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Pathogenesis of Thrombosis in Patients With Malignancy

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Abstract

Cancer cells can contribute to activation of the clotting system by their capacity to produce and release procoagulant/fibrinolytic substances and inflammatory cytokines, and by their interaction with host cells (endothelial, monocytes, platelets, and neutrophils). Moreover, anticancer drugs (chemotherapy/hormone therapy) may greatly affect the risk of thromboembolic complications in cancer patients by similar mechanisms, eg, through the release of procoagulants by tumor cells, through endothelial damage, or stimulation of tissue factor production by host cells. The interactions between cancer/metastatic processes and thrombosis have been reviewed here from the pathogenetic viewpoint. We hope that better knowledge of these pathogenetic pathways will lead to the development of more targeted strategies to prevent thromboembolism in cancer patients. *Int J Hematol.* 2001;73:137-144.

Key words: Thrombosis; Cancer; Metastasis; Tumor procoagulants; Cancer chemotherapy

1. Introduction

Thrombosis is a frequent complication of malignancy and represents the second most important cause of death in cancer patients. Postmortem studies have shown an increased incidence of thromboembolic disease in cancer, particularly in patients who died of mucinous carcinoma of the pancreas, lung, and gastrointestinal tract [1,2]. Histological analyses have also documented the presence of fibrin or platelet plugs in and around many types of tumors, suggesting a local activation of coagulation [3].

Thrombosis often represents the earliest clinical manifestation of cancer, and it may be triggered in these patients by surgery or other predisposing conditions (chemotherapy, radiotherapy, long-term bedrest, or trauma). Thromboembolic disorders in cancer patients include venous and arterial thrombosis, migratory thrombophlebitis, pulmonary embolism, thrombocytic nonbacterial endocarditis, and the clinical manifestations of disseminated intravascular clotting, ie, thrombotic microangiopathy and disseminated intravascular coagulation (DIC). Severe DIC is generally associated with acute leukemia and is characterized by life-threatening hemorrhages caused by an excessive consumption of clotting factors and platelets [4].

However, even in the absence of obvious thrombosis, cancer patients with solid tumors and leukemias commonly present with abnormalities in laboratory coagulation tests, characterized by varying degrees of clotting activation, indicating a subclinical hypercoagulable condition [5-7]. The results of laboratory tests demonstrate that fibrin formation and removal is continuous during the development of malignancy. It is important to remember that fibrin formation can play a role not only in thrombogenesis but also in tumor progression [8]. Fibrin deposits on potentially metastatic blood-borne malignant cells mediate the attachment of these cells to the vascular endothelium, favoring extravasation and the formation of distant metastases. Pharmacologic interventions to block thrombogenicity in malignancy could be important not only for reducing thromboembolic complications, but also for improving the treatment of cancer.

The purpose of this brief survey is to outline the development of our knowledge about the interactions between the hemostatic system and malignant disease, considering the recent progress in cell biology and biochemistry of tumor cell activities, to provide useful information on the pathogenesis of thrombotic complications in the cancer patient.

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2. Clinical Conditions

Thromboembolic disease can be the earliest clinical sign of a tumor. This observation, originally reported by the French clinicians Armand Trousseau over a century ago, was first confirmed by anecdotal reports and retrospective studies. In recent years, however, controlled prospective clinical trials have clearly shown that in the absence of conventional risk factors (ie, idiopathic thrombosis), patients with deep vein thrombosis (DVT) have a substantial likelihood of having cancer [9], which may be occult or at a very early stage [10]. This raises the question of whether aggressive diagnostic screening for cancer in patients with idiopathic DVT may lead to improved management of malignancy in these patients [11]. Recently, a prospective multicenter study called SOMIT (Screening for Occult Malignancy in patients with venous Thromboembolism) has been conducted in Italian centers to compare the clinical importance of an extensive cancer screening versus routine examinations for patients who present with DVT and no apparent risk factors [12]. The trial is now closed and the results are under evaluation.

On the other hand, in patients diagnosed with cancer who therefore have a high risk of thrombosis, the occurrence of clinically overt thrombosis depends on a number of variables, such as the type of tumor (eg, adenocarcinomas of the gastrointestinal tract or ovary are considered particularly high-risk malignancies), the median survival period of patients with different tumors, and the types of therapy administered. Both surgery and chemotherapy increase the cancer-associated risk of developing thrombosis [5].

Surgery, the first choice of therapy for most localized solid tumors, is a precipitating factor for thromboembolic disease, because it activates the hemostatic system [13]. The risk of postoperative thrombosis is increased approximately 2-fold in patients with malignancy compared to patients without malignancy. A prospective study suggests that preoperative evaluation of thrombosis markers, such as the plasma level of thrombin–antithrombin complex, may be useful to identify those patients at higher risk for postoperative DVT [14].

The role of medical interventions (ie, chemo-, hormone-, and radiotherapy), in increasing the thrombotic risk in malignancy has been demonstrated by retrospective and prospective clinical studies [15-17]. For several years, thrombotic complications have been described in association with specific chemotherapeutic agents, such as L-asparaginase, mitomycin C, cisplatin, and high-dose chemotherapy conditioning regimens for bone marrow transplantation [15]. Prospective controlled studies have shown that conventional chemotherapy commonly used for breast cancer can increase the risk of thromboembolism [16] and that prophylaxis with low-dose warfarin in these patients can significantly decrease this risk [18]. In addition, hormone therapy with tamoxifen is a risk factor for thrombosis in breast cancer. In premenopausal women, the combination of chemotherapy with tamoxifen has been associated with more thrombotic complications than chemotherapy alone [17]. Also, the use of hematopoietic growth factors [ie, granulocyte colony-stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF)] may be implicated in hypercoagulation and clot formation in breast cancer [19,20].

Finally, it is important to consider that the majority of cancer patients (without any clinical sign of thrombosis and before any therapy) have abnormal laboratory coagulation test results that are consistent with a hypercoagulable state or low-grade DIC. The development of novel, more sensitive laboratory tests has enabled detection of subtle alterations in the hemostatic system. Plasma markers of clotting activation such as thrombin–antithrombin complex, prothrombin fragment 1 + 2, fibrinopeptide A, and D-dimer are all elevated in patients with cancer [21-25]. These results clearly demonstrate that thrombin generation and fibrin formation are constantly found in studies of these patients who are at increased risk of thromboembolic complications. However, despite numerous studies describing all types of hemostatic abnormalities, few attempts have been made to assess the clinical utility of laboratory testing for predicting thrombosis in these patients.

3. Pathogenetic Mechanisms

Many factors, including general factors, tumor-specific factors, and chemotherapy-related factors can contribute to the activation of blood coagulation and thrombotic diathesis in patients with cancer.

General mechanisms for clotting activation in malignancy are related to the host response to the tumor and include the acute-phase reaction, paraprotein production, inflammation, necrosis, and hemodynamic disorders. However, tumor-specific clot-promoting mechanisms have a prominent role in the activation of the hemostatic system in cancer. Of particular interest are a number of procoagulant effects triggered by chemotherapy.

3.1. Tumor Cell Prothrombotic Mechanisms

Tumor cell prothrombotic mechanisms are schematically represented in Figure 1. Malignant cells can interact with the hemostatic system in multiple ways. The principal prothrombotic properties of tumor cells are: the ability to interact with host cells, including endothelial cells lining the blood vessels, monocytes, platelets, and neutrophils; the ability to produce and release procoagulant and fibrinolytic substances, as well as inflammatory cytokines such as interleukin 1β (IL-1β), tumor necrosis factor (TNF)-α, and vascular endothelial growth factor (VEGF).

3.1.1. Tumor Cell–Host Cell Interactions

Studies of laboratory parameters demonstrate the activation/perturbation of several cellular systems in vivo in the cancer patient. Indeed, findings of elevated plasma levels of endothelial markers (eg, von Willebrand factor, thrombomodulin, soluble E-selectin, tissue-type plasminogen activator [tPA], and PA inhibitor [PAI]) [26] indicate the activation of hemostasis at the endothelial level, especially during chemotherapy [27-29]. Further, the increase in tissue factor (TF) procoagulant activity (PCA) expressed by circulating mononuclear cells shows that this cellular compartment becomes activated [30]. Finally, the detection by flow cytometry of high levels of platelet membrane–specific glycopro-

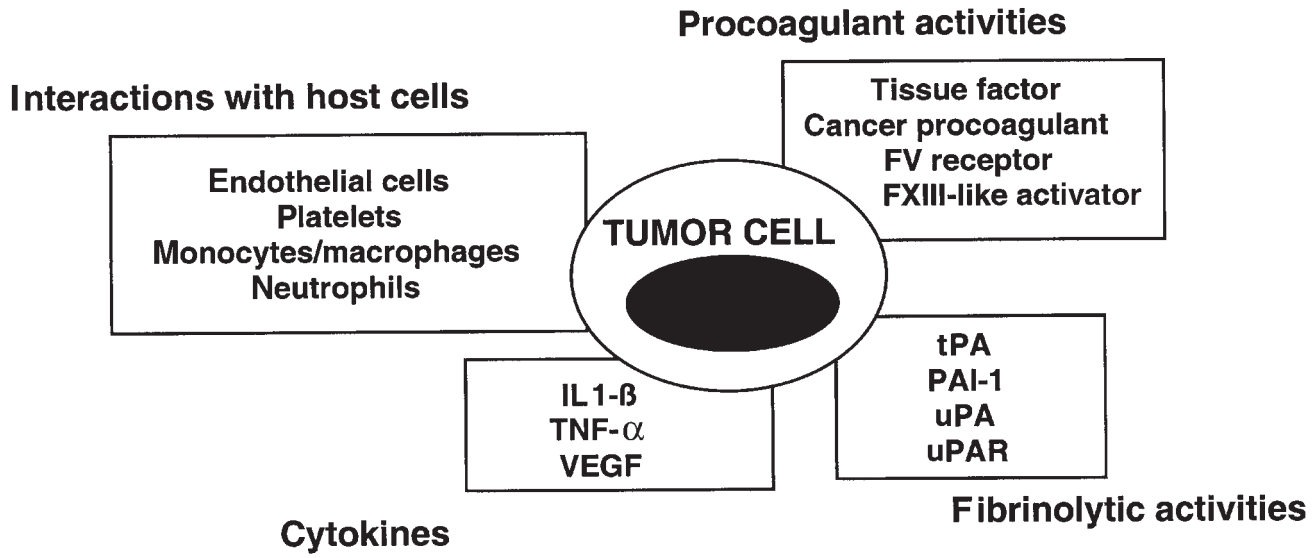

Figure 1. Principal pathways of tumor cell interactions with the hemostatic system. Tumor cells express the cellular procoagulants: TF, CP, a Factor V receptor, and a Factor XIII–like substance that activate the clotting cascade; fibrinolysis proteins: uPA, tPA, and PAI; receptor: uPAR; and cytokines, including IL-1β, TNF-α, and VEGF, that induce the endothelium thrombogenicity. They also interact (directly or through soluble mediators) with other blood cells, ie, endothelial cells, monocytes, platelets, and neutrophils. TF indicates tissue factor; CP, cancer procoagulant; uPA, urokinase-type plasminogen activator; tPA, tissue-type plasminogen activator; PAI, plasminogen activator inhibitor; uPAR, urokinase-type plasminogen activator receptor; IL, interleukin; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

teins which are exposed upon activation of the hemostatic system, provides evidence for in vivo platelet activation in malignant conditions [31]. Recently, neutrophil activation in patients with myeloproliferative disorders has also been demonstrated by different tests such as increased cell membrane CD11b expression and increased content and release of endogenous elastase and cathepsin G, which possess prothrombotic properties [32].

3.1.1.1. Interactions of Tumor Cells With the Vascular Wall

Tumor cells can interact with the endothelium through their ability to synthesize and release inflammatory cytokines. Tumor cell–derived inflammatory cytokines, including TNF-α and IL-1β, can induce the expression of TF procoagulant activity by endothelial cells (Figure 2) [33]. They also downregulate the expression of endothelial cell thrombomodulin (TM), the surface high-affinity receptor for thrombin. The TM–thrombin complex activates the protein C system, which functions as a potent anticoagulant. TF upregulation and TM downregulation lead to a prothrombotic condition of the vascular wall [34,35]. The same cytokines stimulate endothelial cells to produce the fibrinolysis inhibitor PAI-1. Inhibition of fibrinolysis further contributes to the prothrombotic potential of endothelial cells [36].

Tumor cells are also able to directly adhere to endothelial cells and to the extracellular matrix through membrane adhesion molecules [37,38]. Endothelial cells activated by IL-1β or TNF-α increase the exposure on their membranes of counterreceptors for the tumor-adhesion molecules. Malignant cells attached to the vessel wall may play a key role in promoting localized clotting activation and thrombus formation by releasing cytokines and favoring the adhesion and arrest of other cells, including leukocytes and platelets. The adhesion of tumor cells to each other or to vascular cells may also facilitate cell migration and extravasation. In addition, the TF-induced expression of VEGF by endothelial cells may have implications in tumor neovascularization [39].

3.1.1.2. Interaction of Tumor Cells With Platelets

Platelets can be activated by cells from human and experimental tumors. Upon activation, they aggregate and release their granule contents, as shown by in vitro and in vivo studies [40]. Compared with those in complete remission, patients with active malignant disease have elevated levels of β-thromboglobulin and platelet factor 4 [41]. Circulating activated platelets have also been demonstrated in cancer patients by detection of the platelet membrane antigens CD62 and CD63 [42]. Tumor cells or membrane vesicles that have been shed spontaneously from tumor cells can directly aggregate platelets in vitro [43], and can induce platelet aggregation through the release of proaggregatory mediators including adenosine diphosphate, thrombin, and a cathepsin-like cysteine proteinase [44].

3.1.1.3. Interaction of Tumor Cells With Monocyte-Macrophages

Monocyte-macrophages circulate in the bloodstream or localize on the vascular wall in response to inflammatory

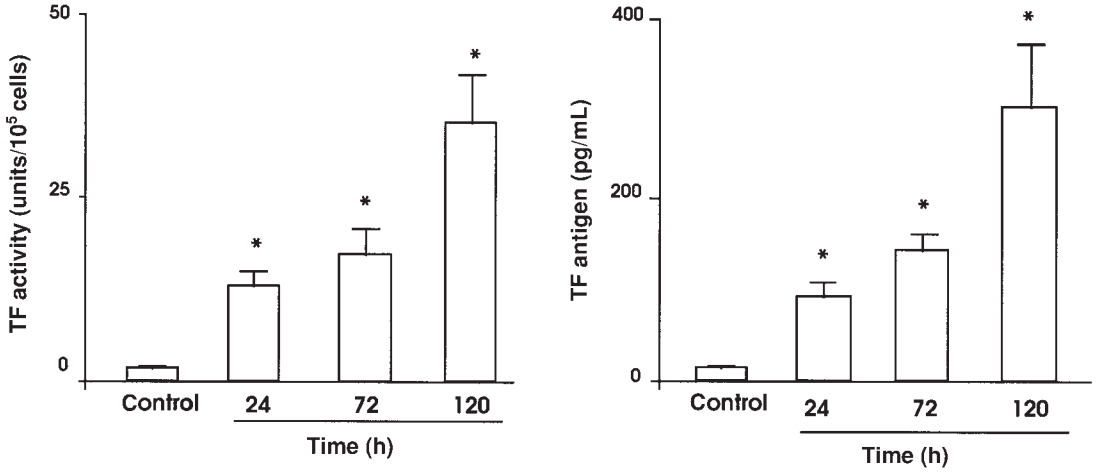

Figure 2. NB4 leukemic cells induce the expression of endothelial cell tissue factor. NB4 cells were grown, up to 72 hours, and the conditioned media (CM) was collected every 24 hours. Endothelial cell monolayers were exposed to NB4-CM for 4 hours at 37°C. After incubation, the expression of TF by endothelial cells was measured both by activity (left panel), using the 1-stage clotting assay of normal human plasma, and by antigen concentration (right panel), using an enzyme-linked immunosorbent assay (ELISA). The asterisk indicates statistically significant differences from the control. TF indicates tissue factor.

stimuli. They also constitute an integral part of the lymphoreticular infiltrate of the tumor mass. Like endothelial cells, mononuclear phagocytes do not constitutively express TF, but generate and expose this procoagulant on their surface in response to different stimuli, including bacterial endotoxins, inflammatory particles, complement proteins, immune complexes, and lymphokines. In vitro studies show that tumor cells and/or tumor cell products can induce the expression of monocyte TF [45]. Mononuclear cell activation may occur in vivo as well. Indeed, tumor-associated macrophages harvested from experimental and human tumors express significantly more TF than control cells [45,46]. This mechanism might contribute to the activation of the hemostatic system and deposition of fibrin within tumor tissues. Furthermore, circulating monocytes from patients with different types of cancer have been shown to express increased TF activity [30]. The generation of procoagulant substances by monocyte-macrophages in vivo is a possible mechanism of clotting activation in malignancy [45]. Also, there is evidence that tumor-associated macrophages respond to tumor-derived mediators not only by exposing TF, but also by increasing their fibrinolytic enzyme production [47].

3.1.2. Procoagulant Activities

These procoagulant properties of tumor cells enable them to promote fibrin formation at sites of extravasation and in the tumor microenvironment at the extracellular level [48,49]. Tumor cells may express different types of procoagulants, of which TF and cancer procoagulant (CP) are the best characterized (Figure 3). Other tumor cell procoagulants are a factor V receptor associated with vesicles shed from tumor cell plasma membranes, which binds factor V, thus facilitating the assembly of prothrombinase complex [50], and a factor XIII–like substance that promotes the cross-linking of fibrin [51].

3.1.2.1. Tissue Factor

TF is a 47-kd transmembrane glycoprotein that forms a complex with factor VII (FVII)/FVIIa. The TF/FVII complex triggers blood coagulation by proteolytically activating factors IX and X [52,53]. TF is the cellular procoagulant found in normal cells, including endothelial cells and monocyte-macrophages. However, as mentioned, these cells do not express TF in normal resting conditions, but expose this procoagulant in response to proinflammatory stimuli, ie, the cytokines IL-1β and TNF-α and bacterial endotoxins [45,53]. In contrast to normal cells, malignant cells express TF constitutively. It is noteworthy that in addition to activating blood coagulation, TF has the capacity to regulate VEGF expression in tumor cells and in vascular endothelium [54], which represents an important proangiogenetic mechanism.

3.1.3. Cancer Procoagulant

CP is a 68-kd cysteine proteinase (EC 3.4.22.26) with 674 amino acid residues and no detectable carbohydrate [55]. It activates factor X independently of FVII and cleaves the factor X heavy chain at a different site compared with other known factor X activators [56,57]. CP has been found in extracts of neoplastic cells and in amnion–chorion tissues, but not in extracts of normally differentiated cells [55,58-61]. Using enzyme-linked immunosorbent assay (ELISA), CP antigen has been identified in the sera of cancer patients and has been found to be elevated in 85% of the study subjects [62]. These findings are consistent with the results of tests determining the procoagulant activity of CP in the sera of patients with cancer [63]. TF and CP have been identified in several human and animal tumor tissues [50,64]. The 2 proteins are different in nature and therefore different methods are required to analyze them [49].

In recent years, many studies have focused on the PCA expressed by leukemic cells. Several authors have identified TF in these cells [52,65,66]. Donati et al, Falanga et al, and Alessio et al [61,67,68] have reported CP in blasts of various acute myelogenous leukemia phenotypes, with the greatest expression in acute promyelocytic leukemia (APL). Interestingly, in patients with acute myeloid leukemias, CP was detected in the bone marrow mononuclear cells at the onset of the disease, but not in cells from the same subjects upon complete remission [61]. These findings support the hypothesis that CP may be expressed by undifferentiated cells, but is repressed once normal differentiation occurs. Recent data show that differentiating treatment with all-*trans*-retinoic acid (ATRA) of cultured APL cells in vitro can influence their PCA. Indeed, the ATRA-induced cell differentiation downregulates the expression of both CP [69] and TF [65,66] in APL cells. ATRA also inhibits the leukemic cell PCA in vivo: levels of both TF and CP in marrow blasts are progressively reduced in patients given ATRA for remission-induction therapy of APL [21]. The demonstration that this effect parallels the improvement of the plasma coagulation parameters provides the first in vivo evidence of a role of tumor cell PCA in the hemostatic complications associated with malignancy [70]. Falanga et al [60] have also demonstrated that after treatment with ATRA, CP activity is virtually abolished only in NB4 cells sensitive to ATRA-induced cytodifferentiation and not in the differentiation-resistant cells, whereas TF activity is significantly reduced in all cell lines regardless of their sensitivity to ATRA [60]. These results are in agreement with the characteristics of the 2 procoagulants and provide support for the concept that CP is a differentiation-dependent protein, which might provide a new tool (marker) to monitor cancer cell maturation, although TF regulation is more complex and involves either normal or tumor cells.

3.1.4. Fibrinolytic Activities

Tumor cells can express all the proteins regulating the fibrinolytic system, including the urokinase-type PA (uPA), tPA, and the fibrinolysis inhibitors PAI-1 and PAI-2 [71]. Among the activators, uPA is the most widely expressed within malignant lesions [54]. Furthermore, cancer cells can carry the specific PA receptor (uPAR) on their membranes. The presence of these receptors favors the assembly of all the fibrinolytic components on tumor cell membranes, facilitating the activation of the fibrinolytic cascade [72], and is believed to play a role in the pathogenesis of the bleeding symptoms characteristic of malignant disease [70]. The finding of impaired plasma fibrinolytic activity (by global tests, ie, the euglobulin lysis area on fibrin plates) in patients with solid tumors represents another tumor-associated prothrombotic mechanism [73]. Recent data strongly suggest that in addition to their activities in the regulation of fibrinolysis, PAs and their inhibitors may play a role in tumor invasion, tumor cell proliferation, and metastasis. In addition, they are under evaluation as potentially valuable predictors of disease-free intervals and long-term survival in malignant disease [71].

3.1.5. Cytokines

Tumor cells produce inflammatory cytokines, including TNF-α, IL-1β, and VEGF. The role of TNF-α and IL-1β in regulating a number of hemostatic functions of endothelial cells and monocytes (ie, the expression of TF, TM, and adhesion molecules), has been elucidated (see section 3.1.1.1.). In addition to those cytokine activities, the production of VEGF by malignant cells may significantly affect the functions of the microvasculature in proximity to the tumor and may play an important role in tumor neoangiogenesis [39,74]. Furthermore, VEGF is chemotactic for macrophages and can induce TF procoagulant activity of monocytes and endothelial cells [75]. Interestingly, the expression of TF by tumor cells upregulates the transcription of VEGF in these cells [76]. Finally, TF modulates the expression of VEGF by endothelial cells, a function that can have important implications in tumor neovascularization [39]. Regulation of VEGF synthesis by TF in malignant cells and vascular cells provides an important link in cancer patients between activation of coagulation, inflammation, and thrombosis and tumor progression and metastasis [77].

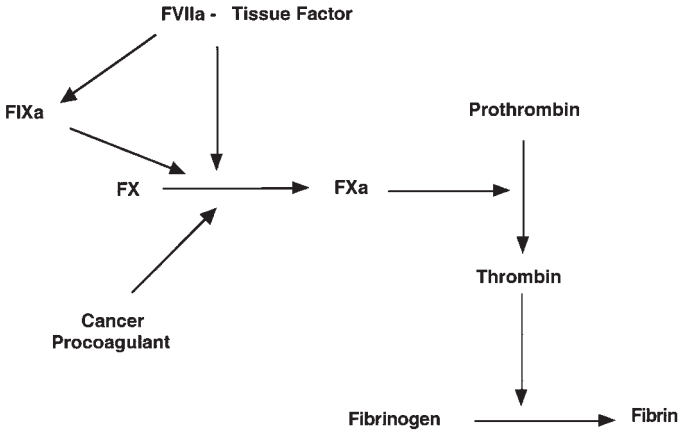

Figure 3. The mechanism of intervention in the clotting cascade of the 2 best studied tumor procoagulants: tissue factor and cancer procoagulant.

3.2. Antitumor Drug Prothombotic Mechanisms

In recent years, there has been increasing evidence that anticancer therapy can affect the risk of thromboembolic complications in this disease [15,16,18]. A number of mechanisms for anticancer drug–related thrombosis have been identified. The first mechanism is due to the release of procoagulants and cytokines by tumor cells that have been damaged by chemotherapy. This mechanism is considered to be responsible for the exacerbation of DIC in acute leukemias which is evident from laboratory and clinical data upon starting chemotherapy [70]. In addition, the downregulation of TF and CP in blasts of patients with APL, paralleled by improvement in laboratory and clinical signs of hypercoagulation in these subjects, provides strong evidence of a role for procoagulant activities in the pathogenesis of DIC [21]. Also, the release of cytokines in response to chemotherapy may have important implications for increasing thrombotic risk. This was suggested by Bertomeu et al [78], who demonstrated that plasma sam-

ples collected from women with breast cancer postchemotherapy contained higher levels of mediators (probably cytokines) that were able to increase the reactivity of endothelial cells to platelets.

Another mechanism involves the direct damage exerted by chemoradiotherapy on vascular endothelium. Using an in vitro assay to detect the sublethal effects of drugs on endothelial integrity in tissue culture, 2 categories of chemotherapeutic agents have been identified [79]. The differences may depend on the drugs' different mechanisms of cytotoxicity. Also, radiation therapy can cause endothelial injury, as demonstrated by the release of von Willebrand protein from human umbilical vein endothelial cells irradiated with doses of up to 40 Gy [80]. In animal studies, bleomycin has been implicated in morphological damage to vascular endothelium of the lung, which may result in pulmonary thrombosis and fibrosis. Furthermore, in some experimental models, adriamycin has been shown to directly affect glomerular cells, impairing their permeability and leading to nephrotic syndrome accompanied by hypercoagulation and increased thrombotic tendency [81]. Although this mechanism has not been confirmed in human studies, profound changes in plasma levels of markers of endothelial damage have been reported in patients receiving chemotherapy [27-29].

A third prothrombotic mechanism of antitumor therapy is the direct stimulation of expression of TF-procoagulant activity in macrophages and monocytes by some chemotherapeutic agents [82]. Therefore, chemotherapy can induce a procoagulant response from host cells.

The last mechanism involves the reduction in the plasma levels of anticoagulant proteins (antithrombin, protein C, and protein S), a consistent finding after chemotherapy [83] and a well known risk factor for thrombosis. This defect in naturally occurring anticoagulants is likely to be a consequence of direct hepatotoxity of radio- and chemotherapy.

4. Summary and Conclusions

The pathogenesis of hemostatic disorders in cancer is complex and reflects the interaction of different mechanisms involving the activation of various hemostatic components such as the coagulation and fibrinolytic systems, vascular endothelium, monocytes, and platelets. The results of laboratory tests clearly demonstrate that the rate of fibrin formation and dissolution are accelerated in patients with cancer, predisposing to an increased risk of secondary thrombosis. Also, fibrin formation is involved in metastatic processes.

Tumor cells interact with all parts of the hemostatic system. They can directly activate the coagulation cascade by producing their own procoagulant factors or they can stimulate the prothrombotic properties of other blood cell components. Additional mechanisms of clotting activation are initiated by chemotherapy or other cancer therapies.

In the last 10 years, research has greatly improved our knowledge of tumor-promoted prothrombotic functions. Understanding the molecular basis of the underlying mechanisms may help to identify more targeted strategies to prevent thromboembolism in cancer patients, particularly when surgery or medical therapies are planned.

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