

PDGF ANTAGONISTS IN THE TREATMENT OF MALIGNANCIES

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Platelet-derived growth factor (PDGF) is a family of isoforms which stimulate the growth, survival and migration of fibroblasts, smooth muscle cells and other cell types (reviewed in Heldin et al., 2002). PDGF isoforms have important roles during the embryonal development, where they are of crucial importance for the development of certain types of mesenchymal cell types. In the adult, PDGF stimulates wound healing and regulates the interstitial fluid pressure in tissues. Overactivity of PDGF has been implicated in certain malignancies, as well as in other diseases involving excessive cell growth, including atherosclerosis and fibrotic conditions.

The fact that PDGF is involved in several serious diseases has made PDGF antagonists highly desirable, and the first clinically useful antagonists are now available. This review will focus on the preclinical and initial clinical studies in which PDGF antagonists have been used in the treatment of malignancies.

PDGF isoforms and receptors

There are four homologous PDGF polypeptide chains, which form five different disulfide-bonded dimeric isoforms, PDGF-AA, -AB, -BB, -CC, and -DD (reviewed in Heldin et al., 2002). The PDGF isoforms exert their cellular effects by binding to alpha- and beta-tyrosine kinase receptors. Ligand binding induces dimerization of the receptors. The resulting composition of the receptor complexes depends on the stimulating PDGF isoform and on the receptor types expressed by the target cell. Alpha-alpha receptor homodimers

can be induced by all isoforms except PDGF-DD, alpha-beta receptor heterodimers by all isoforms except PDGF-AA, and beta-beta receptor homodimers by PDGF-BB and -DD.

Ligand-induced receptor dimerization is followed by receptor autophosphorylation on specific tyrosine residues in the intracellular domains of the receptors. Thereby, docking sites for SH2-domain-containing signaling molecules are formed. At least ten different types of SH2 domain proteins can bind to and are activated by PDGF receptors, including the tyrosine kinase Src, phosphatidylinositol-3'-kinase, the Grb2/Sos1 complex which activates Ras and the Erk MAP kinase pathway, the tyrosine phosphatase SHP-2, and members of the STAT family of transcription factors. Activation of these pathways induces PDGF's cellular responses, including cell proliferation, survival, actin reorganization and chemotaxis.

Autocrine effect of PDGF in malignancies

The first indication that PDGF has a transforming capacity was the finding 20 years ago that the sis oncogene is derived from the PDGF B-chain gene (Waterfield et al., 1983; Doolittle et al., 1983). Since then, it has become clear that PDGF is often expressed in human tumors, and several examples of co-expression of PDGF and PDGF receptors by tumor cells have been reported, suggesting autocrine stimulation of tumor cell growth (reviewed in Heldin and Westermark, 1999).

There are rare examples of genetic alterations involving PDGF or PDGF receptor genes, which cause constitutive activation of PDGF receptors and contribute to specific types of malignancies (Table 1). A subset of high-grade glioblastomas show amplification (Fleming et al., 1992; Kumabe et al., 1992) or activating deletion

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Table 1: Genetic alterations that give rise to dysregulated PDGF receptor signaling in malignancies. For references, see the text.

Genetic alteration	tumor type
<i>Amplification of the PDGF alpha receptor gene</i>	<i>glioblastoma</i>
<i>Activating point mutations in the PDGF alpha-receptor gene</i>	<i>gastrointestinal stroma tumors</i>
<i>Fusion of the PDGF alpha-receptor gene with the FIPL1 gene</i>	<i>idiopathic hypereosinophilic syndrome</i>
<i>Fusion of the PDGF beta-receptor gene With the genes for Tel or rabaptin</i>	<i>chronic myelomonocytic leukemia</i>
<i>Fusion of the PDGF B-chain gene with the gene for collagen 1A1</i>	<i>dermatofibrosarcoma protuberans</i>

mutation (Clarke and Dirks, 2003) of the PDGF alpha-receptor gene. Patients with chronic myelomonocytic leukemia (CMML) show fusions of the PDGF beta-receptor gene with the genes for the transcription factor Tel or rabaptin-5, which causes constitutive dimerization and activation of the receptor kinase (Golub et al., 1994; Magnusson et al., 2001). Also, a constitutively active FIPIL1-PDGF alpha-receptor fusion protein has been identified in patients with idiopathic hypereosinophilic syndrome (Cools et al., 2003a). Moreover, activating point mutations in the alpha-receptor gene have been described in a subset of patients with gastrointestinal stroma tumor (GIST; Heinrich et al., 2003), which alternatively can have activating mutations in the related stem cell factor receptor. Dermatofibrosarcoma protuberans (DFSP) is an example of a disease in which a PDGF ligand gene is altered; the PDGF B-chain gene is fused to the collagen 1A1 gene, leading to the production of large amounts of a collagen-PDGF fusion protein which is processed to PDGF-BB (O'Brian et al., 1998; Shimizu et al., 1999; Simon et al., 1997).

In addition to the defined specific genetic alterations leading to constitutive activation of PDGF receptors, there is a more general upregulation of PDGF and PDGF receptors,

possibly through epigenetic mechanisms, in glioblastomas (Hermanson et al., 1988) and sarcomas (Smits et al., 1992), suggesting that autocrine PDGF stimulation occur in these tumor types. The reasons for the increased synthesis of PDGF and PDGF receptors in these cases are not known.

PDGF antagonists in the treatment of human malignancies

The most well-characterized PDGF antagonists are on one hand macromolecules (antibodies, soluble receptor domains or DNA aptamers) which bind to PDGF isoforms and prevent them from binding to receptors, and on the other hand low molecular weight receptor kinase inhibitors (reviewed in Östman and Heldin, 2001). The macromolecular antagonists have the advantage of being specific, but are expensive and cumbersome to administer. In contrast, the available kinase inhibitors are not perfectly specific for PDGF receptors, but are relatively inexpensive and easy to administer. The most well-characterized PDGF receptor kinase inhibitor is STI571 (imatinib, Glivec), which inhibits, in addition to PDGF alpha- and beta-receptors, also the kinases of the stem cell factor receptor, Abl and Arg (reviewed in Capdeville et al., 2002). The initial

clinical use of STI571 has revealed only mild and tolerable side effects. Another PDGF receptor kinase inhibitor which have shown promising results in phase I trials is SU11248 (Mendel et al., 2003).

STI571 has through its ability to inhibit Abl been used in the treatment of chronic myeloic leukemia, with very encouraging results. In addition, it has shown promising effects in the treatment of malignancies involving genetic alteration of PDGF and PDGF receptors, including CMML (Apperly et al., 2002; Magnusson et al., 2002), DFSP (Maki et al., 2002; Rubin et al., 2002) and idiopathic hypereosinophilic syndrome (Cools et al., 2003b). In addition, good results have been obtained with STI571 treatment of GIST, which shows activation of PDGF alpha-receptor or stem cell receptor, both of which is sensitive to STI571 (van Oosterom et al., 2001). Clinical trials in which glioblastoma patients are treated with PDGF antagonists are ongoing, but no results have yet been reported.

Paracrine effects of PDGF in tumors

PDGF receptors are generally not expressed in epithelial cells or in tumor cells of epithelial origin. However, PDGF isoforms are often produced in such tumors, and may thus have roles in paracrine stimulation of normal cell types in the tumor which express PDGF receptors. An effect of PDGF in the recruitment and growth of stroma has been demonstrated in animal models; transfection of PDGF B-chain into tumor cells devoid of PDGF receptors led to the formation of a well vascularized stroma without necroses, when these cells were

grown in mice (Forsberg et al., 1993; Skobe and Fuxe, 1998). In such tumors, tumor cell growth may be enhanced through improved nutrition, as well as by paracrine effects of trophic factors produced by stroma cells.

Most solid tumors express PDGF receptors on fibroblasts and myofibroblasts, as well as on endothelial cells or perivascular cells (Hermanson et al., 1988; Sundberg et al., 1993; Pietras et al., 2003). Angiogenic effects of PDGF have been demonstrated in the chick chorioallantoic membrane assay (Risau et al., 1992), the mouse corneal pocket assay (Cao et al., 2002) and in tumors (Li et al., 2003). Important roles for PDGF in pericyte recruitment (Hellström et al., 1999) and smooth muscle cell growth (Crosby et al., 1998), are well established. Whether, in addition, PDGF has angiogenic effects via direct action on endothelial cells, remains to be elucidated.

Although the importance of tumor-stroma interactions are not yet fully characterized, targeting the stroma is an interesting treatment modality which deserves further exploration. In this context, the combination of PDGF antagonists with anti-angiogenic treatment might be a specially efficient complement to the targeting of tumor cells directly.

Another aspect of paracrine stimulation of PDGF in tumors relates to the regulation of tumor interstitial fluid pressure (IFP). PDGF has been shown to be of crucial importance for the regulation of the IFP in the skin (Rodt et al., 1996). Solid tumors often have an increased IFP, which is an obstacle in chemotherapy, since it prevents efficient uptake of chemotherapeutical drugs. Evidence was recently obtained that PDGF contributes to

Table 2: Paracrine effects of PDGF on different types of normal cells in tumors.

<i>Cell type</i>	<i>Consequence of PDGF stimulation</i>
<i>Stromal fibroblasts or myofibroblasts</i>	<i>increased tumor interstitial fluid pressure</i>
<i>Endothelial cells</i>	<i>angiogenesis?</i>
<i>Pericytes and vascular smooth muscle cells</i>	<i>maturation and enforcement of vessels</i>

the increased IFP of tumors in animal models, and that treatment with PDGF antagonists can lower the IFP of tumors (Pietras et al., 2001). Importantly, the decrease in tumor IFP was accompanied by an increased uptake of chemotherapeutic drugs and an increased treatment effect (Pietras et al., 2002; 2003). Future clinical studies will reveal whether PDGF antagonists can enhance chemotherapy also in patients.

Perspectives

The initial results of PDGF antagonist treatment of malignancies caused by a specific mutations in PDGF or PDGF receptor genes, are encouraging. Whether PDGF antagonists will also be useful in treatment of tumors in which PDGF or PDGF receptors are upregulated through epigenetic mechanisms, remains to be elucidated. It will be important to carefully select patients for treatment with PDGF antagonists; preferentially, only patients with activated PDGF receptor should be included. The ability to select patients suitable for treatment

will depend on the development of tools to monitor expression of PDGF and PDGF receptors, e.g. by immunohistochemistry. In this context, phospho-specific antibodies which specifically recognize activated PDGF receptors, would be particularly useful.

The unique importance of PDGF for the development and maintenance of tumor stroma, makes PDGF antagonists potentially useful in attempts to explore anti-stromal therapy of cancers. Such an approach, maybe combined with conventional anti-angiogenic therapy, could be a useful complement to direct targeting of tumor cells. A problem when tumor cells are directly targeted by signal transduction inhibitors is that mutants insensitive to the inhibitors are selected for, which frequently leads to tumor recurrence, despite good initial treatment effect. One advantage of targeting normal cell types in the tumor, is that in this case selection for treatment insensitivity is not anticipated. Additional pre-clinical and clinical studies to explore the possible use of PDGF antagonists in tumor therapy are highly warranted.

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