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Hemostatic biomarkers in cancer progression

Anna Falanga*, Marina Marchetti

Division of Immunohematology and Transfusion Medicine, Hospital Papa Giovanni XXIII, Bergamo, Italy

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ABSTRACT

Malignant disease is characterized by a hemostatic imbalance, usually shifted towards a procoagulant direction, and a high incidence of thrombotic complications. The mechanisms of hemostasis that are critically involved in thrombosis are also implicated in tumor progression, angiogenesis, and metastatic spread. As there is a close relationship between cancer and the clotting system, circulating biomarkers of activation of various hemostasis compartments (i.e. coagulation, fibrinolysis, platelets, endothelium, and other blood cells) have been extensively studied to predict cancer outcomes along with predicting the thrombotic risk. In this review, we will summarize the results of published studies and will focus on ongoing research and future directions of clotting activation bioproducts as biomarkers of cancer disease and progression.

1. The biological significance of hemostatic biomarkers for cancer progression

The National Institute of Health defines a biomarker as a cellular, biochemical, and/or molecular entity that can be objectively measured and serve as an indicator of ongoing normal or pathogenic biological processes, or pharmacological responses to therapeutic interventions. In the context of malignant disease, biomarkers can play a crucial role in aiding the diagnosis of early stage cancers (*diagnostic biomarker*), estimation of tumor aggressiveness, predict the likelihood of patient survival in the absence of treatment (*prognostic biomarker*), and predict patient response to antitumor therapy (*predictive biomarker*). Despite the remarkable advances in tumor biology research and in "omics" technologies, limited tumor biomarkers have been adopted successfully into routine clinical care of oncologic patients over the last 30 years [1, 2]. The requirement for new biomarkers is continuously increasing for personalized medicine, the growing of the therapeutic armamentarium, and the evidence that early cancer detection in otherwise asymptomatic patients does improve both survival and quality of life.

Patients with cancer are commonly characterized by abnormalities in laboratory coagulation tests, underlying a subclinical hypercoagulable condition [3]. The results of laboratory tests demonstrate that a process of fibrin formation and removal is very active during the development of malignancy. Histopathological analyses demonstrate the presence of fibrin and platelet aggregates in and around various types of tumors, indicating local blood clotting activation. Different molecular mechanisms can cause the onset of a hypercoagulable state. In cancer, the interaction between tumor cells with the vascular endothelium, and constituents of the coagulation cascade is responsible for the conversion of the intravascular milieu into a prothrombotic, proinflammatory, and proadhesive environment (Fig. 1) [4, 5]. Hypercoagulability in cancer increases the risk of thromboembolic complications, which actually occur at a high rate compared to a non-cancer population [6]. Accordingly, a number of hemostatic biomarkers have been evaluated with regard to their capacity to predict primary or recurrent venous thromboembolism (VTE) in these patients, and some of these have been integrated in risk score models for the evaluation of individual VTE risk [7, 8].

However, hypercoagulability also influences the biology of the tumor, favoring its growth and development of metastasis [5]. Activation of blood coagulation results in a selective advantage for cancer cells. Indeed fibrin, the final product of the coagulation cascade, provides a scaffold for tumor cell anchorage and invasion, and protects the tumor cell from immune system recognition and destruction. Further, while activated coagulation proteases (i.e. thrombin, FVIIa, FXa) induce receptor-mediated intracellular signals promoting invasive growth [9] [10]. Furthermore, the cancer-associated hypercoagulable state can be linked to known oncogenetic lesions causal for the onset and progression of malignancy [11].

Due to the relationship between cancer and hemostasis, the biomarkers of hemostatic system activation can be a promising tool in predicting cancer outcomes. Several laboratory abnormalities of hemostasis have been described in cancer, including prolonged and shortened prothrombin time (PT), partial thromboplastin time (PTT), increased and decreased levels of thrombin, FV, FVIII, FIX, FXI, FXII,

* Corresponding author at: Division of Immunohematology and Transfusion Medicine, Hospital Papa Giovanni XXIII, Piazza OMS, 1, 24127 Bergamo, Italy.
E-mail address: afalanga@asst-pg23.it (A. Falanga).

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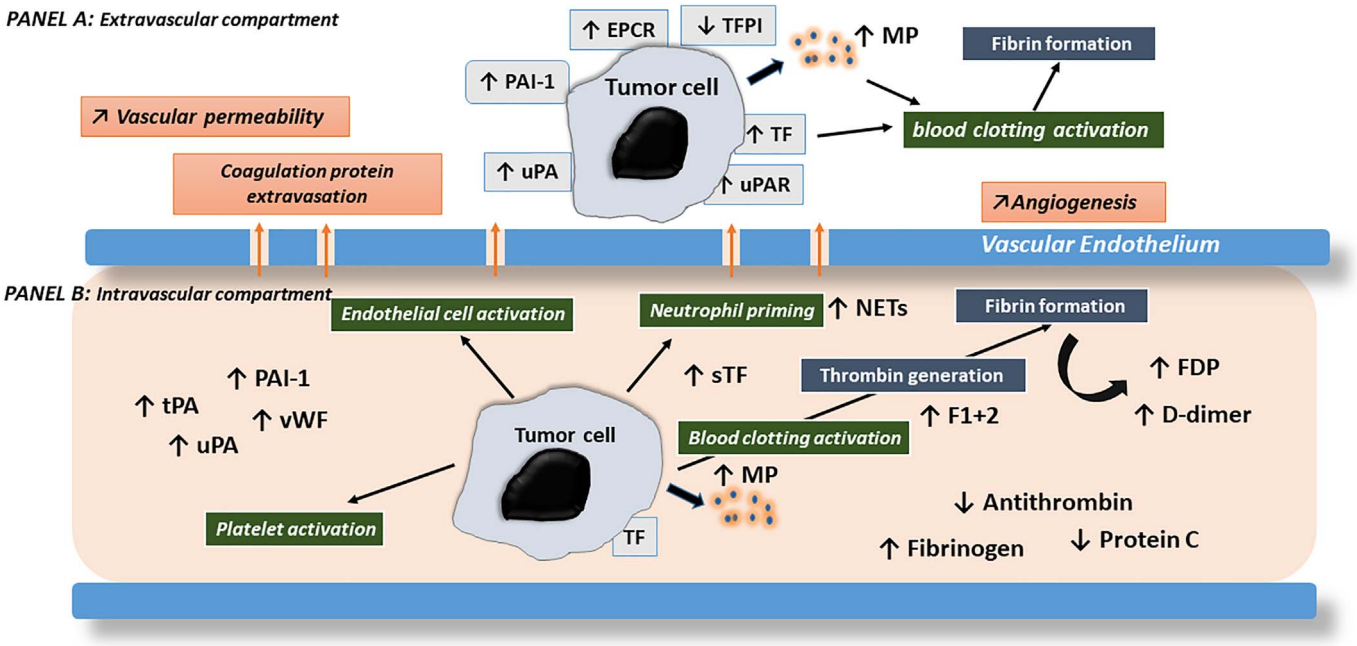

Fig. 1. Tumor cell associated biomarkers during cancer development and dissemination.

PANEL A. *Extravascular activation of blood coagulation by tumor cells*. Tumor-associated vasculature is characterized by an enhanced permeability induced by tumor cell derived proangiogenic factors. This permeability allows the extravasation of coagulation proteins into the tumor microenvironment, where they interact with tumor-associated TF. The assembly of coagulation proteins on tumor cells causes the local activation of blood clotting cascade, which is potentiated by the TF-bearing MP released by the tumor cells, and the low levels of TFPI, and that culminates in thrombin generation and fibrin formation. The fibrin formed around tumors serves as a temporary matrix for tumor cell migration, which is supported by the proteins of the fibrinolytic system expressed by the tumor tissues (i.e. uPA, tPA, uPAR, and PAI-1).

PANEL B. *Intravascular activation of blood coagulation by tumor cells.* Once in circulation, the tumor cell can activate, as well as blood coagulation, induce the prothrombotic features of endothelium and platelets, and NETosis by neutrophils. Reductions in the levels of natural anticoagulant (Protein C, Antithrombin), the increased thrombin generation, fibrin degradation products (D-dimer, FDP), and in fibrinogen levels are some of the hemostatic biomarkers modifications induced by the tumor cells. These alterations have a significant impact on both tumor metastasis and cancer-associated thrombosis.

Abbreviations: MP = microparticles, uPA = urokinase plasminogen activator, t-PA = tissue plasminogen activator, PAI-1, plasminogen activator inhibitor –1, TF = Tissue Factor, TFPI = TF pathway inhibitor, FDP fibrin degradation products, =NETs = neutrophil extracellular traps, EPCR = endothelial protein C receptor, F1 + 2 = prothrombin fragment 1 + 2.

fibrinogen, fibrinogen/fibrin degradation products, the thrombin-antithrombin complex (TAT), and thrombocytosis. These laboratory abnormalities increase with cancer progression, which are consistent with a tight relationship between tumor burden and clotting deregulation [12]. Accordingly, consistent evidence emerging from the literature demonstrates that plasma levels of thrombotic biomarkers are significantly lower in patients with early cancer compared to those with advanced cancer. These observations have pathophysiological and clinical implications.

In the last three decades, many studies have been performed with the aim to evaluate the levels of hemostatic markers in patients with advanced, as well as limited, disease in relation to the overall survival (OS), disease specific survival (DSS), disease free survival (DFS), progression free survival (PFS), and in relation to tumor response to therapies (Fig. 2). Many hemostatic biomarkers have been involved in these studies, ranging from standard clotting tests to more sophisticated hemostatic assays, evaluated alone or in combination of two or more.

In this review, we will focus on hemostasis biomarkers associated with tumor progression in specific cancer types, from those extensively investigated (i.e. D-Dimer, fibrinogen, plasminogen activator proteins) to those, which are novel with limited evaluation (i.e. plasma microparticles, endogenous thrombin potential, neutrophil extracellular traps or NETs).

2. Extensively investigated thrombotic biomarkers for cancer progression

2.1. D-DIMER AND FIBRINOGEN

Fibrinogen and D-dimer are the most studied hemostatic biomarkers in relation to cancer disease features, likely due to the availability of these assays in most hospitals, their low cost, and their use in pre-operative routine screening. Overall, data from these studies agree on a significant association between elevated plasma levels of D-dimer and/or fibrinogen and advanced tumor stage and poor prognosis in patients with different types of cancer, including lung, colorectal, gastric, esophageal, ovarian, renal, pancreatic and breast cancer. Furthermore, the finding of majority of these studies highlights a strong association of these biomarkers with cancer outcomes and patient survival, even independently from VTE.

2.1.1. Lung cancer

Elevated D-dimer and fibrinogen plasma levels have been frequently reported in lung cancer patients in association with large tumor burden, clinical progression and poor prognosis [13–16]. In 70 patients with non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) a significant prognostic role of D-dimer for OS was found, independent of tumor stage, histological type, performance status and tumor size [15]. In 2003, the results of the first large retrospective study of 826 consecutive patients with a new diagnosis of lung cancer [14], reported the median survival periods were shorter in patients with abnormally elevated compared to normal values (154 days vs. 308 days; $p < .01$). The difference was larger in patients with adenocarcinoma and in those with earlier stages of the disease (i.e., Stage T1 tumors). In the subsequent years, new studies reinforce previous evidences. In 100 newly diagnosed lung cancer patients (87% with NSCLC), D-dimer levels predicted OS independently of the clinical stage of disease, histologic tumor type and performance status (HR: 5.1, $p = .013$) [16]. Similarly, the pre-operative plasma D-dimer level was an important prognostic biomarker for 1-year OS, independently of VTE, in 232 patients with operable NSCLC [13]. Particularly, for patients with high D-dimer