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REVIEW

The prothrombotic activity of cancer cells in the circulation

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ABSTRACT

The hemostatic system is often subverted in patients with cancer, resulting in life-threatening venous thrombotic events. Despite the multifactorial and complex etiology of cancer-associated thrombosis, changes in the expression and activity of cancer-derived tissue factor (TF) – the principle initiator of the coagulation cascade – are considered key to malignant hypercoagulopathy and to the pathophysiology of thrombosis. However, many of the molecular and cellular mechanisms coupling the hemostatic degeneration to malignancy remain largely uncharacterized. In this review we discuss some of the tumor-intrinsic and tumor-extrinsic mechanisms that may contribute to the prothrombotic state of cancer, and we bring into focus the potential for circulating tumor cells (CTCs) in advancing our understanding of the field. We also summarize the current status of anticoagulant therapy for the treatment of thrombosis in patients with cancer.

1. Introduction

In 1865, Armand Trousseau was the first to consider thrombotic events as reflective of malignant burden and indicative of a poor outcome for patients with cancer [1]. Today, Trousseau's syndrome is considered intrinsically related in part to cancer progression as the consequence of an aberrant thrombotic activity exploited by malignant tumors. As a result, patients diagnosed with malignancy following an initial episode of thromboembolism have a lower survival rate and higher mortality risk in comparison to those patients without an underlying thrombotic condition [2,3]. Indeed, complications from venous thromboembolism (VTE), including pulmonary embolism, are the second leading cause of death for patients with cancer, with the risk of VTE elevated from 7-fold to up to 28-fold in patients with cancer as compared to patients without cancer [3]. This variability in VTE risk is correlated with the type of malignancy, patient demographics, treatment regimen, duration of follow-up period, period of study, and method of detecting and reporting VTE [4]. Several mechanisms have been suggested to contribute in part and in combination to the increased thrombotic complications observed in patients with cancer: 1) the expression of tissue factor (TF) by circulating tumor cells (CTCs); 2) the shedding of procoagulant microparticles by malignant cells; 3) the interaction of cancer cells with blood platelets; 4) the generation of neutrophil extracellular traps (NETs); and 5) the secondary deleterious effects of anti-cancer therapies [3]. Despite the tremendous strides made in defining the molecular and genetic drivers of tumorigenesis, much remains to be learned about the mechanisms underlying the observed increase of thrombotic events in patients with malignancy. In this review, we will summarize the complex biological processes related to cancer-associated thrombosis and we will bring to the fore some of the questions that we believe need to be addressed to better understand the prothrombotic biology of cancer.

2. The procoagulant phenotype of tumor cells and microparticles

Tissue factor is widely considered to be the major molecular driver of cancer-associated coagulopathy and thromboembolic disorders [8]. TF is a membrane receptor and protein co-factor required for initiating the extrinsic coagulation cascade necessary for physiological hemostasis [4–7]. TF exposure following tissue injury converts the zymogen coagulation factor VII (FVII) to activated factor VIIa (FVIIa), thus potentiating its catalytic efficiency in converting factor X to activated factor X (FXa) and factor IX to activated factor IX (FIXa). The catalytic efficiency of these reactions is increased by several orders of magnitude by the presence of calcium (Ca^{2+}) and an anionic phospholipid surface. For instance, FIXa forms the tenase complex with its protein cofactor FVIIIa on the surface of phosphatidylserine (PS)-containing cell membranes in the presence of calcium ions to generate FXa. The protease FXa produced on phosphatidylserine (PS)-expressing cell membranes reversibly associates, in a Ca^{2+}-dependent manner, with the cofactor factor Va (FVa), forming the prothrombinase complex [19]. The latter converts pro-thrombin (FII) into the serine protease thrombin (FIIa). Thrombin is a promiscuous enzyme which cleaves fibrinogen into insoluble fibrin,

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activates platelets and amplifies its own production through direct activation of factor XI (FXI) and the cofactors FVIII and FV [8,9]. TF-dependent reactions are negatively regulated by the tissue factor plasma protein inhibitor (TFPI), which forms a quaternary complex with TF/FVIIa and FX to inhibit thrombin generation [20].

Membrane bound- and intravascular-TF expression and activity has been shown to be upregulated in many human cancers compared with normal tissues, often correlating with thromboembolic complications and poor prognosis [9–15]. The highest levels of TF expression have been reported in cancers which are most strongly associated with a high incidence of thrombotic events such as cancers of the pancreas, brain, lung, stomach, ovaries and gliomas [10–12,14,16–18]. In vitro, cancer cells exhibit highly procoagulant surfaces characterized by prominent expression of TF and PS which are able to generate thrombin [3–5,11]. Additionally, cultured cancer cells have been shown to endogenously synthetize FVIIa, which is capable of sustaining TF catalytic activity and thus, the amplification of the procoagulant activity of cancer cells [12]. Moreover, TF-expressing microparticles (MPs) shed from growing tumors have been demonstrated to enhance thrombus propagation in a mouse model of vessel injury [15]. This finding is analogous to the evidence that TF-positive MPs derived from leukocytes trigger the activation of the coagulation cascade following incorporation into thrombi. Specifically, the localization of TF-MPs into a developing thrombus has been shown to be mediated by the selective interaction of PGSL-1 on the MPs with P-selectin expressed on the membrane of activated platelets [13–15]. Consequently, anti-P-selectin blocking antibodies have been shown to markedly reduce thrombotic events in mice with solid tumors [16]. Furthermore, in vivo studies of carcinoma cells implanted in mice have shown that the release of TF-MPs was proportional to the size of the primary tumor and its TF-expression levels, supporting the hypothesis that primary cancer cells may be a predominant source of TF-MPs [18,19]. Subsequent experiments demonstrated that the ability of cancer cells and MPs to form a thrombus in vitro could be abrogated by TF-blocking antibodies or by annexin V, which binds to the membrane-exposed PS to inhibit activation of FX [4].

The increased expression of TF in tumor is considered to be the result of the activation of dominant-acting oncogenes or loss of recessive tumor suppressors rather than dictated by genetic aberrations of the TF gene, as different genetic loci regulate the levels of TF in cancer. For example, in colorectal cancer cells (CRC), the proto-oncogene kRAS and the tumor suppressor p53 have been shown to cooperate to cause TF regulation at a transcriptional and translational level [17]. Similarly, loss of PTEN, a lipid phosphatase known to be essential for tumor suppression, has been found to be associated with profound upregulation of TF in cultured tumor cells, and promote their procoagulant activity [18]. In addition, in a mouse model of tumorigenesis, the oncoprotein MET has been demonstrated to enhance the pathological procoagulant activity of cancer cells via upregulation of the hemostatic plasminogen activator inhibitor type 1 (PAI-1) and cyclooxygenase-2 (COX-2) genes [19]. Furthermore, transforming growth factor β (TGF_β) has been reported to regulate TF expression through the induction of an epithelial to mesenchymal transition (EMT) in cancer cells [20].

To date, whilst the mechanisms describing the genetics underlying TF expression in malignancy have been largely described, is still unclear whether TF on cancer cells functions in a regulated fashion. Cell culture studies have shown that resting intravascular cells, such as monocytes, express a membrane-bound encrypted form of TF, with negligible procoagulant response, which can be subsequently decrypted to locally activate FX [21]. The molecular determinants underlying the conversion of the encrypted TF into its procoagulant form (decryption) remain ill-defined, if not controversial [23]. Several in vitro studies have proposed that the increase in membrane exposure of negative charged lipids, such as phosphatidylserine, is a key cellular determinant of the conversion of encrypted TF towards its active form [24–26]. Moreover, mutational studies in which cysteines of the TF extracellular disulfide loop (Cys186–Cys209) were substituted with serines or alanines uncovered the importance of the disulfide isomerization for TF decryption [27,28]. In this context, the disulfide exchange to form a disulfide bond within TF has been shown to be regulated by the targeted action of the protein disulfide isomerase (PDI) [22]. Adding to this complexity is the fact that tumor cells may exhibit extensive intra- and inter-procoagulant phenotypic heterogeneity, potentially deriving from stochastic events in TF protein expression and microenvironment signals [23]. Along these lines, a number of studies have shown the role of the microenvironment as a key mediator of TF activation and function on endothelial cells, vascular smooth muscle cells, monocytes and macrophages [29]. For instance, the vascular expression of TF as well as its procoagulant potential are known to be regulated by reactive oxygen species (ROS), inflammatory cytokines (e.g., tumor necrosis factor-α), biogenic amines (e.g., serotonin) and molecular activators (e.g., thrombin) [29,30]. However, whether tumor cells possess a cryptic form of TF and whether its activation is controlled by microenvironment-derived paracrine signals or internal cellular structural rearrangements is not known.

It is important to note that the physiological activation of the coagulation system in blood and plasma by triggers such as bacteria can only be achieved when the surface concentration of procoagulant stimuli is greater than a threshold value [44,45]. In the setting of cancer, it is unclear whether a threshold value of procoagulant activity is required to initiate the coagulation cascade and generate sufficient thrombin production to form fibrin and activate platelets. Moreover, the distinct activity of TF seems to be influenced by the physiological variance in the levels of coagulation factors, specifically coagulation factor IX (FIX) and factor X (FX) [50]. These findings suggest that a diverse set of extracellular procoagulant mediators as well as exposure to different microenvironment niches may signal to influence the activity of tumor-derived TF, with potential profound prognostic implications for cancer-related thrombosis.

The key question remains: to what degree does the microenvironment contribute to the procoagulant phenotype of cancer cells and thus to the risk of developing thrombotic events? Indeed, the procoagulant tendency of cancer cells is considered as one of the causes responsible for the prothrombotic state of patients with cancer. It is perhaps intuitive that a contact between TF-bearing tumor cells (and/or microparticles) and the blood system would trigger the coagulation cascade in malignancy. Cancer cells shed from primary tumor and wandering in the bloodstream, referred to as circulating tumor cells (CTCs), could represent a “vector” of cancer-associated thrombosis. Fig. 1 depicts the potential causes and consequences of procoagulant CTCs. It is also worth mentioning that CTCs can be found as a multi-cellular cluster in homotypic and/or heterotypic (associated with platelets and white blood cells) aggregates [24–26]. However, the contribution of single CTCs or CTC clusters to thrombotic events remains ill-defined. Certainly, the possibility to isolate and analyze CTCs and CTC clusters may provide insights into mechanisms of thrombosis in cancer. Indeed, the biophysical, molecular and genetic profiling of CTCs from patient samples may be used to define their thrombotic potential and act as biomarkers to develop recommendations for the use of anticoagulant therapies to prevent or block the onset of vascular events in patients with cancer. Also, given the heterogeneous character of cancer, there might be subpopulations within individual tumors that possess different procoagulant phenotypes. It remains to be defined whether subpopulations of CTCs activate the coagulation cascade and platelet responses responsible for thrombosis, and whether hematogenous CTC clusters have a higher prothrombotic potential compared to single CTCs.

The morphological abnormalities of tumor blood vessels consisting of excessive and haphazard branching, marked permeability, leakage of plasma, and clotting of extravasated fibrinogen and chronic vascular damage may exacerbate thrombotic events in patients with malignancy [28–30]. Indeed, many kinds of carcinomas, including those of the breast and stomach, often succeed in stimulating extravascular coagulation in active angiogenic sites by upregulating TF expression [31,32].

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