Upsala J Med Sci 109: 165–178, 2004

Development and possible clinical use of antagonists for PDGF and TGF-β

Carl-Henrik Heldin

Ludwig Institute for Cancer Research, Box 595, Biomedical Center, S-751 24 Uppsala, Sweden

ABSTRACT

A free full-text copy of this article can be found at the web page of Upsala J Med Sci: http://www.ujms.se

Platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β) are examples of signaling molecules which control the growth, survival motility and differentiation of cells. PDGF stimulates the growth mainly of connective tissue cells, whereas TGF-β inhibits the growth of most cell types. PDGF and TGF-β exert their cellular effects by vinding to receptors equipped with tyrosine and serine/threonine kinase activities, respectively. Both factors have important roles e.g. during the embryonal development and in wound healing.

Overactivity of PDGF or PDGF receptors contributes to the development of certain diseases characterized by excessive cell growth including fibrotic disorders, atherosclerosis and malignancies. Overactivity of TGF-β also contributes to fibrotic conditions, since TGF-β promotes accumulation of extracellular matrix molecules. In cancer, TGF-β is initially a tumor suppressor due to its ability to inhibit cell growth, however, at later stages of tumor progression TGF-β has tumor promoting activity by enhancing the invasive properties of tumor cells and by suppressing the immune system and promoting angiogenesis.

The involvement of PDGF in TGF-β in serious diseases makes clinically useful antagonists highly desirable. A low molecular weight receptor kinase inhibitor of the PDGF receptor kinase is now tested clinically, and TGF-β antagonists are under development. The present review discusses the development and possible clinical use of antagonsts for PDGF and TGF-β.

INTRODUCTION

The growth, survival, motility and differentiation of cells are regulated in part by signals the cell receives from its environment. Such signals are important, e.g. during embryonal development, wound healing, hematopoiesis and during the immune

Received 18 March 2004
Accepted 25 March 2004

response, and may come from interactions with other cells or the extracellular matrix, or from stimulation by specific soluble growth regulatory factors.

Accumulating evidence suggest that the lack of growth control of tumor cells is due to constitutive activation of components, which in the normal cell is controlled by growth stimulatory factors, or by loss of functional activity of components in growth inhibitory pathways. Modulation of signaling pathways involved in cell growth and survival is therefore an interesting strategy to explore in the treatment of cancer.

The present review focuses on two important growth regulatory factors, *i.e.* platelet-derived growth factor (PDGF), a major mitogen for connective tissue cells, and transforming growth factor-β (TGF-β), which inhibits the growth of most cell types. The possibility to use knowledge about the signaling mechanisms of these factors in the development of drugs for the treatment of patients with cancer or other diseases, will be discussed.

PLATELET-DERIVED GROWTH FACTOR

Mechanism of action of PDGF

PDGF is a family of disulfide-bonded dimers of structurally similar polypeptide chains, which occur as homodimers (PDGF-AA, -BB, -CC and -DD) or as a heterodimer (PDGF-AB) (reviewed in 1). The PDGF isoforms exert their cellular effects by binding to structurally similar α- and β-tyrosine kinase receptors. Ligand binding induces receptor dimerization which is a key event in the activation of the receptors. Since the A-, B- and C-chains of PDGF bind to the α-receptor, whereas the B- and D-chains bind to the β-receptor, different types of homo- and heterodimeric receptor complexes can be formed depending of the stimulating PDGF isoform and depending on which of the receptors the target cell expresses (Fig. 1).

Ligand-induced receptor dimerization brings the intracellular parts of the receptors close to each other, so that the kinase domain of one receptor can phosphorylate specific tyrosine residues in the other, and vice versa. The autophosphorylation serves two important functions; phosphorylation of the activation loop of the kinase domain enhances the kinase activity, and phosphorylation of tyrosine residues outside the kinase domain creates specific docking sites for SH2-domain-containing signaling molecules. About 10 different types of SH2-domain-containing proteins bind to the PDGF receptors. The specificity in binding is determined by the amino acid residues downstream of the phosphorylated tyrosine. The docking of the signaling molecules activates a cascade of signaling pathways, which ultimately leads to initiation of DNA synthesis and cell division, survival and migration (reviewed in 2).

Among the signaling pathways activated after PDGF stimulation of cells, phosphatidylinositol-3'-kinase (PI3-kinase) and phospholipase-Cγ (PLCγ) are particularly important for the motility effects of PDGF, and, in the case of PI3-kinase, for the survival effect. Important mediators of the mitogenic effect of PDGF are Ras, which is activated by the docking of the adapter Grb2 in complex with the exchange factor Sos1 to the receptor and which activates the Erk MAP kinase pathway, and

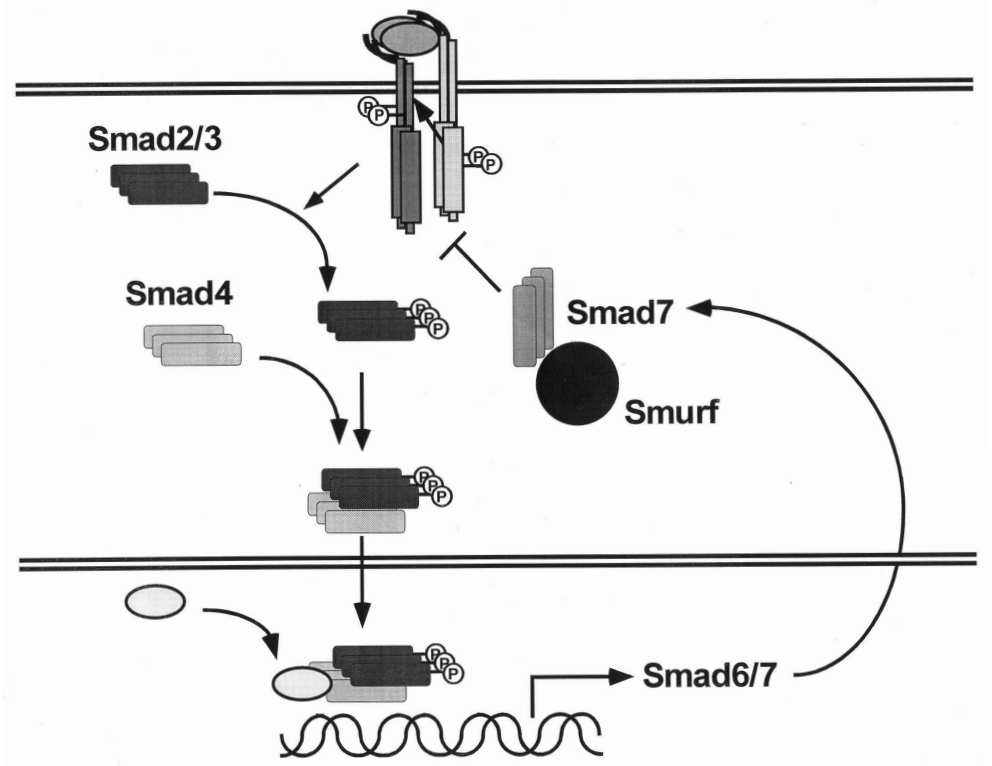

Fig. 1. Five PDGF isoforms induce the formation of three dimeric receptor complexes. The picture schematically illustrates the binding specificities of PDGF isoforms. The α- and β-receptors for PDGF each contains 5 immunoglobulin-like domains extracellularly, and a kinase domain intracellularly which is split into two parts by an intervening sequence.

Src, which induces the transcription factor c-Myc. It should be emphasized, however, that intracellular signaling occurs in a network of signaling components, with extensive interactions between different signaling pathways.

In vivo *function of PDGF*

PDGF has important functions during the embryonal development. The ligand is often produced by specific epithelial cells, and act on neighboring connective tissue cells by paracrine stimulation. The specific functions of individual PDGF isoforms and receptors have been elucidated by knock-out studies in the mouse. Thus, inactivation of the genes for the PDGF B-chain (3) or the β-receptor (4), leads to severe defects in the development of the kidneys due to a complete lack of mesangial cells. Also blood vessel development is defect leading to bleedings at the time of birth, which often is the direct cause of death; the reason is that in the absence of PDGF-B or PDGF β-receptor, the vascular smooth muscle cells and pericytes do not develop normally (5).

Knock-out of the A-chain leads to death of the mice at around the age of 3 weeks,

due to a defect development of alveolar smooth muscle cell progenitors (6). In addition, the mucosal lining of the gastrointestinal tract is defect with a decreased number of villus clusters (7). Knock-out of the α-receptor gives a severe phenotype with cranial malformations and a deficiency in myotome formation (8).

In the adult, PDGF promotes wound healing. PDGF stimulates chemotaxis and growth of many cell types involved in the healing process, including fibroblasts, smooth muscle cells, neurotrophils and macrophages. Clinical studies have shown that topical administration of PDGF stimulates wound healing in patients with decubitus ulcers (9).

The importance of PDGF for the connective tissue compartment is also illustrated by the fact that PDGF regulates the interstitial fluid pressure (10). The exact mechanism involved is not known, but probably involves increased interactions between fibroblasts of the connective tissue and matrix molecules, and a stimulation of contraction of fibroblasts through effects on the actin filament system of such cells.

PDGF in disease

Overactivity of PDGF has been implicated in several types of serious disorders, including malignant diseases, as well as atherosclerosis and fibrotic diseases.

In atherosclerosis, injury to the endothelial cell layer leads to deposit of PDGF from platelets, macrophages and other cells adhering to the injured site. PDGF then stimulates migration of smooth muscle cells from the media into the intima of the vessel, where they proliferate. This intimal thickening causes a narrowing of the vessel lumen and is an early stage in the atherosclerotic process, which subsequently is followed by lipid deposition and plaque formation (11).

Given the important role of PDGF for the growth and development of different types of connective tissue cells, it is not surprising that overactivity of PDGF can contribute to fibrosis. There are evidence for the involvement of PDGF in lung fibrosis, glomerulonephritis, liver cirrhosis and myelofibrosis (reviewed in 12).

In malignancies, PDGF has two principally different effects. On one hand, activation of PDGF receptors, by mutational events or by constitutive autocrine stimulation by ligand, may drive the proliferation and survival of tumor cells. On the other hand, PDGF produced in the tumor may act in a paracrine manner on the non-malignant cells in the tumor, *i.e.* fibroblast-like cells in the stroma, and smooth muscle cells, pericytes and, possibly, endothelial cells of blood vessels (reviewed in 13).

The first demonstration that autocrine stimulation by PDGF can have a transforming effect, came through the discovery that the *sis* oncogene of simian sarcoma virus is derived from the PDGF B-chain gene (14; 15). Subsequent studies have demonstrated that production of PDGF by tumor cells that possess PDGF receptors is common in glioblastoma (16) and sarcoma (17). Experimental proof that PDGF overproduction can cause glioblastoma has come from studies showing that such tumors are formed after intracranial injection of simian sarcoma virus into marmosets (18), or a PDGF B-chain containing retrovirus into mice (19).

There are some examples of chromosomal translocations in patients which lead

to constitutive activation of PDGF receptors. Thus, the skin tumor dermatofibrosarcoma protuberance (DFSP) is associated with a translocation which fuses the collagen 1A1 gene with the PDGF B-chain gene (20). This results in the production of large quantities of a fusion protein, which after processing to PDGF-BB causes autocrine stimulation of growth and cell transformation (21; 22).

In the case of the rare disease chronic monomyelocytic leukemia (CMML), the part of the β-receptor gene that encodes the kinase domain is fused to certain genes, *e.g*. the Tel gene, that have in common that they encode proteins that can dimerize (23–25). This results in constitutively dimerized and activated receptors, which drive tumor cell proliferation and survival. A similar situation prevails in the case of hypereosinophilic syndrome (HES); in this disease the PDGF α-receptor gene has been fused to the FIP1L1 gene, leading to constitutive dimerization and activation of the receptor kinase activity (26).

In the gastrointestinal stromal tumor (GIST), the α-receptor for PDGF often have point mutations in the activation loop, or elsewhere, causing activation of the receptor kinase which drives tumor cell growth and survival (27). Interestingly, GIST alternatively can have mutations in the structurally related tyrosine kinase receptor for stem cell factor (28).

Finally, a subset of gliomas shows amplification of the α-receptor gene, leading to an increased susceptibility to ligand stimulation or activation in the complete absence of ligand (29; 30).

Tumors of epithelial cell types generally do not express PDGF receptors. However, PDGF produced in such tumors may stimulate non-tumor cells in blood vessels and in the stroma, in a paracrine manner. Whereas PDGF does not have as potent effect in the early stages of angiogenesis as the classical angiogenesis factors of the vascular endothelial cell factor or fibroblast growth factor families, PDGF may have important roles at later stages in vessel maturation through its effects on smooth muscle cell development and pericyte recruitment (5; 31). Interestingly, PDGF receptor-deficient melanoma cells transfected with PDGF-B or -D were found to grow faster and to have an increased pericyte coverage of their vessels, compared to untransfected melanoma cells (32).

Another important effect of paracrine PDGF stimulation relates to the regulation of the interstitial fluid pressure (IFP) in tumors. Many solid tumors have increased IFP (33), which is an obstacle in tumor treatment, since it leads to an inefficient uptake of chemotherapeutical drugs from the circulation. There is evidence that PDGF, through its action on stromal myofibroblasts, may be one of the factors causing the increased tumor IFP (34).

PDGF antagonists for clinical use

Given the involvement of PDGF overexpression in serious diseases, clinically useful PDGF antagonists are highly warranted. Among the different antagonists that have been developed are molecules that prevent ligand activation of receptors, including antibodies, soluble extracellular receptor domains or DNA aptamers

(reviewed in 35). The advantage of these types of antagonists is that they are often highly specific, however, since they are macromolecules, they are expensive and cumbersome to administrate.

Another type of antagonists is low molecular weight inhibitors of the PDGF receptor kinase. These are less expensive and easier to administer, but are not absolutely specific. For instance, imatinib (STI571, Glivec) inhibits in addition to the α- and β-receptors for PDGF, also the kinases of stem cell factor receptor, Abl and Arg; it is already used clinically in the treatment of chronic myeloid leukemia which is characterized by overactivity of Abl (36; 37), and of GIST which is characterized by activation of stem cell factor receptor or PDGF α-receptor (38).

Initial clinical studies in which Glivec has been used on patients with malignancies characterized by activation of PDGF receptors, have shown encouraging results. Thus, reduction of tumor mass has been reported for patients with GIST (28; 38), CMML (39), HES (26) and DFSP (40; 41). However, whereas treatment of nude mice with intracranially growing glioblastoma has been shown to reduce tumor growth (42), it remains to be determined whether patients with glioblastoma benefit from treatment with PDGF antagonists.

PDGF antagonists may also potentially be useful in order to inhibit paracrine PDGF stimulation of normal cells in the tumor. Thus, PDGF antagonists may have an anti-angiogenic effect, or may enhance the anti-angiogenic effect of *e.g*. vascular endothelial growth factor antagonists (43).

Another interesting possibility is to lower the tumor IFP by treatment with PDGF antagonists and thereby achieve a higher uptake and efficiently of chemotherapy. Encouraging results have been obtained in animal models, where a significant enhancement of chemotherapy has been observed in the presence of Glivec treatment (44).

The initial clinical studies using PDGF antagonists have given encouraging results. It will be a challenge to develop selective PDGF antagonists and explore their usefulness for the treatment, not only of malignant diseases, but also for the treatment of atherosclerosis and fibrotic conditions.

TRANSFORMING GROWTH FACTOR-β

Mechanism of action of TGF-β

TGF-β is a disulfide-bonded dimer, and is a prototype for a family of about 30 members in humans, including TGF-β:s, activins, bone morphogenetic proteins (BMP:s) and growth and differentiation factors (GDF:s). TGF-β family members regulate cell proliferation, differentiation and apoptosis, and have important functions during embryonal development (reviewed in 45). They exert their cellular effects by forming a heteromeric complex of type I and type II serine/threonine kinase receptors. Within such complexes, the constitutively active type II receptor phosphorylates and activates the kinase activity of the type I receptor.

Important substrates for the activated type I receptor kinase, include members of

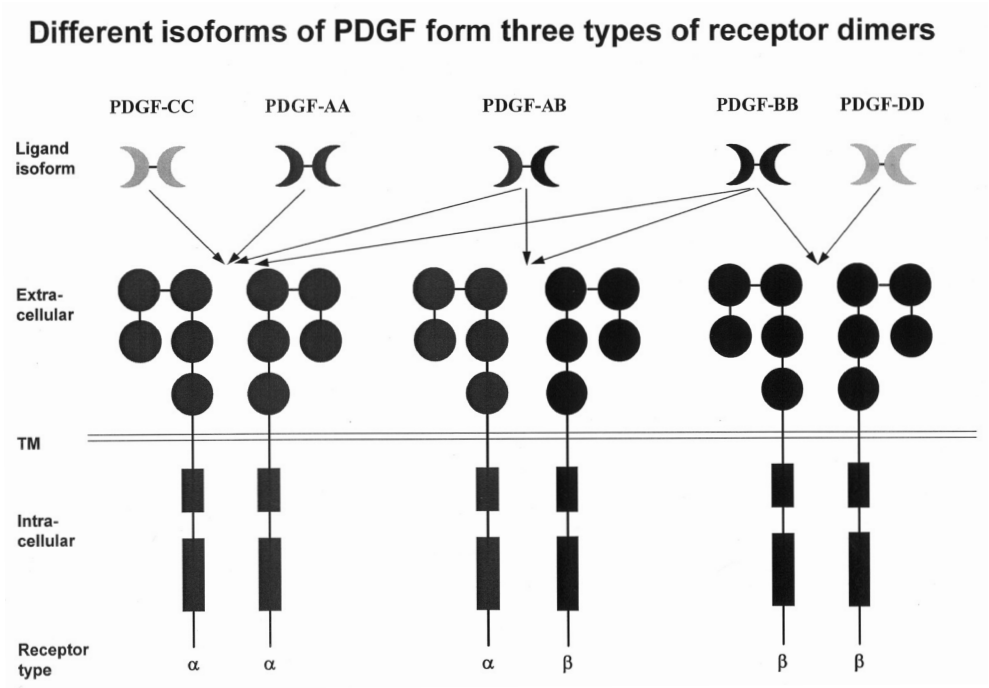

Fig. 2. Involvement of Smad molecules in TGF-β signaling. The picture illustrates that TGF-β induces a heteromeric receptor complex consisting of two type I and two type II receptors. Within this complex the constitutively active type II receptor (light blue) phosphorylate the type I receptor (dark blue) in a region just upstream of the kinase domain, whereby the type I receptor kinase is activated. The type I receptor then phosphorylates R-Smads (Smad2 and 3) which then forms complex with the Co-Smad (Smad4); the complex is then translocated into the nucleus, where it in conjunction with other nuclear proteins (yellow) binds to the promoter regions of specific genes. Among the target genes are I-Smads (Smad6 and 7). The induced I-Smads act in a negative feedback mechanism by competing with R-Smads for binding to the receptors and, in the case of Smad7, by bringing the ubiquitin ligase Smurf to the receptor.

the Smad family of signal transduction molecules. The Smad family consists of three subfamilies. Receptor-activated Smad (R-Smads) are phosphorylated by type I receptors in a C-terminal -Ser-Xaa-Ser motif (Smad2 and 3 for TGF-β and activin receptors, and Smad1, 5 and 8 for BMP receptors). Thereafter, R-Smads form complexes with the common-mediator Smad (Co-Smad; Smad4) which are translocated into the nucleus, where they regulate transcription by direct or indirect binding to the promoter regions of specific genes. Among the target genes for R-Smad/Co-Smad complexes are inhibitory Smads (I-Smads; Smad6 and 7), which are induced by TGF-β or BMP stimulation and act in a negative feed back loop to suppress Smad signaling. I-Smads bind to the receptors and thereby prevent interaction and phosphorylation of R-Smads; in addition, Smad7 binds the ubiquitin ligase Smurf, which thereby is recruited to the receptors and targets them for ubiquitin-mediated degradation in proteosomes (Fig. 2) (reviewed in 46).

Smad molecules have two conserved domains, the C-terminal MH2 domain which is present in all Smads, and the N-terminal MH1 domain which is present in R- and Co-Smads, but not in I-Smads. The MH1 domain, in certain Smads, regulates nuclear import and transcription by direct binding to DNA, as well as via binding to other nuclear proteins. The MH2 domain is responsible for interactions with receptors and for intermolecular interactions within Smad complexes. It also mediates interactions with cytoplasmic anchors, like SARA, and with a number of nuclear transcription factors. In the linker region connecting the MH1 and MH2 domains, there is a PY motif which binds Smurf ubiquitin ligases. In addition to the activating phosphorylation of R-Smads in the C-terminal tail by receptors, the activity of Smad molecules are also modulated by phosphorylation in other parts of the molecules by other kinases, including Erk MAP kinase, protein kinase C and calmodulin-dependent kinase II. Thus, Smad molecules are involved in a multitude of interactions, which regulate their activation, subcellular localization, stability and nuclear roles as transcription factors (reviewed in 47).

The nuclear partners of Smad molecules include the histone acetyl-transferase domain-containing co-activators of the p300 and P/CAF families. Certain Smads bind directly to 5'-GAGA-3' motifs in DNA, but this binding is of low affinity, and interactions with other DNA binding transcription factors are important for Smad function. Smad complexes also repress the transcription of specific genes, *e.g.* through interaction with co-repressors associated with histone deacetylase activity, such as TGIF, Ski and SnoN.

Although Smad molecules have crucial importance in TGF-β signaling, also other signaling pathways are induced after TGF-β stimulation of cells. These include activation of the Erk, JNK and p38 MAP kinase pathways and PI3-kinase (reviewed in 48). The importance of these signaling pathways for the effects of TGF-β on cells, remains to be elucidated, however, evidence has been presented that JNK and p38 activation are important for induction of apoptosis (reviewed in 49).

In vivo *function of TGF-β*

All TGF-β family members have roles during embryonal development. Knock-out of their receptors or signaling Smad molecules generally leads to embryonic lethality (reviewed in 50).

In the adult, TGF-β stimulates wound healing through its ability to cause accumulation of matrix molecules; TGF-β both stimulate production of extracellular matrix molecules, and inhibit the degradation of matrix through the production of protease inhibitors.

TGF-β in disease

Overactivity of TGF-β results in fibrotic diseases, including lung fibrosis, glomerulonephritis and liver cirrhosis. Whereas PDGF promotes fibrosis by stimulation of

connective tissue cells to migrate and proliferate, TGF-β's involvement in fibrosis is through its ability to cause accumulation of extracellular matrix.

In cancer, TGF-β has a complicated role. Initially TGF-β is a tumor suppressor because of its abilities to inhibit cell proliferation and promote apoptosis of cells. In certain tumors loss of function mutations in components in the TGF-β pathway are seen. Thus, mutations in the TGF-β receptor genes are seen in colorectal cancers (51), and the Smad4 gene is mutated in 50% of pancreatic carcinoma (52). However, at later stages of tumor progression, TGF-β has tumor promoter activity by inducing epithelial-to-mesenchymal transdifferentiation and thereby promoting invasiveness and metastasis of tumor cells that have retained responsiveness to TGF-β. Moreover, the effects of TGF-β on the non-malignant cells in the tumor, suppressing the immune response and promoting angiogenesis, also promote tumorigenesis (reviewed in 53). The dual role of TGF-β in tumorigenesis was clearly demonstrated in a keratinocyte tumorigenesis model; overexpression of TGF-β in keratinocytes initially inhibited the formation of benign skin tumors in response to exposure to a chemical carcinogen, but the benign tumors that eventually were formed rapidly progressed to invasive spindle-cell carcinomas (54).

TGF-β antagonists for clinical use

TGF-β antagonists could potentially be useful in the treatment of various fibrotic diseases, as well as in advanced forms of cancer. Inhibition of TGF-β by a soluble extracellular domain of the type II TGF-β receptor in mouse models for breast cancer and melanoma led to a clear reduction in metastasis and inhibition of angiogenesis (55–57). Moreover, low molecular weight inhibitors of the TGF-β type I receptor kinase have been developed (58–61), which offers an alternative way of inhibiting TGF-β signaling.

However, the dual role of TGF-β in tumorigenesis makes use of TGF-β antagonists complicated. Whereas inhibition of TGF-β signaling, *e.g.* through sequestration of TGF-β or inhibition of TGF-β receptor kinases, could have beneficial effects in fibrotic conditions or late stage cancers, such treatment would be associated with a risk of promoting recruitment of novel tumor cells due to inhibition of the tumor suppressive effects of TGF-β. An interesting and appealing strategy would therefore be to develop selective TGF-β inhibitors, which inhibit only those intracellular pathways that are involved in tumor progression, while leaving those that are involved in the tumor suppressive effects unperturbed. Recent work on the transcription factor Yin Yang-1 (YY1) has illustrated that this may be a possible strategy. YY1 binds to Smad molecules and represses several of their responses, however, YY1 does not affect TGF-β- or BMP-induced growth inhibition (62). An inhibitor that mimics the effect of YY1 on TGF-β signaling, would thus be an interesting candidate for evaluation in treatment of advanced cancers. However, such a strategy would clearly require additional work to elucidate in detail which pathways are responsible for the various effects of TGF-β on cells.

FUTURE PERSPECTIVES

Since many diseases involve overactivity of various growth regulatory factors, inhibitors of signal transduction are likely to become important future drugs. The first inhibitors of this type are currently used clinically with encouraging results.

PDGF antagonists have proven to be useful in certain rather uncommon malignancies that are dependent on a constitutively active PDGF receptor. However, it is possible that PDGF antagonists can be used in a more general manner to target normal cell types in solid tumors, in order to lower the interstitial fluid pressure and thereby enhance the effect of chemotherapy, and to inhibit angiogenesis and thereby starve the tumor cells. Moreover, PDGF antagonists can potentially be used also in the treatment of atherosclerosis and fibrotic conditions. Which type of antagonist that will be most useful, remains to be determined. The advantage with antibodies, soluble receptor domains or aptamers is the high specificity, on the other hand, the less specific but more convenient low molecular weight inhibitors have shown rather modest side effects. The most noticeable side effect of Glivec treatment has been peri-orbital edema (63), maybe reflecting that PDGF has an important role in the control of the interstitial fluid pressure in loose connective tissue.

The first generation TGF-β antagonists, a soluble extracellular TGF-β type II receptor and low molecular weight receptor kinase inhibitors, are now tested in animal models of advanced cancers. Initial results have shown positive treatment effects, and have encouraged continued work to produce clinically useful TGF-β antagonists. It remains to be seen, however, if the side effects during treatment with TGF-β antagonists will be tolerable. Since TGF-β has an important controlling effect of the immune system, inhibition of TGF-β may be accompanied by excessive immune reactions. Moreover, TGF-β inhibits the growth of most cell types; inhibition of TGF-β may thus promote cell growth and development of tumors. An important future task will be to explore the side effects of TGF-β antagonists, and to elucidate whether it is possible to develop selective TGF-β antagonists which inhibit only those responses that cause disease progression.

Although it is too early to say how useful antagonists for PDGF and TGF-β will be for the treatment of human disease, the results achieved so far are clearly encouraging, and provide a stimulation for further work to develop and explore the clinical utility of such antagonists.

ACKNOWLEDGEMENTS

I thank Ingegärd Schiller for skilful secretarial work.

REFERENCES

1. Heldin C-H, Eriksson U, Östman A (2002). New members of the platelet-derived growth factor family of mitogens. Arch. Biochem. Biophys. 398: 284–290.
2. Heldin C-H, Östman A, Rönnstrand L (1998). Signal transduction via platelet-derived growth factor receptors. Biochim. Biophys. Acta 1378: F79–F113.

3. Levéen P, Pekny M, Gebre-Medhin S, Swolin B, Larsson E, Betsholtz C (1994). Mice deficient for PDGF B show renal, cardiovascular, and hematological abnormalities. Genes Dev. 8: 1875–1887.
4. Soriano P (1994). Abnormal kidney development and hematological disorders in PDGF β-receptor mutant mice. Genes Dev. 8: 1888–1896.
5. Lindahl P, Johansson BR, Levéen P, Betsholtz C (1997). Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. Science 277: 242–245.
6. 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, Törnell J, Heath JK, Betsholtz C (1996). PDGF-A signaling is a critical event in lung alveolar myofibroblast development and alveogenesis. Cell 85: 863–873.
7. Karlsson L, Lindahl P, Heath JK, 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.
8. Soriano P (1997). The PDGFα receptor is required for neural crest cell development and for normal patterning of the somites. Development 124: 2691–2700.
9. Robson MC, Phillips LG, Thomason A, Robson LE, Pierce GF (1992). Platelet-derived growth factor BB for the treatment of chronic pressure ulcers. Lancet 339: 23–25.
10. Rodt SÅ, Åhlén K, Berg A, Rubin K, Reed RK (1996). A novel physiological function for platelet-derived growth factor-BB in rat dermis. J. Physiol. 495: 193–200.
11. Ross R (1999). Atherosclerosis – an inflammatory disease. N. Engl. J. Med. 340: 115–126.
12. Heldin C-H, Westermark B (1999). Mechanism of action and in vivo role of platelet-derived growth factor. Physiol. Rev. 79: 1283–1316.
13. Pietras K, Sjöblom T, Rubin K, Heldin C-H, Östman A (2003). PDGF receptors as cancer drug targets. Cancer Cell 3: 439–443.
14. Waterfield MD, Scrace GT, Whittle N, Stroobant P, Johnsson A, Wasteson Å, Westermark B, Heldin C-H, Huang JS, Deuel TF (1983). Platelet-derived growth factor is structurally related to the putative transforming protein $p28^{sis}$ of simian sarcoma virus. Nature (London) 304: 35–39.
15. Doolittle RF, Hunkapiller MW, Hood LE, Devare SD, Robbins KC, Aaronson SA, Antoniades HN (1983). Simian sarcoma virus *onc* gene, v-*sis*, is derived from the gene (or genes) encoding a platelet-derived growth factor. Science 221: 275–277.
16. Hermanson M, Funa K, Hartman M, Claesson-Welsh L, Heldin C-H, 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: 3213–3219.
17. Smits A, Funa K, Vassbotn FS, Beausang-Linder M, af Ekenstam F, Heldin C-H, Westermark B, Nistér M (1992). Expression of platelet-derived growth factor and its receptors in proliferative disorders of fibroblastic origin. Am. J. Pathol. 140: 639–648.
18. Deinhardt F (1980). The biology of primate retrovirus. In: G Klein (ed. Viral Oncology. Raven Press, New York, pp. 359–398.
19. Uhrbom L, Hesselager G, Nistér M, Westermark B (1998). Induction of brain tumors in mice using a recombinant platelet-derived growth factor B-chain retrovirus. Cancer Res. 58: 5275–5279.
20. Simon M-P, Pedeutour F, Sirvent N, Grosgeorge J, Minoletti F, Coindre J-M, Terrier-Lacombe M-J, Mandahl N, Craver RD, Blin N, Sozzi G, Turc-Carel C, O'Brien KP, Kedra D, Fransson I, Guilbaud C, Dumanski JP (1997). Deregulation of the platelet-derived growth factor B-chain gene via fusion with collagen gene *COL1A1* in dermatofibrosarcoma protuberans and giant-cell fibroblastoma. Nature Genet. 15: 95–98.
21. Greco A, Fusetti L, Villa R, Sozzi G, Minoletti F, Mauri P, Pierotti MA (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.
22. Shimizu A, O'Brien KP, Sjöblom T, Pietras K, Buchdunger E, Collins VP, Heldin C-H, Dumanski JP, 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.

23. Carroll M, Tomasson MH, Barker GF, Golub TR, Gilliland DG (1996). The TEL/platelet-derived growth factor β receptor (PDGFβR) fusion in chronic myelomonocytic leukemia is a transforming protein that self-associates and activates PDGFbR kinase-dependent signaling pathways. Proc. Natl. Acad. Sci. USA 93: 14845–14850.
24. Jousset C, Carron C, Boureux A, Quang CT, Oury C, Dusanter-Fourt I, Charon M, Levin J, Bernard O, 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-PDGFRβ oncoprotein. EMBO J. 16: 69–82.
25. Sjöblom T, Boureux A, Rönnstrand L, Heldin C-H, Ghysdael J, Östman A (1999). Characterization of the chronic myelomonocytic leukemia associated TEL-PDGFβR fusion protein. Oncogene 18: 7055–7062.
26. 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.
27. 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: 708–710.
28. Heinrich MC, Corless CL, Demetri GD, Blanke CD, von Mehren M, Joensuu H, McGreevey LS, Chen CJ, Van den Abbeele AD, Druker BJ, Kiese B, Eisenberg B, Roberts PJ, Singer S, Fletcher CD, Silberman S, Dimitrijevic S, Fletcher JA (2003). Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. J. Clin. Oncol. 21: 4342–4349.
29. Fleming TP, Saxena A, Clark WC, Robertson JT, Oldfield EH, Aaronson SA, Ali IU (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.
30. Kumabe T, Sohma Y, Kayama T, Yoshimoto T, Yamamoto T (1992). Amplification of α-platelet-derived growth factor receptor gene lacking an exon coding for a portion of the extracellular region in a primary brain tumor of glial origin. Oncogene 7: 627–633.
31. Crosby JR, Seifert RA, Soriano P, Bowen-Pope DF (1998). Chimaeric analysis reveals role of PDGF receptors in all muscle lineages. Nat. Genet. 18: 385–388.
32. Furuhashi M, Sjöblom T, Abramsson A, Ellingsen J, Micke P, Li H, Bergsten-Folestad E, Eriksson U, Heuchel R, Betsholtz C, Heldin C-H, Östman A (2004). PDGF production by B16 melanoma cells leads to increased pericyte coverage of vessels and an associated increase in tumor growth rate. Cancer Res. (in press).
33. Jain RK (1987). Transport of molecules in the tumor interstitium: a reivew. Cancer Res. 47: 3039–3051.
34. Pietras K, Östman A, Sjöquist M, Buchdunger E, Reed RK, Heldin C-H, Rubin K (2001). Inhibition of platelet-derived growth factor receptors reduces interstitial hypertension and increases transcapillary transport in tumors. Cancer Res. 61: 2929–2934.
35. Östman A, Heldin C-H (2001). Involvement of platelet-derived growth factor in disease: development of specific antagonists. Adv. Cancer Res. 80: 1–38.
36. Druker BJ, Sawyers CL, Kantarjian H, Resta DJ, Reese SF, Ford JM, Capdeville R, Talpaz M (2001). Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N. Engl. J. Med. 344: 1038–1042.
37. Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, Lydon NB, Kantarjian H, Capdeville R, Ohno-Jones S, Sawyers CL (2001). Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N. Engl. J. Med. 344: 1031–1037.
38. van Oosterom AT, Judson I, Verweij J, Stroobants S, Donato di Paola E, Dimitrijevic S, Martens M, Webb A, Sciot R, Van Glabbeke M, Silberman S, Nielsen OS (2001). Safety and efficacy of imatinib (STI571) in metastatic gastrointestinal stromal tumours: a phase I study. Lancet 358: 1421–1423.
39. Apperley JF, Gardembas M, Melo JV, Russell-Jones R, Bain BJ, Baxter EJ, Chase A, Chessells JM, Colombat M, Dearden CE, Dimitrijevic S, Mahon FX, Marin D, Nikolova Z, Olavarria E, Silberman S, Schultheis B, Cross NC, Goldman JM (2002). Response to imatinib mesylate in

patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor beta. N. Engl. J. Med. 347: 481–487.
40. Maki RG, Awan RA, Dixon RH, Jhanwar S, Antonescu CR (2002). Differential sensitivity to imatinib of 2 patients with metastatic sarcoma arising from dermatofibrosarcoma protuberans. Int. J. Cancer 100: 623–626.
41. Rubin BP, Schuetze SM, Eary JF, Norwood TH, Mirza S, Conrad EU, Bruckner JD (2002). Molecular targeting of platelet-derived growth factor B by imatinib mesylate in a patient with metastatic dermatofibrosarcoma protuberans. J. Clin. Oncol. 20: 3586–3591.
42. Kilic T, Alberta JA, Zdunek PR, Acar M, Iannarelli P, O'Reilly T, Buchdunger E, Black PM, Stiles CD (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.
43. Erber R, Thurnher A, Katsen AD, Groth G, Kerger H, Hammes HP, Menger MD, Ullrich A, 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: 338–40.
44. Pietras K, Rubin K, Sjöblom T, Buchdunger E, Sjöquist M, Heldin C-H, Östman A (2002). Inhibition of PDGF receptor signaling in tumor stroma enhances antitumor effect of chemotherapy. Cancer Res. 62: 5476–5484.
45. Moustakas A, Souchelnytskyi S, Heldin C-H (2001). Smad regulation in TGF-β signal transduction. J. Cell Sci. 114: 4359–4369.
46. Shi Y, Massagué J (2003). Mechanisms of TGF-β signaling from cell membrane to the nucleus. Cell 113: 685–700.
47. ten Dijke P, Miyazono K, Heldin C-H (2000). Signaling inputs converge on nuclear effectors in TGF-β signaling. Trends. Biol. Sci. 25: 64–70.
48. Derynck R, Zhang YE (2003). Smad-dependent and Smad-independent pathways in TGF-beta family signalling. Nature 425: 577–84.
49. ten Dijke P, Goumans M-J, Itoh F, Itoh S (2002). Regulation of cell proliferation by Smad proteins. J. Cell. Physiol. 191: 1–16.
50. Goumans MJ, Mummery C (2000). Functional analysis of the TGFb receptor/Smad pathway through gene ablation in mice. Int. J. Dev. Biol. 44: 253–265.
51. Markowitz S, Wang J, Myeroff L, Parsons R, Sun L, Lutterbaugh J, Fan RS, Zborowska E, Kinzler KW, Vogelstein B, Brattain M, Willson JKV (1995). Inactivation of the type II TGF-β receptor in colon cancer cells with microsatellite instability. Science 268: 1336–1338.
52. Hahn SA, Schutte M, Hoque ATMS, Moskaluk CA, da Costa LT, Rozenblum E, Weinstein CL, Fischer A, Yeo CJ, Hruban RH, Kern SE (1996). *DPC4*, a candidate tumor suppressor gene at human chromosome 18q21.1. Science 271: 350–353.
53. Siegel PM, Massagué J (2003). Cytostatic and apoptotic actions of TGF-β in homeostasis and cancer. Nat. Rev. Cancer 3: 807–821.
54. Cui W, Fowlis DJ, Bryson S, Duffie E, Ireland H, Balmain A, Akhurst RJ (1996). TGFβ1 inhibits the formation of benign skin tumors, but enhances progression to invasive spindle carcinomas in transgenic mice. Cell 86: 531–542.
55. Bandyopadhyay A, Zhu Y, Malik SN, Kreisberg J, Brattain MG, Sprague EA, Luo J, Lopez-Casillas F, Sun LZ (2002). Extracellular domain of TGFβ type III receptor inhibits angiogenesis and tumor growth in human cancer cells. Oncogene 21: 3541–3551.
56. Muraoka RS, Dumont N, Ritter CA, Dugger TC, Brantley DM, Chen J, Easterly E, Roebuck LR, Ryan S, Gotwals PJ, Koteliansky V, Arteaga CL (2002). Blockade of TGF-β inhibits mammary tumor cell viability, migration, and metastases. J. Clin. Invest. 109: 1551–1559.
57. Yang YA, Dukhanina O, Tang B, Mamura M, Letterio JJ, MacGregor J, Patel SC, Khozin S, Liu ZY, Green J, Anver MR, Merlino G, Wakefield LM (2002). Lifetime exposure to a soluble TGF-β antagonist protects mice against metastasis without adverse side effects. J. Clin. Invest. 109: 1607–1615.
58. Callahan JF, Burgess JL, Fornwald JA, Gaster LM, Harling JD, Harrington FP, Heer J, Kwon C, Lehr R, Mathur A, Olson BA, Weinstock J, Laping NJ (2002). Identification of novel inhibitors of the transforming growth factor b1 (TGF-β1) type 1 receptor (ALK5). J. Med. Chem. 45: 999–1001.

59. DaCosta Byfield S, Major C, Laping NJ, Roberts AB (2004). SB-505124 is a selective inhibitor of transforming growth factor-beta type I receptors ALK4, ALK5, and ALK7. Mol. Pharmacol. 65: 744–752.
60. Inman GJ, Nicolas FJ, Callahan JF, Harling JD, Gaster LM, Reith AD, Laping NJ, Hill CS (2002). SB-431542 is a potent and specific inhibitor of transforming growth factor-β superfamily type I activin receptor-like kinase (ALK) receptors ALK4, ALK5, and ALK7. Mol. Pharmacol. 62: 65–74.
61. Laping NJ, Grygielko E, Mathur A, Butter S, Bomberger J, Tweed C, Martin W, Fornwald J, Lehr R, Harling J, Gaster L, Callahan JF, Olson BA (2002). Inhibition of transforming growth factor (TGF)-β1-induced extracellular matrix with a novel inhibitor of the TGF-β type I receptor kinase activity: SB-431542. Mol. Pharmacol. 62: 58–64.
62. Kurisaki K, Kurisaki A, Valcourt U, Terentiev AA, Pardali K, ten Dijke P, Heldin C-H, Ericsson J, Moustakas A (2003). Nuclear factor YY1 inhibits transforming growth factor β- and bone morphogenetic protein-induced cell differentiation. Mol. Cell. Biol. 23: 4494–4510.
63. Capdeville R, Buchdunger E, Zimmermann J, Matter A (2002). Glivec (STI571, imatinib), a rationally developed, targeted anticancer drug. Nat. Rev. Drug Discov. 1: 493–502.

About the Author

Professor Carl-Henrik Heldin is director of the Ludvig Institute for Cancer Research in Uppsala, Sweden, and professor of Molecular Cell Biology at Uppsala University. Professor Heldin received the 2003 Rudbeck Award for his pioneering studies of the structure and function of PDGF (Platelet Derived Growth Factor) and its applications to various new treatments of cancer. He has received a number of prestigious scientific awards and is the author of more than 480 research articles and reviews in international journals.

Corresponding author: Professor Carl-Henrik Heldin
Ludvig Institute for Cancer Research
P.O. Box 595
SE-751 24 Uppsala
Sweden