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Review

Platelet-derived Growth Factor Signaling and Human Cancer

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Platelet-derived growth factor (PDGF) is a critical regulator of mesenchymal cell migration and proliferation. The vital functions of PDGFs for angiogenesis, as well as development of kidney, brain, cardiovascular system and pulmonary alveoli during embryogenesis, have been well demonstrated by gene knock-out approaches. Clinical studies reveal that aberrant expression of PDGF and its receptor is often associated with a variety of disorders including atherosclerosis, fibroproliferative diseases of lungs, kidneys and joints, and neoplasia. PDGF contributes to cancer development and progression by both autocrine and paracrine signaling mechanisms. In this review article, important features of the PDGF isoforms and their cell surface receptor subunits are discussed, with regards to signal transduction, PDGF-isoform specific cellular responses, and involvement in angiogensis, and tumor-stromal interactions.

Keywords: Platelet-derived growth factor, PDGF recepter (PDGF)

Introduction

Platelet-derived growth factor (PDGF) was described approximately 30 years ago as a major mitogenic component of whole blood, but was absent in cell-free serum. PDGF was purified from the alpha-granules of the platelets, and was thought to be produced mainly by megakaryocytes, the precursor cells of platelets (Kohler and Lipton, 1974; Ross *et al*., 1974; Westermark and Wasteson, 1976). However, subsequent studies revealed that PDGF is also produced by the epithelium, endothelium, and many other cell types which are in close apposition to mesenchymal cells, which express PDGF receptors. These expression patterns suggest PDGF-mediated paracrine signaling (Ataliotis and Mercola, 1997; DiCorleto and Bowen-Pope, 1983; Bronzert *et al*., 1990).

PDGF is a potent mitogen for mesenchymal cells including fibroblasts, smooth muscle cells, and glial cells (Ross *et al*., 1986; Deuel, 1987; Heldin, 1992). PDGF also induces other diverse and important cellular processes including chemotaxis, survival, apoptosis (programmed cell death) and transformation *in vitro* (Deuel *et al*., 1982; Deuel *et al*., 1983; Doolittle *et al*., 1983; Senior *et al*., 1983; Waterfield *et al*., 1983; Williams *et al*., 1983; Huang *et al*., 1984; Bejcek *et al*., 1989; Barres *et al*., 1992; Kim *et al*., 1995). These activities correlate with the proposed functional roles of PDGF *in vivo* during embryonic development, inflammation, and wound healing (Deuel and Huang, 1984; Tzeng *et al*., 1985; Deuel, 1987; Pierce *et al*., 1988; Raff *et al*., 1988; Pierce *et al*., 1989; Yeh *et al*., 1991). Additionally, a causative role in tumorigenesis was suggested when v-sis (the oncogene of simian sarcoma virus) was found to be 92% homologous to PDGF-B. (Deuel *et al*., 1983; Doolittle *et al*., 1983; Waterfield *et al*., 1983; Deuel, 1987). Clinical studies revealed that aberrant expression of PDGF and its receptor was associated with a variety of disorders including atherosclerosis, fibroproliferative diseases of lungs, kidneys and joints, and neoplasia (Deuel *et al*., 1983; Doolittle *et al*., 1983; Waterfield *et al*., 1983; Huang *et al*., 1984; Ross *et al*., 1986; Deuel, 1987; Raff *et al*., 1988; Bejcek *et al*., 1989; Heldin, 1992; Kim *et al*., 1994), suggesting a role for PDGF in the development and progression of certain diseases. In this article, we review important features of the PDGF isoforms and their cell surface receptor subunits with regards to signal transduction, PDGF-isoform specific cellular responses, and involvement in human cancer development and progression.

Platelet-derived Growth Factor Isoforms

Biosynthesis and processing of PDGF isoforms PDGF belongs to the PDGF/VEGF (vascular endothelial growth factor) family, which is characterized by eight strictly conserved cysteine residues with similar spacing in between

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(Joukov *et al.*, 1997; Heldin and Westermark, 1999). The A-, B-, C-, and D-chain genes of PDGF are localized to the chromosomes 7p22, 22q13, 4q31, and 11q22, respectively, and their expression is independently regulated (Tzeng *et al.*, 1985; Deuel, 1987; Raff *et al.*, 1988; Yeh *et al.*, 1991; Heldin, 1992; Betsholtz *et al.*, 2001; LaRochelle *et al.*, 2001; Uutela *et al.*, 2001; Dijkmans *et al.*, 2002). Two forms of the PDGF A-chain, containing 196 and 211 amino acid residues resulting from differential splicing of the transcript, are synthesized, dimerized, proteolytically processed in the N-terminus, and secreted from the cell as a ~30 kDa dimer (Bonthron *et al.*, 1988; Rorsman *et al.*, 1988). The PDGF B-chain encoding 241 amino acid residues is dimerized, processed by additional proteolysis, and secreted as a 24 kDa dimer (Ostman *et al.*, 1988; Ostman *et al.*, 1992). The homodimers PDGF AA, BB, and the heterodimer AB contain three intrachain disulfide bonds made between the 1st & 6th, 3rd & 7th, and 5th & 8th cysteine residues from the N-terminus as diagrammed in Fig. 1b. The 2nd and 4th cysteine residues are asymmetrically connected by two interchain disulfide bridges (Heldin *et al.*, 1993).

Recently, the PDGF C- and D-chains were discovered in a BLAST search of the expressed-sequence tag (EST) databases at the National Center for Biotechnology Information (Bergsten *et al.*, 2001; LaRochelle *et al.*, 2001; Li *et al.*, 2000). Independently, PDGF C has also been identified as fallotein (Tsai *et al.*, 2000) and spinal-cord-derived growth factor (SCDGF) (Hamada *et al.*, 2000). The full-length PDGF C- and D proteins contain 345 and 370 amino acid residues respectively, and both have a unique two-domain structure with a N-terminal CUB domain and a C-terminal PDGF/VEGF domain. The CUB domain, composed of approximately 110 amino acids, shares sequence homology with the CUB domains of the complement subcomponents C1r/C1s as well as bone morphogenetic protein 1 (BMP-1) (Bork, 1991). Proforms of PDGF C and D are secreted as an approximately 85 kDa homodimer after cleavage of the N-terminal 22 signal peptide residues. Currently, it is unknown whether PDGF C- and D-chains form heterodimers between each other or with other PDGF chains. Whereas secreted PDGF AA, BB, and AB can readily activate their cell surface receptors, proteolytic removal of the CUB domain is required for the growth factor domain of PDGF CC and DD to activate the cell surface receptors α-PDGFR and β-PDGFR. Interestingly, although phylogenetic analysis of the growth factor domains of PDGF C and D revealed more similarity to VEGFs than to PDGFs, the growth factor domains of PDGF CC and DD do not activate the VEGF receptors.

While the growth factor domain of PDGF C contains all 8 cysteine residues strictly conserved among VEGF/PDGF family members, the PDGF D growth factor domain lacks the fifth conserved cysteine residue in this motif. In addition to the 8 conserved cysteine residues, the growth factor domain of PDGF C has four additional cysteine residues located between invariant cysteines 3 and 4, 5 and 6, 6 and 7 and an additional cysteine after the invariant cysteine 8. The growth factor domain of PDGF D also bears these additional cysteine residues found in PDGF C, except for the cysteine between invariant 5 and 6, as shown in Fig. 1a. Previous studies showed that the disulfide bonds between invariant cysteine residues 1 & 6, and 3 & 7, are critical for the biological activity of PDGF BB, whereas the disulfide bridge between invariant cysteine residues 5 & 8 is not (Giese *et al.*, 1987; Sauer and Donoghue, 1988; Ostman *et al.*, 1992). Thus, missing the 5th invariant cysteine residue in the PDGF D growth factor domain may not affect its biological activity as a PDGF ligand, although it may alter protein stability and/or its binding affinity due to the changes in three-dimensional structure. Interestingly, it was reported that PDGF DD growth factor domain is a ~3 fold less efficient competitor than PDGF BB in a ligand binding assay (Bergsten *et al.*, 2001). It should be mentioned that at present it is unknown which cysteine residues in the PDGF C and D peptides are utilized for disulfide bonds to form dimers. Detailed structural analysis of PDGF C and D dimers will help us understand common and unique features of these isoforms compared to the previously well characterized PDGF AA and BB.

Functions of PDGFs PDGF is expressed throughout an animals embryo development and adult life. The vital functions of PDGFs during embryonic development have been well demonstrated by gene knock-out approaches {reviewed in (Betsholtz *et al.*, 2001)}. PDGF A-deficient mice die either before embryonic day 10 or undergo premature death after birth. Histological analysis of these mice showed loss of alveolar smooth muscle cells resulting in defective pulmonary alveoli development, mesenchymal defects in the skin, and reduced oligodendrocytes in the brain which lead to dysmyelination and motorial defects (Bostrom *et al.*, 1996; Fruttiger *et al.*, 1999). Deletion of the PDGF B gene is lethal during late gestation. Lethality of PDGF-B deficiency is caused by abnormal renal development resulting from the absence of mesangial cells, a major structural and functional component of kidney glomeruli. Lethality is also caused by failure of the pericyte to migrate to the new blood vessels which then leads to abnormal blood vessel formation and defective cardiovascular system development (Leveen *et al.*, 1994; Soriano, 1994; Lindahl *et al.*, 1997).

Studies of PDGFs and PDGF receptors expression patterns in different tissues during embryogenesis as well as pathological processes have provided clues for differential roles of PDGFs in diverse cellular processes. In development, timing of expression can vary for each PDGF isoform in the same developing organ. For example, analysis of PDGF expression in the developing murine kidney reveal differential timing of expression between PDGF-A and PDGF-C. PDGF-C expression is highest in the early stages of nephron development, while PDGF-A is preferentially expressed in the later stages, even though α-PDGFR is expressed throughout development. (Li *et al.*, 2000) Additional to temporal and

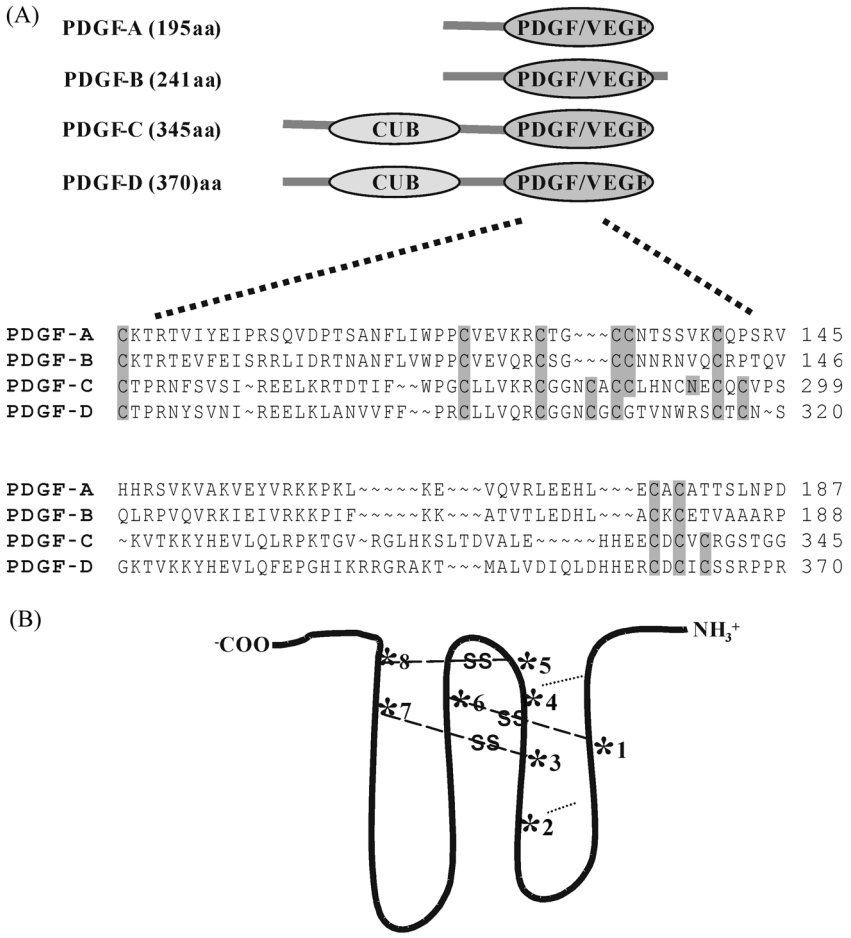

Fig. 1. Comparison of Growth Factor Domains among the PDGF isoforms. (A) Cartoon representation of the domains of each PDGF isoform. Sequence of each PDGF/VEGF domain starts at the first cysteine of the motif. Cysteines shaded in dark gray are the 8 invariant cysteines common to all members of the PDGF/VEGF family members. The 5th invariant cysteine residue has been replaced by glycine in PDGF-D. Residues shaded light gray are additional cysteines conserved among isoforms PDGF-C and PDGF-D. Numbers are in context of the full-length protein of the isoforms. (B) Cartoon of the 3-loop structure of the PDGF-B monomer, illustrating the intrachain disulfide bonds formed between the invariant cysteines 1 and 6, 3 and 7 and 5 and 8. Cysteines 2 and 4 (shaded gray) form interchain disulfide bonds to create the PDGF dimer.

spatial differences in expression, *in vitro* studies indicate that different PDGF isoforms may induce intracellular signal transduction pathways differently in cells with the same genetic background. For example, although PDGF AA and BB are equally potent mitogens, only PDGF BB has transforming ability in murine fibroblasts in vitro (Beckmann *et al.*, 1988; Bejcek *et al.*, 1989; Kim *et al.*, 1994). An important question to be addressed is whether PDGF BB induces unique positive signals for transformation, or if PDGF AA and BB differentially regulate negative signaling critical for "fine tuning" of PDGF-mediated cellular processes (This issue is further discussed in the "PDGF receptors and signal transduction" section below).

PDGF, especially AA and BB, exert direct mitogenic and/or chemoattractive activities on mesenchymal cells, such as fibroblast, vascular smooth muscle cells, glial cells, macrophages and chondroncytes, which express high affinity receptors (Ross *et al.*, 1986; Deuel, 1987; Heldin, 1992). In addition, PDGF amplifies its initial proliferative signals by increasing the expression levels of PDGF itself and other mitogens (Clemmons *et al.*, 1981; Clemmons and Van Wyk, 1985; Paulsson *et al.*, 1987). PDGF also regulates the production of collagen (Canalis, 1981; Narayanan and Page, 1983), fibronectin (Blatti *et al.*, 1988), proteoglycan (Schonherr *et al.*, 1991), hyaluronic acid (Heldin *et al.*, 1989) and collagenase (Chua *et al.*, 1985). These results indicate a role for PDGF in active connective tissue remodeling through recruiting essential cellular components and regulating ECM turnover.

PDGF functions as a "competent" factor that regulates the cell cycle during the G_0/G_1 transition (Stiles *et al.*, 1979; Pledger *et al.*, 1981). For PDGF-stimulated cells to progress beyond late G_1 phase and transit the cell cycle and divide, progression factors such as insulin and insulin-like growth factor-1 are required (Stiles, 1983; Olashaw *et al.*, 1987).

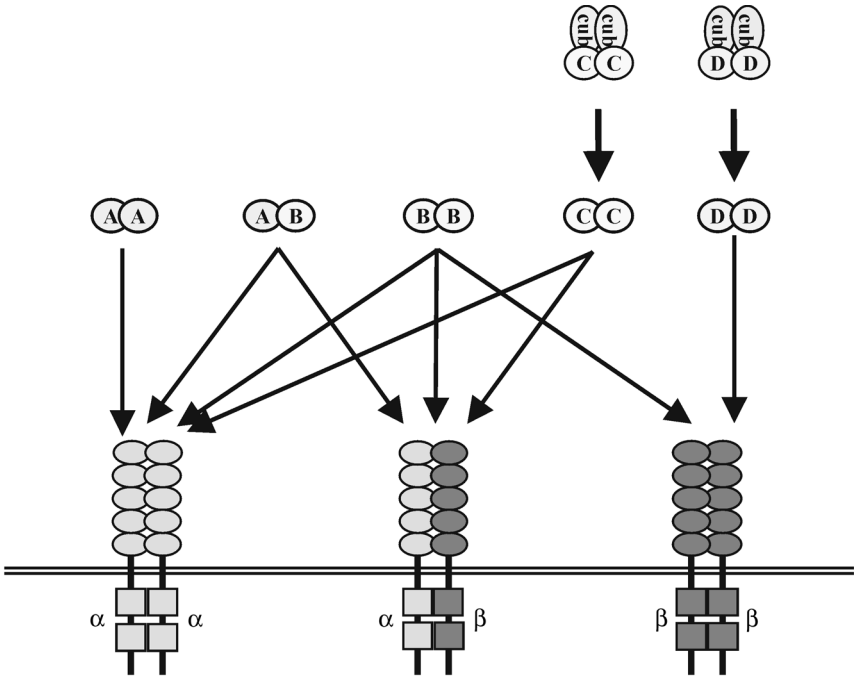

Fig. 2. PDGF dimer interactions with PDGF receptors. Each PDGF homodimer differs in their interactions with the PDGFRs. PDGF-AA activates αα-PDGFR, while PDGF-BB activates αα-, ββ-, or ββ-PDGFR. PDGF-CC activates either αα- or αβ-PDGFR, but PDGF-DD can activate only ββ-PDGFR. Only one heterodimer, PDGF-AB, has been identified to date. PDGF-AB activates either αα- or αβ-PDGFR

Seemingly paradoxical to PDGFs role in mitogenesis, high levels of PDGF expression have been observed in tissues within unfavorable environments for cell growth, raising the important question of whether the responses of cells to PDGF vary depending upon the genetic programs that can be activated by extracellular factors. In fact, we previously demonstrated that serum-deprived normal rat kidney fibroblast (NRK) cells undergo apoptotic cell death following chronic stimulation with either PDGF AA or PDGF BB. We also showed that PDGF-induced apoptosis is inhibited by cell cycle progression factors such as insulin. (Kim *et al.*, 1995). Consistent with our observation, other investigators have reported during the past decade that constitutive activation of protein-tyrosine kinase receptors, including PDGFR and epidermal growth factor receptor (EGFR), causes growth arrest and/or apoptosis in vitro (Armstrong *et al.*, 1994; Eastman, 1995; Unlu *et al.*, 1998; Kottke *et al.*, 1999). This evidence suggests that polypeptide growth factors *in vivo* may signal cell fate positively or negatively depending upon intracellular and extracellular signaling context, providing the molecular basis for the checkpoints of growth factor signaling.

Platelet-derived Growth Factor Receptors and Signal Transduction

PDGF receptors PDGF isoforms exert their cellular effects by activating two structurally related cell surface receptor tyrosine kinases (α-PDGFR and β-PDGFR). The α-PDGFR and β-PDGFR genes are localized on chromosomes 4q12 and 5q33, respectively. Both PDGFRs contain five extracellular immunoglobulin-like domains, a transmembrane domain, a juxtamembrane domain, splitted kinase domains, a kinase insert domain, and a cytoplasmic tail. These two receptors share 31% identity in the ligand binding domain, 27% identity in the kinase insert and 28% identity in the C-terminus, whereas they are 85% and 75% identical in the two halves of the kinase insert domain (Matsui *et al.*, 1989; Rosenkranz and Kazlauskas, 1999). The three dimeric PDGF receptors (αα, αβ, ββ) mediate PDGF isoform-specific signal transduction. PDGF AA effectively activates only αα-PDGFR, PDGF AB can activate either αα-PDGFR or αβ-PDGFR, while PDGF BB activates all three dimeric PDGF receptors (Claesson-Welsh *et al.*, 1988; Matsui *et al.*, 1989; Claesson-Welsh, 1994). The growth factor domain of PDGF CC activates both the αα-PDGFR and αβ-PDGFR, and the growth factor domain of PDGF DD activates only the ββ-PDGFR (Li *et al.*, 2000; Bergsten *et al.*, 2001; Gilbertson *et al.*, 2001; LaRochelle *et al.*, 2001). At present, it is unclear whether the PDGF DD growth factor domain can activate αβ-PDGFR.

Similar to the PDGF ligands, the critical functions of the PDGF receptors during embryonic development have been well demonstrated by gene targeting approaches {reviewed in (Betsholtz *et al.*, 2001)}. Compared to PDGF A- and PDGF B-deficient mice, the phenotype of α-PDGFR deficiency revealed unique features including cleft face, spina bifida, and

skeletal defects, resulting in embryonic death between E8 and E16 (Soriano, 1997). The unique phenotypes of the α-PDGFR knockout mouse suggest that PDGF C may play a unique role during embryonic development through α-PDGFR activation. Histological analysis of PDGF B- and β-PDGFR-deficient mice performed so far revealed identical phenotypes, suggesting that PDGF D-mediated β-PDGFR signaling may not play a major role during embryonic development. A complete understanding of α- and β-PDGFRs functions awaits thorough analysis of PDGF C- and D-mediated signal transduction and their cellular effects during embryogenesis as well as both physiological and pathological processes in adults.

PDGFR-mediated intracellular signal transduction

Differential interactions of PDGF isoforms with PDGF receptor subunits establish one basis for the diverse PDGF isoform-specific functions. Dimerization and autophosphorylation of PDGFR occur upon receptor-ligand interaction. Phosphorylated tyrosine residues, in the context of specific amino acid residues, interact with Src homology 2 domains (SH2) of intracellular signaling molecules. These include phospholipase γ (PLC-γ), Ras GTPase-activating protein (RAS-GAP), p85 subunit of phosphatidylinositol 3-kinase (PI-3K), growth-factor receptor-bound protein 2 (Grb 2), Syp (tyrosine-specific phosphatase), Src homology and collagen protein (Shc), Crk (a group of adaptor proteins) and Src, a family of non-receptor tyrosine kinases (Claesson-Welsh, 1994). These signaling molecules further transduce signal transduction pathways by activating downstream signaling molecules such as mitogen activated protein kinase family members (ERKs, JNKs), and focal adhesion kinase (FAK, a mediator of integrin signaling pathway) among others. These signals enter the nucleus and stimulate expression of a set of immediate-early-response genes that mediate PDGF-induced cellular processes including cell cycle, cell migration, and transformation.

The similarities and differences in the functions of the two PDGFR subunits result from the specific interactions between the flanking amino acid residues of the phosphotyrosine in the PDGF receptor subunit and the SH2 domain of intracellular signaling molecules (Heidaran *et al.*, 1993; Kazlauskas *et al.*, 1993; Lechleider *et al.*, 1993; Bazenet and Kazlauskas, 1994; Bazenet *et al.*, 1996; DeMali *et al.*, 1997; Yokote *et al.*, 1998; Rosenkranz and Kazlauskas, 1999). Src, PI-3K, and PLC-γ bind both receptors with Src at the juxtamembrane domain, PI-3K at the kinase insert, and PLC-γ at the cytoplasmic tail. Only β-PDGFR, but not α-PDGFR, effectively interacts with Ras-GAP, a negative regulator of Ras. Conversely, Crks possess very high binding affinities for α-PDGFR, but not for β-PDGFR. Interestingly, SHP-2 binds to the carboxyl-terminal of α-PDGFR instead of the kinase insert domain of β-PDGFR.

Although interaction of the above mentioned signaling molecules with PDGF receptors were shown to further activate downstream signaling pathways, little is known about which signaling pathways are α- or β-PDGFR-specific, and how these signal transduction pathways are agonized or antagonized to regulate specific cellular processes. To address this issue, we previously established NIH3T3 clones in which α-PDGFR signaling is inhibited by a dominant-negative α-PDGFR, or an anti-sense construct of α-PDGFR (Yu *et al.*, 2000). We showed that inhibition of α-PDGFR signaling enhanced PDGF BB-mediated phenotypic transformation, suggesting that α-PDGFR antagonizes β-PDGFR-induced transformation. While both α- and β-receptors effectively activate ERKs, α-PDGFR, but not β-PDGFR, activates stress-activated protein kinase-1/c-Jun NH_2-terminal kinase-1 (SAPK1/JNK-1). Inhibition of JNK-1 activity using a dominant-negative JNK-1 mutant markedly enhanced PDGF BB-mediated anchorage-independent cell growth, demonstrating an antagonistic role for JNK-1 in PDGF-induced transformation. These results revealed a striking feature of PDGF signaling: the specificity and the strength of the PDGF-growth signal is modulated by α-PDGFR-mediated simultaneous activation of growth stimulatory and inhibitory signals. As depicted in Fig. 3, activation of α-PDGFR may transduce both positive and negative signaling for cell transformation, while β-PDGFR mainly induces positive signaling for cell transformation. PDGF BB activation of both receptors shifts the balance of signaling to favor the transformation pathway, while PDGF AA activation of α-

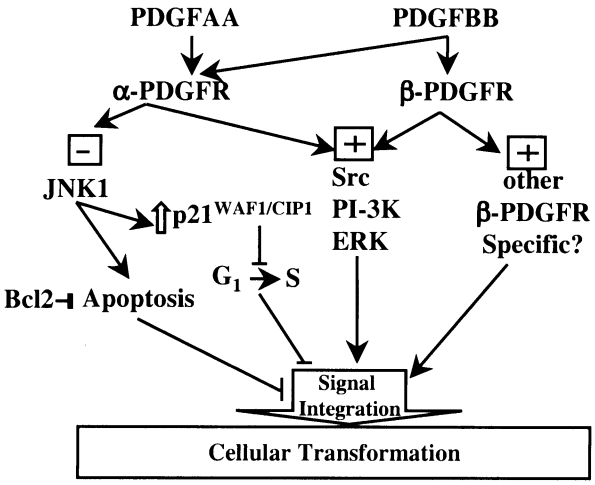

Fig. 3. A Model for PDGF Regulation of Cellular Transformation. The scheme depicts PDGFR-activated signaling molecules (such as JNK, ERK, PI-3K and Src) critical for transformation regulation. PDGF AA or BB activation of α-PDGFR induces both pro- and anti-transformation pathways, while PDGF BB activation of β-PDGFR promotes pro-transformation pathways. α-PDGFR-mediated anti-transformation pathway includes JNK-1 activation which leads to induction of $p21^{WAF1/CIP1}$ and/or apoptosis. When the pro-apoptotic pathway is downregulated by anti-apoptotic gene products such as Bcl-2, α-PDGFR promotes cellular transformation.

PDGFR alone does not. Consistent with this model, we previously demonstrated that α-PDGFR activation can result in phenotypic transformation when α-PDGFR-mediated negative signaling is inhibited by bcl-2 (Kim *et al.*, 1994). Our on-going studies further show that the α-PDGFR/JNK-1 pathway is critical for PDGF-regulation of apoptosis as well as PDGF-induced expression of $p21^{WAF1/CIP1}$, an inhibitor of cyclin-dependent kinases critical for G_1/S checkpoint (Yu and Kim, manuscript in preparation). This suggests that the loss of α-PDGFR-induced negative signaling (such as JNK-1) may be as critical as gain of positive oncogenic signaling (such as ERKs) for tumor development and/or progression.

α-PDGFR-mediated simultaneous activation of both positive and negative signaling has also been demonstrated in cell migration. In smooth muscle cells, PDGF BB induces cell migration more effectively than PDGF AA. Inhibition of α-PDGFR using a neutralizing monoclonal antibody to α-PDGFR enhanced β-PDGFR-induced smooth muscle cell migration (Koyama *et al.*, 1994). Additionally, it was shown that α-PDGFR has an intrinsic ability to mediate both positive and negative chemotactic signaling (Yokote *et al.*, 1996). In fibroblast cells, however, we previously showed that both α- and β-PDGFRs promote cell migration with equal effectiveness, and their effects are additive (Yu *et al.*, 2001).

Taken together, cell-type and PDGFR subunit specific-intracellular signal transduction pathways are responsible for PDGF isoform-specific regulation of diverse cellular processes. Of particular importance, α-PDGFR-mediated agonistic and antagonistic activities for cell growth and motility provide a molecular basis for "fine-tuning" of PDGF signaling depending on genetic background of the cells and additional extracellular factors. At present, little is known about signal transduction pathways mediated by PDGF C- and PDGF D-activated PDGFRs. Differences in the affinity and duration of PDGF isoform-binding to the PDGFR subunit may result in subtle, but critical, differences in signal transduction pathways. The unique structure of PDGF C and D, requiring proteolytic cleavage of the CUB domain to allow growth factor domains to activate PDGFRs, implements another level of regulation in PDGF signaling. Further analysis of overlapping and distinct signal transduction pathways mediated by α-PDGFR following interactions with PDGF A or PDGF C, as well as by β-PDGFR upon binding of PDGF B or PDGF D will lead us to better understanding of PDGF functions *in vivo*.

PDGF and Human Cancer

Relevance of PDGF signaling to human tumor progression Studies during the past two decades clearly indicate the significance of PDGF in human tumors including glioma (Hermanson *et al.*, 1992; Plate *et al.*, 1992; Westermark *et al.*, 1995), dermatofibrosarcoma (Shimizu *et al.*, 1999; Greco *et al.*, 2001), neurofibroma (Kadono *et al.*, 2000), myelomonocytic leukemia (Golub *et al.*, 1994), osteoblastoma (Sulzbacher *et al.*, 2000), and osteosarcoma (Allam *et al.*, 1992; Liang *et al.*, 1996). *In vitro*, overexpression of the v-sis oncogene product (p28v-sis) or PDGF B in cells that express their receptors enhances transformation, indicating an autocrine mechanism in tumorigenesis (Beckmann *et al.*, 1988; Uhrbom *et al.*, 2000). In accord with the strong transforming ability of β-PDGFR, most malignant mesothelioma cell lines express β-PDGFR, whereas normal mesothelial cells predominantly express α-PDGFR (Langerak *et al.*, 1996). Unlike β-PDGFR, in vitro studies showed that α-PDGFR transduces both positive and negative signals for cell transformation, and that high α-PDGFR or PDGF A expression exerts none or weak transforming ability in normal or pre-malignant cells. However, in clinical studies, α-PDGFR as well as PDGF-A expression is more prominent in high-grade tumors such as glioma. (Mapstone, 1991; Hermanson *et al.*, 1996; Rosenkranz and Kazlauskas, 1999; Smith *et al.*, 2000; Yu *et al.*, 2000). Taking *in vitro* and clinical studies together, we speculate that the perturbations of α-PDGFR-mediated negative signaling may occur and accumulate from the early stages of tumor progression in some tumors (analogous to tumor promoters), and at later stages, α-PDGFR signaling mainly promotes tumor cell growth and disease progression.

In addition to the autocrine mechanism, recent studies revealed a critical role for paracrine PDGF signaling in carcinogenesis through the regulation of epithelial-stromal interactions. Using nude mice, it was demonstrated that PDGF activation of stromal cells results in tumorigenic conversion of immortal human keratinocytes (Skobe and Fusenig, 1998). Mounting evidence suggests that aberrant interaction between stroma and epithelium is critical for the neoplastic progression of breast epithelium (Zoltowska, 1997; Moinfar *et al.*, 2000). Genetic alterations occur both in epithelial and mesenchymal cells of mammary carcinoma (Moinfar *et al.*, 2000). Furthermore, evidence suggests that genetic alterations in the stromal cells may precede genetic changes in the epithelial cells (Moinfar *et al.*, 2000). Enhanced PDGF immunostaining was detected in soft tissue tumors and advanced breast tumors (Bronzert *et al.*, 1987; Palman *et al.*, 1992; Seymour and Bezwoda, 1994; Coltrera *et al.*, 1995). The significance of PDGF signaling for breast epithelial-stromal interaction was supported by in situ hybridization and immunohistochemical analysis. Expression of PDGF B-protein and mRNA was restricted to the breast epithelium and tumor cells, whereas membranous PDGF receptor immunostaining was detected in stromal cell populations in all of the breast tissues examined (Coltrera *et al.*, 1995). PDGF receptor staining was particularly localized in the periepithelial stroma of breast carcinoma, suggesting a paracrine stimulation of adjacent stromal tissue by breast tumor cells (Coltrera *et al.*, 1995). Furthermore, PDGF was shown to initiate the human breast carcinoma desmoplasia (Shao *et al.*, 2000; Walker, 2001). Similar to breast cancer, paracrine mechanisms were also

observed in colorectal cancer and small cell lung carcinoma (Kawai *et al.*, 1997; Sundberg *et al.*, 1997). The significance of PDGF signaling in prostate cancer is also well recognized, and a multi-institutional phase II study was successfully completed to test the efficacy of a PDGF receptor inhibitor for patients with hormone-refractory cancer (Ko *et al.*, 2001). Taken together, it is clear that PDGF signaling is critical for the development of many types of human cancers.

PDGFs' role in angiogenesis and tumor metastasis The fatal phenotypes of PDGF- or PDGFR-deficient mice include cardiovascular and hematological defects. PDGFs produced by endothelial cells in vessels promote recruitment and proliferation of vascular smooth muscle cells/pericyte progenitors expressing PDGFR (Betsholtz *et al.*, 2001). Chemotactic and mitogenic activities mediated by the PDGF/PDGFR paracrine signaling loop are crucial for the formation, branching and maintenance of blood vessels. As in embryogenesis, PDGF plays a critical role for angiogenesis in human tumors. Tumor angiogenesis, required for tumor outgrowth and metastasis, is a complex and highly regulated process involving many different cell types and extracellular factors. Endothelial cells and smooth muscle cells are the major components of blood vessels, and VEGF/PDGF super family members are among the critical mediators of tumor angiogenesis. Clinical studies revealed a correlation between vascular counts and expression frequency of VEGF and PDGF in tumors (Anan *et al.*, 1996). PDGFs directly and indirectly stimulate the angiogenic processes. PDGF released by the tumor cells induce migration of endothelial cells and vascular smooth muscle cells (vSMC), and also stimulate proliferation of these cells, suggesting a direct role of PDGFs in angiogenesis (Thommen *et al.*, 1997). PDGFs were shown to induce transcription and secretion of VEGF by β-PDGFR expressing endothelial cells, suggesting an indirect role for PDGF induced angiogenesis (Wang *et al.*, 1999). PDGFs also mediate the paracrine signaling loop between endothelial cells and vSMC/pericytes during tumor angiogenic processes. While PDGF-BB, -AB, and the growth factor domain of PDGF-CC induce indistinguishable angiogenic responses in mouse cornea assay, PDGF-AA stimulates only a weak response (Cao *et al.*, 2002). This suggests that α-PDGFR and β-PDGFR may differently regulate angiogenic processes. Interestingly, α-PDGFR, a pro-angiogenic component, was shown to inhibit basic fibroblast growth factor-mediated angiogenesis in the chick embryo chorioallantoic membrane assay and also in matrigel plugs subcutaneously injected in mice (De Marchis *et al.*, 2002). This re-emphasizes the ability of the α-PDGFR to simultaneously activate both agonistic and antagonistic signals for a specific cellular process.

The ability of tumor cells to colonize distant organs is determined by the inherent metastatic ability of cancer cells including inducibility of angiogenesis, invasiveness, motility, and increased cell survival. Importantly, it has been well recognized that certain types of cancer cells have preferred end target organs for metastasis. For example, breast cancers cells colonize preferentially to the lung, liver and bone, while the bone is the most common organ of prostate cancer cell metastasis (Yoneda, 1998; Cher, 2001). This suggests that interactions between cancer cells and the host environment of the end organ are also critical for directing cancer cells to the metastatic site, allowing them to survive and colonize the organ. Considering that PDGFs released by the tumor cells are among the potent chemoattractants and mitogens for host mesenchymal cells, it may be reasonable to speculate PDGFs roles for cancer metastasis at the preferential organ sites. This is an under-investigated, but potentially critical, area of PDGF research.

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