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Oncogenic derivatives of platelet-derived growth factor receptors

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Abstract. Platelet-derived growth factor receptors (PDGFRs) and their ligands, platelet-derived growth factors (PDGFs) play critical roles in mesenchymal cell migration and proliferation. In embryogenesis the PDGFR/PDGF system is essential for the correct development of the kidney, cardiovascular system, brain, lung and connective tissue. In adults, PDGFR/PDGF is important in wound healing, inflammation and angiogenesis. Abnormalities of PDGFR/PDGF are thought to contribute to a number of human diseases, and especially malignancy. Constitutive activation of the PDGFRα or PDGFRβ receptor tyrosine kinases is seen in myeloid malignancies as a consequence of fusion to diverse partner genes, and activating mutations of PDGFRα are seen in gastrointestinal tumours (GISTs). Autocrine signalling as a consequence of PDGF-B overexpression is clearly implicated in the pathogenesis of dermatofibrosarcoma protruberans (DFSP) and overexpression of PDGFRs and/or their ligands has been described in many solid tumours. PDGFR signalling is inhibited by imatinib mesylate, and this compound has clear clinical activity in patients with myeloid malignancies, GIST and DFSP.

Key words. PDGFRA; PDGFRB; imatinib.

The structure and function of platelet-derived growth factor receptors

Receptor and ligand isoforms

The PDGFR/PDGF system includes two receptors (PDGFRα and PDGFRβ) and four ligands (PDGF-A, B, C and D). The receptors are plasma membrane spanning proteins with an extracellular ligand binding domain consisting of five immunoglobulin-like structures, a single transmembrane domain, a regulatory juxtamembrane domain and an intracellular catalytic domain. PDGFRα are PDGFRβ related in sequence (30% amino acid similarity) and are members of the class III subtype of receptor tyrosine kinases (RTKs), a group that shares a characteristic insertion sequence between two conserved elements of the tyrosine kinase (TK) domain. Other class III RTKs are KIT (stem cell factor receptor), FLT3 (FLT3-ligand receptor) and FMS (macrophage colony stimulating factor receptor) [1].

PDGFRα is encoded by the gene *PDGFRA* at chromosomal band 4q12 and is transcribed as a 6.4-kb messenger RNA (mRNA). PDGFRβ is encoded by *PDGFRB* at 5q33, and its message is 5.5 kb [2, 3]. After cleavage of the signal peptides, PDGFRα consists of 1063 amino acid residues and PDGFRβ 1067 amino acids. Precursor proteins are converted in the endoplasmic reticulum to 140 kDa (α receptor), and 160 kDa (β receptor) forms, and each receptor is glycosylated through N-linked and O-linked sugar groups.

The four PDGF ligands A, B, C and D are encoded by genes at chromosome bands 7p22, 22q13, 4q31 and 11q22, respectively. The A and B isoforms may form homodimers or heterodimers, and their activities are well characterised. In contrast, relatively little is known about the C and D isoforms [4]. PDGFRβ is capable of binding strongly to PDGF-BB and DD homodimers, but weakly to AB heterodimers and not at all to PDGF-AA. PDGFRα binds to A, B and C PDGF homodimers and AB heterodimers with roughly equal affinity but scarcely to D homodimers [5–8]. Different ligands are capable of in-

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Table 1. Summary of PDGFR/PDGF abnormalities in malignancy.

Mechanism of activation		Disease	Molecular abnormality	Refs
PDGFR fusion	reciprocal translocation	myeloproliferative disorders including chronic myelomonocytic leukaemia +/– eosinophilia, atypical chronic myeloid leukaemia	*ETV6-PDGFRB*	[61]
			H4-PDGFRB	[78, 79]
			RABEP1-PDGFRB	[84]
			PDE4DIP-PDGFRB	[87]
			CEV14-PDGFRB	[72]
			HIP1-PDGFRB	[75]
			BCR-PDGFRA	[90]
	interstitial deletion	systemic mastocytosis with eosinophilia, hypereosinophilic syndrome	*FIP1L1-PDGFRA*	[96]
PDGF fusion	translocation	dermatofibrosarcoma protuberans	*CO1A1-PDGF-B* results in excess PDGF-B and autocrine/paracrine stimulation	[50]
Activating PDGFR mutation		gastrointestinal stromal tumour	*PDGFRA* point mutations	[51, 111]
Overexpression of normal receptor and/or ligand		human brain tumours	*PDGFRA* amplification	[45, 46]
		diverse malignancies	Autocrine PDGF/PDGFR stimulation?	[13, 34–40]

ducing different signals; for example, PDGF-AA and BB are equally potent mitogens, but only BB transforms murine fibroblasts in vitro [9–11]. These different signalling capabilities and the fact that expression of both receptors and all four ligands is under independent control endows the PDGFR/PDGF system with a high degree of combinatorial flexibility. Abnormalities of PDGFR/PDGF are associated with diverse human diseases, including atherosclerosis, various fibrotic conditions, predisposition to neural tube defects and cancer [4, 12–15]. The roles of PDGFR/PDGF in cancer are detailed below and summarised in table 1.

PDGFR signal transduction

Binding of PDGF ligand dimers induces receptor dimerisation and conformational changes that enable autophosphorylation of specific tyrosine residues in the receptor cytoplasmic domains. Specific binding of intracellular signalling molecules to these phosphorylated tyrosines via SH2, PTB or other phosphotyrosine interaction modules initiates a cascade of signalling, culminating in the ligand-induced phenotype [5, 16].

Site-directed mutagenesis of intracellular tyrosine residues to phenylalanine and subsequent biochemical analysis has been used to define signal transduction pathways activated by PDGFRs (e.g. [5, 17, 18]). Figure 1 shows more than 10 different SH2-domain-containing molecules found to bind different PDGFR autophosphorylation sites. Signal transduction molecules with enzymatic activity include phosphotidylinositol 3′ kinase (PI3-kinase), phospholipase C-γ (PLCγ), the Src family of tyrosine kinases, the tyrosine phosphatase SHP-2, and Ras GTP-activating protein (GAP). Adapter molecules that link the activated receptor to other signalling components include Grb2, Shc, Nck, Grb7 and Crk [19]. Signal transducers and activators of transcription (STATs) are recruited and phosphorylated by activated PDGFRs and subsequently migrate to the nucleus as dimers and directly activate transcription of target genes [20]. Recruitement and phosphorylation may be direct, as in the case of STAT1, or indirect, as in the case of STAT3 [21].

Since excessive or uncontrolled signalling may be dangerous, several mechanisms have been uncovered that damp down and limit the signal transduced by tyrosine kinases. First, binding of ligand leads to internalisation and degradation of the ligand-receptor complex in the endosome and after fusion with lysosomes, they are degraded within 30–60 min. Second, activated receptors may also undergo ubiquitination, which targets them for cytoplasmic degradation in proteosomes [19]. Third, tyrosine kinases are subject to negative regulatory feedback signals in the form of proteins such as protein tyrosine phosphatases (PTPs) and the suppressor of cytokine signalling (SOCS) family [22, 23].

Normal functions of PDGFRs

Stimulation by PDGF leads to a varied set of cellular responses, including mitogenesis, protection from apoptosis [24], increased calcium mobilisation [25], activation

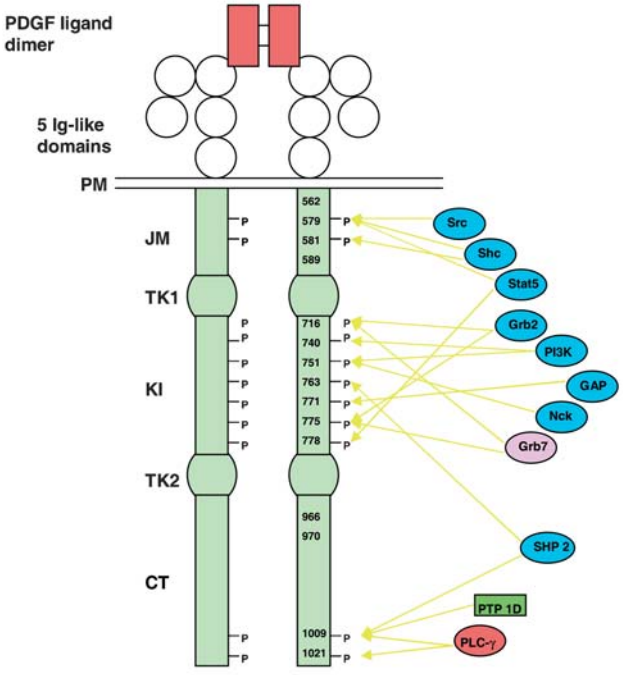

Figure 1. Signal transduction by PDGFRs. Illustrated is the structure of PDGFRβ, which has five extracellular immunoglobulin (Ig)-like domains, a transmembrane domain that traverses the plasma membrane (PM), a juxtamembrane domain (JM), a bipartite catalytic tyrosine kinase domain (TK1 and TK2) separated by a kinase insert region (KI) and a C-terminal tail (CT). Upon binding of PDGF ligand dimers, specific tyrosine residues (indicated by amino acid number and a 'P') within the cytoplasmic domain are autophosphorylated. Known interactions between specific signalling proteins and phosphotyrosines are indicated. Signalling by PDGFRα is broadly similar, although some differences have been identified [5, 19].

of the hexose monophosphate shunt [26] and promotion of inter-cell signaling by inducing phosphorylation of gap junction proteins [27].

Targeted disruption of *PDGF-B* or *PDGFRB* results in highly similar embryonic lethal mutant phenotypes, including disorders of the vascular system, microvessel leakage, haemorrhage and microaneurysm formation, anaemia and thrombocytopenia [28]. *PDGFRA* expression is necessary for the normal patterning and formation of neural crest cells that develop into vertebrae, ribs and sternum [29]. Transient expression of *PDGFRA* in the neural tube at the point of tube closure and throughout the mesoderm directs formation of bone, skin, connective tissue, heart and muscle. This function of *PDGFRA* has been well characterised in the naturally occurring mouse mutant *Patch* (*Ph*). Heterozygotes for a complete deletion of the *PDGFRA* locus have a white patch on their trunks, resulting from a failure of melanocytes to migrate to the midline of their body during development. In contrast to heterozygotes whose spinal cords develop normally, one-third of *Patch* homozygotes survive to full term and display craniofacial abnormalities, including cleft face and severe spina bifida occulta [30, 31]. In humans, specific combinations of naturally occurring *PDGFRA* promoter haplotypes strongly affect neural tube genesis [14]. PDGFRA is also expressed in the testis and localized in Leydig and Sertoli cell cytoplasm. Leydig cell production of testosterone under luteinizing hormone (LH) stimulation is a well known phenomenon. PDGF-A –/– mice show a lack of adult Leydig cell development with a parallel decrease of testosterone production, suggesting a pivotal role of PDGF-A and its cognate receptor, PDGFRα, in Leydig cell differentiation and/or survival [32] .

By mediating the involvement and chemotactic responses of monocytes, macrophages and platelets in inflamed tissue, PDGFRβ is implicated in tissue interstitial pressure and inflammation [33]. PDGFRβ signals modulate the tension between cells and the extracellular matrix, of normal blood vessels and proliferative vascular diseases. Injured blood vessel wall cells overexpress PDGFRβ and are induced to proliferate [12], thus implicating this receptor in the deposition of atherosclerotic plaques.

Abnormalities of PDGFRs in solid tumours

Autocrine and paracrine loops

Co-expression of PDGFRs and PDGF ligands, which suggests autocrine stimulation, has been described in a wide range of malignancies, including meningioma, melanoma, breast cancer, ovarian cancer, pancreatic cancer, lung cancer, prostate cancer, soft tissue malignant fibrous histiocytoma, osteoblastoma, glioblastoma and astrocytoma [13, 34–40]. Paracrine stimulation of PDGFRs may also play an important role in the abnormalities of epithelial cell/stromal interactions that characterise many solid tumours [40–43] and also haematological disorders associated with bone marrow fibrosis, notably idiopathic myelofibrosis [44]. However, although these associations are intriguing and may well be very important, the precise role that PDGF/PDGFR autocrine and paracrine loops play in malignancy is not clear.

Overexpression of *PDGFRs* in solid tumours

PDGFRA is amplified and overexpressed in a subset of glial tumours [13, 45, 46], and gene expression profiling identified significant upregulation of *PDGFRB* (originally erroneously identified as *PDGFRA*) in metastatic medulloblastoma [47]. As above, it is unclear whether overexpression of PDGFRs is really a primary cause of these diseases, but overexpression of tyrosine kinases is a feature of several malignancies and is believed to result in receptor activation by crowding at the plasma membrane and consequent ligand-independent dimerisation.

Overexpression of PDGF ligands

The *v-sis* oncogene from simian sarcoma virus is related to PDGF-B, thus establishing that this ligand family may play a role in malignancy [48]. Although apparent overexpression or aberrant expression of PDGFs has been found in several malignancies [13], the clearest example of ligand overexpression is in dermatofibrosarcoma protruberans (DFSP), a rare slow growing dermal neoplasm of intermediate malignancy [49]. Most cases of DFSP are characterised by the presence of a translocation between chromosome bands 17q12 and 22q13, usually either in the form of an unbalanced translocation or a supernumerary ring chromosome. This abnormality fuses *COL1A1* on chromosome 17 to *PDGF-B* on chromosome 22 [50], and thus brings *PDGF-B* under control of the widely expressed *COL1A1* promoter. Although the size of the fusion protein is quite large (~116 kDa), it is cleaved and processed to yield normal PDGF-B ligand. It is thus likely therefore that the principal consequence of the fusion serves is inappropriate expression of PDGF-B, resulting in an autocrine growth loop.

Activation of *PDGFRs* by point mutation or deletions

Most gastrointestinal stromal tumours (GISTs) have activating mutations of *KIT,* but recently it has emerged that approximately half of *KIT* mutation-negative cases have activating mutations of *PDGFRA* [51]. Mutations in *PDGFRA* and *KIT* are mutually exclusive and result in the activation of common signalling pathways [51]. Changes that have been described to date are D842V, D846Y and Δ842–845DIMH within the activation loop and V561D in the juxtamembrane domain [51, 52]. These mutations are at analogous positions to some of the changes in *KIT* or *FLT3* seen in patients with GIST, mastocytosis and acute myeloid leukaemia (AML) [53]. Gain-of-function mutations in the activation loop are believed to switch the catalytic domain into its active conformation, a change that is normally induced by ligand binding. Mutations in the juxtamembrane region interfere with a WW-like domain that is thought to restrain receptor activity in the absence of ligand [54, 55]. Activating mutations in the catalytic domain and the juxtamembrane domain also appear to induce or facilitate receptor self association [56]. In addition to the point mutations seen in GIST, a transforming variant of *PDGFRA* with an in-frame deletion of exons 8 and 9 was found to be amplified and overexpressed in a glioblastoma tumour [57].

PDGFR fusion genes in haematological malignancies

Overview

Several rare gene fusions involving *PDGFRA* or *PDGFRB* have been described in haematological malignancies, specifically chronic myeloproliferative disorders (CMPD), myelodysplastic/myeloproliferative syndromes (MDS/MPD) and also acute myeloid leukaemia (AML). Patients have a very wide age range and most present with an atypical chronic myeloid leukaemia/chronic myelomonocytric leukaemia (CML/CMML)-like disease, often with pronounced eosinophilia and splenomegaly. Transformation to acute leukaemia occurs in a minority of cases with variable latency, but death may occur from other complications [58]. Remarkably, and currently inexplicably, the great majority of affected individuals are male; for example, in a recent review of 34 patients with the t(5;12), only two were female [58].

Fusion gene structure

PDGFR gene fusions arise as a consequence of specific acquired chromosomal translocations and individual fusions are described below. In general though, the breakpoints are such that the resultant fusion proteins retain the intracellular catalytic domains of PDGFRα or PDGFRβ, but the extracellular domains of these receptors are replaced by the N-terminal part of the partner protein. The structure of some representative examples are shown on figure 2. The chimaeric proteins are no longer responsive to PDGF but they have constitutive tyrosine kinase activity, i.e. they are continuously sending proliferative and anti-apoptotic signals to the cell in which they reside. Structurally and functionally, these fusion proteins are very similar to BCR-ABL in CML. Since the chromosomal translocations that generate these fusions are balanced, a reciprocal fusion (i.e. *ABL-BCR*, *PDGFRB-TEL* etc.) is also generated and is detectable by reverse transcription-polymerase chain reaction (RT-PCR) in some cases. However, it is believed that these reciprocal chimaeric genes do not play an important pathogenetic role.

Role of the partner proteins

Although the partner proteins are unrelated in sequence and function, they share two common properties. First, they provide specific domains capable of homotypic interaction that result in oligomerization of the fusion protein. This mimics normal ligand-induced PDGFR dimerization and explains, at least in part, why the fusion proteins are constitutively active. Second, the upstream control elements that drive the expression of the partner gene also drive the expression of the fusion gene. In order to result in myeloid expansion, the fusion gene must be expressed in hamopoietic progenitor cells, and in fact most partner genes are universally or widely expressed.

In addition to the *PDGFR* fusions considered in this review, analogous fusions involving other tyrosine kinases such as *FGFR1*, *JAK2*, *ABL1*, *ABL2*, *SYK* and *ALK* are

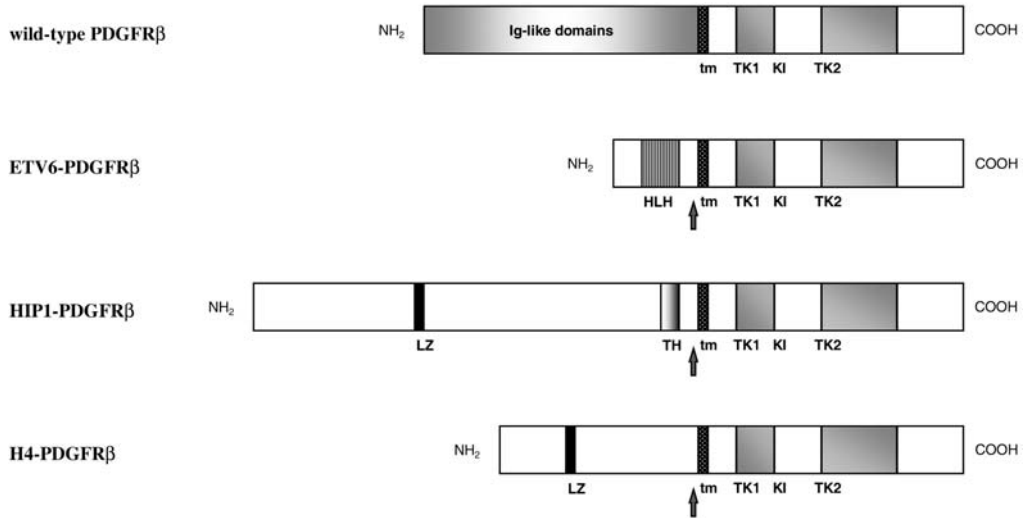

Figure 2. Structure of selected PDGFRβ fusion proteins. tm, transmembrane domain; TK1 and TK2, tyrosine kinase domains; KI, kinase insert; HLH, helix-loop-helix; LZ, leucine zipper; TH, talin homology domain. The arrows indicate the point of fusion in each case.

seen in haematological malignancies [59]. Tyrosine kinase fusions readily give rise to leukaemia in mouse models and are believed to early, and probably primary, determinants of the disease process.

ETV6-PDGFRB

The t(5;12)(q33;p13) was first described in 1987 [60], and at least 34 cases have been described to date in association with atypical CML, AML, CMML, chronic eosinophilic leukaemia (CEL) or unclassified myeloproliferative disorders [58]. The t(5;12) was cloned in 1994 and shown to fuse the then novel gene *ETV6* (previously named *TEL*) at 12p13 to *PDGFRB* [61]. *ETV6* encodes a member of the ETS transcription factors and was subsequently shown to be required for the establishment of haemopoiesis of all lineages in the bone marrow [62] and to be widely involved in leukaemia by fusion to multiple partner genes. The *ETV6-PDGFRB* gene product consists of the amino-terminal 154 amino acids of *ETV6* harbouring a helix-loop-helix (HLH; also known as pointed) domain fused to the transmembrane and cytoplasmic domains of PDGFRβ. *ETV6-PDGFRB* was the first PDGFR fusion gene to be identified and is currently the best characterised.

ETV6-PDGFRβ transforms Ba/F3 cells to growth factor independence. Transformation of Ba/F3 cells requires the HLH domain and kinase activation of PDGFRβ [63, 64], *myc* [65], NFκB [66], PI3 kinase [67], PLCγ and activation of STAT5 [68]. More recently, using a tetracycline-inducible construct, it was shown that ETV6-PDGFRβ partially protected Ba/F3 cells from apoptosis on interleukin (IL)-3 withdrawal but did not generate factor-independent clones, in contrast to cells that continuously expressed the fusion. Surprisingly, rather than augmenting proliferation in response to IL-3, ETV6-PDGFRβ expression led to a dramatic decrease in IL-3-induced proliferation as a consequence of increased apoptosis [69]. Thus the interaction of ETV6-PDGFRβ and cytokine signalling may be an important component of the disease process.

Studies using a mouse bone marrow transplant (BMT) assay have shown that ETV6-PDGFRβ causes a rapidly fatal myeloproliferative disorder (MPD) that closely recapitulates CMML [70]. Mutations of tyrosine residues within the PDGFRβ moiety did not abrogate transformation in Ba/F3 cells, but tyrosines 579 and 581 were essential for induction of the murine disease, illustrating the limitations of cell lines in modelling the effects of oncogenes [70]. A fatal MPD was efficiently induced in mice that genetically lacked GM-CSF and IL-3, demonstrating that these cytokines are not necessary for the development of disease, at least in this model system [70].

ETV6-PDGFRβ was originally identified in a patient with CMML who later developed AML with acquisition of the t(8;21)(q22;q22), thus providing an example of an early mutation in the multistep pathogenesis of AML [61]. Recently, it has been demonstrated formally that *ETV6-PDGFRB* co-operates with *AML1-ETO* to induce AML in mice [71].

CEV14-PDGFRB

In 1997 Abe et al. described a patient with AML and a t(7;11)(p15;p15) at initial diagnosis, who later relapsed, exhibiting marked eosinophilia, hepatosplenomegaly and an additional cytogenetic abnormality, a t(5;14)-(q33;q32). The t(5;14) fused the intracellular domains of

PDGFRB to a novel gene, *CEV14* (now known as *TRIP11*), which is predicted to encode a very long central coiled-coil domain, two leucine zipper motifs and a putative thyroid hormone receptor interacting domain [72]. *CEV14* is expressed in a wide range of tissues and is found in association with the Golgi apparatus and microtubules. It has been proposed that *CEV14* serves to link the cis-Golgi network to the minus ends of centrosome-nucleated microtubules [73]. Thus far no other *CEV14-PDGFRB* positive cases have been described.

HIP1-PDGFRB

A t(5;7)(q33;q11.2) was identified in a patient with CMML/eosinophilia and shown to fuse *HIP1* to *PDGFRB* [74]. All but the 18 C-terminal amino acids of the HIP1 protein, including the leucine-zipper and talin homology domains, were found to be fused to PDGFRβ. The HIP1- PDGFRβ fusion protein oligomerises, is constitutively tyrosine-phosphorylated and transforms Ba/F3 cells to IL-3-independent growth. Oligomerisation and kinase activation required the 55-amino acid carboxyl-terminal talin homology region but not the leucine zipper domain [75]. A second case with *HIP1-PDGFRB* has recently been described [76].

HIP1 is a clathrin-associated protein that normally interacts with Huntingtin, the protein mutated in Huntington's disease. Recently, HIP1 has been found to be overexpressed in epithelial tumours and identified as an oncogene in its own right by altering receptor trafficking [77].

H4-PDGFRB

Fusion of *H4/D10S170* to *PDGFRB* was reported in two patients with atypical CML [78, 79]. A third case has been reported recently [80], and we have identified two additional cases [unpublished observations]. *H4/D10S170* is a ubiquitously expressed gene that is phosphorylated by serum stimulation and may play a role in apoptotic signalling [81]. H4/D10S170 protein is suggested to be cytoskeletal, has a predicted α-helical conformation similar to the myosin heavy chain tail, a putative SH3 domain at the C-terminus and two putative leucine zipper domains [82]. *H4/D10S170* was originally identified as a gene that fuses to the *RET* tyrosine kinase in approximately 20% of papillary thyroid cancers as a consequence of the inv(10)(q11.2q21) [82, 83].

Retroviral transduction of *H4-PDGFRB*, but not a kinase-inactive mutant, conferred factor-independent growth to Ba/F3 cells. Mutational analysis showed that the amino-terminal H4 leucine zipper domain as well as H4 amino acids 101–386 was required for efficient transformation. Tryptophan-to-alanine substitutions in the PDGFRβ WW-like domain or substitution of tyrosines 579/581 by phenylalanine impaired factor-independent growth of Ba/F3 cells [79]. In a mouse BMT model, *H4-PDGFRB* gave rise to T-cell lymphoblastic lymphoma rather than the expected myeloproliferative disorder, probably due to the use of relatively low retroviral titres [79].

RABEP1-PDGFRB

A single CMML patient has been reported with an acquired t(5;17)(q33;p13) and in frame fusion between *RABEP1*, the gene encoding Rabaptin-5, and *PDGFRB* [84]. *RABEP1-PDGFRB* encodes a chimaeric protein that includes more than 85% of the native Rabaptin-5, retaining three of the four predicted coiled-coil domains. Transduction with a retroviral vector expressing the fusion transformed Ba/F3 cells to growth factor independence and caused a fatal myeloproliferative disease in mice that was indistinguishable from the disease induced by *ETV6-PDGFRB* [84].

Rabaptin-5 is a well-characterized protein that plays a critical role in the regulation of endocytosis of activated growth factor receptors through regulation of the small Ras-family GTPases Rab4 and Rab5 [85, 86]. Rabaptin-5 is localized mainly in the cytosol, but is recruited to early endosomes through GTP-dependent interaction with Rab5. Homodimerisation of Rabaptin-5 is mediated by coiled-coil domains, and these domains are also predicted to dimermerise the PDGFRB fusion protein.

PDE4DIP-PDGFRB

A t(1;5)(q23;33) was cloned from a 11-month-old girl with an MPD associated with eosinophilia and found to fuse *PDE4PIP* (encoding phosphodiesterase4D interacting protein; also known as myomegalin) to *PDGFRB* [87]. Interestingly, the t(1;5) has been reported before in infants [88], perhaps suggesting an association between this abnormality and some sort of prenatal DNA damage. *PDE4DIP* is widely expressed and mostly composed of α-helical, coiled-coil structures and a leucine-zipper domain, and it has domains shared with microtubule-associated proteins. It has been proposed that this protein functions as an anchor to localize components of the cyclic AMP (cAMP)-dependent pathway to the Golgi/centrosomal region of the cell [89].

BCR-PDGFRA

The first reported fusion gene involving *PDGFRA* was cloned from two patients with atypical *BCR-ABL*-negative CML, both of whom had a t(4;22)(q12;q11) [90]. A third patient has been reported [91], and we are aware of three additional cases. One patient progressed to B-cell acute lymphoblastic leukaemia, and another had T-lymphoid extramedullary disease, suggesting a stem cell dis-

order. The breaks within *BCR* were variable, and unusually the genomic breakpoints in two of the three characterised cases fell within a *PDGFRA* exon, with *BCR* intron sequences being incorporated into the mature fusion mRNA [90].

FIP1L1-PDGFRA

Idiopathic hypereosinophilic syndrome (HES), as its name suggests, is the unexplained, persistent elevation of eosinophil counts. It has been known for many years that a subset of HES patients evolve to acute leukaemia, suggesting that the eosinophilia was in fact symptomatic of an underlying MPD [92, 93]. Unexpectedly, some HES patients were found to be responsive to imatinib mesylate [94, 95], indicating that the underlying lesion was likely to be an activated tyrosine kinase. HES by definition is associated with a normal karyotype, but Cools and colleagues were fortunate in identifying a patient with persistent eosinophilia and a t(1;4). Cloning of this translocation revealed a fusion between *FIP1L1* and *PDGFRA* [96]. *FIP1L1* is a newly described human gene with homologues in a number of genera, including the *Saccharomyces cerevisiae* gene *Fip1*, known to be a component of the mRNA polyadenylation apparatus [97]. *FIP1L1-PDGFRA* was also identified independently using proteomic techniques in the EOL-1 cell line [98]. *FIP1L1* is located on chromosome 4, only 800 kb proximal to *PDGFRA* [96], and therefore *FIP1L1-PDGFRA* is formed by a small, cytogenetically invisible interstitial deletion, the t(1;4) being incidental. The *FIP1L1* moiety of the fusion is subject to complex alternative splicing, and RT-PCR usually detects multiple fusion transcripts in affected individuals. Not all of these transcripts are in frame, but at least one functional fusion is identifiable in each patient [96]. As described above for *BCR-PDGFRA*, the *PDGFRA* breakpoints fall within an exon and disrupt the region encoding the WW-like domain. The unusual nature of the breakpoints suggest that loss of part of this domain has been selected for, which is somewhat surprising given the fact that the WW-like domain is almost identical between PDGFRα and PDGFRβ, and that this domain is fully retained in PDGFRβ fusions. *FIP1L1-PDGFRA* transforms Ba/F3 cells to IL-3 independence and induces an MPD in a mouse BMT model [96, 98, 99].

In the original series, *FIP1L1-PDGFRA* was identified in more than 50 % of HES patients [96]. We have found the incidence to be lower at ~20 % [unpublished observations], but this still means that *FIP1L1-PDGFRA* is the most common known *PDGFR* fusion. Currently there is an ongoing debate as to the precise haematological and morphological features associated with *FIP1L1-PDGFRA*, which have been described as 'systemic mast cell disease with associated eosinophilia' [95] or 'myeloproliferative variant of HES' [100]. The term *FIP1L1-PDGFRA* positive CEL has been suggested as a term to encompass all patients who are positive for this fusion [101].

Other *PDGFR* fusions and diagnostic issues

Two-colour FISH analysis has demonstrated disruption of *PDGFRB* in patients with a t(1;3;5)(p36;p21;q33), t(2;12;5)(q37;q22;q33), t(3;5)(p21;q31), and t(5;14)-(q33;q24) respectively, suggesting that several *PDGFRB* partner genes remain to be characterised [102]. Indeed, new *PDGFRB* partners at chromosome bands 3p22 (2 loci), 12q13, and 15q22 have recently been identified [F. Grand and N. C. P. Cross, unpublished observations]. A number of patients with a CML-like CMPD have been described with a translocation of 4q11–12 that may or may not involve *PDGFRA* [90].

Rapid identification of *PDGFR*-rearranged patients is clearly important because most of these individuals will respond to imatinib (see below). To date, all patients that have been described with *PDGFRB* fusions have had a translocation involving 5q31–33 by standard cytogenetic analysis. However, there are several other translocations that target this region in myeloid disorders that do not involve *PDGFRB* [58], and so molecular confirmation of *PDGFRB* involvement is necessary. FISH can be used to detect *PDGFRB* disruption, although in some cases false negative results may arise due to the fact that some translocations that appear to be simple reciprocal changes down the microscope are in fact complex at the molecular level [78]. We normally use RT-PCR whenever possible, but variable breakpoints could also lead to false negative results.

As for *PDGFRA* rearrangements, fusion to *BCR* is associated with the t(4;22), but fusion to *FIP1L1* is not visible using standard cytogenetic banding techniques. FISH can be employed to detect deletion of the *CHIC2* locus at 4q12 as a surrogate for *FIP1L1-PDGFRA* [103], and RT-PCR used to detect fusion mRNA [96]. Representative FISH results are shown on figure 3. Since the breakpoints are clustered in most cases, rearrangements can also be detected by Southern blotting for both *PDGFRA* and *PDGFRB*.

Targeted therapy of PDGFR abnormalities

Imatinib mesylate

Imatinib selectively inhibits the tyrosine kinase activity of ABL, ARG, KIT, PDGFRα and PDGFRβ [104]. Following the dramatic success of this compound for treatment of patients with *BCR-ABL*-positive CML, there has been considerable interest in extending the clinical use of imatinib to diseases in which *PDGFR* abnormalities have been implicated.

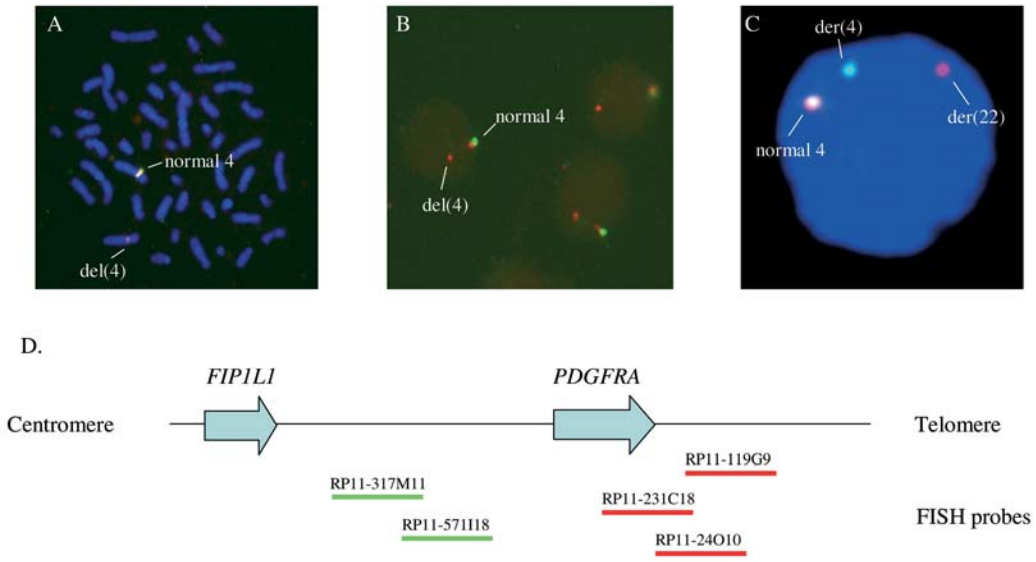

Figure 3. Detection of *PDGFRA* rearrangements by FISH. (*A*) and (*B*) Metaphase and three interphase cells from a *FIP1L1-PDGFRA* patient showing deletion of BACs RP11-317M11 and RP11-571I18 (green signal) from one copy of chromosome 4. (*C*) Disruption of PDGFRA (separated red and green signals) in an interphase cell from a patient with *BCR-PDGFRA*. (*D*) Configuration of FISH probes.

Activity of imatinib in patients with *PDGFRB* fusions

Imatinib was shown to be active against the ETV6-PDGFRβ fusion protein in cell lines [105] and in a mouse model [106]. Subsequently, three *ETV6-PDGFRB*-positive CMPD patients, plus a fourth individual with a *PDGFRB* rearrangement but unknown fusion, were treated with imatinib. All four patients achieved complete cytogenetic remission, and one patient achieved molecular remission [107]. Response to imatinib has been documented in a further *ETV6-PDGFRB* patient [108] and also individuals with *RABEP1-PDGFRB* [109], *H4-PDGFRB* [80] and *PDE4DIP-PDGFRB* [87].

Activity of imatinib in patients with *PDGFRA* fusions or activating mutations

Several patients with *FIP1L1-PDGFRA* have exhibited equally dramatic responses to imatinib, many of them achieving molecular remission [96, 110]. Responses may be achieved with relatively low doses of imatinib (100 mg/day) compared to *BCR-ABL*-positive CML patients (typically 400 mg/day), since this compound has a higher specific activity (lower IC_{50}) towards PDGFRs compared to ABL [96]. One *FIP1L1-PDGFRA*-positive patient relapsed with an acquired mutation in the kinase domain that conferred imatinib resistance [96]. In a mouse model this mutant responded to another small molecule inhibitor, PKC412, currently in phase II trials for treatment of AML patients with FLT3 abnormalities [99].

Clinical response to imatinib has also been described in an atypical CML patient with *BCR-PDGFRA* [91] and some patients with *PDGFRA* mutation-positive GIST [111, 112]. Notably, GIST patients with the *PDGFRA* D842V mutation were resistant to imatinib. This change is analogous to the well-described, imatinib-resistant, D816V *KIT* mutation, seen in a majority of patients with systemic mastocytosis.

Activity of imatinib in other diseases

Clear inhibitory effects of imatinib have been documented in vitro and in vivo against DFSP with overexpression of PDGF-B as a consequence of the *COL1A1-PDGFB* fusion [113–115]. Despite the association of PDGFRs with fibrosis, imatinib is largely ineffective against myelofibrosis [116, 117]. Inhibitory effects that may or may not be related to inhibition of the PDGFR/PDGFRB axis have been described using cell lines or model systems for lung cancer [118], prostate cancer [119], neuroblastoma [120] and ovarian cancer [121]. Finally, it has been shown that inhibition of PDGFRs by imatinib may enhance the uptake of chemotherapeutic drugs by lowering tumour interstitial hypertension [122]. Potentially this could be used as a general strategy to enhance the effects of chemotherapy.

Alternative compounds and strategies

Several other molecules have been developed that inhibit PDGFRs, but few of these have evaluated clinically. As mentioned above, PKC412 is active against PDGFRs and may be useful for treatment of imatinib-resistant cases [99]. Another compound, SU101, was tested in a phase II trial of patients with hormone-refractory prostate cancer.

Despite the fact that many patients had advanced phase disease and been heavily pretreated, some modest benefits were achieved [123]. As an alternative to targeting PDGFRs themselves, strategies that block downstream signalling components may also be effective in blocking aberrant PDGFR signals. Perhaps the most advanced of these are the farnesyltransferase inhibitors, but thus far there are no data regarding the effectiveness of these compounds in patients with pathogenetic abnormalities of the PDGF/PDGFR system.

Note added in proof: Two additional PDGFRB gene fusions have been recently published in MPD: *NIN-PDGFRB* and *HCMOGT1-PDGFRB*.

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