Minireview

Simultaneous induction of stimulatory and inhibitory signals by PDGF

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Abstract Platelet-derived growth factor (PDGF) exerts its effects on cells via binding to structurally similar α- and β-tyrosine kinase receptors. Ligand binding induces receptor dimerization and autophosphorylation which allows docking of SH2 domain containing signal transduction molecules. At least 10 different SH2 domain molecules bind in a specific manner to 11 identified autophosphorylated tyrosine residues in the PDGF β-receptor, thereby initiating signaling pathways leading to cell growth and motility. Available information indicates that there is considerable cross-talk between different signaling pathways, and that stimulatory and inhibitory signals often are initiated in parallel.

Key words: Platelet-derived growth factor; Stimulatory signal induction; Inhibitory signal induction

1. Introduction

Platelet-derived growth factor (PDGF) is a family of signaling molecules of particular importance for growth and motility of connective tissue cells (reviewed in [1,2]). Structurally, PDGF isoforms are disulfide-bonded dimers of homologous A- and B-polypeptide chains, arranged as homodimers (PDGF-AA and PDGF-BB) or a heterodimer (PDGF-AB). PDGF isoforms have important functions during the embryonal development. Mice with the B-chain gene inactivated show a striking deficiency of mesangial cells of the kidney glomeruli leading to a defect filtration [3]. PDGF A-chain gene knock-out resulted in generalized lung emphysema due to a lack of alveolar myofibroblasts [4]. In the adult, PDGF stimulates wound healing [5], and overactivity of PDGF is sometimes associated with tumorigenesis and fibrotic disease (reviewed in [1]). These findings are consistent with an important function of PDGF in connective tissue development.

PDGF isoforms exert their effects on target cells by binding to two structurally related protein tyrosine kinase receptors. The α-receptor binds both the A- and B-chains of PDGF, whereas the β-receptor binds only the B-chain; since the ligand is bivalent, the different PDGF isoforms induce specific dimeric complexes of PDGF receptors. Both α- and β-receptors induce mitogenic responses. Both receptors also stimulate actin reorganization in the form of edge ruffling and loss of stress fibers; the β-receptor, but not the α-receptor, in addition stimulates the formation of circular ruffles on the dorsal surface of the cell. Moreover, whereas the β-receptor mediates stimulation of chemotaxis, the α-receptor does not; in fact the α-receptor inhibits the chemotaxis of certain cell types (reviewed in [1]). The present review focuses on the intracellular signal transduction pathways that are involved in PDGF-induced cell growth and motility, and will discuss the fact that often stimulatory and inhibitory pathways are induced in parallel.

2. Activation of PDGF receptors

Receptor dimerization is an important event in signal transduction via PDGF receptors, as is the case for most if not all tyrosine kinase receptors [6]. The juxtaposition of the kinase domains of two receptors allows phosphorylation *in trans* between the receptors, often referred to as autophosphorylation. The autophosphorylation serves two important functions: it increases the catalytic activity of the kinase and it provides docking sites for downstream signal transduction molecules.

The known autophosphorylated tyrosine residues in the β-receptor for PDGF are illustrated in Fig. 1. One site is a conserved tyrosine residue localized inside the kinase domain (Tyr^{857}). Mutation of this residue to a phenylalanine residue gives a receptor with a lowered kinase activity, suggesting that phosphorylation of Tyr^{857} is important for activation of the kinase [7]. The other autophosphorylation sites are spread out over the cytoplasmic part of the receptor; a total of 11 of the 15 tyrosine residues in the noncatalytic part of the receptor are phosphorylated (reviewed in [8]; Rönnstrand et al., unpublished observations). Also the α-receptor becomes phosphorylated on several tyrosine residues, but the mapping data are less complete for this receptor.

3. Interaction between the activated PDGF β-receptor and SH2 domain proteins

An important aspect of signal transduction is regulated protein-protein interactions between components in the different signaling pathways. Such interactions are directed by different types of domains, e.g. SH2 and PTB domains which recognize phosphorylated tyrosine residues in specific environments (reviewed in [9]). At least 10 different SH2 domain containing molecules bind to different phosphorylated tyrosine residues in the PDGF β-receptor in a specific manner (Fig. 1); the specificity is primarily determined by the character of the 3–6 amino acid residues downstream of the phosphorylated tyrosine. No PTB domain is known to bind to the receptor. The SH2 domain proteins that interact with the PDGF β-receptor fall into two categories, i.e. molecules with enzymatic or other activity, and adaptor molecules which serve to connect the receptor with other molecules.

One class of molecules that bind to the receptor are themselves tyrosine kinases, i.e. members of the Src family, which bind to phosphorylated Tyr^{579}, and with lower affinity to Tyr^{581}, in the juxtamembrane domain [10]. The binding leads to activation of the Src kinase, after which Src phosphorylates

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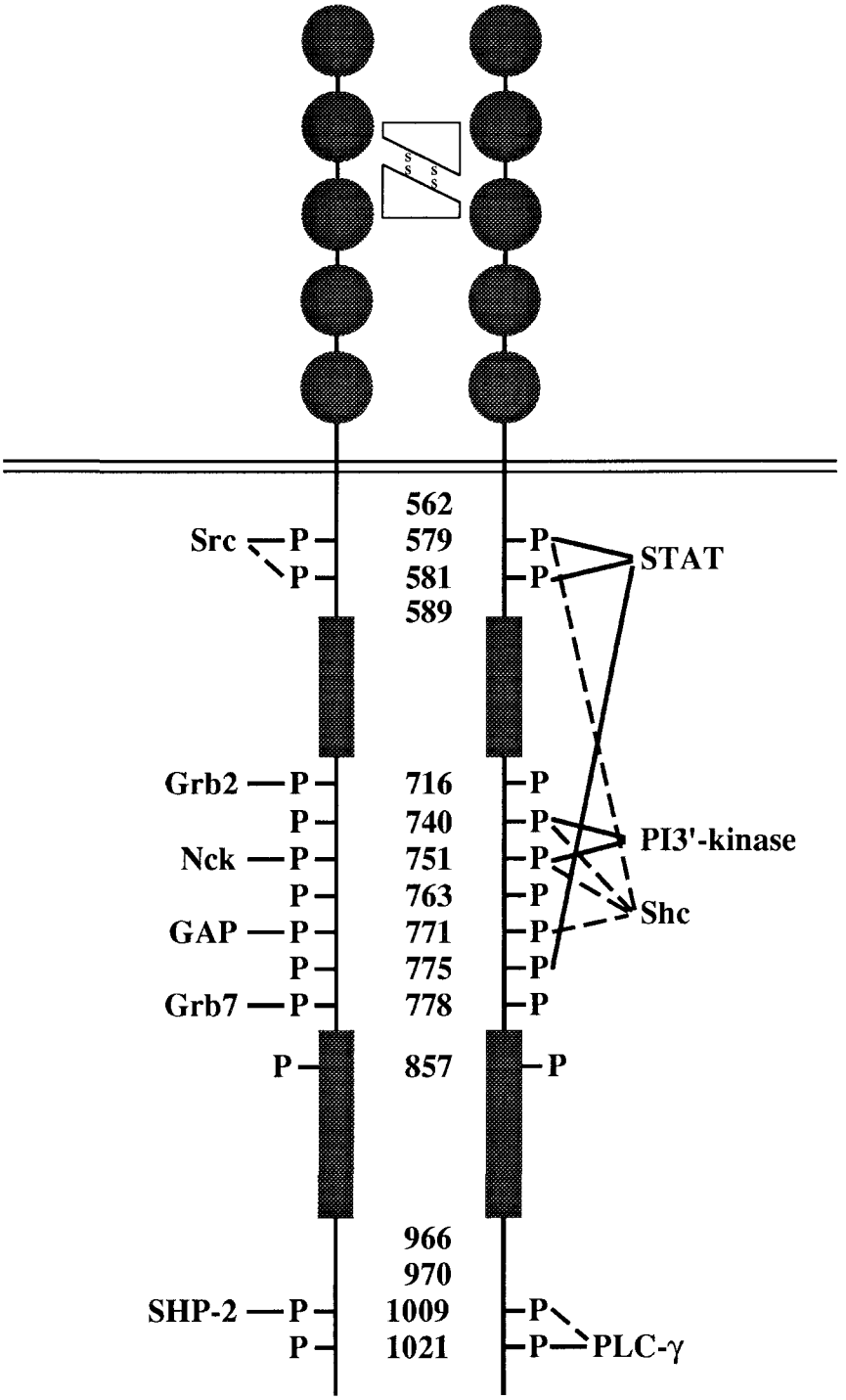

Fig. 1. Schematic illustration of the interaction between the autophosphorylated PDGF β-receptor and downstream signal transduction molecules. A ligand-induced dimeric receptor complex is shown. Ig-like domains in the extracellular part are illustrated by filled circles. Tyr^{857} inside the C-terminal part of the kinase domain (filled rectangles) is indicated, as well as the numbers of all intracellular tyrosine residues outside the catalytic domain; tyrosine residues known to be autophosphorylated are indicated with P. The specificity in the interaction between SH2 domain molecules and autophosphorylated tyrosine residues is indicated; solid lines indicate high affinity interactions and broken lines interactions of lower affinity. Note that it is not known how many SH2 domain proteins that can bind to the receptor dimer simultaneously.

the PDGF receptor (see further below), as well as other substrates. Members of the Src family have important roles in PDGF-induced mitogenicity [11], but the exact mechanism for their involvement remains to be elucidated.

An important signaling molecule, which binds to the PDGF β-receptor, is phosphatidylinositol 3′-kinase (PI 3′-kinase). The regulatory subunit, p85, binds to phosphorylated Tyr^{740} or Tyr^{751} in the kinase insert of the receptor [12,13]. Hereby, the activity of the catalytic subunit, p110, increases, leading to the phosphorylation of phosphatidylinositol(4,5)bisphosphate ($PI(4,5)P_2$) to $PI(3,4,5)P_3$. The downstream effectors of PI 3′-kinase include the serine/threonine kinase Akt, certain mem-

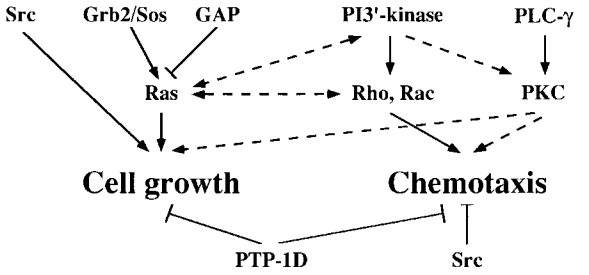

Fig. 2. Schematic illustration of signaling pathways downstream of the PDGF β-receptor that are important for cell growth and chemotaxis. Stimulatory (→) and inhibitory (—ı) pathways are indicated with solid lines. Accessory pathways are indicated with broken lines.

bers of the protein kinase C (PKC) family, as well as the small GTP binding proteins Rho, Rac and Ras (reviewed in [14]). Studies of tyrosine to phenylalanine mutants of the PDGF β-receptor which have been rendered specifically unable to activate the PI 3′-kinase, as well as studies using wortmannin and other PI 3′-kinase inhibitors, have indicated that PI 3′-kinase has an important role in signaling pathways leading to chemotaxis and actin reorganization [15,16], and in certain cell types it is also of importance for other PDGF responses, including cell growth [12,13].

Phospholipase C-γ (PLC-γ) is an enzyme which also uses $PI(4,5)P_2$ as substrate, and cleaves it into inositoltrisphosphate which mobilizes Ca^{2+} from intracellular stores and diacylglycerol which activates members of the PKC family. PLC-γ binds to phosphorylated Tyr^{1021} and, with lower affinity, Tyr^{1009}, in the C-terminal tail of the PDGF β-receptor [17]. This leads to tyrosine phosphorylation of PLC-γ, which increases its catalytic activity. Mutation of Tyr^{1021} to a phenylalanine residue, which leads to an inability of the mutant receptor to phosphorylate and activate PLC-γ, has no dramatic effect on signaling via the PDGF β-receptor. However, when signaling from the receptor is severely compromised by mutation of several tyrosine residues, reintroduction of Tyr^{1021}, and thereby of the ability to activate PLC-γ, is associated with reappearance of some mitogenic signaling [18]. Moreover, PLC-γ has been shown under certain circumstances to be involved also in chemotactic signaling [19,20].

Another SH2 domain molecule which binds to the C-terminal tail of the β-receptor is SHP-2, a protein tyrosine phosphatase which binds to phosphorylated Tyr^{1009} [21]. The interaction leads to activation of the enzymatic activity with potential ability to dephosphorylate the autophosphorylated receptors.

Activation of the small GTP binding protein Ras is an important event in PDGF signaling. Ras is converted to the GTP-bound form, and thereby activated, by the nucleotide exchange factor Sos1 which forms a complex with the SH2 domain containing adaptor molecule Grb2. The activating event is the translocation of the Grb2/Sos1 complex to the inside of the cell membrane, where Ras is located, through direct binding to the PDGF β-receptor to phosphorylated Tyr^{716} [22], or indirectly via the adaptor Shc [23] or the tyrosine phosphatase SHP-2 [24], which are both phosphorylated on tyrosine residues by the PDGF receptor. The downstream activators of Ras include the MAP kinase cascade which is important in PDGF-mediated stimulation of cell growth [25,26].

Ras is deactivated when the bound GTP is dephosphorylated to GDP, a process which is catalyzed by the GTPase activating protein (GAP) of Ras. GAP binds to phosphorylated Tyr^{771} of the PDGF β-receptor [12,13], an interaction which potentially regulates Ras activation.

Signal transducers and activators of transcription (STATs) are of particular importance for signaling via cytokine receptors (reviewed in [27]). However, certain STAT members are also phosphorylated and activated by PDGF receptors [28]. They interact with phosphorylated Tyr^{579}, Tyr^{581} and Tyr^{775} [29], but their significance in PDGF signaling remains to be determined.

Grb2 and p85 of PI 3′-kinase are two adaptor molecules with well characterized binding partners, i.e. Sos1 and p110, respectively. However, a number of other adaptor molecules, for which the interactive partners are less well known, also bind to the PDGF β-receptor. These include Nck which binds to phosphorylated Tyr^{751} [30], Shc which binds with rather low affinity to several of the autophosphorylated tyrosine residues in the receptor [31], and Grb7 which is structurally related to Grb2 and binds to phosphorylated Tyr^{775} [32].

4. Cross-talk between signaling pathways leading to cell growth and chemotaxis

The available information regarding the involvement of different signaling pathways downstream of PDGF β-receptors in stimulation of cell growth and chemotaxis is summarized in Fig. 2. As discussed above, activation of Ras, via direct or indirect binding of the Grb2/Sos1 complex to the receptor is important for stimulation of cell growth [25,26]. Src is also important for stimulation of cell growth in a pathway involving the transcription factor c-Myc [33]. Activation of PI 3′-kinase is of major importance for stimulation of chemotaxis, presumably via activation of Rac or other small GTP binding proteins [34]. PLC-γ appears not to be of major importance for signaling cell growth or chemotaxis, but may in certain situations stimulate both cell growth [18] and chemotaxis [19,20], presumably via activation of PKC or increase in the cytoplasmic Ca^{2+} concentration.

Recent studies have given several examples of cross-talk between different signaling pathways. For instance, a direct physical interaction between Ras and PI 3′-kinase has been demonstrated as well as activation of PI 3′-kinase by Ras [35]. Certain PI 3′-kinase responses have also been shown to be mediated by Ras [36]. Moreover, the small GTP binding proteins Ras, Rho and Rac have been shown to activate each other [37–39]. Such cross-talk may be the reason why Ras activation induces not only cell proliferation, but also under certain conditions chemotaxis [40], and may also explain why PI 3′-kinase in addition to its major role in stimulation of actin reorganization and chemotaxis also in some cell types stimulates cell growth [12,13].

5. Stimulatory and inhibitory signals for cell growth and chemotaxis

An interesting observation is that activation of PDGF receptors often appears to induce stimulatory and inhibitory signals for cell growth as well as motility in parallel. Some examples will be discussed below.

The tyrosine phosphatase SHP-2 binds to activated and

autophosphorylated PDGF receptors, an interaction which increases the phosphatase activity of SHP-2 [41]. Moreover, it has been shown that the autophosphorylated receptor is a substrate for SHP-2 [42]. Thus, it is an interesting possibility, which remains to be elucidated, that the recruitment of SHP-2 to the autophosphorylated PDGF receptors serves a feedback control function leading to a dephosphorylation of the receptor and thus a decreased interaction with other SH2 domain proteins. It should be noted that SHP-2 may also be involved in stimulatory pathways [24].

Another, more specific, feedback mechanism could be exerted by GAP. Ras is localized at the cell membrane and is activated after translocation of the Grb2/Sos1 complex to the autophosphorylated PDGF β-receptor. The juxtaposition at the receptor of GAP which deactivates Ras could serve as a feedback control mechanism; however, this possibility needs to be experimentally verified. Interestingly, GAP binds to the β-receptor but not to the α-receptor [43], and this may be one explanation for the differences in signaling between the two receptors.

Binding of members of the Src family of tyrosine kinases to the juxtamembrane domain of the PDGF β-receptor leads to their activation [10]. The Src kinase then phosphorylates the receptor on Tyr^{934} located within the C-terminal part of the kinase domain [19], as well as other substrates. Mutation of Tyr^{934} to a phenylalanine gives a receptor with decreased ability to mediate cell growth, but an increased ability to mediate cell motility [19]. This finding is consistent with the notion that Src phosphorylation of Tyr^{934} is important for stimulation of cell growth, and also suggests that this phosphorylation exerts a negative effect on motility signals.

In contrast to the β-receptor, the α-receptor for PDGF inhibits chemotaxis induced by other agents, e.g. in human fibroblasts [44], smooth muscle cells [45], and transfected PAE cells [46]. PI 3′-kinase, which has been shown to have an important role in stimulation of chemotaxis by the β-receptor, also binds to the α-receptor [47]. Yokote et al. [46] explored the possibility that inhibitory signals also are initiated after activation of the α-receptor. Analysis of a series of tyrosine to phenylalanine residue mutants revealed three that were able to stimulate chemotaxis, i.e. receptor mutants in which Tyr^{768}, Tyr^{993} or Tyr^{1018} were replaced with phenylalanine residues. Tyr^{768} and Tyr^{1018} are autophosphorylation sites, which then may serve as docking sites for molecules which inhibit chemotaxis. Tyr^{993} is not autophosphorylated, but its mutation leads to an increased phosphorylation of the neighboring Tyr^{988}, which may be involved in stimulation of chemotaxis.

6. Concluding remarks

Research during the recent years has revealed several signal transduction pathways that are activated by PDGF. However, the information about the importance of individual pathways in PDGF-stimulated cell growth and motility is still incomplete. Two striking general principles have appeared from these studies so far: there is considerable cross-talk between individual pathways, and often stimulatory signals are initiated in parallel to inhibitory ones. This organization of intracellular signal transduction allows modulation and fine tuning. Since individual signal transduction molecules may be present at different amounts in different cell types, another consequence is that PDGF stimulation may give different effects in different PDGF responsive cell types.

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