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Targeting clotting proteins in cancer therapy – progress and challenges

Wolfram Ruf[a,b*], Andrea S. Rothmeier[b], Claudine Graf[a,c]

[a] Center for Thrombosis and Hemostasis, University Medical Center, Mainz, Germany
[b] Department of Immunology and Microbial Science, The Scripps Research Institute, La Jolla, CA, USA
[c] 3rd Medical Department, University Medical Center, Mainz, Germany

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ABSTRACT

Cancer-associated thrombosis remains a significant complication in the clinical management of cancer and interactions of the hemostatic system with cancer biology continue to be elucidated. Here, we review recent progress in our understanding of tissue factor (TF) regulation and procoagulant activation, TF signaling in cancer and immune cells, and the expanding roles of the coagulation system in stem cell niches and the tumor microenvironment. The extravascular functions of coagulant and anti-coagulant pathways have significant implications not only for tumor progression, but also for the selection of appropriate target specific anticoagulants in the therapy of cancer patients.

Introduction

The clinical association of the hemostatic system and cancer progression has long been recognized from the high incidence of venous thromboembolism, including Trousseau's syndrome, in patients with advanced cancers [1]. Cancer cells express tissue factor (TF) and, upon exposure to blood, activate the plasmatic coagulation cascade and platelets during metastasis [2]. Consequently, metastatic disease is associated with an increased risk for thrombosis. A large body of experimental evidence has delineated a multitude of interactions between cancer cells, the blood and the vascular endothelium that contribute to the efficiency of metastatic tumor cell dissemination [3,4]. However, tumor cell TF expression is not the sole determinant for cancer-associated thrombosis and cancer types display marked differences in the incidence of clinically relevant thromboembolism [5,6]. Contributing factors are direct activations of platelets [7] and the contact phase [8] by which tumor cells independently promote intravascular coagulation and thrombosis. Moreover, while tumor cell TF expression is sufficient to cause signs of systemic coagulation activation, it does not predict the release of TF bearing microvesicles (MV) with measurable procoagulant activity into the circulation of tumor bearing mice [9]. The cellular mechanisms of TF activation, regulation, and incorporation into procoagulant MV remain active areas of research and recent advances on the functional regulation of the TF pathway in untransformed and transformed cells will be discussed in this review.

Understanding the cellular and tumor specific mechanisms of coagulation activation is pivotal for therapy and prevention of cancer-associated thrombosis, a major complication of cancer progression as well as cancer therapy [10]. However, the last two decades have provided us with a wealth of new information demonstrating that the hemostatic system influences substantially and directly aspects of tumor cell biology ranging from angiogenesis, cancer stem cell maintenance implicated in therapy resistance, to the immune cell composition of the tumor micro-environment. While cancer cell proangiogenic effects of TF had originally established the pathophysiological relevance of upstream coagulation signaling [11], recent progress has elucidated additional novel roles of the coagulant and anti-coagulant balance in physiological innate immune signaling and hematopoiesis. These new directions of research have potentially far reaching implications for cancer progression and immune evasion. As discussed below, the broader roles of the hemostatic system in the response to injury and infection may contribute to the characteristics of cancers as "wounds that do not heal", a term originally coined to describe the similarities of tumor and regenerative angiogenesis in the context of a deposited transitional fibrin-rich extracellular matrix [12]. Most important, a better understanding of the physiological and pathophysiological functions of coagulant signaling in stem cell niches and tumor microenvironments may provide additional guidance for choices of Vitamin K antagonists and target-selective oral anticoagulants in adjuvant cancer therapy.

Regulation of TF activity and procoagulant MV release

The procoagulant activity of TF is crucial for metastasis and a unique property of full-length, but not alternatively spliced (as) TF, a circulating, soluble isoform of TF with a unique C-terminus

* Corresponding author at: Center for Thrombosis and Hemostasis, Langenbeckstr. 1; D-55131 Mainz, Germany.
E-mail address: ruf@uni-mainz.de (W. Ruf).

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replacing the cytoplasmic and trans-membrane domains of full-length TF [13]. Untransformed cells maintain TF mainly in a non-coagulant state on the cell surface and require activating signals to convert TF to a procoagulant receptor. Non-coagulant, cryptic TF in non-tumorogenic epithelial cells binds FVIIa with low affinity, but fully supports proteolytic signaling of the TF-FVIIa complex through protease activated receptor 2 (PAR2) [14]. TF is maintained in a cryptic state by protein disulfide isomerase (PDI) dependent thiol-disulfide exchange reactions that modify an allosteric disulfide bond in the TF extracellular domain [14,15]. Activating stimuli, including $Ca2^{+}$ fluxes [15,16], can convert cryptic TF to a high affinity receptor for FVIIa and fully procoagulant state in the context of cell surface exposure of negatively charged phosphatidylserine (PS) [17]. Subsequent studies have shown that TF prothrombotic activity and fibrin formation in vivo is indeed dependent on PDI activity [18-20].

While initial studies employed rather non-physiological stimuli, including strong oxidizing substances, to activate cell surface TF, pathophysiological relevant scenarios of TF activation on untransformed hematopoietic and vascular cells have been delineated, including platelet-leukocyte interactions [20], cell injury signals activating the purinergic P2X7 receptor [18], and activation of the complement cascade [21] (Fig. 1). In the latter case, conversion of monocyte TF to a procoagulant form depends on both, redox changes of surface PDI caused by complement factor C5 activation as well as PS exposure by the subsequent membrane insertion of activated C7. Aggressive cancer cells do not regulate TF activity through PDI and thiol-disulfide exchange pathways in the same way as non-malignant cells do [22], which may contribute to the latent procoagulant state in cancer patients.

Another important aspect of TF activation on cells is the coupling of these events to molecular pathways that culminate in the generation of procoagulant MV. A hallmark of cancer cells is the spontaneous release of MV bearing TF [23]. Procoagulant MV generation, rather than cancer cell procoagulant activity per se, strongly correlates with metastatic behavior of syngeneic mouse tumor cells [24]. TF is released on MV that also incorporate adhesion receptors, including P-selectin glycoprotein ligand-1 (PSGL1) and β1 integrins [18], and remodeling of the actin cytoskeleton plays a crucial role in facilitating TF insertion into MV. The TF cytoplasmic domain interacts with the actin binding protein filamin A [25] which is crucial for targeting of TF to MV released from PAR2-stimulated cancer cells [26,27]. A different role for cytoskeletal anchoring of TF emerged from the study of macrophages that tightly control TF activity dependent on cell adhesion [28]. In primed macrophages, filamin A restricts mobility of TF located primarily in glycosphingolipid-rich raft domains and thus prevents TF recruitment onto highly procoagulant MV, unless filamin A is proteolytically cleaved by calpain.

Macrophage calpain is activated as the consequence of a series of events that results from triggering of the purinergic P2X7 receptor by high concentrations of ATP, a cell injury signal that may be released from tumor cells under hypoxic stress or chemotherapeutic insult. P2X7 receptor activation, dependent on endosomal reactive oxygen species (ROS) and activation of thioredoxin reductase, causes extracellular release of thioredoxin and thereby reductive changes of cell surface proteins, PS exposure and TF activation [28]. While the allosteric TF disulfide can be reduced by thioredoxin to inactivate TF [29], TF procoagulant activation and release of TF on MV also requires PDI [18,28]. The release of TF and other cell surface receptors on these highly procoagulant and prothrombotic MV requires trafficking of TF to the tips of filopodia that form during macrophage activation by P2X7 receptor signaling. The thiol disulfide exchange reactions following this injury response also trigger activation of the inflammasome and the effector caspase 1 that not only promotes the release of pro-inflammatory IL1β, but also -through actin destabilization- the final severing and release of highly procoagulant MV. Thus, the P2X7 receptor triggered

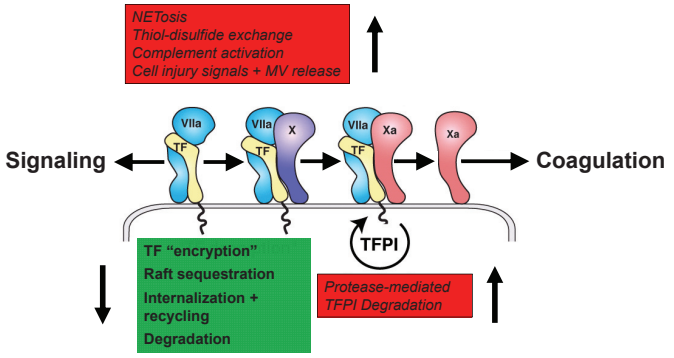

Fig. 1. Cellular regulation of TF with activating pathways in red and inhibitory mechanisms in green.

activation of TF procoagulant activity is an apparently evolutionary conserved mechanism to couple coagulation and inflammation.

The finding that cell adhesion is required for primary macrophages to maintain TF in a non-coagulant state is in line with the known crosstalk of TF and its cytoplasmic domain with integrin and cell adhesion signaling [25,30,31], but the precise mechanism by which normal cells control cell surface TF procoagulant activity remains to be elucidated. It is tempting to speculate that the same pathways that control coagulation may in turn serve signaling functions of the TF-FVIIa complex in extravascular locations. Conversely, deregulated cell adhesion and integrin function of tumor cells may disable adhesion-dependent control of TF activity and thus favor prothrombotic MV release. However, tumor cells release also TF with little procoagulant activity into the circulation [9] and these forms of TF may primarily serve signaling roles after uptake by vascular and perivascular cells [32-34], similar to recently elucidated integrin-dependent exosome preconditioning of metastatic niches [35].

There is also expanding genetic and experimental evidence that in addition to TF expression, regulation of TF procoagulant activity is a relevant determinant for cancer progression. In addition to the demonstrated role of the anticoagulant protein C (PC) pathway in controlling intravascular tumor dissemination [13,36-39], endothelial TF pathway inhibitor (TFPI) expression limits TF-dependent experimental metastasis [40]. Tumor cell expression of TFPI is regulated by hypoxia inducible factor (HIF) 1α [41] and TFPI expression by tumor cells is correlated with improved outcome in breast cancer [42]. Genetic polymorphisms associated with breast cancer furthermore show that traditional prothrombotic risk factors are not the sole determinant for tumor progression and that additional hemostatic components, i.e. FX and the endothelial protein C receptor (EPCR), may be contributing to the development of cancer [43].

TF signaling in tumor cells and angiogenesis

The coagulation cascade influences many facets of cancer development beyond the metastatic process, including angiogenesis, immune evasion and tumor growth. Simon Karpatkin proposed that thrombin is a key effector protease that facilitates the transition from tumor dormancy [4], but a wealth of additional data implicate direct TF signaling as a central pathway promoting tumor progression. Oncogenic mutations in a number of key signaling pathways cause the constitutive upregulation of TF, including activating mutations in the phosphatidylinositol 3 (PI-3) kinase pathway, the epidermal growth factor receptor (EGFR), and the hepatocyte growth factor receptor (MET proto-oncogene), as well as loss of tumor suppressors [44]. TF is frequently upregulated in concert with its main cell signaling receptor, PAR2, and hypoxia through induction of HIF2α, epigenetic changes in chromatin structure, and androgen receptor signaling can induce tumor cell autonomous synthesis of FVIIa [45-