Platelet-Derived Growth Factor Signaling in the Lung
From Lung Development and Disease to Clinical Studies

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Abstract

Platelet-derived growth factors (PDGFs) and their receptors (PDGFRs) represent one of the most intensively studied families of growth factors in the last four decades. PDGF signaling plays an essential role in cell proliferation, differentiation, migration, and survival. *In vivo* studies have documented an important role of PDGF signaling in the normal development of several organs, such as the kidney, eye, or lung. PDGF signaling is essential for the formation of intact mesenchymal cells during embryogenesis. Recently, this knowledge has been extended to a role of PDGF signaling in diseases in general, such as cancer and atherosclerosis, and more importantly in lung diseases, including pulmonary arterial hypertension, lung cancer, and lung fibrosis. In this review, we provide an up-to-date overview of PDGF signaling, including tissue- and cell-type–specific expression patterns and effects. We highlight current therapeutic approaches modifying PDGF signaling in lung diseases and summarize clinical trials in which PDGF signaling has been inhibited. In conclusion, although PDGF inhibition has been used in multiple clinical trials, we suggest that more elaborate and specific approaches for spatio-temporal control of PDGF signaling are required for developing personalized approaches involving PDGF signaling in lung disease.

Keywords: receptor isotype; lung fibrosis; lung cancer; signal transduction

Clinical Relevance

We provide an up-to-date overview of platelet-derived growth factor (PDGF) signaling, including tissue- and cell-type–specific expression patterns, and its role during development and disease. We highlight current therapeutic approaches modifying PDGF signaling in lung diseases, summarize clinical trials, and give an outlook about specific approaches needed for future interventions of PDGF signaling in lung diseases.

The platelet-derived growth factor (PDGF) signaling pathway is found in most cell types. Five different ligand isoforms (PDGF-AA, PDGF-BB, PDGF-CC, PDGF-DD, and PDGF-AB) and two PDGF-receptor isotypes (PDGFRα and PDGFRβ) have been described. The PDGFRs belong to the family of transmembrane tyrosine-kinase receptors, which are activated after ligand binding (PDGFs) to their extracellular domain, causing dimerization and subsequent autophosphorylation of the intracellular PDGFR domains. This leads to activation and signaling of downstream intracellular pathways. PDGF signaling plays an essential role in embryonic and normal lung development. PDGF signaling controls cell proliferation, differentiation, migration, and survival via ligand-specific activation (1–3), suggesting that this pathway plays a critical role in disease pathomechanisms (4). Indeed, overexpression of PDGFRs is associated with several diseases, including lung, skin, kidney fibrosis, and cancer (5–7). In the present review, we provide up-to-date information on PDGF signaling pathways and assess the importance of PDGF signaling in the lung. We summarize and compare results from studies in human and small animal model systems regarding signal transduction, lung development, and pulmonary diseases and review clinical studies that modify PDGF signaling in lung disease.

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Tissue- and Cell-Specific Localization

PDGF Ligands

Five PDGF ligands (PDGF-AA, PDGF-BB, PDGF-CC, PDGF-DD, and PDGF-AB) have been described to date. PDGF was originally identified as a constituent of whole blood that was not detected in cell-free, plasma-derived serum and was subsequently purified from human platelets (8–13). Purified PDGF was identified as a heterodimer consisting of PDGF-A and PDGF-B chains. In 2000 and 2001, PDGF-C and PDGF-D were described (14–16). Since then, PDGF-A and PDGF-B have been considered as the classical PDGFs, whereas PDGF-C and PDGF-D are novel ones, encoded by *pdgf-a*, *pdgf-b*, *pdgf-c*, and *pdgf-d* genes, respectively (17, 18).

PDGF-A and PDGF-B form disulfide-bonded homodimeric (PDGF-AA and PDGF-BB) and heterodimeric (PDGF-AB) isoforms. After dimerization of the PDGF-A and PDGF-B chains within the cell, the dimers are proteolytically cleaved and are thereby activated inside the producing cells (1, 19, 20). The main protease responsible for the conversion of pro–PDGF-A to active PDGF-A is the proprotein convertase furin (21). The enzyme for the conversion of pro–PDGF-B to active PDGF-B is unknown, but it is assumed to be a related proprotein convertase (20). On the other hand, PDGF-C and PDGF-D are synthesized as inactive precursor molecules. Proteolytical cleavage by extracellular proteases is needed for binding and activating PDGFRs (22). Tissue-type plasminogen activator has been identified as a candidate protease involved in the extracellular activation of pro–PDGF-C to active PDGF-C form (20, 22). Moreover, urokinase-type PA and matriptase have been suggested as potential proteases involved in the activation of PDGF-D chains (23, 24). PDGF-CC and PDGF-DD act as homodimers and are not able to form heterodimers (20, 25).

PDGF ligands can be detected in many different tissues and cell types as outlined in Table 1, but early investigations have largely looked at RNA expressions and have not determined the expression of different dimeric isoforms. It has been shown that PDGF-A and PDGF-B in humans are expressed in most cell types, such as fibroblasts, vascular smooth muscle cells, endothelial cells, neurons, macrophages, B and T cells, and platelets (26–35). PDGF-B is expressed in vascular endothelial cells, neurons, and megakaryocytes. PDGF-A and PDGF-C are expressed in epithelial cells, muscle, and neuronal progenitors (36). Furthermore, in humans, PDGF-C expression was observed in vascular endothelial cells and smooth muscle cells (37). PDGF-D expression is less characterized, but it has been detected in fibroblasts and smooth muscle cells (36).

The synthesis of PDGF ligands is a dynamic and sensitive system. Various external stimuli may influence the level of PDGF expression, such as low oxygen (hypoxia), thrombin, or stimulation with different cytokines and growth factors, including PDGF itself (36, 38, 39).

PDGF Receptors

The two receptor isoforms (PDGFRα and PDGFRβ) are encoded by the genes *pdgfr-a* and *pdgfr-b*, respectively, and show similar structural patterns. Their extracellular domain consists of five immunoglobulin-like domains recognized by individual ligands. There are three known dimeric combinations of these receptors: homodimeric PDGFRα/PDGFRα and PDGFRβ/PDGFRβ forms and the heterodimeric PDGFRα/PDGFRβ form (12, 40). Receptor dimerization occurs after ligand binding to the Ig-like domains 2 and 3 of their extracellular part, which leads to stabilization of their intracellular parts by receptor–receptor interactions. Once the intracellular parts are in close proximity, cross-autophosphorylation of receptor kinase domains leads to activation and downstream signal transduction (41, 42).

The synthesis of PDGFRs is rapidly increased during inflammation (43, 44). Estrogen triggers increased expression of PDGFRα and PDGFRβ in mouse uterus (45), whereas basic fibroblast growth factor increases the expression of PDGFRα in vascular and bronchial smooth muscle cells. Moreover, transforming growth factor (TGF)-β, LPS, TNF-α, and IL-1α stimulation influences the level of expression of individual PDGFR isotypes (46–50).

PDGFRs are expressed in most cell types but are highly expressed and active in mesenchymal cells, such as fibroblasts and smooth muscle cells. Additionally, the expression of PDGFRα and PDGFRβ in mice has been observed in stromal organizer cells present in Peyer's patch anlagen of the ileum (51). The expression of PDGFRα is specifically detected in subtypes of mesenchymal precursor cells located in the lung, skin, and intestine, whereas PDGFRβ is expressed in pericytes and vascular smooth muscle cells (36). Other cells expressing PDGFRα are human platelets, mouse megakaryocytes, and rat liver endothelial cells (52–54); PDGFRβ is expressed in mouse capillary endothelial cells (55), T cells, and macrophages (35, 56–58). Cultured monocytes (59) and natural killer (NK) cells (60) also express PDGFRβ. An overview of the tissue- and cell type–specific expression pattern of PDGFRs is given in Table 2.

Regulation of PDGF Receptor and PDGF Ligand Expression

Although the expression of PDGFRs is regulated on the transcriptional and post-transcriptional level, detailed information mainly exists for transcriptional regulation. Basal expression of *pdgfr-b* is regulated by the transcription factors NF-Y and Sp1 via the consensus CCAAT motif and an upstream Sp1 binding site, respectively, within its promoter region (61–63). Three NF-Y subunits (NF-YA, NF-YB, and NF-YC) are necessary for the initialization of *pdgfr-b* transcription (64). These NF-Y subunits control the level of PDGFRβ expression during different phases of the cell cycle (65). The proteins c-Myc and p73, on the other hand, repress *pdgfr-b* expression on the transcriptional level (66–69) through their interaction with NF-Y. Yang and colleagues (70) showed that the tumor suppressor p53 binds and represses the activity of the *pdgfr-b* promoter. They also observed that, in the presence of c-Myc, p73 affects the activity of p53 on the *pdgfr-b* promoter. Additionally, transcription of *pdgfr-b* is repressed by deletion of the GC-rich regions localized 100 bp upstream of the transcriptional start site (63). In contrast, little is known about the mechanisms regulating PDGFRα expression. Bonello and colleagues showed that peroxide-inducible Ets-1 controls the transcription of the *pdgfr-a* gene in vascular smooth muscle cells by binding its promoter (71). Zhang

Table 1. Tissue and Cell Types Expressing Platelet-Derived Growth Factor Ligands in Human and Mouse

PDGF Ligand	Tissue	References	Cell Type	References
PDGF-A	Kidney	(14)	Fibroblasts	(26)
	Brain, neural tissue	(238)	Vascular smooth muscle cells	(27, 28)
	Heart	(14)	Vascular endothelial cells	(29)
	Liver	(14)	Neurons	(238)
	Testis	(14)	Macrophages	(30)
	Uterus	(239)	Platelets/megakaryocytes	(31, 241)
	Vascular system	(27, 29)	Epithelial cells	(4, 36, 158, 242)
	Skeleton	(240)	Uterus endometrium/myometrium	(239)
	Lung	(4, 158)	Skeletal myoblasts	(240)
			tLTs	(164, 165)
			B cells	(33)
PDGF-B	Vascular system	(29)	Fibroblasts	(27, 28)
	Kidney	(243)	Vascular smooth muscle cells	(244)
	Neural tissue	(244)	Vascular endothelial cells	(29)
	Uterus	(245)	Neurons	(244)
			Macrophages	(30)
			Platelets/megakaryocytes	(241)
			Uterus endometrium/myometrium	(245)
			tLTs	(164, 165)
PDGF-C	Heart	(14)	Epithelial cells	(36)
	Pancreas	(14)	Skeletal myoblasts	(36)
	Kidney	(1, 37)	Neurons	(36)
	Ovary	(14)	Vascular endothelial cells	(37)
	Adrenal glands	(247)	Vascular smooth muscle cells	(37)
	Prostate	(246)		
	Uterus, placenta	(247)		
	Nervous system	(248)		
PDGF-D	Heart	(20)	Fibroblasts	(36)
	Pancreas	(20)	Smooth muscle cells	(36)
	Kidney	(249)		
	Ovary	(20)		
	Adipose tissue	(16, 88)		
	Stomach	(16, 88)		
	Bladder	(16, 88)		
	Testis	(16, 88)		
	Lung	(14)		

Definition of abbreviations: PDGF, platelet-derived growth factor; tLT, tertiary lymphoid structure.

and colleagues described functional c-Fos and Ying Yang 1 binding elements in the promoter sequence of PDGFRα. TNF-α, released after fibroblast injury, reduced the transcription of *pdgfr-a* by stimulating complex formation of the transcription factors c-Fos and Ying Yang 1, together with an enriched presence of negative regulatory activity by histone deacetylase (72).

Zinc finger transcription factors, such as Sp1, Sp3, or Egr-1, activate the *pdgf-a* promoter in vascular smooth muscle and endothelial cells (73–75). Wilms' tumor suppressor gene (WT-1) represses the activity of the *pdgf-a* promoter via its binding to Sp1/Sp3/Egr-1 binding sites in murine fibroblasts and human kidney cells (76, 77). *Pdgf-a* gene transcription is repressed via interaction of DNA-binding repressors NF1/X and Sp1, a basal regulator of *pdgf-a* transcription, which prevents Sp1 binding to its specific promoter binding site (78). The GC factor 2 also represses *pdgf-a* transcription, probably by competition of the GC factor 2 with basal transcription factors for promoter binding (79).

A recent study showed that transcription of *pdgf-b* is regulated by SDF-1α by inducing the binding of ELK-1 to its *pdgf-b* promoter sequence (80).

Pdgf-c transcription is controlled by early growth response-1 (Egr-1) and fibroblast growth factor (FGF)-2, which stimulates *pdgf-c* mRNA expression through ERK (81). Furthermore, angiotensin II (AngII) induced PDGF-C expression via the receptor AT1 and Egr-1 activation (82).

Pdgf-d transcription in vascular smooth muscle cells is regulated via binding of Ets-1 and/or SP1 to the GGAT binding sites within the *pdgf-d* promoter (83).

Studies on post-transcriptional/translational regulation of PDGF/PDGFR isoforms are limited. Wang and colleagues showed that activation of p38 mitogen-activated protein kinase (MAPK) by IL-1β leads to stabilization of *pdgfr-a* mRNA, resulting in its increased expression in rat myofibroblasts (84, 85).

PDGF Signaling

Aberrant PDGF signaling is associated with several human diseases, such as glioblastoma; gastrointestinal stromal tumor; breast cancer; atherosclerosis; retinal vascular disease; pulmonary arterial hypertension (PAH); lung cancer; and fibrosis of the kidney, liver, heart, and lung.

Table 2. Tissue and Cell Types Expressing Platelet-Derived Growth Factor Receptors in Human and Mouse

PDGF Receptor	Tissue	References	Cell Type	References
PDGFRα	Lung	(36, 250)	Fibroblasts	(90)
	Skin	(251)	Kidney mesangial cells	(252)
	Intestine	(53)	Platelets	(52, 53)
	Kidney	(252)	Neurons	(253)
	Lung mesenchyme	(4)	Fibrocytes	(187)
	Peyer's patch	(51)	Stromal organizer cells	(51)
			Megakaryocytes	(54)
PDGFRβ	Brain	(55)	Fibroblasts	(90)
	Kidney	(252, 254)	Vascular smooth muscle cells	(36, 90)
	Peyer's patch	(51)	Pericytes	(36, 255, 256)
			Capillary endothelial cells	(55)
			Neurons	(257)
			Macrophages	(56, 57)
			Kidney mesangial cells	(252)
			Fibrocytes	(187)
			Stromal organizer cells	(51)
			T cells	(35, 58)
			Cultured monocytes	(59)
			Natural killer cells	(60)

Definition of abbreviation: PDGF, platelet-derived growth factor.

In the following sections, we outline current mechanisms and downstream processes of PDGF signaling.

Receptor–Ligand Interactions

The basic principles of PDGF signaling are conserved from invertebrates to vertebrates, including mammals (36). PDGF signaling is initiated by the binding of distinct PDGF ligands to specific dimeric receptors. Three different PDGFR dimers have been described: PDGFRαα, PDGFRαβ, and PDGFRββ. The ligands of these receptors (PDGF-AA, PDGF-BB, PDGF-CC, PDGF-DD, and PDGF-AB) show different binding specificities *in vitro* and *in vivo* to these receptor pairs (Figure 1). *In vitro*, PDGF-AA binds with high affinity to PDGFRα homodimers, whereas PDGF-BB shows binding affinity to all three PDGFR dimers. Activated PDGF-CC is a ligand of PDGFRα homodimers, whereas PDGF-DD shows affinity for PDGFRβ homodimers. Several studies have indicated that the novel ligands PDGF-CC and -DD also activate the heterodimer PDGFRαβ (86–88). *In vivo*, PDGF ligands showed different binding affinities: PDGF-AA and PDGF-CC signal via PDGFRαα, whereas PDGF-BB signals through PDGFRββ. For PDGF-DD and -AB, binding affinity toward the different PDGF receptors is largely unknown and is a subject of current research.

PDGF Signaling Pathways

PDGFRα and PDGFRβ are receptor tyrosine kinases (RTKs) with an extracellular domain, a transmembrane spanning region, and an intracellular part containing a tyrosine kinase domain with a specific amino acid sequence (11, 89). Dimeric PDGF ligands bind two monomeric receptors at the same time, thereby inducing dimerization of PDGF receptors and autophosphorylation of the tyrosine residues within its intracellular domain. This phosphorylation results in transduction of signals via recruitment of surrounding proteins containing Src homology region 2 (SH2) domains. Several proteins interfering and binding to the intracellular domains of PDGFRα and PDGFRβ have been described; some of them act like adaptor proteins (e.g., Grb2,

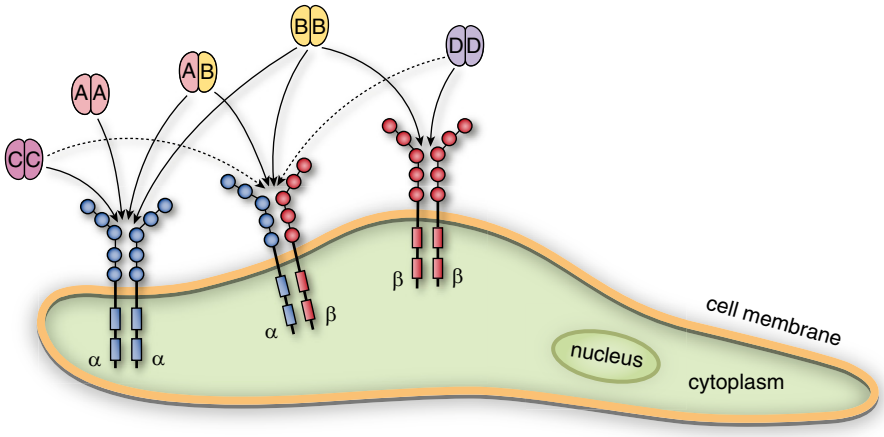

Figure 1. Platelet-derived growth factor receptors (PDGFRs) and ligand binding patterns. PDGFRs are transmembrane proteins expressed on most cell types. The extracellular domain contains five Ig-like domains, of which ligand binding occurs at Ig domains 2 and 3. The intracellular part consists of tyrosine kinase domains. PDGFRs form three dimeric forms: homodimeric PDGFRαα and ββ and heterodimeric PDGFRαβ. Five different ligand isoforms are known to bind to PDGFRs. The ligands bind to receptors with different affinities. The *black solid arrows* indicate documented binding interactions from *in vitro* data. The *dotted arrows* display potential binding interactions.

Grb7, and Shc), whereas others show enzymatic activities (PI3K, PLC-γ, SHP-2, or GTPase-activating protein [GAP] for Ras) and are involved in further downstream signaling (36). In addition, phosphotyrosine-binding domain–containing proteins, such as c-ErbB2, recognize and bind phosphorylated tyrosine residues (90, 91).

There are two main intracellular signaling pathways activated by PDGF signaling (Figure 2): (*1*) the phosphatidylinositol 3′-kinase/Akt/mammalian target of rapamycin (PI3K/Akt/mTOR) pathway and (*2*) the MAPK cascade pathway.

The PI3K/Akt/mTOR pathway tightly controls cellular survival, growth, proliferation, and metabolic activity (92–94). The PDGF ligand–mediated activation of PDGFR recruits PI3K, which converts phosphatidylinositol-4,5-biphosphate (PIP2) into phosphatydilinositol-3,4,5-triphosphate (PIP3). This process is actively controlled by tumor-suppressor phosphatase PTEN (phosphatase and tensin homolog deleted on chromosome 10) by dephosphorylating PIP3 back to its PIP2 form. PIP3 activates phosphoinositide-dependent kinase-1, which phosphorylates and activates Akt. Once Akt is activated, it leads to the activation of multiple downstream proteins, including mTOR. Specifically, mTOR is constitutively activated through suppression of the tuberous sclerosis 1 and 2 (TSC1/TSC2) complex (95–97). The TSC1/TSC2 complex functions as a GAP, which in turn inhibits (Rheb)-GTP (*Ras* homolog enriched in brain). Phosphorylated Akt activates TSC2, which causes separation of the TSC1/TSC2 complex from the cell membrane. Loss of the TSC1/TSC2 complex leads to the elevated level of GTP-bound Rheb and activation of mTOR (96–99). Zhang and colleagues observed that activation of Akt led to a significant reduction of PDGFR expression in mouse embryonic fibroblasts (MEFs), suggesting a negative feedback loop (100). On the other hand, inhibition of mTOR after rapamycin treatment restored the synthesis of PDGFR in MEFs. Although it has not been entirely elucidated how mTOR suppresses the synthesis of PDGFR, these data suggest that suppression occurs on the transcriptional level by reduced PDGFR transcription. Aberrant activation of this pathway by mutations of any member is known to appear in several types of human cancer (101–103).

The MAPK cascade signaling pathway is important in stimulating cell migration, differentiation, or proliferation. MAPK-dependent signal transduction is initiated by growth factor receptor–bound protein 2 (Grb2), an adaptor protein that directly binds to autophosphorylated PDGF receptors via its SH2 domain. Moreover, Grb2 is indirectly activated through Shc or SHP-2. These molecules become phosphorylated on their tyrosine residues and are recognized by the SH2 domain of Grb2. Subsequently, the SH3 domain of Grb2 binds to SOS, which is a nucleotide exchange factor for Ras, leading to the conversion of Ras-GDP to activated Ras-GTP (104). Once Ras is activated, the signal is transduced by Raf-1 and MAPK cascade members, such as MEK or ERK, leading to cell growth, proliferation, differentiation, and migration (105, 106).

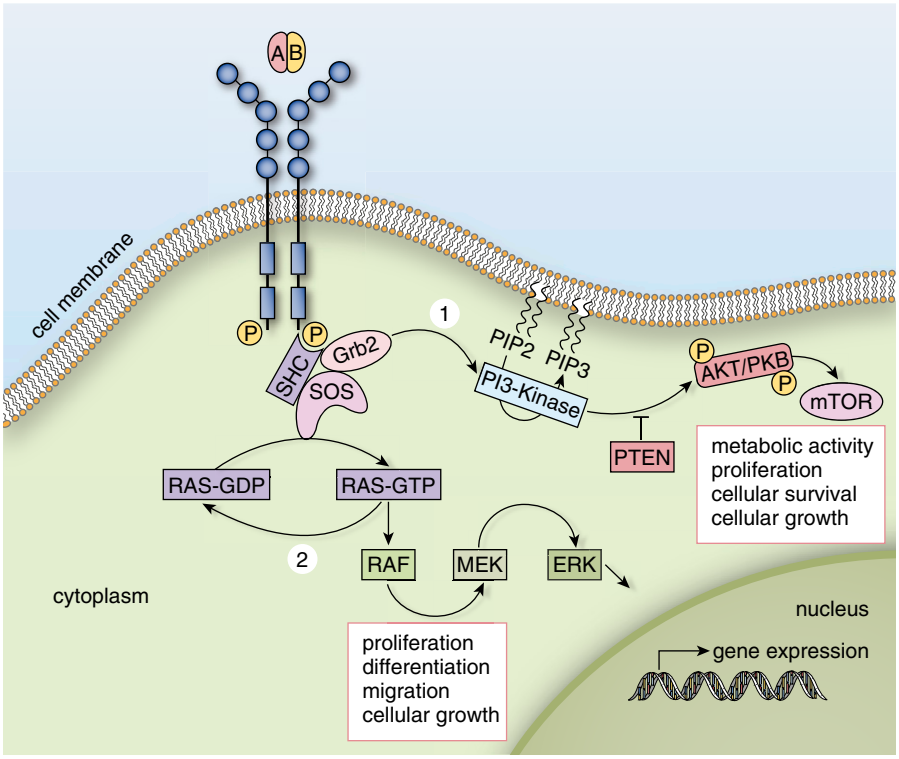

Figure 2. Platelet-derived growth factor (PDGF) signaling pathways. PDGF ligand binding to its respective receptor dimer leads to conformational changes within the receptor and generates close proximity of adjacent tyrosine residues. Autophosphorylation then leads to further signal transduction via two main pathways: (*1*) the PI3K pathway, which mediates Akt signaling for the promotion of cell survival, and (*2*) the MAPK cascade, which is an evolutionary-conserved mechanism of signal transduction. Hydrolytic conversion of RAS–guanosine diphosphate (GDP) to RAS–guanosine triphosphate (GTP) leads to an activation of the mitogen-activated protein kinase cascade, resulting in gene target transcription. This pathway takes part in cell proliferation, differentiation, migration, and cell growth. ERK, extracellular signal–regulated kinase; MEK, mitogen-activated protein kinase kinase.

Regulation of PDGF Signaling

Signaling via PDGF receptors is a carefully controlled process. One of these mechanisms involves the degradation of MAPK phosphatase 3, which normally dephosphorylates and inactivates ERK signaling. Therefore, this process enhances ERK-MAPK cascade activation (107). On the contrary, there are also mechanisms that negatively regulate PDGFR signaling. Dephosphorylation and inactivation of PDGFRs by tyrosine phosphatases, such as protein tyrosine phosphatase 1B or T cell protein tyrosine phosphatase, is one example of negative feedback regulation of PDGF signaling (108, 109). Another mechanism of negative regulation includes docking of Ras-GAP to the activated PDGFRβ. This counteracts activation of Ras, which occurs via simultaneous docking of the Grb2-SOS complex (110). Moreover,

PDGFRα does not bind Ras-GAP and therefore activates ERK signaling more efficiently than PDGFRβ (111). An alternative modulation of PDGF signaling is triggered via interaction of PDGFRs with other cell surface receptors, such as the RTK epidermal growth factor receptor (EGFR) (112) or non–tyrosine-kinase receptors, including integrin receptors (113). Saito and colleagues (112) proposed that the interactions between PDGF and EGF receptors are mediated by reactive oxygen species and members of the Src kinases family. It leads to the formation of a heterodimeric PDGFRβ–EGFR complex under basal conditions (112). This complex may provide a structure for other molecules required for transactivation and subsequently the evidence of PDGFRβ–EGFR cross-talk.

Ligand binding by PDGFRs initializes not only downstream signaling but also internalization of PDGFR to endosomes, which are translocated to the cytoplasm (114). In this respect, PDGFRβ internalization is a tightly controlled process dependent on its interaction with PI3K and on its kinase activity (115, 116). Upon activation of PDGFRβ, signaling occurs at cell surfaces and in endosomes. Active signaling continues in endosomes until PDGFRs are degraded by endosome–lysosome fusion (117) or by ubiquitination-mediated proteasomal degradation (118). Alternatively, receptors are sorted to transport recycling vesicles and are then presented on the cell surface. Transfer of signaling activity into the endosome represents a mechanism for modulating the intensity of PDGF signaling and subsequent influences on the cellular response (119). Kawada and colleagues suggested an important role of PDGFRβ internalization in the regulation of cell migration because the adaptor protein Grb2 formed a complex with DOCK4, which is a regulator of cell migration, and Dynamin2, which is involved in regulation of receptor endocytosis (120–122). Grb2-DOCK4-Dynamin2 has been described to interact with activated PDGFRβ, thereby triggering PDGF-dependent cell migration in the absence of proliferation.

Functional Role of PDGF Ligands

A large number of *in vitro* and *in vivo* systems have been used to study the functional role of PDGF signaling. Since the 1970s, the classical ligands PDGF-A and PDGF-B have been intensively studied. PDGF-A is involved in proliferation of cardiac fibroblasts and plays an essential role in lung alveolar septal formation, alveogenesis, and alveolar myofibroblast development (4, 123). There are two conserved isoforms of PDGF-A, PDGF-A^{short} and PDGF-A^{long}, that arise due to alternative mRNA splicing. The highest total levels of PDGF-A (both short and long isoform) and the highest levels of PDGF-A^{long} were found in the lung (124). Along with this finding, it was shown that the long isoform of PDGF-A appears to be important in the development and homeostasis of the lung because mice lacking the long isoform demonstrated dilated distal airways, reduced numbers of alveoli, and the development of emphysema-like histology at the age of 3 months (124). Moreover, it was shown that the long PGDF-A isoform rather than the amount of PDGFRα is responsible for the observed phenotype (124). The important role of PDGF-B ligand in the ontogeny of kidney and the development of vasculature is shown in various publications (15, 125, 126). Additionally, Koehler and colleagues observed an increased expression of PDGF-A and PDGF-B in peripheral blood leukocytes and lymphocytes in experimental allergic encephalomyelitis, an animal model of multiple sclerosis (34). Expression of PDGF-A and PDGF-B was also detected in T cells, NK cells, and NK T cells. Their findings indicate that PDGF-A and PDGF-B synthesized by lymphocytes may influence the response and activity of T cells in demyelinating diseases, which could be important for future therapeutic strategies (34). An important role for the proliferation and differentiation of B cells was also shown for PDGF-A (33). PDGF-C and PDGF-D have only recently been published (1, 16, 88), and their biological functions are the object of ongoing investigations. Several studies have been performed, and PDGF-C was involved in all phases of wound healing, such as inflammation, proliferation, and remodeling (127). Moreover, recent studies indicated that PDGF-C is a potent neuroprotective factor critical for neuronal survival (128). PDGF-C may also play a role in fibrotic renal disease, as indicated by recent *in vivo* experiments (129). PDGF-D is thought to stimulate extracellular matrix deposition and angiogenesis. Studies also indicate involvement in hepatic and renal fibrosis (130). Moreover, PDGF-C and PDGF-D expression was associated with tumor development and progression (18, 131–133).

Differences in Signaling via PDGFRα and PDGFRβ Receptors

Studying the precise functional role of PDGFRs is technically limited because specific blockers/inhibitors of single receptor isotypes are lacking and because specific deletion of the *pdfgr-a* or *pdgrf-b* gene leads to embryonic lethality or serious defects in early development. Signaling pathways activated by PDGF-B ligand are mediated by one specific receptor dimer, as described recently using genetically defined MEFs with knockouts of PDGFRα, PDGFRβ, or both (134). NF-κB and IL-6 activation is mediated by the PDGFRαβ heterodimer. Furthermore, PDGFRα activates the biosynthesis of the C21-steroid hormone, and PDGFRβ takes part in the activation of EGFR signaling pathway and angiogenesis. On a transcriptome level, a set of 33 genes was specifically activated by PDGFRα homodimers, whereas a set of 15 genes was activated by PDGFRβ homodimers exclusively, and a set of 25 genes was activated by the PDGFRαβ heterodimeric receptor (134).

Further *in vitro* studies indicated that PDGFRα signaling leads to inhibition of fibroblast and smooth muscle cell chemotaxis and that PDGFRβ potentially stimulates fibroblast chemotaxis (90, 135). *In vivo* studies using transgenic mice lacking either PDGFRα or PDGFRβ revealed essential functions during development because mice with either knockouts died at an early stage of embryogenesis. PDGFRβ−/− embryos are deficient in vascular smooth muscle cells and pericytes. PDGFRα−/− embryos showed deficiency in a large number of mesenchymal cells, such as smooth muscle cell progenitors (136). The different phenotypes of mice lacking PDGFRα or PDGFRβ suggest unique roles and distinct spatial and temporal expression patterns *in vivo* (134). The effects of PDGFRαα and PDGFRββ homodimers on target cells could also be explained by divergent interaction with distinct SH2 domain proteins. In addition, PDGFRαβ heterodimeric receptors may demonstrate different intracellular autophosphorylation patterns compared with α- and β-homodimeric receptors (137).

PDGF Cross-Talk with Other Signaling Pathways

It has been thought for a long time that PDGFs and other growth factors exclusively transduce signals via their specific receptors. Several studies indicate, however, that PDGF signaling also cross-talks with other signaling pathways, which can occur via specific ligand-binding other than PDGF to PDGFR or via intracellular cross-activation or cross-inhibition between PDGFR and other transmembrane receptor types.

For the former, cross-talk of PDGFR with ligands of other RTKs has been observed for VEGF, EGF, and TGF-β. Ball and colleagues described VEGF-A–induced PDGFR signal transduction using bone marrow–derived human adult mesenchymal stem cells deficient in VEGFRs. They observed that VEGF-A induced the phosphorylation of both homodimeric PDGFRs, suggesting that VEGF-A may directly stimulate PDGFR signaling. They also observed that the VEGF-A/PDGF axis seems to play a critical role in cellular invasion and proliferation during tissue repair and tumorigenesis (138).

Mendelson and colleagues (139) introduced a possible cross-talk between PDGFRβ and EGFR signaling pathways. In mouse embryonic fibroblasts, PDGF-B/PDGFRβ signaling activated metalloproteinase ADAM17, which in turn stimulated the release of EGF ligands. Thus, EGFR/ERK-mediated signal transduction was responsible for extended ERK1/2 phosphorylation, which is also critical for PDGF-B–induced cell migration (139). Porsch and colleagues (140) investigated a potential interaction of the glycoprotein CD44 with PDGFRβ and TGF-β type I (TβRI) receptor signaling, suggesting a negative regulation for both receptors. Moreover, they observed that TGF-β and PDGF-BB stimulated the phosphorylation of Smad2, a downstream signaling molecule of the TGF-β signaling pathway.PDGF-BB may directly bind to the TGF-β receptor because PDGFs and TGF-β showed a similar topology (141). Additionally, PDGFRβ and TβRI interaction was independent of ligand and receptor kinase stimulation, and the interaction occurred via the extracellular or transmembrane part of PDGFRβ (140). Cell migration induced by PDGFRβ signaling was decreased in the presence of TβRI kinase inhibitor, suggesting that PDGF-BB–triggered migration depends on the activity of the TβRI kinase.

There is also increasing knowledge about downstream cross-reaction between PDGFRs and G protein–coupled receptors (GPCRs). One example is the Wnt receptor Frizzled (142), in which PDGF signaling mediated cooperation of Wnt2-Wnt7 and thus increased the level of Wnt activity required for proper lung development. Inhibition of PDGF signaling reduced Wnt2-Wnt7 coordinated signaling and smooth muscle cell development in lung explant cultures.

A prominent example of cross-activation of the PDGFR pathway is AngII signaling. Several studies showed phosphorylation of PDGFRβ tyrosine residues via either binding of PDGF-BB or prior activation of AngII type 1 receptor (Figure 3). The latter is independent of Ca^{2+} and requires reactive oxygen species (143–145) yet exhibits differences in the transactivation of PDGFRβ. In particular, AngII-stimulated PDGFRβ signaling shows a distinct phosphorylation mechanism and subcellular localization of the Shc-PDGFRβ complex compared with PDGF-BB–induced signaling. Moreover, phosphorylation of Shc-PDGFRβ by AngII was more effective than by PDGF-BB alone (146).

Sciaccaluga and colleagues recently described a functional cross-talk between PDGFRβ and CXCR4 signaling in human glioblastoma cells, which was essential for cell migration (147). CXCR4 belongs to the family of GPCRs and requires CXCL12 ligand binding for signal activation. The inhibition of CXCR4 also decreased PDGF-BB/PDGFRβ–induced cell migration, which underlined the functional importance of this cross-talk (147). Moreover, there is evidence that this cross-talk is specific for PDGFR because several studies demonstrated that inhibition of EGFR activity did not influence cell

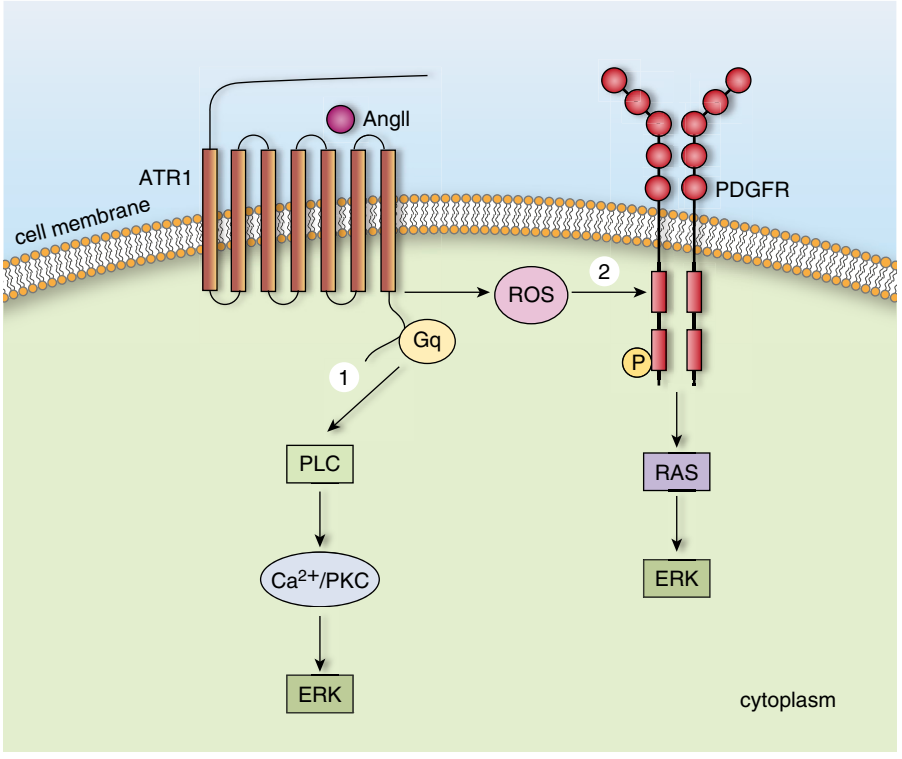

Figure 3. Activation of PDGF signaling by heterodimeric G protein–coupled receptor ATR1. (*1*) Angiotensin II (AngII)-activated ATR1 interacts with Gq, a heterodimeric G protein that activates phospholipase C (PLC) and simultaneously increases the deposition of intracellular Ca^{2+}. Protein kinase C (PKC) stimulates further downstream signaling via phosphorylation of ERK. (*2*) The interaction of active ATR1 with the heterodimeric Gq protein leads to the production of second-messenger reactive oxygen species (ROS), which is necessary for PDGFR phosphorylation and subsequent ERK activation. By that, AngII-activated ATR1 mediates transactivation of PDGFR signaling.

migration triggered by CXCL12/CXCR4 (147, 148). Finally, multiple studies have confirmed cross-activation of PDGFR by GPCRs, such as CXCR4, S1PRs, or P2Y2 (147, 149–151), but the exact mechanisms of cross-activation and their precise role in lung tissue remains to be fully elucidated.

The Role of PDGF Signaling in Lung Development

The importance of PDGF signaling in embryonic development *in vivo* was initially documented by the discovery that *pdgf-a*–null mouse embryos died during embryogenesis or shortly after birth. Surviving *pdgf-a*–null mice developed arrested lung development resembling emphysema due to a lack of alveolar septation that was caused by the absence of myofibroblasts (4). A similar study investigated, in detail, the lung morphology of *pdgfr-a*–null mice and showed a reduction in lung size, indicating a crucial role of PDGFRα signaling in lung growth (2). Overexpression of PDGF-A in the lung epithelium also resulted in perinatal death caused by fetal lung enlargement, failure of airspace development, and mesenchymal cell hyperplasia during lung development (152). Homozygous *PDGF-b*−/− mice displayed a number of anatomical and histological abnormalities during embryogenesis, such as reduced liver volume, reduced size of kidneys, empty urinary bladders, or perinatal death (153). Moreover, the heart and some of the large arteries distended later in embryogenesis, and hemorrhages occurred, with both leading to prenatal death. The *pdgf-c* null mice died perinatally because of feeding and breathing complications due to malformation of palate fusion/cleft palate and deformation of the dorsal spinal cord (154). The knock-out of *pdgf-c* null mice overlapped with *pdgf-a* gene deletion. To our knowledge, deletion of *pdgf-d* genes has not been reported. However, the expression of *pdgf-d* mRNA is detected in the developing rat eye. After inhibition of PDGF-D expression in intact rat eye organ cultures, the proliferation of lens epithelial cells was reduced by 75%. This observation indicates that PDGF-D plays a major role *in vivo* in the strictly coordinated growth of eye tissue (155). The role of PDGF-D during lung development has not been determined.

Similar knock-out studies exist for the PDGF receptors. The inactivation of the *pdgfr-a* gene led to cranial malformations and deficiency in myotome formation (156), leading to intrauterine death by embryonic day (E)10.5 (157). Mice that survived early organogenesis showed multiple defects, including reduced growth and dilation of the pericardium. It was also noted that mesenchymal cells expressing *pdgfr-a* mRNA are essential for postnatal alveolar septation. Mice lacking PDGFRα or fibroblast growth factor receptor signaling show deficiency in alveolarization and the absence of (or defective) interstitial myofibroblast differentiation (4). During lung morphogenesis, the accumulation of *pdgfr-a*–positive cells is detected at sites of future epithelial folding. Later in embryogenesis, lung *pdgfr-a*–positive mesenchymal cells undergo PDGF-A–mediated migration to the site of alveolar septation, which does not occur in the absence of *pdgfr-a*–positive cells (158).

The phenotype of embryos that are homozygous negative for *pdgf-b* or *pdgfr-b* results in very similar phenotypes. In both types of mice, sudden onset of edema formation and dilatation of the heart and large blood vessels occur after E16 to E19 (159). Moreover, in *pdgf-b*−/− and *pdgfr-b*−/− mice, several kidney abnormalities were observed (153). Most *pdgfr-b*–null mutant mice developed perinatally lethal microvascular bleedings caused by a shortage of vascular mural cells (159, 160).

PDGF Signaling in Lung Disease

The impact of PDGF signaling on lung diseases, such as PAH, lung cancer, or interstitial lung disease, has been recently investigated in several studies. PAH is characterized by vasoconstriction, vascular cell proliferation, and small pulmonary vessel remodeling, which leads to a progressive increase in pulmonary vascular resistance and often death due to right ventricular failure (161). Experimental data imply that PDGF signaling plays an important role in PAH by influencing pulmonary vascular remodeling (162, 163). Perros and colleagues uncovered overexpression of PDGF-A, PDGF-B, PDGFRα, and PDGFRβ in the pulmonary arteries of PAH lungs compared with control subjects (164). Moreover, immunohistochemistry showed activation of the PDGFRβ pathway in PAH vascular lesions associated with cellular proliferation. Confirmation of *in vitro* PDGF-induced migration and proliferation of pulmonary artery smooth muscle cells supported a role for PDGF signaling in pulmonary vascular remodeling in PAH (164). Additionally, PDGF-A and -B expression was detected in perivascular tertiary lymphoid structures (tLTs), which contain areas of B and T cells. This study showed local production of PDGF-A, indicating its importance in preserving tLTs in idiopathic PAH (164, 165). Zhao and colleagues confirmed PDGF-BB–induced proliferation and survival of pulmonary artery smooth muscle cells and showed that this effect was mediated by JNK pathway activation (166). Moreover, Xing and colleagues demonstrated that PDGF signaling plays a role in cigarette smoke–induced PAH and observed higher expression levels of PDGF-B and PDGFRβ in pulmonary arteries of rats exposed to cigarette smoke compared with control rats (167).

Aberrant PDGF signaling or overexpression of PDGF ligands/receptors has been detected in several tumors, such as breast, prostate, and liver cancer; brain tumor; leukemia; lung adenocarcinoma; and non–small cell lung cancer (NSCLC) (19, 168, 169). The expression of PDGFRα/β and PDGF-A/B in NSCLC is associated with poor prognosis (170–172). PDGF signaling is important for tumor growth, angiogenesis, and lymphangiogenesis *in vivo*; this was affected by coexpression of PDGF-BB and VEGF-C (or VEGFR3) in NSCLC, also resulting in poor prognosis (172–174). The angiogenic properties of PDGF ligands are supported by the finding that PDGF-AA regulates VEGF expression in an autocrine way and facilitates the malignant process of transforming precancerous lesions to advanced cancer (175). Genetic alterations, such as point mutations in, deletion of, or gene rearrangements of *pdgfr* genes (176–178), have been detected in several cancers, such as gastrointestinal stromal tumor, leukemia, or glioblastoma (19). Mutations in *pdgfr-a* genes amplifying PDGFRα expression led to an elevated number of receptors and to subsequent initiation of ligand-independent PDGF signaling in human glial tumors, lung adenocarcinoma, and NSCLC

(169, 179–182). Also, the amplification of the *pdgf-c* gene is a frequent finding in NSCLC lines, whereas silencing of PDGFRα or PDGF-C in these lines resulted in reduced cell proliferation (182). Moreover, it was shown that paracrine expression of PDGF-A and -C is involved in the recruitment of cancer-associated fibroblasts into the tumor mass, further contributing to tumor growth (172, 183). Inhibition of PDGFRα and -β in NSCLC led to reduced tumor mass, highlighting the importance of the PDGF/PDGFR axis for tumor growth (184). However, PDGFR expression patterns vary significantly in different NSCLC subtypes (170), implying a cell- and disease-specific expression of PDGF and PDGFR family members.

In addition to PAH and lung cancer, fibrotic diseases affecting the lung, kidney, liver, skin, or heart have been linked with elevated PDGF signaling (85, 185, 186). All of these fibrotic diseases are characterized by active tissue remodeling involving the accumulation of extracellular matrix components (e.g., collagen) and proliferation of mesenchymal cell types (e.g., (myo)fibroblasts), leading to progressive scaring and loss of organ function. Fibrocytes, which are abundant in the lungs of bleomycin-treated mice, are highly migratory when treated with PDGF ligands *in vitro*, suggesting a recruiting effect to the lung by the PDGF/PDGFR chemotactic gradient (187). Moreover, Lo Re and colleagues observed in a mouse model of silica-induced lung fibrosis that TGF-β autocrine signal transduction promotes the expression of PDGF-B by $CD4^+$ $Foxp3^+$ regulatory T cells (T_{reg}), which stimulated PDGF-B–mediated fibroblast proliferation, increased collagen deposition, and increased the extent of lung fibrosis (188). Fibroblast proliferation induced by T_{regs} was completely suppressed by imatinib mesylate, an inhibitor of PDGF-B/TGF-β signaling (188). Furthermore, neutralizing T_{reg} activities led to an increased number of T effector (T_{eff}) cells and IL-4–driven fibrogenesis, indicating that T_{regs} play a critical role in controlling T_{eff} cell function during inflammation and fibrosis (188). PDGF activity was also observed in a mouse model of radiation-induced fibrosis, which documents an important role of PDGF signaling in the development of pulmonary fibrosis (189). PDGF-C is involved in the progression of pulmonary and cardiac fibrosis (190, 191), and PDGF-C and PDGF-D have been associated with the development of renal fibrosis (192). PDGFRα- and PDGFRβ-mediated signals play a role in pulmonary fibrosis (36, 187, 193, 194). Inflammatory mediators, such as TGF-β and IL-1β, have distinct effects on the expression of PDGFRα and PDGFRβ. In lung mesenchymal cells, PDGFRα expression is decreased by TGF-β, whereas it is increased by IL-1β (195, 196). In contrast, PDGFRα expression is increased by TGF-β in fibroblasts isolated from patients with scleroderma (197, 198). TGF-β– and/or PDGF-stimulated idiopathic pulmonary fibrosis (IPF) fibroblasts showed an increased expression of IL-16, whereas in normal lung fibroblasts, TGF-β, but not PDGF, down-regulated IL-16 gene expression (199). Moreover, several environmental factors, such as asbestos or air pollutants, stimulate the expression of PDGFRα in a small animal model (200–203). PDGFRβ expression in the lung appears constant and without regulation by inflammatory stimuli. Nevertheless, PDGFRβ may play a role in the proliferative responses alone or in the heterodimeric conformation with PDGFRα (36).

Clinical Relevance of Targeting PDGF Signaling in Human Diseases

Aberrant PDGF signaling has been convincingly documented in a large variety of pulmonary diseases. Therefore, targeting PDGF signaling by inhibiting PDGF ligands and/or receptors represents therapeutic options. An overview of PDGF signaling inhibitors recently used in clinical trials is provided in Table 3.

PDGF inhibitors currently in use include DNA aptamers, neutralizing antibodies, or decoy receptors that sequester PDGFs and thus prevent their binding to and activation of PDGF receptors (204). Alternatively, the activity of PDGFRs can be inhibited by neutralizing antibodies (205). One of the most effective ways to block PDGF signaling, however, is to inhibit the activity of the intracellular PDGF receptor kinases (Figure 4). Several potent inhibitors of PDGF receptor kinases have been tested, including imatinib, sunitinib, and sorafenib (19, 187, 206). Imatinib mesylate is a tyrosine kinase inhibitor targeting PDGFRs, discoidin domain receptors (DDR1 and DDR2), c-kit, and c-Abl (207–211).

The inhibition of PDGF signaling was initially investigated in the treatment of PAH. After the publication of a promising case report (212), the recent clinical trial Imatinib in Pulmonary Arterial Hypertension, a Randomized, Efficacy Study (IMPRES) was performed to investigate the safety and efficacy of imatinib in PAH. Despite various adverse effects, such as nausea, edema, diarrhea, and subdural hematoma, an improvement of hemodynamics and functional capacity (e.g., in the 6-minute walk distance) has been observed (213). As discussed in an accompanying editorial by Marc Humbert, more sufficient data about the long-term efficacy and safety of imatinib for patients with PAH are required (214). The safety and tolerability of sorafenib has also been tested in patients with advanced but stable PAH. The results showed a good tolerance for the lower dose, although several adverse effects, such as skin reactions occurring on the hands and feet, could be detected (215). Future studies will have to clarify whether the potential therapeutic effects of PDGFR kinase inhibition outweighs the significant side effects reported.

The latest preclinical trial in NSCLC documented that inhibition of PDGF signaling with imatinib improved angiogenesis and supported chemotherapy. Clinical trials following up on this initial report, however, showed only little improvement, and progression of the disease was unaffected (216). Another clinical study targeting PDGFR by imatinib was designed to facilitate the effects of paclitaxel, a cytostatic drug used for the treatment of cancer, on tumor cells in patients with NSCLC. This study had several limitations, such as the absence of a randomized control group exposed to paclitaxel alone. More importantly, several adverse effects, including cardiac events, fatigue, and infection, were observed, and no improvement in general and progression-free survival was observed after treatment (217).

Imatinib also has anti-inflammatory properties. Moreover, imatinib showed antitumor effects against melanoma lung metastases through the stimulation of a subset of dendritic cells named "interferon-producing killer dendritic cells" (218). Additionally, *in vitro* studies demonstrated the inhibitory properties of imatinib on the differentiation and

Table 3. Pharmacological Inhibitors Targeting Platelet-Derived Growth Factor Signaling in Human Diseases

Inhibitor	Drug Name	Target	Condition	Status, Phase, Study Design	Outcome	Notes	Clinicaltrials.gov Identification Number
DNA aptamers	E10030 (anti-PDGF pegylated aptamer) plus lucentis	Anti–PDGF-B aptamer, strongly binds to PDGF-B	Age-related macular degeneration	Completed, phase 2, randomized, double-masked, controlled trial (n = 449)	Waiting for multicenter analyses before publishing results	—	NCT01089517
	Fovista (formely E10030, anti-PDGF pegylated aptamer) in combination with lucentis	Anti–PDGF-B aptamer, strongly binds to PDGF-B	Age-related macular degeneration	Recruiting, phase 3, randomized, double-masked, controlled trial, estimated enrollment (n = 622)	No study results published yet, still recruiting	—	NCT01940900
Decoy receptors	NA	NA	NA	NA	Not used in the clinical trials yet	*In vitro* studies	NA
Neutralizing antibodies (PDGF ligands)	NA	NA	NA	NA	Not used in the clinical trials yet	*In vitro* studies	NA
Neutralizing antibodies (PDGFRα)	IMC-3G3 versus Ramucirumab	Human anti-PDGFRα monoclonal antibody (IMC-3G3)	Brain and central nervous system tumors	Ongoing, not recruiting, phase 2, open-label interventional study (n = 80)	Still active, not recruiting anymore	—	NCT00895180
	IMC-3G3 with paclitaxel/carboplatin versus paclitaxel/carboplatin alone	Human anti-PDGFRα monoclonal antibody (IMC-3G3)	Non–small cell lung cancer	Ongoing, not recruiting, phase 2, open-label, randomized, crossover assignment (n = 137)	Still active, not recruiting anymore	—	NCT00918203
	IMC-3G3	Human anti-PDGFRα monoclonal antibody	Gastrointestinal stromal tumor	Completed, phase 2, nonrandomized, interventional study (n = 21)	No study results published yet	—	NCT01316263
	Olaratumab, IMC-3G3	Fully human anti-PDGFRα monoclonal antibody	Solid tumors	Completed, phase 1, open-label, pharmacodynamics study, single group assignment (n = 20)	Well tolerated, showed preliminary antitumor activity, phase 2 ongoing (258)	—	NCT00768391

(Continued)

Table 3. (*Continued*)

Inhibitor	Drug Name	Target	Condition	Status, Phase, Study Design	Outcome	Notes	Clinicaltrials.gov Identification Number
	MEDI-575	Fully human anti-PDGFRα monoclonal antibody	Cancer	Completed, phase 1, nonrandomized, multicenter, open-label, single-arm, dose-escalation study (*n* = 35)	No study results published yet	—	NCT00816400
Imatinib	Gleevec	PDGFR, bcr-abl, c-kit	Thymic carcinoma	Completed, phase 1, nonrandomized, open-label, single group assignment (*n* = 11)	No study results published yet	—	NCT00314873
	Gleevec in combination with Taxotere (docetaxel)	PDGFR, c-kit	Lung cancer	Completed, phase 2, open-label, nonrandomized, single group assignment (*n* = 23)	Nonhematologic toxicity, no improvement observed, one partial improvement, 13 progressions of the disease observed in patients (216)	—	NCT00222144
	Gleevec in combination with paclitaxel	PDGFR	Non–small cell lung cancer	Terminated, phase 2, single stage, open-label (*n* = 34)	Primary endpoint in response rate observed; however, no improvement in overall and progression-free survival after treatment (217)	—	NCT01011075
	Imatinib, scleroglivec	PDGFR, bcr-abl, c-kit	Localized/systemic scleroderma	Completed, phase 2, randomized, double-blind, parallel assignment (*n* = 28)	No study results published yet	—	NCT00479934
	Imatinib mesylate, Gleevec, STI571	PDGFR, bcr-abl, c-kit	Fallopian tube cancer, ovarian cancer, primary peritoneal cavity cancer	Completed, phase 2, open-label, interventional study (*n* = not provided)	No study results published yet	—	NCT00039585

(Continued)

Table 3. (*Continued*)

Inhibitor	Drug Name	Target	Condition	Status, Phase, Study Design	Outcome	Notes	Clinicaltrials.gov Identification Number
	Imatinib mesylate	PDGFR, bcr-abl, c-kit	Hypereosinophilic syndrome	Recruiting, phase 2, open-label, single group assignment, estimated enrollment (n = 50)	No study results published yet, still recruiting	—	NCT00044304
	Imatinib mesylate	PDGFR, bcr-abl, c-kit	Idiopathic pulmonary fibrosis	Completed, phase 2 and phase 3, double-blind, placebo-controlled, randomized, parallel assignment (n = 120)	No change in primary end-point between treatment and placebo control (230)	—	NCT00131274
Imatinib	Imatinib mesylate (QTI571)	PDGFR, bcr-abl, c-kit	Pulmonary arterial hypertension	Completed, phase 3, multinational, multicenter, double-blind, placebo-controlled, randomized (n = 202)	Improvement in hemodynamics, improvement in functional capacity (6MWD test), various adverse effects (213)	—	NCT00902174
Nintedanib	BIBF (1120)	PDGFR, VEGF, bFGF	Idiopathic pulmonary fibrosis	Completed, phase 2, prospective, double-blind, placebo-controlled, randomized (n = 432)	Trend toward a reduction in the decline in lung function (235)	—	NCT00514683
	BIBF (1120)	PDGFR, VEGF, bFGF	Idiopathic pulmonary fibrosis	Completed, phase 2 and phase 3, prospective, double-blind, placebo controlled, randomized (n = 515 and n = 551)	Trend toward a reduction in the decline in lung function (233, 236)	—	NCT01335464, NCT01335477
	BIBF (1120)	PDGFR, VEGF, bFGF	Thyroid cancer	Recruiting, phase 2, double-blind, randomized, parallel assignment, estimated enrollment (n = 143)	No study results published yet	—	NCT01788982

(*Continued*)

Table 3. (*Continued*)

Inhibitor	Drug Name	Target	Condition	Status, Phase, Study Design	Outcome	Notes	Clinicaltrials.gov Identification Number
Sorafenib	Sorafenib	PDGFR and VEGF	Pulmonary arterial hypertension	Completed, phase 1, nonrandomized, open-label, safety study (n = 12)	No study results published yet	—	NCT00452218
	Sorafenib	PDGFR and VEGF	Pulmonary arterial hypertension	Completed, 16-wk, phase 1b, single-center, open-label (n = 22)	Tested dose was well tolerated, adverse effects include moderate skin reactions (hands and feet) (215)	Registered under IND no. 75, 684; held by the University of Chicago	—
	Sorafenib	PDGFR and VEGF	Uveal melanoma	Recruiting, phase 2, randomized discontinuation, double-blind, placebo-controlled, estimated enrollment (n = 200)	No study results published yet, still recruiting	—	NCT01377025
Sunitinib	SU11248, Sutent	PDGFR and VEGF	Prostate cancer	Completed, phase 2, open label, nonrandomized, single group assignment (n = 36)	Few confirmed 50% post-treatment declines in PSA in men with CRPC (259)	—	NCT00299741
	Sunitinib malate	PDGFR and VEGF	Von Hippel-Lindau syndrome	Phase 1, after phase 2 terminated; inability to recruit an adequate number of participants, open-label, nonrandomized, single group assignment (n = 2)	No statistical analysis due to the small number of participants	—	NCT00673816
	Sunitinib	PDGFR and VEGF	Non–small cell lung cancer	The recruitment status of this study is unknown because the information has not been verified recently, open-label, single group assignment (n = 16)	No study results published yet	—	NCT01019798

Definition of abbreviations: 6MWD, 6-minute walk distance; bFGF, basic fibroblast growth factor; CRPC, castration-resistant prostate cancer; NA, not applicable; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; PSA, prostate-specific antigen; VEGF, vascular endothelial growth factor.

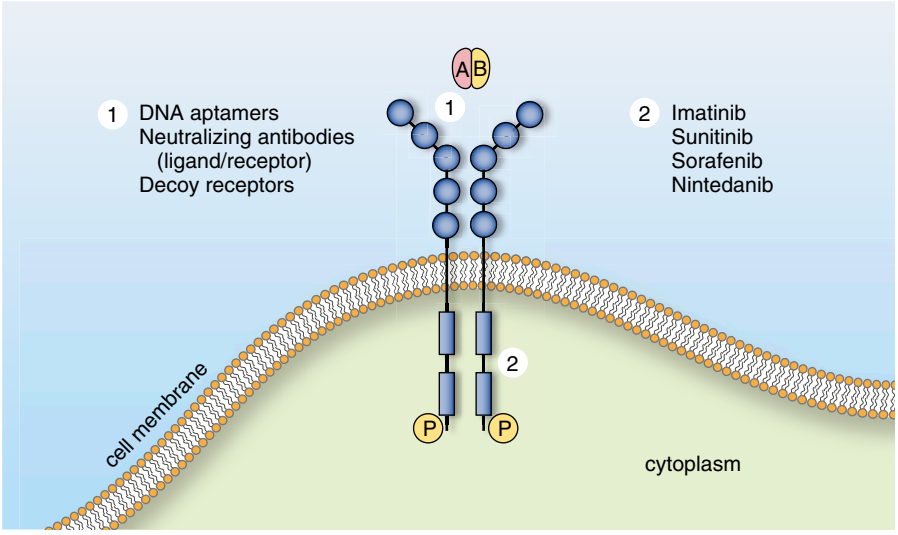

Figure 4. Inhibition of PDGF signaling. Pharmacological inhibition of PDGF signaling may be achieved by (*1*) inhibition of ligand binding by, for example, DNA aptamers, neutralizing antibodies, or decoy receptors, and (*2*) different classes of kinase inhibitors with various specificity acting on the intracellular part of the receptors.

functional activities of dendritic cells, as well as monocytes and macrophages, from bone marrow progenitors (219, 220). Furthermore, Divekar and colleagues (221) demonstrated that imatinib reduced IL-4–producing T cells but increased $CD4^+$ T cells in the lungs of patients with systemic sclerosis. Therefore, targeting PDGF signaling to control immune cell populations in lung diseases might also be important.

Several recent studies have focused on the inhibition of mTOR, a downstream molecule of the PI3K/Akt/mTOR pathway, because it is known that mutations within the components of the PI3K/Akt/mTOR pathway lead to various forms of cancer (100, 102). Inhibition of mTOR may be achieved by rapamycin in a concentration-dependent manner. Based on *in vitro* and *in vivo* studies, rapamycin induces growth arrest in the G_1 phase of the cell cycle and in some cases induces apoptosis in several tumor cell lines (101, 222–225). Moreover, additional inhibitors of mTOR that have similar target profiles, such as CCI-779, RAD001, and AP23573, have been developed (226). Combined treatment with RAD001 and the PDGFRα inhibitor pazopanib showed an antitumor effect in xenograft models in synovial sarcoma cells (227). Additionally, a recent clinical trial testing the target effects and safety profile of RAD001 was performed in patients diagnosed with NSCLC. This study is ongoing, and results are awaited.

Early clinical trials using rapamycin analogs (CCI-779 and RAD001) have been promising, and striking antitumor activities against certain malignancies have been observed in the absence of limiting side effects (226, 228, 229). Current studies are determining the right dosing and are identifying the patient groups for which this treatment will be the most effective and beneficial. Recent clinical trials have investigated the safety and efficacy of imatinib in patients with IPF. This study was the first to investigate the safety and feasibility of tyrosine kinase inhibitors in the treatment of fibrotic lung diseases. A clinical phase 2 study of imatinib versus placebo, however, found no benefit to lung function improvement or survival in IPF (230).

Nintedanib (synonym BIBF 1120) is a potent inhibitor of multiple tyrosine kinase receptors, PDGF, vascular endothelial growth factor (VEGF), and basic fibroblast growth factor receptor (231). Results obtained from the recent phase 2 TOMORROW (To Improve Pulmonary Fibrosis with BIBF 1120) trial have determined the most effective dose of nintedanib. Based on these results, double-blinded, phase 3 clinical trials (INPULSIS-1 and INPULSIS-2) have been conducted using a large cohort of participants within 24 countries covering North and South America, Europe, Asia, and Australia. INPULSIS-1 showed a significant reduction of decline in the FVC in the nintedanib group but did not show a significant difference in the time to first acute exacerbation. INPULSIS-2 also showed a reduction in the decline of FVC in the nintedanib group, but this was not significant. On the other hand, in INPULSIS-2, a significant increase to first acute exacerbation was observed. Additionally, St. George's Respiratory Questionnaire, a questionnaire of quality of life, was unchanged in INPULSIS-1 but showed a significant difference in INPULSIS-2 in favor of the nintedanib group (232–236).

Although these positive results have shed new hope for patients with IPF, tyrosine kinase inhibition remains largely unspecific and affects a number of (un) known targets. This may lead to side effects like nausea rash, fatigue, and edema (19), and the extent of side effects and therapeutic efficiency will have to be individually evaluated in the future (193, 230, 234, 237).

Conclusion

PDGFs are a family of homo- or heterodimeric proteins binding to specific receptors present on the cell surface of most cells. PDGF signaling is mediated by PDGF ligand/PDGFR interaction; activates the MAPK or PI3K/Akt/mTOR pathway; and controls cell growth, proliferation, metabolism, differentiation, migration, tissue remodeling, and suppression of cell death. Several experimental studies have been performed to investigate the interaction of PDGFRα- and PDGFRβ-mediated downstream processes, demonstrating overlapping activities in *in vivo* and *in vitro* models. Based on *in vivo* studies, strong evidence supports an essential role of PDGF signaling in embryogenesis, specifically in normal organogenesis of the brain, kidney, and lung. Although the detailed mechanisms of PDGF signaling during development are poorly understood, PDGF inhibition and deletion of the receptors (PDGFRα or PDGFRβ) or ligands (PDGF-A, PDGF-B, PDGF-C, or PDGF-D) lead to embryonic lethality or severe developmental defects in early development, such as lung developmental abnormalities as

a consequence of arrested alveolar septation.

In adults, the role of PDGF signaling is highlighted by formation of *de novo* connective tissue during wound healing. On the other hand, aberrant expression and signaling of PDGF ligands and receptors is associated with several tissue disorders, including the lung diseases PAH, lung cancer, or IPF. Several clinical trials have recently focused on targeting PDGF signaling by inhibiting PDGF ligands, receptors, and/or PDGFR kinase activity. The most recent positive trials testing the safety and efficacy of nintedanib, an inhibitor of RTKs IMPULSIS-1 and IMPULSIS-2, showed an improvement in lung function in patients with IPF, giving hope to patients with this deadly disease.

The increasing knowledge about the signal transduction pathways of PDGF RTKs, overlapping cross-activities with alternate pathways, and the evidence that PDGF plays an important role in embryonic development and wound healing and in various disease manifestations make PDGF one of the most studied and therapeutically addressed growth factors. Future studies will outline these cross-activating activities and evaluate whether they constitute chances or barriers for future clinical trials.

Therefore, a concentrated effort in understanding the regulation of PDGF signaling, including studies of receptor cross-talk and overlapping intracellular signaling, is required to improve specific spatio-temporal targeting of this pathway and to reduce side effects for new therapeutic options. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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