Thromboembolic Events in Patients Treated with Anti-Angiogenic Drugs

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Abstract: Induction of neo-angiogenesis is a fundamental step in many pathological conditions. The therapeutic value of inhibiting angiogenesis is an interesting area of research in oncology, with vascular endothelial growth factor (VEGF) being the most suitable anti-angiogenic target. In the last decade a number of anti-VEGF drugs have demonstrated, especially in combination with standard chemotherapy, clinical efficacy in the treatment of different solid tumor types. As data from clinical trials on anti-VEGF drugs are becoming available, it is increasingly recognized that VEGF, in addition to being a permeability, proliferation, and migration factor, is also a maintenance and protection factor for endothelial cells, being capable of regulating multiple biological functions, i.e. the production of vasoactive mediators and the expression of components of the thrombolytic and coagulation pathways. Consequently, the disturbance of vascular homeostasis by blocking VEGF may lead to endothelial dysfunction and adverse vascular effects, such as venous and arterial thromboembolic events. In preclinical models angiogenesis and the increased expression of VEGF has been associated to altered expression of proinflammatory genes. These genes may be regulated in a biphasic manner, and it is possible that anti-VEGF therapy may disrupt a negative feedback loop that leads to potential *in situ* thrombus formation. Accordingly, combination treatment with bevacizumab and chemotherapy, compared with chemotherapy alone, was recently associated with an increased risk of thromboembolism.

The present review considers the biological mechanisms and clinical impact of thromboembolic complications during anti-angiogenic treatments in cancer patients.

Keywords: Vascular endothelial growth factor, oxidant stress, nitric oxide, bevacizumab, endothelial dysfunction, haemostatic activation.

BIOLOGICAL PROPERTIES OF THE ENDOTHELIUM

The endothelium is a dynamic organ that plays a critical role in maintaining vascular homeostasis. The endothelial cell (EC), in fact, behaves as a receptor-effector structure which senses different physical or chemical stimuli that occur inside the vessel and, therefore, modifies the vessel shape or releases the mediators to counteract the effect of the stimulus and maintain homeostasis. The net effect is maintenance of normal vascular tone, but the endothelium also maintains normal blood viscosity, prevents abnormal blood clotting, and prevents abnormal bleeding in terms of a balance between tissue plasminogen activator and its inhibitor. In addition, it limits inflammation of the vasculature and it can suppress smooth muscle cell proliferation. These are functions of the normal endothelium. The opposite occurs in the presence of a dysfunctional endothelium, in which abnormally functioning ECs cause vasoconstriction, increased inflammation and thrombosis (Fig. (**1**)) [1].

Our increased understanding of the endothelium's role began in the early eighties with the studies carried out by the Nobel P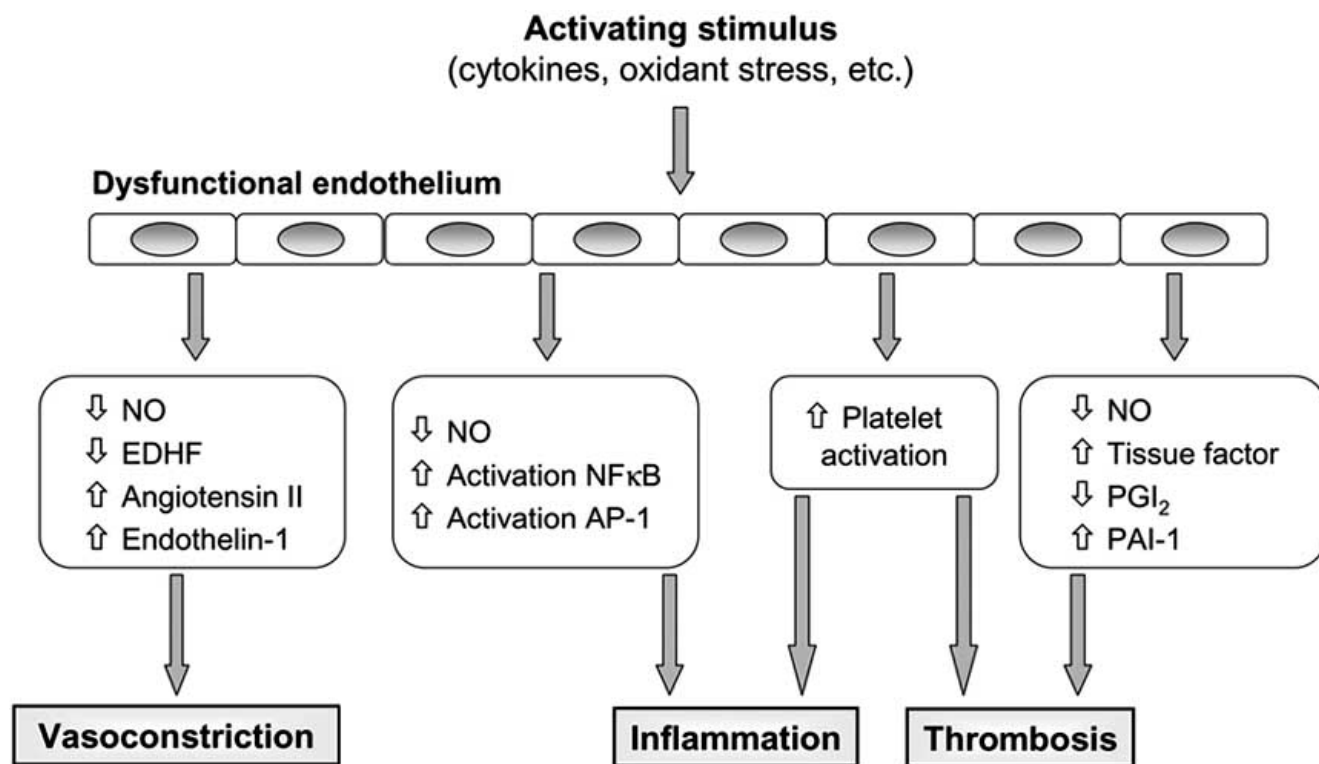rize winner Furchgott, who hypothesized that the EC had an important role in smooth muscle relaxation [2]. The findings obtained in this pioneering work represented a considerable advance, because the causative compound called endothelial-derived relaxing factor (EDRF) had yet to be identified and, in fact, remained unidentified for years until its chemical structure was characterized as nitric oxide (NO).

NO is continuously synthesized by the endothelium and has a wide range of biological properties that maintain vascular homeostasis. It is a potent vasodilator and inhibitor of platelet aggregation and thus has an important protective role. Accordingly, endothelial dysfunction is generally regarded as a decreased NO bioavailability [3].

Oxidative stress also contributes to homeostasis in vascular cells [3]. Oxidant products [referred as reactive oxygen species (ROS)] are produced as a consequence of normal aerobic metabolism, but are highly reactive with other biological molecules. Under normal physiological conditions, ROS production is balanced by an efficient system of antioxidants, molecules that are capable of neutralizing them and thereby preventing oxidant damage. In pathological states, ROS may be present in relative excess and this shift of balance in favor of oxidation (oxidative stress) may have detrimental effects on cellular and tissue function [3, 4].

Several enzyme systems contribute to production of ROS in vascular tissues. Among them, the NO synthase (NOS), and in particular the endothelial isoform of NOS (eNOS) is now recognized as an important source of superoxide in sev-

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Activating stimulus
(cytokines, oxidant stress, etc.)

Dysfunctional endothelium

⬇ NO
⬇ EDHF
⬆ Angiotensin II
⬆ Endothelin-1

⬇ NO
⬆ Activation NFκB
⬆ Activation AP-1

⬆ Platelet activation

⬇ NO
⬆ Tissue factor
⬇ PGI$_2$
⬆ PAI-1

Vasoconstriction   **Inflammation**   **Thrombosis**

Fig. (1). An activating stimulus, such as cytokines, or a condition of increased oxidant stress, would lead to activation of endothelial cells (EC) and platelets, which causes a switch toward a pro-thrombotic, pro-inflammatory condition contributing to increased cardiovascular risk. EDHF: endothelium derived hyperpolarizing factor; NF-κB: nuclear transcription factor-κB; AP-1: activator protein-1; STATs: signal transducers and activators of transcription; PGI$_2$: prostacyclin; PAI-1: plasminogen activator inhibitor-1.

eral clinical settings [5]. Furthermore, eNOS can generate superoxide rather than NO in response to pathogenic stimuli. These findings have led to the concept of "NOS uncoupling", where the activity of the enzyme for NO production is decreased, in association with an increase in NOS-dependent superoxide production, such that both superoxide and NO are produced simultaneously. Under this circumstance, eNOS may become a peroxynitrite generator, leading to a dramatic increase in oxidative stress, since peroxynitrite formed by the NO-superoxide reaction, has additional detrimental effects on vascular function [4].

Beside their direct attack to various cellular targets (i.e. nucleic acids, lipids, etc.), ROS are also capable of activating other signaling molecules, such as protein kinase C (PKC) and nuclear transcription factor-κB (NF-κB) leading to transcription of genes encoding cytokines, growth factors and adhesion molecules [6]. These molecular changes are ultimately responsible for the phenotypic switch of the endothelium from a non-adhesive, non-thrombogenic cellular interface to one that expresses and secretes several adhesion molecules and chemoattractants capable of recruit and activate other vascular cell types [4, 6].

In recent years it has become increasingly recognized that ROS can act as signaling molecules not only in the inflammatory response, but also in many aspects of growth factor-mediated physiological responses. In fact, at moderate, non-toxic concentrations, ROS act as physiological signal transduction messengers, and a variety of natural stimuli works by changing the cellular redox (oxidation/reduction) state as a part of the normal intracellular signaling network [7].

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF): ANGIOGENIC AND VASCULAR PROTECTIVE ACTIONS

Recent reports suggest that ROS play an important role in angiogenesis [8]. The underlying molecular mechanism(s) remain largely unknown, but numerous reports have demonstrated that the intracellular redox state is closely associated with the pattern of VEGF expression [9-12].

VEGFs and their Receptors

Angiogenesis and lymphangiogenesis are regulated predominantly by several different growth factors and their associated receptor tyrosine kinases. Foremost among these is the VEGF family, which consists of several members, mainly VEGF-A, VEGF-B, VEGF-C and VEGF-D, sharing a common structure of 8 characteristically spaced cysteine residues in a VEGF homology domain [13]. VEGF-A (hereinafter referred to as VEGF), initially designated VPF (vascular permeability factor), is essential for angiogenesis in health and pathophysiology, and it is currently a major focus for drug targeting in the development of novel treatments for different human diseases [13-16]. Other members of this family might be involved in inflammatory angiogenesis (VEGF-B) [17], or may act as lymphangiogenic growth factors (VEGF-C and VEGF-D) [17, 18]. VEGF signal in arterial and venous ECs is transduced through 2 main receptors with tyrosine kinase activity, namely, VEGFR1 and VEGFR2 [19]. There is considerable evidence that VEGFR-2 is the major mediator of VEGF-driven responses in endothelial cells and it is a crucial signal transducer in both

physiologic and pathologic angiogenesis [20]. The VEGF gene has its expression regulated by ROS and additional data support the hypothesis that VEGF mRNA is up-regulated by hydrogen peroxide in a dose- and time-dependent manner [21, 22]. Furthermore, hydrogen peroxide is also capable of inducing a significant VEGFR2 expression [23].

The binding of VEGF to 1 of its transmembrane tyrosine kinase receptors, which are predominantly found on endothelial cells, results in receptor dimerisation, activation and autophosphorylation of the tyrosine kinase domain. This triggers a cascade of complex downstream signaling pathways (Fig. (**2**)).
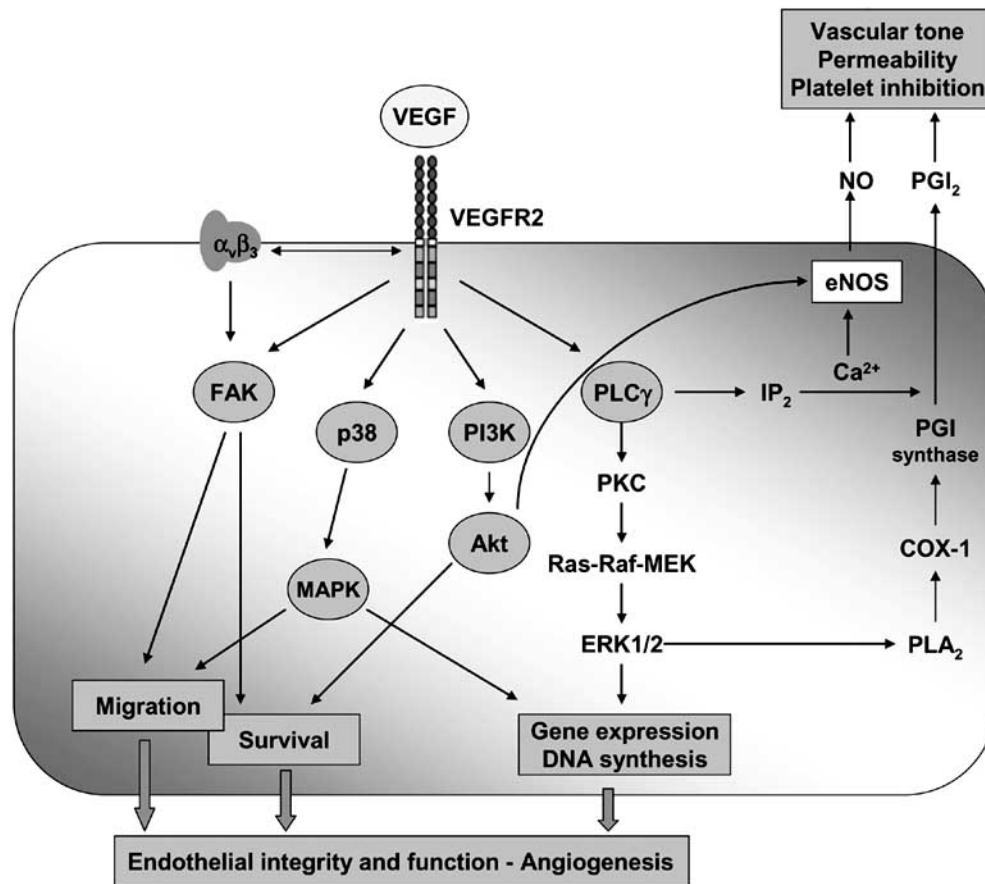
VEGF SIGNALING IN ENDOTHELIAL CELL BIOLOGY

Regulation of Vascular Permeability

VEGF was originally identified as a factor which increased vascular permeability [24, 25]. However, the signaling mechanisms underlying this effect remain largely unknown. Induction of endothelial fenestrations may be an important mechanism by which VEGF modulates vascular permeability [26]. The signaling pathways mediating VEGF-induced fenestration are unclear, though a permissive environment involving changes in the composition of the ex-

tracellular matrix may be required [26, 27]. VEGF-induced formation of fenestrations is associated with caveolae. These are a subset of lipid raft domains seen as morphologically distinct, flask-shaped invaginations that are particularly abundant and critical in the cardiovascular system [28]. In vascular endothelial cells, caveolae represent approximately 95% of cell surface vesicles and have multiple functions in organizing and regulating signaling cascades, controlling cell motility, and serving as endocytic carriers [28].

VEGF also induces the appearance of vesicular-vacuolar organelles [29, 30] and the role for NO in mediating VEGF-induced vascular permeability is supported by the observations that the eNOS inhibitor L-NAME inhibited permeability changes induced by VEGF *in vivo* [31]. Furthermore, the close coupling between activated VEGFR2 and eNOS is brought about by their colocalization within caveolae/lipid rafts and direct associations with caveolin-1. The activation of caveolar eNOS depends on caveolin-1 dissociation [reviewed in 28]. In the absence of VEGFR2 activation, caveolin-1 tightly binds to a motif in the oxygenase domain of eNOS maintaining the enzyme in an inactive state within caveolae. VEGF stimulation leads to Src kinase–mediated phosphorylation of caveolin-1 [32]. Consequently, eNOS dissociates from caveolin-1 and becomes activated by binding to calmodulin, dynamin-2a, and several heat shock pro-

Fig. (2). Vascular endothelial growth factor (VEGF) signaling *via* its receptor (VEGFR2). VEGF binding to VEGFR2 initiates a number of signaling cascades leading to cell survival, migration, and proliferation of endothelial cells. NO: nitric oxide; PGI$_2$: prostacyclin; eNOS: endothelial NO synthase; PLCγ; PKC: protein kinase C; ERK: extracellular regulated kinase; MAPK: mitogen activated protein kinase; FAK: focal adhesion kinase; PI3-K: phosphatidylinositol 3' kinase; Akt/PKB: protein kinase B; COX-1: cyclooxygenase-1; PLA$_2$: phospholipase-2.

teins [33]. Taken together, the experimental data now available support a model in which endocytosis and trafficking contribute to the rapid and transient activation of eNOS. A detailed discussion of the events leading to internalization and recycling of VEGFR2 and NO is beyond the scope of this review and has been the objective of a recent article [28].

Other molecules have been implicated as effectors of VEGF-induced venular permeability, such as PLC-γ tyrosine phosphorylation, mobilization of intracellular calcium and PKC activation [34]. VEGF increased phosphorylation of components of intercellular endothelial adherens and tight junctions may mediate disruption of cell–cell adhesions, leading to increased vasopermeability [35-37]. Nonetheless, some evidence has been published against the involvement of VEGFR2 in the ability of VEGF to trigger vascular permeability [38]. Thus, the issue is still an open matter.

Cell Survival and Proliferation

A fundamental cellular mechanism by which VEGF promotes the formation of new blood vessels and maintains their integrity is the activation of EC survival or anti-apoptotic signaling [39]. Long-term effects of VEGF on EC survival are mediated through upregulation of components of the anti-apoptotic cellular machinery. VEGF, in fact, is capable of inhibiting apoptosis by several mechanisms, including activation of the anti-apoptotic kinase, Akt/PKB [22, 40, 41], induction of the antiapoptotic proteins Bcl-2 and A1 [42] and upregulation of survivin and the X-chromosome-linked inhibitor of apoptosis (XIAP) [43]. VEGF also stimulates DNA synthesis and proliferation through the activation of extracellular-regulated kinase (ERK), which is mediated by Ras-Raf-MEK-ERK pathway [44, 45]. The mitogen activated protein kinase (MAPK) pathway is also implicated in cell proliferation in response to VEGF [46]. All these phenomena are mediated by VEGFR2 and VEGF binding to VEGFR1 is not involved in VEGF-mediated cell survival [22].

Other signaling pathways may also play a role in endothelial cell survival functions of VEGF. For example, integrins/cell adhesion receptors such as the endothelium specific adhesion molecule αvβ3 also play a role in VEGF signal transduction. The cell adhesion molecule VE-Cadherin interacts with VEGFR2 forming a complex with β-catenin and PI3-Kinase to promote cell survival [47]. Surprisingly, the same part of VE-cadherin mediates effects that in one case support VEGFR2 signaling (antiapoptosis, survival effects) and in the other case inhibit VEGFR2 signaling (proliferative effects) [48]. Furthermore, it has been recently demonstrated that semaphorin (sema) 3C promotes EC survival and proliferation and stimulates cell adhesion, migration, and tube formation *in vitro* by inducing β1 integrin serine phosphorylation and VEGF secretion *via* NP/plexin signaling [49].

VEGF-induced proliferation was reported to be dependent on a NO-mediated reduction PKCδ activity [50]. Notably, PKCδ transduces a principal signal for the upregulation of vasohibin, a negative regulator of angiogenesis genetically programmed in ECs [51]. Vasohibin is dominantly expressed in ECs, induced by the stimulation with VEGF or fibroblast

growth factor-2 (FGF-2), and selectively affects on ECs and inhibits angiogenesis. However, the mechanism of how vasohibin inhibits angiogenesis remains to be elucidated [recently reviewed in 52].

Cell Migration

VEGF induces cell migration by increasing tyrosine phosphorylation and focal adhesion association of FAK (focal adhesion kinase) [53-55] and also *via* the PI3 Kinase/Akt pathway. FAK activation is mediated by the c-terminal region of VEGFR2 [56]. VEGF activation of the p38/MAPK stress pathway is also implicated in cell migration and p38 inhibitors decrease cell migration [57]. Using VEGF mutants it was determined that only VEGFR2 and not VEGFR1 resulted in p38 phosphorylation suggesting that VEGFR2 is the main mediator of cell migration in ECs [58].

Other mechanisms have been involved in EC migration. Among these, the capability of VEGF of inducing the expression of matrix-degrading metalloproteinases, which are likely to play an essential permissive role in VEGF-induced migration *in vivo* [59]. In addition, it has been suggested that NO production may play a role in VEGF-induced endothelial cell migration. It is well known that VEGF induces NO production and NO is implicated in non-chemotactic scalar movement (podokinesis) of ECs and as a permissive factor in VEGF-induced endothelial cell migration [60, 61] and angiogenesis [62, 63]. NO has been reported to regulate focal adhesion integrity and FAK tyrosine phosphorylation in endothelial cells [61] and Akt-dependent phosphorylation of eNOS was shown to be required for VEGF-induced cell migration [64].

NO and Prostacyclin (PGI$_2$) Production

As reported above, VEGF increases both eNOS expression and NO and PGI$_2$ production by ECs [31, 65-69]. VEGF-induced PGI$_2$ production results from PKC-mediated ERK activation and ERK-mediated phosphorylation and activation of phospholipase A$_2$ (PLA$_2$) [65], a pathway that is unaffected by inhibitors of eNOS, suggesting that VEGF signaling leading to NO and PGI$_2$ generation bifurcates upstream of ERK [70, 71].

In common with other activators of eNOS, short-term NO production induced by VEGF probably involves calcium mobilization and activation of the constitutive isoform [71], but VEGF *via* activation of VEGFR2, is also capable of upregulating eNOS mRNA and protein expression [72-74] providing a mechanism for prolonged VEGF-induced NO production [71].

NO and PGI$_2$ are best known as vasodilators, but they have several vascular protective effects, including anti-platelet actions, and in the case of NO, inhibition of leukocyte adhesion [71]. Accordingly, VEGF may have similar vascular protective effects through enhanced NO and PGI production [71]. Evidence that VEGF has NO-dependent vascular protective effects independent of angiogenesis or endothelial cell proliferation, comes from preclinical and clinical studies addressing the role of this cytokine in atherosclerosis and its complications [75, 76]. Moreover, an anti-thrombotic effect of VEGF may result both from NO and

PGI$_2$-mediated inhibition of platelet aggregation, and from VEGF-induced expression and activation of the serine proteases, urokinase and tissue type plasminogen activator [77], which cleave plasminogen to generate the key thrombolytic enzyme plasmin. As an apparent paradox, VEGF also has potentially pro-thrombogenic effects, including the induction of endothelial von Willebrand factor (vWF) secretion [71, 78], which is not only essential for platelet adhesion to subendothelial collagen, but increases also endothelial cell adhesion, thus cooperating in the maintenance of endothelial integrity [25].

Vascular Protective Roles of VEGF in the Cardiovascular System

Increasing understanding of the multiplicity of actions exerted by VEGF has provided new insights in the comprehension of the vascular protective role of VEGF [10, 11] and might help to unravel the molecular mechanisms underlying vascular complications observed in patients using treatments that inhibit the VEGF signaling pathway. Although it is not yet fully elucidated how angiogenesis inhibitors upset normal hemostasis, it is likely that disruption of the function and/or integrity of vascular endothelium may lead to an increased risk for thrombosis and/or hemorrhage [79, 80].

In this context, the capability of VEGF of increasing NO production by ECs is of particular significance, considering that endothelial dysfunction is generally regarded as a decreased NO bioavailability and increased oxidative stress. Of particular interest are the findings by González-Pacheco *et al.* who suggested that, while high concentrations of hydrogen peroxide elicit clear-cut damaging effects, mild oxidative stress might act as a protective mechanism in ECs [23]. These effects involved significant changes in VEGF and VEGFR2 gene expression, the latter being mainly driven through a nuclear factor κB (NF-κB)-dependent pathway [23]. In addition, the cytoprotective effect exerted by low doses of hydrogen peroxide was shifted to an EC-damaging pattern by means of specific VEGF blockade, therefore revealing a major role of autologous VEGF and leading the authors to hypothesize that autocrine VEGF makes ECs more resistant to injury by oxidative agents [23]. Thus, one could postulate that the increased rate of thrombotic events observed in trials of combined anti-VEGF chemotherapeutic protocols might result from the blockade of such cytoprotective effect, which is even more crucial considering the increased oxidative stress [81, 82] and haemostatic activation [83] generally associated to chemotherapy.

Predisposition to thrombosis after inhibition of VEGF signaling may, indeed, reflect the multiplicity of actions of VEGF on vascular walls and perhaps on components of the coagulation system. VEGF not only stimulates endothelial cell proliferation, but also promotes ECs survival and helps maintain vascular integrity [84]. Inhibition of VEGF could thereby diminish the regenerative capacity of ECs and cause defects that expose pro-coagulant phospholipids on the luminal plasma membrane or underlying matrix, leading to thrombosis or haemorrhage [85]. In addition, the loss of antiplatelet activity due to reduced NO and PGI$_2$ after inhibition of VEGF signaling may predispose to thromboembolic events.

Although the prevailing rationale for thrombosis in antiangiogenic therapies is that VEGF blockade leads to vascular inflammation and clotting, yet another mechanism by which anti-VEGF treatment may predispose to arterial thrombosis relies in the possibility that monoclonal antibodies targeting VEGF can induce platelet aggregation, degranulation and thrombosis through complex formation with VEGF and activation of the platelet FcgammaRIIa receptor [86].

Platelets are an important *in vivo* source of VEGF, both in health [87] and cancer [88-90], and thrombin generation plays a central role being capable of activating platelets, thus causing VEGF release, and ECs, thus increasing their expression of VEGFR2 and proinflammatory cytokines (Fig. (**3**)) [91]. Moreover, activation of thrombin produces prothrombin fragment 1+2 (F1+2) that act to dampen the positive feedback effect of thrombin to help finely control the angiogenic response [92]. Disruption of these regulatory mechanisms is of utmost importance especially during cancer chemotherapy, since changes in serum VEGF usually coincide with drug-induced thrombocytopenia and the subsequent rebound of platelets might have an unwanted effect of tumor through chemotherapy-induced endothelial damage, platelet activation and further VEGF release, supporting regrowth of the tumor during the second half of the cycle [88]. To avoid the undesired effects of VEGF delivered by platelets, especially during their rebound, there might be a place for drugs preventing platelet activation and aggregation during platelet recovery, as already described in other clinical settings [93].

Thromboembolic Events in Patients Treated with Anti-Angiogenic Drugs

Bevacizumab-Based Therapies

The pivotal role of VEGF in cancer development and dissemination has begun to be elucidated since the late eighties. Several publications have demonstrated [94-96] that VEGF block can induce tumor shrinkage and prevent metastases. The next logical step has been to design specific VEGF pathway inhibitors to interfere with tumor angiogenesis. The first drug proved to have antineoplastic properties by selectively inhibiting VEGF function has been a humanized monoclonal immunoglobulin G targeting soluble VEGF-A (bevacizumab, Avastin®).

Bevacizumab is currently approved in combination with fluoropyrimidine-based chemotherapy for the treatment of patients with metastatic colorectal cancer in both first and second-line regimens [97-101]. It is also approved for the first-line treatment of patients with metastatic breast cancer in combination with paclitaxel [102], of patients with metastatic non-squamous non-small cell lung cancer in addition to platinum-based chemotherapy [103, 104] and of patients with metastatic renal cell cancer in combination with interferon alfa-2a [105].

In the pivotal phase III trial of first-line metastatic colorectal cancer treatment, bevacizumab in combination with standard irinotecan/fluorouracil chemotherapy gave a 10% increase in tumor response rate and a significant lengthening of progression-free and overall survival times (4.4 and 4.7
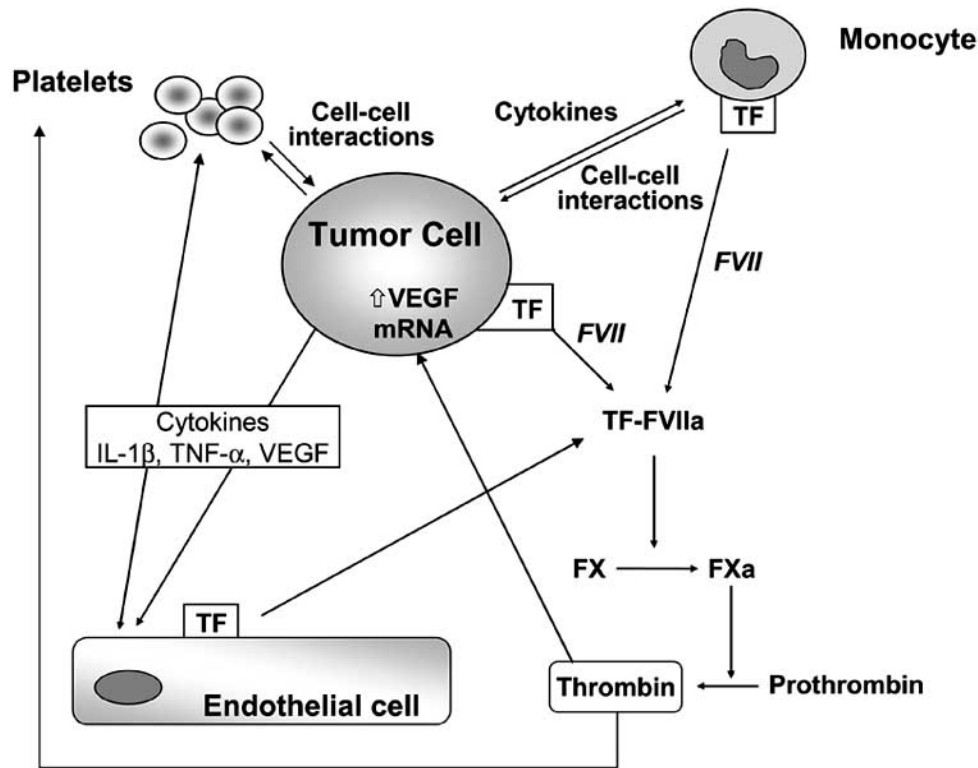
Fig. (3). Tumor cells may induce a procoagulant status either directly, through exposure of tissue factor (TF) or indirectly, through VEGF production and endothelial cell activation. Moreover, tumor cell and/or host cell produced cytokines [i.e., interleukin-1β (IL-1β) or tumor necrosis factor-α (TNF-α)] will sustain a pro-inflammatory, prothrombotic environment leading to further rounds of activation, release of platelet VEGF, or TF exposure and cytokine release by monocytes or tumor associated macrophages. Coagulation activation will ultimately lead to generation of thrombin, which may promote additional tumor VEGF mRNA induction.

month improvement, respectively, as median survival times) [97]. In this study, an increased incidence of thrombotic events was found in bevacizumab arm (393 patients) compared to the control arm (397 patients), but this was not significant (19.4% *vs.* 16.2%, respectively) [97].

Since the first approval for clinical use in metastatic colorectal cancer patients in 2004, bevacizumab has been proven effective in several cancer types other than colorectal, and its indications, as above-mentioned, have been broadened now to include renal, breast and lung cancer (www.fda.gov, www.emea.europa.eu).

Until now six large randomized phase III studies specifically exploring the advantage of adding bevacizumab to standard treatment have been fully published [97, 98, 104-106], and data on thrombotic events have been reported for all the studies. The increased risk of thromboembolic events with bevacizumab use is now well-recognized (Table **1**). However, except for the study by Hurwitz *et al.*, their incidence remains relatively low with P values not reaching significance.

To specifically address questions on bevacizumab-related thromboembolic risk, Genentech provided 2 subset analyses: the first has been recently published and refers to a retrospective pooled analysis of 5 randomized Genentech trials regarding different solid cancer types [107], the second one has been presented at the 2007 American Society of Clinical Oncology Annual Meeting and is a pre-planned analysis of

the BRiTE study[1], an observational cohort study involving 248 study sites in 49 states where bevacizumab had to be used as first line treatment for metastatic colorectal cancer patients in combination with a chemotherapy regimen chosen at investigator's discretion.

In the former study, adverse events analysis was performed on 1745 patients (782 treated with bevacizumab+chemotherapy, 963 with chemotherapy only) pooled from the phase III study by Hurwitz *et al.* [97] and from 4 other randomized phase II studies, 2 involving colorectal cancer patients [108, 109], 1 lung cancer patients [110] and 1 breast cancer patients [106]. Overall incidence of thrombotic events was calculated for the control and bevacizumab group and the possible impact of pre-existent risk factors for cardiovascular morbidity were evaluated. While no relevant differences were found for venous events (VTEs) between the 2 treatment groups, a nearly 2-fold increase of arterial thromboembolic events (ATEs) was demonstrated for patients receiving bevacizumab (3.8 % *vs.* 1.7%) with a Hazard Ratio (HR) of 2.0 (95% CI 1.05-3.75, P= 0.031) in favor of chemotherapy-only group. ATEs were considered as 1 of the following 7 events: angina pectoris, arterial thrombosis, cerebral infarct, cerebral ischemia, cerebrovascular accident, myocardial infarction or myocardial ischemia. When base-

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[1] Sugrue MM. Serious arterial thromboembolic events (sATE) in patients (pts) with metastatic colorectal cancer (mCRC) treated with bevacizumab (BV): Results from the BRiTE registry. 2007 ASCO Annual Meeting. Abstract No: 4136.

Table 1.   Incidence of Thromboembolic Events in Randomized Phase III Studies

Study	Regimen	No. of Patients	TE Incidence	VTE Incidence	ATE Incidence
Hurwitz 2004 [98]	IFL+BEV *vs.* IFL	402 411	19.4% 16.2%	-	-
Miller 2005 [107]	Capecitabine/BEV *vs.* Capecitabine	229 215	7.4% 5.6%	-	-
Sandler 2006 [105]	Carboplatin/Paclitaxel/BEV *vs.* Carboplatin/Paclitaxel	434 444	-	-	-
Escudier 2007 [106]	Interferon alfa-2a/BEV *vs.* Interferon alfa-2a	325 316	-	3% 1%	1.5% 0.7%
Miller 2007 [103]	Paclitaxel/BEV *vs.* Paclitaxel	365 346	2.2% 1.4%	-	-
Saltz 2008 [99]	Oxaliplatin/fluoropirimidin/BEV *vs.* Oxaliplatin/fluoropirimidin	694 675	-	8% 5%	2% 1%

TE (any thromboembolic event), VTE (venous thromboembolic events), ATE (arterial thromboembolic events), BEV (bevacizumab), IFL: irinotecan/fluorouracil based chemotherapy.

line risk factors for thrombotic events (age, sex, hypertension, asymptomatic atherosclerosis, diabetes and history of arterial thromboembolism, myocardial infarction, stroke, transient ischemic attack, or venous thrombosis) were included in a multivariate analysis together with bevacizumab use, variables independently influencing the risk of ATEs remain bevacizumab use, age older than 65 years and history of other ATEs at study entry (HR 1.95 p = 0.04, HR 2.17 p = 0.01, HR 3.65 p < 0.001, respectively). Furthermore, the majority of ATEs occurred within 3 months of treatment [107].

Impact of aspirin use was also analyzed. Consumers of more than 325 mg/day aspirin were excluded from the trial, while lower doses were allowed. In the pooled population only a minority of patients were taking low dose aspirin (13%) and no significant differences in terms of ATEs were detected in this subset of patients between subjects receiving bevacizumab and subject treated with chemotherapy only, however the incidence was higher in the bevacizumab arm despite the use of a concomitant anti-thrombotic drug (1.2% *vs.* 5.1%, p = 0.159) [107].

Of note the incidence of ATEs in the breast cancer trial was low and did not differ between control and bevacizumab arms (1 event per arm) [106]. However, the proportion of premenopausal women, a well-known low risk subpopulation for arterial cardiovascular diseases, and presence of other potentially protective hormonal factors were not specified in the analysis.

Similar results have been achieved in the "less-controlled" prospective BRiTE trial, where bevacizumab was prevalently given in combination with fluorouracil/ ox-

aliplatin regimen (60.7% of patients). In the ATEs analysis of BRiTE registry similar definitions of ATEs and baseline risk factors, respect to Genentech study, were applied, with small differences (addition of "hypercholesterolemia requiring medication" in risk factors).

In the BRiTE trial, unlike Genentech study and other prospective bevacizumab trials, patients with a recent history (< 12 months) of myocardial infarction (MI) and cerebrovascular accidents (CVA) were not excluded. Overall incidence of ATEs in BRiTE was 1.8%, with the majority events being MI and CVA (74%). Most ATEs occurred within the first 6 months of therapy. Multivariate risk factors analysis confirmed history of ATEs at study entry, together with ECOG performance status ≥ 1, to be an independent risk factor of developing ATEs during study treatment with bevacizumab, with a HR of 2.46 (p = 0.025).

The BEATrial[2] was a similarly designed prospective observational study, conducted in Europe, that enrolled 1,915 patients from 40 countries for whom the most commonly used chemotherapy regimen in combination with bevacizumab was oxliplatin/fluoruracil-based (47%). Final efficacy and safety data have recently been presented at the 2008 American Society of Clinical Oncology (ASCO) annual meeting. Reported incidence of ATEs was comparable to that of BRiTE registry (1.3%).

As far as VTEs are concerned, a detailed analysis of their occurrence in patients pooled from the studies by Kabbi-

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[2] Berry SR, Van Cutsem E, Kretzschmar A, *et al.* Final efficacy results for bevacizumab plus standard first-line chemotherapies in patients with metastatic colorectal cancer: First BEAT. 2008 ASCO Annual Meeting. Abstract No: 4025.

navar *et al.* [109] and Hurwitz *et al.* [97] was presented at the 2004 ASCO Annual Meeting by Novotny *et al*[3]. Overall, no substantial differences were detected in VTEs rate between bevacizumab-containing and chemotherapy-only groups, with 74 VTEs in the 492 bevacizumab-treated patients and 74 VTEs in the 500 chemotherapy-only treated patients (15% for both). Most recently, Nalluri *et al.* performed a systematic review and meta-analysis of published randomized controlled trials to assess the overall risk of VTEs associated with the use of bevacizumab [111]. A total of 7956 patients with a variety of advanced solid tumors from 15 randomized controlled trials were identified and included for analysis. The results obtained showed that patients treated with bevacizumab had a significantly increased VTEs risk with a RR of 1.33 (95% CI, 1.13-1.56; P<0.001) compared with controls. The risk was significantly increased for both all grade and high-grade VTE. In addition, the risk was similarly increased for bevacizumab at low or high dose [111], leading the Authors to conclude that the so called low dose of bevacizumab may be already reaching the saturation level to induce thrombosis; alternatively, the difference between the high and low doses of bevacizumab in thrombogenesis may be too small to detect [111].

Other Angiogenesis Inhibitors

Additional angiogenesis inhibitors have recently been approved by the US and European drugs regulatory agencies (FDA and EMEA) for the treatment of solid and hematological malignancies (lenalidomide, thalidomide, sunitinib, sorafenib). All these agents inhibit VEGF signaling by blocking VEGF ligand or VEGF receptor functions and for some of them (e.g. sunitinib) an inhibition of other important tyrosine-kinase proteins, such as platelet-derived growth factor receptor (PDGFR), has been also demonstrated.

An increased incidence of ATEs has been also reported with the use of such inhibitors. Lenalidomide and thalidomide are immunomodulatory drugs, structurally related to each other, with pleiotropic activities, including antiangiogenic and antineoplastic properties. It has been observed a surprisingly higher incidence of VTEs in multiple myeloma patients treated with these drugs, but how much part can be attributable to their antiangiogenic activity remains unclear [112-114]. In a recently published pooled analysis by Menon *et al.* [115] with multiple myeloma patients enrolled in 3 distinct lenalidomide-based clinical trials, 8% of the 125 analyzed patients developed deep vein thrombosis despite the fact that half of them were taking primary thromboprophylactic therapy. There was a trend to a higher incidence of thrombosis in patients receiving concomitant high-dose corticosteroid therapy.

Sunitinib and sorafenib are 2 small molecules inhibiting the tyrosin-kinase activity of vascular endothelial growth factor receptor (VEGFR). Sunitinib has been proven effective in the treatment of gastrointestinal stromal tumors and renal cell carcinomas [116, 117], sorafenib provides significant improvement in progression-free survival in patients

with renal cell carcinoma [118] and hepatocellular carcinoma[4]. While thromboembolic events have not yet been specifically analyzed for the 2 drugs, a 3% incidence of VTEs for sunitinib and a 2.9% incidence of treatment-emergent cardiac ischemia/infarction events are reported in the respective European Public Assessment Reports (http://www.emea.europa.eu).

Although data with respect to incidence and management of cardiovascular and cerebrovascular events from bevacizumab are more mature than those from the various VEGFR tyrosine kinase inhibitors, it is conceivable that, considering the fact that the VEGF inhibitory effects of the various classes of angiogenesis inhibitors are comparable, the inherent risks for any such event is theoretically comparable. As a matter of fact, the apparent differences in incidence of any cardiovascular thromboembolic event observed between bevacizumab and small molecules may be related to the yet relatively small number of patients that have been exposed to the latter [119].

CONCLUSIONS AND PERSPECTIVES

The clinical use of bevacizumab is rapidly increasing and we have now sufficient experience and number of treated patients to draw reasonable conclusions on its toxic profile. The thrombogenic effect of the drug seems to be exerted mainly in arteries, with the risk of venous thrombotic events being comparable to that of patients treated with standard chemotherapy. Some unclear points remain on the definition and grading of VTE among different trials and rate of asymptomatic VTE may be underestimated[5].

The incidence of ATEs attributable to bevacizumab remains low (2-3%) but it is double in size respect to that observed in chemotherapy-only treated patients with a significant difference. However, increased risk of ATEs in bevacizumab-treated patients seems to be mainly related to age, history of previous ATE and ECOG performance status, variables that have to be taken into account before starting an antiangiogenic treatment.

Moreover, undetectable thrombotic phenomena of the microvasculature seem to have a role also on pathogenesis of other side effects typically associated with bevacizumab, such as hypertension and proteinuria [120, 121].

On the other hand, the protective role of other factors such as concomitant use of antihrombotic drugs and hormonal factors still has to be elucidated. Considering the significant impact of history of ATEs of new ATEs on bevacizumab treatment, exclusion of patients with recent episodes (within 12 months) of relevant cardiovascular events should be maintained in future bevacizumab-involving trials. The real impact of thromboembolic toxicity of other antiangiogenic agents such as sunitinib and sorafenib needs further investigations and larger study populations.

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The critical issues emerging from the available studies are, firstly, how to best prevent these complications and, secondly, how to best manage the haemostatic complications of antiangiogenic agents in cancer patients, particularly those undergoing concurrent treatment with standard chemotherapy [79]. The American Society of Clinical Oncology's guidelines do not recommend routine prophylaxis in ambulatory cancer patients receiving chemotherapy with the exception of myeloma patients receiving thalidomide or lenalidomide because of potential bleeding and the relatively low incidence of VTEs in this setting [122]. However, the absolute risk of VTEs in patients treated with bevacizumab may be comparable and, thus, prophylaxis may be conducted accordingly [79,111]. Future studies, specifically designed to address this issue, are urgently required to better define the causal association of antiangiogenic drugs with haemostatic complications and to establish the best prophylactic strategy.

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