

Thrombin induces tumor growth, metastasis, and angiogenesis: Evidence for a thrombin-regulated dormant tumor phenotype

Mary Lynn Nierodzik¹ and Simon Karpatkin^{1,*}

¹New York University School of Medicine, Department of Medicine/Hematology, 550 First Avenue, New York, New York 10016

*Correspondence: simon.karpatkin@med.nyu.edu

Summary

The association of idiopathic venous thrombosis with occult cancer is generally recognized. However, it has not been fully appreciated that thrombin generated during thrombosis can augment the malignant phenotype. Thrombin activates tumor cell adhesion to platelets, endothelial cells, and subendothelial matrix proteins; enhances tumor cell growth; increases tumor cell seeding and spontaneous metastasis; and stimulates tumor cell angiogenesis. These mechanisms are reviewed. Evidence is also presented to support the hypothesis that thrombin serves to preserve dormant tumor cells in individuals, preventing host eradication. It is proposed that tumor malignancy may be regulated by a procoagulant/anticoagulant axis.

Introduction

The association of venous thrombosis and cancer has been recognized for 140 years, and its first description is attributed to Armand Trousseau (Prins and Otten, 2004). The association of thrombosis and cancer has a prevalence rate of 10%–20% (Prins and Otten, 2004). Of particular interest is the association of occult cancer with venous thromboembolism (VTE) in individuals aged 40 years and older of 4%–10% (Prins and Otten, 2004). Most tumor cells have constitutively active tissue factor on their surface capable of generating thrombin in plasma by combining with factor VIIa to activate factor IX to IXa and X to Xa on the activated platelet surface, resulting in the conversion of prothrombin to thrombin. Thus, malignancy initiates a “vicious cycle” in which greater tumor burden supplies greater thrombin and platelet-tumor interaction.

The purpose of this review is to provide evidence that (1) thrombin (thrombosis) contributes to a more malignant phenotype by activating platelet-tumor aggregation, tumor adhesion to subendothelial matrix, tumor growth and metastasis, and tumor-associated angiogenesis; and (2) thrombin serves to preserve and/or activate dormant tumor cells residing in the host.

Role of platelets in experimental pulmonary metastasis

The requirement of platelets for experimental pulmonary metastasis (induced by tail vein injection of tumor cells) and the ability of tumor cells to aggregate platelets was first recognized by Gasic and coworkers (Gasic et al., 1968). Numerous studies have demonstrated a role for platelets in the hematogenous dissemination of animal tumors. Many tumor cells require platelets for the development of metastasis (Camerer et al., 2004; Gasic et al., 1973; Pearlstein et al., 1980); ultrastructural studies have demonstrated arrested tumor emboli surrounded by platelets (Jones et al., 1971); several tumor cell lines induce thrombocytopenia in vivo (Gasic et al., 1973) and aggregate platelets in vitro (Gasic et al., 1973; Pearlstein et al., 1980); a correlation exists between the ability of some tumor cells to aggregate platelets in vitro and their requirement for experimental pulmonary metastasis in vivo (Gasic et al., 1973; Pearlstein et al., 1980).

The rationale for the platelet requirement in tumor metastasis can be explained by thrombin-activated platelets or tumor cells interacting with each other to form platelet-tumor emboli, and in so doing prolonging tumor survival in the circulation (Jones et al.,

1971). For example, it has been shown that under basal conditions >98% of labeled tumor cells injected intravenously into mice disappear within 24 hr (Fidler, 1970). Sequestration of tumor cells by platelets protects tumor cells from immunologic host surveillance by impeding natural killer cell-mediated elimination of tumor cells (Nieswandt et al., 1999). Platelets also secrete tumor cell growth and angiogenesis factors, such as platelet-derived growth factor (PDGF) (Kepner and Lipton, 1981), vascular endothelial growth factor (VEGF) (Mohle et al., 1997), and Angiopoietin-1 (ANG-1) (Li et al., 2001). Platelet-derived lysophosphatidic acid (LPA) promotes tumor cell proliferation by binding the LPA₁ receptor on breast (MDA-BO2) and ovarian (CHO) cancer cells. LPA also contributes to bone metastasis by stimulating the release of the bone osteoclast resorption stimulators interleukin IL-8 and IL-6 (Boucharaba et al., 2004).

Platelet-tumor aggregation

At least two separate mechanisms of tumor-induced platelet aggregation have been identified (Lerner et al., 1983): (1) requirement for serum complement activation, a stable plasma cofactor other than fibrinogen, divalent cation, and the sialo-lipo-protein vesicular component of the tumor membrane (seen in murine SV40 transformed 3T3 fibroblasts and possibly a rat renal sarcoma, PW20); (2) requirement for the generation of thrombin and a phospholipid component of the tumor plasma membrane (seen in the human colon carcinomas LoVo and HCT8 and an anaplastic murine cell line, HUT-20).

Inhibition of experimental tumor metastasis with anti-platelet-aggregating agents has been controversial because the specific anti-platelet-aggregating agents, aspirin and ticlopidine, as well as prostaglandin I₂, were unsuccessful (Karpatkin et al., 1984, 1988). These disappointing results prompted a reevaluation of Gasic's original observation, that platelets were necessary for metastasis. It was confirmed employing three different tumor cell lines (CT26, Lewis lung, B16 amelanotic melanoma). The new experiments demonstrated that the experimental platelet requirement was early (within the first 6 hr after injection of anti-platelet Ab) and that human platelets could reconstitute metastasis in mice initially protected by the induction of thrombocytopenia (Pearlstein et al., 1984). Focus was therefore directed toward adhesion, which is not inhibited by these agents.

Platelet-tumor adhesion

Early studies designed to measure the adhesion of tumor cells to platelets under static conditions revealed the requirement of platelet glycoprotein (GP) IIb/IIIa (integrin $\alpha_{IIb}\beta_3$), fibronectin, VWF, and the RGDS domain of the adhesive proteins for adhesion. Experimental murine tumor metastasis with CT26, B16a, and T241 Lewis bladder cancer is blocked 45%–65% in vivo with an anti-VWF antibody (without inducing thrombocytopenia), and a monoclonal antibody against platelet GPIIb-IIIa blocks the platelet reconstitution effect in mice protected from pulmonary metastasis with thrombocytopenia (Karpatkin et al., 1988). Soluble fibrin monomer also augments platelet-tumor cell adherence in vitro and in vivo and also requires platelet GPIIb-IIIa, as well as tumor ICAM-1 (Biggerstaff et al., 1999).

A recent study employing human colon carcinomas LS174HT and COLO205 has extended these observations for adhesion of tumor cells to platelets under dynamic flow conditions. A two phase sequential process for tumor adhesion to platelets under flow was demonstrated: platelet P-selectin mediates tumor cell tethering and rolling followed by stable adhesion initiated by GPIIb-IIIa and VWF, via an RGD-dependent mechanism (McCarty et al., 2000). Other recent studies have demonstrated the presence of the P-selectin receptors PSGL-1 (P-selectin glycoprotein ligand-1) and CD24 (GPI-linked surface mucin) in KS breast cancer cells (Aigner et al., 1998), the presence of distinct ligands for selectins on colon carcinoma mucin-type glycoproteins, and attenuation of tumor growth and metastasis in P-selectin-deficient mice in association with absence of in vivo platelet-tumor aggregates (Kim et al., 1998).

The role of a required "GPIIb-IIIa-like" integrin on either the platelet, tumor cell, or other structure is supported by additional data. In experimental pulmonary metastases, the pentapeptide GRGDS blocks B16F10 melanoma metastases by 97% without impairing cellular tumorigenicity. This is due to inhibition of retention of tumor cells in the vasculature. Five times more cells are retained in control mice at 7 hr. This difference increases with time (Humphries et al., 1986). Similar results were noted in a second report in which platelets appeared not to be responsible, i.e., thrombocytopenia did not inhibit the RGDS effect (Humphries et al., 1988), suggesting that tumor cells may bind via an RGDS mechanism to structures other than platelets. The disintegrin Albolabrin, an RGD-containing peptide, inhibits attachment of B16F10 mouse melanoma to fibronectin or laminin when immobilized on plastic in vitro and inhibits experimental pulmonary metastasis by 90% in vivo (Soszka et al., 1991).

It is likely that other adhesive ligands such as laminin (Terranova et al., 1984), vitronectin (Cheresh et al., 1989), type IV collagen (Kramer and Marks, 1989), and thrombospondin (Roberts et al., 1987), as well as other integrin receptors (Soszka et al., 1991), $\alpha_3\beta_1$ (Klepfish et al., 1993), $\alpha_5\beta_1$ (Klepfish et al., 1993), and $\alpha_v\beta_3$ (Cheresh et al., 1989) are also involved in tumor adhesion (Roberts et al., 1987; Tuszynski et al., 1987), platelet-tumor interaction (Roberts et al., 1987; Tuszynski et al., 1987), and metastases (Iwamoto et al., 1987; Tuszynski et al., 1987).

Thrombin induces platelet-tumor adhesion and experimental pulmonary metastasis

Thrombin both activates and enhances exposure of GPIIb-IIIa on the platelet membrane surface, as well as tumor surface (see below). Thrombin also induces the release of platelet fibronectin and VWF onto the platelet surface, suggesting bridging between

platelets and tumor cells via fibronectin and VWF. Thrombin activates tumor-platelet adhesion in vitro and metastasis in vivo. Thrombin treatment of platelets enhances tumor adhesion 2- to 4-fold in six tumor cell lines (HM54 hamster melanoma; human HCT8 colon cancer and SK-Mel-28 melanoma; and murine B16a melanoma, KLN205 squamous cell, and CT26 colon cancer) (Nierodzik et al., 1991).

Importantly, in vivo experiments with thrombin substantiate these in vitro observations. When 0.2–0.5 units of thrombin are injected together with tumor cells intravenously (a dose titrated not to reduce the platelet count), pulmonary metastases increase 4- to 413-fold with two different syngeneic tumor cell lines (CT26 and B16a) (Nierodzik et al., 1991).

Thrombin-activated tumor cells enhance adhesion to naive platelets and endothelial cells

A direct effect of thrombin on tumor cells was next described in which a 3-fold increased adhesion of thrombin-treated tumor cells to platelets was noted. When thrombin-treated platelets and thrombin-treated tumor cells are added together, no additive effect is obtained (Nierodzik et al., 1991), suggesting that thrombin-treated tumor cells and thrombin-treated platelets are operating through the same mechanism. Five of seven tumor cell lines from three different species are activated by thrombin (B16a, HCT-8, Lewis lung, HM29, SK-Mel-28) to adhere to platelets. The thrombin effect is maximum after 1 hr of incubation and is not inhibited by the protein synthesis inhibitor cycloheximide or the thrombin competitive inhibitor DAPA [dansyl-arginine N-(3-ethyl-1, 5 pentanediy) amide] added after thrombin activation, indicating that thrombin induces a secondary event (Nierodzik et al., 1991). Thrombin-activated tumor cells (SKMel-28 and HM29) also enhance their adhesion to bovine aortic and capillary endothelial cells 2.1- to 2.3-fold under static conditions (Klepfish et al., 1993). Similar observations occur under flow conditions in which thrombin increases the adhesion of human melanoma 397 cells to endothelial cells 2.2-fold and under conditions in which adhesion is blocked by antibodies to GPIIb-IIIa as well as P-selectin (Dardik et al., 1998); ^{51}Cr -labeled tumor cells (HELA or HT29) have greatly increased adhesion to endothelial cells in culture in the presence of both platelets and thrombin compared to platelets or thrombin alone (Helland et al., 1997).

Thrombin also serves to disrupt endothelial cell junctions supported by vascular endothelial cadherin (VE cadherin) and β -catenin (Konstantoulaki et al., 2003). Ab against VE cadherin inhibits neovasculature and tumor growth (Liao et al., 2000).

Of particular interest is the observation that the thrombin-treated melanoma tumor cells studied also have a "GPIIb-IIIa-like" receptor on their surface (inhibited by anti-GPIIb-IIIa and RGDS) (Boukerche et al., 1989; McGregor et al., 1989; Nierodzik et al., 1992). M3Dau melanoma cells, which react with platelets, bind a GPIIb-IIIa-specific monoclonal antibody, synthesize "GPIIb-IIIa-like" glycoproteins, and do not react with Glanzmann thrombasthenia platelets (congenital absence of GPIIb-IIIa) (Boukerche et al., 1989). When preincubated with anti-GPIIb-IIIa antibody, M3Dau tumor cell growth is dramatically inhibited following subcutaneous implantation into nude mice (Boukerche et al., 1989). Sixteen of twenty-one human malignant melanoma frozen biopsies bind to an anti-GPIIb-IIIa antibody, whereas negative results are obtained with 15 benign human melanocyte specimens and 73 of 75 other human carcinomas (McGregor et al., 1989). GPIIb-IIIa has been reported in 17 tumor cell lines of different histologi-

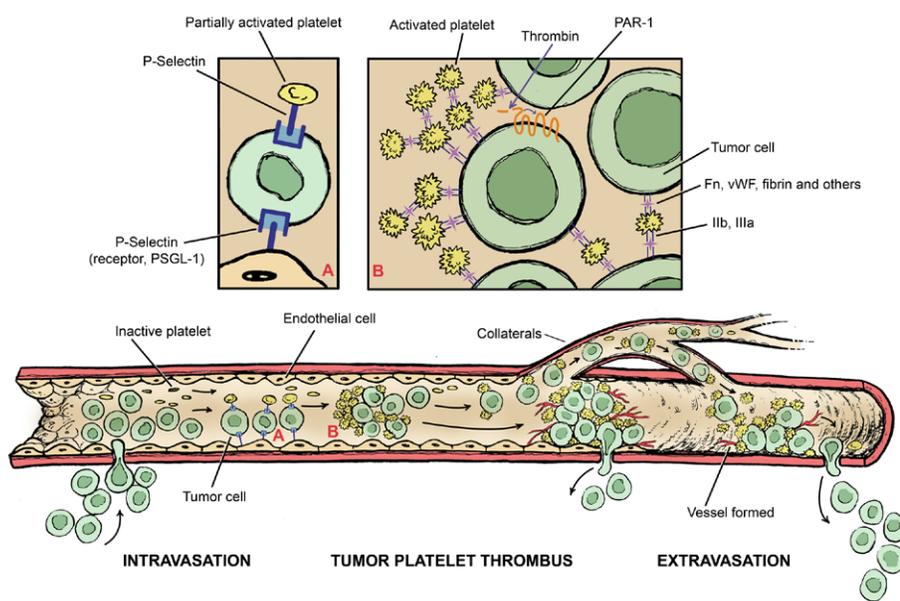


Figure 1. Summary figure: Events occurring following tumor intravasation, tumor-platelet-endothelial cell binding and platelet-tumor thrombus formation, tumor-platelet embolization, angiogenesis, and extravasation

(1) Migration of tumor cells into the vasculature is stimulated by thrombin, which is induced by tissue factor on the surface of most tumor cells. Other factors stimulating migration also play a role. (2) Thrombin and other tumor-secreted agents activate endothelial cells, so that they now have P-selectin on their surface. P-selectin binds weakly to tumor cells containing the P-selectin ligand receptor. Weakly activated platelets also bind tumor cells via P-selectin on their surface. This induces weak tethering of tumor to endothelium and platelets. (3) A tighter combination of platelets and tumor cells develops, which produces thrombin at a more rapid rate, since platelets provide a catalytic surface for thrombin generation from coagulant proteins. This leads to a firm bond between platelets and tumor cells, mediated by platelet integrin IIb-IIIa binding to tumor integrins via VWF, fibronectin, and other RGDS ligands. This also leads to angiogenesis via thrombin-stimulated synthesis and secretion of VEGF and GRO- α from tumor cells—as well as release of PDGF, VEGF, and ANG-1 from platelets, and increased ANG-2 and KDR in endothelial cells.

Activated platelets and tumor cells secrete proangiogenic growth factors, which dominate over antiangiogenic factors. (4) Platelet-tumor aggregates protect tumor cells from NK cells and embolize downstream, leading to ischemic and endothelial cell denudation. Tumor cells and platelets bind more avidly to subendothelial basement membrane and matrix. (5) Tumor emboli lead to tumor extravasation into the parenchyma and neovascularization.

cal origin: skin, blood, lung, liver, kidney, cervix, colon, bladder, breast, and prostate.

Genetic confirmation of the role of circulating platelets, platelet-tumor cell activation, and fibrinogen in experimental pulmonary metastasis has recently been studied in a B16 melanoma model in *NF-E2^{-/-}* knockout mice with virtually no circulating platelets, and in *PAR-4^{-/-}* knockout mice (the major thrombin receptor in mice) with platelets that fail to respond to thrombin. Similar results were noted with fibrinogen *Fib^{-/-}* knockout mice, indicating that fibrinogen is also necessary for metastasis (Camerer et al., 2004). Marked reduction in experimental pulmonary metastasis is seen in all three groups (6%, 14%, and 24% of wild-type, respectively). Of considerable interest are the recent observations obtained with *G α ^{-/-}* platelet knockout mice, a critical G protein required for platelet activation. Experimental and spontaneous pulmonary metastasis obtained with syngeneic Lewis lung as well as B16-BL6 tumor cells are decreased by \sim 100-fold and \sim 60-fold, respectively (Palumbo et al., 2005). However, *G α ^{-/-}* platelets have no effect on subcutaneous tumor growth in vivo.

Thrombin-activated PAR-1 on tumor cells enhances experimental pulmonary metastasis

Important in vivo experiments with thrombin-activated tumor cells (CT26, B16F1, B16F10) revealed experimental pulmonary metastases that were enhanced 10- to 156-fold (Nierodzik et al., 1992). This demonstrated for the first time a cause and effect relationship between in vivo thrombin generation and cancer.

Thrombin activates its G-coupled seven transmembrane protease-activated receptor (PAR-1) by cleaving the receptor's N-terminal end. The tethered end folds back on the second extracellular loop and activates the cell. Similar activation can be obtained with the six N-terminal amino acids of the new tethered end, SFLLRN. PAR-1 is present on seven of seven tumor cells examined by immunoblot and four additional cell lines examined by RT-PCR. Seven of eleven lines respond to the PAR-1 thrombin

receptor activation peptide (TRAP), by a 2- to 3-fold enhanced adhesion to platelets (Nierodzik et al., 1996). Two TRAP-treated murine cell lines, B16F10 and CT26, enhance their experimental pulmonary metastasis 17- to 320-fold, despite having no effect on enhanced adhesion to platelets, suggesting that thrombin stimulates other tumor growth/metastasis-promoting properties, as well as adhesion. Activation of PAR-1 stimulates chemokinesis of melanoma and prostate tumor cells (Shi et al., 2004). Overexpression of PAR-1 in B16 malignant melanoma cells leads to a 5-fold enhancement of experimental pulmonary metastasis (Nierodzik et al., 1998). A precedent for thrombin-induced enhanced oncogenesis is supported by the observations that thrombin can act as a mitogenic agent for mesenchymal tissue: fibroblasts, endothelial cells, and smooth muscle cells (Carney et al., 1984; Chen and Buchanan, 1975; Gospodarowicz et al., 1978). Indeed, PAR-1 has been recognized as a possible potent oncogene in a cDNA expression library screen for genes that induce focus-forming activity, and transformation of NIH 3T3 cells in vitro. This was associated with Rho-mediated signaling pathways, loss of anchorage independence, and serum-dependent growth (Martin et al., 2001).

It is likely that thrombin could be acting as a growth-stimulatory signal through its activation of PAR-1, leading to downstream mitogenic signaling events. It is unlikely that thrombin induces a malignant phenotype in primary cells, but more likely that persistent thrombin signaling acts as an additional tumorigenic event in malignant cells or cells programmed to become malignant, through combinations of various events (Hanahan and Weinberg, 2000). Thrombin also contributes to the malignant phenotype by the induction of angiogenesis following upregulation of vascular growth factors released from the tumor as well as platelets, which stimulate endothelial cell tumor formation (see below).

Thrombin can upregulate genes capable of inducing or contributing to oncogenesis and angiogenesis (see below). For example, in an analysis of two thrombin-treated murine tumor cell lines

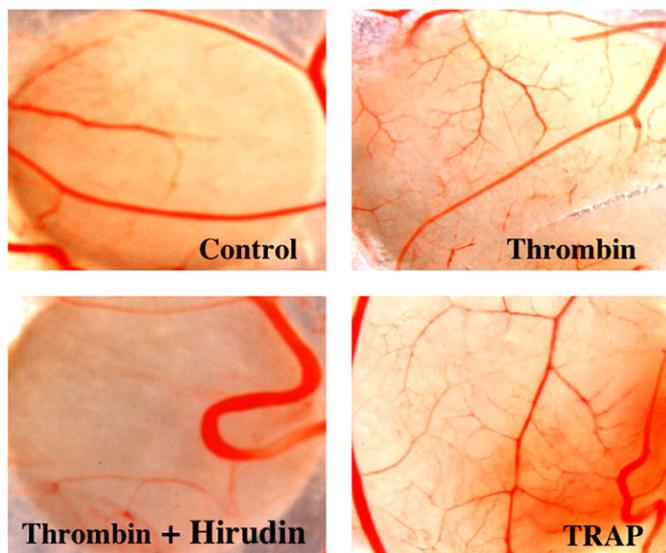


Figure 2. Neoangiogenesis in the chorioallantoic membrane

Ten-day-old chick embryos were prepared with filter disks and treated with either PBS, thrombin, thrombin and hirudin, or PAR-1 receptor against TRAP at 24 and 48 hr. Neoangiogenesis was evaluated at 72 hr. Adapted from Caunt et al. (2003).

(B16F10 and UMCL) we have observed that thrombin upregulates several of these genes by 2- to 168-fold: GRO- α (Caunt et al., 2006), Twist, metalloproteinase 1 and 2, osteopontin, S100 calcium-binding proteins, and tetraspanin CD151 (T. Tang and S.K., unpublished data). These genes are capable of rendering tumor cells more virulent and/or metastatic following transfection. Indeed, Twist has recently been shown to be responsible for mammary tumor metastasis as opposed to growth (Yang et al., 2004). Specific breast gene signatures have been shown to have a propensity for metastasis (Minn et al., 2005). It is suggested that events similar to these could be enhanced by thrombin.

Thrombin may also be contributing to the malignant phenotype by affecting other essential alterations in cell physiology heretofore undescribed, such as self-sufficiency in growth signals, insensitivity to antigrowth signals, resistance to apoptosis, and unlimited replication potential, as well as already documented sustained angiogenesis and tissue invasion and metastasis.

These events are summarized in Figure 1.

Angiogenesis

The requirement of angiogenesis for tumor growth and metastasis is well recognized. Recent studies have revealed that thrombin has a significant stimulatory effect on angiogenesis in that it can induce vascular growth factors, VEGF-A in tumor cells (Huang et al., 2000; Ollivier et al., 2000), or receptor VEGFR2 (Tsopanoglou and Maragoudakis, 1999) and Angiopoietin-2 (ANG-2) in endothelial cells (Huang et al., 2002). Thrombin can also stimulate the release of VEGF-A (Mohle et al., 1997) and ANG-1 from platelets (Li et al., 2001) as well as induce tube formation of endothelial cells in a matrigel membrane system (Haralabopoulos et al., 1997). Direct application of as little as 0.05–0.1 U/ml of thrombin to a chorioallantoic chick membrane induces angiogenesis 2- to 3-fold over a 72 hr period (Figure 2). This is associated with the upregulation of VEGF-A and ANG-2 and is blocked with hirudin (thrombin inhibitor), as well as a soluble decoy, VEGF-A recep-

tor inhibitor (VEGFR2-Fc), and Angiopoietin receptor inhibitor (Tie2-Fc). It is initiated by PAR-1-specific peptide (TRAP) and requires activation of protein kinase C, MAP kinase, and PI3 kinase (Caunt et al., 2003). Recent studies reveal that thrombin upregulates and enhances secretion of the angiogenesis and tumor-promoting chemokine GRO- α in human MCF7 breast and PC3 prostate tumor cells 25- to 64-fold and is also upregulated in human umbilical vein (HUVEC) endothelial cells as well as human brain microendothelial cells. This upregulation is pivotal in thrombin-induced angiogenesis, since thrombin-induced upregulation of angiogenesis, angiogenesis growth factors VEGF, KDR, ANG-2, MMP-1, MMP-2, HUVEC tube formation, and cell growth is inhibited by anti-GRO- α Ab, or siRNA knockdown of tumor as well as HUVEC cell GRO- α . There is recent evidence that the tissue factor VIIa complex not only induces the production of thrombin, but also activates angiogenesis by cleaving (activating) protease-activated receptor 2 (PAR-2) (in synergy with platelet PDGF-BB) (Belting et al., 2004).

Platelets contain both angiogenic (VEGF-A, ANG-1, PDGF, bFGF) and antiangiogenic growth factors (thrombospondin, PF-4, endostatin). However, despite the presence of antiangiogenic factors, the overall effect of platelets or their thrombin-induced releasate on angiogenesis is stimulatory, suggesting that this is the predominant mode (Brill et al., 2004, 2005). In addition, there is evidence that platelet agonist ligands may have different effects on pro- versus antiangiogenesis. This is supported by recent work showing that specific activation of protease-activated receptors 1 and 4 counter-regulates endostatin and VEGF release from platelets when stimulated by their proteolytically induced agonists—not thrombin per se (which stimulates both receptors). The PAR-4 agonist (ATPGKF) suppresses VEGF-A release but enhances endostatin release. The PAR-1 agonist (TFLLR) induces VEGF-A release but inhibits endostatin release (Ma et al., 2005) (Figure 1).

Effect of endogenous thrombin on tumor growth and metastasis: Critique of previous in vitro and in vivo experiments

Animal studies in which tumor cells are treated with exogenous thrombin do not represent the true pathophysiologic situation since (1) they give no information on endogenous thrombin production/concentration at the tumor host interface; and (2) experimental tail vein tumor pulmonary metastasis is a highly artificial situation in which 1×10^5 – 1×10^6 tumor cells are injected as a bolus into the tail vein of a mouse, and primary pulmonary entrapment and tumor nodule appearance are monitored. Spontaneous metastasis in the host represents the release of considerably fewer cells into the circulation, with an as yet unknown fraction of these cells actually implanting and growing.

This criticism prompted a study designed to circumvent these objections (Hu et al., 2004). This was accomplished by utilizing a spontaneously metastasizing murine breast tumor, 4T1. Pretreating tumor-implanted mice with hirudin (a highly potent thrombin inhibitor, Ki 0.05 pM) inhibited implanted tumor growth in mice 4- to 11-fold (again highly artificial). However, it also inhibited seeding into the blood and spontaneous metastasis, and it increased mouse survival (Hu et al., 2004). The effect on seeding into the blood was dramatic, varying from complete to 15- to 32-fold protection with two different tumor cell lines. Viable seeding into the lung was also measured by culturing surviving tumor cells in the lung at 7 days. This was similarly protected 17- to 395-fold. The protective effect on pulmonary metastasis was >2.2-fold. All

Table 1. Cancer survival in cancer patients treated with anticoagulants

Study	Protocol	Results
Zacharski et al., 1984; confirmed by Lebeau et al., 1994 (277 patients with heparin); Altinbas et al., 2004 (84 patients with LMWH)	randomized, 50 patients with SCLC, coumadin for 26 weeks	survival, 50 versus 23 weeks, $p = 0.018$, includes 12 patients with disseminated disease no effect in 381 other non-SCLC cancer patients
FAMOUS (Fragmin Advanced Malignancy Outcome Study) (Kakkar et al., 2004)	randomized, double-blind, 385 patients with cancer, LMWH versus placebo, 1 year	survival at 1, 2, and 3 years, $p = 0.19$ subgroup of 102 alive at 17 months: 44 versus 24 months median survival, $p = 0.03$ 2 years: 78% versus 55%, $p = 0.03$ 3 years: 60% versus 35%, $p = 0.03$
MALT (Malignancy and LMWH Treatment) (Klerk et al., 2005)	randomized, double-blind, 302 patients with cancer, LMWH versus placebo, 6 weeks	median survival, 8 versus 6.6 months, hazard ratio = 0.75 subgroup of 164 with ≥ 6 months life expectancy: 15.4 versus 9.4 months, $p = 0.01$ 12 months survival: 39% versus 27%, $p = 0.02$ 24 months survival: 21% versus 11%, $p = 0.02$
CLOT (Comparison of LMWH versus Oral Anticoagulant Treatment) (Lee et al., 2005)	randomized, 602 patients, cancer + VTE, LMWH versus coumadin, 6 months	no difference in 12 months survival subgroup of 150 without metastasis: 80% versus 64% survival, $p = 0.03$ in favor of LMWH

five treated animals were alive at 40 days, whereas all five control animals were dead.

The above observations come closer to defining an endogenous role for thrombin in the induction of a promalignant phenotype but could still be criticized for the requirement of injecting 1×10^5 – 1×10^6 tumor cells into the flank of an animal—considerably higher than the amount of tumor seeded into the blood from a tumor nodule. Studies on the effect of hirudin in mice that develop spontaneous tumors with time should circumvent this problem. Preliminary studies in such a spontaneous mouse prostate tumor model (TRAMP) reveal that hirudin treatment inhibits tumor development 4-fold over a 17 week period of observation.

Extrapolating from these murine experimental data, it is likely that low-grade thrombin generation may be harmful to some patients with malignancies, because it may predispose to enhanced growth and metastatic progression of the lesion. Many tumor cells have constitutively active tissue factor on their surface, which can activate the coagulation system on the catalytic platelet surface with generation of thrombin. Low-grade intravascular coagulation, as diagnosed by increased fibrinogen turnover (Yoda and Abe, 1981), increased plasma levels of fibrinogen/fibrin-related antigen (Yoda and Abe, 1981), and increased plasma levels of fibrinopeptide A have been observed in most patients with solid tumors (Rickles and Edwards, 1983). One study noted elevated fibrinopeptide A levels in 60% of patients at time of disease. Persistent elevation was associated with a poor prognosis (Rickles and Edwards, 1983). Thus a “vicious” autocrine cycle is established. It is of interest that tissue factor expression correlates with hematogenous metastasis in melanoma cells (Fisher et al., 1995; Mueller et al., 1992) and is associated with the leading edge of invasive breast carcinomas (Contrino et al., 1996). In addition, enzymatically active thrombin has been reported to be present on surgically removed tumor specimens, including malignant melanoma, by affinity-ligand histochemical analysis (Zacharski et al., 1995). Thrombin-receptor (PAR-1) overexpression has been reported in human breast metastatic tissue in vivo (Even-Ram et al., 1998).

Clinical studies with anticoagulants

The first convincing randomized study was the pioneering work of Zacharski and coworkers (Zacharski et al., 1984), who treated

441 cancer patients with coumadin for 26 weeks. Clinical improvement was noted in a subset of 50 patients with SCLC (small cell carcinoma of the lung); survival was prolonged from 23 weeks in control patients to 50 weeks in treated patients ($p = 0.018$). This work was confirmed by Lebeau et al. (1994), who used heparin instead of warfarin and by Altinbas et al., who used low-molecular weight heparin (LMWH) (Altinbas et al., 2004; Table 1).

Recently, Kakkar et al. (2004) reported the first large double-blind study of 385 patients with cancer, employing LMWH versus placebo for 1 year. Although no beneficial effect was noted in all patients, a subset of 102 patients alive at 17 months showed a significantly improved survival of 44 versus 24 months ($p = 0.03$), which persisted at 3 years (60% versus 35%), $p = 0.03$. Similar results were noted in a second large double-blind study of 302 patients reported by Klerk et al. (2005), in which patients were treated with 6 weeks of LMWH versus placebo. Again the major effect noted was in a subgroup of 164 patients with a predicted 6 months or greater survival. The overall improvement was 15.4 versus 9.4 months ($p = 0.01$). A greater effect was noted at 2 years (21% versus 11%) ($p = 0.02$). These two latter studies strongly suggested that the initial tumor burden was inversely rate limiting with respect to benefit with LMWH. The choice of anticoagulant—coumadin versus LMWH—was examined in a randomized study of 602 cancer patients with venous thrombosis in which patients were treated for 6 months with LMWH versus coumadin (Lee et al., 2005). Again, although no overall benefit was noted at 12 months, a subgroup of 150 patients without metastasis had a better survival with LMWH compared to coumadin (80% versus 64%; $p = 0.03$). The benefit of LMWH versus coumadin could be explained on the basis of heparin/LMWH having beneficial effects other than that of anticoagulation. Indeed, inhibition of P-selectin binding of tumor cells to platelets/endothelial cells by heparin has been described (Stevenson et al., 2005). It is also of interest that the major effect of LMWH is on Xa, although it does inhibit thrombin as well, but to a lesser extent. In addition, hirudin, a specific inhibitor of thrombin, is similarly effective, and Xa is required for the production of thrombin. However, an independent effect of Xa cannot be ruled out. These recent clinical studies demonstrate for the first time that the numerous murine studies cited in this review may have human relevance.

Table 2. Tumor dormancy

Supporting data	Reference
Fatal melanoma in two kidney transplant recipients, 16 years after "surgical cure" in donor; 13 other similar reports 6 months–8 years after donor cure	MacKie et al., 2003
Thyroid cancer in 36% of 101 consecutive autopsies; 6%–28% in six other series of 1558 autopsies	Harach et al., 1985
Breast cancer in 20% of 101 consecutive autopsies; 37% at ages 40–54	Nielsen et al., 1987
Prostate cancer in 5%–30% of autopsies, which increased with age	Konety et al., 2005
CLL clones in "normal" elderly men and close relatives	Rawstron et al., 2002
Elderly individuals with MGUS have a 1%/year conversion rate to multiple myeloma or lymphoma	Kyle, 1997

Effect of thrombin on tumor dormancy

Of particular interest are the observations of Shulman and Lindmarker (2000), who followed 854 patients who had been treated for 6 months versus 6 weeks with coumadin for deep vein thrombosis for the ensuing 6 years. Those patients treated for 6 months developed significantly less cancer during the 6 year observation period than those exposed to anticoagulant for 6 weeks with an odds ratio of 1.6 (95% CI 1.1–2.4; $p = 0.02$), and when confined to 40 patients with urogenital tumors, the odds ratio increased to 2.5 (95% CI 1.3–5.0). It is intriguing to speculate that these data, as well as recent animal observations, suggest that thrombin may be contributing to preserving tumor dormancy, with later growth stimulated by critical concentration of thrombin. Other factors, such as immune surveillance and angiogenesis, are also likely to play a role. One can further speculate that tumor cells lie dormant and/or grow slowly in systemic organs of many cancer-prone individuals and that this dormancy is regulated by thrombin as well as endogenous anticoagulants (anti-thrombin III, protein C, thrombomodulin, α_2 macroglobulin, tissue factor pathway inhibitor [TFPI]) and other factors. Indeed, it has been estimated that it takes 2–8 years for cancer cells to be detectable after transformation to malignant cells, considering the doubling time and volume required for detection.

What is the evidence for tumor dormancy (Table 2)? It is well recognized that many if not all subjects harbor tumor cells at various states of dedifferentiation (Table 1). Fatal melanoma has been described in two kidney transplant recipients 16 years after surgical cure in the donor (MacKie et al., 2003). Autopsies of individuals dying from accidents or nonmalignant disease often reveal microscopic or in situ cancer. Thyroid cancer is detected in 38% of 101 consecutive autopsies (Harach et al., 1985). Breast cancer is detected in 20% of 110 consecutive autopsies (Nielsen et al., 1987). Prostate cancer is detectable in 15%–30% of autopsies, with increasing incidence with age (Konety et al., 2005). Chronic lymphatic leukemia (CLL) clones are found in "normal" elderly men as well as close-degree relatives of patients with CLL (Rawstron et al., 2002). Elderly individuals with monoclonal nonspecific gammopathy of undetermined significance (MGUS) have a 1%/year conversion rate to develop multiple myeloma or lymphoma (Kyle, 1997).

It is particularly intriguing to comment on the Second Northwick Park Heart Study (Miller et al., 2004), which prospectively studied hypercoagulability once a year for 4 years with follow-up of 11 years in a cohort of 3052 middle-aged men, clinically free of malignancy, for the development of myocardial infarction.

Hypercoagulability was defined as two yearly consecutive positive measurements of both increased prothrombin activation fragments 1+2 as well as fibrinopeptide A, exceeding the upper quartiles of the population. This was found in 111 men. Although no greater incidence of myocardial infarction was noted, total mortality from cancer was higher in those with persistent activation than the other group, 11.3% versus 5.1%, respectively, with a relative risk of 2.2 ($p = 0.015$). This was due largely to higher mortality from gastrointestinal cancer, 6.3% versus 1.9%, with a relative risk of 3.26 ($p < 0.001$). This was also associated with an earlier diagnosis of gastrointestinal cancer as well as a more rapid course to death. This prospective study is remarkably powerful in supporting the role of thrombin as a contributing factor to cancer development and/or progression.

The experimental animal data, as well as the intriguing observation of Shulman and Lindmarker (2000) and the recent report of Miller et al. (2004), provide compelling evidence for the use of an appropriate anti-thrombin agent for the adjuvant treatment of patients with newly diagnosed cancer. The particular tumor or dosing regimen and duration of therapy remain to be determined. But it is likely that this approach would be efficacious in patients immediately after the diagnosis of cancer, rather than after the tumor burden becomes too great to be responsive. A second concern is the deleterious effect of anti-thrombotic agents on bleeding, although this was not a major complication in the double-blind FAMOUS and MALT trials (e.g., 4% versus 2% in control groups). Since the major anti-thrombin effect appears to be on its reaction with PAR-1, agents that inhibit this reaction may be safer than those inhibiting blood coagulation.

References

- Aigner, S., Ramos, C., Hafezi-Moghadam, A., Lawrence, M., Friederichs, J., Altevogt, P., and Ley, K. (1998). CD24 mediates rolling of breast carcinoma cells on P-selectin. *FASEB J.* 12, 1241–1251.
- Altinbas, M., Coskun, H., Er, O., Ozkan, M., Eser, B., Unal, A., Cetin, M., and Soyuer, S. (2004). A randomized clinical trial of combination chemotherapy with and without low-molecular-weight heparin in small cell lung cancer. *J. Thromb. Haemost.* 2, 1266–1271.
- Belting, M., Dorrell, M.I., Sandgren, S., Aguilar, E., Ahamed, J., Dorfleutner, A., Carmeliet, P., Mueller, B.M., Friedlander, M., and Ruf, W. (2004). Regulation of angiogenesis by tissue factor cytoplasmic domain signaling. *Nat. Med.* 10, 502–509.
- Biggerstaff, J.P., Seth, N., Amirhosravi, A., Amaya, M., Fogarty, S., Meyer, T.V., Siddiqui, F., and Francis, J.L. (1999). Soluble fibrin augments platelet/tumor cell adherence in vitro and in vivo, and enhances experimental metastasis. *Clin. Exp. Metastasis* 17, 723–730.
- Boucharaba, A., Serre, C., Gres, S., Saulnier-Blache, J., Bordet, J., Guglielmi, J., Clezardin, P., and Peyruchaud, O. (2004). Platelet-derived lysophosphatidic acid supports the progression of osteolytic bone metastases in breast cancer. *J. Clin. Invest.* 114, 1714–1725.
- Boukerche, H., Berthier-Vergnes, O., Tabone, E., Dore, J., Leung, L., and McGregor, J. (1989). Platelet-melanoma cell interaction is mediated by the glycoprotein IIb-IIIa complex. *Blood* 74, 658–663.
- Brill, A., Elinav, H., and Varon, D. (2004). Differential role of platelet granular mediators in angiogenesis. *Cardiovasc. Res.* 63, 226–235.
- Brill, A., Dashevsky, O., Rivo, J., Gozal, Y., and Varon, D. (2005). Platelet-derived microparticles induce angiogenesis and stimulate post-ischemic revascularization. *Cardiovasc. Res.* 67, 30–38.
- Camerer, E., Qazi, A., Duong, D., Cornelissen, I., Rommel, A., and Coughlin, S. (2004). Platelets, protease-activated receptors, and fibrinogen in hematogenous metastasis. *Blood* 104, 397–401.

- Carney, D., Stiernberg, J., and Fenton, J. (1984). Initiation of proliferative events by human α -thrombin requires both receptor binding and enzymatic activity. *J. Cell. Biochem.* *26*, 181–195.
- Caunt, M., Huang, Y., Brooks, P., and Karparkin, S. (2003). Thrombin induces neoangiogenesis in the chick chorioallantoic membrane. *J. Thromb. Haemost.* *1*, 2097–2102.
- Caunt, M., Hu, L., Tang, T., Brooks, P., Ibrahim, S., and Karparkin, S. (2006). Growth-regulated oncogene is pivotal in thrombin-induced angiogenesis. *Cancer Res.* *66*, 4125–4132.
- Chen, L., and Buchanan, J. (1975). Mitogenic activity of blood components. I. Thrombin and prothrombin. *Proc. Natl. Acad. Sci. USA* *72*, 131–135.
- Cheresh, D., Smith, W., and Cooper, H. (1989). A novel vitronectin receptor integrin (avb α) is responsible for distinct adhesive properties of carcinoma cells. *Cell* *57*, 59–69.
- Contrino, J., Haiar, G., Kreutzer, D., and Rickles, F. (1996). In situ detection of tissue factor in vascular endothelial cells: Correlation with the malignant phenotype of human breast disease. *Nat. Med.* *2*, 209–215.
- Dardik, R., Savion, N., Kaufmann, Y., and Varon, D. (1998). Thrombin promotes platelet-mediated melanoma cell adhesion to endothelial cells under flow conditions: Role of platelet glycoproteins P-selectin and GPIIb-IIIa. *Br. J. Cancer* *77*, 2069–2075.
- Even-Ram, S., Uziely, B., Cohen, P., Grisaru-Granovsky, S., Maoz, M., Ginzburg, Y., Reich, R., Vlodaysky, I., and Bar-Shavit, R. (1998). Thrombin receptor overexpression in malignant and physiological invasion processes. *Nat. Med.* *4*, 909–914.
- Fidler, I. (1970). Metastases: Quantitative analysis of distribution and fate of tumor emboli labeled with 125 I-5-iodo-2'-deoxyuridine. *J. Natl. Cancer Inst.* *45*, 773–782.
- Fisher, E., Ruf, W., and Mueller, B. (1995). Tissue factor-initiated thrombin generation activates the signaling thrombin receptor on malignant melanoma cells. *Cancer Res.* *55*, 1629–1632.
- Gasic, G., Gasic, T., and Stewart, C. (1968). Antimetastatic effects associated with platelet reduction. *Proc. Natl. Acad. Sci. USA* *61*, 46–52.
- Gasic, G., Gasic, T., Galanti, N., Johnson, T., and Murphy, S. (1973). Platelet-tumor-cell interactions in mice. The role of platelets in the spread of malignant disease. *Int. J. Cancer* *11*, 704–718.
- Gospodarowicz, D., Brown, K., Birdwell, C., and Zetter, B. (1978). Control of proliferation of human vascular endothelial cells. Characterization of the response of human umbilical vein endothelial cells to fibroblast growth factor, epidermal growth factor, and thrombin. *J. Cell Biol.* *77*, 774–788.
- Hanahan, D., and Weinberg, R.A. (2000). The hallmarks of cancer. *Cell* *100*, 57–70.
- Harach, H., Franssila, K., and Wasenius, V. (1985). Occult papillary carcinoma of the thyroid: A "normal" finding in Finland. A systematic autopsy study. *Cancer* *56*, 531–538.
- Haralabopoulos, G., Grant, D., Kleinman, H., and Maragoudakis, M. (1997). Thrombin promotes endothelial cell alignment in Matrigel in vitro and angiogenesis in vivo. *Am. J. Physiol.* *273*, C239–C245.
- Helland, I., Klemensten, B., and Jorgensen, L. (1997). Addition of both platelets and thrombin in combination accelerates tumor cells to adhere to endothelial cells in vitro. *In Vitro Cell. Dev. Biol.* *33*, 182–186.
- Hu, L., Lee, M., Campbell, W., Perez-Soler, R., and Karparkin, S. (2004). Role of endogenous thrombin in tumor implantation, seeding and spontaneous metastasis. *Blood* *104*, 2746–2751.
- Huang, Y.Q., Li, J.-J., Hu, L., and Karparkin, S. (2000). Thrombin induces the synthesis of VEGF and Angiopoietin-2 (Ang-2). *Blood* *96*, 34A.
- Huang, Y., Li, J., Hu, L., Lee, M., and Karparkin, S. (2002). Thrombin induces increased expression and secretion of angiopoietin-2 from human umbilical vein endothelial cells. *Blood* *99*, 1646–1650.
- Humphries, M., Olden, K., and Yamada, K. (1986). A synthetic peptide from fibronectin inhibits experimental metastases of murine melanoma cells. *Science* *232*, 467–470.
- Humphries, M., Olden, K., and Yamada, K. (1988). Investigation of the biological effect of anti-cell adhesive synthetic peptides that inhibit experimental metastasis of B16F10 murine melanoma cells. *J. Clin. Invest.* *81*, 782–790.
- Iwamoto, Y., Robey, F., and Graf, J. (1987). YIGSR, a synthetic laminin pentapeptide, inhibits experimental metastasis formation. *Science* *238*, 1132–1134.
- Jones, J., Wallace, A., and Fraser, E. (1971). Sequence of events in experimental and electron microscopic observations. *J. Natl. Cancer Inst.* *46*, 493–504.
- Kakkar, A.K., Levine, M.N., Kadziola, Z., Lemoine, N.R., Low, V., Patel, H.K., Rustin, G., Thomas, M., Quigley, M., and Williamson, R.C. (2004). Low molecular weight heparin, therapy with dalteparin, and survival in advanced cancer: the fragmin advanced malignancy outcome study (FAMOUS). *J. Clin. Oncol.* *22*, 1944–1948.
- Karparkin, S., Ambrogio, C., and Pearlstein, E. (1984). Lack of effect of in vivo prostacyclin on the development of pulmonary metastases in mice following intravenous injection of CT26 colon carcinoma, Lewis lung carcinoma, or B16 amelanotic melanoma cells. *Cancer Res.* *44*, 3880–3883.
- Karparkin, S., Pearlstein, E., Ambrogio, C., and Collier, B. (1988). Role of adhesive proteins in platelet tumor interaction in vitro and metastasis formation in vivo. *J. Clin. Invest.* *81*, 1012–1019.
- Kepner, N., and Lipton, A. (1981). A mitogenic factor for transformed fibroblasts from human platelets. *Cancer Res.* *41*, 430–432.
- Kim, Y., Borsig, L., Varki, N., and Varki, A. (1998). P-selectin deficiency attenuates tumor growth and metastasis. *Proc. Natl. Acad. Sci. USA* *95*, 9325–9330.
- Klepfish, A., Greco, M., and Karparkin, S. (1993). Thrombin stimulates melanoma tumor-cell binding to endothelial cells and subendothelial matrix. *Int. J. Cancer* *53*, 978–982.
- Klerk, C.P., Smorenburg, S.M., Otten, H.M., Lensing, A.W., Prins, M.H., Piovello, F., Prandoni, P., Bos, M.M., Richel, D.J., van Tienhoven, G., and Buller, H.R. (2005). The effect of low molecular weight heparin on survival in patients with advanced malignancy. *J. Clin. Oncol.* *23*, 2119–2120.
- Konety, B., Bird, V., Deorah, S., and Dahmouh, L. (2005). Comparison of the incidence of latent prostate cancer detected at autopsy before and after the prostate specific antigen era. *J. Urol.* *174*, 1785–1788.
- Konstantoulaki, M., Kouklis, P., and Malik, A. (2003). Protein kinase C modifications of VE-cadherin, p120, and β -catenin contribute to endothelial barrier dysregulation induced by thrombin. *Am. J. Physiol. Lung Cell. Mol. Physiol.* *285*, L434–L442.
- Kramer, R., and Marks, N. (1989). Identification of integrin collagen receptors on human melanoma cells. *J. Biol. Chem.* *264*, 4684–4688.
- Kyle, R. (1997). Monoclonal gammopathy of undetermined significance and solitary myeloma. *Hematol. Oncol. Clin. North Am.* *11*, 71–87.
- Lebeau, B., Chastang, C., Brechot, J., Capron, F., Dautzenberg, B., Delaisements, C., Mornet, M., Brun, J., Hurdeboucq, J., and Lemarie, E. (1994). Subcutaneous heparin treatment increases survival in small cell lung cancer. *Cancer* *74*, 38–45.
- Lee, A., Rickles, F., Julian, J., Gent, M., Baker, R., Bowden, C., Kakkar, A., Prins, M., and Levine, M. (2005). Randomized comparison of low molecular weight heparin and coumarin derivatives on the survival of patients with cancer and venous thromboembolism. *J. Clin. Oncol.* *23*, 2123–2129.
- Lerner, W., Pearlstein, E., Ambrogio, C., and Karparkin, S. (1983). A new mechanism for tumor-induced platelet aggregation: Comparison with mechanism shared by other tumors with possible pharmacologic strategy toward prevention of metastases. *Int. J. Cancer* *31*, 463–469.
- Li, J.-J., Huang, Y.-Q., Basch, R., and Karparkin, S. (2001). Thrombin induces the release of angiopoietin-1 from platelets. *Thromb. Haemost.* *85*, 204–206.
- Liao, F., Li, Y., O'Connor, W., Zanetta, L., Bassi, R., Santiago, A., Overholster, J., Hooper, A., Mignatti, P., Dejana, E., et al. (2000). Monoclonal antibody to VE-cadherin is a potent inhibitor to angiogenesis, tumor growth and metastasis. *Cancer Res.* *60*, 6805–6812.
- Ma, L., Perini, R., McKnight, W., Dickey, M., Klein, A., Hollenberg, M.D., and Wallace, J.L. (2005). Proteinase-activated receptors 1 and 4 counter-regulate endostatin and VEGF release from human platelets. *Proc. Natl. Acad. Sci. USA* *102*, 216–220.

- MacKie, R., Reid, R., and Junor, B. (2003). Fatal melanoma transferred in a donated kidney 16 years after melanoma surgery. *N. Engl. J. Med.* *348*, 567–568.
- Martin, C., Mahon, G., Klinger, M., Kay, R., Symons, M., Der, C., and Whitehead, I. (2001). The thrombin receptor, PAR-1, causes transformation by activation of Rho-mediated signaling pathways. *Oncogene* *20*, 1953–1963.
- McCarty, O., Mousa, S., Bray, P., and Konstantopoulos, K. (2000). Immobilized platelets support human colon carcinoma cell tethering, rolling, and firm adhesion under dynamic flow conditions. *Blood* *96*, 1789–1797.
- McGregor, B., McGregor, J., Weiss, L., Wood, G., Hu, C., Boukerche, H., and Warnke, R.A. (1989). Presence of cytoadhesins (IIb-IIIa-like glycoproteins) on human metastatic melanomas but not on benign melanocytes. *Am. J. Clin. Pathol.* *92*, 495–499.
- Miller, G., Bauer, K., Howarth, D., Cooper, J., Humphries, S., and Rosenberg, R. (2004). Increased incidence of neoplasia of the digestive tract in men with persistent activation of the coagulant pathway. *J. Thromb. Haemost.* *2*, 2107–2114.
- Minn, A.J., Gupta, G.P., Siegel, P.M., Bos, P.D., Shu, W., Giri, D.D., Viale, A., Olshen, A.B., Gerald, W.L., and Massague, J. (2005). Genes that mediate breast cancer metastasis to lung. *Nature* *436*, 518–524.
- Mohle, R., Green, D., Moore, M., Nachman, R., and Rafii, S. (1997). Constitutive production and thrombin-induced release of VEGF by human megakaryocytes and platelets. *Proc. Natl. Acad. Sci. USA* *94*, 663–668.
- Mueller, B., Reisfeld, R., Edgington, T., and Ruf, W. (1992). Expression of tissue factor by melanoma cells promotes efficient hematogenous metastasis. *Proc. Natl. Acad. Sci. USA* *89*, 832–836.
- Nielsen, M., Thomsen, J., Primdahl, S., Dyreborg, U., and Andersen, J. (1987). Breast cancer and atypia among young and middle-aged women: A study of 110 medicolegal autopsies. *Br. J. Cancer* *56*, 814–819.
- Nierodzik, M., Plotkin, A., Kajumo, F., and Karparkin, S. (1991). Thrombin stimulates tumor-platelet adhesion *in vitro* and metastasis *in vivo*. *J. Clin. Invest.* *87*, 229–236.
- Nierodzik, M., Kajumo, F., and Karparkin, S. (1992). Effect of thrombin treatment of tumor cells on adhesion of tumor cells to platelets *in vitro* and metastasis *in vivo*. *Cancer Res.* *52*, 3267–3272.
- Nierodzik, M., Bain, R., Liu, L.-X., Shivji, M., Takeshita, K., and Karparkin, S. (1996). Presence of the seven transmembrane thrombin receptor on human tumour cells: Effect of activation on tumour adhesion to platelets and tumour tyrosine phosphorylation. *Br. J. Haematol.* *92*, 452–457.
- Nierodzik, M., Chen, K., Takeshita, K., Li, J., Huang, Y., Feng, X., D'Andrea, M., Andrade-Gordon, P., and Karparkin, S. (1998). Protease-activated receptor 1 (PAR-1) is required and rate-limiting for thrombin-enhanced experimental pulmonary metastasis. *Blood* *92*, 3694–3700.
- Nieswandt, B., Hafner, M., Echtenacher, B., and Mannel, D. (1999). Lysis of tumor cells by natural killer cells in mice is impeded by platelets. *Cancer Res.* *59*, 1295–1300.
- Ollivier, V., Chabbat, J., Herbert, J.M., Hakim, J., and de Prost, D. (2000). Vascular endothelial growth factor production by fibroblasts in response to factor VIIa binding to tissue factor involves thrombin and factor Xa. *Arterioscler. Thromb. Vasc. Biol.* *20*, 1374–1381.
- Palumbo, J.S., Talmage, K.E., Massari, J.V., La Jeunesse, C.M., Flick, M.J., Kombrinck, K.W., Jirouskova, M., and Degen, J.L. (2005). Platelets and fibrin(ogen) increase metastatic potential by impeding natural killer cell-mediated elimination of tumor cells. *Blood* *105*, 178–185.
- Pearlstein, E., Salk, P., Yogeewaran, G., and Karparkin, S. (1980). Correlation between spontaneous metastatic potential, platelet-aggregating activity of cell surface extracts, and cell surface sialylation in 10 metastatic-variant derivatives of a rat renal sarcoma cell line. *Proc. Natl. Acad. Sci. USA* *77*, 4336–4339.
- Pearlstein, E., Ambrogio, C., and Karparkin, S. (1984). Effect of anti-platelet antibody on the development of pulmonary metastases following injection of CT26 colon adenocarcinoma, Lewis lung carcinoma and B16 amelanotic melanoma tumor cells in mice. *Cancer Res.* *44*, 3884–3887.
- Prins, M., and Otten, H.-M.M. (2004). Thrombosis and cancer: A short history of Trousseau's syndrome. In *Thrombosis and Cancer*, G. Lugassy, A. Falanga, A. Kakkar, and F. Rickles, eds. (London, New York: Taylor and Francis), pp. 1–10.
- Rawstron, A., Yuille, M., Fuller, J., Cullen, M., Kennedy, B., Richards, S., Jack, A., Matutes, E., Catovsky, D., Hillmen, P., and Houlston, R. (2002). Inherited predisposition to CLL is detectable as a subclinical monoclonal B-lymphocyte expansion. *Blood* *100*, 2289–2290.
- Rickles, F., and Edwards, R. (1983). Activation of blood coagulation in cancer: Trousseau's syndrome revisited. *Blood* *62*, 14–31.
- Roberts, D., Sherwood, J., and Ginsburg, V. (1987). Platelet thrombospondin mediates attachment and spreading of human melanoma cells. *J. Cell Biol.* *104*, 131–139.
- Shi, X., Gangadharan, B., Brass, L., Ruf, W., and Mueller, B. (2004). Protease-activated receptors (PAR1 and PAR2) contribute to tumor cell motility and metastasis. *Mol. Cancer Res.* *2*, 395–402.
- Shulman, S., and Lindmarker, P. (2000). Incidence of cancer after prophylaxis with warfarin against recurrent venous thromboembolism. Duration of anticoagulation trial. *N. Engl. J. Med.* *3432*, 1953.
- Soszka, T., Knudsen, K., Beviglia, L., Rossi, C., Poggi, A., and Niewiarowski, S. (1991). Inhibition of murine melanoma cell-matrix adhesion and experimental metastasis by Albolabrin, an RGD-containing peptide isolated from the venom of *Trimeresarus albolabris*. *Exp. Cell Res.* *196*, 6–12.
- Stevenson, J., Choi, S., and Varki, A. (2005). Differential metastasis inhibition by clinically relevant levels of heparins—Correlation with selectin inhibition, not anti-thrombotic activity. *Clin. Cancer Res.* *11*, 7003–7011.
- Terranova, V., Williams, J., and Liotta, L. (1984). Modulation of metastatic activity of melanoma cells by laminin and fibronectin. *Science* *226*, 982–984.
- Tsopanoglou, N., and Maragoudakis, M. (1999). On the mechanism of thrombin-induced angiogenesis. Potentiation of vascular endothelial growth factor activity on endothelial cells by up-regulation of its receptors. *J. Biol. Chem.* *274*, 23969–23976.
- Tuszynski, G., Gasic, T., Rothman, V., Knudsen, K., and Gasic, G. (1987). Thrombospondin, a potentiator of tumor cell metastasis. *Cancer Res.* *47*, 4130–4133.
- Yang, J., Mani, S., Donaher, J., Ramaswamy, S., Itzykson, R., Come, C., Savagner, P., Gitelman, I., Richardson, A., and Weinberg, R. (2004). Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell* *117*, 927–939.
- Yoda, Y., and Abe, T. (1981). Fibrinopeptide A (FPA) level and fibrinogen kinetics in patients with malignant disease. *Thromb. Haemost.* *46*, 706–709.
- Zacharski, L., Memoli, V., Morain, W., Schlaeppi, J.-M., and Rousseau, S. (1995). Cellular localization of enzymatically-active thrombin in intact tissue by hirudin binding. *Thromb. Haemost.* *73*, 793–797.
- Zacharski, L.R., Henderson, W.G., Rickles, F.R., Forman, W.B., Cornell, C.J., Jr., Forcier, R.J., Edwards, R.L., Headley, E., Kim, S.H., O'Donnell, J.F., et al. (1984). Effect of warfarin anticoagulation on survival in carcinoma of the lung, colon, head and neck, and prostate. Final report of VA Cooperative Study #75. *Cancer* *53*, 2046–2052.

DOI 10.1016/j.ccr.2006.10.002