

Role of placenta growth factor in cancer and inflammation

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Abbreviations: FGF2, fibroblast growth factor 2; MMPs, matrix metalloproteinases; oxLDL, oxidized low-density lipoprotein; PDGFB, platelet-derived growth factor- β ; PIGF, placenta growth factor; RA, rheumatoid arthritis; SRs, scavenger receptors; TAMs, tumor-associated macrophages; TLRs, toll-like receptors; VCAM-1, vascular cell adhesion molecule-1; VEGFR, vascular endothelial growth factor receptor

Abstract

Accumulating evidences have documented that angiogenesis is closely linked to inflammation and regulators of angiogenesis play key roles in various inflammatory conditions. PIGF is an angiogenic protein belonging to the VEGF family and is upregulated mainly in pathologic conditions. Recently, PIGF was discovered having a proinflammatory role in inflammatory arthritis and its serum level drew attention not only as a useful surrogate biomarker but also a potential therapeutic target in atherosclerosis and various cancers. Particularly, PIGF has attractive clinical values because endogenous PIGF is redundant for vascular development and physiological vessel maintenance in healthy adults. However, there have been conflicting results about the efficacy of PIGF inhibition depending on the experimental and clinical settings. Further close investigations for resolving the puzzle of PIGF biology are required.

Keywords: angiogenesis inducing agents; inflammation; neoplasms; neovascularization, pathologic; placenta growth factor

Introduction

Inflammation is a complex set of interaction between soluble factors and cells that can arise in any tissue in response to traumatic, infectious, post-ischemic, toxic or autoimmune injuries. If targeted destruction and assisted repair are not properly phased, inflammation can lead to persistent tissue damage (Nathan, 2002). Angiogenesis is closely coupled with inflammation in many chronic inflammatory conditions with distinct etiopathogeneses, such as atherosclerosis, rheumatoid arthritis, and cancer. Regulators of angiogenesis can modulate inflammatory response and inflammatory response can modulate regulators of angiogenesis, which indicates that angiogenesis and inflammation are components of a common signaling pathway (Imhof and Aurrand-Lions, 2006). In this review, we discuss the distinctive role of PIGF in the mediation of angiogenic and inflammatory signaling in cancer and atherosclerosis, in which inflammation plays a key role as a positive or negative regulator, and rheumatoid arthritis, a representative of chronic inflammatory diseases.

Role of PIGF in cancer

Angiogenesis is a hallmark of tumor pathogenesis and contributes to the progression of cancer from a dormant *in situ* lesion to a life-threatening metastatic disease (Carmeliet, 2005; Kerbel, 2008). The major mediators of tumor angiogenesis are VEGF family and its receptors, and targeted therapies against mediators of tumor angiogenesis are currently under clinical trial (Kerbel, 2008). Angiogenesis inhibitors that target VEGF signaling pathways have proven to be successful clinical treatments in part for various types of cancer. However, a substantial fraction of tumors are resistant to current anti-angiogenic therapies and relapses are not even uncommon after a progression-free period (Hurwitz *et al.*, 2004; Bergers and Hanahan, 2008; Burris and Rocha-Lima, 2008). Moreover, VEGF-A is a trophic factor or survival signal that maintains the quiescent endothelial cells of healthy vessels, and therefore anti-angiogenic therapies cause grave side effects that can lead to life-threatening conditions in a subset of cancer patients (Verheul and Pinedo, 2007). Hypothyroidism and leukopenia as well as vascular complications such as bleeding, disturbed wound healing and thrombotic

events are included in this wide spectrum, which emphasizes the importance of VEGF signaling during physiologic homeostasis of many organ systems (Verheul and Pinedo, 2007). The recently considered attractive alternative for overcoming these problems is the modulation of PIGF-VEGFR1 (also known as Flt1) signaling (Verheul and Pinedo, 2007; Fischer *et al.*, 2008; Loges *et al.*, 2009). The nub of the reason is that PIGF expression, unlike that of VEGF, is undetectable in most organs in healthy conditions, but is highly upregulated in tumor conditions, contributing to the angiogenic and inflammatory switch (Marrony *et al.*, 2003; Fischer *et al.*, 2008).

The first investigation conducted on PIGF expression in tumors was undertaken by Takahashi *et al.* (1994), who demonstrated that PIGF is expressed in hypervascular renal cell carcinoma. Since then, clinical and experimental research on the significance and role of PIGF in tumor progression has been pursued extensively. Accumulating reports have suggested that PIGF might be a useful prognostic marker of cancer progression. Plasma PIGF levels are upregulated and correlate with tumor grade and survival in patients with renal-cell carcinoma (Matsumoto *et al.*, 2003b), and a preoperative high PIGF serum level is a useful prognostic indicator of recurrence and survival in colorectal cancer (Wei *et al.*, 2009). In addition, PIGF mRNA and protein in tumor tissues have been found to be correlated with tumor stage in lung cancer (Zhang *et al.*, 2005), with disease progression and patient survival in colorectal cancer (Wei *et al.*, 2005), with tumor stage and patient survival in gastric cancer (Chen *et al.*, 2004), with recurrence, metastasis and mortality in breast cancer (Parr *et al.*, 2005), and even with post-operative early recurrence in hepatocellular carcinoma (Ho *et al.*, 2007). In tumors, PIGF is not only produced by malignant cells, but also by endothelial cells, smooth-muscle cells, pericytes, cancer-associated fibroblasts, tumor-associated macrophages, and various other inflammatory cells in tumor stroma (Yonekura *et al.*, 1999; Carmeliet *et al.*, 2001; Fischer *et al.*, 2007). However, conflicting data have documented that PIGF expression differs in lung, colorectal, prostate, and thyroid cancers, as it appears to be lower than in matched normal tissues (Viglietto *et al.*, 1995; Matsumoto *et al.*, 2003a; Xu *et al.*, 2006). Thus, it remains to be determined whether PIGF acts as a positive regulator of cancer growth and metastasis in man.

When highly upregulated PIGF transmits its signal through Flt1 by stimulating the phosphorylations of specific Flt1 tyrosine residues, and thus induces

the expression of distinct downstream target genes. PIGF also amplifies VEGF-mediated signal transduction through VEGFR2 (also known as Flk1) by inducing the transphosphorylations of the tyrosine residues of Flk1 (Autiero *et al.*, 2003b). Furthermore, PIGF displaces VEGF-A from Flt1, which releases VEGF-A and allows it to activate Flk1 and enhance VEGF-driven angiogenesis (Park *et al.*, 1994). PIGF has also been reported to form heterodimers with VEGF *in vivo*, and PIGF-VEGF heterodimer formation could deplete VEGF-A intracellular pool, and thus, reduce the formation of angiogenic VEGF-A-VEGF-A homodimers (Cao *et al.*, 1996; Eriksson *et al.*, 2002).

PIGF induces various biological effects by affecting a wide range of different cell types. PIGF can stimulate vessel growth and maturation directly by affecting endothelial and mural cells, and indirectly by recruiting pro-angiogenic cell types. For example, PIGF stimulates the growth, migration and survival of endothelial cells (Ziche *et al.*, 1997; Carmeliet *et al.*, 2001; Adini *et al.*, 2002; Fischer *et al.*, 2007), increases the proliferations of fibroblasts and smooth-muscle cells, induces vasodilatation, and stimulates collateral vessel growth (Yonekura *et al.*, 1999; Bellik *et al.*, 2005). It also promotes the recruitment and maturation of angiogenesis-competent myeloid progenitors to growing sprouts and collateral vessels (Hattori *et al.*, 2002; Luttun *et al.*, 2002b; Pipp *et al.*, 2003; Rafii *et al.*, 2003; Scholz *et al.*, 2003). In addition, PIGF activates and attracts macrophages that release angiogenic and lymphangiogenic molecules (Selvaraj *et al.*, 2003), and inhibits the differentiation of dendritic cells. Malignant tumors take advantage of these pleiotropic functions of PIGF to promote growth, angiogenesis, and resistance toward antiangiogenic treatments. PIGF signaling participates directly in the tumoral angiogenic switch by stimulating the proliferation of endothelial cells and acting on mural cells, including pericytes and smooth muscle cells, and indirectly by upregulating the expressions of VEGF-A, FGF2, PDGFB, MMPs, and other angiogenic factors (Roy *et al.*, 2005; Marcellini *et al.*, 2006). PIGF also recruits Flt1-positive hematopoietic progenitor cells and macrophages to growing tumor sites, and thus, contributes to neovascularization and lymphangiogenesis (Hattori *et al.*, 2002; Luttun *et al.*, 2002b; Schoppmann *et al.*, 2002; Pipp *et al.*, 2003; Rafii *et al.*, 2003; Cursiefen *et al.*, 2004; Murakami *et al.*, 2008). In particular, tumor-associated macrophages (TAMs), representing up to 50% of tumor mass, play a major role in cancer-related inflammation and angiogenesis (Solinas *et al.*, 2009). TAMs preferentially localizes in hypoxic regions of tumor mass and secretes several angio-

genic factors and angiogenesis-modulating enzymes, that sustain angiogenesis and reorganizes tumor vasculature. Moreover, TAMs promotes lymphangiogenesis by secreting VEGF-C and possibly, by promoting the transdifferentiation of peritumoral stroma cells to lymphatic endothelial cells in lymph vessels (Schoppmann *et al.*, 2002; Cursiefen *et al.*, 2004). Indeed, anti-PIGF therapy suppressed lymphangiogenesis, which might be dependent on macrophage inhibition because lymphatic endothelial cells do not express detectable levels of Flt1 (Carmeliet *et al.*, 2001; Fischer *et al.*, 2007). Furthermore, PIGF play a direct role in the control of tumor cell motility and invasiveness (Taylor and Goldenberg, 2007).

As mentioned above, current anti-angiogenic strategies mainly focus on inhibiting the signaling of VEGF-A and Flk1, which pose some challenging problems. One is that a substantial proportion of cancer patients are resistant to VEGF-based therapy. Various mechanisms contribute to this resistance, and allow escape from anti-VEGF therapy. One is angiogenic rescue, a process involving the increased expressions of other pro-angiogenic factors (Bergers and Hanahan, 2008). In particular, after anti-VEGF therapy, PIGF-Flt1 signaling provides a major route for angiogenic rescue (Willett *et al.*, 2005; Kerbel, 2008). Therefore, the inhibition of PIGF could provide an attractive means of supplementing the deficient

anti-VEGF therapy. In fact, Fisher *et al.* have demonstrated that anti-PIGF blocking antibody inhibits the growth and metastasis of various tumors in mice, including those resistant to VEGFR inhibitors, and that it enhances the efficacy of chemotherapy and of VEGFR inhibitors (Fischer *et al.*, 2007). Remarkably, anti-PIGF antibody has also been reported to inhibit angiogenesis, lymphangiogenesis, and tumor cell motility without switching on the angiogenic rescue program and affecting healthy vessels (Fischer *et al.*, 2007). Additional and supportive evidences for the efficacy of PIGF inhibition in cancer were adduced by Van De Viere *et al.* (2010). However, Bais *et al.* (2010) took issue with these results and showed that neutralization of PIGF, genetic ablation of host PIGF receptor signaling, or combined anti-PIGF treatment and loss of host Flt1 tyrosine kinase signaling do not inhibit angiogenesis during primary tumor growth. Xu *et al.* (2006) even found that blocking PIGF could aggravate tumor progression by demonstrating that PIGF overexpression impairs tumor growth and angiogenesis in xenograft models. Although it has been recently suggested that expression of a functional Flt1 in tumor cells is a major determinant of anti-PIGF antibodies efficacy (Yao *et al.*, 2011), no clear reason for result discrepancies has been provided to date. Experimental observations regarding the *in vivo* role of PIGF in mouse models of tumors

Table 1. Summary of controversial evidence regarding the effect of PIGF on tumor biology

Model	Tumor subtype	PIGF manipulation	Tumor growth	Angiogenesis	Metastasis	Reference
Pro-promoting evidences						
C57Bl/6 mice	B16 melanoma / Pancreatic Panc02 adenocarcinoma / CT26 colon carcinoma	Anti-PIGF blocking antibody	↓	↓	↓	Glaser <i>et al.</i> , 2011
Balb/c nude mice	B16F10 melanoma	Anti-PIGF blocking antibody	↔	n.d.	↓	Pipp <i>et al.</i> , 2003
C57Bl/6 PIGF ^{-/-} mice	Skin papilloma	PIGF knockout	↓	↓	n.d.	Pope, 2002
C57Bl/6/ASV-B mice	Hepatocellular carcinoma	PIGF silencing	↓	↓	n.d.	Pope, 2002
C57Bl/6 Rip1Tag2 × PIGF ^{-/-} mice	Pancreatic β cell carcinoma	PIGF knockout	↔	↔	n.d.	Pope, 2002
Pro-inhibitory evidences						
C57BL/6 mice	Murine fibrosarcoma	PIGF overexpression	↓	↓	n.d.	Kerbel, 2008
C57Bl/6 mice	Murine Lewis lung carcinoma	PIGF overexpression	↓	↓	n.d.	Zhang <i>et al.</i> , 2005
C57Bl/6 Rip1 PIGF-1 transgenic mice	Pancreatic β cell carcinoma	PIGF overexpression	↓	↓	n.d.	Ziche <i>et al.</i> , 1997
SCID mice	Human tumor cell lines (HCT116, A549, and U87-MG)	PIGF overexpression	↓	↓	n.d.	Hattori <i>et al.</i> , 2002

n.d., not determined; ↓, inhibition; ↔, not inhibition.

are summarized in Table 1. Further meticulous investigations are required to clarify the efficacies of anti-PIGF therapies in cancer patients.

Role of PIGF in chronic inflammatory conditions

Atherosclerosis

Atherosclerosis is a chronic inflammatory disease characterized by lipid-containing inflammatory lesions in large and medium-sized arteries (Hansson and Libby, 2006). Accumulating evidence shows that angiogenesis plays a key role in atherogenesis and acute lesion instability of progressive atherosclerosis, and is intimately associated with inflammation (Luttun *et al.*, 2002b; Herrmann *et al.*, 2006) and a variety of pro- and anti-angiogenic factors are involved in the angiogenesis of athero-

genesis (Herrmann *et al.*, 2006).

PIGF is upregulated in early and advanced atherosclerotic lesions and is detectable in adventitial cells and in the shoulders and caps of plaque (Luttun *et al.*, 2002a). In particular, PIGF is mainly located at the shoulders of atherosclerotic plaque (Roncal *et al.*, 2010), where high density microvessels exist with marked macrophage infiltration. PIGF promotes atherosclerotic intimal thickening and macrophage accumulation and induces neo-vascularization and endothelial activation (Khurana *et al.*, 2005). PIGF seems to act more effectively during the early phase of atherogenesis, because anti-PIGF antibody treatment significantly inhibits early lesions, but is ineffective during the more advanced stages of plaque development (Roncal *et al.*, 2010). Inhibition of Flt1 failed to affect angiogenesis in plaque (Luttun *et al.*, 2002b). Plaque microvessels were not found in the early lesions of ApoE knockout mice regardless of the presence of

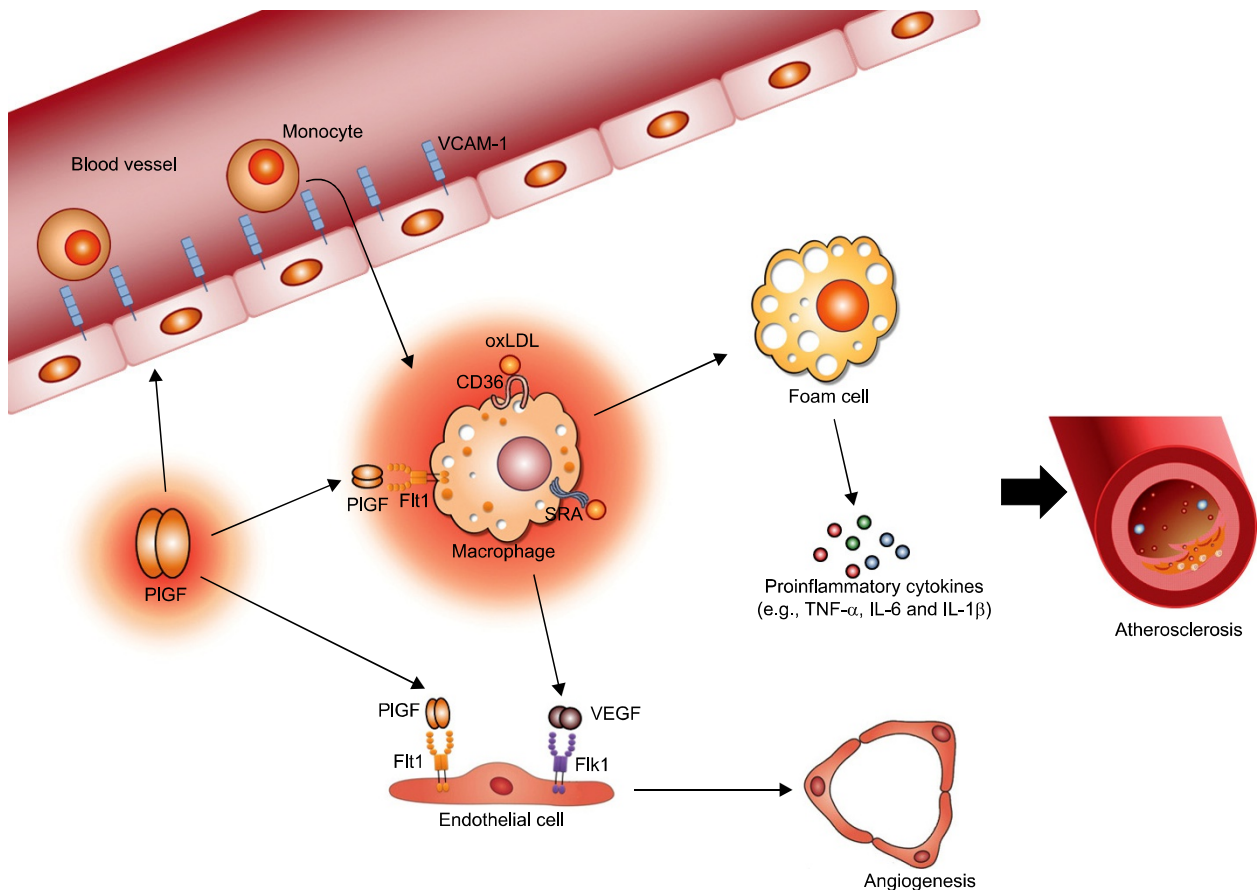


Figure 1. Role of PIGF in atherosclerosis. PIGF is upregulated in plaque shoulders and caps of atherosclerotic lesions. PIGF recruits monocytes by increasing the expression of VCAM-1, a key molecule in the processes of monocyte/macrophage adhesion and rolling at the luminal side of arteries via the activation of Flt1 in endothelial cells. Inflowing monocytes differentiate into macrophages, accumulate oxidized LDL (oxLDL) via scavenger receptors (SRA) and are further activated by PIGF, and form foam cells. Activated macrophages and foam cells produce proinflammatory and angiogenic mediators, which promote inflammatory response, stimulate lesion progression, and increase the risk of plaque rupture.

PlGF, and PlGF deficiency caused a significant reduction in size and macrophage content of early atherosclerotic plaques in ApoE knockout mice compared with mice deficient only in ApoE (Khurana *et al.*, 2005). Thus, the suppressive effect of anti-PlGF on plaque growth and vulnerability might be dependent on inhibition of macrophage infiltration and activation rather than on the direct inhibition of angiogenesis. PlGF recruits macrophages by augmenting the expression of VCAM-1, a key molecule in the monocyte/macrophage adhesion and rolling processes on activated endothelial cells in the luminal side of arteries (Selvaraj *et al.*, 2003; Roncal *et al.*, 2010).

Regulation of monocyte/macrophage recruitment by PlGF is important because monocyte/macrophage is the predominant cell type present within atherosclerotic vessels and is involved in all stages of atherosclerosis development (initiation, progression, and rupture) (Saha *et al.*, 2009). Recruited monocytes differentiate into macrophages with upregulated expression of scavenger receptors (SRs) and innate immune recognition receptors such as Toll-like receptors (TLRs) under the influence of locally produced factors like monocyte colony-stimulating factor and other stimuli. SRs mediate the

macrophage uptake of oxidized LDL (oxLDL) particles, which leads to intracellular cholesterol accumulation and the formation of foam cells. Innate immune recognition receptors like TLRs interact with oxLDL and microbial components, such as LPS, Hsp60, and other ligands, and results in macrophage activation and the productions of proinflammatory mediators. Macrophages also activate T cells by presenting specific antigens and modulate T-cell responses by producing cytokines, such as IL-18 and IL-12. These inflammatory mediators, in concert with T-cell-produced cytokines, promote inflammatory responses, stimulate lesion progression, and increase the risk of plaque rupture (Figure 1) (Yan and Hansson, 2007). Furthermore, activated macrophages also stimulate angiogenesis, which can recruit more inflammatory cells and increases angiogenesis (Moulton *et al.*, 2003).

Circulating levels of PlGF are undetectable in normal individuals, but are elevated in patients with atherosclerotic or ischemic heart disease. In patients with acute coronary syndrome, a high plasma PlGF level within 12 h of symptom onset has been shown to predict a poor prognosis in the short and long-term (Heeschen *et al.*, 2004; Lenderink *et al.*, 2006;

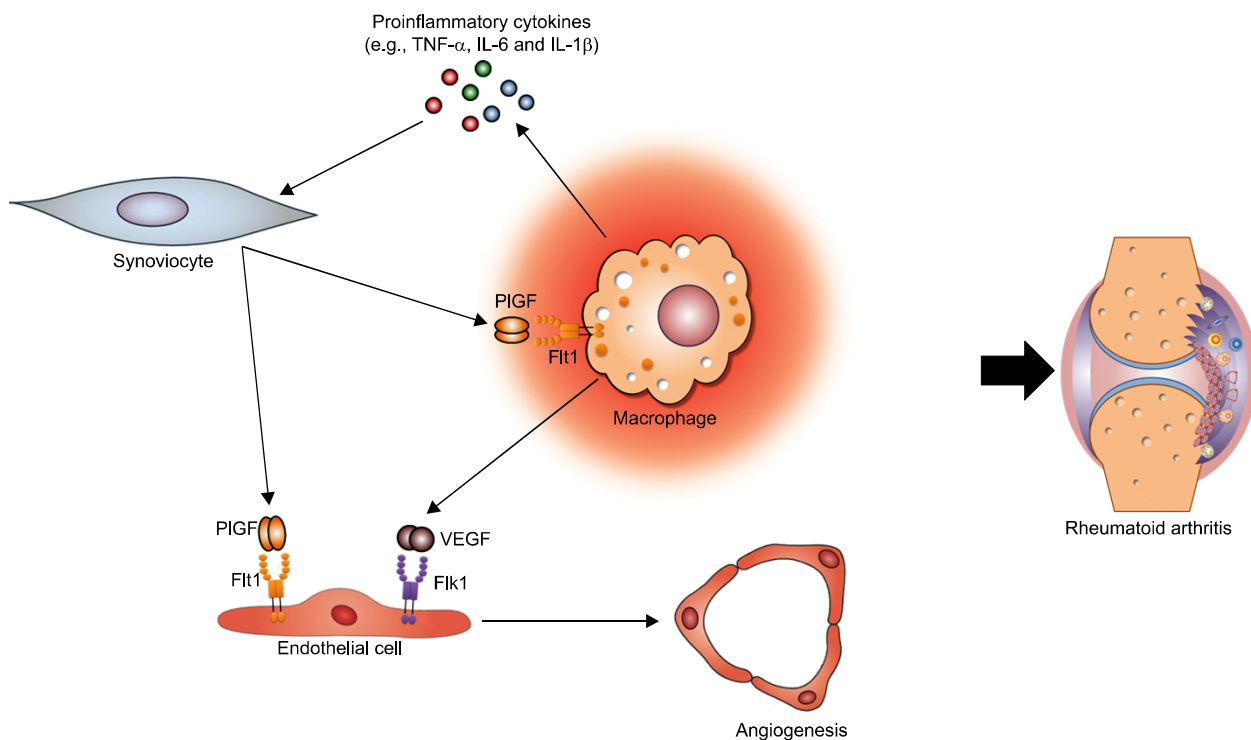


Figure 2. Role of PlGF in rheumatoid arthritis. PlGF, predominantly produced by rheumatoid synoviocytes, promotes angiogenesis and stimulates vascular endothelial cell permeability. Newly employed macrophages produce TNF-α and IL-6 when stimulated by PlGF/Flt-1 binding or by contact with activated endothelial cells. TNF-α and IL-6, in turn, further enhance PlGF secretion by macrophages and synoviocytes, and thus create a vicious cycle of inflammation.

Markovic *et al.*, 2010; Glaser *et al.*, 2011). Furthermore, a single initial measurement of plasma PIGF appears to enhance the predictive and prognostic abilities of traditional markers, such as, B type-natriuretic peptide and troponin I (Glaser *et al.*, 2011).

However, the pathophysiologic role of PIGF during the late symptomatic phase of acute coronary syndrome could differ from that during early disease. Thickened atherosclerotic plaque results in hypoxia of vessel walls and myocardium, and hypoxia is one of the most potent angiogenic stimuli (Bjornheden *et al.*, 1999). Under hypoxic conditions, PIGF is upregulated in cardiomyocytes and fibroblasts, and contributes to myocardial angiogenesis and tissue healing (Green *et al.*, 2001; Torry *et al.*, 2009). PIGF is also known to promote the mobilization, chemotaxis, and recruitment of bone marrow-derived endothelial progenitor cells to ischemic tissues to facilitate the healing of injured vessels (Li *et al.*, 2006). Thus, depending on the stage of atherosclerosis progression, PIGF may be beneficial or deleterious. In this regard, the timely controlled properties of PIGF should be taken into account when considering PIGF a potential therapeutic target in atherosclerotic heart disease.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic inflammatory synovitis that is dominated by the presence of macrophages, lymphocytes and synovial fibroblasts, which cause bone and cartilage destruction (Pope, 2002). The deformed synovial architecture of RA, called pannus, is characterized by high density of sub-lining blood vessels. These new blood vessels can maintain the chronic inflammatory state by transporting inflammatory cells to sites of inflammation and by supplying nutrients and oxygen to proliferating inflamed tissues (Marrelli *et al.*, 2011). Indeed, increases in blood vessel numbers has been shown to be correlated with synovial cells hyperplasia and mononuclear cell infiltration and with the indices of joint tenderness (Rooney *et al.*, 1988; Rosu *et al.*, 2008), which suggests that a close association exists between angiogenesis and inflammation in the synovium of RA.

Angiogenesis is finely tuned by balances between pro-angiogenic and anti-angiogenic mediators such as growth factors, cytokines, chemokines, cellular adhesion molecules and others (Szekanecz *et al.*, 2010). Of these, VEGF and its receptors are key regulators of the angiogenic process also in inflammatory synovium of RA. Recently, we found that PIGF, a member of the VEGF family, is also highly expressed in the layer that lines the hyperplastic synovium of RA joints and that it is also in-

creased in the synovial fluid of patients (Bottomley *et al.*, 2000; Yoo *et al.*, 2009). As a specific ligand for Flt1, PIGF has potent angiogenic properties and also induces growth and migration of endothelial cells (Luttun *et al.*, 2002a; Autiero *et al.*, 2003a). PIGF is copiously highly produced by fibroblast-like synoviocytes exposed to proinflammatory cytokines such as IL-1 β and TNF- α , and it, in turn, increases the productions of TNF- α , IL-1, IL-6, and VEGF by mononuclear cells in RA (Bottomley *et al.*, 2000; Selvaraj *et al.*, 2003; Yoo *et al.*, 2009). Furthermore, monocytes from patients with active RA exhibit an exaggerated response to PIGF because Flt1 expression is elevated in the monocytes from these patients (Yoo *et al.*, 2009). Finally, a chain of these processes creates a vicious cycle that encourages the perpetuation of chronic joint inflammation (Figure 2) (Yoo *et al.*, 2008). Therefore, synovial inflammation and angiogenesis would seem to be controllable if the action of PIGF were interrupted. In fact, arthritis seldom develops in response to anti-collagen II antibodies in PIGF knockout mice, which suggests that PIGF is a critical mediator of the inflammation of RA (Yoo *et al.*, 2009). Furthermore, PIGF induces VEGF release from mononuclear cells, and the binding of PIGF to Flt1 leads to intermolecular crosstalk between Flt1 and Flk1, which amplifies Flk1 signaling and consequently enhances VEGF-driven response (Bottomley *et al.*, 2000; Autiero *et al.*, 2003b). Therefore, the inhibition of PIGF could suppress both VEGF-driven inflammation and angiogenesis. Another possible mechanism responsible for the effects of anti-PIGF treatment is that more VEGF would be trapped by Flt1 due to loss of competition from PIGF, which would reduce binding opportunity between VEGF and Flk1 (Park *et al.*, 1994), a more potent pro-angiogenic receptor than Flt1, and reduce angiogenesis.

Taken together, enhanced expression and signaling by PIGF-Flt1 system potentiates rheumatoid inflammation both directly and indirectly by encouraging inflammation and angiogenesis. Since PIGF is redundant in terms of vascular development and physiological vessel maintenance in healthy adults (Carmeliet *et al.*, 2001), it represents a promising novel target for the control of RA with negligible side effects.

Concluding remarks

PIGF lies dormant in the normal healthy state and becomes active in the presence of pathologic conditions, such as, those associated with chronic inflammatory diseases. PIGF induces angiogenesis

directly by activating endothelial cells but it also promotes inflammation by recruiting and activating macrophages *via* Flt1 signaling. Macrophages, stimulated by PIGF, secrete proinflammatory cytokines like TNF- α and IL-6 and angiogenic factors like VEGF and perpetuate inflammation and angiogenesis, which are critical for tumour progression, plaque vulnerability, and pannus formation in rheumatoid arthritis. Although blocking or inhibiting the PIGF-Flt1 system has successfully controlled inflammation and angiogenesis in many animal studies of disease models, observed effects should be interpreted with caution for two reasons. First, PIGF can have beneficial or harmful effects that depend on the stage of disease progression in some diseases such as atherosclerosis. Second, modulation of PIGF-Flt1 system can have an influence on expression and action of VEGF family system or other angiogenesis-modulating factors, which might give rise to unexpected responses in *in vivo* system. The dimerization pattern of PIGF and VEGF-A varies, and PIGF may antagonize or enhance VEGF-A function (Xu *et al.*, 2006; Cao, 2009). And the possibility cannot be excluded that anti-PIGF therapy could induce angiogenic rescue programme through stimulating expression of various pro-angiogenic factors as in anti-VEGF therapy. Indeed, anti-PIGF induces a small but angiogenic escape effect (Fischer *et al.*, 2007), and which could be another reason for failure of PIGF blockade to inhibit angiogenesis in primary tumor growth in different experimental systems (Bais *et al.*, 2010). Therefore, further investigation on the temporal expression pattern and functional property of PIGF in relation to VEGF family members and their receptors or other angiogenic factors is warranted in disease models, before the efficacy of PIGF-Flt1 system inhibition is confirmed clinically in patients with chronic inflammatory diseases or conditions.

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References

Adini A, Kornaga T, Firoozbakht F, Benjamin LE. Placental growth factor is a survival factor for tumor endothelial cells and macrophages. *Cancer Res* 2002;62:2749-52

Autiero M, Lutun A, Tjwa M, Carmeliet P. Placental growth factor and its receptor, vascular endothelial growth factor

receptor-1: novel targets for stimulation of ischemic tissue revascularization and inhibition of angiogenic and inflammatory disorders. *J Thromb Haemost* 2003a;1:1356-70

Autiero M, Waltenberger J, Communi D, Kranz A, Moons L, Lambrechts D, Kroll J, Plaisance S, De Mol M, Bono F, Kliche S, Fellbrich G, Ballmer-Hofer K, Maglione D, Mayr-Beyrle U, Dewerchin M, Dombrowski S, Stanimirovic D, Van Hummelen P, Dehio C, Hicklin DJ, Persico G, Herbert JM, Shibuya M, Collen D, Conway EM, Carmeliet P. Role of PIGF in the intra- and intermolecular cross talk between the VEGF receptors Flt1 and Flk1. *Nat Med* 2003b;9:936-43

Bais C, Wu X, Yao J, Yang S, Crawford Y, McCutcheon K, Tan C, Kolumam G, Vernes JM, Eastham-Anderson J, Haughney P, Kowanzetz M, Hagenbeek T, Kasman I, Reslan HB, Ross J, Van Bruggen N, Carano RA, Meng YJ, Hongo JA, Stephan JP, Shibuya M, Ferrara N. PIGF blockade does not inhibit angiogenesis during primary tumor growth. *Cell* 2010;141:166-77

Bellik L, Vinci MC, Filippi S, Ledda F, Parenti A. Intracellular pathways triggered by the selective FLT-1-agonist placental growth factor in vascular smooth muscle cells exposed to hypoxia. *Br J Pharmacol* 2005;146:568-75

Bergers G, Hanahan D. Modes of resistance to anti-angiogenic therapy. *Nat Rev Cancer* 2008;8:592-603

Bjornheden T, Levin M, Evaldsson M, Wiklund O. Evidence of hypoxic areas within the arterial wall *in vivo*. *Arterioscler Thromb Vasc Biol* 1999;19:870-6

Bottomley MJ, Webb NJ, Watson CJ, Holt L, Bukhari M, Denton J, Freemont AJ, Brenchley PE. Placenta growth factor (PIGF) induces vascular endothelial growth factor (VEGF) secretion from mononuclear cells and is co-expressed with VEGF in synovial fluid. *Clin Exp Immunol* 2000;119:182-8

Burriss H 3rd, Rocha-Lima C. New therapeutic directions for advanced pancreatic cancer: targeting the epidermal growth factor and vascular endothelial growth factor pathways. *Oncologist* 2008;13:289-98

Cao Y. Positive and negative modulation of angiogenesis by VEGFR1 ligands. *Sci Signal* 2009;2:re1

Cao Y, Chen H, Zhou L, Chiang MK, Anand-Apte B, Weatherbee JA, Wang Y, Fang F, Flanagan JG, Tsang ML. Heterodimers of placenta growth factor/vascular endothelial growth factor. Endothelial activity, tumor cell expression, and high affinity binding to Flk-1/KDR. *J Biol Chem* 1996;271:3154-62

Carmeliet P. Angiogenesis in life, disease and medicine. *Nature* 2005;438:932-6

Carmeliet P, Moons L, Lutun A, Vincenti V, Comperolle V, De Mol M, Wu Y, Bono F, Devy L, Beck H, Scholz D, Acker T, DiPalma T, Dewerchin M, Noel A, Stalmans I, Barra A, Blacher S, Vandendriessche T, Ponten A, Eriksson U, Plate KH, Foidart JM, Schaper W, Charnock-Jones DS, Hicklin DJ, Herbert JM, Collen D, Persico MG. Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. *Nat Med* 2001;7:575-83

Chen CN, Hsieh FJ, Cheng YM, Cheng WF, Su YN, Chang

- KJ, Lee PH. The significance of placenta growth factor in angiogenesis and clinical outcome of human gastric cancer. *Cancer Lett* 2004;213:73-82
- Cursiefen C, Chen L, Borges LP, Jackson D, Cao J, Radziejewski C, D'Amore PA, Dana MR, Wiegand SJ, Streilein JW. VEGF-A stimulates lymphangiogenesis and hemangiogenesis in inflammatory neovascularization *via* macrophage recruitment. *J Clin Invest* 2004;113:1040-50
- Eriksson A, Cao R, Pawliuk R, Berg SM, Tsang M, Zhou D, Fleet C, Tritsarlis K, Dissing S, Leboulch P, Cao Y. Placenta growth factor-1 antagonizes VEGF-induced angiogenesis and tumor growth by the formation of functionally inactive PIGF-1/VEGF heterodimers. *Cancer Cell* 2002;1:99-108
- Fischer C, Jonckx B, Mazzone M, Zacchigna S, Loges S, Pattarini L, Chorianopoulos E, Liesenborghs L, Koch M, De Mol M, Autiero M, Wyns S, Plaisance S, Moons L, van Rooijen N, Giacca M, Stassen JM, Dewerchin M, Collen D, Carmeliet P. Anti-PIGF inhibits growth of VEGF(R)- inhibitor-resistant tumors without affecting healthy vessels. *Cell* 2007;131:463-75
- Fischer C, Mazzone M, Jonckx B, Carmeliet P. FLT1 and its ligands VEGFB and PIGF: drug targets for anti-angiogenic therapy? *Nat Rev Cancer* 2008;8:942-56
- Glaser R, Peacock WF, Wu AH, Muller R, Mockel M, Apple FS. Placental growth factor and B-type natriuretic peptide as independent predictors of risk from a multibiomarker panel in suspected acute coronary syndrome (Acute Risk and Related Outcomes Assessed With Cardiac Biomarkers [ARROW]) study. *Am J Cardiol* 2011;107:821-6
- Green CJ, Lichtlen P, Huynh NT, Yanovsky M, Laderoute KR, Schaffner W, Murphy BJ. Placenta growth factor gene expression is induced by hypoxia in fibroblasts: a central role for metal transcription factor-1. *Cancer Res* 2001;61:2696-703
- Hansson GK, Libby P. The immune response in atherosclerosis: a double-edged sword. *Nat Rev Immunol* 2006;6:508-19
- Hattori K, Heissig B, Wu Y, Dias S, Tejada R, Ferris B, Hicklin DJ, Zhu Z, Bohlen P, Witte L, Hendrikx J, Hackett NR, Crystal RG, Moore MA, Werb Z, Lyden D, Rafii S. Placental growth factor reconstitutes hematopoiesis by recruiting VEGFR1(+) stem cells from bone-marrow microenvironment. *Nat Med* 2002;8:841-9
- Heeschen C, Dimmeler S, Fichtlscherer S, Hamm CW, Berger J, Simoons ML, Zeiher AM. Prognostic value of placental growth factor in patients with acute chest pain. *JAMA* 2004;291:435-41
- Herrmann J, Lerman LO, Mukhopadhyay D, Napoli C, Lerman A. Angiogenesis in atherogenesis. *Arterioscler Thromb Vasc Biol* 2006;26:1948-57
- Ho MC, Chen CN, Lee H, Hsieh FJ, Shun CT, Chang CL, Lai YT, Lee PH. Placenta growth factor not vascular endothelial growth factor A or C can predict the early recurrence after radical resection of hepatocellular carcinoma. *Cancer Lett* 2007;250:237-49
- Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R, Kabbinavar F. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004;350:2335-42
- Imhof BA, Aurrand-Lions M. Angiogenesis and inflammation face off. *Nat Med* 2006;12:171-2
- Kerbel RS. Tumor angiogenesis. *N Engl J Med* 2008;358:2039-49
- Khurana R, Moons L, Shafi S, Luttun A, Collen D, Martin JF, Carmeliet P, Zachary IC. Placental growth factor promotes atherosclerotic intimal thickening and macrophage accumulation. *Circulation* 2005;111:2828-36
- Lenderink T, Heeschen C, Fichtlscherer S, Dimmeler S, Hamm CW, Zeiher AM, Simoons ML, Boersma E. Elevated placental growth factor levels are associated with adverse outcomes at four-year follow-up in patients with acute coronary syndromes. *J Am Coll Cardiol* 2006;47:307-11
- Li B, Sharpe EE, Maupin AB, Teleron AA, Pyle AL, Carmeliet P, Young PP. VEGF and PIGF promote adult vasculogenesis by enhancing EPC recruitment and vessel formation at the site of tumor neovascularization. *FASEB J* 2006;20:1495-7
- Loges S, Schmidt T, Carmeliet P. "Antimyoangiogenic" therapy for cancer by inhibiting PIGF. *Clin Cancer Res* 2009;15:3648-53
- Luttun A, Tjwa M, Carmeliet P. Placental growth factor (PIGF) and its receptor Flt-1 (VEGFR-1): novel therapeutic targets for angiogenic disorders. *Ann N Y Acad Sci* 2002a;979:80-93
- Luttun A, Tjwa M, Moons L, Wu Y, Angelillo-Scherrer A, Liao F, Nagy JA, Hooper A, Priller J, De Klerck B, Compornolle V, Daci E, Bohlen P, Dewerchin M, Herbert JM, Fava R, Matthys P, Carmeliet G, Collen D, Dvorak HF, Hicklin DJ, Carmeliet P. Revascularization of ischemic tissues by PIGF treatment, and inhibition of tumor angiogenesis, arthritis and atherosclerosis by anti-Flt1. *Nat Med* 2002b;8:831-40
- Marcellini M, De Luca N, Riccioni T, Ciucci A, Orecchia A, Lacal PM, Ruffini F, Pesce M, Cianfarani F, Zambruno G, Orlandi A, Failla CM. Increased melanoma growth and metastasis spreading in mice overexpressing placenta growth factor. *Am J Pathol* 2006;169:643-54
- Markovic M, Ignjatovic S, Dajak M, Majkic-Singh N. Placental growth factor as short-term predicting biomarker in acute coronary syndrome patients with non-ST elevation myocardial infarction. *South Med J* 2010;103:982-7
- Marrelli A, Cipriani P, Liakouli V, Carubbi F, Perricone C, Perricone R, Giacomelli R. Angiogenesis in rheumatoid arthritis: A disease specific process or a common response to chronic inflammation? *Autoimmun Rev* 2011;10:595-8
- Marrony S, Bassilana F, Seuwen K, Keller H. Bone morphogenetic protein 2 induces placental growth factor in mesenchymal stem cells. *Bone* 2003;33:426-33
- Matsumoto K, Suzuki K, Koike H, Hasumi M, Matsui H, Okugi H, Shibata Y, Ito K, Yamanaka H. Placental growth factor gene expression in human prostate cancer and benign prostate hyperplasia. *Anticancer Res* 2003a;23:3767-73
- Matsumoto K, Suzuki K, Koike H, Okamura K, Tsuchiya K, Uchida T, Takezawa Y, Kobayashi M, Yamanaka H.

- Prognostic significance of plasma placental growth factor levels in renal cell cancer: an association with clinical characteristics and vascular endothelial growth factor levels. *Anticancer Res* 2003b;23:4953-8
- Moulton KS, Vakili K, Zurakowski D, Soliman M, Butterfield C, Sylvain E, Lo KM, Gillies S, Javaherian K, Folkman J. Inhibition of plaque neovascularization reduces macrophage accumulation and progression of advanced atherosclerosis. *Proc Natl Acad Sci USA* 2003;100: 4736-41
- Murakami M, Zheng Y, Hirashima M, Suda T, Morita Y, Ooehara J, Ema H, Fong GH, Shibuya M. VEGFR1 tyrosine kinase signaling promotes lymphangiogenesis as well as angiogenesis indirectly *via* macrophage recruitment. *Arterioscler Thromb Vasc Biol* 2008;28:658-64
- Nathan C. Points of control in inflammation. *Nature* 2002; 420:846-52
- Park JE, Chen HH, Winer J, Houck KA, Ferrara N. Placenta growth factor. Potentiation of vascular endothelial growth factor bioactivity, *in vitro* and *in vivo*, and high affinity binding to Flt-1 but not to Flk-1/KDR. *J Biol Chem* 1994;269:25646-54
- Parr C, Watkins G, Boulton M, Cai J, Jiang WG. Placenta growth factor is over-expressed and has prognostic value in human breast cancer. *Eur J Cancer* 2005;41:2819-27
- Pipp F, Heil M, Issbrucker K, Ziegelhoeffer T, Martin S, van den Heuvel J, Weich H, Fernandez B, Golomb G, Carmeliet P, Schaper W, Clauss M. VEGFR-1-selective VEGF homologue PIGF is arteriogenic: evidence for a monocyte-mediated mechanism. *Circ Res* 2003;92:378-85
- Pope RM. Apoptosis as a therapeutic tool in rheumatoid arthritis. *Nat Rev Immunol* 2002;2:527-35
- Rafii S, Avecilla S, Shmelkov S, Shido K, Tejada R, Moore MA, Heissig B, Hattori K. Angiogenic factors reconstitute hematopoiesis by recruiting stem cells from bone marrow microenvironment. *Ann N Y Acad Sci* 2003;996:49-60
- Roncal C, Buyschaert I, Gerdes N, Georgiadou M, Ovchinnikova O, Fischer C, Stassen JM, Moons L, Collen D, De Bock K, Hansson GK, Carmeliet P. Short-term delivery of anti-PIGF antibody delays progression of atherosclerotic plaques to vulnerable lesions. *Cardiovasc Res* 2010;86: 29-36
- Rooney M, Condell D, Quinlan W, Daly L, Whelan A, Feighery C, Bresnihan B. Analysis of the histologic variation of synovitis in rheumatoid arthritis. *Arthritis Rheum* 1988; 31:956-63
- Rosu A, Ciurea P, Simionescu C, Margaritescu C, Musetescu AE, Ciurea R, Vreju AF. The study of angiogenesis in early rheumatoid arthritis—clinical, immunohistochemical and immunological correlations. *J Med Life* 2008;1:287-94
- Roy H, Bhardwaj S, Babu M, Jauhainen S, Herzig KH, Bellu AR, Haisma HJ, Carmeliet P, Alitalo K, Yla-Herttuala S. Adenovirus-mediated gene transfer of placental growth factor to perivascular tissue induces angiogenesis *via* upregulation of the expression of endogenous vascular endothelial growth factor-A. *Hum Gene Ther* 2005;16: 1422-8
- Saha P, Modarai B, Humphries J, Mattock K, Waltham M, Burnand KG, Smith A. The monocyte/macrophage as a therapeutic target in atherosclerosis. *Curr Opin Pharmacol* 2009;9:109-18
- Scholz D, Elsaesser H, Sauer A, Friedrich C, Lutun A, Carmeliet P, Schaper W. Bone marrow transplantation abolishes inhibition of arteriogenesis in placenta growth factor (PIGF) *-/-* mice. *J Mol Cell Cardiol* 2003;35:177-84
- Schoppmann SF, Birner P, Stockl J, Kalt R, Ullrich R, Caucig C, Kriehuber E, Nagy K, Alitalo K, Kerjaschki D. Tumor-associated macrophages express lymphatic endothelial growth factors and are related to peritumoral lymphangiogenesis. *Am J Pathol* 2002;161:947-56
- Selvaraj SK, Giri RK, Perelman N, Johnson C, Malik P, Kalra VK. Mechanism of monocyte activation and expression of proinflammatory cytochemokines by placenta growth factor. *Blood* 2003;102:1515-24
- Solinas G, Germano G, Mantovani A, Allavena P. Tumor-associated macrophages (TAM) as major players of the cancer-related inflammation. *J Leukoc Biol* 2009;86: 1065-73
- Szekanecz Z, Besenyei T, Paragh G, Koch AE. New insights in synovial angiogenesis. *Joint Bone Spine* 2010;77:13-9
- Takahashi A, Sasaki H, Kim SJ, Tobisu K, Kakizoe T, Tsukamoto T, Kumamoto Y, Sugimura T, Terada M. Markedly increased amounts of messenger RNAs for vascular endothelial growth factor and placenta growth factor in renal cell carcinoma associated with angiogenesis. *Cancer Res* 1994;54:4233-7
- Taylor AP, Goldenberg DM. Role of placenta growth factor in malignancy and evidence that an antagonistic PIGF/Flt-1 peptide inhibits the growth and metastasis of human breast cancer xenografts. *Mol Cancer Ther* 2007;6:524-31
- Torry RJ, Tomanek RJ, Zheng W, Miller SJ, Labarrere CA, Torry DS. Hypoxia increases placenta growth factor expression in human myocardium and cultured neonatal rat cardiomyocytes. *J Heart Lung Transplant* 2009;28:183-90
- Van de Veire S, Stalmans I, Heindryckx F, Oura H, Tijeras-Raballand A, Schmidt T, Loges S, Albrecht I, Jonckx B, Vinckier S, Van Steenkiste C, Tugues S, Rolny C, De Mol M, Dettori D, Hainaud P, Coenegrachts L, Contreres JO, Van Bergen T, Cuervo H, Xiao WH, Le Henaff C, Buyschaert I, Kharabi Masouleh B, Geerts A, Schomber T, Bonnin P, Lambert V, Haustraete J, Zacchigna S, Rakic JM, Jimenez W, Noel A, Giacca M, Colle I, Foidart JM, Tobelem G, Morales-Ruiz M, Vilar J, Maxwell P, Viores SA, Carmeliet G, Dewerchin M, Claesson-Welsh L, Dupuy E, Van Vlierberghe H, Christofori G, Mazzone M, Detmar M, Collen D, Carmeliet P. Further pharmacological and genetic evidence for the efficacy of PIGF inhibition in cancer and eye disease. *Cell* 2010;141:178-90
- Verheul HM, Pinedo HM. Possible molecular mechanisms involved in the toxicity of angiogenesis inhibition. *Nat Rev Cancer* 2007;7:475-85
- Viglietto G, Maglione D, Rambaldi M, Cerutti J, Romano A, Trapasso F, *et al.* Upregulation of vascular endothelial growth factor (VEGF) and downregulation of placenta growth factor

(PIGF) associated with malignancy in human thyroid tumors and cell lines. *Oncogene* 1995;11:1569-79

Wei SC, Tsao PN, Yu SC, Shun CT, Tsai-Wu JJ, Wu CH, Su YN, Hsieh FJ, Wong JM. Placenta growth factor expression is correlated with survival of patients with colorectal cancer. *Gut* 2005;54:666-72

Wei SC, Liang JT, Tsao PN, Hsieh FJ, Yu SC, Wong JM. Preoperative serum placenta growth factor level is a prognostic biomarker in colorectal cancer. *Dis Colon Rectum* 2009;52:1630-6

Willett CG, Boucher Y, Duda DG, di Tomaso E, Munn LL, Tong RT, Kozin SV, Petit L, Jain RK, Chung DC, Sahani DV, Kalva SP, Cohen KS, Scadden DT, Fischman AJ, Clark JW, Ryan DP, Zhu AX, Blaszkowsky LS, Shellito PC, Mino-Kenudson M, Lauwers GY. Surrogate markers for antiangiogenic therapy and dose-limiting toxicities for bevacizumab with radiation and chemotherapy: continued experience of a phase I trial in rectal cancer patients. *J Clin Oncol* 2005; 23:8136-9

Xu L, Cochran DM, Tong RT, Winkler F, Kashiwagi S, Jain RK, Fukumura D. Placenta growth factor overexpression inhibits tumor growth, angiogenesis, and metastasis by depleting vascular endothelial growth factor homodimers in orthotopic mouse models. *Cancer Res* 2006;66:3971-7

Yan ZQ, Hansson GK. Innate immunity, macrophage activation, and atherosclerosis. *Immunol Rev* 2007;219: 187-203

Yao J, Wu X, Zhuang G, Kasman IM, Vogt T, Phan V, Shibuya M, Ferrara N, Bais C. Expression of a functional VEGFR-1 in tumor cells is a major determinant of anti-PIGF antibodies efficacy. *Proc Natl Acad Sci USA* 2011;108:11590-5

Yonekura H, Sakurai S, Liu X, Migita H, Wang H, Yamagishi S, Nomura M, Abedin MJ, Unoki H, Yamamoto Y, Yamamoto H. Placenta growth factor and vascular endothelial growth factor B and C expression in microvascular endothelial cells and pericytes. Implication in autocrine and paracrine regulation of angiogenesis. *J Biol Chem* 1999;274:35172-8

Yoo SA, Kwok SK, Kim WU. Proinflammatory role of vascular endothelial growth factor in the pathogenesis of rheumatoid arthritis: prospects for therapeutic intervention. *Mediators Inflamm* 2008;2008:129873

Yoo SA, Yoon HJ, Kim HS, Chae CB, De Falco S, Cho CS, Kim WU. Role of placenta growth factor and its receptor flt-1 in rheumatoid inflammation: a link between angiogenesis and inflammation. *Arthritis Rheum* 2009;60:345-54

Zhang L, Chen J, Ke Y, Mansel RE, Jiang WG. Expression of Placenta growth factor (PIGF) in non-small cell lung cancer (NSCLC) and the clinical and prognostic significance. *World J Surg Oncol* 2005;3:68

Ziche M, Maglione D, Ribatti D, Morbidelli L, Lago CT, Battisti M, Paoletti I, Barra A, Tucci M, Parise G, Vincenti V, Granger HJ, Viglietto G, Persico MG. Placenta growth factor-1 is chemotactic, mitogenic, and angiogenic. *Lab Invest* 1997; 76:517-31