Structural and Functional Properties of Platelet-Derived Growth Factor and Stem Cell Factor Receptors

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The receptors for platelet-derived growth factor (PDGF) and stem cell factor (SCF) are members of the type III class of PTK receptors, which are characterized by five Ig-like domains extracellularly and a split kinase domain intracellularly. The receptors are activated by ligand-induced dimerization, leading to autophosphorylation on specific tyrosine residues. Thereby the kinase activities of the receptors are activated and docking sites for downstream SH2 domain signal transduction molecules are created; activation of these pathways promotes cell growth, survival, and migration. These receptors mediate important signals during the embryonal development, and control tissue homeostasis in the adult. Their overactivity is seen in malignancies and other diseases involving excessive cell proliferation, such as atherosclerosis and fibrotic diseases. In cancer, mutations of PDGF and SCF receptors—including gene fusions, point mutations, and amplifications—drive subpopulations of certain malignancies, such as gastrointestinal stromal tumors, chronic myelomonocytic leukemia, hypereosinophilic syndrome, glioblastoma, acute myeloid leukemia, mastocytosis, and melanoma.

The type III tyrosine kinase receptor family consists of platelet-derived growth factor (PDGF) receptor α and β , stem cell factor (SCF) receptor (Kit), colony-stimulating factor-1 (CSF-1) receptor, and Flt-3 (Blume-Jensen and Hunter 2001). Members of this receptor family are characterized by five Ig-like domains in their extracellular part, a single transmembrane domain, and an intracellular part consisting of a rather well-conserved juxtamembrane domain, a tyrosine kinase domain with a characteristic inserted sequence without homology

with kinases, and a less well-conserved carboxy-terminal tail. The ligands for these receptors are all dimeric molecules, and on binding they induce receptor dimerization. Although the overall mechanisms for the activation of the type III tyrosine kinase receptors and the signaling pathways they induce are similar, the receptors are expressed on different cell types and thus have different functions in vivo.

Here we will describe the structural and functional properties of the PDGF receptors and Kit.

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PDGF RECEPTORS

Ligand-Binding Specificities of PDGF Receptors

The PDGF family consists of five members (i.e., disulfide-bonded dimers of homologous A-, B-, C-, and D-polypeptide chains, and the AB heterodimer) (Heldin and Westermark 1999). The PDGF- α receptor binds all PDGF chains except the D chain, whereas the β receptor binds PDGF-B and -D; thus, the different PDGF isoforms can induce $\alpha\alpha$ -, $\alpha\beta$ -, or $\beta\beta$ -receptor dimers (Fig. 1). The ligand-binding sites are located in Ig-like domains 2 and 3 (Heidaran et al. 1990; Lokker et al. 1997; Miyazawa et al. 1998; Shim et al. 2010); however, ligand-induced receptor dimerization is stabilized by direct receptor–receptor interactions in Ig-like domains 4 and 5 (Omura et al. 1997; Yang et al.

2008). The latter interactions are important because they orient the receptors so that their activation by autophosphorylation in *trans* is facilitated. Binding of vascular endothelial growth factor (VEGF)-A to PDGFR- α and PDGFR- β has been reported (Ball et al. 2007), but the physiological significance of this finding remains to be elucidated.

Ligand stimulation results in homo- as well as heterodimerization of PDGF- α and - β receptors; the different dimeric receptor complexes have overlapping but slightly different signaling capacities (see further below). However, PDGF receptors can also form complexes with other tyrosine kinase receptors, such as the epidermal growth factor (EGF) receptor (Saito et al. 2001) and fibroblast growth factor (FGF) receptor-1 (Faraone et al. 2006), but also with non-kinase receptors, such as integrins (Sundberg

Figure 1. Ligand-binding specificities of PDGF and SCF receptors. The different ligands are depicted above the respective receptor dimers they bind to. Binding of PDGF-CC and PDGF-DD to $\alpha\beta$ -heterodimeric PDGF receptors have also been described, but the functional significance of such complexes remains to be determined.

and Rubin 1996; Schneller et al. 1997), CD44 (Li et al. 2006), the low-density lipoprotein receptor-related protein (LRP) (Boucher et al. 2002; Loukinova et al. 2002), and the poliovirus receptor Necl-5 (Minami et al. 2010). Such interactions modulate signaling via PDGF receptors.

Activation of PDGF Receptor Kinases

PDGF-induced receptor dimerization leads to autophosphorylation of certain tyrosine residues in the intracellular parts of the receptors. Thus, the α and β receptors have 10 and 11 autophosphorylation sites, respectively (Fig. 2) (Heldin et al. 1998). The autophosphorylation serves two important functions: It leads to changes in the conformation of the intracellular parts of the receptors promoting their activation, and it provides docking sites for SH2-domain-containing signal transduction molecules.

There are at least three mechanisms involved in activation of PDGF receptor kinases.

Like most tyrosine kinase receptors (Hubbard 1997), the PDGF receptors are autophosphorylated in the activation loop of the kinases (residues Tyr849 and Tyr857 in the α and β receptors, respectively). Phosphorylation of this residue of the β receptor has been shown to be necessary for full activation of the receptor kinase (Baxter et al. 1998). Probably phosphorylation causes a change in conformation of the activation loop, which opens up the active site of the kinase and allows access of ATP and protein substrate. Moreover, truncation of the carboxy-terminal tail of the β receptor causes receptor activation (Chiara et al. 2004). This suggests that the carboxyl terminus in the resting state is folded over the kinase domain keeping the kinase inactive; autophosphorylation in the carboxyl terminus is likely to relieve the inhibition. Finally, in the resting state, the juxtamembrane domain of several tyrosine kinase receptors has been shown by X-ray crystallography to be folded over and inhibit the kinase domain; autophosphorylation causes a change in conformation, which

Figure 2. Binding of SH2-containing signaling molecules to phosphorylation sites in PDGF and SCF receptors. The known phosphorylated tyrosine residues and the molecules that bind to them are indicated. Y849, Y857, and Y823 in the α receptor, β receptor, and Kit, respectively, are located in the activation loops of the kinase domains; no molecules are known to bind to these phosphorylation sites. Y934 and Y900 in the β receptor and Kit, respectively, are not autophosphorylation sites, but phosphorylated by Src.

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relieves the inhibition (Wybenga-Groot et al. 2001; Hubbard 2004). A similar inhibitory function has been shown in the PDGF- β receptor (Irusta et al. 2002). Moreover, in the context of oncogenic fusion proteins of the PDGF receptors, the juxtamembrane domains have been shown to have an inhibitory function (see further below), supporting the notion that the juxtamembrane domain has an inhibitory function also in full-length PDGF receptors. Together, these mechanisms cooperate to keep the kinase inactive; autophosphorylation of several tyrosine residues is necessary for full activation of the receptor kinase.

Activation of Intracellular Signal Transduction Pathways by PDGF Receptors

The second important function of autophosphorylation of the PDGF receptors is to allow binding of signaling molecules containing SH2 domains, which recognize phosphorylated tyrosine residues (Heldin et al. 1998). Because different SH2 domains have different preferences regarding the three to six amino acid residues downstream from the phosphorylated tyrosine, there is a certain specificity in binding. The PDGF receptors have been reported to bind about 10 different families of SH2-domain-containing molecules, which initiates the activation of different signaling pathways (Fig. 2). Because the autophosphorylation pattern of PDGF- α and - β receptors differ depending on whether the receptors occur in homo- or heterodimeric complexes, each of the three dimeric PDGF receptor complexes have distinct signaling properties (Ekman et al. 1999).

Certain of the SH2-domain signaling molecules that bind to PDGF receptors have intrinsic enzymatic activities (i.e., members of the Src family of tyrosine kinases, the GTPase-activating protein [GAP] for Ras, the tyrosine phosphatase SHP-2, and phospholipase C- γ [PLC- γ]) (reviewed in Heldin and Westermark 1999). The respective enzymatic activities are activated by binding to the receptors or by phosphorylation by the receptor kinases. Alternatively, the enzymes are constitutively active and just

brought to the inner leaflet of the plasma membrane by the activated receptors.

There are also examples of SH2-domain-containing adaptor molecules, including Nck, Shc, and Crk, which bind to activated PDGF receptors. They act by mediating interactions with different downstream signaling molecules. Some of them form stable complexes with enzymes, such as Grb2, which form a complex with the nucleotide exchange molecule SOS1 activating Ras. Moreover, the α - or β -regulatory p85 subunits of the phosphatidylinositol 3'-kinase bind to the receptors and to the α - or β -p110 catalytic subunits. In addition, certain members of the STAT family of transcription factors bind to and are activated by PDGF receptors (reviewed by Heldin and Westermark 1999).

The intracellular parts of the PDGF receptors also interact with certain signaling molecules independent of autophosphorylation (e.g., the PDZ-domain protein NHERF), which binds to the end of the carboxy-terminal tail of the PDGF receptors and enhances receptor signaling (Maudsley et al. 2000; Demoulin et al. 2003; Takahashi et al. 2006; Theisen et al. 2007), and the adaptor molecule Alix, which binds to the region around Tyr1021 of the carboxy-terminal tail and facilitates binding of the ubiquitin ligase Cbl (Lennartsson et al. 2006).

In order for initiation of signaling via PDGF receptors to be efficient, tyrosine phosphatases need to be inactivated (Sundaresan et al. 1995). This is achieved by a PI3-kinase-dependent oxidation of a cysteine residue in the active site of phosphatases (Bae et al. 2000).

Much effort has been put into the elucidation of which signaling pathways mediate the various effects of PDGF on cells (i.e., cell proliferation, survival, chemotaxis, and actin reorganization). Although cell-type differences have been reported, in general, PI3 kinase has been found to be important for the antiapoptotic and motility responses of PDGF. Src via activation of the transcription factor Myc, and Ras via activation of the Erk MAP kinase pathway, are important for the growth-stimulating effects. It should be noted, however, that there is an extensive cross talk between different signaling pathways. Thus, each of the many signaling pathways in-

duced by the activated receptor can, to different extents and in a cell-type-specific manner, contribute to most of the cellular effects of PDGF.

Modulation of Signaling via PDGF Receptors

Both $\alpha\alpha$ - and $\beta\beta$ -receptor complexes induce powerful mitogenic signals. However, whereas $\beta\beta$ -receptor homodimers and $\alpha\beta$ heterodimers induce chemotaxis, the $\alpha\alpha$ homodimer inhibits chemotaxis, at least in certain cell types (Eriksson et al. 1992). The $\alpha\beta$ -heterodimeric receptor complex appears to induce the most potent mitogenic signal. A mechanism for this difference could be that Tyr771, to which RasGAP binds, is efficiently phosphorylated in the $\beta\beta$ homodimer, but not in the $\alpha\beta$ heterodimer (Ekman et al. 1999). Thus, RasGAP, which deactivates Ras, cannot bind to the $\alpha\beta$ -receptor heterodimer leading to a more efficient activation of Ras by the $\alpha\beta$ -receptor heterodimer.

The simultaneous binding to activated PDGF receptors of the Grb2/SOS1 complex, which activates Ras, and of RasGAP, which inactivates Ras, provides an example of how signaling via PDGF receptors can be modulated. Another example is the binding of the tyrosine phosphatase SHP-2, which dephosphorylates the β receptor and its substrates (Lechleider et al. 1993). However, in addition to negatively modulating PDGF receptor signaling via dephosphorylation, SHP-2 also positively contributes to signaling through dephosphorylation of the carboxy-terminal tyrosine residue in Src, whereby Src is activated, and by functioning as an adaptor, which can bind Grb2/SOS1, thus promoting activation of Ras (Dance et al. 2008).

Internalization and Sorting of PDGF Receptors

Following ligand binding, PDGF receptors are accumulated in coated pit areas at the cell membrane, and then internalized in a clathrin- and dynamin-dependent manner, in a process that partly depends on the kinase activity of the receptors (Sorkin et al. 1991). Signaling continues in endosomes (Wang et al. 2004), until the pH decreases enough to cause dissociation of PDGF

from its receptors. Most of the internalized PDGF receptors are degraded by fusion of endosomes with multivesicular bodies and lysosomes, or by degradation in proteasomes, processes that are promoted by polyubiquitination of the receptors (Heldin et al. 1982; Mori et al. 1992). Ubiquitination of the PDGF- β receptor can be performed by the ubiquitin ligase Cbl (Miyake et al. 1999); degradation of Cbl is promoted by the adaptor molecule Alix, which binds to the PDGF- β receptor, thereby preventing ubiquitination of the receptor and thus stabilizing it (Lennartsson et al. 2006).

Although most of PDGF receptors are degraded after ligand-induced internalization, there are mechanisms that affect sorting and promote recycling of the receptor to the plasma membrane. One such mechanism was shown to involve overactivation of PLC- γ and its downstream effectors PKC α in cells deficient of the tyrosine phosphatase TC-PTP (Karlsson et al. 2006); TC-PTP dephosphorylates preferentially Tyr1021 in the PDGF- β receptor, which is the binding site of PLC- γ . The overactivated PLC- γ was found to promote recycling in a protein kinase C-dependent manner (Hellberg et al. 2009). Another mechanism was found to operate in Ras-transformed fibroblasts; in these cells, PI3-kinase is overactivated leading to internalization of receptors via macropinocytosis, which is accompanied by increased recycling (Schmees et al. 2012). In both these cases, the induction of recycling was associated with an increased PDGF signal strength and duration. Thus, modulation of intracellular sorting mechanisms can affect PDGF signaling.

Function of PDGF and PDGF Receptors during Embryonal Development

Primarily based on gene knockout studies in mice, PDGF and PDGF receptors have been shown to have important roles to promote proliferation, migration, and differentiation of specific cell types during the embryonal development (reviewed by Andrae et al. 2008). A common theme that has emerged from these studies is that PDGF isoforms, secreted by epithelial or endothelial cells, act in a paracrine

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manner on nearby mesenchymal cells, such as fibroblasts, pericytes, and smooth muscle cells (Hoch and Soriano 2003; Andrae et al. 2008).

Knockout of PDGFR- α and PDGF-A was found to affect early mesenchymal derivatives in both embryo and extraembryo tissues, and a proportion of these mice die before or at embryonic day 10.5 (Hoch and Soriano 2003). Knockout of PDGFR- α also causes defects in neural crest mesenchyme derivatives, including the cardiac outflow tract, the thymus and skeletal components in the facial and other regions, and in the development of the palate and teeth (Soriano 1997; Tallquist et al. 2000; Tallquist and Soriano 2003; Xu et al. 2005).

PDGF-A knockout mice that survive birth develop lung emphysema, because PDGFR- α -positive alveolar myofibroblast precursors do not migrate to the alveolar saccules (Boström et al. 1996; Lindahl et al. 1997b). Knockout of PDGF-A or PDGFR- α in mice also leads to abnormal development of gastrointestinal villi (Karlsson et al. 2000), skin blistering (Soriano 1997), reduced hair development (Karlsson et al. 1999), and defect development of Leydig cells of the testis (Gnessi et al. 2000; Brennan et al. 2003). In each case, the interaction between the PDGFR- α expressed by mesenchymal cells of different kinds, and the ligand expressed by neighboring epithelial cells, is perturbed.

PDGFR- α also mediates signals needed for proliferation and differentiation of oligodendrocyte progenitor cells (Calver et al. 1998) and retinal astrocytes (Fruttiger et al. 1996), and determines the number of oligodendrocyte progenitor cells in the adult brain (Woodruff et al. 2004). PDGF-AA is constitutively secreted from neuronal cell bodies but not from axons (Fruttiger et al. 2000).

PDGFR- β is expressed on pericytes and vascular smooth muscle cells and mediates recruitment of these cells to newly formed vessels in response to PDGF-BB secreted by endothelial cells (Lindahl et al. 1997a; Hellström et al. 1999; Bjarnegård et al. 2004), and in particular by the tip cells that lead the angiogenic sprout (Gerhardt et al. 2003). PDGFR- β knockout embryos progressively develop abnormal glomeruli in the kidney owing to defect development

of mesangial cells (Levéen et al. 1994; Soriano 1994; Lindahl et al. 1998), capillary microaneurysm (Lindahl et al. 1997a), cardiac defects (Van den Akker et al. 2008), and placental defects (Ohlsson et al. 1999), and die at E16–E19 from hemorrhage. A role for PDGFR- β in the early development of hematopoietic/endothelial precursors has also been shown; activation of PDGFR- β on these cells drives differentiation toward endothelial cells (Rolny et al. 2006).

It is thus clear that PDGFR- α and PDGFR- β have different functions during embryonal development. To determine whether the differences are owing to different expression patterns or to different signaling capacities, the intracellular parts of the receptors were swapped (Klinghoffer et al. 2001). Whereas loss of the cytoplasmic part of PDGFR- α could be rescued by the cytoplasmic part of PDGFR- β , loss of the intracellular part of PDGFR- β was only partly rescued by the intracellular part of PDGFR- α . A partial rescue was also obtained when the PDGFR- α kinase domain was replaced with a more distant kinase domain (Hamilton et al. 2003). These findings suggest that both expression patterns and signaling capacities account for the differences in function of the two PDGF receptors (Klinghoffer et al. 2001).

Function of PDGF and PDGF Receptors in the Adult

In the adult, activation of PDGFR- β controls the intestinal fluid pressure of tissues and prevents formation of edema (Rodt et al. 1996). A probable mechanism is that fibroblasts and myofibroblasts, which express PDGFR- β , make contact with extracellular components via their integrins; activation of PDGFR- β induces contraction of the cells, which controls the interstitial fluid pressure (Lidén et al. 2006).

PDGF has also been shown to stimulate wound healing (Robson et al. 1992). PDGF receptors are expressed by several cell types involved in wound healing, such as fibroblasts, smooth muscle cells, neutrophils, and macrophages; on PDGF stimulation these cells are recruited to the wounded area (e.g., by PDGF released from platelets). PDGF also contributes

to wound healing by stimulating the production of different matrix molecules (reviewed by Heldin and Westermark 1999).

A specific function for PDGFR- α to promote proliferation of insulin-promoting β cells in juvenile pancreatic islets was recently elucidated (Chen et al. 2011).

Role of PDGF Receptor Activation in Diseases

PDGF receptor activation has been observed in cancer and in other diseases involving excess cell proliferation, such as atherosclerosis and fibrotic conditions.

Overactivity of PDGF Receptors in Tumor Cells

Certain malignancies are characterized by mutations in PDGF receptor genes. Thus, 5% of gastrointestinal stromal tumors (GIST) show point mutations in the PDGFR- α gene (Heinrich et al. 2003); in this tumor type, mutations in the Kit gene are even more common (see below). The mutations affect the control mechanisms involved in keeping the receptor kinase inactive and lead to a constitutively active kinase. Similar activating point mutations in PDGFR- α have also been observed in hypereosinophilic syndrome (Elling et al. 2011).

In chronic myelomonocytic leukemia (CMML) the intracellular part of the PDGFR- β gene has been found to be fused to the TEL gene (Golub et al. 1994) or other genes that have in common that they encode proteins that can dimerize or oligomerize (Magnusson et al. 2001). Similarly, the PDGFR- α gene is fused to the FIP1L1 gene in hypereosinophilic syndrome (Cools et al. 2003; Griffin et al. 2003) and in systemic mastocytosis (Pardanani et al. 2003). The resulting fusion proteins have constitutively active kinases as a result of juxtaposition of the kinases of the receptors, as well as by the loss of regulatory sequences in the juxtamembrane (Stover et al. 2006) and transmembrane (Toffalini et al. 2010) domains.

The PDGFR- α gene has been found to be amplified in subsets of glioblastomas (Fleming

et al. 1992; Kumabe et al. 1992; Puputti et al. 2006), anaplastic oligodendrogliomas (Smith et al. 2000), esophageal squamous cell carcinoma (Arai et al. 2003), and pulmonary artery intimal sarcoma (Zhao et al. 2002). The increased number of receptors on such cells makes the cells very sensitive to PDGF stimulation; moreover, at a sufficiently high receptor density, ligand-independent activation may occur. In addition, an activated deletion mutant of PDGFR- α has been found in a human glioblastoma (Clarke and Dirks 2003).

PDGF receptors may be activated also as a consequence of mutation of ligand genes. Thus, in the rare skin tumor dermatofibrosarcoma protuberans (DFSP), the PDGF-B gene is fused to the collagen 1A1 gene (Simon et al. 1997; O'Brien et al. 1998), resulting in the production of large amounts of a fusion protein, which after processing becomes similar to mature PDGF-BB that activates its receptors in an autocrine manner (Shimizu et al. 1999).

Epithelial tumors can undergo epithelial-mesenchymal transition (EMT), whereby they lose their epithelial characteristics and start to express mesenchymal components, such as PDGF receptors (Thiery et al. 2009). Thus, whereas epithelial tumors generally do not respond to PDGF, they may do so after they have undergone EMT (Jechlinger et al. 2003). EMT correlates with increased invasiveness and metastasis; interestingly, inhibition of PDGF signaling was shown to inhibit metastasis in mouse models for breast cancer (Jechlinger et al. 2006), hepatocellular carcinoma (Gotzmann et al. 2006), and prostate cancer (Dolloff et al. 2005; Russell et al. 2009). PDGFR- α appears to be more important than PDGFR- β in the promotion of metastasis of epithelial tumors.

A malignancy-dependent increase in expression of PDGF isoforms and receptors has been observed in glioblastoma tumors. Thus, in this tumor type, PDGF appears to be involved in autocrine and paracrine stimulation, both in the tumor cells via PDGFR- α and in cells of the stroma via PDGFR- β (Hermanson et al. 1992). Moreover, in basal cell carcinoma, activation of the sonic hedgehog signaling pathway induces the expression of PDGFR- α (Xie et al. 2001).

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Activation of PDGF Receptors in the Stromal Compartment of Tumors

In addition to direct effects on the tumor cells, PDGF made by tumor cells and other cell types in solid tumors acts in a paracrine manner on various cell types in the vasculature and in the interstitial stroma of tumors (Pietras et al. 2008). Thus, PDGF promotes tumor angiogenesis by stimulating perivascular progenitor cells, pericytes, and vascular smooth muscle cells (Song et al. 2005), and by recruiting protumorigenic inflammatory cells. Moreover, overactivity of PDGF contributes to the increased interstitial fluid pressure that characterizes a majority of solid tumors, which leads to a decreased transcapillary transport and is therefore an obstacle in the treatment of tumors with chemotherapeutical drugs (Heldin et al. 2004).

Activation of PDGF Receptors in Atherosclerosis

PDGF isoforms and receptors occur at increased levels in atherosclerotic lesions. According to the response to injury hypothesis, PDGF isoforms released by platelets at sites of endothelial cell injury stimulate vascular smooth muscle cells to migrate from the vessel media into the intima and to proliferate (Ross 1993). Chronic inflammation involving release of PDGF from different types of immune cells also contributes to the narrowing of the vessel lumen (Hansson 2005). PDGF and other growth factors and cytokines released then maintain the local inflammation in a vicious circle.

Activation of PDGF Receptors in Fibrotic Diseases

Increased PDGF signaling has also been linked to various fibrotic conditions, such as lung fibrosis, myelofibrosis, glomerulonephritis, and liver cirrhosis. During chronic inflammation, activated macrophages secrete PDGF isoforms as well as other inflammatory cytokines, which up-regulate PDGF receptors on mesenchymal cells, thus promoting their proliferation and production of matrix molecules (Bonner 2004). In support of the notion that overactive PDGF

signaling drives fibrosis, knockin of constitutively active mutants of PDGFR- α was found to lead to increased connective tissue growth and a progressive fibrotic phenotype in multiple organs (Olson and Soriano 2009). Knockin of a similar PDGFR- β mutant caused an enhanced wound healing response in the skin and the liver (Krampert et al. 2008).

PDGF Receptors as Binders of Viruses

PDGFR- α has been shown to be a receptor for adeno-associated virus type 5 (Di Pasquale et al. 2003) and cytomegalovirus (Soroceanu et al. 2008). Thus, PDGFR- α facilitates infections by these viruses.

DEVELOPMENT AND CLINICAL USE OF PDGF ANTAGONISTS

Because overactivity of PDGF signaling is connected to many serious diseases, efforts have been made to develop antagonists of PDGF signaling. Several types of inhibitors are now available, including DNA aptamers binding PDGF isoforms, inhibitory antibodies against the receptors, and low molecular weight inhibitors of PDGF receptor kinases (e.g., imatinib, sunitinib, and sorafenib) (Demetri 2011). Beneficial effects have been observed by treatment of the rare malignancies that are driven by mutations in genes for PDGF and PDGF receptors (i.e., CMML, DFSR, hypereosinophilic syndrome, and GIST). However, treatment of glioblastoma that also often is characterized by overactivity of PDGF receptors, with PDGF antagonists, has been less successful; probably these tumors have undergone too many other genetic or epigenetic alterations and inhibition of PDGF receptor signaling is therefore not enough. There are indications that anti-PDGF treatment could be of more general application for the targeting of the stroma compartment of solid tumors, to inhibit metastasis (Pietras et al. 2003; Catena et al. 2010), and to lower the interstitial fluid pressure and thereby increase the transcapillary flow and drug delivery (Heldin et al. 2004). The side effects of anti-PDGF therapy have been relatively mild, but include a tendency to develop

edema, reflecting the important role of PDGF in regulation of the interstitial fluid pressure (Rodbart et al. 1996), and heart failure, reflecting an important role of PDGF in stress-induced cardiac angiogenesis (Chintalgattu et al. 2010). In patients with advanced ovarian cancer, treatment with the inhibitory Fab fragment CDP860 led to the development of significant ascites (Jayson et al. 2005).

STEM CELL FACTOR RECEPTOR (Kit)

Activation of Kit by SCF

The biologically active SCF is a homodimeric protein that is primarily produced by fibroblasts and endothelial cells, and exists both as a soluble and membrane-bound form owing to alternative splicing of exon 6 (Huang et al. 1992). Both forms of SCF are initially produced as transmembrane proteins, but the protein containing exon 6 becomes cleaved into the soluble SCF and the form lacking this exon remains membrane associated. Several proteases have been suggested to be involved in the processing of SCF, including MMP9, members of the ADAM family, and Chymase-1 (Longley et al. 1997; Heissig et al. 2002; Kawaguchi et al. 2007).

The SCF receptor is also called Kit, because it was first identified as an oncoprotein derived from the Hardy-Zuckerman 4 feline sarcoma virus (Besmer et al. 1986). It is expressed in primitive hematopoietic cells and mast cells, but also in melanocytes and basal cells in the skin, germ cells, interstitial cells of Cajal, and in certain regions of the brain. There are four human isoforms of Kit. Two splice forms are characterized by the presence or absence of a four-amino-acid sequence in the extracellular juxtamembrane region (Reith et al. 1991). These two splice forms display different signaling abilities with the shorter form being more strongly tyrosine phosphorylated and more powerful in activation of downstream signaling, whereas the longer Kit splice form signals less intensely but more persistently (Caruana et al. 1999; Voytyuk et al. 2003). Kit isoforms are also characterized by the absence or presence of a serine residue in the kinase insert region (Crosier et al. 1993).

The process by which SCF activates Kit has been studied both at a biochemical and structural level, and occurs in a manner similar to that described above for the PDGF receptor; the dimeric SCF ligand brings two Kit monomers in close proximity of each other, which enables interactions between the extracellular domains of the receptors generating a stable dimer (Fig. 1) (Lemmon et al. 1997; Zhang et al. 2000; Yuzawa et al. 2007).

The crystal structure of the Kit kinase domain revealed that the juxtamembrane region is inserted between the two lobes of the kinase domain thereby suppressing kinase activity, and that this inhibition is released on phosphorylation of tyrosine residues within the juxtamembrane segment (Mol et al. 2003, 2004). A kinetic study of the autophosphorylation process showed that the juxtamembrane region is first to be phosphorylated, consistent with the role of this region in suppressing the kinase activity (DiNitto et al. 2010).

Activation of Intracellular Pathways by Kit

The intracellular region of Kit contains seven autophosphorylation sites that are involved in the activation of the Kit kinase and can act as docking sites for intracellular signaling proteins (Fig. 2). The signaling pathways activated downstream from Kit overlap to a large extent with those described for the PDGF receptor (see above), and include PI3-kinase, Src kinases, MAP kinase pathways, and phospholipase C and D. Furthermore, it is well established that there exists a comprehensive cross talk between different pathways. For example, Src kinases contribute to Erk1/2 activation, and Src in combination with PI3-kinase promotes Jnk activation, in response to SCF (Timokhina et al. 1998; Lennartsson et al. 1999).

Attempts to study the role of individual signaling proteins include mutation of all tyrosine residues in the intracellular region of Kit and then adding them back one at a time. Using this approach, it was found that the Src docking site on Kit is important for the migratory, survival, and, partially, the proliferative response to SCF, whereas restoring coupling to PI3-kinase

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only had a small effect on the survival and migration of cells (Hong et al. 2004). In addition, by mutating individual tyrosine residues in Kit, it was found that Src and PI3-kinase binding are essential for cell migration (Ueda et al. 2002). These studies identify Src and PI3-kinase signaling as especially important in SCF-induced responses in vitro. Also, in vivo studies have confirmed the role of the Src and PI3-kinase in Kit-mediated functions. Mice expressing Kit lacking the Src binding site display primarily defects in the immune system, whereas lack of the PI3-kinase binding site generated fertility deficiencies (Blume-Jensen et al. 2000; Kissel et al. 2000; Agosti et al. 2004; Kimura et al. 2004).

The absence of clear functions of the other autophosphorylation sites in Kit probably reflects redundancy masking the role of binding of signaling proteins to these sites. Furthermore, the role of individual signaling pathways may vary between different cell types illustrated by the specific defects described above in mice expressing different Kit mutations, in which some tissues are strongly affected, whereas others are not, although they all express the same Kit mutant.

Down-Regulation of Kit

There are several mechanisms that act in parallel to down-regulate Kit signaling ensuring an appropriate signal intensity and duration. These include Kit ubiquitination and internalization, dephosphorylation, and PKC-dependent serine phosphorylation. Because all these processes require Kit activation for their initiation they function as negative-feedback loops limiting the signaling output from the receptor once it has been activated.

As described above for PDGF receptors, the activated Kit is internalized through clathrin-coated pits. Ubiquitination of Kit, which occurs in concert with the internalization process, depends on the ubiquitin ligase Cbl that can bind either directly to Kit or indirectly through adaptor proteins, such as Grb2, p85, or CrkL (Wisniewski et al. 1996; Sattler et al. 1997; Masson et al. 2006). In addition, a SOCS6-containing ubiquitination complex has been found to in-

teract with Kit and promote its down-regulation (Bayle et al. 2004; Zadjali et al. 2011). The internalized Kit is sorted inside the cell toward degradation, which occurs in both lysosomes and proteasomes (Miyazawa et al. 1994; Zeng et al. 2005).

The activity of many kinases is suppressed by the action of phosphatases. In the case of Kit, the phosphatase SHP1 has been shown to associate and negatively regulate the activated Kit (Paulson et al. 1996; Kozlowski et al. 1998).

Protein kinase C (PKC) is activated downstream from Kit and phosphorylates Kit on serine residues in the kinase insert region, which leads to reduced Kit kinase activity (Blume-Jensen et al. 1993, 1995). Moreover, activation of PKC also results in shedding of the extracellular domain of Kit limiting the ability of SCF to stimulate cells (Yee et al. 1994).

Function of Kit

There are a large number of mouse loss-of-function mutations both in the Kit (*W* loci) and SCF (*Sl* loci) genes with varying degrees of severity. From the study of these mutant mice it was found that hematopoiesis, pigmentation, fertility, peristaltic movement, and certain aspects of the nervous system, are regulated by Kit signaling.

Hematopoiesis

Kit has been found to be expressed in primitive hematopoietic cells but the expression declines during differentiation, except for mast cells that retain high Kit expression also as fully differentiated cells (Ogawa et al. 1991; Oliveira and Lukacs 2003). Kit promotes cell survival and proliferation of primitive hematopoietic cells, often in synergy with other cytokines.

Pigmentation

Kit is expressed on melanocytes and has been found to be important for the migration of these cells from the neuronal crest to the dermis during development (Wehrle-Haller 2003). Defects in Kit signaling interferes with melanocyte mi-

gration and gives rise to the pigmentation defects found in patients with piebaldism (see below).

Fertility

The reduced fertility of *W* and *Sl* mutant mice was confirmed by knockin experiments with a Kit mutant unable to activate PI3-kinase; this resulted in sterile mice or mice with reduced fertility depending on gender (Blume-Jensen et al. 2000; Kissel et al. 2000). Because Kit signaling through PI3-kinase has been found to be important for survival (Blume-Jensen et al. 1998), a likely reason for the reduced fertility is an inability of mutant Kit to support viability of germ cells. A truncated cytoplasmic form of Kit (tr-Kit) exists in sperm owing to usage of an alternative promoter (Rossi et al. 2003). Tr-Kit has been found to be important for activation of the oocyte after fertilization (Sette et al. 1997).

Peristaltic Movement

Interstitial cells of Cajal (ICC) function as pacemaker cells and can communicate with both smooth muscle cells and nerves. It was found that *W* and *Sl* mice have a constipation phenotype, which is correlated with a reduced number of ICC (Huizinga et al. 1995; Ward et al. 1995). Also, in humans, a loss of ICC has been associated with defective gut movement and constipation (Lyford et al. 2002).

Nervous System

Kit expression has been found in neurons and *W* and *Sl* mutant mice have defects in spatial learning (Motro et al. 1996; Katafuchi et al. 2000). Furthermore, neuroproliferative zones in adult rat have been found to be positive for Kit (Jin et al. 2002).

Role of Kit in Diseases

Acute Myeloid Leukemia

Acute myeloid leukemia (AML) is characterized by an uncontrolled increase in the number of

cells from the myeloid lineage that accumulate in the bone marrow and interfere with normal hematopoiesis. Kit expression is found in 60%–80% of AML cells and treatment with SCF results in increased cell proliferation (Ikeda et al. 1991; Odenike et al. 2011). Furthermore, activating mutations in Kit have been found in AML patients, although not very frequently (Odenike et al. 2011).

GIST

GISTs derive from ICC, which are pacemaker cells for peristaltic movement (Wang et al. 2000). These tumors are frequently driven by mutations in Kit, or PDGFR- α , often located in the juxtamembrane region, although mutations in the kinase domain and extracellular region have also been found (Antonescu 2011).

Mastocytosis

Mastocytosis is a disease characterized by abnormal accumulation of mast cells in tissues. Mast cells are among the few differentiated hematopoietic cells that retain Kit expression and patients with mastocytosis frequently harbor activating mutations within the kinase domain (Ustun et al. 2011).

Melanoma

Melanoma is a malignant disease of melanocytes, which can be located in different parts of the body, although most frequently in the skin. Normally, melanocytes express Kit, but the role of this receptor in melanoma is complicated; Kit has been found to be down-regulated in cutaneous melanoma (Easty and Bennett 2000), but activating mutations have been reported in other types of melanoma (Curtin et al. 2006; Ashida et al. 2009).

Small-Cell Lung Cancer

Small-cell lung cancer (SCLC) is an aggressive form of lung cancer that is often associated with smoking (Jackman and Johnson 2005). It was shown that SCLC cells coexpress Kit and SCF

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suggesting the presence of an autocrine loop (Hibi et al. 1991). However, immunohistochemical staining of tumors for Kit expression yielded varying results both regarding Kit expression and correlation with clinical outcome (Fischer et al. 2007). Furthermore, no activating Kit mutations could be found in SCLC tumors. Although imatinib inhibits growth of SCLC cell lines, it failed to show efficiency in animal models (Wolff et al. 2004). However, combining imatinib with chemotherapy (Decaudin et al. 2005), or using SU5416, which inhibits several kinases including Kit, produced more positive results in experimental models (Litz et al. 2004).

Piebaldism

In cancer, Kit is activated by gain-of-function mutations or by autocrine stimulation. In contrast, piebaldism involves germline loss-of-function mutations in Kit leading to patches of skin and hair that are depigmented owing to lack of melanocytes (Spritz 1994).

Clinically Used Kit Antagonists

Overactivity of Kit has been found in several malignant diseases (see above) and inhibiting Kit kinase activity is an approach to treat these conditions. Imatinib, also discussed in the PDGF receptor context above, inhibits Kit and has been used clinically. Because imatinib does not efficiently inhibit Kit with activating mutations within the kinase domain (Frost et al. 2002; Ma et al. 2002), other drugs have been developed that can inhibit Kit with such mutations, such as dasatinib and nilotinib (Demetri 2011). Thus, depending on the mutational status of Kit, different drugs should be selected. Neither of the drugs mentioned above exclusively inhibit Kit, but inhibit also other kinases and this may contribute to their clinical effectiveness. Other multiselective drugs whose targets include Kit are sunitinib and sorafenib. Masitinib is a potent inhibitor that targets Kit among other kinases and is approved for treating mast cell tumors in a veterinary setting.

FUTURE PERSPECTIVE

Studies over the last 30 years have revealed much about the structural properties, signaling capacities, normal functions, and role in diseases of PDGF and SCF receptors. Moreover, treatment regimens for malignant diseases in which these receptors are overactive are used routinely in the clinic. Future studies will aim at elucidating mechanisms for control of receptor signaling, including internalization, intracellular sorting, and termination of signaling. It is anticipated that such control mechanisms will include post-translational modifications of the receptors and their signaling mediators, other than the already characterized phosphorylation and ubiquitination. Additional information is also needed regarding cooperation of the receptors with coreceptors at the cell membrane, as well as the importance of interactions with the receptors and intracellular molecules, to enhance or control their signaling capacities. It will also be important to determine exactly where in the cell the receptors induce their different signals: at the plasma membrane, in endosomes, or in other intracellular organelles. In addition, whereas some information is available regarding the involvement of PDGF and SCF receptors in disease, a more detailed analysis of these receptors in malignant as well as nonmalignant diseases is highly warranted, as are more efficient and specific methods to treat diseases in which the receptors are overactive.

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