Structural and functional aspects of platelet-derived growth factor*

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Summary

The finding that PDGF production is common in normal as well as transformed cells indicate that PDGF has a function in autocrine and paracrine stimulation of cells in several physiological and pathological conditions. The expression of mRNA for the two chains of PDGF are independently regulated. The fact that the different dimeric forms of PDGF have different functional effects, indicate that the cellular response to PDGF may be more complex than previously thought, and may involve binding to more than one receptor. The cellular effects ascribed to PDGF, growth stimulation, ruffling and chemotaxis, seems to be mainly associated with B chain containing dimers. The function of PDGF-AA remains to be elucidated.

Platelet-derived growth factor (PDGF) is a major mitogen for connective tissue cells and glial cells cultured in vitro (for reviews see Heldin et al., 1985; Ross et al., 1986). Its in vivo function has not been elucidated, but is localization to the blood platelets in combination with the facts that PDGF stimulates not only proliferation, but also matrix production and chemotaxis of connective tissue cells, has led to the assumption that PDGF has a role in wound healing. However, recent findings that PDGF does not only occur in platelets, but is produced by a variety of different normal and transformed cell types, indicate that PDGF may have wider functions, mediating cell proliferation in a number of normal and pathological conditions.

PDGF exerts its mitogenic effect via binding to a specific 170–185 kD cell surface receptor (reviewed in Heldin & Rönnstrand, 1988). The receptor is a transmembrane protein with an external ligand binding domain, and an internal domain with a protein tyrosine kinase activity that becomes activated after ligand binding (Ek et al., 1982; Yarden et al., 1986).

The mechanism whereby the mitogenic signal is transmitted from the activated receptor and further into the cell, is largely unknown. The fact that several growth factor receptors and oncogene products are protein tyrosine kinases (Hunter, 1987), indicates that tyrosine phosphorylation of specific substrates is important in stimulation of cell growth. In spite of extensive investigations (Cooper et al., 1982; Ek & Heldin, 1984; Frackelton et al., 1984), no substrate for the PDGF receptor kinase, with a proven role in the mitogenic pathway has yet been found. Other signals that have been discussed as part of the PDGF-stimulated mitogenic pathway, include turnover of phosphatidylinositol, with subsequent elevation of the cytoplasmic Ca2+ concentration (Moelenaar et al., 1984), and stimulation of protein kinase C (Rozengurt et al., 1983), as well as induction of specific genes (Cochran et al., 1983, Kelly et al., 1983; Kruijer et al., 1984; Müller et al., 1984, Greenberg & Ziff, 1984).

This review will focus on structural and functional aspects of PDGF-like factors, as well as their possible involvement in autocrine or paracrine stimulation of cell growth.

Structures of PDGF

PDGF is a 30 kD dimeric molecule, composed of two disulphide-bonded polypeptide chains denoted A and B (Johnsson et al., 1984). The PDGF B chain is almost identical to p24sp. the transforming protein of simian sarcoma virus (SSV) (Waterfield et al., 1983; Doolittle et al., 1983; see further below). Analyses of cDNAs of the A (Betscholtz et al., 1986b) and the B (Josephs et al., 1984; Collins et al., 1985; Rao et al., 1986) chain of PDGF

Figure 1 Schematic illustration of the different dimeric forms of PDGF. Both the A and B chains are synthesized as precursor molecules (upper part of the figure) which undergo proteolytic processing (arrows). Amino acid sequence identities are marked by black colour. The two polypeptide chains are assembled into homodimers (PDGF-AA or PDGF-BB) or heterodimers (PDGF-AB) (lower part of the figure). The dimers are held together by disulphide bonds. Note, that it is not known exactly which cysteine residues that are involved in inter-chain disulphide bonds.

revealed that both are synthesized as precursor molecules with hydrophobic signal sequences indicating that they are secretory products. The two chains, which are proteolytically processed after dimerization, are homologous to each other; within the mature parts the amino acid sequence similarity is 60% (Figure 1) (Johnsson et al., 1984; Betscholtz et al., 1986b).

Evidence was recently obtained that a major part of PDGF purified from human platelets is PDGF-AB, i.e. a heterodimer of one A chain and one B chain (Hammacher et al., 1988a). PDGF purified from porcine platelets (Stroobant & Waterfield, 1984), as well as the transforming protein of SSV (Robbins et al., 1983) have been identified as PDGF-BB. Finally, structural analyses of PDGF-like factors purified from the conditioned media of human osteosarcoma (Heldin et al., 1986b) melanoma (Westermark et al., 1986d) and glioma (Hammacher et al., 1988b) cell lines, revealed the existence also of PDGF-AA. Thus, all possible dimeric combinations of PDGF chains have been found (Figure 1).

SSV-transformation is exerted by externalized PDGF-BB

The structural homology between p24sp and PDGF (Waterfield et al., 1983; Doolittle et al., 1983; Robbins et al., 1983), led to the hypothesis that a PDGF-like growth factor is involved in SSV-transformation. This hypothesis has received support from several subsequent observations. First, SSV-transformed cells in vitro produce PDGF-like growth factors which bind to and activate the PDGF receptor (Deuel et al., 1983; Bowen-Pope et al., 1984; Owen et al., 1984; Garrett et al., 1984; Huang et al., 1984; Johnsson et al., 1985). Second, only cell types that respond to PDGF, i.e. have PDGF receptors, can be transformed by SSV (Deinhardt, 1980; Leal et al., 1985). Finally, SSV-transformation can be reverted by agents that prevent the binding

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of PDGF to its receptor, e.g. PDGF antibodies (Johnsson et al., 1985) and suramin (Betsholtz et al., 1986a). The morphological and functional characteristics of acutely SSV-transformed human fibroblasts indicate that the infected cell receives a powerful autocrine stimulation to grow, but other features that have been associated with the malignant phenotype, e.g. immortalization, do not occur (Johnsson et al., 1986). In conclusion, studies on SSV-transformed cells in vitro are fully compatible with a simplistic model for SSV-transformation, involving the autocrine action of a factor similar to PDGF-BB. However, to explain the malignant glioblastomas and fibrosarcomas that develop in marmoset monkeys infected by SSV (Deinhardt, 1980), one has to propose that additional genetic alterations, which are responsible for the development of the fully malignant phenotype, occur in the infected cells (Westermark et al., 1986a).

**Production of PDGF in human tumour cells**

Expression of PDGF A or B chain mRNA, or production of PDGF receptor competing activity, have been found in a variety of human tumour cell lines (reviewed in Heldin et al., 1986a). No correlation has been found between the expressions of the A and B chains, indicating that they are independently regulated.

Several cell lines of connective tissue cell origin or glial origin have been found to produce PDGF. Since the normal counterparts of these cells respond to PDGF, it is possible that the endogenous growth factor production has an autocrine effect and drives tumour cell growth. PDGF production is common in human glioma cell lines; 23 and 17 cell lines, out of 23 investigated, express A and B chain mRNA, respectively (Nilsson et al., 1986b).

Cell lines derived from cell types that do not respond to PDGF have also been found to produce PDGF. Since these tumour cells lack PDGF receptors it is highly unlikely that the endogenous PDGF production has any impact on tumour cell growth. PDGF production is common also in these tumour types; 8 and 9 mammary carcinoma cell lines, out of 10 investigated, were found to express the A and B chain mRNA, respectively (Perez et al., 1987). If PDGF production also occurs in carcinomas in vivo, it is possible that this contributes to the stimulation of stroma cell proliferation, which is a common finding in many carcinomas.

**PDGF in autocrine and paracrine stimulation of normal cells**

Smooth muscle cells (Seifert et al., 1984; Nilsson et al., 1985) and placental cytotrophoblasts (Goustein et al., 1985) have been found to produce PDGF. Since these cell types also respond to PDGF, it is possible that the PDGF production serves an autocrine function. This suggests a role of PDGF in pathophysiological reactions in the vessel wall and in placental growth. Clearly such autocrine systems in normal cells must be subjected to regulation, but little is known about the factors that exert such control functions. It was recently found that stimulation of human fibroblasts by mitogens led to expression of PDGF A chain mRNA and production of PDGF receptor competing activity (Paulsson et al., 1987). Though the interpretation of this finding is not clear, it suggests the presence of a positive feed-back mechanism which may amplify the mitogenic signal.

Endothelial cells (DiCorleto & Bowen-Pope, 1983) and activated macrophages (Shimokado et al., 1985; Martinet et al., 1986) are examples of normal cells that produce PDGF but do not have PDGF receptors. Though it is unlikely that the PDGF production in these cases has any autocrine function, it is possible that the factor stimulates neighbouring cells in a paracrine fashion. Thus, PDGF secreted by regenerating endothelial cells in the vessel wall could stimulate the proliferation of underlying smooth muscle cells, and PDGF secreted by activated macrophages could be involved in the stimulation of connective tissue cells that is often seen in chronic inflammatory processes.

**Difference in the functional activities of different dimeric forms of PDGF**

PDGF-like growth factors were recently purified from the conditioned medium of a human glioma cell line, U-343 MGa CI 2:6 (Hammacher et al., 1986b), which expresses both PDGF A and B chain mRNA (Betsholtz et al., 1986b). All three possible dimeric forms of PDGF were identified, but PDGF-AA was by far the predominant species. Analyses of PDGF-AA in several functional assays revealed differences compared to PDGF-AB; PDGF-AA had lower mitogenic activity, lower ability to stimulate actin reorganization and membrane ruffling, and no chemotactic activity (Nistér et al., 1986e). Receptor binding experiments suggested that there exists more than one PDGF receptor class. The observed dissimilarities in functional activities of different dimeric forms of PDGF, might be explained by differences in ligand binding specificity between these receptor classes.

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