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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; PlGF, 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. PlGF is an angiogenic protein belonging to the VEGF family and is upregulated mainly in pathologic conditions. Recently, PlGF 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, PlGF has attractive clinical values because endogenous PlGF is redundant for vascular development and physiological vessel maintenance in healthy adults. However, there have been conflicting results about the efficacy of PlGF inhibition depending on the experimental and clinical settings. Further close investigations for resolving the puzzle of PlGF 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 PlGF 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 PlGF 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 PlGF-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 PlGF 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 PlGF expression in tumors was undertaken by Takahashi *et al*. (1994), who demonstrated that PlGF is expressed in hypervascular renal cell carcinoma. Since then, clinical and experimental research on the significance and role of PlGF in tumor progression has been pursued extensively. Accumulating reports have suggested that PlGF might be a useful prognostic marker of cancer progression. Plasma PlGF levels are upregulated and correlate with tumor grade and survival in patients with renal-cell carcinoma (Matsumoto *et al*., 2003b), and a preoperative high PlGF serum level is a useful prognostic indicator of recurrence and survival in colorectal cancer (Wei *et al*., 2009). In addition, PlGF 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, PlGF 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 PlGF 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 PlGF acts as a positive regulator of cancer growth and metastasis in man.

When highly upregulated PlGF transmits its signal through Flt1 by stimulating the phosphorylations of specific Flt1 tyrosine residues, and thus induces the expression of distinct downstream target genes. PlGF 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, PlGF displaces VEGF-A from Flt1, which releases VEGF-A and allows it to activate Flk1 and enhance VEGF-driven angiogenesis (Park *et al*., 1994). PlGF has also been reported to form heterodimers with VEGF *in vivo*, and PlGF-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).

PlGF induces various biological effects by affecting a wide range of different cell types. PlGF can stimulate vessel growth and maturation directly by affecting endothelial and mural cells, and indirectly by recruiting pro-angiogenic cell types. For example, PlGF 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, PlGF 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 PlGF to promote growth, angiogenesis, and resistance toward antiangiogenic treatments. PlGF 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). PlGF 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-PlGF 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, PlGF 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, PlGF-Flt1 signaling provides a major route for angiogenic rescue (Willett *et al*., 2005; Kerbel, 2008). Therefore, the inhibition of PlGF could provide an attractive means of supplementing the deficient anti-VEGF therapy. In fact, Fisher *et al*. have demonstrated that anti-PlGF 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-PlGF 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 PlGF 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 PlGF, genetic ablation of host PlGF receptor signaling, or combined anti-PlGF 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 PlGF could aggravate tumor progression by demonstrating that PlGF 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-PlGF 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 PlGF in mouse models of tumors

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

Model	Tumor subtype	PlGF manipulation	Tumor growth	Angiogenesis	Metastasis	Reference
Pro-promoting evidences						
C57Bl/6 mice	B16 melanoma / Pancreatic Panc02 adenocarcinoma / CT26 colon carcinoma	Anti-PlGF blocking antibody	↓	↓	↓	Glaser *et al*., 2011
Balb/c nude mice	B16F10 melanoma	Anti-PlGF blocking antibody	↔	n.d.	↓	Pipp *et al*., 2003
C57Bl/6 PlGF-/- mice	Skin papilloma	PlGF knockout	↓	↓	n.d.	Pope, 2002
C57Bl6/ASV-B mice	Hepatocellular carcinoma	PlGF silencing	↓	↓	n.d.	Pope, 2002
C57Bl/6 Rip1Tag2 × PlGF-/- mice	Pancreatic β cell carcinoma	PlGF knockout	↔	↔	n.d.	Pope, 2002
Pro-inhibitory evidences						
C57BL/6 mice	Murine fibrosarcoma	PlGF overexpression	↓	↓	n.d.	Kerbel, 2008
C57Bl/6 mice	Murine Lewis lung carcinoma	PlGF overexpression	↓	↓	n.d.	Zhang *et al*., 2005
C57Bl/6 Rip1 PlGF-1 transgenic mice	Pancreatic β cell carcinoma	PlGF overexpression	↓	↓	n.d.	Ziche *et al*., 1997
SCID mice	Human tumor cell lines (HCT116, A549, and U87-MG)	PlGF overexpression	↓	↓	n.d.	Hattori *et al*., 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 atherogenesis (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 neovascularization 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

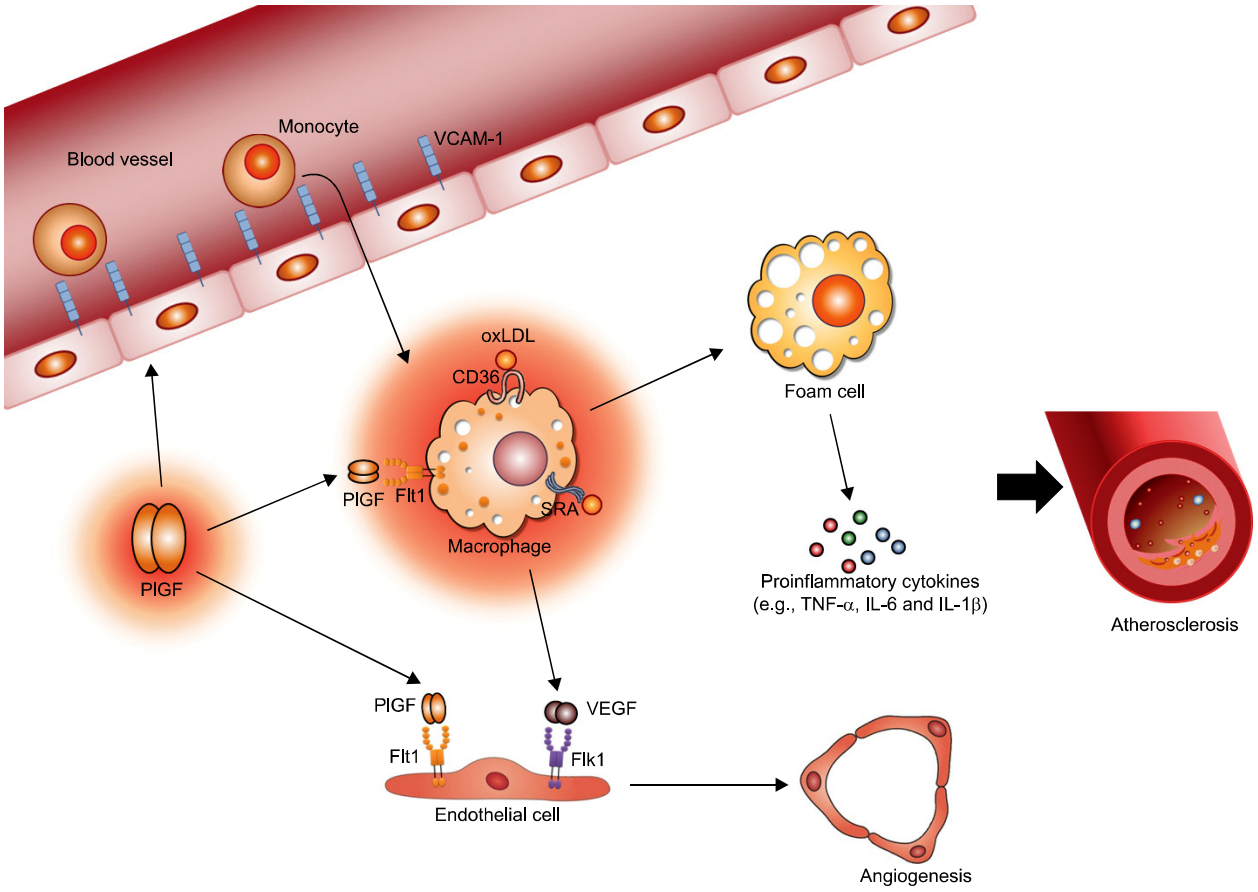

Figure 1. Role of PIGF in atherosclerosis. PIGF is upregulated in plaque shoulders and caps of atherosclerotic lesions. PIGF recruits monocytes by increasing the expression of VCAM-1, a key molecule in the processes of monocyte/macrophage adhesion and rolling at the luminal side of arteries *via* the 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 plague 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;

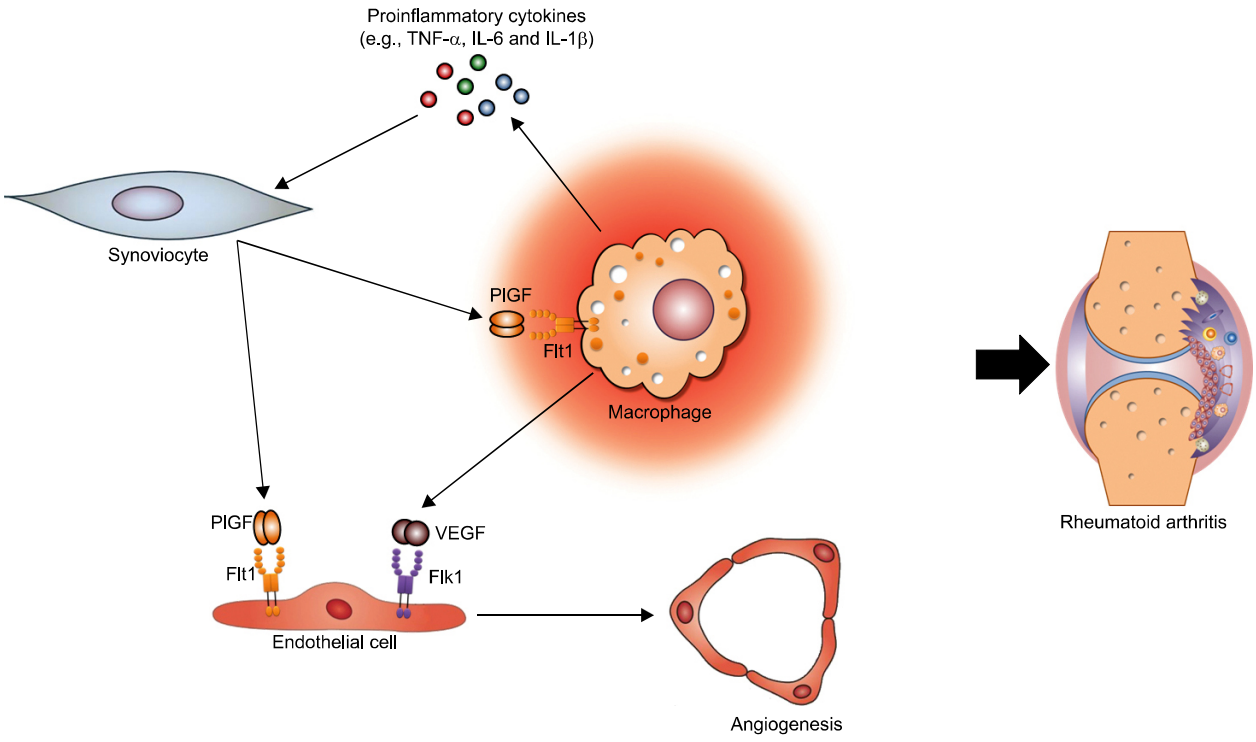

Figure 2. Role of PlGF in rheumatoid arthritis. PlGF, predominantly produced by rheumatoid synoviocytes, promotes angiogenesis and stimulates vascular endothelial cell permeability. Newly employed macrophages produce TNF-α and IL-6 when stimulated by PlGF/Flt-1 binding or by contact with activated endothelial 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 PlGF 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 PlGF 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, PlGF is upregulated in cardiomyocytes and fibroblasts, and contributes to myocardial angiogenesis and tissue healing (Green *et al*., 2001; Torry *et al*., 2009). PlGF 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, PlGF may be beneficial or deleterious. In this regard, the timely controlled properties of PlGF should be taken into account when considering PlGF 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 PlGF, 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 increased in the synovial fluid of patients (Bottomley *et al*., 2000; Yoo *et al*., 2009). As a specific ligand for Flt1, PlGF has potent angiogenic properties and also induces growth and migration of endothelial cells (Luttun *et al*., 2002a; Autiero *et al*., 2003a). PlGF is copiously highly produced by fibroblast-like synoviocytes exposed to proinflammatory cytokines such as IL-1β 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 PlGF 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 PlGF were interrupted. In fact, arthritis seldom develops in response to anti-collagen II antibodies in PlGF knockout mice, which suggests that PlGF is a critical mediator of the inflammation of RA (Yoo *et al*., 2009). Furthermore, PlGF induces VEGF release from mononuclear cells, and the binding of PlGF to Flt1 leads to intermolecular crosstalk between Flt-1 and Flk1, which amplifies Flk1 signaling and consequently enhances VEGF-driven response (Bottomley *et al*., 2000; Autiero *et al*., 2003b). Therefore, the inhibition of PlGF could suppress both VEGF-driven inflammation and angiogenesis. Another possible mechanism responsible for the effects of anti-PlGF treatment is that more VEGF would be trapped by Flt1 due to loss of competition from PlGF, 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 PlGF-Flt1 system potentiates rheumatoid inflammation both directly and indirectly by encouraging inflammation and angiogenesis. Since PlGF 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

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

directly by activating endothelial cells but it also promotes inflammation by recruiting and activating macrophages *via* Flt1 signaling. Macrophages, stimulated by PlGF, 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 PlGF-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, PlGF can have beneficial or harmful effects that depend on the stage of disease progression in some diseases such as atherosclerosis. Second, modulation of PlGF-Flt1system 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 PlGF and VEGF-A varies, and PlGF may antagonize or enhance VEGF-A function (Xu *et al*., 2006; Cao, 2009). And the possibility cannot be excluded that anti-PlGF therapy could induce angiogenic rescue programme through stimulating expression of various pro-angiogenic factors as in anti-VEGF therapy. Indeed, anti-PlGF induces a small but angiogenic escape effect (Fischer *et al*., 2007), and which could be another reason for failure of PlGF 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 PlGF in relation to VEGF family members and their receptors or other angiogenic factors is warranted in disease models, before the efficacy of PlGF-Flt1 system inhibition is confirmed clinically in patients with chronic inflammatory diseases or conditions.

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