Hemostatic Regulators of Tumor Angiogenesis: A Source of Antiangiogenic Agents for Cancer Treatment?

Martina E. Daly, Andreas Makris, Malcolm Reed, Claire E. Lewis

The maintenance of vascular integrity and control of blood loss are regulated by a sophisticated system of circulating and cell-associated hemostatic factors. These factors control local platelet aggregation, the conversion of soluble fibrinogen to an insoluble fibrin polymer, and the dissolution of fibrin. However, hemostatic factors are also involved in a number of physiologic processes, including development, tissue remodeling, wound repair, reproduction, inflammation, and angiogenesis. In this review, we outline ways in which angiogenesis is coordinated with and regulated by hemostasis. We focus on inhibitors of angiogenesis contained within platelets or harbored as cryptic fragments of hemostatic proteins and assess the experimental and preclinical evidence for their ability to inhibit tumor angiogenesis and, thus, their potential to be anticancer agents. Finally, we review the results of recent clinical trials involving angiogenesis inhibitors and the evidence that antiangiogenic therapy may be associated with hemostatic complications. [J Natl Cancer Inst 2003;95:1660–73]

Angiogenesis, the development of new blood vessels by the sprouting of the existing vasculature, occurs in various diseases, including cancer, but in only a limited range of healthy adult tissues such as the ovary and endometrium during the menstrual cycle and in wound healing. With these exceptions, the vasculature is quiescent in adults, being tightly regulated by a balance of pro- and antiangiogenic factors. Similarly, the coagulation cascade, which usually controls blood loss during vascular damage (i.e., hemostasis), is normally inactive in adults because of the balance between pro- and anticoagulant proteins in the bloodstream. However, when damage to the vascular endothelium occurs, exposure of the subendothelial matrix mobilizes both the hemostatic and angiogenic systems to prevent excessive bleeding into the surrounding tissues and to repair the defect in the vessel wall.

For tumor growth and metastasis to occur, the tight regulatory balance between pro- and antiangiogenic factors under physiologic conditions is disturbed in favor of angiogenic stimulators. Coagulation is activated concomitantly, and the patient develops an increased risk of a thrombotic event. During the past two decades, it has become evident that, in addition to their recognized role in the regulation of coagulation, plasma- and platelet-derived hemostatic factors or fragments thereof also contribute to the regulation of angiogenesis. Because tumor growth and metastasis depend on angiogenesis, negative regulators of angiogenesis are of particular interest as potential anticancer agents. In this review, the antiangiogenic properties of a variety of plasma- and platelet-derived hemostatic proteins will be outlined and their potential as inhibitors of tumor angiogenesis in anticancer therapies will be discussed.

INTERPLAY BETWEEN ANGIOGENESIS AND HEMOSTASIS UNDER PHYSIOLOGIC CONDITIONS

The coagulation cascade is initiated when damage to the endothelia results in exposure of tissue factor (TF) and fibrillar collagen present in the subendothelial matrix (Fig. 1). TF forms a complex with activated factor VII (factor VIIa), small amounts of which are present in the circulation, to catalyze the activation of factors X and IX to factors Xa and IXa, respectively. The activity of the TF–factor VIIa complex is inhibited in vivo by TF pathway inhibitor in a factor Xa–dependent manner. Factor Xa is also generated through an alternative reaction that is catalyzed by factor IXa and that requires activated factor VIII (factor VIIIa) as a cofactor. Factor Xa generated through either pathway activates factor V. Activated factor V (factor Va) then acts as a cofactor for factor Xa in the conversion of circulating and platelet-bound prothrombin to the enzyme thrombin (Fig. 1) (1). Thrombin is essential for normal hemostasis, directly promoting clot formation by catalyzing the conversion of soluble fibrinogen to insoluble fibrin monomers and then activating circulating factor XIII, which in turn catalyzes the cross-linking of fibrin monomers to form a fibrin mesh. Through its activation of factors V, VIII, and XI, thrombin also has positive feedback effects on coagulation. Thus, factors Va and VIIIa are essential cofactors for factors Xa and IXa, respectively, whereas factor XIa promotes coagulation by catalyzing the activation of additional factor IX. In addition to its procoagulant role in plasma-based coagulation, thrombin is a potent agonist of platelets, inducing their activation through protease-activated receptor-1 and the glycoprotein (GP) Ib–V–IX complex (Fig. 2) (2).

Platelets can also become activated after adhesion to collagen exposed at the site of injury. Platelets adhere to collagen either directly, via the collagen receptor GPVI, or indirectly, through von Willebrand factor, which binds to both collagen and the platelet receptor GPIb–V–IX. The interaction with collagen

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stimulates platelet activation, resulting in the exposure of high-affinity binding sites for fibrinogen on GPIbIIa that allows the cross-bridging of activated platelets and formation of platelet aggregates (Fig. 2). The observation that many coagulation factors can bind to receptors on resting and activated platelets and can participate in the assembly of the factor X– and prothrombin-activating complexes also supports a role for platelets in the initiation and propagation phases of blood coagulation, independent of TF and factor VIIa (2).

Angiogenesis is initiated in concert with coagulation, when the increased permeability of the damaged vessel results in extravasation of adhesive plasma glycoproteins, such as fibrinogen and fibronectin, and the formation of a temporary scaffold for migrating endothelial cells (3). Platelet activation at the site of injury results in the release of the intra-platelet granule contents, including several activators and inhibitors of angiogenesis (Table 1 and Fig. 2). In particular, platelets are an important in vivo source of vascular endothelial growth factor (VEGF), the most potent angiogenic factor known (4,5). VEGF also induces endothelial cell release of von Willebrand factor, further facilitating platelet adhesion (6). VEGF promotes endothelial cell proliferation and acts as an indirect procoagulant by increasing vascular permeability and increasing TF expression on endothelial cells, which in turn activates the coagulation cascade and promotes platelet adhesion and activation (7–9). Thrombin also induces angiogenesis in vivo by cleaving the amino-terminal tails of protease-activated receptors on endothelial cells, thereby inducing their activation and the secretion of angiogenic factors.

Fig. 1. Pathways involved in hemostasis. Procoagulant and fibrinolytic pathways are indicated by solid arrows. Anticoagulant and antifibrinolytic pathways are indicated by broken arrows. Coagulation factors are represented by roman numerals with the activated and inactivated forms indicated by the suffixes 'a' and 'i,' respectively. Hemostatic proteins having antiangiogenic activity, or from which antiangiogenic fragments are derived, are shown in boxes. TF = tissue factor; F = factor; TFPI = tissue factor pathway inhibitor; PC = protein C; APC = activated protein C; PAI-1 = plasminogen activator inhibitor-1; uPA = urokinase-type plasminogen activator; tPA = tissue-type plasminogen activator.

Fig. 2. Contribution of platelets to hemostasis and angiogenesis. Thrombin is generated from its precursor, prothrombin, by activated factor X (FXa) in the presence of activated factor V (FVa). Thrombin activates platelets through its protease-activated receptor (PAR)-1. Platelets can also be activated by binding to collagen, which is mediated directly through glycoprotein VI (GPVI) or indirectly via von Willebrand factor (VWF) binding to GPIb–V–IX. Signal transduction from any of these receptors leads to phospholipase C recruitment, which mediates calcium mobilization, platelet shape change, degranulation, and activation of GPIbIIa to allow binding of fibrinogen and platelet–platelet interactions. Degranulation results in the release of both positive and negative regulators of angiogenesis. VEGF = vascular endothelial growth factor; FGF = fibroblast growth factor; HGF = hepatocyte growth factor; PDGF = platelet-derived growth factor; EGF = epidermal growth factor; IGF = insulin-like growth factor; PF-4 = platelet factor 4; TGFβ-1 = transforming growth factor β-1; PAI-1 = plasminogen activator inhibitor-1; TIMP = tissue inhibitor of metalloproteinase; HMK = high-molecular-weight kininogen. Solid lines indicate direct interactions. Broken lines indicate multi-step processes that occur on activation of platelets and result in platelet shape change, mobilization of calcium, release of granule contents, and activation of GPIbIIa.
plasminogen activator, metalloproteinase, chymase, and heparanase families of proteinases (10). These enzymes catalyze local degradation of basement membranes and matrix proteins and promote angiogenesis by activating or releasing growth factors sequestered within the matrix, including VEGF. Activation of endothelial cells by thrombin also increases expression of VEGF receptors (11). These events result in the migration of proliferating endothelial cells from the clot margin into the fibrin mesh to reinstate the vessel wall. The adhesion and spreading of the migrating endothelial cells are mediated through the binding of the endothelial cell \( \alpha_v \beta_3 \) integrin to arginine–glycine–aspartic acid (RGD) amino acid sequences located on the fibrin mesh (12). Cell migration also requires localized pericellular fibrinolysis. This process is initiated by growth factor–stimulated expression of the endothelial cell plasminogen activator receptor (uPAR) and urokinase-type plasminogen activator (uPA), which catalyze the generation of the fibrinolytic enzyme plasmin from plasminogen (13,14).

**ACTIVATION OF THE COAGULATION SYSTEM DURING TUMOR ANGIOGENESIS**

Tumor growth and metastasis are dependent on sustained angiogenesis (15,16). The switch to an angiogenic phenotype occurs when a small, in situ tumor begins to recruit a blood supply. This switch is often characterized by increased expression of angiogenic proteins such as VEGF (17–19). Results of studies (18) with transgenic mice suggest that the switch occurs early in tumor development as a result of genetic changes and/or as a response of tumor cells to local stresses such as hypoxia and low pH. Within the tumor microenvironment, hypoxia increases levels of the transcription factors hypoxia-inducible factor-1 (HIF-1) and HIF-2 in tumor cells and in stromal cells such as macrophages and fibroblasts; HIF-1 and HIF-2 then promote the transcription of genes for VEGF and several other proangiogenic factors (20–22). Hypoxia also decreases levels of antiangiogenic factors such as thrombospondin-1 (TSP1) (23), altering the balance in favor of proangiogenic activities.

Activation of the hemostatic system has long been recognized to occur in cancer patients (24). Cancer patients are at increased risk of venous thromboembolisms; indeed, thrombosis may be the first symptom of occult malignancy. Many procoagulant factors, such as TF, are expressed and secreted by tumor cells and by stromal cells within tumors. Moreover, tumor angiogenesis results in the formation of abnormal blood vessels; the consequent damaged or irregular endothelium/blood flow also activates the hemostatic system (24). Factors related to treatment (e.g., surgery, chemotherapy, hormone therapy, indwelling central venous catheters) are likely to contribute to the association between malignancy and risk of thromboembolism. However, activation of the coagulation system occurs also during tumor angiogenesis. Many tumor cells constitutively express TF, which, when exposed to circulating factor VIIa, activates factor X, and the coagulation cascade (Fig. 1) (25). Tumor cells also express other activators of factor X, such as mucin and a cysteine protease known as cancer procoagulant (26,27). The local thrombin generation and the fibrin deposition increase endothelial cell motility, promote angiogenesis, and activate platelets.

The contribution of platelets to tumor angiogenesis first became evident when Gasic et al. (28) related the inhibition of pulmonary metastasis to the presence of an antiplatelet antibody. It has been proposed that the hyperpermeability of the tumor vasculature, caused by tumor-derived angiogenic factors, may result in exposure of the subendothelium and thus facilitate platelet adhesion (29). In addition, tumor-derived VEGF can stimulate the release of endothelial stores of von Willebrand factor, supporting platelet adhesion. Activation of adhered platelets then results in the release of potent stimulators and inhibitors of angiogenesis. The presence of activated platelets within the dense vasculature of soft tissue sarcomas supports the hypothesis that platelets contribute to tumor-induced angiogenesis (30).

**PLASMA-DERIVED HEMOSTATIC FACTORS KNOWN TO INHIBIT ANGIOGENESIS**

**Angiostatin**

In 1994, O’Reilly et al. (31) reported that metastases in a murine model of Lewis lung carcinoma are suppressed by a circulating angiogenesis inhibitor. The inhibitor, angiostatin, was a 38-kd protein that corresponded to the first four kringle domains of murine plasminogen. The equivalent fragment of human plasminogen inhibited neovascularization and growth of lung metastases in the same murine model and specifically inhibited endothelial cell proliferation in vitro (31). Kringle domains are autonomous regions of approximately 80 amino acids that are characterized by a triple loop structure with three disulfide bonds and cysteine residues at the N and C termini. Kringle domains are found in several blood-clotting and fibrinolytic proteins, and proteolytic cleavage of plasmin and its precursor, plasminogen, at various sites is now known to result in a variety of kringle domain–containing fragments that are collectively known as angiostatin. Kringle domains 1, 2, and 3 each possess inhibitory activity similar to angiostatin in endothelial cell proliferation assays, whereas kringle domain 4 lacks inhibitory activity (32). The smallest physiologic antiangiogenic fragment derived from plasminogen is a 22-kd fragment encompassing kringle domain 1 and part of kringle domain 2 (33). How angiostatin is generated in vivo is unclear. Because tumor cells

<table>
<thead>
<tr>
<th>Proangiogenic factors</th>
<th>Antiangiogenic factors</th>
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<tr>
<td>Vascular endothelial growth factor-A, -C</td>
<td>Thrombospondin-1</td>
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<tr>
<td>Fibroblast growth factor-2</td>
<td>Transforming growth factor ( \beta )-1</td>
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<td>Hepatocyte growth factor</td>
<td>Platelet factor-4</td>
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<tr>
<td>Angiopoietin-1</td>
<td>Hepatocyte growth factor–derived fragments</td>
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<tr>
<td>Platelet-derived growth factor</td>
<td>Plasminogen (precursor of angiostatin)</td>
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<tr>
<td>Epidermal growth factor</td>
<td>( \alpha_v )-antiplasmin</td>
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<tr>
<td>Insulin-like growth factor-1, -2</td>
<td>Plasminogen activator inhibitor-1</td>
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<td>Insulin-like growth factor binding protein-3</td>
<td>Heparin binding fibronectin fragment</td>
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<td>Vitronectin</td>
<td>Epidermal growth factor fragment</td>
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<td>Fibrinectin</td>
<td>Endostatin</td>
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<td>Fibrinogen</td>
<td>High-molecular-weight kininogen (precursor of kininostatin)</td>
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<td>Heparanase</td>
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<td>Thymidine phosphorylase</td>
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<td>Sphingosine 1-phosphate</td>
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**Table 1.** Platelet-derived pro- and antiangiogenic factors
do not express plasminogen mRNA, they are unlikely to produce angiostatin directly. Several proteinases, including macrophage metalloelastase (34) and metalloproteinases 3, 7, and 9 (35,36) can process angiostatin from systemic plasminogen. Angiostatin can also be generated by reductase treatment of plasmin. In the presence of a free sulfhydryl donor, plasmin serves as both substrate and enzyme for angiostatin generation (37,38). Matrix metalloproteinase-2 catalyzes the generation of angiostatin in the Lewis lung carcinoma model (39).

In addition to inhibiting endothelial cell proliferation, angiostatin also induces endothelial cell apoptosis in vitro and inhibits fibroblast growth factor (FGF)-2 and VEGF-induced endothelial cell migration and endothelial cell tube formation, possibly by binding to tissue plasminogen activator (tPA) and preventing plasminogen activation of its receptor, uPAR (40–42). At least two binding sites for angiostatin have been characterized on endothelial cells, a cell surface transmembrane adenosine triphosphate synthase (43) and angiostatin, binding to which leads to the internalization of angiostatin and an increase in focal adhesion kinase activity (44).

Angiostatin suppresses primary and metastatic tumor growth in various animal models with no evidence of toxicity or drug resistance (45), demonstrating the potential benefits of angiostatin-based gene therapy in the treatment of tumor growth and metastases. Growth of lung metastases was suppressed in mice implanted with fibrosarcoma cells expressing a murine angiostatin cDNA, even after removal of the primary tumor (46), and growth of a renal tumor was suppressed in mice implanted with a renal cell carcinoma (RCC) cell line expressing an angiostatin transgene (47). Retroviral and adenoviral vectors expressing the angiostatin gene also inhibited angiogenesis in vivo (48). In a rat model of glioma, delivery of an adeno-associated viral (AAV) vector expressing angiostatin suppressed tumor growth and improved long-term survival in 40% of the treated rats, whereas the combination of the AAV-angiostatin vector with an AAV vector carrying the suicidal thymidine kinase gene improved long-term survival in 55% of the treated rats (49). Liposome-delivered angiostatin delayed tumor growth and inhibited the appearance of lung metastases in a transgenic model of breast carcinoma (50) and also inhibited growth of human squamous cell carcinoma xenografts (51). Transduction of Lewis lung carcinoma cells with a retroviral vector expressing porcine pancreatic elastase suppressed tumor growth at the injection sites and in the lungs, presumably because the secreted elastase digested systemic plasminogen and generated angiostatin (52). Angiostatin also potentiated the antitumor effects of ionizing radiation in mice bearing Lewis lung carcinomas (53).

**Prothrombin-Derived Fragments**

Regulation of thrombin activity is essential to prevent unopposed fibrin deposition. In contrast to its role in promoting fibrin formation at sites of exposed subendothelium, thrombin performs an anticoagulant role at the periphery of the clot, where it binds to thrombomodulin on the endothelium and activates circulating protein C (Fig. 1). VEGF may play a positive regulatory role in this anticoagulant mechanism by dose dependently increasing endothelial cell expression of thrombomodulin (54). Activated protein C catalyzes the proteolytic inactivation of factors VIIIa and Va (Fig. 1) (55). In addition, cryptic fragments of the thrombin precursor prothrombin possess anticoagulant and antiangiogenic properties.

The functional domains of prothrombin include a γ-carboxyglutamic acid (Gla) domain (amino acids 1–44), two contiguous kringles domains (amino acids 45–273), and a catalytic domain (amino acids 274–581) (56). Factor Xa activates prothrombin, releasing a fragment encompassing the N-terminal Gla and kringle domains. Further cleavage by thrombin releases fragment 1, corresponding to the Gla domain and kringle 1 (amino acids 1–155), and fragment 2, corresponding to kringle 2 (amino acids 156–273) (56,57). Fragment 2 may act as a negative regulator of coagulation by inhibiting factor Xa-catalyzed prothrombin activation (58). Fragment 2 may also have antiangiogenic properties. A recombinant rabbit prothrombin kringle 2 domain also suppressed endothelial cell proliferation in vitro and inhibited angiogenesis in the chick chorioallantoic membrane (CAM) assay (59). Subsequently, fragments corresponding to kringle domains 1 and 2 of human prothrombin were shown to inhibit endothelial cell growth in vitro and prevent angiogenesis in the CAM assay (60). The identification of a nonapeptide sequence in kringle 2 that inhibits both the activity of factor Xa and angiogenesis suggests that the antiangiogenic activity of fragment 2 may be related to its ability to inhibit thrombin generation (61).

**Antithrombin Conformations**

O’Reilly et al. (62) demonstrated the antiangiogenic activity of cleaved antithrombin after screening small-cell lung cancer cell lines for their in vivo ability to inhibit growth of secondary tumors. Antithrombin is the major inhibitor of thrombin and factor Xa in plasma (63). The inhibitory activity of antithrombin, a member of the serine protease inhibitor (serpin) family, depends on the integrity of its reactive site loop (64,65). Antithrombin binding of heparin in vitro, or heparin sulfate in vivo, leads to exposure of the loop and an increase in the rate of thrombin inhibition (63,66). Cleavage within the loop causes a conformational change, trapping thrombin and resulting in an inactive complex that dissociates slowly, releasing cleaved antithrombin (65). Heat treatment generates at least two other antithrombin conformations: the inactive latent conformation and the pre-latent conformation, which has heparin affinity and thrombin inhibitory properties similar to those of native antithrombin (67,68).

The cleaved, latent, and pre-latent forms of antithrombin suppressed growth factor–stimulated proliferation of endothelial cells and inhibited angiogenesis in the CAM assay, although native antithrombin had negligible effects in these assays (62,68). Cleaved and latent antithrombin, but not native antithrombin, inhibited the growth of a neuroblastoma cell line in mice (62), and pre-latent antithrombin reduced tumor volume by 75% (relative to control-treated mice) in a murine fibrosarcoma model (68). In a murine fibrosarcoma model, latent antithrombin increased the frequency of terminal deoxynucleotidyltransferase-mediated UTP end-labeling (TUNEL)-positive tumor cells, indicative of increased apoptosis, and induced apoptosis of endothelial cells in vivo (69). In the presence of FGF-2, latent antithrombin also reduced endothelial cell migration and prevented formation of focal adhesion contacts (69). The low affinity of latent antithrombin for heparin, and its inability to bind thrombin suggest that its antiangiogenic effects are not mediated through interaction with heparan sulfate or through the throm-
endothelial cells and to GPIIbIIIa on activated platelets (78). It is therefore possible that the antiangiogenic effects of these non-inhibitory serpins and of the inhibitory serpin antithrombin are mediated through a shared mechanism related to a common structural feature.

Fibrinogen Fragments

Fibrinogen is a 340-kd protein that is found predominantly in plasma but is also bound to the surface of circulating platelets. Fibrinogen, which is composed of two sets of three nonidentical chains (α, β, and γ) held together by disulfide bonds, consists of a trinodular structure with one central domain, the E domain, and two identical outer D domains. Fibrinogen is cleaved by thrombin, resulting in the formation of fibrin monomers that, in the presence of factor XIIIa, polymerize into a fibrin meshwork—a crucial step in the formation of a blood clot.

In both healing wounds and growing tumors, the deposition of fibrinogen or fibrin in the extracellular matrix serves as a scaffold to bind growth factors and to promote the adhesion, proliferation, and migration of endothelial cells during angiogenesis. However, plasmin cleavage of the carboxyl termini of the paired α-, β-, and γ-chains of fibrinogen results in formation of a 50-kd fragment containing the central domain of human fibrinogen (fibrinogen E fragment) that has potent antiangiogenic activity in vitro and in vivo (73,74). This fragment inhibits VEGF-induced migration and tubule formation by human dermal microvascular endothelial cells in vitro (73) and inhibits tumor growth in a syngeneic murine model by selectively disrupting the tumor endothelium and inducing widespread intravascular thrombosis (74). This antiangiogenic activity contrasts with the angiogenic effects of a related fragment, fibrin E fragment, which is formed after fibrinogen is cleaved by thrombin yielding fibrin monomers that are then cleaved by plasmin. Fibrin E fragment has been shown to stimulate the migration and tubule formation of human dermal microvascular endothelial cells in vitro (75) and vessel formation in the CAM assay (76).

PLATELET-DERIVED INHIBITORS OF ANGIOGENESIS

In addition to being a source of VEGF and other angiogenic factors, the α-granules of platelets contain several antiangiogenic factors, which are released on activation and which limit endothelial cell proliferation and migration to the site of wound repair (Table 1).

TSP1 and Transforming Growth Factor-β1

TSP1 is a trimeric molecule, of which each subunit includes an N-terminal heparin-binding domain, a linker domain, a pro-collagen homology domain, three propeptide-like type 1 repeats, three epidermal growth factor-like type 2 repeats, seven calcium-binding type 3 repeats, and a globular C-terminal domain (77). TSP1 participates in the formation and resolution of the fibrin clot by binding to fibrin, plasminogen, and urokinase through its heparin-binding domain and mediates platelet–platelet and platelet–endothelium interactions at sites of vascular injury by binding to CD36 and the integrin αβ₃ on activated endothelial cells and to GPIIbIIIa on activated platelets (78). TSP1 also plays a major role in localizing transforming growth factor (TGF)-β1 at the site of wound repair (79). Activated platelets release both TSP1 complexed with active TGF-β1 and TSP1 devoid of TGF-β1. The latter converts the latent precursor of TGF-β1 secreted by endothelial cells to its biologically active form (80). TGF-β1 is central to the wound repair process because it recruits inflammatory cells, stimulates capillary angiogenesis and the deposition of a new matrix, and stimulates the resolution of these processes (81).

Platelet-derived TSP1 inhibits the proliferation of endothelial cells from various tissues and prevents their organization into capillary-like structures. TSP1 has also been shown to inhibit neovascularization in the rat cornea (82). TSP1 has a concentration-dependent effect on endothelial cell migration in vitro, inhibiting migration toward basic FGF at low concentrations (ED₅₀ ≈ 1 nM) but promoting migration of murine lung capillary and bovine aortic endothelial cells at higher concentrations (20–100 nM) (82,83). The antiangiogenic activity of TSP1 resides in the second and third type 1 repeats. In particular, two nonoverlapping peptides corresponding to sequences in the 1 repeat—the WSWX motifs and the CSVTCG peptide sequence—suppress angiogenesis independently (84,85). WSWX motifs bind heparan sulfate proteoglycans, but the mechanism underlying their antiangiogenic effects is unknown. TSP1 binds to CD36 via the CSVTCG peptide. The antiangiogenic effect of this peptide is mediated by binding to CD36 on capillary endothelial cells, which activates the intracellular tyrosine kinase p59fyn, leading to increased caspase-3 expression and increased endothelial cell apoptosis (86). At high concentrations (ED₅₀ = 20–40 nM), TSP1 promotes angiogenesis and increases the number and length of microvessels outgrowing aortic ring explants in collagen gels (87). It is possible that this proangiogenic activity is mediated by the N-terminal heparin-binding domain of TSP1, which is angiogenic in vivo (88).

TSP1 expression has been both directly and inversely associated with tumor grade, depending on the type of tumor studied and on whether expression is evaluated in the tumor cells or in the surrounding stromal cells (78). The differences in effects of TSP1 expression in different tumors may be related to the interaction of TSP1 with TGF-β1 and to the concentration of TGF-β1 at the tumor site. Although TGF-β1 has potent inhibitory effects on the proliferation and migration of endothelial cells in the majority of in vitro studies, in vivo studies, depending on the model, TGF-β1 is either angiogenic or antiangiogenic (81). Thus, reduced TGF-β1 expression, resulting from hemizygosity for the TGF-β1 gene, and overexpression of a TGF-β1 transgene have both been associated with accelerated chemically induced carcinogenesis in different transgenic mouse models (89,90).

Platelet Factor 4

Platelet factor 4 (PF4), which is released from the α-granules of activated platelets, promotes coagulation by competing with antithrombin for heparin-like glycosaminoglycans on endothelial cells, which it binds through its C-terminal heparin-binding region. PF4 was identified as an inhibitor of angiogenesis on the basis of the observation that heparin increased migration of capillary endothelial cells in vitro and enhanced tumor angiogenesis in vivo (91). Recombinant human PF4 inhibited angiogenesis dose dependently in a CAM assay. The angiostatic effect
is a result of a specific inhibition of growth factor–stimulated endothelial cell proliferation (92), and these effects, which are mediated by the heparin-binding region of PF4, can be suppressed by heparin (92).

PF4 inhibits angiogenesis either by blocking the low-affinity interaction between heparin-binding growth factors and heparin-like glycosaminoglycans on endothelial cells or by directly neutralizing the heparin-binding region of growth factors. The finding that a PF4 analog that does not bind heparin retains the ability to suppress tumor growth in mice and inhibit angiogenesis in the CAM assay suggested the existence of heparin-independent inhibition pathways (93). Indeed, PF4 inhibits proliferation of vascular endothelial cells induced by VEGF_{121}, an isoform of VEGF that does not bind heparin (94). More recently, PF4 was shown to disrupt FGF-2 signaling through an intracellular mechanism that operates downstream of the FGF receptor (FGFR)-1 (95). PF4 also exerts angiostatic effects by inhibiting angiogenin-stimulated actin polymerization (96) and by blocking endothelial cell cycle entry and progression into S phase through a glycosaminoglycan-independent pathway (97).

Recombinant human PF4 inhibited solid tumor growth in mice (98) and the development of experimental lung metastases in mice injected with B15F10 melanoma cells (99). In an animal model of intracerebral glioma, transduction of tumor cells with vectors expressing a secretable form of PF4 resulted in slow-growing hypovascular tumors and prolonged survival (100). Administration of an adenoviral vector expressing PF4 decreased VEGF secretion in mice with malignant ascites and was more effective when administered in combination with adenoviral vectors expressing angiostatin or endostatin than when administered alone (101). Several PF4-derived fragments, including a peptide corresponding to amino acids 47–70 that contains the heparin-binding site, inhibit angiogenesis in vitro (102,103).

**Fibrinolytic Inhibitors**

Because degradation of basement membrane and matrix proteins is required for cell migration, the role of endogenous inhibitors of proteolysis in the regulation of angiogenesis has been the focus of much attention. Platelets are a rich source not only of tissue inhibitor of metalloproteinase-1 and -2, but also of several inhibitors of fibrinolysis (104). Plasminogen activator inhibitor (PAI)-1, derived predominantly from vascular smooth muscle cells and megakaryocytes, is the primary inhibitor of plasminogen activators in vivo (105,106). PAI-1, a serpin, is present in plasma and also in platelet α-granules, where it is stored in an active conformation bound to the cell-adhesion protein vitronectin (107). The vitronectin–PAI-1 complex inhibits vascular smooth muscle cell migration by blocking binding of the cell surface αvβ3 integrin to vitronectin (108). In the CAM assay, PAI-1 inhibits angiogenesis both by inhibiting proteinase (i.e., uPA and tPA) activity and also by blocking integrin binding to vitronectin (109). Paradoxically, however, increased PAI-1 expression in cancer patients is associated with unfavorable outcome (110–112). Thus, the role of PAI-1 in regulating tumor growth and metastasis remains unclear, although recent studies suggest that, depending on its concentration, PAI-1 can promote or inhibit tumor growth by regulating angiogenesis. In a study (113) of microvessel outgrowth from murine PAI-1-deficient aortic rings, physiologic concentrations of PAI-1 promoted angiogenesis by inhibiting plasminogen activators, whereas higher levels of PAI-1 had antiangiogenic properties. Similarly, Matrigel implant assays in mice showed that PAI-1 could either promote or inhibit tumor growth and angiogenesis, depending on its concentration (114).

Fibrinolysis is also regulated in vivo by the specific plasmin inhibitor α2-antiplasmin. In the presence of angiopoietin-1, α2-antiplasmin can suppress the sprouting of endothelial cells grown in fibrin gels, presumably by inhibiting plasmin, the secretion of which is induced by angiopoietin-1 (115). Through its inhibition of plasmin and other proteinases, platelet-derived α2-macroglobulin may also suppress angiogenesis. Furthermore, α2-macroglobulin can regulate angiogenesis by binding to and modulating the activity of several growth factors. For example, α2-macroglobulin can bind VEGF and inhibit binding to the VEGF receptor (116). Although α2-macroglobulin can inhibit basic FGF-induced endothelial cell proliferation in a dose-dependent manner, it does not affect tubule formation (117).

**Endostatin**

By using a strategy similar to that used to identify angiostatin, O’Reilly et al. (118) identified a second potent angiogenesis inhibitor, endostatin, from culture medium conditioned by a murine endothelial tumor cell line. Endostatin, a 20-kd C-terminal fragment of collagen XVIII, specifically inhibits endothelial cell proliferation and migration in vitro and potently inhibits the vascularization and growth of tumors in animal models (45). The generation of endostatin (or endostatin-like collagen XVIII fragments) by proteolytic enzymes such as cathepsin L and matrix metalloproteases involves cleavage of the peptide bonds within the protease-sensitive hinge regions of the C-terminal domain. The processing of collagen XVIII to endostatin may represent a local control mechanism for regulating angiogenesis.

Endostatin is found throughout the extracellular matrix and in rat platelets. Thrombin, at concentrations that are sufficient to activate the protease-activated receptor 4 but that do not induce platelet aggregation, can induce rat platelets to release endostatin (119–121). Whether human platelets contain endostatin remains to be established.

Other platelet-derived angiogenesis inhibitors have been identified, including a peptide encompassing amino acid residues 33–42 of murine epidermal growth factor (mEGF), which inhibited the stimulatory effects of laminin on both endothelial cell motility and angiogenesis in the CAM assay (122). Two heparin-binding fragments derived from the amino (29 kd) and carboxyl (40 kd) termini of fibronectin also inhibit endothelial cell growth in a dose-dependent manner (123).

**THE CONTACT SYSTEM AND HIGH-MOLECULAR-WEIGHT KININOGEN DOMAIN 5**

Together, the serine proteinases coagulation factor XII, plasma prekallikrein, and their nonenzymatic cofactor high-molecular-weight kininogen (HK) constitute the contact system, which is so named because it was originally believed to require contact with artificial negatively charged surfaces for zymogen activation to occur. The contact system had been thought to be important in hemostasis, because deficiency of any one of the contact factors prolonged clotting time in in vitro coagulation assays. However, the absence of bleeding disorders in individ-
uals having inherited deficiencies of factor XII, plasma prekallikrein, or HK indicates that this system plays only a secondary role in hemostasis. By contrast, it is now recognized that assembly and activation of the contact system takes place in vivo on cell membranes, independently of negatively charged surfaces, and that the proteins in this system contribute to a variety of biologic activities having anticoagulant, profibrinolytic, proinflammatory, and antiadhesive properties (124).

Of particular relevance is HK, a plasma glycoprotein comprising six domains, each possessing distinct properties. Kallikrein cleaves domain 4 of HK, releasing the vasodilator bradykinin and generating a two-chain high-molecular-weight kininogen (HKa) (124). The transition from HK to HKa involves major conformational changes to expose domain 5, which mediates the acquired ability of HKa to compete with vitronectin for binding to uPAR on endothelial cells (124,125). HKa and the fragment corresponding to domain 5 both inhibit proliferation and induce apoptosis of endothelial cells in vitro, with a potency comparable with that of angiotatin (126,127). HKa also inhibits neovascularization of subcutaneously implanted Matrigel plugs and rat corneal angiogenesis in vivo (127). Recombinant domain 5, known as kinostatin, also inhibits migration of endothelial cells on vitronectin and FGF-2-stimulated angiogenesis in the CAM assay (126,128). Although the antiangiogenic effects of HKa may be mediated through an interaction with uPAR, the results of at least one study (127) do not support this mechanism. The recent report (129) that the antiangiogenic effects of HKa can be completely blocked by an antibody to endothelial cell tropomyosin, and that tropomyosin exposure was increased on the surface of proliferating endothelial cells, suggests an alternative mechanism.

**Table 2. Classification of angiogenesis inhibitors**

<table>
<thead>
<tr>
<th>Class</th>
<th>Examples</th>
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</thead>
<tbody>
<tr>
<td>Inhibitors of matrix breakdown</td>
<td>BMS-275291</td>
</tr>
<tr>
<td></td>
<td>Dalteparin (Fragmin)</td>
</tr>
<tr>
<td></td>
<td>Suramin</td>
</tr>
<tr>
<td>Direct inhibitors of endothelial cells</td>
<td>2-Methoxyestradiol (2-ME)</td>
</tr>
<tr>
<td></td>
<td>CC-5013 (thalidomide analog)</td>
</tr>
<tr>
<td></td>
<td>Combretastatin A4 phosphate</td>
</tr>
<tr>
<td></td>
<td>LY317615 (protein kinase beta inhibitor)</td>
</tr>
<tr>
<td></td>
<td>Soy isoflavone (genistein; soy protein isolate)</td>
</tr>
<tr>
<td></td>
<td>Thalidomide</td>
</tr>
<tr>
<td></td>
<td>AE-941 (Neovastat; GW786034)</td>
</tr>
<tr>
<td></td>
<td>Anti-vascular endothelial growth factor (anti-VEGF antibody (bevacizumab; Avastin)</td>
</tr>
<tr>
<td></td>
<td>Interferon-α</td>
</tr>
<tr>
<td></td>
<td>PTK787/ZK 222584</td>
</tr>
<tr>
<td></td>
<td>VEGF-Trap</td>
</tr>
<tr>
<td></td>
<td>ZD6474</td>
</tr>
<tr>
<td>Endothelial-specific integrin/survival signaling inhibitors</td>
<td>EMD 121974</td>
</tr>
<tr>
<td>Drugs with nonspecific mechanism of action</td>
<td>Anti-β integrin antibody (Medi-522; Vitaxin)</td>
</tr>
<tr>
<td></td>
<td>Carboxyamidotriazole (CAI)</td>
</tr>
<tr>
<td></td>
<td>Celcexib (Celebrex)</td>
</tr>
<tr>
<td></td>
<td>Halofuginone hydrobromide</td>
</tr>
<tr>
<td></td>
<td>(Tempostatin)</td>
</tr>
<tr>
<td></td>
<td>Interleukin-12</td>
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<tr>
<td></td>
<td>Rofecoxib (VIOXX)</td>
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**Hemostasis as a Source of Antiangiogenic Agents in the Treatment of Cancer: Results from Clinical Trials to Date**

Proliferation and migration of microvascular endothelial cells are essential for tumor growth and metastasis. Thus, it is not surprising that the tumor vasculature has become an important target for anticancer therapies (16). There may be several advantages of antiangiogenic therapy. For example, because genetic instability is frequently associated with acquired resistance to conventional chemotherapy drugs, targeting the genetically stable endothelial cells may avoid or delay the onset of drug resistance (130). Furthermore, endothelial cells are directly accessible to systemic therapies. Angiogenesis-targeted therapy has potential value for different types of cancers and may be useful for treatment of advanced disease, adjuvant therapy, or prevention.

An overview of antiangiogenic agents and current clinical trials is available from the U.S. National Cancer Institute’s Web site (http://www.cancer.gov/clinicaltrials/developments/anti-angiogenesis) and forms the basis for the classification of angiogenesis inhibitors outlined in Table 2. In addition, there are several anticancer drugs including antioncogene-directed signal transduction inhibitors such as trastuzumab (Herceptin), hormone blockers such as tamoxifen, and conventional chemotherapeutic agents such as cyclophosphamide and methotrexate that possess antiangiogenic properties (131). Of the numerous antiangiogenic agents in various phases of clinical trials, some agents have been associated with unexpected toxicities. However, in general, phase I trials have shown most antiangiogenic agents to be safe and well tolerated and have therefore moved on to phase II and phase III trials.

Although preclinical studies have confirmed the antiangiogenic properties of several hemostatic proteins and their derivatives, few such agents have progressed to clinical application in trials with cancer patients, with the exception of trials involving angiotatin, endostatin, TSP1 mimetics and a synthetic uPA inhibitor (see below). Because VEGF is synthesized by megakaryocytes and by the malignant and stromal cell compartments of solid tumors in cancer patients, and because VEGF has an indirect procoagulant role in increasing vascular permeability, promoting platelet adhesion, and increasing TF expression on endothelial cells, we have also outlined below the efficacy of various anti-VEGF therapies in anticancer clinical trials.

**Angiostatin**

The results of a phase I trial, in which angiostatin was evaluated in 24 patients with advanced cancer treated in three groups of eight at three different dose levels (7.5, 15, or 30 mg/m²) given by twice-daily subcutaneous injection, have been reported in abstract form (132). Angiostatin was well tolerated, even during prolonged therapy. Two patients, however, developed hemorrhages into brain metastases. An extensive panel of coagulation parameters was not influenced by angiostatin. Importantly, the plasma concentrations of angiostatin that were achieved were in the range at which biologic activity was seen in preclinical models. Seven of 24 patients had long-term stable disease of more than 5 months, and four of 24 patients had long-term stable disease of more than 7 months.

**Endostatin**

The results of phase I trials with endostatin were reported recently. Eder et al. (133) administered human recombinant
endostatin (rh-Endo) as a daily 20-minute intravenous infusion to 15 patients with advanced and treatment-refractory solid tumors. The dose was escalated from 15 to 240 mg/m² in successive cohorts of patients. No clinically significant treatment-related toxicities were seen. Although there were no objective responses as measured by conventional criteria, one patient with a pancreatic neuroendocrine tumor achieved a minor response (17% maximum tumor reduction) and two patients (one with colon cancer and one with soft tissue sarcoma) showed stable disease for periods of 3 and 4 months, respectively. The use of the 20-minute daily infusion meant that the systemic exposure to endostatin at the 240 mg/m² dose was 50% lower than that provided by the dosing regimen that afforded maximum growth inhibition in preclinical studies.

Recently, the results of a phase I trial of daily intravenous bolus and continuous infusion of rh-Endo in 28 patients with adult refractory solid tumors were reported (134). Treatments were well tolerated. There were minor responses in two patients with pancreatic neuroendocrine tumors and stable disease in four others lasting more than 6 months. Eder et al. (134) suggested that reductions in circulating endothelial cells may be a potential useful surrogate marker of antiangiogenic activity because reductions were seen in the patients with minor or stable disease, and increases were seen in those with progressive disease.

Herbst et al. (135) treated 25 patients with advanced solid tumors in a phase I trial with rh-Endo administered as a 20-minute daily intravenous infusion at dose levels increased from 15 to 600 mg/m². Treatment was well tolerated, with no dose-limiting toxicity. Pharmacokinetic studies suggested that, at a dose of 300 mg/m², a plasma concentration was achieved that was compatible with antiangiogenic activity and the systemic exposure to endostatin was lower than that provided by the dosing regimen that afforded maximum growth inhibition in preclinical studies.

Thrombospondin Mimetics

Preclinical studies have demonstrated that simple peptide mimetics of TSP1 are effective in vivo antitumor agents in two tumor models—primary bladder cancer and metastatic melanoma (137). In vitro, these peptide mimetics had no effect on cancer cells but inhibited the growth of endothelial cells by inducing CD36 receptor–dependent apoptosis. In vivo, these peptide mimetics were associated with reduced microvessel density and increased apoptosis in both endothelial cells and tumor cells. These compounds are currently being tested in human phase I trials.

In a phase I trial, the TSP1 mimetic protein ABT-510 was administered to 26 adult patients with advanced cancer (138). Patients received ABT-510 daily as a continuous infusion subcutaneously or as a bolus once or twice daily by subcutaneous injection. Because all patients receiving the continuous infusion developed local skin infiltration at the injection site, delivery via continuous infusion was halted. One patient with non–small-cell lung cancer hemorrhaged in a cerebellar metastasis after 32 days of therapy, and a patient with leiomyosarcoma had a transient ischemic attack after 21 days of therapy. Other adverse events including fatigue, nausea, and insomnia were mild and not dose-limiting. No other clinically significant toxicities were reported. Of 23 evaluable patients, nine had stable disease for at least 8 weeks and five had stable disease for more than 16 weeks.

Synthetic uPA Inhibitor

The poor prognosis in patients with tumors expressing high levels of uPA, and the observation that the endogenous uPA inhibitor, type 2 PAI (PAI-2), substantially reduced pulmonary metastases in rats infused with rat mammary cancer cells, suggest that inhibition of uPA might prove to be an effective antitumor therapy (139,140). Recently, Bartz et al. (141) reported on the use of a synthetic uPA inhibitor, WX-UK1, in a double-blind, randomized, three-way crossover phase I study. They administered intravenously increasing doses of WX-UK1 in the range of 0.01–0.3 mg/kg to healthy male volunteers. The pharmacokinetics of WX-UK1 was linear, and the systemic safety profile was highly acceptable. Only minor disturbances were seen in coagulation parameters, and these were not thought to be clinically relevant. On the basis of the safety data in healthy volunteers, a phase I/II trial has been launched in patients with gastric, pancreatic, ovarian, and head and neck cancers.

Antiangiogenic Therapies Targeting VEGF

VEGF acts mainly through VEGF receptor (VEGFR)-1, VEGFR-2, and neuropilin receptors expressed on vascular endothelium to increase microvascular permeability, induce endothelial cell proliferation and migration, and promote endothelial cell survival and angiogenesis (142). Its central role in tumor angiogenesis makes VEGF and its receptors attractive targets for cancer therapy. Several different approaches have been used to block VEGF, including neutralizing anti-VEGF monoclonal antibodies, monoclonal antibodies that block VEGF receptors, and small-molecule tyrosine kinase inhibitors that block VEGF receptor activation and downstream signaling (142).

The recombinant humanized anti-VEGF antibody (rhuMab-VEGF) bevacizumab (Avastin; Genentech, South San Francisco, CA) selectively blocks VEGFR-2 and inhibits the growth of colon cancer liver metastases in vitro and in vivo (143,144). Phase I studies have established the safety of bevacizumab both alone and in combination with chemotherapy (145,146), and phase II trials have demonstrated efficacy in colon, breast, and non–small-cell lung cancers (147–149). Recently, the results of a randomized double-blind, placebo-controlled trial of bevacizumab in patients with metastatic RCC were reported (150). RCC is a logical disease for treatment with bevacizumab because the majority of RCCs secrete high levels of VEGF as a consequence of mutations in the von Hippel–Lindau tumor suppressor gene. Bevacizumab resulted in statistically significant prolongation of time to progression with minimal toxicity, although tumor regression was rarely seen (150). Bevacizumab has also been studied in lung and colorectal cancers. In a randomized phase II trial in patients with non–small-cell lung cancer, patients were treated with carboplatin and paclitaxel with
or without bevacizumab at two different doses. In patients with non–squamous cell histology, patients treated with the highest dose of bevacizumab had a longer time to progression than patients treated with chemotherapy alone (149).

Recently, the results of a randomized phase III trial that compared chemotherapy alone with bevacizumab in combination with chemotherapy as first-line therapy for patients with metastatic colorectal cancer have been reported (151). The addition of bevacizumab statistically significantly prolonged both progression-free survival (10.6 versus 6.2 months) and overall survival (20.3 versus 15.6 months). No increase in thrombosis was observed, but gastrointestinal perforation, although rare, was increased.

SU5416 (Semaxanib; Sugen, South San Francisco, CA) is a small-molecule inhibitor of VEGFR-2, Flt-1, and c-kit. In animal models, SU5416 blocks VEGFR signaling, leading to antitumor activity in multiple tumor types (152). On the basis of data from several phase I trials, a dose of 145 mg/m² administered intravenously twice weekly was chosen to proceed to phase II and III trials both as a single agent and in combination with conventional cytotoxic chemotherapy (153–157). In a phase II trial, nine of 13 treated patients with RCC who were evaluable for response had stable disease when SU5416 was administered in combination with low-dose interferon (158). Toxicity included headaches, fatigue, vomiting, and thromboembolic complications. However, it should be noted that Sugen recently closed a clinical trial involving advanced-stage colorectal cancer patients.

SU6668 (Sugen) is a tyrosine kinase inhibitor that targets several growth factor receptors, including VEGFR-2, FGFR-1, platelet-derived growth factor receptor (PDGFR), and c-kit. SU6668 has antitumor activity against a broad range of tumors and appears to affect tumor growth by multiple mechanisms, including the induction of apoptosis in vascular endothelial cells and in tumor cells (159). Phase I trials in humans have begun using a thrice daily oral schedule (160). Initial results suggest that toxicity associated with SU6668 may be greater than with more selectively targeted tyrosine kinase inhibitors such as PTK787/ZK222584.

PTK787/ZK222584 (Novartis Pharmaceuticals, East Hanover, NJ), an orally active and selective inhibitor of VEGF-mediated KDR/Flk-1 receptor tyrosine kinases, has antitumor and antangiogenic activity in animal models (161,162). In two separate phase I trials evaluating safety, pharmacokinetics, and antitumor activity using dynamic contrast-enhanced magnetic resonance imaging as a surrogate marker for efficacy, Thomas et al. (163) treated 20 patients with liver metastases from colon cancer with PTK787/ZK222584 at dose levels ranging from 50 to 1500 mg/day. Of 16 evaluable patients, eight had stable disease, and dynamic contrast-enhanced magnetic resonance imaging proved useful in predicting response. Drevs et al. (164) reported similar results from a phase I trial of 15 patients with liver metastases from a variety of tumors, including colorectal tumors, in which seven patients achieved stable disease and none of the patients experienced dose-limiting toxicity.

**Hemostatic Complications of Antiangiogenic Therapy**

Because activation of the coagulation system occurs during tumor angiogenesis and VEGF has an indirect role in promoting coagulation, it would be reasonable to predict that antiangiogenic therapies targeting VEGF and its receptors might alleviate the prothrombotic tendency associated with malignancy. The reported increase in thromboembolic events, which led to the abandonment of the trial, in patients with advanced colorectal cancer who received SU5416 in combination with cisplatin and gemcitabine was therefore somewhat unexpected (157). Patients were treated with cisplatin on day 1, gemcitabine on days 1 and 8 repeated every 3 weeks, and SU5416 (85 and 145 mg/m²) intravenously twice weekly. Nine thromboembolic events (three transient ischemic attacks, two cerebrovascular accidents, and four deep venous thromboses) occurred in eight of 19 treated patients, despite attempts to restrict entry onto the trial to patients with low thromboembolic risk. The authors postulated that SU5416 might have prothrombotic effects on endothelial cells and that, because platelets, monocytes, and endothelial cells become activated during treatment with cisplatin (165), a further shift toward a prothrombotic state occurred that resulted in an increased incidence of thromboembolic events. However, other studies (153–157) with SU5416 alone or in combination with other chemotherapy agents, including fluorouracil, irinotecan, carboplatin, and paclitaxel, have not shown any such increase in thrombotic events.

Thrombotic complications have not been restricted to antiangiogenic therapies targeting VEGF. Langmuir et al. (166) observed five deep venous thromboses in 28 patients during prolonged (i.e., >1 year) treatment with bevacizumab. Thromboembolic complications have also been observed in patients treated with thalidomide (167)—a drug with antiangiogenic activity that is increasingly being used in the treatment of refractory multiple myeloma, although it is currently being tested in phase III clinical trials for many other tumor types. Thromboembolic events were observed in seven of 23 patients treated with thalidomide for myeloma over a total of 141.5 patient–treatment-months (167). Of these seven events, five were venous thromboemboli and two were arterial thromboemboli. None of these seven patients had a predisposing pathologic condition for thrombotic events.

Hemorrhagic complications have also been reported in patients undergoing antiangiogenic therapy. In the randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel to carboplatin plus paclitaxel alone in patients with stage IIIb/IV non–small-cell lung cancer, four cases of pulmonary hemorrhage were seen in patients treated with the combined therapy (149). The mechanism by which hemorrhage occurs is not known but it may be related to the disruption of the tumor endothelium by the VEGF targeted therapy. de Vos et al. (138) observed one case of hemorrhage in a patient with an unknown cerebellar metastasis and one episode of transient ischemic attack in a patient with advanced cancer treated with ABT-510. The occurrence of life-threatening bleeding complications suggests that the prophylactic use of anticoagulants for the prevention of thromboembolic events should be approached with caution.

The venous thromboembolisms and tumor hemorrhages observed in patients receiving antiangiogenic therapies, particularly those targeted against VEGF, may partly result from an inhibition of the procoagulant and proangiogenic activities of VEGF. Thus, although inhibition of the indirect procoagulant activities of VEGF will increase the likelihood of bleeding, failure of VEGF to promote endothelial cell proliferation and
migration to reinstate the vessel wall during wound repair will result in an increased thrombolic tendency. Consequently, the overall outcome will be determined by the local concentrations of positive and negative regulators of hemostasis and angiogenesis superimposed on the increased thrombotic risk associated with the underlying malignancy and other therapeutic regimens. Analysis of adverse events from ongoing clinical trials should help to define this relationship further. However, it is important to note that patients with previous venous thromboembolism are often excluded from these trials.

CONCLUDING REMARKS

When vessel injury results in exposure of the thrombogenic subendothelial matrix, angiogenic and coagulation cascades are rapidly mobilized to prevent excessive bleeding and reinstate the vessel wall. This process leads to the production of both potent stimulators of angiogenesis and natural inhibitors of angiogenesis such as angiotatin. This knowledge has allowed researchers to study the coagulation pathway for other potential inhibitors of angiogenesis. It is likely that further investigation will reveal other naturally occurring or laboratory-generated fragments of naturally occurring proteins involved in the coagulation pathway that will also have angiogenic or antivascular activity.

Despite the intense research activity into the development of new antiangiogenic agents and the efficacy of these in preclinical models, few reports have demonstrated convincing efficacy in humans. Indeed, a number of promising agents have been withdrawn from early-phase clinical trials because of a lack of efficacy or an unacceptable toxicity. One possible explanation for the lack of efficacy may be that, like all new anticancer therapies, antiangiogenic agents are initially tested in the advanced cancer setting. Although this testing may be appropriate for cytotoxic chemotherapeutic agents, it may be less relevant for agents likely to produce tumor stasis or a reduction in growth rate, such as angiogenesis inhibitors. In this situation, traditional markers of tumor response to treatment such as a measurable reduction in tumor size may potentially lead to false-negative results in clinical trials. Therefore, the development of new surrogate markers of response may be necessary to accurately test antiangiogenic agents in clinical trials. Such markers may include the evaluation of circulating endothelial cells as a marker of angiogenic activity or positron emission tomography scanning, which reflects the metabolic activity of tumors. In this way, therapeutic responses such as a reduction in tumor growth or stasis, could be more accurately monitored.

REFERENCES


Gentilini G, Kirschbaum NE, Augustine JA, Asther RH, Visentin GP. Inhibition of human umbilical vein endothelial cell proliferation by the CXC chemokine, platelet factor 4 (PF4), is associated with impaired downregulation of p21(Cip1/WAF1). Blood 1999;93:25–33.


growth factor receptor (Flk1/KDR) that inhibits tyrosine kinase catal-


NOTES

Dr. Lewis holds stock in and is currently a director of the University of Sheffield spin-out company Bioacta Ltd. She conducts research sponsored by this company.


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