Acta Biochimica Polonica

Vol. 50 No. 3/2003

647–658

QUARTERLY

Review

Regulatory mechanisms for the expression and activity of platelet-derived growth factor receptor✪

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Received: 30 May, 2003; revised: 19 August, 2003; accepted: 04 September, 2003

Key words: PDGF, receptor, expression, tyrosine kinase, review

PDGF is one of the most potent serum mitogens, and the signalling mechanism by way of its receptor tyrosine-kinase has been extensively studied since its first purification in 1979. The identification of homology between the simian sarcoma virus oncogene, *v-sis*, and the B-chain of PDGF, as well as the frequent over-expression of both the ligands and receptors in various tumours and stroma led to the proposal of the

★Presenter at the XXX Winter School of Faculty of Biotechnology, Jagiellonian University, Kościelisko, Poland, 28th February–4th March, 2003.

✪This work was supported by grants from the Swedish Medical Research Council, the Swedish Cancer Foundation, the Swedish Children's Cancer Foundation, the Inga and Arne Lundberg Foundation, the Jubileum Foundation, and the Swedish Institute. H.U. is supported by the Wenner-Gren Society, the Wenner-Gren Foundations, the Uehara Memorial Life Science Foundation, and the Kanae Foundation for Life & Socio-Medical Science.

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Abbreviations: BMP, bone morphogenetic protein; bFGF, basic fibroblast growth factor; CNS, central nervous system; *COL1A1*, collagen 1 alpha 1 gene; ΔNp73, N-terminal deleted p73; GAP, GTPase-activating protein; GM-CSF1R, granulocyte-macrophage colony stimulating factor-1 receptor; *FIP1L1*, Fip1-like 1 gene; HAT, histone acetyltransferase; HFM, histone-fold motif; IL1β, interleukin-1β; LMW-PTP, low molecular weight phosphotyrosine phosphatase; MAPK, mitogen-activated protein kinase; M-CSF1R, macrophage colony stimulating factor-1 receptor; P/CAF, p300- and CBP-associated factor; PDGF, platelet-derived growth factor; *PDGFRA*, PDGF α-receptor gene; *PDGFRB*, PDGF β-receptor gene; PI3K, phosphatidylinositol 3-kinase; PLCγ, phospholipase Cγ; PNS, peripheral nervous system; PTP, phosphotyrosine phosphatases; SAM, sterile α-motif; SCF, stem cell factor; SH2, src homology domain; SHP-2, src homology protein tyrosine phosphatase-2; SMC, smooth muscle cell; SOS, son of sevenless, Ras-activated nucleotide exchange factor; TGF-β1, transforming growth factor-β1; TNF-α, tumour necrosis factor-α.

PDGF-mediated autocrine and paracrine hypothesis. Consistent with the important roles of PDGF in the growth and survival of cells, the expression and activity of PDGF receptors are tightly controlled by both positive and negative feedback mechanisms at different levels. The deregulation of the control system can result in serious pathological conditions such as chronic inflammation and tumours. Understanding the molecular mechanisms for the regulatory system and the signalling pathway of PDGF is essential in order to find effective therapies in the diseases where PDGF is involved.

PDGF LIGANDS AND THE RECEPTORS

Platelet-derived growth factor (PDGF) is one of the most potent serum mitogens as well as one of the most extensively studied growth factors. PDGF B-chain is a homologue of the simian sarcoma virus transforming protein, v-sis, possessing the activity to render cells competent to enter the G1-S cell cycle phases (Heldin & Westermark, 1999). PDGF exists as a dimer in various combinations, i.e. AA, BB, AB, CC and DD, binding two structurally related tyrosine kinase receptors, α and β, with different affinities (Bergsten *et al.*, 2001; Gilbertson *et al.*, 2001; Heldin *et al.*, 2002). The C- and D-chains are the recently isolated members that are proteolytically cleaved before binding the receptors. The α-receptor binds A-, B- and C-chains, whereas the β-receptor binds B- and D-chains. However, both CC and DD can bind and dimerise the α-β receptor. Binding of PDGF induces dimerisation and mutual phosphorylation of the two receptors, leading to activation of several intracellular molecules, propagating cascades of different signalling pathways (Heldin *et al.*, 1998).

PHYSIOLOGICAL AND PATHOLOGICAL EXPRESSION OF THE RECEPTORS

Although there are slight differences between the signalling molecules activated by the α- and β-receptors, major differences between the functions of the receptors derive from their expression patterns. Expression of the PDGF receptors is increased during development as well as in certain physiological and pathological conditions. The α-receptor plays important roles in the development of neural crest-derived cells and somites (Soriano, 1997; Tallquist & Soriano, 2003), and the β-receptor, of the mural cells of blood vessels (Hellstrom *et al.*, 1999; Lindahl *et al.*, 1997). Neurons and glial cells of the CNS and PNS can express PDGF and its receptors, which are considered to stimulate their proliferation, differentiation and survival (Eccleston *et al.*, 1993; Erlandsson *et al.*, 2001; Funa & Åhgren, 1997; Raff, 1989; Sasahara *et al.*, 1991; Smits *et al.*, 1993; Yeh *et al.*, 1991).

The PDGF β-receptor can be up-regulated in fibroblasts and blood vessels during wound healing and chronic inflammation (Reuterdahl *et al.*, 1991; 1993). Tumour stromal cells including vascular cells express the β-receptor in a paracrine fashion (Hermanson *et al.*, 1988; 1992; Smits *et al.*, 1992). This hypothesis was supported by various experimental tumour models, in which PDGF stimulated tumour growth through the formation of stroma expressing the receptor (Forsberg *et al.*, 1993; Skobe & Fusenig, 1998; Sundberg *et al.*, 1997). Recently, PDGF-specific tyrosine kinase inhibitors were shown to possess anti-angiogenic effects in animal tumour models (Bergers *et al.*, 2003; Uehara *et al.*, 2003). Because of the lack of specific antibodies, it had been thought that endothelial cells expressed the receptor. However, it was later found that mural cells (surrounding the endothelial cells) express the receptors. PDGF has an angiogenic activity (Li *et al.*, 2003; Risau *et al.*, 1992; Smits *et al.*, 1989), and is essential for the formation of normal blood vessels by recruiting pericytes and smooth muscle cells (Lindahl *et al.*, 1997). Consequently, a PDGF kinase inhibitor was shown to block growth of

end-stage tumours, inducing detachment of pericytes and disruption of tumour vascularity (Bergers *et al.*, 2003). Recently, ligand-activated PDGF β-receptor was shown to be involved in the mechanism of increased interstitial fluid pressure in stroma in an experimental tumour model (Pietras *et al.*, 2001; 2002). Hence, administration of a PDGF kinase inhibitor increased tumour drug uptake, and enhanced the effects of cytotoxic drugs (Pietras *et al.*, 2003).

Expression of both receptors is increased in atherosclerotic blood vessels, and the α-receptor was found to be up-regulated by mechanical stress of smooth muscle cells (Fukuoka *et al.*, 1999; Hu *et al.*, 1998; Waltenberger *et al.*, 1996). *In vitro*, it has been shown that PDGF β-receptor expression changes markedly depending on culture conditions, such as density of cells, attachment of cells to matrix, presence of serum, as well as on the differentiation status of cells (Barrett *et al.*, 1996; Terracio *et al.*, 1988; Vaziri & Faller, 1996). The receptor expression is up-regulated when cells grow in suspension and in gel, and also in cells cultured on fibronectin-coated surface. Later, fibronectin has been shown to up-regulate the transcription of *PDGFRB* (Tamura *et al.*, 1998). Furthermore, the expression was shown to be down-regulated when cells are stimulated by growth factors or oncogenes and viral transforming proteins such as Src, Myc, and SV40 large T antigen (Cook *et al.*, 1993; Izumi *et al.*, 2001; Oster *et al.*, 2000; Vaziri & Faller, 1995; Wang *et al.*, 1996; Zhang *et al.*, 1995).

Some tumours gain enhanced mitogenic activity and other growth advantages by alterations of genes in the PDGF-system. Dermatofibrosarcoma protuberans may have a chromosomal translocation placing exon 2 of *PDGF* B-chain under the control of the *COL1A1* promoter (Sirvent *et al.*, 2003). *KIT* and *PDGFRA* mutations appear to be mutually exclusive oncogenic mechanisms in gastrointestinal stromal tumours (Heinrich *et al.*, 2003). The hypereosinophilic syndrome may result from a novel fusion tyrosine kinase, FIP1L1-PDGFRA, that is a consequence of an interstitial deletion (Cools *et al.*, 2003). Expression of PDGF α-receptor is increased in malignant glioma and pulmonary artery intimal sarcoma, which can be a consequence of gene amplification of the receptor, establishing autocrine stimulation (Hui *et al.*, 2001; Zhao *et al.*, 2002). Furthermore, translocation between chromosomes 5 and 12, yielding TEL-PDGFRB fusion protein was shown to be associated with some chronic myelomonocytic leukaemias (Sjöblom *et al.*, 1999). The majority of tumours involving the PDGF-mediated stimulation, however, use over-expression without gene alteration. PDGF α-receptor is activated in medulloblastoma, malignant glioma, and ovarian tumours (Andrae *et al.*, 2002; Henriksen *et al.*, 1993; Hermanson *et al.*, 1992; MacDonald *et al.*, 2001). Consistent with these observations, recent therapeutic trials using PDGF-specific tyrosine kinase inhibitors have shown promising results (Östman & Heldin, 2001).

PDGF RECEPTOR GENES AND THEIR TRANSCRIPTION

The genomic structure and organization of *PDGF* receptor genes are similar. The *PDGFRA* and *PDGFRB* genes span approximately 69 kb and 43 kb, respectively, and contain 23 exons. The 5′-untranslated region of the mRNA is encoded by exon 1, and a large intron of 23 kb separates exon 2 encoding the translation initiator codon AUG and the signal sequence. These two PDGF receptors are also very similar as concerns the locations of exon/intron boundaries in the extracellular immunoglobulin-like domains, the transmembrane domain, the two cytoplasmic tyrosine kinase domains, and the kinase insertion domain. Their organisation also resembles that of the genes encoding stem cell factor (SCF) receptor, KIT, granulocyte-macrophage colony stimulating factor receptor (GM-CSF1R)

and macrophage colony stimulating factor receptor (M-CSF1R). Human *KIT* and *PDGFRA* are located within Ch 4q11-q13, and human *PDGFRB* and *CSF1R* exist in tandem in Ch 5q31-32 and 5q33-35, respectively (Kawagishi *et al.*, 1995); see <www.ncbi.nlm.nih.gov/LocusLink>.

Once the promoters of the receptors had been isolated, *in vitro* studies on the transcriptional regulation of the receptor expression became possible (Afink *et al.*, 1995; Ballagi *et al.*, 1995; Kawagishi *et al.*, 1995; Kitami *et al.*, 1998; Shinbrot *et al.*, 1997; Wang & Stiles, 1994). The transcription start site is approximately 400 bp upstream of the AUG translation initiation codon. The 5′-flanking region of the gene lacks a typical TATA box but contains a typical CCAAT box and GATA motifs. This region also contains potential sites for AP-1 and Sp1.

In this review, we focus on the *PDGFRB* promoter that we study in our laboratory. The *PDGFRB* promoter contains a CCAAT motif that binds the NF-Y transcription factor (Ballagi *et al.*, 1995; Ishisaki *et al.*, 1997). Sp1 binds the upstream GC-rich domain close to the NF-Y binding site and activates the transcription (Molander *et al.*, 2001). NF-Y specifically recognises the CCAAT motif found in various promoters and enhancer regions of many eukaryotic genes (Mantovani, 1998). NF-Y consists of subunits A, B, and C, and the B and C subunits dimerise through the conserved histone-fold motif (HFM) to interact with the subunit A (Kim *et al.*, 1996).

The transcription of *PDGFRB* becomes down-regulated after growth factor stimulation (Oster *et al.*, 2000; Vaziri & Faller, 1995). Growth factor stimulation of cells induces c-Myc expression, which upon binding to the partner protein Max, enters the nucleus. The Myc–Max complex binds to the consensus E-box sequence and stimulates the transcription of several genes necessary for DNA synthesis. Recently, c-Myc was shown to repress the *PDGFRB* transcription (Izumi *et al.*, 2001) through binding and inactivation of NF-Y. The N-terminal transactivation domain of Myc is indispensable for the binding and repression of the transcription. Since PDGF activates Src-kinase, which in turn activates Myc (Barone & Courtneidge, 1995), the Myc-induced receptor repression may be considered a negative feedback following PDGF activation. This mechanism might be involved in the previously reported down-regulation of the PDGF receptor following oncogene expression and changes in the growth conditions of cells.

In addition to c-Myc, the suppressor p73α directly represses the transcription of *PDGFRB* (Hackzell *et al.*, 2002). Although p73 can be induced by Myc (Zaika *et al.*, 2001), and they also bind each other (Uramoto *et al.*, 2002), p73α represses the receptor transcription in Myc-null cells by an independent mechanism. p73 belongs to the p53 tumour suppressor protein family and exists in several C-terminal splicing isoforms (Irwin & Kaelin, 2001). Some of the isoforms exist also as N-terminal deleted variants (ΔNp73) that lack the transactivation domain, acting as dominant-negative molecules through competing with the full-length isoforms for DNA-binding and oligomerisation. The mechanism of the p73α mediated repression was shown to be similar to that used by Myc. The physical interaction of the C-terminal SAM domain of p73α with NF-YB and -YC prevents the NF-Y-mediated transactivation of the *PDGFRB* promoter (Hackzell *et al.*, 2002). In contrast, ΔNp73α activates the *PDGFRB* promoter, which is likely to be one of the reasons for the constitutive elevated expression of PDGF β-receptor in certain neuroblastoma cell lines (Hackzell *et al.*, unpublished observation). However, the exact mechanism for how p73α represses NF-Y activity has not been completely elucidated. Our preliminary results suggest that p73α competes for NF-Y binding with co-activators such as p300 and P/CAF, both possessing histone acetyltransferase (HAT) activity (Uramoto *et al.*, unpublished observation). A similar mechanism

might be involved in the Myc-induced repression. SV40 large T antigen represses the receptor expression also at the transcriptional level, partly through the induction of Myc but also through binding to p53. In fact, p53 seems to be an important activator of the transcription of *PDGFRB* (Uramoto *et al.*, unpublished observation). This tight transcriptional control by several proteins that are essential for cell cycle control may underscore the importance of regulated growth factor stimulation for normal cell growth. Transcriptional regulation seems to be one of the most long-lasting mechanisms to control the activity of the PDGF-receptors.

OTHER POTENTIAL NEGATIVE REGULATORY MECHANISMS FOR THE PDGF β-RECEPTOR

Dimerisation of the receptors leads to autophosphorylation and activation of the receptor kinase. The phosphorylated tyrosine residues create binding sites for the SH2 domains of several intracellular signalling molecules. They are typically phosphatidylinositol 3-kinase (PI3K), the adaptor molecule Grb2 that forms a complex with Sos1, the nucleotide exchange molecule acting on Ras, phospholipase Cγ (PLCγ), the tyrosine kinase Src, the tyrosine phosphatase SHP2, and RasGAP, a GTPase-activating protein (Heldin *et al.*, 1998). These molecules directly or indirectly activate downstream kinases, leading to the activation of transcription factors to initiate target gene transcription. Alternatively, these kinases can directly or indirectly activate or inactivate target molecules involved in apoptosis, cell motility or other cellular functions.

The expression of PDGF receptors is tightly regulated not only at the transcriptional level but also at the protein level. When PDGF binds to the receptor, the dimerised receptors become internalised. These ligand-bound PDGF β-receptors remain activated and tyrosine phosphorylated after internalisation (Sorkin *et al.*, 1993). The ligand-bound receptors are accumulated in both clathrin-coated endosomes and membrane invaginations, caveolae (Kapeller *et al.*, 1993; Yamamoto *et al.*, 1999). The internalisation results in a transient decrease of the receptors on cell membranes. PDGF receptors directly interact with caveolin subtypes, which inhibit the activation of PDGF receptor kinase (Yamamoto *et al.*, 1999). However, it has also been shown that the PDGF-receptor internalisation could be involved in MAPK activation rather than constituting a down-regulation of the signal (Chiarugi *et al.*, 2002; Rakhit *et al.*, 2000).

Cbl is one of the SH2-domain-containing proteins that bind phosphotyrosine of the PDGF receptors. Upon phosphorylation, Cbl, being a proteasomal E3-like ubiquitin ligase for the receptor, leads to a rapid degradation of PDGF receptors *via* a proteasomal pathway (Miyake *et al.*, 1999). However, the overall PDGF-receptor protein degradation upon PDGF stimulation, as estimated by Chiarugi *et al.* (2002), was around 30–40 percent of the total receptors, and the receptors were shown to be functionally active for further stimulation.

Several phosphotyrosine phosphatases (PTPs) bind the phosphotyrosine residues of the receptors through a transient enzyme–substrate interaction as well as through their SH2 domains, rendering dephosphorylation of the PDGF receptors. This accounts for one of the major controlling mechanisms for the activity of the receptors. Several PTPs – PTP-PEST, SHP-2, an active fragment of SHP-2, PTP-1B, and T-cell PTP – have been shown to bind PDGF β-receptor by an in-gel assay (Markova *et al.*, 2003), with SHP-2 being the most efficiently binding PTP. By binding and dephosphorylation of the PDGF receptor, and possibly of its substrates, SHP-2 can act negatively on the signalling. However, SHP-2 is also known to play an important role in mitogenic signalling (Roche *et al.*, 1996). It is possible that the SHP2-bound PDGF receptor can recruit the adaptor protein Grb2, which,

in turn, brings the Ras exchange factor (Sos) to its target, leading to activation of MAP kinases. A similar mechanism was proposed for signal transduction *via* E-selectin (Hu *et al.*, 2001). Cells with the SHP-2 binding mutant PDGF β-receptor (Y763F/Y1009F) were not only decreased in MAP kinase activation, but also significantly decreased in PDGF-BB-induced chemotaxis, without changing the mitogenic response to PDGF (Rönnstrand *et al.*, 1999). Thus, SHP-2 plays a dual role in PDGF signalling, and is implicated in both positive and negative signalling.

Low molecular weight phosphotyrosine phosphatase (LMW-PTP) has also been shown to be able to bind and dephosphorylate Y857 of activated PDGF receptor, inhibiting cell proliferation. Binding of LMW-PTP down-regulates most of the PDGF activated signalling molecules such as PI3-kinase, SHP-2, and PLC-γ1, but not MAP kinases (Chiarugi *et al.*, 1998). The authors also reported that LMW-PTP exists constitutively in the cytosol, associated with the cytoskeleton. Upon PDGF stimulation, c-Src is able to bind and phosphorylate LMW-PTP only in the cytoskeleton-associated fraction. As a consequence of phosphorylation, the catalytic activity of LMW-PTP increases (Chiarugi *et al.*, 2002).

EFFECTS OF VARIOUS STIMULI ON PDGF RECEPTOR EXPRESSION

In addition to the self-regulatory mechanisms elicited by the activation of the receptor, the interaction with other signalling systems is extensive. General conclusions are difficult to draw since conditions and cell types differ among published studies. Some reported findings are described below.

Transforming growth factor-β1 (TGF-β1) has been shown to decrease PDGF α-receptor expression in various cell types such as human primary bronchial SMC (Bonner *et al.*, 1996), lung fibroblasts (Lindroos *et al.*, 1997), a human foreskin fibroblast cell line (Paulsson *et al.*, 1993), and normal mesothelial cells (Langerak *et al.*, 1996). However, the results on the effects of TGF-β on PDGF β-receptor vary in different cell types: in most cell types, no change was observed, but in certain cell types, i.e. human fat-storing cells, up-regulation was reported (Pinzani *et al.*, 1995). The reason for this variability might depend on the growth conditions of cells, as was mentioned before. bFGF increased PDGF α-receptor expression without altering PDGF β-receptor expression in human primary bronchial SMC (Bonner *et al.*, 1996). IL1β did not affect the expression of PDGF receptors on the SMC, but enhanced PDGF α-receptor expression on lung fibroblasts (Lindroos *et al.*, 1997).

In human osteoblastic cells, TNF-α decreased PDGF α-receptor both at the mRNA and at the protein level (Gravestein & Borst, 1998), whereas in human fibroblasts, TNF-α decreased the expression of PDGF β-receptor without affecting PDGF α-receptor (Battegay *et al.*, 1990; Tingström *et al.*, 1992). However, TNF-α did not change the expression of PDGF β-receptor in mouse L1 fibroblasts but suppressed the ligand-stimulated PDGF β-receptor-tyrosine kinase phosphorylation (Molander *et al.*, 2000).

CONCLUSION

More than two decades ago PDGF, A and B, as well as their receptors were isolated. However, new family members continue to be discovered, adding further complexities to the system. Consistent with the essential roles of PDGF in growth and survival of cells, there is a tight control of the expression and activity of PDGF receptors through positive as well as negative feedback systems. Deregulation of the control systems has been implicated in serious pathological conditions such as chronic inflammation and tumours. Understanding of the mechanisms behind the growth factor system may lead to new therapeutic possibilities.

REFERENCES

Afink GB, Nistér M, Stassen BH, Joosten PH, Rademakers PJ, Bongcam-Rudloff E, Van Zoelen EJ, Mosselman S. (1995) Molecular cloning and functional characterization of the human platelet-derived growth factor alpha receptor gene promoter. *Oncogene.*; **10**: 1667–72.

Andrae J, Molander C, Smits A, Funa K, Nistér M. (2002) Platelet-derived growth factor-B and -C and active alpha-receptors in medulloblastoma cells. *Biochem Biophys Res Commun.*; **296**: 604–11.

Ballagi AE, Ishizaki A, Nehlin JO, Funa K. (1995) Isolation and characterization of the mouse PDGF beta-receptor promoter. *Biochem Biophys Res Commun.*; **210**: 165–73.

Barone MV, Courtneidge SA. (1995) Myc but not Fos rescue of PDGF signalling block caused by kinase-inactive Src. *Nature.*; **378**: 509–12.

Barrett TB, Seifert RA, Bowen-Pope DF. (1996) Regulation of platelet-derived growth factor receptor expression by cell context overrides regulation by cytokines. *J Cell Physiol.*; **169**: 126–38.

Battegay EJ, Raines EW, Seifert RA, Bowen-Pope DF, Ross R. (1990) TGF-beta induces bimodal proliferation of connective tissue cells *via* complex control of an autocrine PDGF loop. *Cell.*; **63**: 515–24.

Bergers G, Song S, Meyer-Morse N, Bergsland E, Hanahan D. (2003) Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors. *J Clin Invest.*; **111**: 1287–95.

Bergsten E, Uutela M, Li X, Pietras K, Östman A, Heldin CH, Alitalo K, Eriksson U. (2001) PDGF-D is a specific, protease-activated ligand for the PDGF beta-receptor. *Nat Cell Biol.*; **3**: 512–6.

Bonner JC, Badgett A, Lindroos PM, Coin PG. (1996) Basic fibroblast growth factor induces expression of the PDGF receptor-alpha on human bronchial smooth muscle cells. *Am J Physiol.*; **271**: L880–8.

Chiarugi P, Cirri P, Marra F, Raugei G, Fiaschi T, Camici G, Manao G, Romanelli RG, Ramponi G. (1998) The Src and signal transducers and activators of transcription pathways as specific targets for low molecular weight phosphotyrosine-protein phosphatase in platelet-derived growth factor signaling. *J Biol Chem.*; **273**: 6776–85.

Chiarugi P, Cirri P, Taddei ML, Talini D, Doria L, Fiaschi T, Buricchi F, Giannoni E, Camici G, Raugei G, Ramponi G. (2002) New perspectives in PDGF receptor downregulation: the main role of phosphotyrosine phosphatases. *J Cell Sci.*; **115**: 2219–32.

Cook JR, Wang DY, Chen JK. (1993) Nonresponsiveness of an SV40-transformed Balb/c-3T3 cell line to PDGF mitogenicity is correlated with an irreversible reduction in PDGF receptor number. *Life Sci.*; **53**: 803–10.

Cools J, DeAngelo DJ, Gotlib J, Stover EH, Legare RD, Cortes J, Kutok J, Clark J, Galinsky I, Griffin JD, Cross NC, Tefferi A, Malone J, Alam R, Schrier SL, Schmid J, Rose M, Vandenberghe P, Verhoef G, Boogaerts M, Wlodarska I, Kantarjian H, Marynen P, Coutre SE, Stone R, Gilliland DG. (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**: 1201–14.

Eccleston PA, Funa K, Heldin CH. (1993) Expression of platelet-derived growth factor (PDGF) and PDGF alpha- and beta-receptors in the peripheral nervous system: an analysis of sciatic nerve and dorsal root ganglia. *Dev Biol.*; **155**: 459–70.

Erlandsson A, Enarsson M, Forsberg-Nilsson K. (2001) Immature neurons from CNS stem cells proliferate in response to platelet-derived growth factor. *J Neurosci.*; **21**: 3483–91.

Forsberg K, Valyi-Nagy I, Heldin CH, Herlyn M, Westermark B. (1993) Platelet-derived growth factor (PDGF) in oncogenesis: development of a vascular connective tissue stroma in xenotransplanted human mela-

noma producing PDGF-BB. *Proc Natl Acad Sci U S A.*; **90**: 393–7.

Fukuoka T, Kitami Y, Okura T, Hiwada K. (1999) Transcriptional regulation of the platelet-derived growth factor alpha receptor gene via CCAAT/enhancer-binding protein-delta in vascular smooth muscle cells. *J Biol Chem.*; **274**: 25576–82.

Funa K, Åhgren A. (1997) Characterization of platelet-derived growth factor (PDGF) action on a mouse neuroblastoma cell line, NB41, by introduction of an antisense PDGF beta-receptor RNA. *Cell Growth Differ.*; **8**: 861–9.

Gilbertson DG, Duff ME, West JW, Kelly JD, Sheppard PO, Hofstrand PD, Gao Z, Shoemaker K, Bukowski TR, Moore M, Feldhaus AL, Humes JM, Palmer TE, Hart CE. (2001) Platelet-derived growth factor C (PDGF-C), a novel growth factor that binds to PDGF alpha and beta receptor. *J Biol Chem.*; **276**: 27406–14.

Gravestein LA, Borst J. (1998) Tumor necrosis factor receptor family members in the immune system. *Sem Immunol.*; **10**: 423–34.

Hackzell A, Uramoto H, Izumi H, Kohno K, Funa K. (2002) p73 independent of c-Myc represses transcription of platelet-derived growth factor beta-receptor through interaction with NF-Y. *J Biol Chem.*; **277**: 39769–76.

Heinrich MC, Corless CL, Duensing A, McGreevey L, Chen CJ, Joseph N, Singer S, Griffith DJ, Haley A, Town A, Demetri GD, Fletcher CD, Fletcher JA. (2003) PDGFRA activating mutations in gastrointestinal stromal tumors. *Science.*; **299**: 708–10.

Heldin CH, Östman A, Rönnstrand L. (1998) Signal transduction *via* platelet-derived growth factor receptors. *Biochim Biophys Acta.*; **1378**: F79–113.

Heldin CH, Westermark B. (1999) Mechanism of action and *in vivo* role of platelet-derived growth factor. *Physiol Rev.*; **79**: 1283–316.

Heldin CH, Eriksson U, Östman A. (2002) New members of the platelet-derived growth factor family of mitogens. *Arch Biochem Biophys*; **398**: 284–90.

Hellstrom M, Kalen M, Lindahl P, Abramsson A, Betsholtz C. (1999) Role of PDGF-B and PDGFR-beta in recruitment of vascular smooth muscle cells and pericytes during embryonic blood vessel formation in the mouse. *Development.*; **126**: 3047–55.

Henriksen R, Funa K, Wilander E, Bäckstrom T, Ridderheim M, Öberg K. (1993) Expression and prognostic significance of platelet-derived growth factor and its receptors in epithelial ovarian neoplasms. *Cancer Res.*; **53**: 4550–4.

Hermanson M, Nistér M, Betsholtz C, Heldin CH, Westermark B, Funa K. (1988) Endothelial cell hyperplasia in human glioblastoma: coexpression of mRNA for platelet-derived growth factor (PDGF) B chain and PDGF receptor suggests autocrine growth stimulation. *Proc Natl Acad Sci U S A.*; **85**: 7748–52.

Hermanson M, Funa K, Hartman M, Claesson-Welsh L, Heldin CH, Westermark B, 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**: 3213–9.

Hu Y, Bock G, Wick G, Xu Q. (1998) Activation of PDGF receptor alpha in vascular smooth muscle cells by mechanical stress. *FASEB J.*; **12**: 1135–42.

Hu Y, Szente B, Kiely JM, Gimbrone MA Jr. (2001) Molecular events in transmembrane signaling *via* E-selectin. SHP2 association, adaptor protein complex formation and ERK1/2 activation. *J Biol Chem.*; **276**: 48549–53.

Hui AB, Lo KW, Yin XL, Poon WS, Ng HK. (2001) Detection of multiple gene amplifications in glioblastoma multiforme using array-based comparative genomic hybridization. *Lab Invest.*; **81**: 717–23.

Irwin MS, Kaelin WG. (2001) p53 family update: p73 and p63 develop their own identities. *Cell Growth Differ.*; **12**: 337-49.

Ishisaki A, Murayama T, Ballagi AE, Funa K. (1997) Nuclear factor Y controls the basal transcription activity of the mouse platelet-derived-growth-factor beta-receptor gene. *Eur J Biochem.*; **246**: 142–6.

Izumi H, Molander C, Penn LZ, Ishisaki A, Kohno K, Funa K. (2001) Mechanism for the transcriptional repression by c-Myc on PDGF beta-receptor. *J Cell Sci.*; **114**: 1533–44.

Kapeller R, Chakrabarti R, Cantley L, Fay F, Corvera S. (1993) Internalization of activated platelet-derived growth factor receptor-phosphatidylinositol-3′ kinase complexes: potential interactions with the microtubule cytoskeleton. *Mol Cell Biol.*; **13**: 6052–63.

Kawagishi J, Kumabe T, Yoshimoto T, Yamamoto T. (1995) Structure, organization, and transcription units of the human alpha-platelet-derived growth factor receptor gene, PDGFRA. *Genomics.*; **30**: 224–32.

Kim IS, Sinha S, de Crombrugghe B, Maity SN. (1996) Determination of functional domains in the C subunit of the CCAAT-binding factor (CBF) necessary for formation of a CBF-DNA complex: CBF-B interacts simultaneously with both the CBF-A and CBF-C subunits to form a heterotrimeric CBF molecule. *Mol Cell Biol.*; **16**: 4003–13.

Kitami Y, Fukuoka T, Okura T, Takata Y, Maguchi M, Igase M, Kohara K, Hiwada K. (1998) Molecular structure and function of rat platelet-derived growth factor beta-receptor gene promoter. *J Hypertens.*; **16**: 437–45.

Langerak AW, van der Linden-van Beurden CA, Versnel MA. (1996) Regulation of differential expression of platelet-derived growth factor alpha- and beta-receptor mRNA in normal and malignant human mesothelial cell lines. *Biochim Biophys Acta.*; **1305**: 63–70.

Li H, Fredriksson L, Li X, Eriksson U. (2003) PDGF-D is a potent transforming and angiogenic growth factor. *Oncogene.*; **22**: 1501–10.

Lindahl P, Johansson BR, Leveen P, Betsholtz C. (1997) Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. *Science.*; **277**: 242–5.

Lindroos PM, Coin PG, Badgett A, Morgan DL, Bonner JC. (1997) Alveolar macrophages stimulated with titanium dioxide, chrysotile asbestos, and residual oil fly ash upregulate the PDGF receptor-alpha on lung fibroblasts through an IL-1beta-dependent mechanism. *Am J Respir Cell Mol Biol.*; **16**: 283–92.

MacDonald TJ, Brown KM, LaFleur B, Peterson K, Lawlor C, Chen Y, Packer RJ, Cogen P, Stephan DA. (2001) Expression profiling of medulloblastoma: PDGFRA and the RAS/MAPK pathway as therapeutic targets for metastatic disease. *Nat Genet.*; **29**: 143–52.

Mantovani R. (1998) A survey of 178 NF-Y binding CCAAT boxes. *Nucleic Acids Res.*; **26**: 1135–43.

Markova B, Herrlich P, Rönnstrand L, Böhmer FD. (2003) Identification of protein tyrosine phosphatases associating with the PDGF receptor. *Biochemistry.*; **42**: 2691–9.

Miyake S, Mullane-Robinson KP, Lill NL, Douillard P, Band H. (1999) Cbl-mediated negative regulation of platelet-derived growth factor receptor-dependent cell proliferation. A critical role for Cbl tyrosine kinase-binding domain. *J Biol Chem.*; **274**: 16619–28.

Molander C, Kallin A, Izumi H, Rönnstrand L, Funa K. (2000) TNF-alpha suppresses the PDGF beta-receptor kinase. *Exp Cell Res.*; **258**: 65–71.

Molander C, Hackzell A, Ohta M, Izumi H, Funa K. (2001) Sp1 is a key regulator of the PDGF beta-receptor transcription. *Mol Biol Rep.*; **28**: 223–33.

Oster SK, Marhin WW, Asker C, Facchini LM, Dion PA, Funa K, Post M, Sedivy JM, Penn LZ. (2000) Myc is an essential negative regulator of platelet-derived growth factor beta receptor expression. *Mol Cell Biol.*; **20**: 6768–78.

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

Paulsson Y, Karlsson C, Heldin CH, Westermark B. (1993) Density-dependent inhibitory effect of transforming growth factor-beta 1 on human fibroblasts involves the down-regulation of platelet-derived growth factor alha-receptors. *J Cell Physiol.*; **157**: 97–103.

Pietras K, Östman A, Sjöquist M, Buchdunger E, Reed RK, Heldin CH, Rubin K. (2001) Inhibition of platelet-derived growth factor receptors reduces interstitial hypertension and increases transcapillary transport in tumors. *Cancer Res.*; **61**: 2929–34.

Pietras K, Rubin K, Sjöblom T, Buchdunger E, Sjoquist M, Heldin CH, Östman A. (2002) Inhibition of PDGF receptor signaling in tumor stroma enhances antitumor effect of chemotherapy. *Cancer Res.*; **62**: 5476–84.

Pietras K, Sjöblom T, Rubin K, Heldin CH, Östman A. (2003) PDGF receptors as cancer drug targets. *Cancer Cell.*; **3**: 439–43.

Pinzani M, Gentilini A, Caligiuri A, De Franco R, Pellegrini G, Milani S, Marra F, Gentilini P. (1995) Transforming growth factor-beta 1 regulates platelet-derived growth factor receptor beta subunit in human liver fat-storing cells. *Hepatology.*; **21**: 232–9.

Raff MC. (1989) Glial cell diversification in the rat optic nerve. *Science.*; **243**: 1450–5.

Rakhit S, Pyne S, Pyne NJ. (2000) The platelet-derived growth factor receptor stimulation of p42/p44 mitogen-activated protein kinase in airway smooth muscle involves a G-protein-mediated tyrosine phosphorylation of Gab1. *Mol Pharmacol.*; **58**: 413–20.

Reuterdahl C, Tingström A, Terracio L, Funa K, Heldin CH, Rubin K. (1991) Characterization of platelet-derived growth factor beta-receptor expressing cells in the vasculature of human rheumatoid synovium. *Lab Invest.*; **64**: 321–9.

Reuterdahl C, Sundberg C, Rubin K, Funa K, Gerdin B. (1993) Tissue localization of beta receptors for platelet-derived growth factor and platelet-derived growth factor B chain during wound repair in humans. *J Clin Invest.*; **91**: 2065–75.

Risau W, Drexler H, Mironov V, Smits A, Siegbahn A, Funa K, Heldin CH. (1992) Platelet-derived growth factor is angiogenic in vivo. *Growth Factors.*; **7**: 261–6.

Roche S, McGlade J, Jones M, Gish GD, Pawson T, Courtneidge SA. (1996) Requirement of phospholipase C gamma, the tyrosine phosphatase Syp and the adaptor proteins Shc and Nck for PDGF-induced DNA synthesis: evidence for the existence of Ras-dependent and Ras-independent pathways. *EMBO J.*; **15**: 4940–8.

Rönnstrand L, Arvidsson AK, Kallin A, Rorsman C, Hellman U, Engström U, Wernstedt C, Heldin CH. (1999) SHP-2 binds to Tyr763 and Tyr1009 in the PDGF beta-receptor and mediates PDGF-induced activation of the Ras/MAP kinase pathway and chemotaxis. *Oncogene.*; **18**: 3696–702.

Sasahara M, Fries JWU, Raines EW, Gown AM, Westrum LE, Frosch MP, Bonthron DT, Ross R, Collins T. (1991) PDGF B-chain in neurons of the central nervous system, posterior pituitary, and in a transgenic model. *Cell.*; **64**: 217–27.

Shinbrot E, Liao X, Williams LT. (1997) Isolation and characterization of the platelet-derived growth factor beta receptor promoter. *Dev Dyn.*; **208**: 211–9.

Sirvent N, Maire G, Pedeutour F. (2003) Genetics of dermatofibrosarcoma protuberans family of tumors: From ring chromosomes to tyrosine kinase inhibitor treatment. *Genes Chromosomes Cancer.*; **37**: 1–19.

Sjöblom T, Boureux A, Rönnstrand L, Heldin CH, Ghysdael J, Östman A. (1999) Characterization of the chronic myelomonocytic leukemia associated TEL-PDGF beta R fusion protein. *Oncogene.*; **18**: 7055–62.

Skobe M, Fusenig NE. (1998) Tumorigenic conversion of immortal human keratinocytes through stromal cell activation. *Proc Natl Acad Sci U S A.*; **95**: 1050–5.

Smits A, Hermansson M, Nistér M, Karnushina I, Heldin CH, Westermark B, Funa K. (1989) Rat brain capillary endothelial cells express functional PDGF B-type receptors. *Growth Factors.*; **2**: 1–8.

Smits A, Funa K, Vassbotn FS, Beausang-Linder M, af Ekenstam F, Heldin CH, Westermark B, Nistér M. (1992) Expression of platelet-derived growth factor and its receptors in proliferative disorders of fibroblastic origin. *Am J Pathol.*; **140**: 639–48.

Smits A, Ballagi AE, Funa K. (1993) PDGF-BB exerts trophic activity on cultured GABA interneurons from the newborn rat cerebellum. *Eur J Neurosci.*; **5**: 986–94.

Soriano P. (1997) The PDGF alpha receptor is required for neural crest cell development and for normal patterning of the somites. *Development.*; **124**: 2691–700.

Sorkin A, Eriksson A, Heldin CH, Westermark B, Claesson-Welsh L. (1993) Pool of ligand-bound platelet-derived growth factor beta-receptors remain activated and tyrosine phosphorylated after internalization. *J Cell Physiol.*; **156**: 373–82.

Sundberg C, Branting M, Gerdin B, Rubin K. (1997) Tumor cell and connective tissue cell interactions in human colorectal adenocarcinoma. Transfer of platelet-derived growth factor-AB/BB to stromal cells. *Am J Pathol.*; **151**: 479–92.

Tallquist MD, Soriano P. (2003) Cell autonomous requirement for PDGFR alpha in populations of cranial and cardiac neural crest cells. *Development.*; **130**: 507–18.

Tamura K, Yokote K, Takemoto M, Matsumoto T, Ishisaki A, Funa K, Saito Y, Mori S. (1998) Fibronectin stimulates transcription of the platelet-derived growth factor beta-receptor in cultured rat aortic smooth muscle cells. *Biochem Biophys Res Commun.*; **251**: 677–80.

Terracio L, Rönnstrand L, Tingström A, Rubin K, Claesson-Welsh L, Funa K, Heldin CH. (1988) Induction of platelet-derived growth factor receptor expression in smooth muscle cells and fibroblasts upon tissue culturing. *J Cell Biol.*; **107**: 1947–57.

Tingström A, Reuterdahl C, Lindahl P, Heldin CH, Rubin K. (1992) Expression of platelet-derived growth factor-beta receptors on human fibroblasts. Regulation by recombinant platelet-derived growth factor-BB, IL-1, and tumor necrosis factor-alpha. *J Immunol.*; **148**: 546–54.

Uehara H, Kim SJ, Karashima T, Shepherd DL, Fan D, Tsan R, Killion JJ, Logothetis C, Mathew P, Fidler IJ. (2003) Effects of blocking platelet-derived growth factor-receptor signaling in a mouse model of experimental prostate cancer bone metastases. *J Natl Cancer Inst.*; **95**: 458–70.

Uramoto H, Izumi H, Ise T, Tada M, Uchiumi T, Kuwano M, Yasumoto K, Funa K, Kohno K. (2002) p73 interacts with c-Myc to regulate YB-1 expression. *J Biol Chem.*; **21**: 21.

Vaziri C, Faller DV. (1995) Repression of platelet-derived growth factor beta-receptor expression by mitogenic growth factors and transforming oncogenes in murine 3T3 fibroblasts. *Mol Cell Biol.*; **15**: 1244–53.

Vaziri C, Faller DV. (1996) Down-regulation of platelet-derived growth factor receptor expression during terminal differentiation of 3T3-L1 pre-adipocyte fibroblasts. *J Biol Chem.*; **271**: 13642–8.

Waltenberger J, Akyürek ML, Aurivillius M, Wanders A, Larsson E, Fellström B, Funa K. (1996) Ischemia-induced transplant arteriosclerosis in the rat. Induction of peptide growth factor expression. *Arterioscler Thromb Vasc Biol.*; **16**: 1516–23.

Wang C, Stiles CD. (1994) Platelet-derived growth factor alpha receptor gene expression: isolation and characterization of the promoter and upstream regulatory elements. *Proc Natl Acad Sci U S A.*; **91**: 7061–5.

Wang JL, Nistér M, Bongcam-Rudloff E, Pontén J, Westermark B. (1996) Suppression of platelet-derived growth factor alpha- and beta-receptor mRNA levels in human fibroblasts by SV40 T/t antigen. *J Cell Physiol.*; **166**: 12–21.

Yamamoto M, Toya Y, Jensen RA, Ishikawa Y. (1999) Caveolin is an inhibitor of platelet-derived growth factor receptor signaling. *Exp Cell Res.*; **247**: 380–8.

Yeh H-J, Ruit KG, Wang Y-X, Parks WC, Snider WD, Deuel TF. (1991) PDGF A-chain gene is expressed by mammalian neurons during de-

velopment and in maturity. *Cell.*; **64**: 209–16.

Zaika A, Irwin M, Sansome C, Moll UM. (2001) Oncogenes induce and activate endogenous p73 protein. *J Biol Chem.*; **276**: 11310–6.

Zhang QX, Walker F, Burgess AW, Baldwin GS. (1995) Reduction in platelet-derived growth factor receptor mRNA in v-src- transformed fibroblasts. *Biochim Biophys Acta.*; **1266**: 9–15.

Zhao J, Roth J, Bode-Lesniewska B, Pfaltz M, Heitz PU, Komminoth P. (2002) Combined comparative genomic hybridization and genomic microarray for detection of gene amplifications in pulmonary artery intimal sarcomas and adrenocortical tumors. *Genes Chromosomes Cancer.*; **34**: 48–57.