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PlGF: A Multitasking Cytokine with Disease-Restricted Activity

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Placental growth factor (PlGF) is a member of the vascular endothelial growth factor (VEGF) family that also comprises VEGF-A (VEGF), VEGF-B, VEGF-C, and VEGF-D. Unlike VEGF, PlGF is dispensable for development and health but has diverse nonredundant roles in tissue ischemia, malignancy, inflammation, and multiple other diseases. Genetic and pharmacological gain-of-function and loss-of-function studies have identified molecular mechanisms of this multitasking cytokine and characterized the therapeutic potential of delivering or blocking PlGF for various disorders.

Since the discovery and cloning of placental growth factor (PlGF) in the early 1990s (Maglione et al. 1991), the characteristic features of this VEGF family member have become increasingly identified. PlGF affects not only endothelial cells but also a whole array of other cell types, and its broad spectrum of pleiotropic activities in various biological processes continues to expand. PlGF is redundant for developmental and physological processes but is more important in conditions of disease. Numerous preclinical models have shown that elevating or lowering the expression levels of PlGF can elicit several disease conditions. Precisely because PlGF has a negligible role in health, it has been suggested that PlGF blockade might inhibit these disease processes without affecting normal health. Meanwhile, clinical evaluation of the therapeutic potential of an anti-PlGF monoclonal antibody (mAb) for cancer has commenced. In this review, we highlight key aspects of the biology of PlGF, with attention to its mechanisms of action, interaction with other molecules, and possible clinical implications. We also discuss unresolved or controversial issues about the role and therapeutic potential of PlGF. Rather than providing an encyclopedic survey, we focus primarily on recent discoveries.

PlGF: CELLULAR ACTIVITIES AND MOLECULAR MECHANISMS

The human PlGF gene has been mapped to chromosome 14q24. Its sequence spans an 800-kb-long DNA segment comprising seven exons. In humans, four isoforms have been described—PlGF-1–4 (Maglione et al. 1991; Hauser and Weich 1993; Cao et al. 1997; Yang et al. 2003)—whereas mice only express the equivalent of PlGF-2 (DiPalma et al. 1996). Unlike VEGF, which binds to both VEGF receptor (VEGFR)-1 (also named fms-like tyrosine kinase-1, or

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FLT1) and VEGFR-2 (fetal liver kinase, Flk1/KDR), PlGF binds only to FLT1 and sFLT1, the natural soluble version of the receptor lacking transmembrane and intracellular domains (Kendall and Thomas 1993). PlGF-2 can also bind to neuropilin (NRP)-1 and -2 because of an insertion of 21 basic amino acids at the carboxyl terminus (Migdal et al. 1998; Persico et al. 1999). PlGF-1 and PlGF-3 are diffusible isoforms, whereas PlGF-2 and PlGF-4 have heparin binding domains (Yang et al. 2003).

PlGF: A Pleiotropic Factor

PlGF affects different cell types and regulates various biological responses (Fig. 1). One of the activities of PlGF, identified early on, is its effects on vessel growth and maturation (Ziche et al. 1997; Yonekura et al. 1999; Carmeliet et al. 2001). This proangiogenic activity of PlGF relies on direct effects on endothelial and mural cells, as well as on indirect effects on nonvascular cells with proangiogenic activity (Fig. 1). PlGF enhances the proliferation, migration, and survival of endothelial cells (Ziche et al. 1997; Carmeliet et al. 2001; Adini et al. 2002; Fischer et al. 2007; Schmidt et al. 2011), although some of these effects remain debated (see below). This cytokine also stimulates proliferation of mesenchymal fibroblasts and regulates the contractile response of mural cells, organized around the endothelium during collateral vessel growth (Yonekura et al. 1999; Bellik et al. 2005). In addition, PlGF recruits

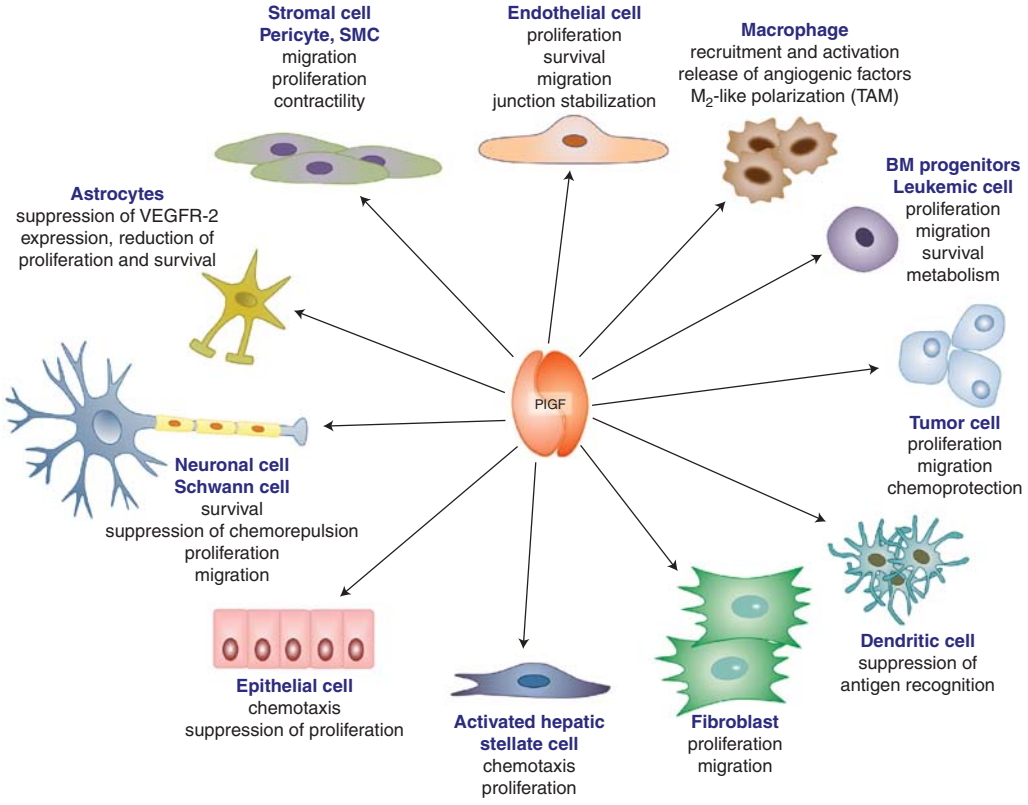

Figure 1. PlGF is a multitasking cytokine affecting various cellular activities. Scheme illustrating the pleiotropic actions of PlGF, including effects on survival, migration, proliferation, metabolism, and activation effects on vascular (endothelial cells, pericytes/smooth muscle cells) as well as nonvascular cells (macrophages, bone marrow–derived progenitors, tumor cells, dendritic cells, fibroblasts, hepatic stellate cells, epithelial cells, neurons, Shwann cells, astrocytes). BM, Bone marrow; SMC, smooth muscle cell; TAM, tumor-associated macrophage.

myeloid progenitors to growing sprouts and collateral vessels (Hattori et al. 2002; Luttun et al. 2002; Pipp et al. 2003; Rafii et al. 2003; Scholz et al. 2003). Futhermore, PlGF activates and attracts macrophages, capable of releasing angiogenic and lymphangiogenic factors (Selvaraj et al. 2003), and interferes with dendritic cell differentiation and accumulation as well as with antigen recognition (Lin et al. 2007; Rolny et al. 2011).

PlGF recruits mesenchymal progenitors in endochondral ossification (Fiedler et al. 2005), stimulates keratinocyte migration in wound healing (Failla et al. 2000), and enhances chemotaxis of retinal pigment epithelial cells (Hollborn et al. 2006). It also promotes survival of cortical neurons (Du et al. 2010), promotes axon growth cone formation of dorsal root ganglion neurons (Cheng et al. 2004), and stimulates proliferation and migration of Schwann cells (Chaballe et al. 2011a). PlGF enhances growth of tumor cells, both of solid and hematological tumors (Fischer et al. 2008; Schmidt et al. 2011).

Molecular Mechanisms

It was initially postulated that membrane-anchored FLT1 functions as a ligand trap, because it has a high affinity for VEGF but weak tyrosine kinase activity (Park et al. 1994). According to this model, PlGF would stimulate endothelial cell growth indirectly via displacement of VEGF from the FLT1 decoy receptor, thereby liberating VEGF for activation of VEGFR-2 (Park et al. 1994). Accumulating evidence suggests additional mechanisms. For instance, PlGF up-regulates the expression of angiogenic factors such as VEGF, basic fibroblast growth factor (FGF2), platelet derived growth factor β (PDGFB), and matrix metalloproteinases (MMPs), among other molecules (Roy et al. 2005; Marcellini et al. 2006). Furthermore, activation of FLT1 by PlGF induces an intermolecular FLT1:VEGFR-2 cross talk that amplifies VEGF/VEGFR-2 signaling, suggesting that endothelial cells are capable of enhancing their own responsiveness to VEGF by releasing PlGF (Autiero et al. 2003).

Via activation of FLT1, PlGF induces signaling pathways different from those induced by VEGF. This is the case for macrophages, which predominantly/exclusively express FLT1 (Clauss et al. 1996; Sawano et al. 2001), but also for endothelial cells (Landgren et al. 1998; Autiero et al. 2003), which up-regulate FLT1 in pathological conditions (Shibuya 2006; Huang et al. 2007; Vanheule et al. 2010; Frysz-Naglak et al. 2011). As shown by mass spectrometry, PlGF and VEGF induce phosphorylation of distinct tyrosine residues of FLT1 (Autiero et al. 2003), further underlining that PlGF and VEGF transmit distinct angiogenic signals through FLT1. Moreover, a recent study reported that FLT1 transmits distinct intracellular signals in response to its different ligands, with only VEGF-B but not VEGF or PlGF stimulating trans-endothelial lipid uptake (Hagberg et al. 2010).

Other studies reported divergent findings about the biological activity of PlGF, and some of its activity profile remains debated. For instance, PlGF has been reported to be ineffective in stimulating endothelial cell growth and migration in vitro, a finding attributed to the weak tyrosine kinase signal activity of FLT1 (Park et al. 1994). It should be noticed, however, that endothelial cells produce abundant PlGF in culture, saturating FLT1 and thereby rendering it unresponsive to exogenous PlGF. Such a hypothesis is supported by findings that endothelial cells from PlGF knockout mice are capable of responding to exogenous PlGF and that loss of PlGF reduces endothelial cell responses (Carmeliet et al. 2001; Schmidt et al. 2011).

PlGF has been also documented to inhibit tumor angiogenesis when overexpressed to supraphysiological levels in tumor cells that coexpress VEGF. In such conditions, VEGF:PlGF heterodimers are formed at the expense of the more proangiogenic VEGF:VEGF homodimers, explaining the reduced angiogenic activity of these tumor cells (Eriksson et al. 2002; Xu et al. 2006; Schomber et al. 2007). Tumor overexpression of a PlGF variant containing an endoplasmic reticulum retention signal similarly promoted VEGF:PlGF heterodimer formation and reduced tumor angiogenesis and growth

(Bjorndahl et al. 2004). Furthermore, a PlGF variant, unable to bind FLT1 but still capable of heterodimerizing with VEGF, also inhibited VEGF- and PlGF-dependent angiogenesis in cancer (Tarallo et al. 2010). It should be noted, however, that the precise role of the VEGF:PlGF heterodimers remains a matter of debate, as several other studies reported *pro*angiogenic—not *anti*angiogenic—effects of these heterodimers in vitro and in vivo (DiSalvo et al. 1995; Cao et al. 1996a,b; Autiero et al. 2003). So far, little/nearly nothing is known about the proangiogenic or antiangiogenic activity of naturally occurring VEGF:PlGF heterodimers in physiological settings in vivo. It thus remains unresolved if the antiangiogenic activity of PlGF:VEGF heterodimers only becomes evident when PlGF is overexpressed at supraphysiological levels.

In any case, it is worth noting that a larger number of studies report a proangiogenic effect for PlGF when it is expressed endogenously by tumor or stroma cells, or when a PlGF transgene is modestly overexpressed by tumor cells (Hiratsuka et al. 2001; Adini et al. 2002; Li et al. 2006; Marcellini et al. 2006; Kerber et al. 2008; Tarallo et al. 2010). Furthermore, genetic neutralization of PlGF *inhibits*—not *stimulates*—vessel growth in adipose tissue, tumors, and other injured, ischemic, or inflamed tissues (Table 1). Final resolution of these issues awaits further investigation.

PlGF, REDUNDANT IN DEVELOPMENT AND HEALTH

Given its pleiotropic activities, it is not surprising that PlGF induces various biological effects in vivo (Table 1). PlGF-deficient mice exhibit normal development, viability, and health, indicating that endogenous PlGF is dispensable for vascular development and homeostasis in the adult (Carmeliet et al. 2001). PlGF is also not required for exercise-induced angiogenesis in the heart or skeletal muscle (Gigante et al. 2004). This redundancy in health is further illustrated by the fact that an sFLT1 trap that neutralizes VEGF, VEGF-B, and PlGF induces a similar phenotype as an anti-VEGF antibody in healthy animals (Malik et al. 2006). A possible explanation for the more important disease-associated role of PlGF is that its expression is low/undetectable in most healthy tissues, but significantly up-regulated in pathologies (Marrony et al. 2003; Fischer et al. 2008). In line herewith, FLT1 expression is also up-regulated in disease (Fischer et al. 2008).

Although PlGF is expressed by trophoblast cells and placental villi in pregnancy (Munaut et al. 2008; Depoix et al. 2011), PlGF-deficient mice are fertile (Carmeliet et al. 2001). However, high sFLT1 levels in the placenta likely neutralize local PlGF (Kendall et al. 1996; Cindrova-Davies et al. 2011; Furuya et al. 2011). PlGF also regulates the maturation of uterine natural killer cells in the endometrium needed for trophoblast invasion (Tayade et al. 2007). Overall, although PlGF is expressed in development, its role is largely redundant.

PlGF, A DISEASE-MODIFYING CANDIDATE

In contrast to its more enigmatic function in development and health, PlGF has a predominant role in the angiogenic and inflammatory "switch" in various diseases, as shown by studies using pharmacological and genetic inhibition or overexpression approaches (Table 1). Part of its biological activity is mediated by molecular effects on the vasculature, but effects on nonvascular cells are also in play (Fig. 1). PlGF is expressed by various cell types in pathological conditions, including vascular cells, fibroblasts, leukocytes, hepatocytes, bone marrow–derived cells, neurons, epithelial cells, and tumor cells (Table 2). Several stimuli up-regulate the expression of PlGF in disease conditions, ranging from hypoxia, growth factors, hormones, and oncogenes to physical stimuli (Table 2). The PlGF receptor FLT1 and its coreceptor NRP-1 are also up-regulated in disease conditions (Barleon et al. 1997; Gerber et al. 1997; Beck et al. 2002; Neufeld et al. 2002; Shibuya 2006). A possible implication of these findings is that PlGF blockade might inhibit disease processes more selectively than physiological homeostasis and thus evoke fewer side effects. We summarize below the findings on PlGF's

Table 1. Phenotypes observed upon gain-of-function and loss-of-function of PlGF

PlGF	Gain of function (GOF)	Loss of function (LOF)
General	ND	Knockout: viable and fertile without vascular defects (Carmeliet et al. 2001)
Placenta	PlGF protein attenuates clinical symptoms in a mouse model of preeclampsia (Suzuki et al. 2009)	Knockout: reduced angiogenesis in ovaries (Carmeliet et al. 2001) Knockout: altered natural killer cell differentiation in endometrium (Tayade et al. 2007) Excessive release of sFLT1 by villous trophoblasts reduces plasma levels of PlGF (and other growth factors) and causes endothelial dysfunction in maternal tissues leading to preeclampsia (Foidart et al. 2009; Furuya et al. 2011)
Heart	PlGF-induced revascularization of ischemic myocardium and vessel enlargement in remote myocardium preserve cardiac performance following infarction (Luttun et al. 2002; Kolakowski et al. 2006; Roncal et al. 2008)	Knockout: impaired angiogenesis and inflammation in infarct border (Carmeliet et al. 2001) Knockout: Normal exercise induces angiogenesis (Gigante et al. 2004)
Skeletal muscle	PlGF protein or gene delivery: enhances angiogenesis, collateral growth, and blood flow in ischemic limb (Luttun et al. 2002; Pipp et al. 2003; Babiak et al. 2004); restores microcirculation in aged dystrophic muscle (Gargioli et al. 2008)	Knockout: impaired collateral growth in ischemic limb (Carmeliet et al. 2001; Scholz et al. 2003; Gigante et al. 2006) Knockout: normal exercise-induced angiogenesis (Gigante et al. 2004)
Eye	PlGF prevents vessel obliteration in hyperoxia without including neovascularization (Shih et al. 2003) Local ocular PlGF protein or gene transfer causes hematoretinal barrier breakdown and edema (Miyamoto et al. 2007; Kowalczuk et al. 2011)	Knockout or aPlGF: impaired choroidal neovascularization (Carmeliet et al. 2001; Rakic et al. 2003; Van de Veire et al. 2010) Knockout or aPlGF does not impair retinal vascularization during development (Carmeliet et al. 2001; Feeney et al. 2003) PlGF gene silencing in RPE cells ablates their proangiogenic potential (in vitro) (Akrami et al. 2011)
Vascular system	PlGF gene delivery increases atherosclerosis, macrophage accumulation, and neovascularization (Khurana et al. 2005) PlGF gene delivery induces angiogenesis in healthy mice (Roy et al. 2005) PlGF overexpression in tumor cells improves tumor vessel normalization (Hedlund et al. 2009) PlGF protein is a vasodilator of resistance arteries (ex vivo) (Osol et al. 2008) hPlGF-1 protein stabilizes adherens junctions in ECs (Cai et al. 2011)	Knockout: normal blood and lymphatic development (Carmeliet et al. 2001) PlGF blockage by knockout, aPlGF, or sFLT1 reduces inflammatory cell infiltration in atherosclerotic lesions and (early) lesion size (Khurana et al. 2005; Onoue et al. 2009; Roncal et al. 2010) Down-regulation of PlGF normalizes tumor vessels, enhancing chemotherapy (Rolny et al. 2011) Knockout: impaired collateral growth in ischemic limb (Carmeliet et al. 2001; Scholz et al. 2003; Gigante et al. 2006)

Continued

Table 1. *Continued*

PlGF	Gain of function (GOF)	Loss of function (LOF)
Skin	PlGF gene delivery accelerates wound healing (Cianfarani et al. 2006) Overexpression of PlGF increases skin vascularization without affecting lymphatics (Wirzenius et al. 2007) Overexpression of PlGF enhances cutaneous delayed-type hypersensitivity response (Oura et al. 2003)	Knockout: normal blood and lymphatic development (Carmeliet et al. 2001) Knockout: impaired skin wound healing (Carmeliet et al. 2001) Knockout: reduced cutaneous delayed-type hypersensitivity response (Oura et al. 2003)
Bone and joints	ND	Knockout: impaired cartilage and bone metabolism during fracture repair (Maes et al. 2006) Knockout of PlGF and PlGF-FLT1 antagonist peptide: suppresses disease development or progression of rheumatoid arthritis (Yoo et al. 2009)
Fat tissue	ND	Knockout or aPlGF reduces de novo adipose tissue development, in part by reducing angiogenesis (Lijnen et al. 2006) Knockout reduces the fraction of brown adipocytes and stimulates white adipocyte hypertrophy, promoting insulin resistance and hyperinsulinemia (Hemmeryckx et al. 2008)
Solid tumors	Overexpression of PlGF in epidermis or tumor cells: increases tumor growth, angiogenesis, and metastasis (Adini et al. 2002; Li et al. 2006; Marcellini et al. 2006; Kerber et al. 2008); redirects premetastatic niche (Kaplan et al. 2005); inhibits tumor angiogenesis and growth via formation of VEGF/PlGF heterodimers (Eriksson et al. 2002; Xu et al. 2006; Schomber et al. 2007; Tarallo et al. 2010) PlGF educates $CD34^+$ progenitors to proangiogenic $CD11b^+$ myelomonocytes in breast cancer (Laurent et al. 2011)	Knockout or aPlGF: blockage of tumor growth, metastasis, lymphangiogenesis, and angiogenesis (Carmeliet et al. 2001; Fischer et al. 2007; Coenegrachts et al. 2010; Van de Veire et al. 2010) PlGF-Flt-1 antagonist peptide blocks growth of human xenograft tumors (Taylor and Goldenberg 2007) Down-regulation of PlGF is implicated in TAM polarization to a tumor-inhibiting M1-like phenotype, anti-tumor immune responses, and tumor vessel normalization, decreasing tumor growth and metastasis and enhancing chemotherapy (Rolny et al. 2011) Silencing of PlGF in tumor cells prevents proangiogenic activity in $CD11b^+$ cells and slows tumor growth (Laurent et al. 2011)
Hematological malignancies	PlGF induces migration and/or proliferation of AML, ALL, and CML cell lines in vitro (Ikai et al. 2005; Casalou et al. 2007; Schmidt et al. 2011)	Knockout or aPlGF prolong survival of imatinib-sensitive and -resistant CML mice (Schmidt et al. 2011)

Continued

Table 1. *Continued*

PlGF	Gain of function (GOF)	Loss of function (LOF)
Bone marrow (BM)/blood, sepsis	PlGF reconstitutes hematopoiesis through stimulation of stem cell differentiation and mobilization (Hattori et al. 2002) PlGF recruits angiogenic BM progenitors and macrophages to neovessels (Lyden et al. 2001; Luttun et al. 2002; Pipp et al. 2003; Kaplan et al. 2005; Li et al. 2006; Carlo-Stella et al. 2007) Viral PlGF gene delivery induces PAI1 plasma levels via mechansms involving HIF1α and miRNA regulation, and promotes a prothrombotic phenotype (Patel et al. 2010, 2011) Overexpression of PlGF in erythroid cells to mimic sickle cell PlGF levels promotes pulmonary hypertension in mice (Sundaram et al. 2010)	Knockout or aPlGF impairs recruitment of BM progenitors and macrophages to neovessels (Carmeliet et al. 2001; Fischer et al. 2007) Knockout reduces PAI1 plasma levels in sickle cell disease (Patel et al. 2010, 2011) Knockout or aPlGF increases morbidity and mortality in sepsis (Yano et al. 2008)
Nervous system	Intramuscular PlGF gene delivery restores diabetic sensory neuropathy (Murakami et al. 2011) PlGF gene: beneficial effects of mesenchymal stem cell (MSC) therapy of cerebral ischemic injury was enhanced upon PlGF gene transduction of the MSCs (Liu et al. 2006) PlGF protein is neuroprotective for primary cortical neurons in vitro (Du et al. 2010) PlGF-2 protein antagonizes chemorepulsive effects in DRG neurons (Cheng et al. 2004)	Knockout of PlGF in astrocytes up-regulates VEGFR2 protecting against oxygen and glucose deprivation (in vitro) (Freitas-Andrade et al. 2008) Knockout accelerates Wallerian peripheral nerve degeneration (Chaballe et al. 2011a)
Liver	ND	Knockout or aPlGF reduces portosystemic collateral formation and portal pressure in portal hypertension (Van Steenkiste et al. 2009) Knockout or aPlGF reduces angiogenesis, arteriogenesis, inflammation, fibrosis, and portal hypertension in liver cirrhosis (Van Steenkiste et al. 2011)
Gut/colon	ND	Knockout aggravates disease course in acute colitis (Hindryckx et al. 2010)
Lung	PlGF overexpression in lung causes emphysema (Tsao et al. 2004)	Knockout: protection against elastase-induced emphysema (Cheng et al. 2009)

ND, Not determined; aPlGF, neutralizing anti-PlGF antibodies; BM, bone marrow; EC endothelial cell; DRG, dorsal root ganglion; MSC, mesenchymal stem cell; PlGF, placental growth factor; RPE, retinal pigment epithelial; sFLT1, soluble FLT1; TAM, tumor-associated macrophage.

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Table 2. PlGF is expressed by various cell types

Cell type	Inducers	References
Endothelial cells	Hypoxia, VEGF, TGF	Yonekura et al. 1999; Carmeliet et al. 2001; Ota et al. 2002; Yao et al. 2005; Fujii et al. 2008; Du et al. 2010; Jiang et al. 2011
SMCs	H_2O_2, BMP2, AngII	Yonekura et al. 1999; Tayade et al. 2007; Pan et al. 2010; Shaw et al. 2011
Fibroblast-like cells: fibroblasts, activated HSC, synoviocytes	Hypoxia (fibroblasts), cytokines IL-1β and TNF (synoviocytes)	Green et al. 2001; Yoo et al. 2009; Van Steenkiste et al. 2011
Leukocytes, lymphocytes, TAMs		Luttun et al. 2002; Tayade et al. 2007; Rolny et al. 2011
Hepatocytes		Van Steenkiste et al. 2011
BM progenitors, erythroid cells, MSC, stromal cells	BMP-2 (MSCs), integrin/VCAM-1 (stroma	Lyden et al. 2001; Hattori et al. 2002; Marrony et al. 2003; Patel et al. 2010; Schmidt et al. 2011
Neuronal and supportive cells: neurons, Schwann cells, astrocytes	Hypoxia (astrocytes)	Beck et al. 2002; Hayashi et al. 2003; Freitas-Andrade et al. 2008; Chaballe et al. 2011b
Cardiomyocytes	Hypoxia	Luttun et al. 2002; Torry et al. 2009
Epithelial cells: RPE, bronchial epithelial cells, type II pneumocytes, keratinocytes	TGF-β, BMP4, light (RPE), nitric oxide (bronchial epithelial cells)	Failla et al. 2000; Cianfarani et al. 2006; Hollborn et al. 2006; Miyamoto et al. 2007; Mohammed et al. 2007; Verhaeghe et al. 2007; Kernt et al. 2010; Akrami et al. 2011; Sands et al. 2011
Osteogenic cells		Coenegrachts et al. 2010
Chondrocytes	Cytokines (IL-1β, TNF)	De Ceuninck et al. 2004
Tumor cells	Oncogenes	Larcher et al. 2003; Parr et al. 2005; Wei et al. 2005; Fischer et al. 2007; Bagley et al. 2011
Thyrocytes	Thyroid-stimulating hormone	Viglietto et al. 1997; Efimenko et al. 2011
Adipose tissue-derived MSC		Viglietto et al. 1997; Efimenko et al. 2011

Cell types are listed with reported information on inducers of PlGF expression where available. AngII, Angiopoietin II; BMP, bone morphogenic protein; HSC, hepatic stellate cell; IL, interleukin; RPE, retinal pigment epithelial; MSC, mesenchymal stem cell; SMC, smooth muscle cell; TAM, tumor-associated macrophage; TGF, transforming growth factor; TNF, tumor necrosis factor.

involvement in several ischemic, inflammatory, or malignant pathologies, with attention to translational studies and clinical implications.

DISEASES AGGRAVATED BY PlGF DEFICIENCY AND IMPROVED BY PlGF THERAPY

Ischemic Cardiovascular Disease

Myocardial ischemia is a major cause of morbidity and mortality. After acute myocardial infarction (AMI), the viable cardiac tissue in the remote area and border zone undergoes hypertrophy in an effort to compensate for the ischemic muscle damage; if not supported by angiogenesis, this hypertrophy can lead to maladaptive decompensation (Shiojima et al. 2005). A role for PlGF in the response to cardiac ischemia is illustrated by findings that PlGF-deficient mice display reduced angiogenesis and inflammation in the border zone of the infarcted myocardium (Carmeliet et al. 2001). Conversely, PlGF gene or protein transfer in infarcted mice stimulates angiogenesis in the infarct border and vessel enlargement in the remote myocardium, improving vascular perfusion, cardiomyocyte hypertrophy, and cardiac recovery (Luttun et al. 2002; Roncal et al. 2008). Unlike systemic treatment with

VEGF (Lee et al. 2000), PlGF treatment did not cause side effects of hypotension or edema formation (Luttun et al. 2002; Roncal et al. 2008). Similar results were obtained with intramyocardial delivery of recombinant PlGF in a rat model of ischemic cardiomyopathy (Kolakowski et al. 2006).

In accordance with these preclinical data, elevated serum levels of the PlGF trap sFLT1 seem to be an independent predictor of mortality in patients with suspected AMI (Hochholzer et al. 2011). Correlation studies in patients and mouse models indicate that cardiac expression of PlGF promotes wound healing after AMI, possibly by inducing mobilization of mononuclear cells and enhancing angiogenesis (Iwama et al. 2006). These data warrant further investigation of whether PlGF therapy may represent a valuable adjunct or alternative to current revascularization strategies for patients with ischemic heart disease.

However, clinical studies also reported correlations between elevated plasma PlGF levels and adverse cardiac outcome during long-term follow-up of patients with acute coronary syndromes, possibly reflecting ongoing inflammatory processes in the coronary vessel wall that may trigger cardiocascular events (Heeschen et al. 2004; Lenderink et al. 2006; Apple et al. 2007; Siervo et al. 2010). The latter is in line with findings that PlGF blockade slows down atherosclerotic progression in preclinical models (see below), warranting caution in PlGF treatment for cardiac disease.

Limb Ischemia

Gene inactivation studies revealed that collateral vessel growth and vascular perfusion in limb ischemia are impaired in PlGF-deficient mice (Carmeliet et al. 2001; Scholz et al. 2003; Gigante et al. 2006). Conversely, PlGF protein or gene administration enhances angiogenesis, collateral vessel formation, and blood flow in surgically induced limb ischemia in mice, resulting in improved performance in endurance tests (Luttun et al. 2002; Babiak et al. 2004). Similar effects were seen in limb ischemia in the rabbit (Pipp et al. 2003). The effect was due in part to smooth muscle cell growth, as well as activation and recruitment of mononuclear cells to the ischemic region (Luttun et al. 2002; Pipp et al. 2003). Endogenous PlGF is also essential for FGF-2-mediated recovery of blood flow in limb ischemia (Fujii et al. 2008).

Nervous System

In cerebral ischemia, PlGF is up-regulated in neurons and vascular cells. NRP-1 expression is also elevated in vessels while its ligand Sema3A is reduced, overall facilitating binding of PlGF (Beck et al. 2002). Intravenous delivery of PlGF-overexpressing mesenchymal stem cells (MSCs) to rats with surgically induced cerebral ischemia reduces lesion size, increases angiogenesis, and attenuates ischemia-induced functional deficits (Liu et al. 2006). PlGF also promotes the survival of cortical neurons in vitro under oxygen and glucose deprivation (Du et al. 2010). On the other side, PlGF deficiency renders astrocytes more resistant to oxygen and glucose deprivation in vitro (Freitas-Andrade et al. 2008). How these differential effects of PlGF in particular cell types regulate the overall outcome of cerebral ischemia remains to be further unraveled. In the peripheral nervous system, PlGF is detectable in axons of the sciatic nerve, and in Schwann cells after axotomy (Chaballe et al. 2011a). Knockout studies indicate that PlGF stimulates proliferation and migration of Schwann cells and promotes an inflammatory response by recruiting macrophages via up-regulation of MCP-1, essential for axonal regeneration (Chaballe et al. 2011a). PlGF also antagonizes chemo-repulsive effects of Sema3A on dorsal root ganglion neurons through NRP binding (Cheng et al. 2004). In contrast, no effect of PlGF deficiency was observed in the course of motoneuron degeneration in rodent models of ALS (P Carmeliet, unpubl.).

Skin Wound Healing

In the skin, PlGF expression is up-regulated during wound healing (Failla et al. 2000; Kagawa et al. 2009), and PlGF-deficient mice

show delayed wound closure (Carmeliet et al. 2001). This finding is corroborated by results that PlGF gene or protein delivery in the skin stimulates angiogenesis, macrophage infiltration, granulation tissue formation, keratinocyte migration and healing of skin wounds in normal mice as well as in diabetic mice, which suffer impaired wound healing (Carmeliet et al. 2001; Cianfarani et al. 2006; Odorisio et al. 2006). Wound vascularization and healing are improved after treatment of skin wounds with dermal fibroblasts containing endothelial progenitor cells, presumably because these cells release PlGF (Hendrickx et al. 2010).

Bone Fracture Repair

Angiogenesis and inflammation are involved in bone fracture healing (Le et al. 2001; Carano and Filvaroff 2003). In a mouse model of semistabilized bone fracture healing, PlGF deficiency impairs healing, cartilage accumulation, angiogenesis, and inflammatory cell recruitment (Maes et al. 2006). In vitro and in vivo data suggest that PlGF contributes to the proliferation and osteogenic differentiation of mesenchymal progenitors, promotes cartilage turnover, and mediates remodeling of newly formed bone through stimulation of osteoclast differentiation (Maes et al. 2006).

Colitis

Inflammation is a key component of inflammatory bowel disease. In an acute colonic injury model, PlGF deficiency impairs mucosal angiogenesis, aggravates epithelial hypoxia, and worsens the disease outcome (Hindryckx et al. 2010). Notably, PlGF gene delivery improves healing of the inflamed colon (Hindryckx et al. 2010).

Sepsis

PlGF levels are elevated in preclinical animal models of sepsis (Yano et al. 2006). PlGF protects liver endothelial cells against septic injury, explaining why sepsis morbidity is increased following genetic or pharmacologic PlGF blockade (Yano et al. 2006, 2008). PlGF blockade in septic conditions should thus be considered with caution.

Preeclampsia

PlGF induces vasodilation of uterine, myometrial, mesenteric, and subcutaneous arteries. This effect is particularly pronounced in uterine arteries during pregnancy, suggesting that PlGF contributes to uterine vascular remodeling during pregnancy (Osol et al. 2008). These findings are consistent with data that utero-placental hypoperfusion and hypertension in preeclampsia patients reduce PlGF plasma levels, because of excessive release of sFLT1, causing endothelial dysfunction in maternal tissue (Foidart et al. 2009; Furuya et al. 2011). Whether PlGF delivery offers novel therapeutic opportunities for preecclampsia remains to be determined.

DISEASES WITH IMPROVED OUTCOME FOLLOWING PlGF BLOCKADE

Ocular Neovascularization

Retinal neovascularization and macular edema, resulting from blood–retinal barrier (BRB) breakdown, are major causes of visual loss in diabetic patients (Morello 2007). PlGF is elevated in the vitreous and retina of diabetic patients and animals (Khaliq et al. 1998; Miyamoto et al. 2007; Kowalczuk et al. 2011). Intraocular delivery of high amounts of PlGF gene or protein in healthy rodents causes retinal vessel disorganization, dilatation, microaneurysm formation, sprouting, rupture of the retinal barrier, and edema, all reminiscent of the changes seen in early diabetic retinopathy (Miyamoto et al. 2007; Kowalczuk et al. 2011).

Choroidal neovascularization (CNV) in patients with the wet form of age-related macular degeneration (AMD) is another prevalent cause of visual loss, especially in the elderly (Mitchell et al. 2010). PlGF is elevated in human AMD and mouse models of laser-induced CNV (Rakic et al. 2003; Huang et al. 2011), whereas PlGF gene deficiency in mice reduces experimental CNV (Rakic et al. 2003). Current treatment of CNV in human AMD by VEGF blockade offers visual improvement but requires intravitreal injection and can increase the risk of stroke, ischemic heart disease, and adverse ocular events (Mitchell et al. 2010). Genetic

blockage of PlGF or pharmacological inhibition of PlGF by systemic administration of an anti-PlGF mAb inhibits laser-induced CNV in mice (Van de Veire et al. 2010). Combination therapy with an anti-PlGF and anti-VEGFR2 mAb is also more effective than monotherapy with anti-PlGF mAb alone. Anti-PlGF not only inhibits angiogenesis and vascular leakiness, but also improves vessel maturation and, unlike anti-VEGFR2 mAb, attenuates ocular inflammation (Van de Veire et al. 2010). Systemic anti-PlGF mAb may thus offer new treatment perspectives for ocular angiogenic disorders.

Atherosclerosis

Loss of PlGF in mice delays atherosclerotic lesion development and inhibits macrophage infiltration, whereas local PlGF gene delivery aggravates atherosclerotic plaque formation in hypercholesterolemic rabbits (Khurana et al. 2005). PlGF is increased in atherosclerotic aortas in apoE$^{-/-}$ mouse models (Roncal et al. 2010). Pharmacological neutralization of PlGF in preclinical mouse models of aggressive atherosclerosis with vulnerable plaques, characterized by inflammatory cell infiltration and limited collagen maturation, slows down plaque development in early phases of atherogenesis but is largely ineffective in more advanced stages. The effect is accompanied by a reduced infiltration of macrophages and T-cells and a lower expression of the adhesion molecule VCAM-1, involved in the recruitment of circulating monocytes to the subendothelial space (Roncal et al. 2010). Thus, anti-PlGF mAb phenocopies the genetic data by reducing early atherogenesis and inflammatory cell infiltration in the lesion.

Arthritis

Rheumatoid arthritis (RA) is a chronic polyarticular inflammation that affects small joints and is characterized by synovial inflammation and formation of a pannus that destroys articular structures. This pannus is highly vascularized, and numerous factors promote angiogenesis in the inflamed joints (Paleolog 2002). Among them, PlGF induces the secretion of VEGF and proinflammatory cytokines from mononuclear cells (Ballara et al. 2001; Yoo et al. 2009). Deficiency of PlGF suppresses RA disease development and progression in mouse models of arthritic joint disease (Yoo et al. 2009). In addition, administration of soluble FLT1 or an FLT1-neutralizing antibody or peptide reduces disease severity and joint destruction (Bottomley et al. 2000; Miotla et al. 2000; Luttun et al. 2002; Yoo et al. 2009).

Liver Cirrhosis

PlGF is also a disease candidate in liver cirrhosis. PlGF levels are increased in cirrhotic patients and in a CCl4-induced cirrhosis model in mice (Van Steenkiste et al. 2011). In the absence of PlGF, liver inflammation, fibrosis, vessel growth and remodeling, and portal hypertension are all less severe in cirrhotic mice. Furthermore, in mice with established cirrhosis, PlGF blockade attenuates disease progression (Van Steenkiste et al. 2011). In cirrhotic rodents, PlGF is markedly up-regulated in hepatic stellate cells, known key players in fibrogenesis. In vitro, PlGF induces ERK1/2 signaling and promotes chemotaxis and proliferation of hepatic stellate cells (Van Steenkiste et al. 2011). Intriguingly, PlGF prolongs the activation of PDGF-Rα and EGFR, possibly because of a transactivation of these receptors by FLT-1. Together, these data suggest a role for PlGF in cirrhosis and illustrate the therapeutic potential of PlGF blockade to attenuate cirrhotic disease. Notably, PlGF accelerates the progression of hepatocellular carcinoma (HCC), a cancer developing frequently in cirrhotic livers (see below).

Cutaneous Delayed-Type Hypersensitivity

PlGF is up-regulated in skin inflammation. In a mouse model of cutaneous delayed-type hypersensitivity, keratinocyte-specific overexpression of PlGF increases the inflammatory response and causes pronounced vascular enlargement (Oura et al. 2003). Opposite phenotypes are observed in PlGF-deficient mice (Oura et al. 2003).

Obesity

Obesity is a major risk factor of metabolic syndrome and ischemic cardiovascular disease. In mouse models of nutritional obesity, PlGF deficiency impairs fat tissue growth partly because of reduced angiogenesis. Anti-PlGF treatment reduces de novo fat pad formation, however, without affecting adipose tissue development, suggesting a role of PlGF in the early stages of adipogenesis (Lijnen et al. 2006). On the other hand, PlGF deficiency reduces the fraction of brown adipocytes while stimulating white adipocyte hypertrophy in mice fed a high-fat diet, changes that promote insulin resistance and hyperinsulinemia (Hemmeryckx et al. 2008).

Pulmonary Emphysema

Increased PlGF levels are observed in patients with chronic obstructive pulmonary disease (COPD) (Cheng et al. 2008). Enforced overexpression of PlGF in lungs of mice causes emphysema due to type II pneumocyte death (Tsao et al. 2004), but PlGF deficiency protects mice against elastase-induced pulmonary emphysema (Cheng et al. 2009). These phenotypes suggest a role of PlGF in the inflammatory process during emphysema, even though the precise role and mechanism of PlGF in pulmonary homeostasis remain to be unraveled.

Besides the aforementioned disease settings, solid and hematologic cancer is emerging as an additional disorder that could benefit from PlGF blockade. We discuss the evidence and controversies in the next sections.

PlGF IN CANCER AND METASTASIS

An "angiogenic switch" promotes tumor growth (Carmeliet and Jain 2011a; Hanahan and Weinberg 2011). In contrast to healthy vessels, tumor vessels have a distorted architecture and display an irregular lining of discontinuous, misshaped endothelial cells, overall impairing perfusion, facilitating tumor cell dissemination, and promoting cancer invasion (Carmeliet and Jain 2011b). Blockade of VEGF signaling offers clinical benefit in several cancer types, but the survival benefit is often limited (in the order of months), and numerous patients develop resistance (Ferrara 2009; Carmeliet and Jain 2011a). Normalizing tumor vessels is emerging as another novel paradigm to reduce metastasis and enhance antitumor chemotherapy (Carmeliet and Jain 2011b).

PlGF in Tumor Pathogenesis

PlGF mRNA and protein levels correlate with tumor stage, invasion and/or metastasis, tumor recurrence, and inversely with survival in several although not all tumor types (Fischer et al. 2008; Bagley et al. 2011). Exceptions include colon and lung carcinoma, where PlGF expression is low because of epigenetic silencing (Xu and Jain 2007). Not only can the tumor cells themselves produce PlGF, but also most types of stromal cells, including endothelial cells, smooth muscle cells, pericytes, cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), and inflammatory cells (Yonekura et al. 1999; Carmeliet et al. 2001; Luttun et al. 2002; Fischer et al. 2007; Bagley et al. 2011; Rolny et al. 2011). Moreover, recent studies document a tumor cell ↔ stroma cross talk, in which tumor cells can "educate" stroma cells to produce PlGF. This was shown for bone marrow stromal cells in preclinical models of bone metastasis of breast cancer and of chronic myeloid leukemia (Coenegrachts et al. 2010; Schmidt et al. 2011). PlGF thereby creates a fertile microenvironmental soil for the seeding tumor cells to foster their survival and expansive growth.

PlGF can promote tumor growth via various distinct mechanisms (Fig. 2). One of them is by stimulating vessel growth and maturation (Adini et al. 2002; Li et al. 2006; Fischer et al. 2007; Van de Veire et al. 2010). Noteworthy, pharmacological or genetic PlGF blockade also promotes vessel normalization (Van de Veire et al. 2010; Rolny et al. 2011). In spontaneous HCC models, PlGF blockade partially restored the abnormally enlarged intercapillary distance, reduced vessel tortuosity, improved vessel patency and tumor oxygenation, and normalized sinusoidal capillarization (Fig. 3) (Van de Veire

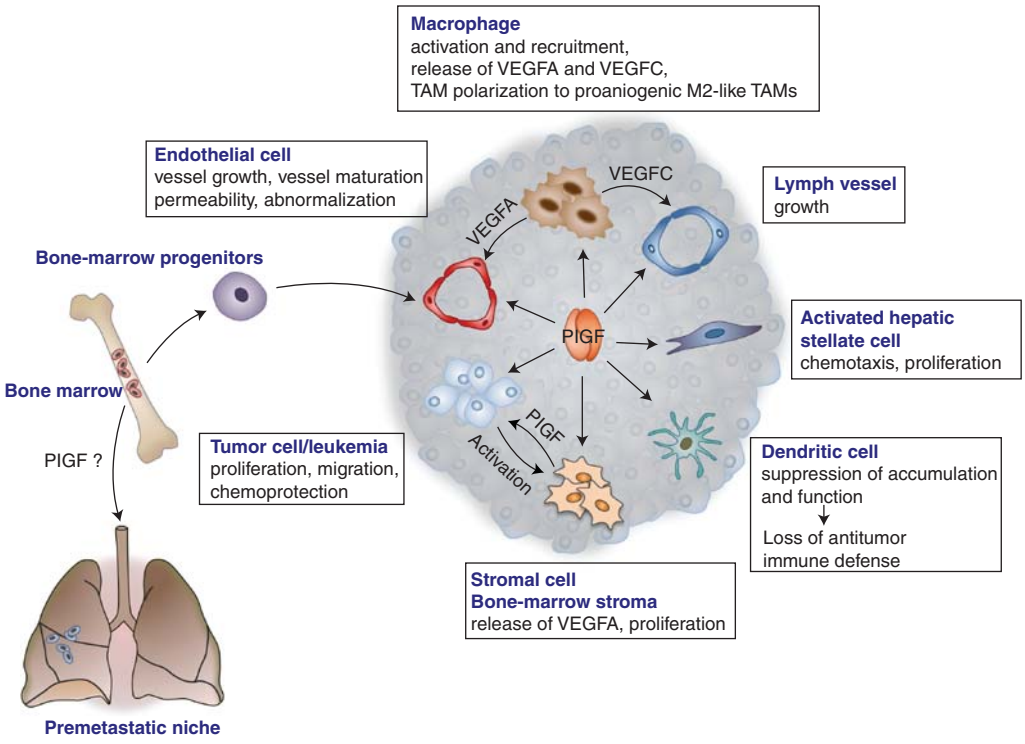

Figure 2. Roles of PlGF in cancer and metastasis. PlGF affects various cellular components and processes in the tumor. It affects angiogenesis by promoting proliferation and migration of endothelial cells, maturation of the vessels by recruiting smooth muscle cells, mobilization of vasculogenic bone-marrow progenitors, and recruitment of macrophages, which produce additional angiogenic and lymphangiogenic factors. PlGF also enhances the "disorganization" of tumor vessels, characterized by an irregular appearance and discontinuous endothelial lining, including "sinusoidal capillarization" in HCC (see also Fig. 3). PlGF also promotes the proliferation and migration of activated hepatic stellate cells in HCC and liver fibrosis. PlGF reduces dendritic cell accumulation and function, thereby suppressing antitumor immune defense responses. PlGF directly stimulates proliferation of tumor cells, which cross talk to and activate stromal cells to produce PlGF (see also Fig. 4). PlGF was also implicated in the mobilization of bone marrow–derived progenitor cells to the "premetastic niche," although conflicting data have been reported. TAM, Tumor-associated macrophage; VEGFA, vascular endothelial growth factor A; VEGFC, vascular endothelial growth factor C.

et al. 2010). Because hypoxia is a driving force for HCC growth (Wu et al. 2007), vessel normalization by PlGF blockade slows down HCC progression. In transplantable tumor models, loss of stromal PlGF normalizes tumor vessels (without altering vascular density), thereby improving tumor oxygenation and responses to chemotherapy, while reducing metastasis (Rolny et al. 2011). In contrast, another study reported vessel normalization in tumors with enforced PlGF overexpression (Hedlund et al. 2009). These apparently divergent results can, however, be reconciled because enforced PlGF overexpression is known to favor the formation of PlGF/VEGF heterodimers at the expense of angiogenic VEGF homodimers that are known to promote vessel disorganization.

PlGF also stimulates inflammatory cell recruitment and activation (Kerber et al. 2008; Ding et al. 2010; Van de Veire et al. 2010; Laurent et al. 2011). It also induces polarization of TAMs to an M2-like proangiogenic phenotype, thereby promoting tumor vessel disorganization (Rolny et al. 2011). PlGF promotes

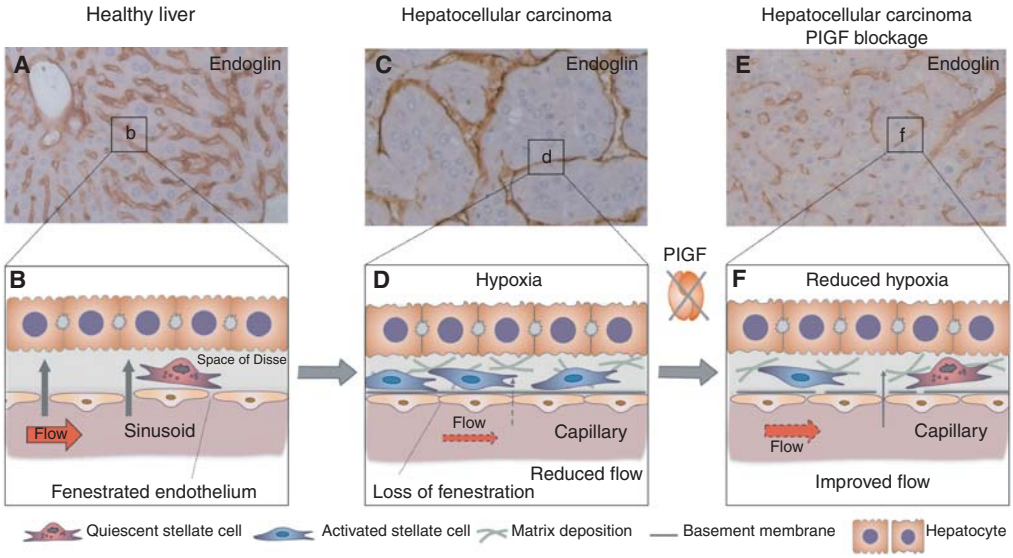

Figure 3. Role of PlGF in sinusoidal disorganization in HCC. (*A*,*B*) In healthy liver, the hepatic microvascular units, the sinusoids (visualized by endoglin immunostaining in *A*), are characterized by a porous fenestrated endothelial lining and absence of a basement membrane, facilitating oxygen and metabolic exchange between the bloodstream and the hepatocytes; the latter are separated from the sinusoids by the perisinusoidal space (space of Disse), where quiescent hepatic stellate cells reside (*B*). (*C*,*D*) In hepatocellular carcinoma, sinusoids undergo "capillarization," characterized by loss of fenestration, increased numbers of activated stellate cells that deposit matrix and release angiogenic factors, change of shape and size of the capillarized sinusoids to more tortuous vessels, and lumen loss in a fraction of the capillaries. These changes result in impaired blood flow (red arrow in *D*) along with reduced oxygen transfer from the blood to the liver parenchyma (gray arrow in *D*), resulting in increased hypoxia, which further promotes tumor cell proliferation resulting in enlarged intercapillary distances (*C*). (*E*,*F*) PlGF blockage by gene silencing or by inhibition with neutralizing antibody partially prevented this disorganization of hepatic sinusoids. The abnormal tortuous appearance, abnormal size, and loss of lumenization of the sinusoids were attenuated (*E*), and sinusoidal "capillarization" was reduced (*F*). This partial "normalization" by PlGF blockage functionally improved tissue oxygenation and reduced proliferation of HCC cells (Van de Veire et al. 2010; Rolny et al. 2011).

growth and migration of tumor cells and protects them against cytotoxic injury by chemotherapeutics (Casalou et al. 2007; Fischer et al. 2007; Taylor et al. 2010; Schmidt et al. 2011). It also suppresses antitumor immune responses by reducing dendritic cell accumulation and function (Lin et al. 2007; Rolny et al. 2011). Furthermore, PlGF/FLT1 promotes mobilization of bone marrow–derived progenitors to future metastatic sites of lung carcinoma, thereby forming a "premetastatic niche" for disseminating metastatic tumor cells (Kaplan et al. 2005, 2009). However, the precise involvement of PlGF/FLT1 in the premetastatic niche formation remains to be further resolved because divergent effects of FLT1 inhibition have been reported (Kaplan et al. 2009; Duda and Jain 2010).

Of note, plasma levels of PlGF are often increased in cancer patients receiving VEGF(R)-inhibition therapy, raising the question of whether PlGF is a factor contributing to the acquistion of "resistance" (evasive escape) against anti-VEGF therapy (Bergers and Hanahan 2008; Jain et al. 2009; Bagley et al. 2011; Carmeliet and Jain 2011a). Experimental models using human tumor xenografts in mice show elevated murine PlGF (but not human PlGF) levels following anti-VEGF treatment, suggesting that PlGF up-regulation by VEGF blockade is at least in part a host reponse (Bagley et al. 2011). Consistent herewith, inhibition or

deletion of host-derived murine PlGF reduces growth of human xenograft or syngeneic tumors (Fischer et al. 2007; Van de Veire et al. 2010; Rolny et al. 2011; Yao et al. 2011). The importance of PlGF in the response of the tumor to VEGF inhibition may, however, be tumor type-specific or context-dependent, as VEGF(R)-inhibitor treatment did not result in an up-regulation of PlGF mRNA or protein in several preclinical tumor models (Bais et al. 2010).

PlGF: A Target for Anticancer Therapy?

Phenocopying the effects seen upon PlGF blockade by gene inactivation or RNA interference (Carmeliet et al. 2001; Van de Veire et al. 2010; Laurent et al. 2011), pharmacological PlGF inhibition by neutralizing antibodies (anti-PlGF antibodies 5D11D4, 3C7A5) or by PlGF/FLT1 peptide antagonists reduced tumor growth, angiogenesis, lymphangiogenesis, inflammation, and metastasis in different ectopically and orthotopically implanted tumors in mouse models, in the absence of adverse side effects (Fischer et al. 2007; Taylor and Goldenberg 2007; Coenegrachts et al. 2010; Bagley et al. 2011). PlGF is also diffusely expressed in medulloblastoma, the most common malignant brain tumor of childhood, and genetic and pharmacologic inhibition of PlGF leads to growth delay and regression of medulloblastoma models in mice as well (Snuderl et al. 2010). Of note, PlGF neutralization by genetic deficiency, anti-PlGF mAb treatment, or small interfering RNA (siRNA)–mediated silencing also reduced the growth of spontaneously arising tumor models, including carcinogen-induced skin papilloma or HCC, as well as a transgenic oncogene-based HCC model (Van de Veire et al. 2010).

In several tumor models, the effects on tumor growth and the underlying mechanisms observed with anti-PlGF treatment phenocopied the effects seen with genetic PlGF deficiency (Van de Veire et al. 2010). For instance, normalization of tumor blood vessels has been observed in the HCC model upon genetic or pharmacological PlGF blockade (Van de Veire et al. 2010; Rolny et al. 2011). In addition, combined anti-VEGFR2/anti-PlGF mAb delivery regimens were superior to the anti-PlGF monotherapies, and anti-PlGF mAb was able to replace anti-VEGFR2 mAb partially without loss of efficacy (Fischer et al. 2007). The 5D11D4 anti-PlGF mAb also blocked the growth of tumors resistant to anti-VEGFR2 blockage (monoclonal antibody DC101 or a VEGF-specific trap, soluble VEGFR2, also termed sFLK1) (Davidoff et al. 2002; Fischer et al. 2007). PlGF blockade also enhanced the effect of chemotherapy in preclinical tumor models and improved vessel normalization and perfusion (Fischer et al. 2007; Rolny et al. 2011). Neutralizing antibodies against human PlGF similarly reduced tumor growth in human xenograft models (Van de Veire et al. 2010; Xu et al. 2010). A humanized anti-human PlGF mAb has entered phase II trials for clinical evaluation of an anticancer benefit.

Nevertheless, not all studies documented antitumor efficacy by blocking PlGF. For instance, not all antibodies with neutralizing capacity in in vitro assays proved to be effective in vivo, either alone as monotherapy or as combination therapy with anti-VEGF mAb, nor did all tumor types respond to anti-PlGF mAb treatment (Bais et al. 2010; Van de Veire et al. 2010; Yao et al. 2011). The precise reasons for these discrepancies remain to be further uncovered (Bais et al. 2010; Van de Veire et al. 2010), but may in part depend on the expression of FLT1 by the tumor cells (Yao et al. 2011). A recent study confirmed that PlGF blockade slowed down chronic myeloid leukemia progression in part by preventing direct growth stimulatory effects of PlGF on leukemia cells, but genetic studies using tyrosine kinase-dead VEGFR1 mice also showed that PlGF signaling in the stromal compartment contributed to leukemia progression (Schmidt et al. 2011). The latter notion is consistent with numerous findings that PlGF affects stromal cells in non-oncology conditions. The relative effect of PlGF on tumor versus stromal cells requires further study.

Also worth mentioning are observations that a soluble FLT1 agent (trapping VEGF, PlGF, and VEGF-B) was not more effective in blocking tumor growth than an anti-VEGF

mAb (Shojaei et al. 2007; Bais et al. 2010). To complicate matters further, divergent tumor growth phenotypes have been reported when implanting tumors in a tyrosine kinase-dead FLT1 mutant mouse model, with some studies reporting a reduction in tumor growth/metastasis (Hiratsuka et al. 2001, 2002; Kerber et al. 2008; Van de Veire et al. 2010; Schmidt et al. 2011) and others showing no effect (Dawson et al. 2009; Bais et al. 2010; Muramatsu et al. 2010). Presumably, the importance of PlGF/FLT1 is context-dependent. Another issue relates to the role of PlGF to angiogenic escape mechanisms of the tumor under anti-VEGF therapy. Indeed, in a recent study using an agent blocking both VEGF and PlGF, reduction of tumor growth was observed while PlGF levels were increased (Bagley et al. 2011). Further exploration is required to resolve these issues.

HEMATOLOGICAL HOMEOSTASIS AND MALIGNANCIES

PlGF promotes the recruitment and differentiation of bone marrow progenitors and regulates hematopoietic reconstitution after myelosuppression (Hattori et al. 2002; Luttun et al. 2002; Carlo-Stella et al. 2007; Loges et al. 2009). PlGF is released by erythroid cells and plasma PlGF is elevated in patients with sickle cell disease (SCD), correlating with hemolysis (Patel et al. 2008; Brittain et al. 2010). PlGF levels are further increased in SCD patients with pulmonary hypertension (Brittain et al. 2010). Consistent herewith, overexpression of PlGF in erythroid cells of healthy mice induces pulmonary hypertension via elevated endothelial production of the vasoconstrictor endothelin-1, which is elevated in SCD patients as well (Patel et al. 2008; Sundaram et al. 2010). The protease inhibitor PAI-1 is similarly elevated in SCD patients and mice. In vitro, PlGF induces PAI-1 expression in endothelial cells and monocytes, in part via HIF-1α-mediated transcriptional regulation and miRNA-regulated posttranscriptional mechanisms, and PlGF deficiency in SCD mice reduces PAI-1 levels, suggesting that PlGF may promote a prothrombotic state in SCD (Patel et al. 2010, 2011). Further data also indicate that erythroid PlGF aggravates inflammation and airway hyperreactivity in SCD via production of proinflammatory leukotriene (Patel et al. 2009; Patel and Kalra 2010).

In vitro, PlGF stimulates the growth of acute lymphoblastic leukemic (ALL) and acute myeloid leukemia (AML) cells, and FLT1 is expressed by human chronic myeloid leukemia (CML) cells (Ikai et al. 2005; Fragoso et al. 2006). However, the role of PlGF in leukemogenesis in vivo remained largely unexplored, and antiangiogenic therapy using VEGF(R) blockers has not met the anticipated success as seen in solid malignancies. A recent study documented that PlGF levels in the bone marrow plasma or peripheral blood correlate with BCR-ABL1$^+$ leukemia load in CML mice and are elevated in CML patients upon disease progression (Schmidt et al. 2011). PlGF produced by the host stroma accelerates disease progression of BCR-ABL1$^+$ leukemia in mouse models of CML. When cocultured with CML cells, bone marrow stromal cells elevated their production of PlGF, a process relying on NF-κB pathway signaling (Fig. 4). By stimulating bone marrow angiogenesis and promoting the growth and glycolytic metabolism of CML cells, PlGF thus appears to create a fertile soil for the CML cells, besides affecting tumor cells themselves.

Furthermore, genetic loss of host PlGF or systemic inhibition with anti-PlGF mAb prolonged the survival of CML mice. Interestingly, PlGF stimulated various signaling pathways in parallel with BCR-ABL1 signaling. The ability of PlGF to stimulate BCR-ABL1-independent signaling in combination with its effects on the CML tumor stroma in the bone marrow likely explains why anti-PlGF mAb treatment added to the anti-CML activity of imatinib in imatinib-sensitive CML mice and prolonged survival in an imatinib-resistant CML model (Schmidt et al. 2011). The ability of malignant cells to induce PlGF in stromal cells was not restricted to CML but was observed with other leukemogenic tumor cells as well, warranting further exploration of targeting the BM stroma or blocking BM angiogenesis in leukemogenic disease (Schmidt et al. 2011).

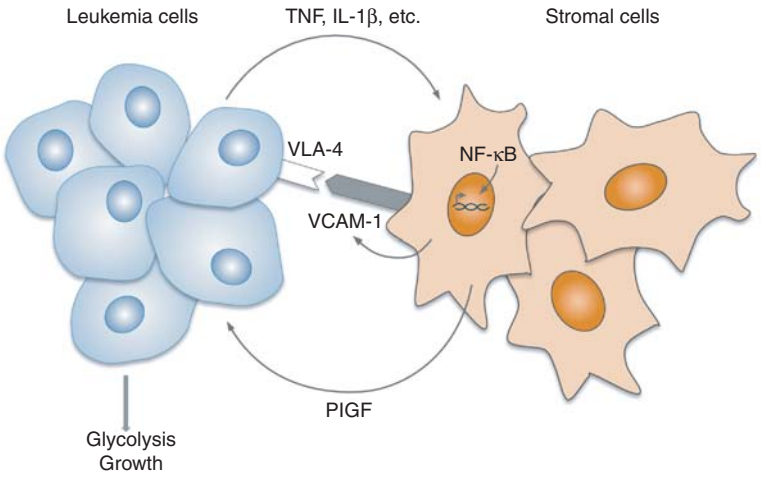

Figure 4. Bidirectional cross talk between leukemia and bone marrow stromal cells. In chronic myeloid leukemia (CML), tumor cells "educate" bone marrow stromal cells to produce PlGF. This induction involves NF-κB activation in the stromal cells and requires leukemia cell/stromal cell contact, which is in part mediated by VLA-4/VCAM-1 interaction. VLA-4/VCAM-1 interaction is reported to activate NF-κB (Zohlnhofer et al. 2000), and NF-κB, in turn, up-regulates VCAM-1 (Rajan et al. 2008), suggesting a positive feedback loop reinforcing $VLA4^+$ CML cell binding to the VCAM-1-expressing stromal cells, thereby ensuring PlGF production. Stromal cell-derived PlGF then promotes proliferation and metabolism of the CML cells. Overall, PlGF creates a fertile microenvironmental soil for the seeding tumor cells to foster cancer cell survival and expansive growth (Schmidt et al. 2011).

CONCLUDING REMARKS

Altogether, the genetic data available to date provide evidence for a role of PlGF in multiple phenotypic traits. A paramount feature is that PlGF appears to be dispensable for development and normal health and that modulation of PlGF expression or action in pathological conditions does not interfere with normal physiological processes. The challenge for the future is to further define the actions of PlGF in normal physiology and disease conditions and to explore if this target can be clinically exploited in an effective, safe manner. Another challenge will be to reconcile the conflicting data obtained by pharmacological PlGF blockade and the more robust PlGF-knockout data.

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P. Carmeliet is named as inventor on patents, claiming subject matter that is partially based on results summarized in this review. The patents are licensed/submitted, which may result in a royalty payment to P. Carmeliet.

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