation, indicates that the most frequent PDGF α-receptor variants in GIST are insensitive to imatinib. However, there are also GIST-associated variants of the PDGF α-receptors which indeed are blocked by imatinib, such as the exon 18 mutations D846Y, N848K, and Y849K; patients with these variants are thus likely to respond to imatinib. Characterization of other PDGF receptor kinase inhibitors, with regard to activity against the more common PDGF α-receptor variants, is an obviously relevant topic for continued studies.

Clinical responses have also been observed in cases of hematologic malignancies with mutational alterations of PDGF receptors (Jones and Cross, 2004; Muller *et al.*, 2006). The first report included demonstration of complete cytogenetic remission in patients with the TEL-PDGF β-receptor rearrangement (Apperley *et al.*, 2002). There have now also been

reports of activity in patients with other PDGF β-receptor rearrangements, including individuals with RABEP1-, H4-, and PDE4DIP-PDGFRB fusion genes (Jones and Cross, 2004). Concerning PDGF α-receptor fusions, striking responses including molecular remissions have been observed in patients with FIP1L1-PDGFRA fusions (Cools *et al.*, 2003), and responses have also been observed in patients with other PDGFRA fusion genes (Score *et al.*, 2006; Trempat *et al.*, 2003).

2. TREATMENT OF GLIOBLASTOMA AND ADVANCED AGGRESSIVE FIBROSIS WITH IMATINIB

Promising results with PDGF inhibitors in cell and animal models of glioblastomas have prompted clinical studies. Initial efforts focused on monotreatment with imatinib in glioblastomas. However, a report indicated that monotreatment with imatinib for this patient group displayed very low activity (Wen *et al.*, 2006). In contrast, very encouraging findings have been made following combination treatment with hydroxyurea and imatinib (Dresemann, 2005; Reardon *et al.*, 2005). Two studies, each encompassing 30 patients with progressive disease, reported 26% and 32% 6-month progression-free survival, which compares very favorable to previous studies in this group of patients. These preliminary studies are now being validated in ongoing studies that include randomization of patients between treatment with hydroxyurea alone and the combination with imatinib. Elucidation of the mechanism underlying the combinatorial effect is highly warranted for further development of this regimen. Furthermore, efforts to identify molecular characteristics of responders and nonresponders should also be prioritized.

A study of 19 patients with AF was also reported in which PDGF receptor targeting was implied as a contributing mechanism to therapeutic effects of imatinib (Heinrich *et al.*, 2006). Three out of 19 patients had a partial response to treatment, and 4 additional patients displayed stable disease that lasted more than 1 year. No evidence for mutational activation of PDGF receptors was obtained, although PDGF β-receptor expression was demonstrated in tumor tissue.

3. PHASE III RENAL CELL CANCER STUDIES WITH MULTIKINASE INHIBITORS WITH PDGF RECEPTOR INHIBITORY ACTIVITY

Sunitinib (Sutent) and sorafenib (Nexavar) both belong to a class of multikinase inhibitors with the ability to block both VEGF and PDGF receptors. Both these drugs have been tested in large phase III studies in

renal cell cancer. Results presented at ASCO 2005 demonstrated impressive effects of both drugs in cytokine-refractory metastatic renal cell cancer (www.asco.org). This was followed in the year 2006 by reports of the first randomized study using sunitinib as first-line treatment in metastatic renal cell cancer (www.asco.org). In this study, sunitinib treatment led to 11 months median progression-free survival, as compared to 5 months for those treated with interferon-α. Furthermore, response rates in the sunitinib and interferon groups were 31% and 6%, respectively. Obviously, the design of these studies and the pleiotrophic effects of sunitinib make it impossible to estimate the contribution of the PDGF receptor inhibition to these results. However, it is very likely that a potent antiangiogenic effect, occurring through combined targeting of VEGF- and PDGF-dependent endothelial cells and pericytes, is a major component of the therapeutic action of this drug.

V. FUTURE PERSPECTIVES

As outlined above, clinical studies have now validated PDGF receptors involved in autocrine growth signaling and in tumor angiogenesis as cancer drug targets.

Concerning the effects in autocrine settings, some key issues for future studies include identification of compounds able to block the imatinib-insensitive variants of PDGF α-receptor associated with GIST. It is also predicted that prolonged treatment with imatinib will lead to the emergence of secondary mutations in the PDGF receptors, and a preparedness to address this situation is motivated. The preliminary observations of imatinib activity in glioblastomas, in combination with hydoxyurea, are encouraging. If confirmed, it will be very valuable to find markers for responders and nonresponders.

The importance of PDGF-dependent pericytes in tumor angiogenesis appears to offer a broad range of therapeutical opportunities. Continued clinical studies with multikinase inhibitors will reveal if different tumor types will display large variations with regard to sensitivity to combined VEGF- and PDGF receptor inhibitors. Also in this context, it will be important to identify characteristics of responders and nonresponders. Furthermore, it will be most interesting to see if the promising effects of stromal PDGF receptor inhibition, with regard to tumor drug-uptake, can be confirmed in clinical settings.

Finally, it should be noted that the available methods for determination of PDGF receptor expression and activation status in tumor tissue are still a limiting factor for rational development of PDGF receptor-based cancer

therapies. Improvement in this area is thus predicted to accelerate this exciting process.

ACKNOWLEDGMENTS

We acknowledge members of our groups and Elisabeth Buchdunger for productive comments. Also Ingegärd Schiller is acknowledged for expert secretarial assistance.

REFERENCES

Abramsson, A., Lindblom, P., and Betsholtz, C. (2003). Endothelial and nonendothelial sources of PDGF-B regulate pericyte recruitment and influence vascular pattern formation in tumors. *J. Clin. Invest.* **112,** 1142–1151.

Alitalo, K., Tammela, T., and Petrova, T. V. (2005). Lymphangiogenesis in development and human disease. *Nature* **438,** 946–953.

Apperley, J. F., Gardembas, M., Melo, J. V., Russell-Jones, R., Bain, B. J., Baxter, E. J., Chase, A., Chessells, J. M., Colombat, M., Dearden, C. E., Dimitrijevic, S., Mahon, F. X., *et al.* (2002). Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor β. *N. Engl. J. Med.* **347,** 481–487.

Apte, S. M., Fan, D., Killion, J. J., and Fidler, I. J. (2004). Targeting the platelet-derived growth factor receptor in antivascular therapy for human ovarian carcinoma. *Clin. Cancer Res.* **10,** 897–908.

Bae, Y. S., Sung, J. Y., Kim, O. S., Kim, Y. J., Hur, K. C., Kazlauskas, A., and Rhee, S. G. (2000). Platelet-derived growth factor-induced H(2)O(2) production requires the activation of phosphatidylinositol 3-kinase. *J. Biol. Chem.* **275,** 10527–10531.

Baranowska-Kortylewicz, J., Abe, M., Pietras, K., Kortylewicz, Z. P., Kurizaki, T., Nearman, J., Paulsson, J., Mosley, R. L., Enke, C. A., and Östman, A. (2005). Effect of platelet-derived growth factor receptor-β inhibition with STI571 on radioimmunotherapy. *Cancer Res.* **65,** 7824–7831.

Basciani, S., Brama, M., Mariani, S., De Luca, G., Arizzi, M., Vesci, L., Pisano, C., Dolci, S., Spera, G., and Gnessi, L. (2005). Imatinib mesylate inhibits Leydig cell tumor growth: Evidence for *in vitro* and *in vivo* activity. *Cancer Res.* **65,** 1897–1903.

Bergers, G., Song, S., Meyer-Morse, N., Bergsland, E., and Hanahan, D. (2003). Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors. *J. Clin. Invest.* **111,** 1287–1295.

Betsholtz, C. (2004). Insight into the physiological functions of PDGF through genetic studies in mice. *Cytokine Growth Factor Rev.* **15,** 215–228.

Boström, H., Willetts, K., Pekny, M., Levéen, P., Lindahl, P., Hedstrand, H., Pekna, M., Hellström, M., Gebre-Medhin, S., Schalling, M., Nilsson, M., Kurland, S., *et al.* (1996). PDGF-A signaling is a critical event in lung alveolar myofibroblast development and alveogenesis. *Cell* **85,** 863–873.

Cao, R., Brakenhielm, E., Li, X., Pietras, K., Widenfalk, J., Östman, A., Eriksson, U., and Cao, Y. (2002). Angiogenesis stimulated by PDGF-CC, a novel member in the PDGF family, involves activation of PDGFR-$\alpha\alpha$ and -$\alpha\beta$ receptors. *FASEB J.* **16,** 1575–1583.

Cao, R., Bjorndahl, M. A., Religa, P., Clasper, S., Garvin, S., Galter, D., Meister, B., Ikomi, F., Tritsaris, K., Dissing, S., Ohhashi, T., Jackson, D. G., *et al.* (2004). PDGF-BB induces intratumoral lymphangiogenesis and promotes lymphatic metastasis. *Cancer Cell* **6,** 333–345.

Capdeville, R., Buchdunger, E., Zimmermann, J., and Matter, A. (2002). Glivec (STI571, imatinib), a rationally developed, targeted anticancer drug. *Nat. Rev. Drug Discov.* **1,** 493–502.

Carroll, M., Tomasson, M. H., Barker, G. F., Golub, T. R., and Gilliland, D. G. (1996). The TEL/platelet-derived growth factor β receptor (PDGF β R) fusion in chronic myelomonocytic leukemia is a transforming protein that self-associates and activates PDGF β R kinase-dependent signaling pathways. *Proc. Natl. Acad. Sci. USA* **93,** 14845–14850.

Choi, M. H., Lee, I. K., Kim, G. W., Kim, B. U., Han, Y. H., Yu, D. Y., Park, H. S., Kim, K. Y., Lee, J. S., Choi, C., Bae, Y. S., Lee, B. I., *et al.* (2005). Regulation of PDGF signalling and vascular remodelling by peroxiredoxin II. *Nature* **435,** 347–353.

Cools, J., DeAngelo, D. J., Gotlib, J., Stover, E. H., Legare, R. D., Cortes, J., Kutok, J., Clark, J., Galinsky, I., Griffin, J. D., Cross, N. C., Tefferi, A., *et al.* (2003). A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. *N. Engl. J. Med.* **348,** 1201–1214.

Corless, C. L., Fletcher, J. A., and Heinrich, M. C. (2004). Biology of gastrointestinal stromal tumors. *J. Clin. Oncol.* **22,** 3813–3825.

Corless, C. L., Schroeder, A., Griffith, D., Town, A., McGreevey, L., Harrell, P., Shiraga, S., Bainbridge, T., Morich, J., and Heinrich, M. C. (2005). PDGFRA mutations in gastrointestinal stromal tumors: Frequency, spectrum and in vitro sensitivity to imatinib. *J. Clin. Oncol.* **23,** 5357–5364.

Dai, C., Celestino, J. C., Okada, Y., Louis, D. N., Fuller, G. N., and Holland, E. C. (2001). PDGF autocrine stimulation dedifferentiates cultured astrocytes and induces oligodendrogliomas and oligoastrocytomas from neural progenitors and astrocytes *in vivo*. *Genes Dev.* **15,** 1913–1925.

Ding, H., Wu, X., Bostrom, H., Kim, I., Wong, N., Tsoi, B., O'Rourke, M., Koh, G. Y., Soriano, P., Betsholtz, C., Hart, T. C., Marazita, M. L., *et al.* (2004). A specific requirement for PDGF-C in palate formation and PDGFR-alpha signaling. *Nat. Genet.* **36,** 1111–1116.

Dolloff, N. G., Shulby, S. S., Nelson, A. V., Stearns, M. E., Johannes, G. J., Thomas, J. D., Meucci, O., and Fatatis, A. (2005). Bone-metastatic potential of human prostate cancer cells correlates with Akt/PKB activation by α platelet-derived growth factor receptor. *Oncogene* **24,** 6848–6854.

Dong, J., Grunstein, J., Tejada, M., Peale, F., Frantz, G., Liang, W. C., Bai, W., Yu, L., Kowalski, J., Liang, X., Fuh, G., Gerber, H. P., *et al.* (2004). VEGF-null cells require PDGFR α signaling-mediated stromal fibroblast recruitment for tumorigenesis. *EMBO J.* **23,** 2800–2810.

Dresemann, G. (2005). Imatinib and hydroxyurea in pretreated progressive glioblastoma multiforme: A patient series. *Ann. Oncol.* **16,** 1702–1708.

Ekman, S., Kallin, A., Engström, U., Heldin, C.-H., and Rönnstrand, L. (2002). SHP-2 is involved in heterodimer specific loss of phosphorylation of Tyr771 in the PDGF β-receptor. *Oncogene* **21,** 1870–1875.

Enge, M., Bjarnegard, M., Gerhardt, H., Gustafsson, E., Kalen, M., Asker, N., Hammes, H. P., Shani, M., Fassler, R., and Betsholtz, C. (2002). Endothelium-specific platelet-derived growth factor-B ablation mimics diabetic retinopathy. *EMBO J.* **21,** 4307–4316.

Erber, R., Thurnher, A., Katsen, A. D., Groth, G., Kerger, H., Hammes, H. P., Menger, M. D., Ullrich, A., and Vajkoczy, P. (2004). Combined inhibition of VEGF and PDGF signaling enforces tumor vessel regression by interfering with pericyte-mediated endothelial cell survival mechanisms. *FASEB J.* **18,** 338–340.

Eriksson, A., Siegbahn, A., Westermark, B., Heldin, C.-H., and Claesson-Welsh, L. (1992). PDGF α- and β-receptors activate unique and common signal transduction pathways. *EMBO J.* **11,** 543–550.

Fleming, T. P., Saxena, A., Clark, W. C., Robertson, J. T., Oldfield, E. H., Aaronson, S. A., and Ali, I. U. (1992). Amplification and/or overexpression of platelet-derived growth factor receptors and epidermal growth factor receptor in human glial tumors. *Cancer Res.* **52,** 4550–4553.

Forsberg, K., Valyi-Nagy, I., Heldin, C.-H., Herlyn, M., and Westermark, B. (1993). Platelet-derived growth factor (PDGF) in oncogenesis: Development of a vascular connective tissue stroma in xenotransplanted human melanoma producing PDGF-BB. *Proc. Natl. Acad. Sci. USA* **90,** 393–397.

Fredriksson, L., Li, H., Fieber, C., Li, X., and Eriksson, U. (2004). Tissue plasminogen activator is a potent activator of PDGF-CC. *EMBO J.* **23,** 3793–3802.

Furuhashi, M., Sjöblom, T., Abramsson, A., Ellingsen, J., Micke, P., Li, H., Bergsten-Folestad, E., Eriksson, U., Heuchel, R., Betsholtz, C., Heldin, C.-H., and Östman, A. (2004). Platelet-derived growth factor production by B16 melanoma cells leads to increased pericyte abundance in tumors and an associated increase in tumor growth rate. *Cancer Res.* **64,** 2725–2733.

Gnessi, L., Basciani, S., Mariani, S., Arizzi, M., Spera, G., Wang, C., Bondjers, C., Karlsson, L., and Betsholtz, C. (2000). Leydig cell loss and spermatogenic arrest in platelet-derived growth factor (PDGF)-A-deficient mice. *J. Cell Biol.* **149,** 1019–1026.

Gollob, J. A., Wilhelm, S., Carter, C., and Kelley, S. L. (2006). Role of Raf kinase in cancer: Therapeutic potential of targeting the Raf/MEK/ERK signal transduction pathway. *Semin. Oncol.* **33,** 392–406.

Golub, T. R., Barker, G. F., Lovett, M., and Gilliland, D. G. (1994). Fusion of PDGF receptor β to a novel ets-like gene, tel, in chronic myelomonocytic leukemia with t(5;12) chromosomal translocation. *Cell* **77,** 307–316.

Gotzmann, J., Fischer, A. N., Zojer, M., Mikula, M., Proell, V., Huber, H., Jechlinger, M., Waerner, T., Weith, A., Beug, H., and Mikulits, W. (2006). A crucial function of PDGF in TGF-β-mediated cancer progression of hepatocytes. *Oncogene* **25,** 3170–3185.

Greco, A., Fusetti, L., Villa, R., Sozzi, G., Minoletti, F., Mauri, P., and Pierotti, M. A. (1998). Transforming activity of the chimeric sequence formed by the fusion of collagen gene COL1A1 and the platelet derived growth factor b-chain gene in dermatofibrosarcoma protuberans. *Oncogene* **17,** 1313–1319.

Guo, P., Hu, B., Gu, W., Xu, L., Wang, D., Huang, H. J., Cavenee, W. K., and Cheng, S. Y. (2003). Platelet-derived growth factor-B enhances glioma angiogenesis by stimulating vascular endothelial growth factor expression in tumor endothelia and by promoting pericyte recruitment. *Am. J. Pathol.* **162,** 1083–1093.

Hägerstrand, D., Hesselager, G., Achterberg, S., Wickenberg Bolin, U., Kowanetz, M., Kastemar, M., Heldin, C.-H., Isaksson, A., Nistér, M., and Östman, A. (2006). Characterization of an imatinib-sensitive subset of high-grade human glioma cultures. *Oncogene* **25,** 4913– 4922.

Heidaran, M. A., Mahadevan, D., and Larochelle, W. J. (1995). β PDGFR-IgG chimera demonstrates that human β PDGFR Ig-like domains 1 to 3 are sufficient for high affinity PDGF BB binding. *FASEB J.* **9,** 140–145.

Heinrich, M. C., Corless, C. L., Duensing, A., McGreevey, L., Chen, C. J., Joseph, N., Singer, S., Griffith, D. J., Haley, A., Town, A., Demetri, G. D., Fletcher, C. D., *et al.* (2003). PDGFRA activating mutations in gastrointestinal stromal tumors. *Science* **299,** 708–710.

Heinrich, M. C., McArthur, G. A., Demetri, G. D., Joensuu, H., Bono, P., Herrmann, R., Hirte, H., Cresta, S., Koslin, D. B., Corless, C. L., Dirnhofer, S., van Oosterom, A. T., *et al.* (2006).

Clinical and molecular studies of the effect of imatinib on advanced aggressive fibromatosis (desmoid tumor). *J. Clin. Oncol.* **24,** 1195–1203.
Heldin, C.-H., and Westermark, B. (1999). Mechanism of action and *in vivo* role of platelet-derived growth factor. *Physiol. Rev.* **79,** 1283–1316.
Heldin, C.-H., Östman, A., and Rönnstrand, L. (1998). Signal transduction via platelet-derived growth factor receptors. *Biochim. Biophys. Acta* **1378,** F79–F113.
Heldin, C.-H., Eriksson, U., and Östman, A. (2002). New members of the platelet-derived growth factor family of mitogens. *Arch. Biochem. Biophys.* **398,** 284–290.
Heldin, C.-H., Rubin, K., Pietras, K., and Östman, A. (2004). High interstitial fluid pressure-an obstacle in cancer therapy. *Nat. Rev. Cancer* **4,** 806–813.
Hermanson, M., Funa, K., Hartman, M., Claesson-Welsh, L., Heldin, C.-H., Westermark, B., and Nistér, M. (1992). Platelet-derived growth factor and its receptors in human glioma tissue: Expression of messenger RNA and protein suggests the presence of autocrine and paracrine loops. *Cancer Res.* **52,** 3213–3219.
Hesselager, G., Uhrbom, L., Westermark, B., and Nistér, M. (2003). Complementary effects of platelet-derived growth factor autocrine stimulation and p53 or Ink4a-Arf deletion in a mouse glioma model. *Cancer Res.* **63,** 4305–4309.
Heuchel, R., Berg, A., Tallquist, M., Åhlén, K., Reed, R. K., Rubin, K., Claesson-Welsh, L., Heldin, C.-H., and Soriano, P. (1999). Platelet-derived growth factor β receptor regulates interstitial fluid homeostasis through phosphatidylinositol-3′ kinase signaling. *Proc. Natl. Acad. Sci. USA* **96,** 11410–11415.
Hwang, R. F., Yokoi, K., Bucana, C. D., Tsan, R., Killion, J. J., Evans, D. B., and Fidler, I. J. (2003). Inhibition of platelet-derived growth factor receptor phosphorylation by STI571 (Gleevec) reduces growth and metastasis of human pancreatic carcinoma in an orthotopic nude mouse model. *Clin. Cancer Res.* **9,** 6534–6544.
Jain, R. K. (2001). Delivery of molecular and cellular medicine to solid tumors. *Adv. Drug Deliv. Rev.* **46,** 149–168.
Jayson, G. C., Parker, G. J., Mullamitha, S., Valle, J. W., Saunders, M., Broughton, L., Lawrance, J., Carrington, B., Roberts, C., Issa, B., Buckley, D. L., Cheung, S., *et al.* (2004). Blockade of platelet-derived growth factor receptor-β by CDP860, a humanized, PEGylated di-Fab', leads to fluid accumulation and is associated with increased tumor vascularized volume. *J. Clin. Oncol.* **23**(5), 973–981.
Jechlinger, M., Sommer, A., Moriggl, R., Seither, P., Kraut, N., Capodiecci, P., Donovan, M., Cordon-Cardo, C., Beug, H., and Grunert, S. (2006). Autocrine PDGFR signaling promotes mammary cancer metastasis. *J. Clin. Invest.* **116,** 1561–1570.
Jones, A. V., and Cross, N. C. (2004). Oncogenic derivatives of platelet-derived growth factor receptors. *Cell. Mol. Life Sci.* **61,** 2912–2923.
Jousset, C., Carron, C., Boureux, A., Quang, C. T., Oury, C., Dusanter-Fourt, I., Charon, M., Levin, J., Bernard, O., and Ghysdael, J. (1997). A domain of TEL conserved in a subset of ETS proteins defines a specific oligomerization interface essential to the mitogenic properties of the TEL-PDGFR β oncoprotein. *EMBO J.* **16,** 69–82.
Karlsson, L., Lindahl, P., Heath, J. K., and Betsholtz, C. (2000). Abnormal gastrointestinal development in PDGF-A and PDGFR-(α) deficient mice implicates a novel mesenchymal structure with putative instructive properties in villus morphogenesis. *Development* **127,** 3457–3466.
Kilic, T., Alberta, J. A., Zdunek, P. R., Acar, M., Iannarelli, P., O'Reilly, T., Buchdunger, E., Black, P. M., and Stiles, C. D. (2000). Intracranial inhibition of platelet-derived growth factor-mediated glioblastoma cell growth by an orally active kinase inhibitor of the 2-phenylaminopyrimidine class. *Cancer Res.* **60,** 5143–5150.
Kim, S. J., Uehara, H., Yazici, S., Busby, J. E., Nakamura, T., He, J., Maya, M., Logothetis, C., Mathew, P., Wang, X., Do, K. A., Fan, D., *et al.* (2006). Targeting platelet-derived growth

factor receptor on endothelial cells of multidrug-resistant prostate cancer. *J. Natl. Cancer Inst.* **98,** 783–793.

Kiyan, J., Kiyan, R., Haller, H., and Dumler, I. (2005). Urokinase-induced signaling in human vascular smooth muscle cells is mediated by PDGFR-β. *EMBO J.* **24,** 1787–1797.

Klinghoffer, R. A., and Kazlauskas, A. (1995). Identification of a putative Syp substrate, the PDGF β receptor. *J. Biol. Chem.* **270,** 22208–22217.

Klinghoffer, R. A., Mueting-Nelsen, P. F., Faerman, A., Shani, M., and Soriano, P. (2001). The two PDGF receptors maintain conserved signaling *in vivo* despite divergent embryological functions. *Mol. Cell* **7,** 343–354.

Kovalenko, M., Gazit, A., Böhmer, A., Rorsman, C., Rönnstrand, L., Heldin, C.-H., Waltenberger, J., Böhmer, F. D., and Levitzki, A. (1994). Selective platelet-derived growth factor receptor kinase blockers reverse sis-transformation. *Cancer Res.* **54,** 6106–6114.

Kovalenko, M., Denner, K., Sandström, J., Persson, C., Gross, S., Jandt, E., Vilella, R., Böhmer, F., and Östman, A. (2000). Site-selective dephosphorylation of the platelet-derived growth factor β-receptor by the receptor-like protein-tyrosine phosphatase DEP-1. *J. Biol. Chem.* **275,** 16219–16226.

Leppänen, O., Miyazawa, K., Bäckström, G., Pietras, K., Sjöblom, T., Heldin, C.-H., and Östman, A. (2000). Predimerization of recombinant platelet-derived growth factor receptor extracellular domains increases antagonistic potency. *Biochemistry* **39,** 2370–2375.

Lev, D. C., Kim, S. J., Onn, A., Stone, V., Nam, D. H., Yazici, S., Fidler, I. J., and Price, J. E. (2005). Inhibition of platelet-derived growth factor receptor signaling restricts the growth of human breast cancer in the bone of nude mice. *Clin. Cancer Res.* **11,** 306–314.

Leveen, P., Pekny, M., Gebre-Medhin, S., Swolin, B., Larsson, E., and Betsholtz, C. (1994). Mice deficient for PDGF B show renal, cardiovascular, and hematological abnormalities. *Genes Dev.* **8,** 1875–1887.

Li, L., Heldin, C.-H., and Heldin, P. (2006). Inhibition of platelet-derived growth factor-BB-induced receptor activation and fibroblast migration by hyaluronan activation of CD44. *J. Biol. Chem.* **281,** 26512–26519.

Li, X., and Eriksson, U. (2003). Novel PDGF family members: PDGF-C and PDGF-D. *Cytokine Growth Factor Rev.* **14,** 91–98.

Li, X., Tjwa, M., Moons, L., Fons, P., Noel, A., Ny, A., Zhou, J. M., Lennartsson, J., Li, H., Luttun, A., Pontén, A., Devy, L., *et al.* (2005). Revascularization of ischemic tissues by PDGF-CC via effects on endothelial cells and their progenitors. *J. Clin. Invest.* **115,** 118–127.

Liden, A., Berg, A., Nedrebo, T., Reed, R. K., and Rubin, K (2006). Platelet-derived growth factor BB-mediated normalization of dermal interstitial fluid pressure after mast cell degranulation depends on beta3 but not beta1 integrins. *Circ. Res.* **98,** 635–641.

Lindblom, P., Gerhardt, H., Liebner, S., Abramsson, A., Enge, M., Hellström, M., Bäckström, G., Fredriksson, S., Landegren, U., Nyström, H. C., Bergström, G., Dejana, E., *et al.* (2003). Endothelial PDGF-B retention is required for proper investment of pericytes in the microvessel wall. *Genes Dev.* **17,** 1835–1840.

Loizos, N., Xu, Y., Huber, J., Liu, M., Lu, D., Finnerty, B., Rolser, R., Malikzay, A., Persaud, A., Corcoran, E., Deevi, D. S., Balderes, P., *et al.* (2005). Targeting the platelet-derived growth factor receptor α with a neutralizing human monoclonal antibody inhibits the growth of tumor xenografts: Implications as a potential therapeutic target. *Mol. Cancer Ther.* **4,** 369–379.

Matei, D., Chang, D. D., and Jeng, M. H. (2004). Imatinib mesylate (Gleevec) inhibits ovarian cancer cell growth through a mechanism dependent on platelet-derived growth factor receptor α and Akt inactivation. *Clin. Cancer Res.* **10,** 681–690.

McArthur, G. A. (2006). Dermatofibrosarcoma protuberans: A surgical disease with a molecular savior. *Curr. Opin. Oncol.* **18,** 341–346.

McArthur, G. A., Demetri, G. D., van Oosterom, A., Heinrich, M. C., Debiec-Rychter, M., Corless, C. L., Nikolova, Z., Dimitrijevic, S., and Fletcher, J. A. (2005). Molecular and clinical analysis of locally advanced dermatofibrosarcoma protuberans treated with imatinib: Imatinib target exploration consortium study b2225. *J. Clin. Oncol.* **23,** 866–873.

Motzer, R. J., Hoosen, S., Bello, C. L., and Christensen, J. G. (2006). Sunitinib malate for the treatment of solid tumours: A review of current clinical data. *Expert Opin. Investig. Drugs* **15,** 553–561.

Muller, A. M., Martens, U. M., Hofmann, S. C., Bruckner-Tuderman, L., Mertelsmann, R., and Lubbert, M. (2006). Imatinib mesylate as a novel treatment option for hypereosinophilic syndrome: Two case reports and a comprehensive review of the literature. *Ann. Hematol.* **85,** 1–16.

Nilsson, B., Bumming, P., Meis-Kindblom, J. M., Oden, A., Dortok, A., Gustavsson, B., Sablinska, K., and Kindblom, L. G. (2005). Gastrointestinal stromal tumors: The incidence, prevalence, clinical course, and prognostication in the preimatinib mesylate era—a population-based study in western Sweden. *Cancer* **103,** 821–829.

Östman, A. (2004). PDGF receptors-mediators of autocrine tumor growth and regulators of tumor vasculature and stroma. *Cytokine Growth Factor Rev.* **15,** 275–286.

Östman, A., and Heldin, C.-H. (2001). Involvement of platelet-derived growth factor in disease: Development of specific antagonists. *Adv. Cancer Res.* **80,** 1–38.

Pardanani, A., Brockman, S. R., Paternoster, S. F., Flynn, H. C., Ketterling, R. P., Lasho, T. L., Ho, C. L., Li, C. Y., Dewald, G. W., and Tefferi, A. (2004). FIP1L1-PDGFRA fusion: Prevalence and clinicopathologic correlates in 89 consecutive patients with moderate to severe eosinophilia. *Blood* **104,** 3038–3045.

Persson, C., Sävenhed, C., Bourdeau, A., Tremblay, M. L., Markova, B., Böhmer, F. D., Haj, F. G., Neel, B. G., Elson, A., Heldin, C.-H., Rönnstrand, L., Östman, A., *et al.* (2004). Site-selective regulation of platelet-derived growth factor β receptor tyrosine phosphorylation by T-cell protein tyrosine phosphatase. *Mol. Cell. Biol.* **24,** 2190–2201.

Pietras, K., and Hanahan, D. (2005). A multitargeted, metronomic, and maximum-tolerated dose "chemo-switch" regimen is antiangiogenic, producing objective responses and survival benefit in a mouse model of cancer. *J. Clin. Oncol.* **23,** 939–952.

Pietras, K., Östman, A., Sjöquist, M., Buchdunger, E., Reed, R. K., Heldin, C.-H., and Rubin, K. (2001). Inhibition of platelet-derived growth factor receptors reduces interstitial hypertension and increases transcapillary transport in tumors. *Cancer Res.* **61,** 2929–2934.

Pietras, K., Rubin, K., Sjöblom, T., Buchdunger, E., Sjöquist, M., Heldin, C.-H., and Östman, A. (2002). Inhibition of PDGF receptor signaling in tumor stroma enhances antitumor effect of chemotherapy. *Cancer Res.* **62,** 5476–5484.

Pietras, K., Sjöblom, T., Rubin, K., Heldin, C.-H., and Östman, A. (2003a). PDGF receptors as cancer drug targets. *Cancer Cell* **3,** 439–443.

Pietras, K., Stumm, M., Hubert, M., Buchdunger, E., Rubin, K., Heldin, C.-H., McSheehy, P., Wartmann, M., and Östman, A. (2003b). STI571 enhances the therapeutic index of epothilone B by a tumor-selective increase of drug uptake. *Clin. Cancer Res.* **9,** 3779–3787.

Reardon, D. A., Egorin, M. J., Quinn, J. A., Rich, J. N., Sr., Gururangan, I., Vredenburgh, J. J., Desjardins, A., Sathornsumetee, S., Provenzale, J. M., Herndon, J. E., II, Dowell, J. M., Badruddoja, M. A., *et al.* (2005). Phase II study of imatinib mesylate plus hydroxyurea in adults with recurrent glioblastoma multiforme. *J. Clin. Oncol.* **23,** 9359–9368.

Risau, W., Drexler, H., Mironov, V., Smits, A., Siegbahn, A., Funa, K., and Heldin, C.-H. (1992). Platelet-derived growth factor is angiogenic *in vivo*. *Growth Factors* **7,** 261–266.

Roberts, W. G., Whalen, P. M., Soderstrom, E., Moraski, G., Lyssikatos, J. P., Wang, H. F., Cooper, B., Baker, D. A., Savage, D., Dalvie, D., Atherton, J. A., Ralston, S., *et al.* (2005). Antiangiogenic and antitumor activity of a selective PDGFR tyrosine kinase inhibitor, CP-673,451. *Cancer Res.* **65,** 957–966.

Robson, M. C., Phillips, L. G., Thomason, A., Robson, L. E., and Pierce, G. F. (1992). Platelet-derived growth factor BB for the treatment of chronic pressure ulcers. *Lancet* **339,** 23–25.

Rodt, S. A., Ahlén, K., Berg, A., Rubin, K., and Reed, R. K. (1996). A novel physiological function for platelet-derived growth factor-BB in rat dermis. *J. Physiol.* **495**(Pt. 1), 193–200.

Rolny, C., Nilsson, I., Magnusson, P., Armulik, A., Jakobsson, L., Wentzel, P., Lindblom, P., Norlin, J., Betsholtz, C., Heuchel, R., Welsh, M., and Claesson-Welsh, L. (2006). Platelet-derived growth factor receptor-β promotes early endothelial cell differentiation. *Blood* **108,** 1877–1886.

Score, J., Curtis, C., Waghorn, K., Stalder, M., Jotterand, M., Grand, F. H., and Cross, N. C. (2006). Identification of a novel imatinib responsive KIF5B-PDGFRA fusion gene following screening for PDGFRA overexpression in patients with hypereosinophilia. *Leukemia* **20,** 827–832.

Shao, Z. M., Nguyen, M., and Barsky, S. H. (2000). Human breast carcinoma desmoplasia is PDGF initiated. *Oncogene* **19,** 4337–4345.

Shimizu, A., O'Brien, K. P., Sjöblom, T., Pietras, K., Buchdunger, E., Collins, V. P., Heldin, C.-H., Dumanski, J. P., and Östman, A. (1999). The dermatofibrosarcoma protuberans-associated collagen type Iα1/platelet-derived growth factor (PDGF) B-chain fusion gene generates a transforming protein that is processed to functional PDGF-BB. *Cancer Res.* **59,** 3719–3723.

Sihto, H., Sarlomo-Rikala, M., Tynninen, O., Tanner, M., Andersson, L. C., Franssila, K., Nupponen, N. N., and Joensuu, H. (2004). KIT and platelet-derived growth factor receptor α tyrosine kinase gene mutations and KIT amplifications in human solid tumors. *J. Clin. Oncol.* **23,** 49–57.

Simon, M. P., Pedeutour, F., Sirvent, N., Grosgeorge, J., Minoletti, F., Coindre, J. M., Terrier-Lacombe, M. J., Mandahl, N., Craver, R. D., Blin, N., Sozzi, G., Turc-Carel, C., *et al.* (1997). Deregulation of the platelet-derived growth factor B-chain gene via fusion with collagen gene COL1A1 in dermatofibrosarcoma protuberans and giant-cell fibroblastoma. *Nat. Genet.* **15,** 95–98.

Sjöblom, T., Boureux, A., Rönnstrand, L., Heldin, C.-H., Ghysdael, J., and Östman, A. (1999). Characterization of the chronic myelomonocytic leukemia associated TEL-PDGF β R fusion protein. *Oncogene* **18,** 7055–7062.

Sjöblom, T., Shimizu, A., O'Brien, K. P., Pietras, K., Dal Cin, P., Buchdunger, E., Dumanski, J. P., Östman, A., and Heldin, C.-H. (2001). Growth inhibition of dermatofibrosarcoma protuberans tumors by the platelet-derived growth factor receptor antagonist STI571 through induction of apoptosis. *Cancer Res.* **61,** 5778–5783.

Skobe, M., and Fusenig, N. E. (1998). Tumorigenic conversion of immortal human keratinocytes through stromal cell activation. *Proc. Natl. Acad. Sci. USA* **95,** 1050–1055.

Song, S., Ewald, A. J., Stallcup, W., Werb, Z., and Bergers, G. (2005). PDGFRβ+ perivascular progenitor cells in tumours regulate pericyte differentiation and vascular survival. *Nat. Cell Biol.* **7,** 870–879.

Soriano, P. (1994). Abnormal kidney development and hematological disorders in PDGF β-receptor mutant mice. *Genes Dev.* **8,** 1888–1896.

Soriano, P. (1997). The PDGF α receptor is required for neural crest cell development and for normal patterning of the somites. *Development* **124,** 2691–2700.

Sundaresan, M., Yu, Z. X., Ferrans, V. J., Irani, K., and Finkel, T. (1995). Requirement for generation of H2O2 for platelet-derived growth factor signal transduction. *Science* **270,** 296–299.

Sundberg, C., Ljungström, M., Lindmark, G., Gerdin, B., and Rubin, K. (1993). Microvascular pericytes express platelet-derived growth factor-β receptors in human healing wounds and colorectal adenocarcinoma. *Am. J. Pathol.* **143,** 1377–1388.

Takahashi, Y., Morales, F. C., Kreimann, E. L., and Georgescu, M. M. (2006). PTEN tumor suppressor associates with NHERF proteins to attenuate PDGF receptor signaling. *EMBO J.* **25,** 910–920.

Tomasson, M. H., Williams, I. R., Hasserjian, R., Udomsakdi, C., McGrath, S. M., Schwaller, J., Druker, B., and Gilliland, D. G. (1999). TEL/PDGFβR induces hematologic malignancies in mice that respond to a specific tyrosine kinase inhibitor. *Blood* **93,** 1707–1714.

Tomasson, M. H., Williams, I. R., Li, S., Kutok, J., Cain, D., Gillessen, S., Dranoff, G., Van Etten, R. A., and Gilliland, D. G. (2001). Induction of myeloproliferative disease in mice by tyrosine kinase fusion oncogenes does not require granulocyte-macrophage colony-stimulating factor or interleukin-3. *Blood* **97,** 1435–1441.

Trempat, P., Villalva, C., Laurent, G., Armstrong, F., Delsol, G., Dastugue, N., and Brousset, P. (2003). Chronic myeloproliferative disorders with rearrangement of the platelet-derived growth factor α receptor: A new clinical target for STI571/Glivec. *Oncogene* **22,** 5702–5706.

Uhrbom, L., Hesselager, G., Östman, A., Nistér, M., and Westermark, B. (2000). Dependence of autocrine growth factor stimulation in platelet-derived growth factor-B-induced mouse brain tumor cells. *Int. J. Cancer* **85,** 398–406.

Ustach, C. V., and Kim, H. R. (2005). Platelet-derived growth factor D is activated by urokinase plasminogen activator in prostate carcinoma cells. *Mol. Cell. Biol.* **25,** 6279–6288.

Ustach, C. V., Taube, M. E., Hurst, N. J., Jr., Bhagat, S., Bonfil, R. D., Cher, M. L., Schuger, L., and Kim, H. R. (2004). A potential oncogenic activity of platelet-derived growth factor d in prostate cancer progression. *Cancer Res.* **64,** 1722–1729.

Uutela, M., Wirzenius, M., Paavonen, K., Rajantie, I., He, Y., Karpanen, T., Lohela, M., Wiig, H., Salven, P., Pajusola, K., Eriksson, U., and Alitalo, K. (2004). PDGF-D induces macrophage recruitment, increased interstitial pressure, and blood vessel maturation during angiogenesis. *Blood* **104,** 3198–3204.

Vassbotn, F. S., Östman, A., Langeland, N., Holmsen, H., Westermark, B., Heldin, C.-H., and Nistér, M. (1994). Activated platelet-derived growth factor autocrine pathway drives the transformed phenotype of a human glioblastoma cell line. *J. Cell. Physiol.* **158,** 381–389.

Walz, C., Curtis, C., Schnittger, S., Schultheis, B., Metzgeroth, G., Schoch, C., Lengfelder, E., Erben, P., Muller, M. C., Haferlach, T., *et al.* (2006). Transient response to imatinib in a chronic eosinophilic leukemia associated with ins(9;4)(q33;q12q25) and a CDK5RAP2-PDGFRA fusion gene. *Genes Chromosomes Cancer* **45,** 950–956.

Wen, P. Y., Yung, W. K., Lamborn, K. R., Dahia, P. L., Wang, Y., Peng, B., Abrey, L. E., Raizer, J., Cloughesy, T. F., Fink, K., Gilbert, M., Chang, S., *et al.* (2006). Phase I/II study of imatinib mesylate for recurrent malignant gliomas: North American brain tumor consortium study 99–08. *Clin. Cancer Res.* **12,** 4899–4907.

Xian, X., Håkansson, J., Ståhlberg, A., Lindblom, P., Betsholtz, C., Gerhardt, H., and Semb, H. (2006). Pericytes limit tumor cell metastasis. *J. Clin. Invest.* **116,** 642–651.

Xu, L., Tong, R., Cochran, D. M., and Jain, R. K. (2005). Blocking platelet-derived growth factor-D/platelet-derived growth factor receptor beta signaling inhibits human renal cell carcinoma progression in an orthotopic mouse model. *Cancer Res.* **65,** 5711–5719.

Yi, B., Williams, P. J., Niewolna, M., Wang, Y., and Yoneda, T. (2002). Tumor-derived platelet-derived growth factor-BB plays a critical role in osteosclerotic bone metastasis in an animal model of human breast cancer. *Cancer Res.* **62,** 917–923.

Zwerner, J. P., and May, W. A. (2001). PDGF-C is an EWS/FLI induced transforming growth factor in Ewing family tumors. *Oncogene* **20,** 626–633.

Zwerner, J. P., and May, W. A. (2002). Dominant negative PDGF-C inhibits growth of Ewing family tumor cell lines. *Oncogene* **21,** 3847–3854.