The Role of Hemostatic System Inhibitors in Malignancy

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ABSTRACT

Malignancy is associated with alterations in the hemostatic system that present as thromboembolic or bleeding complications. Antineoplastic treatment further escalates blood coagulation and fibrinolytic abnormalities. Moreover, hemostatic system inhibitors play a role in tissue maintenance or, contrarily, contribute to cancer progression. The inhibitors regulate migration, proliferation, apoptosis, angiogenesis, and distant metastases formation, as well as interfere with host defense system mechanisms. They exhibit different functions depending on tumor type, histologic grade, and clinical stage of the disease. The activity of coagulation inhibitors underlies the pathomechanisms of some complications resulting from therapeutic procedures, such as radiation injury to normal tissues. Because coagulation activation is widely recognized to influence cancer growth and distant dissemination, numerous attempts were made to introduce various forms of coagulation inhibitors to antineoplastic treatment. This review summarizes up-to-date information on preclinical and clinical benefits and pitfalls of hemostatic system inhibitors administration in cancer, with special emphasis on tumor biology and prophylaxis and treatment of various complications observed in the course of malignant disease.

KEYWORDS: Hemostatic system inhibitors, cancer, TFPI, TFPI-2, AT III, protein C system, protein Z, ZPI, fibrinolysis inhibitors, heparin

Numerous clinical observations, laboratory analyses, and experimental studies revealed the association between malignant disease and alterations in the hemostatic system, which are tightly interrelated, multifactorial pathologic processes.

More than a century ago, thromboembolic complications were recognized to occur in the cancer patient population with higher frequency than in healthy individuals.1,2 From that time, various types of blood coagulation and fibrinolytic system dysfunctions were described in patients with malignancy: from subtle laboratory abnormalities to overt thrombosis or severe hemorrhages.2–4 Thromboembolic disorders include, among others, Trousseau's syndrome, marantic endocarditis, deep vein thrombosis (DVT), and pulmonary embolism (PE).2–5 It has to be stressed that different types of malignant disease are associated with distinct tendency to thrombotic or hemorrhagic complications. Such episodes may be diagnosed at various stages of the disease (prior to diagnosis of cancer, during the course of the disease, or during anticancer treatment). The complications are most often observed at the terminal stage.
of malignancy.\textsuperscript{3,4} Thrombotic episodes are one of the main causes of death of cancer patients.\textsuperscript{3,4} Strong evidence exists that tumor cells are the source of procoagulants or cytokines, which directly or indirectly trigger the coagulation cascade.\textsuperscript{6,7} On the contrary, several types of malignant tumors activate the fibrinolytic system.\textsuperscript{6,7} However, disorders in hemostasis may also be a result of a shift in the plasma concentration and/or activity of coagulation/fibrinolysis inhibitors.\textsuperscript{6,7} In this regard, increased levels of tissue factor pathway inhibitor (TFPI) and decreased concentrations of antithrombin (AT) and heparin cofactor II (HCII) were detected in plasma of cancer patients, particularly at an advanced stage of the disease.\textsuperscript{8–12} Radical antineoplastic treatment (surgery, radiotherapy, or chemotherapy) frequently leads to the normalization of plasma concentration of hemostatic inhibitors.\textsuperscript{13,14} On the other hand, anticancer treatment may contribute to iatrogenic thromboembolic complications, partially via influence on plasma concentration of coagulation inhibitors. Tamoxifen was demonstrated to decrease plasma TFPI and thrombomodulin (TM) levels, whereas asparaginase reduced AT and HCII plasma concentrations.\textsuperscript{15,16} Surgery performed in gynecologic cancer patients leads to a more pronounced increase of free plasma TFPI and a greater decrease of plasma AT and protein C (PC) than in women undergoing surgical procedures due to benign disease.\textsuperscript{17} More sophisticated procedures employed in oncologic patients are also associated with an increased risk for developing thromboembolic complications. For example, in patients undergoing bone marrow transplantation, decreased plasma concentrations of PC and AT, concurrent with an increased plasminogen activator inhibitor-1 (PAI-1) level, precede the occurrence of hepatic venoocclusive disease (VOD).\textsuperscript{18,19} Changes in the plasma concentrations of hemostatic system inhibitors may also serve as markers of endothelial cell (EC) dysfunction, which contributes to anticancer treatment complication (e.g., increase in soluble TM and PAI-1 in patients with transplant-related complications).\textsuperscript{20,21}

There is ample evidence that alterations in the hemostatic system components not only contribute to thromboembolic and bleeding complications in cancer patients but also play a pivotal role in cancer progression.

**TISSUE FACTOR PATHWAY INHIBITOR**

One of the most important inhibitors of blood coagulation is TFPI.\textsuperscript{22} It inhibits the activity of both coagulation factor Xa and tissue factor/factor VIIa (TF/VIIa) complex.\textsuperscript{22} In patients suffering from colon, gastric, pancreatic, breast, prostate, renal, vesicular cancer, as well as multiforme glioblastoma and various types of hematologic malignancies, increased plasma concentrations of TFPI were detected in comparison with healthy individuals.\textsuperscript{8,11,12,23} This finding was particularly pronounced in patients presenting at a terminal stage of the disease.\textsuperscript{8} Additionally, surgical resection of pancreatic carcinoma leads to normalization of plasma TFPI concentration, whereas progression of the disease correlates with its increased levels.\textsuperscript{9} Of particular interest is the fact that heparin administration resulted in higher release of TFPI in patients with malignancy than in noncancer patients.\textsuperscript{8} Furthermore, an increased concentration of coagulation factor Xa/TFPI complexes were detected in patients with solid tumors than in healthy subjects.\textsuperscript{24} The increase in plasma TFPI concentration may represent a potential host compensatory mechanism driven by procoagulant state (that contributes to cancer progression) in patients with malignancy. Of interest, experimental studies demonstrated an antimetastatic function of TFPI. Namely, intravenous administration of TFPI to mouse, prior to injection of tumor cells, abrogates tumor cell–induced coagulation activation and decreases blood-borne lung metastases formation.\textsuperscript{25} Both free and tumor-associated TFPI were documented to play a role in reduction of hematogenous metastasis.\textsuperscript{25} Moreover, intraperitoneal administration of TFPI, subsequent to injection of tumor cells, also leads to a significant decrease of B16 melanoma lung metastases.\textsuperscript{26} Thus, it seems that TFPI exerts its main effect during the early stage of metastatic spread when tumor cells are circulating in the bloodstream. TFPI may neutralize TF-dependent metastases. It was documented that both the activity of the TF cytoplasmic domain and the proteolytic activity of TF/VIIa complex contribute to the metastatic process.\textsuperscript{27,28} Tumor cell–TF-mediated thrombin generation and platelet activation and aggregation (tumor cell–induced platelet activation, TCIPA) facilitate dissemination via multiple mechanisms.\textsuperscript{29–31} Inhibition of TF/VIIa or TF/VIIa/Xa complex activity by TFPI is considered one of the mechanisms for its antimetastatic function, although the precise role of Xa activity reduction in TFPI antimetastatic effect is questionable and remains under investigation.\textsuperscript{26,31} Several studies demonstrated that anticoagulants, particularly low-molecular-weight heparins (LMWHs), inhibit metastasis and increase overall survival of cancer patients.\textsuperscript{32–37} Apart from factor Xa and thrombin inhibitory properties, LMWH releases TFPI from ECs and downregulates the activities of enzymes in the extracellular matrix (ECM).\textsuperscript{38} In this regard, of interest are the results of a study on non-anticoagulant LMWH, which retains TFPI-releasing properties.\textsuperscript{38} Its administration in the mouse model of melanoma reduces lung metastases similar to enoxaparin (exhibiting unchanged anticoagulant properties) without influencing blood clotting.\textsuperscript{39–41} Tissue factor was demonstrated to mediate angiogenesis both directly via TF-signaling function as well as indirectly through activation of blood coagulation and subsequent thrombin generation and fibrin formation, known for their proangiogenic roles.\textsuperscript{39–41} Activation of
the TF–cytoplasmic domain results in cell migration, and protease activated receptor (PAR) activation by TF/VIIa or TF/VIIa/Xa complex stimulates the angiogenic process.42–44 Thus, TFPI, being an inhibitor of coagulation, may interfere with TF activity and downregulate the TF-mediated proangiogenic effect. Interestingly, EC-bound TFPI regulates TF-signaling events exerted through activation of PARs.45 Moreover, the inhibitor is present in plasma mainly coupled with lipoproteins, hence it may also play distinct roles that depend on its binding to the very-low-density lipoprotein (VLDL) receptors.46 Thus, it is not surprising that TFPI inhibits proliferation of ECs in vitro independent of TF.46,47

Contradictory results concerning TFPI presence in loco in tumor tissue have been published.47–51 Werling et al47 failed to demonstrate the expression of TFPI in cancer cells of non–small cell lung cancer (NSCLC), renal, colon, and breast cancer, and lymphoma tissue. Similarly, there was no TFPI presence in tumor cells in gastric or laryngeal cancers,50–53 whereas minimal and inconsistent expression of TFPI was demonstrated in pancreatic tumor cells.59 Contrary to the above results, another study revealed the presence of TFPI in cancer cells in most cases of colon cancer; however, expression of the inhibitor was heterogeneous as it was observed only in selected areas of examined specimens.48 The differences in the results of immunohistochemical (IHC) studies may result from methodological discrepancies. Tumor cell–associated TFPI may be of blood-borne origin or may be synthesized by cancer cells per se. The rationale for the latter is the demonstration of TFPI mRNA and protein in tumor cells of breast cancer.52 TFPI mRNA and protein was also demonstrated in breast, colon, and pancreatic cancer cell lines.53 The absence, or weak expression, of TFPI in some tumor cells may indicate a relative deficiency of TFPI, which may facilitate blood coagulation activation. It is also conceivable that the consistent TFPI presence in cancer cells may result from the absence of appropriately configured TF/VIIa/Xa complex required for TFPI binding. An experimental study revealed that TFPI does not inhibit tumor cell proliferation.54 TFPI binds to factor Xa and TF/VIIa, which leads to the formation of a tertiary complex.55 The latter is then transported to the caveolae, localized in the cell membrane.56 Such a translocation of this complex abolishes the proteolytic activity of its components.54 However, caveolae are a rich source of intracellular signaling molecules (e.g., tyrosine kinases, protein G–binding receptors, as well as calcium membrane pumps).55,56 Thus, it is conceivable that TFPI indirectly stimulates TF–mediated intracellular signaling cascade.56 The presence of TFPI was also demonstrated in connective tissue stroma adjacent to cancer cells in ECs of small blood vessels supplying the neoplasms, as well as in tumor-infiltrating macrophages (TAMs).47,51,57,58 It cannot be excluded that in cancer tissue, contrary to TFPI’s role in the circulation, the inhibitor may exert a pro-neoplastic function. In this regard, of interest are results of a study demonstrating that ECM-bound TFPI cooperates with TF/VIIa complex in facilitating cancer cell adhesion and migration.79 It was also documented that the procoagulant function of TF in highly aggressive melanoma is regulated by TFPI, and this activity is essential for perfusion of vasulogenic mimicry channels formed by TF-expressing melanoma cells.60 These channels play an essential role in supplying the nascent tumor.

Tissue factor pathway inhibitor-2

TFPI-2, although structurally similar to its homologue TFPI, displays distinct inhibitory spectrum from the latter. In contrast with TFPI, TFPI-2 is only a weak inhibitor of TF/VIIa.22,61–64 The essential TFPI-2 role is inhibition of serine proteinases including plasmin,62,64–67 plasma kallikrein,62 trypsin, chymotrypsin,62 as well as factor Xa.62

TFPI-2 is synthesized mainly in ECs of small blood vessels and thereafter is secreted (60 to 90%) into the ECM.69 It is present in most normal tissues.68,70,71 In malignant tumor, the expression of the TFPI-2 gene is significantly decreased compared with normal tissues.72–74 Moreover, an inverse correlation between TFPI-2 distribution and the degree of malignancy in human neoplasms is well documented.71,75,76 In tumors with the highest degree of malignancy (e.g., in glioblastomas), TFPI-2 was undetected.75 Similarly, the absence of TFPI-2 mRNA was also observed in choriocarcinoma, fibrosarcoma,78 and pancreatic cancer79 cell lines. Of interest, several highly aggressive cancers delete the locus for the TFPI-2 gene in chromosome 7q region, which results in a complete lack of TFPI-2 protein expression in these cells.79–81 Recently, numerous studies revealed the phenomenon of epigenetic inactivation of TFPI-2 synthesis via aberrant methylation of TFPI-2 promoter cytosine–phosphorothioate–guanine (CpG) islands during cancer progression.72,74,79,82–85 The finding was associated with increased cancer growth, invasion, and dissemination.7,2,74,79,82–85 Methylation-associated silencing of several components of pathways regulating TFPI-2 presence in cancer cells86 as well as an aberrant–splicing of TFPI-2 mRNA leading to formation of untranslated variant of TFPI-2 mRNA were discovered.87 The role of extracellular metalloproteinases in modification of the binding properties of TFPI-2 that alter the extracellular location of the protein and disrupt its function was also suggested.88

Being a product of a tumor suppressor gene, TFPI-2 is implicated to play a role in maintaining the stability of the tumor environment and in the inhibition of the growth of neoplasms.71,75 This ECM–derived inhibitor decreases invasion of tumor cells of several
cancer cell lines.\textsuperscript{75,89–94} The key step in cancer invasion is proteolysis of ECM and basement membrane degradation.\textsuperscript{95} Importantly, TFPI-2 is a potent inhibitor of plasmin regardless of whether the latter is associated with the tumor cells or ECM.\textsuperscript{62,96} Downregulation of TFPI-2 protein concentration leads to increased plasmin- and trypsin-mediated ECM proteolysis and subsequently enhances the cancer cell’s ability to degrade the ECM.\textsuperscript{62,92,96,97} The effect is partially mediated by the inhibitory effect of TFPI-2 on the plasmin– or trypsin-dependent activation of pro-matrix metalloproteinase (MMP)-1 and pro-MMP-3, as well as MMP-2 formation.\textsuperscript{78,92,97} Contradictory results exist concerning direct inhibitory effect of TFPI-2 toward MMP-1, MMP-13, MMP-2, and MMP-9.\textsuperscript{98,99} Of interest, restoration of TFPI-2 led to an inhibition of invasion and tumor growth of several cancer lines.\textsuperscript{79,100,101}

Reduced apoptosis of cancer cells contributes to tumor progression. In this regard, TFPI-2–mediated induction of apoptosis further proves the hypothesis that TFPI-2 deficiency facilitates tumor growth.\textsuperscript{102,103} TFPI-2–positive multiforme glioblastoma cell line (SNB-19) and low-grade glioma cell line (Hs683) revealed an increased rate of apoptosis in contrast with TFPI-2–negative glioblastoma cell lines and normal glial tissue—where no apoptosis is observed.\textsuperscript{75,78,102} Of interest, restoration of TFPI-2 in human glioblastoma cell line activates both intrinsic and extrinsic caspase-mediated, proapoptotic signaling pathways and induces apoptosis.\textsuperscript{103}

It is widely known that coagulation system components contribute to cancer biology, and TF is the key procoagulant in malignancy.\textsuperscript{3,7,8,27,39,42,55,104,105} Because the expression of TF is enhanced whereas TFPI-2 is decreased with increasing degree of malignancy, and the fact that TFPI-2 is a weak inhibitor of TF/VIIa complex activity, it does not seem conceivable that TFPI-2 influences the function of hemostatic system components on tumor growth. However, there exists a unique observation that TFPI-2 contributes to cancer invasion via binding to TF/VIIa complex on tumor cells in hepatocellular carcinoma.\textsuperscript{106}

TFPI-2 was shown to inhibit angiogenesis in experimental models.\textsuperscript{107,108} TFPI-2 contributes to the maintenance and integrity of the ECM and basement membrane of small blood vessels, thus preventing initiation of angiogenesis. Moreover, TFPI-2 mediates EC adhesion to the ECM.\textsuperscript{69} Interestingly, TFPI-2 in ECs is upregulated by vascular endothelial growth factor (VEGF), which suppresses proliferation of ECs\textsuperscript{109} and thus negatively regulates VEGF activity. Recently, TFPI-2 was demonstrated to strongly contribute to vasculogenic mimicry, a vessel-like network formation by cancer cells themselves.\textsuperscript{110} These features are typical for some highly aggressive neoplasms (melanoma as well as breast, lung, prostate, and ovarian cancer).\textsuperscript{110}

### PROTEIN Z-DEPENDENT PROTEINASE INHIBITOR AND PROTEIN Z

It is noteworthy that tumor cells synthesize and release procoagulants that lead to multifactorial activation of factor X and subsequent thrombin generation and fibrin formation, which in turn contributes to tumor growth and metastasis. The activity of factor Xa is inhibited mainly by TFPI.\textsuperscript{22} However, another mechanism of direct inhibition of factor Xa, which involves the protein Z (PZ)/protein Z–dependent proteinase inhibitor (ZPI) system, has been described.\textsuperscript{112} ZPI inhibits the coagulation response prior to the formation of the prothrombinase complex.\textsuperscript{113} It attenuates factor Xa activity in the presence of calcium and phospholipids.\textsuperscript{112} Protein Z, a 62-kDa, vitamin K–dependent plasma nonpeptidase glycoprotein, augments its activity by more than 1000-fold.\textsuperscript{111,112}

Interestingly, expression of ZPI was observed in colon, breast, gastric, renal, and endometrial cancer cells, pancreatic cancer, NSCLC, and glial neoplasms.\textsuperscript{113–116} The staining intensity for ZPI was irregular: both strong and weak expression of ZPI was observed. Moreover, various percentages of ZPI-positive cancer cells were revealed in different specimens of examined tissues. In colon cancer, expression of ZPI was also observed in TAMs.\textsuperscript{114–116}

PZ was detected in colon, breast, gastric, renal, and endometrial cancer cells, pancreatic cancer, NSCLC, and glial neoplasms.\textsuperscript{114–116} Staining intensity for PZ was more pronounced in less differentiated cancer cells of anaplastic gliomas. In contrast, more differentiated cancer cells of gastric cancer revealed stronger staining than did more malignant ones. Tumor cells of NSCLC and renal cancer were characterized by weak staining intensity for PZ. In the case of pancreatic and renal cancers, as well as malignant melanoma, the intensity of staining for PZ in cancer cell bodies was irregular: both high and low levels of PZ were observed independently of the degree of malignancy. Similar to ZPI, the expression of PZ was revealed in TAMs in colon cancer, NSCLC, and malignant melanoma. Furthermore, PZ was localized in small blood vessels of the above-mentioned tumors.\textsuperscript{114–116}

Double-staining immunohistochemical studies revealed coexpression of both proteins in cancer cells in all the above tumor types, although areas of tumor foci, where only one protein (ZP or ZPI) was localized, were also revealed. It seems likely that ZP and ZPI, apart from being the inhibitors of coagulation, may additionally play a regulatory role in tumor progression.\textsuperscript{115}

### ANTITHROMBIN

Another essential inhibitor of blood coagulation is AT (synonymous with antithrombin III [AT III]). It inhibits thrombin proteolytic activity via thrombin-antithrombin (TAT) complex formation.\textsuperscript{117} Moreover, it
downregulates the activity of several other blood coagulation proteases (e.g., factor IXa, Xa, XIa, XIIa, and, in the presence of heparin, factor VIIa). In breast cancer, gastric cancer, and melanoma patients, no difference in the plasma AT concentration was observed in relation to healthy individuals. However, decreased activity of AT was demonstrated in the plasma of colon, ovarian, and prostate cancer patients in comparison with noncancer individuals. Low preoperative plasma AT concentration and activity was normalized in the postoperative period in prostate cancer patients. In laryngeal cancer patients undergoing radiotherapy, both pre- and postradiation plasma concentrations of AT were lower than in the normal population. Furthermore, low plasma AT concentration in laryngeal and lung cancer (NSCLC and small cell lung cancer [SCLC]) patients correlated with short overall survival. Moreover, decreased AT activity related to the progression of malignant disease was demonstrated. In contrast, significantly higher AT complex concentrations, which reflect a procoagulant state, was revealed in advanced cancer patients compared with individuals who presented at earlier stages of the disease. Increased plasma TAT levels in patients with active breast cancer significantly correlated with elevated concentrations of blood breast cancer marker CA 15–3.

Thrombin is mitogenic for cancer cells. It stimulates proliferation, migration, metastases formation, and contributes to TCIPA. Thrombin also exerts a proangiogenic effect via inducing vessel permeability, synthesis of VEGF and its receptors, and stimulating proteolytic events.

In most cases of colon cancer, the absence or scanty AT expression in cancer cells was revealed, and AT was readily detected in only 15% of colon cancer cases. In light of the important role of thrombin, a deficiency in circulating AT and in nascent tumor tissue is of particular interest. This may contribute to blood coagulation activation and, as a consequence, facilitate tumor growth and the processes of angiogenesis and metastasis. It is noteworthy that AT blocks the mitogenic activity of thrombin in ECs and induces apoptosis of ECs via damage of cell-to-cell junctions and between these cells and ECM components, as well as through its influence on apoptosis inhibitors’ gene expression, ultimately exerting an antiangiogenic effect. Furthermore, distinct forms of AT molecules (e.g., inactive form of AT molecule, designated as latent antithrombin) or fragments formed as a result of its limited proteolysis function as inhibitors of angiogenesis (e.g., antangiogenic AT [aaAT]). Thus, AT deficiency, or its absence, in colon cancer tissue may facilitate angiogenesis and, subsequently, colon cancer progression. In contrast, in cases of colon cancer patients characterized by positive AT staining of cancer cells, this inhibitor may contribute to tissue maintenance and a better prognosis.

The presence of mononuclear cells is a frequent finding in tumor burden. These cells contribute to tumor growth, serving as an additional source of coagulation factors, their receptors, as well as various cytokines. Antithrombin, independent of its anticoagulant function, also exerts an anti-inflammatory effect. It protects mononuclear cells from activation and cytokine release.

**PROTEIN C SYSTEM**

The PC system represents another important coagulation inhibitory mechanism. In cancer patients, an inverse correlation between plasma PC concentration and the risk of thromboembolic complications in the postoperative period was demonstrated. Intravascular coagulation and increased concentration of procoagulants is often observed in patients with malignancy. Moreover, these patients may exhibit activated PC (APC) resistance, which is not due to factor V Leiden mutation. The findings revealed a high prevalence of APC resistance in gastrointestinal cancer patients, compatible with an acquired defect in the APC pathway, ultimately contributing to thrombosis. Acquired APC resistance was also detected in breast cancer, myeloma, and primary non–Hodgkin’s cerebral lymphoma patients. Moreover, in multiple myeloma patients, APC resistance appears to be a transitional disorder that may be related to the disease status.

Under normal conditions, thrombin, via binding with TM localized in the cell membrane, leads to PC activation. The latter reaction is markedly enhanced when PC is bound to its receptor, endothelial cell protein C receptor (EPCR). Activated protein C, after binding with its cofactor, protein S (PS), inactivates coagulation factors Va and VIIIa.

The presence of PC was documented in cancer cells of SCLC, gastric cancer, and pancreatic cancer. Contrary to the above, the lack or weak expression of the protein was revealed in tumor cells of melanoma and prostate cancer. Interestingly, in cancer patients, the increase of D-dimer concentration was significantly correlated with the monocyte APC content but not with monocyte TF levels or TAT complexes, which reflects a local, rather than systemic, thrombin and fibrin formation. It is suggested that the APC formed locally enters the circulation and binds to peripheral blood monocytes. APC bound to monocyte is known to inhibit cytokine production by these cells and therefore may be involved in regulatory responses of monocytes in cancer patients.

The presence of PC system components in various types of tumor tissue suggests its role in tumor biology. Interestingly, it was revealed that these proteins
may exert a stimulatory effect on cancer progression (e.g., angiogenesis). Namely, APC was demonstrated to exert antiapoptotic activity toward ECs.\textsuperscript{143} Of interest, in patients with cerebral ischemia, the activity of APC was particularly strong in hypoxic tissue.\textsuperscript{144} Similar hypoxic conditions are observed in tumor tissue, particularly at the host-tumor interface, where the most intensive tumor growth and the most pronounced angiogenesis are observed.\textsuperscript{43} Recently, APC was documented to inhibit apoptosis of neurons, subsequently decreasing the intensity of neurodegenerative processes in the brain.\textsuperscript{145} The latter APC activity is mediated via PAR-1.\textsuperscript{145} The presence of PAR-1 (primarily designated as “tethered ligand” thrombin receptor, or TLTR) was detected on cancer cells as well.\textsuperscript{146} Expression of PAR-1 was observed in prostate cancer, breast cancer, high-grade endometrial carcinoma, NSCLC, melanoma, head and neck cancers, ovarian cancer, pancreatic adenocarcinoma, as well as in numerous cell lines, such as human renal carcinoma cells, human colon cancer cell lines, in permanent hepatocellular carcinoma (HCC) cell lines, primary HCC cell cultures, and human glioblastoma cell lines.\textsuperscript{147–158} This suggests that APC may also exert an antiapoptotic effect toward human cancer cells. It cannot be excluded that APC, via its interaction with PAR-1, may exert other pro-neoplastic effects characteristic of PAR-1 (e.g., facilitate metastasis).\textsuperscript{159} However, these need to be further elucidated. A case of a patient treated with APC for localized colon cancer complicated by sepsis was described.\textsuperscript{160} The patient developed a massive neoplastic invasion of bone marrow shortly after APC administration, which suggested that APC may have contributed to the progression of cancer.\textsuperscript{160} Recently, APC was documented to promote breast cancer cell migration through interactions with EPCR and PAR-1.\textsuperscript{150}

Moreover, APC, similar to thrombin, directly stimulates transformation of inactive progelatinase A into its active form, gelatinase A (MMP-2).\textsuperscript{161} The enzyme plays an essential role in proteolytic processes during angiogenesis,\textsuperscript{161} local tumor invasion, and metastases formation.\textsuperscript{162} Expression of MMP-2 was observed in colon, gastric, pancreatic, and oral cancer.\textsuperscript{162–165} Moreover, MMP-2 also enhances vascular permeability by stimulation of the signaling cascade through both PAR-1 and sphingosine 1-phosphate receptor-1.\textsuperscript{144} Thus, it may contribute to new vessel formation in the tumor burden. APC was also demonstrated to stimulate EC proliferation in vitro and angiogenesis in vivo.\textsuperscript{166} However, the coordinated interplay between particular components of the PC system is a prerequisite for APC formation and its optimal activity. The absence or deficiency of at least one of the PC system components may result in disturbances of its function. In this regard, observations of inconsistent and heterogeneous expression of PC, PS, and TM were observed in colon cancer tissue.\textsuperscript{48} The results of experimental studies performed on various types of malignancy indicate that only the coexistence of TM and EPCR leads to significant PC activation.\textsuperscript{167} However, in the absence of TM, activation of PC may result from PC binding to EPCR.\textsuperscript{152} The latter was detected on almost half of the primary tumor cell lines, including melanoma, as well as renal and colon carcinomas, and its expression in the tumor cells was detected, although this was a rare event.\textsuperscript{168} High EPCR expression was found in a small panel of human tumors, particularly in ECs.\textsuperscript{168} EPCR was also upregulated in the peritoneum of the patients with epithelial ovarian cancer.\textsuperscript{169} The functional significance of EPCR overexpression on tumor cells and ECs is still unclear.

The activity of PC is inhibited by protein C inhibitor (PCI).\textsuperscript{170} Under normal conditions, PCI is synthesized by hepatocytes and the urinary and reproductive systems. Its presence was documented in tumor cells of laryngeal cancer and SCLC (necrotic cells).\textsuperscript{57,171} Higher expression of PCI was demonstrated in renal cell carcinoma in comparison with normal renal tissue.\textsuperscript{172} In vitro studies revealed that overexpression of PCI in renal carcinoma cells is associated with a reduction of tumor growth.\textsuperscript{171} There was no correlation in breast cancer patients between the PCI protein and mRNA expression in the tumor burden and lymph node involvement.\textsuperscript{173}

The presence of TM in cancer cells was also revealed.\textsuperscript{167} However, heterogeneous expression of TM was observed in cancers of similar histopathologic types, which grow in various organs.\textsuperscript{174} In lung cancer patients, weak expression of TM in tumor cells correlated with a pessimistic prognosis.\textsuperscript{175–178} In lung and esophageal cancer patients, expression of TM in lymph node metastatic cells was a significantly less frequent finding than in primary tumors, suggesting that reduction of TM expression may play a role in the metastatic process.\textsuperscript{176–178} Similarly, intense expression of TM in tumor cells of oral squamous cell carcinoma was associated with a lower frequency of lymph node metastasis and significantly more favorable patient survival compared with no expression of TM in cancer cells.\textsuperscript{179} There was no difference in the expression of TM in cancer cells between primary lesions versus lymph node metastases in pharyngeal or laryngeal squamous cell carcinoma.\textsuperscript{180} However, the low-grade carcinomas revealed only scanty staining for TM.\textsuperscript{180} Low TM expression in breast cancer cells significantly correlated with a high relapse rate.\textsuperscript{181} In colon cancer, one fourth of examined specimens exhibited TM expression, whereas in the remainder of these neoplasms, TM expression was weak or absent.\textsuperscript{48,182} TM expression declined with increasing clinical stage of the disease and tumor size, as well decreasing tumor cell differentiation.\textsuperscript{182} Its presence is
protective for colorectal cancer and correlates with longer overall survival. Although primary malignant ovarian tumors and metastases localized in the omentum and intestine contain TM, its concentration is much lower than in benign ovarian tumors. In contrast, malignant ascitic fluid of patients with advanced ovarian cancers is characterized by elevated concentrations of soluble TM (sTM) compared with benign peritoneal exudates. Contrary results were obtained for various experimental pancreatic cancer cell lines, which were mostly TM mRNA and protein positive.

There is no agreement in relation to the presence of TM in vessels supplying glial tumors. Namely, it was reported that small vessels of normal brain and low-grade but not high-grade gliomas revealed intense staining for TM whereas another study demonstrated the absence of TM in vessel walls of normal brain, and increased expression of this molecule in vessels of gliomas of different degree of malignancy, as well as in vessels localized in brain adjacent to the tumor.

The exact mechanism of TM’s biological activity is not fully recognized. It was documented that this protein regulates cancer growth independently of its anticoagulant activity. Experimental studies revealed that TM overexpression in tumor cell was associated with reduced proliferation in vitro and tumor growth in vivo. The cytoplasmic domain of TM was implicated in mediating tumor biology. Thrombomodulin functions as a cell-to-cell adhesion molecule. This is also supported by the observation that experimental esophageal squamous cell carcinoma, characterized by low TM expression, exerted greater invasiveness than the high-TM-expressing clone of the carcinoma. Furthermore, TM inhibits intrahepatic spread in human hepatocellular carcinoma. Moreover, the antitumorigenic effect of TM was demonstrated in islet cell–derived tumors, and human sTM inhibited invasion and metastasis of murine melanoma (B16F10). It is also conceivable that TM-lacking metastatic cells are more susceptible to the procoagulant state, which contributes to cancer progression.

The effectiveness of radiation therapy is limited by the risk of injury to normal tissues present in the irradiated area, which is a dose-limiting factor. Microvascular damage is a prominent feature of both early (inflammatory) as well as delayed (fibroproliferative) radiation injuries in the intestine and in many other normal tissues. There is ample evidence suggesting that endothelial dysfunction, particularly deficiency of endothelial TM, plays an essential role in the pathogenesis of these radiation responses. Inadequate levels of TM results in loss of vascular thromboresistance, excessive activation of cellular thrombin receptors by thrombin, and insufficient activation of PC, a protein exhibiting anticoagulant, anti-inflammatory, and cytoprotective properties. These changes are presumed to be critically involved in many aspects of early intestinal radiation toxicity and may contribute to the fibroproliferative processes that lead to delayed intestinal dysfunction, fibrosis, and clinical manifestation of postradiation complications.

Another component of the PC system is PS. Expression of PS was demonstrated in primary brain tumors, as well as in SCLC. Experimental studies revealed that lung cancer cell lines, and brain tumor cell lines per se, are capable of synthesizing and releasing PS. In contrast, in melanoma, prostate cancer, gastric cancer, and pancreatic cancer, no or subtle PS expression was observed. Similar to PC, PS exerts antiapoptotic activity toward hypoxic neurons as well. However, the mechanism responsible for this activity is obscure. It is unknown if PS contributes to cancer cell apoptosis, and more studies are needed to assess this possibility. On the other hand, it was documented that PS induces phagocytosis in the early phase of apoptosis of malignant lymphoma cells. Efficient elimination of apoptotic cells prevents the inflammatory response, as well as the host immunologic reaction. In malignant tumors, the growth of neoplasms is a result of proliferation of cells, as well as their apoptotic death. Thus, the presence of PS in cancer cells may prevent cancer from the host defense system response. PS binds with cells undergoing apoptosis via phosphatidylserine. In plasma, roughly 60% of PS exists in complex with C4 binding protein (C4BP). Binding of C4BP to PS attenuates the activity of the latter while localizing C4BP (which is involved in inhibition of activation of complement system) in the direct neighborhood of apoptotic cells. Protein S also binds to a membrane tyrosine kinase receptor Tyro3, a member of the Axl family. The presence of this receptor was demonstrated in tumor cells of lung cancer cell lines. Tyro3 activity mediates immunologic reactions, phagocytosis, and cell adhesion.

HEPARIN COFACTOR II

HCII is a glycoprotein synthesized in the liver and circulates in the bloodstream. It inhibits thrombin and clot-bound thrombin but not other proteinases. HCII exerts inhibitory activity toward thrombin in the presence of various polyanionic molecules, including the glycosaminoglycans heparin and dermatan sulfate. The inhibitor contributes to 20 to 30% thrombin inhibition during blood coagulation. Decreased concentrations of HCII are detected in cancer patients,
particularly at an advanced stage of the disease, which may contribute to thromboembolic disease.\textsuperscript{8–10} Additionally, chemotherapy (e.g., asparaginase treatment) may lead to reduction of HCII plasma content.\textsuperscript{15,16} However, upregulation of HCII in the peritoneum of epithelial ovarian cancer patients was observed.\textsuperscript{169} Of interest, in patients suffering from acute promyelocytic leukemia-associated disseminated intravascular coagulation, a decrease in plasma HCII was not accompanied by a proportional reduction in AT concentration, which suggests preferential consumption of HCII by thrombin and may spare consumption of AT.\textsuperscript{205} To date, information on the role of HCII in tumor biology is obscure.

FIBRINOLYTIC SYSTEM INHIBITORS

It is widely recognized that fibrinolytic system components contribute to cancer progression.\textsuperscript{95} Apart from their role in fibrin degradation, they facilitate proteolytic events, tumor cell motility, metastatic dissemination, and angiogenesis. The influence of fibrinolytic components on cancer biology depends not only on plasmin activity but also results from reciprocal interactions of particular proteins (e.g., urokinase plasminogen activator [uPA], urokinase plasminogen activator receptor [uPAR], PAI-1, PAI-2, PAI-3, proteinase nexin-1, α\textsubscript{2}-antiplasmin, thrombin activatable fibrinolysis inhibitor [TAFI]).

PAI-1

Enhanced activity of uPA has been demonstrated in numerous malignant tumors, a property frequently associated with high histologic malignancy and unfavorable prognosis.\textsuperscript{208–213} Similarly, high expression of tissue plasminogen activator (t-PA) in cancer tissue implicated poor prognosis.\textsuperscript{214,215} Thus, it would be expected that PAI-1, serving as inhibitor of plasminogen activator (PAs), interferes with cancer progression. However, an interesting, albeit paradoxical discovery, that PAI-1 overexpression correlates with unfavorable prognosis,\textsuperscript{208,213,214} prompted a revision of our understanding of how the plasminogen system functions in cancer biology. It seems that the multifactorial interactions of proteins of the fibrinolytic system, as well as other factors, influence the net effect on cancer progression. It is thought that an optimal level of specific proteolytic activity, not an absolute lack or supraoptimal stimulation of a particular activity of the plasminogen system components, is responsible for aggressive tumor behavior.\textsuperscript{216–219} PAI-1 is involved not only in pericellular events but also in the regulation of intracellular signals.

Increased PAI-1/uPA tissue content is associated with higher histologic grade of breast cancer,\textsuperscript{219} whereas high tissue PAI-1 protein or gene expression is associated with lymph node involvement (breast, colorectal, gastric cancer),\textsuperscript{220,221} higher incidence of distant metastases (colorectal, gastric, esophageal cancer),\textsuperscript{221–223} and increased stage of the disease (breast cancer, melanoma, NSCLC).\textsuperscript{221,224,225} There exist contradictory data obtained from studies performed on experimental models concerning the role of PAI-1 in invasion, migration and metastasis. However, these data should be interpreted with caution, as even subtle differences in experimental conditions and model types results in a failure to reproduce the results.\textsuperscript{226} PAs-mediated proteolytic activity focused on the leading edge of cancer cells (or ECs with regard to angio genesis) is dependent on uPAR distribution.\textsuperscript{227,228} PAI-1 accelerates internalization of uPA/uPAR complex and consequently increases the rate of uPAR redistribution, which may contribute to the more aggressive biology of a tumor.\textsuperscript{227,228} PAI-1 regulates the proteolytic process during alternate detachment and adhesion of different edges of cancer cells, which leads to cell migration. In this regard, PAI-1 binding to vitronectin prevents it from PA-dependent proteolytic degradation that facilitates adhesion, whereas on the opposite edge of the cell, vitronectin bound to uPAR localizes the cell to the ECM.\textsuperscript{229,230} PAI-1–mediated dissociation of vitronectin from uPAR facilitates cell detachment.\textsuperscript{231,232}

PAI-2

Plasma PAI-2 concentration may serve as a marker of monocytic leukemia.\textsuperscript{233} It was documented that high tumor PAI-2 concentration is a positive prognostic factor in several malignances (e.g., ovarian cancer, lung cancer, laryngeal cancer).\textsuperscript{234–236} However, in endometrial cancer, PAI-2 content is associated with an unfavorable prognosis.\textsuperscript{237–239} Subtle immunohistochemical expression of PAI-2 was demonstrated in cancer cells, ECs, and tumor stroma in gastric, pancreatic, and laryngeal cancer.\textsuperscript{49,50,240} Inhibition of uPA proteolytic activity by PAI-2 results in the reduction of tumor growth and metastatic dissemination (highly aggressive M24met melanoma animal model, HT1080 sarcoma cell lines).\textsuperscript{241,242} Extracellular activity of PAI-2 inhibits monocyte proliferation and differentiation.\textsuperscript{243} Of interest, the inhibitor exists mainly in an intracellular form.\textsuperscript{244} Such a localization of PAI-2, along with the lack of uPA in the cytosol, suggests a distinct biological activity of the former. It is conceivable that the intracellular pool of protein derives from dead or dying cells.\textsuperscript{245} PAI-2 binds to the retinoblastoma (Rb) protein in the cell nucleus, which results in the inhibition of oncogenesis.\textsuperscript{246} Other mechanisms may function in tumor biology. Contradictory results were reported on the role of PAI-2 in the process of apoptosis.\textsuperscript{246–248} However, recently published data revealed susceptibility to apoptotic stimuli, both in cells overexpressing and lacking PAI-2.\textsuperscript{249}
PAI-3
PAI-3 is also known as PCI. Its role in malignancy was discussed earlier (see “Protein C System”).

Protease Nexin-1
The inhibitor protease nexin-1 (PN-1) is localized in the extracellular space, where it serves as a PA, plasmin, trypsin, and thrombin inhibitor. Glycosaminoglycan-bound PN-1 is an essential thrombin inhibitor in the pericellular space. PN-1 inhibits PA-mediated proteolytic events and prevents cell detachment and apoptosis. However, in a pancreatic cancer animal model, PN-1 overexpression increased tumor invasiveness via enhanced synthesis of ECM components (e.g., collagen I, laminin, and fibronectin).

α2-Antiplasmin
A member of the serpin family, α2-antiplasmin (α2-AP) is a plasmin inhibitor. In breast and gastric cancer, as well melanoma patients, a decreased concentration of plasma α2-AP was detected compared with healthy individuals. However, increased plasma concentrations of plasmin/α2-AP complexes were documented in breast and lung cancer patients in comparison with a control group, which indicates a profibrinolytic state in patients suffering from the above-mentioned neoplasms. The inhibitor antagonizes proteolytic events mediated by plasmin. Experimental studies demonstrated that α2-AP reduced motility of neoplastic cells of oral squamous cell carcinoma, which resulted from the inhibition of cell adhesion protein degradation.

Thrombin Activatable Fibrinolysis Inhibitor
Thrombin activity leads to the activation of an inhibitor of fibrinolysis, thrombin activatable fibrinolysis inhibitor (TAFI). It attenuates plasminogen conversion to plasmin and thus may interfere with plasmin-mediated proteolysis of ECM components and play a role in invasion and angiogenesis. To date, experimental studies performed on animal models revealed that TAFI influences neither primary nor metastatic tumors. However, abnormalities in plasma concentration of TAFI may contribute to hemostatic complications in the course of malignancy. Namely, in acute promyelocytic leukemia, a malignant neoplasm known for its high potential for early hemorrhagic death, TAFI activity is significantly reduced, which may facilitate hemorrhagic diathesis. In turn, in lung cancer patients, an increased concentration of TAFI was detected, which may contribute to thromboembolic complications in this population. Of interest, the increase in TAFI concentration is particularly pronounced in SCLC compared with NSCLC patients, which is in agreement with a higher incidence of thromboembolic episodes observed in the former group of patients.

HEMOSTATIC SYSTEM INHIBITORS IN ANTICANCER TREATMENT
Contemporary oncology faces the necessity for introducing tailored antineoplastic treatment contingent on detailed analysis of not only histopathologic type and grade of malignancy but also a spectrum of molecular characteristics of the tumor, which distinguishes the individual cancer patient. Hemostatic system proteins affect tumor growth and metastatic spread via their pro- and anti-coagulant activity, as well as by biological effects exerted by individual proteins, independent of their role in hemostasis. In fact, the past 2 decades provided new and interesting information related to the functions of the blood coagulation/fibrinolytic system components in cancer biology. As a consequence, based on the mutual interactions within the two intersecting fields, novel therapeutic possibilities emerge, including compounds interfering with hemostasis to be tested in cancer patients.

TF, Factor VIIa, and Factor Xa Inhibitors
The role of TF in cancer biology suggests that interfering with its activity may lead to improved cancer patient survival. In experimental mouse models, recombinant forms of TF/VIIa complex inhibitors (e.g., rTFPI or recombinant nematode anticoagulant protein c2 [rNAPc2]) decrease primary tumor growth and reduce metastases (for review, see Ref. 39). Moreover, rNAPc2 is an inhibitor of angiogenesis (for review, see Ref. 39). Administration of monoclonal antibodies directed to TF or drugs decreasing TF cellular expression (e.g., retinoids, pentoxifylline, or statins) may serve as another option of adjunct therapy in cancer patients.

Increased synthesis of TF in cancer cells is ascribed to the activity of oncogenes. In this regard, interfering with intracellular signaling pathways may prove a valuable form of treatment. Interestingly, epidermal growth factor receptor (EGFR) agonists (e.g., anti-EGFR monoclonal antibody C225 [IMC-C225; cetuximab]), apart from their antineoplastic and VEGF-reducing activity, lead to inhibition of TF synthesis. It is conceivable that other drugs interfering with EGFR signaling (e.g., erlotinib, gefitinib, ABX-EGF), tyrosine kinase antagonists, and small molecular inhibitors (farnesyl transferase inhibitors) may indirectly down-regulate TF synthesis and thus exert antineoplastic activity.
patients. Administration of active site–blocked factor VIIa (FFR-rFVIIa, FVIIai), which attenuates activation of blood coagulation while not increasing the risk of bleeding complications, seems yet another attractive option.271,272

Recently, an active site–directed inhibitor of factor Xa, MCM09, inhibited melanoma lung colonies in mice.273 However, nematode–derived selective factor Xa inhibitor (rNAP5) did not exhibit anticancer activity (for review, see Ref. 39).

**Thrombin Inhibitors**

It is widely known that thrombin strongly contributes to cancer progression. In brief, it induces migration of cancer cells and ECs, angiogenesis, and facilitates metastases formation (for review, see Refs. 39 and 129).

Argatroban is a derivative of arginine, which binds competitively to the active site of thrombin and exerts an anticoagulant effect.274 Its effectiveness was demonstrated in the treatment of pulmonary embolism after surgery for lung cancer.275 There are data suggesting that this oral thrombin inhibitor inhibits the effects of thrombin on tumor progression. Namely, it prevents B16BL6 melanoma cell migration, as well as bone metastasis in mice.276 Thrombin was demonstrated to stimulate angiogenesis, among others, via induction of the main proangiogenic factor VEGF and its receptors.277,278 The increased permeability of small blood vessels is one of the VEGF-mediated effects.41 Interestingly, intracerebral infusion of argatroban leads to reduction of brain edema, inhibited tumor growth, and diminished tumor–related neurologic deficits in glioma rat models.279 Furthermore, systemic administration of argatroban reduced tumor mass, attenuated neurologic deficits, and prolonged survival in rats with gliomas as well.280–282 Moreover, argatroban completely abrogated thrombin–induced VEGF synthesis in an experimental model.283

Another specific thrombin inhibitor, hirudin, inhibited metastases formation in a wide variety of human tumor models.33,283–286 It also reduces by almost one half the amount of platelet–derived VEGF released at the site of the thrombus formation and inhibits metastasis in experimental models.287 However, because hirudin and its fragments exert an inhibitory activity toward the thrombin–fibrinogen interaction, this drug has been less intensively investigated in preclinical studies.

Another option of anticancer treatment is the administration of a biologically active form of APC, which does not increase the risk of bleeding complications.143,145 Recently, in a phase III study (ENHANCE US), it was revealed that recombinant APC (drotrecogin α) significantly improved the results of treatment of patients with sepsis.288

Most thrombin effects are exerted via its binding to thrombin receptor, PAR. Both PAR-1 and PAR-2 were documented to mediate cancer cell motility and metastasis formation.289 PAR-1 is the dominant receptor that regulates angiogenesis, an essential step in tumor progression.290 Thus, another option in anticancer treatment consists of introducing small molecular inhibitors of PARs, such as RWJ-58259.291 This compound binds PAR-1 resulting in a strong antithrombotic effect in vivo.291 Of interest, PAR-1 inhibitors (e.g., SCH-79797, RWJ-56110) were documented to inhibit angiogenesis in cancer patients.292

**Heparins**

The highly negatively charged polysulfated glycosaminoglycan heparin is synthesized by mast cells and possibly ECs.292 Commercially available forms of heparin include unfractionated heparin (UFH; a mixture of glycosaminoglycans with molecular weights ranging from approximately 5 to 30 kDa) and LMWH (molecular weight of 4 to 6 kDa). The latter form of heparin derives from cleavage of UFH at unsulfated glucosamine residues. The anticoagulant effect of both forms of heparin requires binding to AT, which leads to a conformational change and increased serine proteinase–inhibitory activity of the latter. Longer chains of UFH form a tertiary complex with AT and thrombin resulting in the inhibition of both factor Xa and thrombin. To the contrary, LMWHs exert their inhibitory activity selectively toward factor Xa. Moreover, heparin administration leads to TFPI and t-PA release from ECs and increased expression of cell–surface P–selectin. More predictable pharmacokinetics of LMWH improves safety and offers convenience with its prolonged administration in clinical settings.

Several meta-analyses of randomized clinical trials comparing the advantage of UFH and LMWHs for treatment of thromboembolic disease published in the 1990s revealed improved survival in the subpopulation of cancer patients undergoing LMWH therapy.293–296 Recent results of randomized clinical trials focused on the role of LMWH in patients with malignancy attract particular attention. Namely, in nonthrombosis cancer patients, 1–year dalteparin administration prolonged survival in relation to placebo-treated patients (FAMOUS study).37 A 2-week period of therapeutic doses of nadroparin, with a subsequent 4-week period of half-therapeutic dose of the drug administration, improved overall survival in cancer patients with no history of thrombosis (MALT study).297 The addition of prophylactic doses of dalteparin, along with chemotherapy administration, in SCLC patients improved progression–free survival.298 Furthermore, secondary prophylaxis of thrombotic disease with dalteparin in cancer patients results in prolonged overall survival compared with oral anticoagulant therapy (CLOT study).299 In the above-mentioned trials, the beneficial effect of LMWH
on cancer patient survival was particularly pronounced in patients with either a limited disease or a good prognosis. A pilot study in advanced melanoma patients demonstrated that long-term enoxaparin therapy increases survival in comparison with chemotherapy administration or best supportive care.35

Numerous biological properties of LMWH were demonstrated that influence cancer biology. The anti-cancer effects of LMWH may be attributed to its anti-coagulant activity as hemostatic proteins contribute to local growth and metastatic dissemination of cancer.

LMWH inhibits TF expression on ECs,300 which may inhibit the multifaceted TF effect on cancer progression (described earlier). Moreover, LMWH up-regulates TFPI expression on ECs,300,301 TFPI is known to exert an inhibitory effect on early stages of metastasis and exhibits inhibitory activity on angiogenesis. Heparin-mediated TFPI release from ECs is thought to contribute to the latter process.301 However, it was widely documented that the role of LMWH by far exceeds its function in hemostasis. Interactions of platelets and tumor cells mediated by P-selectin are inhibited by LMWH, limiting the metastatic process.287 Recent results from experimental studies provide evidence that the LMWH-dependent effect on cancer progression inhibition results mainly from its antimetastatic function.302 Of interest, LMWH was documented to inhibit heparanase, an enzyme endowed with heparin sulfate degradation properties.303 Heparanase is overexpressed in tumors with a high degree of malignancy.303 Its activity leads to an increased invasion of tumor cells.303 Heparan sulfate–bound proangiogenic factors are relatively inactive and protected from proteolytic degradation.304 Heparanase releases proangiogenic factors from components of the tumor environment, facilitating new vessel network formation.303 Furthermore, binding of LMWH to growth factors may prevent binding to respective receptors.304,305 Heparin fragments of fewer than 18 glycoside residues decrease VEGF activity, whereas heparin fragments of fewer than 10 glycoside residues reduce basic fibroblast growth factor (bFGF) activity.306 In fact, LMWHs inhibit VEGF- and bFGF-induced angiogenesis in experimental models.307,308 Interestingly, in vitro experiments in high-grade glioma models demonstrated that LMWH stimulates tumor cell apoptosis and inhibits tumor growth.309

Platelet Inhibitors
It has been amply documented that blood platelets perform multidirectional functions in tumor progression.29 Various studies were conducted to establish the role of interfering with platelet activity as anticancer therapy.

Platelet surface glycoproteins (e.g., GPIIb-IIIa complex) were shown to play an indispensable role in the process of metastatic dissemination.310 Administration of specific antibodies directed to GPIIb–IIIa complex on the platelet surface results in the inhibition of fibrinogen–platelet binding followed by a reduction in platelet aggregates formation and was demonstrated to exert an antineoplastic effect.311 Several candidates for such therapy, including murine antibody 7E3, chimeric human/murine antibody ReoPro (abciximab) (Eli Lilly & Co.), and cyclic heptapeptide Integrin (eptifibatide) (Millenium Pharmaceuticals, Inc.), are currently under investigation.311 A monoclonal antibody, 10E5, inhibited platelet GPIIb–IIIa with subsequent inhibition of lung colonization by cancer cells.312 Abciximab inhibits platelet aggregation and angiogenesis.313 The murine version of abciximab markedly diminished tumor cell–induced thrombocytopenia and inhibited formation of lung metastases in a rat model.314 Moreover, the monoclonal antibody 7E3 F(ab′)2 reduced platelet-stimulated sprouting of ECs.292,315 Of interest is a new class of drugs, namely small molecular peptidomimetic inhibitors of GPIIb–IIIa.314 These oral agents were documented to exert an anti-invasive effect in vitro and antimetastatic activity in vivo.314 Furthermore, snake venom Arg-Gly-Asp–containing peptides (e.g., triflavin, rhodostomin, trigramin) proved to inhibit TCIPA in experimental models.315–318 This effect is mediated principally by binding of the peptides to fibrinogen receptor associated with GP IIb/IIIa complex on platelet surface.315–318 Moreover, heparin can directly bind GPIIb–IIIa.319,320 Activated platelets express P-selectin on their membrane, which regulates platelet interactions with mononuclear cells and cancer cells. Heparin abrogates cancer cell–EC and cancer cell–platelet interactions mediated by selectins.287,321 It was demonstrated that heparin inhibited P-selectin–based platelet interactions with tumor cell surface mucins and reduced metastatic dissemination.287,321 Recently, several clinical trials have proved the advantageous effects of LMWH administration on cancer patient survival (the topic is widely described elsewhere in this issue).

Hemostatic System Inhibitors in Treatment of Radiation Therapy Complications
Normal tissue tolerance is a dose-limiting factor in radiation therapy. Endothelial dysfunction and thrombosis play a role in the pathophysiology of radiation injury. Inhibitors of the hemostatic system not only contribute to acute and chronic complications but also modulate normal tissue recovery process. Short-term thrombin inhibition with hirudin attenuates intestinal radiation toxicity in rats,322 whereas administration of heparin or warfarin leads to recovery of nervous system function in humans.323 However, experimental studies conducted in rat models revealed exacerbation of acute intestinal toxicity after nadroxiaparin administration that resulted from reduced anti-inflammatory mechanisms
Antiplatelet drugs (ticlopidine, clopidogrel) prevent or ameliorate radiation enteropathy.\textsuperscript{325,326} Ticlopidine was demonstrated to inhibit the prothrombotic effects of thrombopoietin and to ameliorate survival after supralethal body irradiation.\textsuperscript{327} Despite these promising data, inhibitors of the hemostatic system need to be further tested in clinical trials prior to introducing them to the clinic for the treatment of radiation injury.

**CONCLUSION**

Recent discoveries have provided new insight into the nature of interactions between hemostasis and cancer, although the first observation of an increased frequency of thrombosis in cancer patients dates back to the 19th century. Mounting evidence of a reciprocal interplay among hemostatic system (coagulation, fibrinolysis, blood platelets) functions, cancer coagulopathy, and processes vital for tumor biology (such as tumor cell proliferation, differentiation, migration, angiogenesis, proteolysis, invasion, and metastasis) has been accumulated. New light was shed on antineoplastic and pro-apoptotic (with regard to tumor cells) functions of hemostatic proteins. Clinical trials with the use of anti-coagulants in cancer patients proved the effectiveness of LMWH. As a consequence, novel strategies of prevention and treatment of venous thromboembolic episodes in this subset of patients have been formulated. However, of paramount importance, it has been realized that anti-coagulants may offer much more (i.e., they may prolong cancer patient survival). Recently, a spectrum of new-generation anticoagulants has been introduced into clinical practice. Unfortunately, up to now, none of them has been tested in well-designed clinical trials. However, data compiled on the interaction between hemostasis and malignancy warrants clinical trials with the use of new compounds interfering with hemostasis. Would they hold the promise of creating a novel, effective and safe form of adjunct treatment of cancer patients? It seems worthwhile to address this question in a clinical setting.

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**ABBREVIATIONS**

\begin{tabular}{ll}
\(\alpha_2\)-AP & \(\alpha_2\)-antiplasmin \\
aaAT & antiangiogenic AT \\
APC & activated PC \\
AT & antithrombin \\
AT III & antithrombin III \\
bFGF & basic fibroblast growth factor \\
C4BP & C4 binding protein \\
CpG & cytosine-phosphorothioate-guanine \\
DVT & deep vein thrombosis \\
EC & endothelial cell \\
ECM & extracellular matrix \\
EGFR & epidermal growth factor receptor \\
EPCR & endothelial cell protein C receptor \\
GP & glycoprotein \\
HCII & heparin cofactor II \\
HCC & hepatocellular carcinoma \\
ICH & immunohistochemical \\
LMWH & low-molecular-weight heparins \\
MMP & matrix metalloproteinase \\
NSCLC & non–small cell lung cancer \\
PA & plasminogen activator \\
PAI-1 & plasminogen activator inhibitor-1 \\
PAR & protease activated receptor \\
PC & protein C \\
PCI & protein C inhibitor \\
PE & pulmonary embolism \\
PN-1 & protease nexin-1 \\
PS & protein S \\
PZ & protein Z \\
Rb & retinoblastoma \\
rNAPc2 & recombinant nematode anticoagulant protein c2 \\
SCLC & small cell lung cancer \\
sTM & soluble TM \\
TAFI & thrombin activatable fibrinolysis inhibitor \\
TAMs & tumor-infiltrating macrophages \\
TAT & thrombin–antithrombin \\
TCIPA & tumor cell-induced platelet activation \\
TF/VIIa & tissue factor/factor VIIa \\
TFPI & tissue factor pathway inhibitor \\
TLTR & “tethered ligand” thrombin receptor \\
TM & thrombomodulin \\
t-PA & tissue plasminogen activator \\
UFH & unfractionated heparin \\
uPA & urokinase plasminogen activator \\
uPAR & urokinase plasminogen activator receptor \\
VEGF & vascular endothelial growth factor \\
VLDL & very-low-density lipoprotein \\
VOD & venoocclusive disease \\
ZPI & protein Z–dependent proteinase inhibitor
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