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Tissue factor and cancer

Wolfram Ruf

Department of Immunology and Microbial Science, The Scripps Research Institute, La Jolla, CA

Summary

The hemostatic system is involved in multiple interactions with transformed cells that progress from a dormant, non-vascularized tumor to highly metastatic phenotypes. Oncogenic transformations up regulate not only the initiator of the coagulation cascade, tissue factor (TF), but also induce other molecules that are required for TF's direct cell signaling activity, including the protease activated receptor (PAR) 2 and factor VIIa. TF-dependent signaling is a major driver for primary tumor progression, whereas TF-initiated coagulation and other components of the hemostatic system support metastasis. Basic research continues to identify pivotal molecular interactions in these processes and provides potential leads for targeting specific tumor promoting pathways associated with hemostasis and thrombosis.

Introduction

A prothrombotic state is one of the hallmarks of malignancy and cancer-associated thrombosis is a major contributor to morbidity and mortality in patients with advanced cancers (1). Tissue factor (TF), the cellular initiator of the coagulation cascade, triggers remote thrombotic complications involving procoagulant TF⁺ microparticles shed from tumor cells (2), while other procoagulants stimulate platelet- and neutrophil-dependent thrombotic events (3; 4). TF is responsible for local thrombin generation and fibrin deposition in the tumor microenvironment and thereby influences multiple cellular interactions of tumor and host cells (5). An expanding body of literature furthermore points to important roles of direct, TF-mediated cell signaling in promoting tumor growth and angiogenesis involving the TF cytoplasmic domain coupled to proteolytic activation of the protease activated receptor (PAR) 2 or non-proteolytic integrin ligation (6–11). In addition, tumor cell TF procoagulant activity is crucial for successful metastatic tumor dissemination (12; 13) and metastasis is significantly influenced by mutations and pharmacological interventions that induce a prothrombotic states in animal models. This brief summary reviews recent developments concerning roles of the hemostatic system in tumor progression.

A crucial role for direct TF signaling in tumor cell-induced angiogenesis

Overexpression of TF promotes primary tumor growth (11; 14–16) and oncogenic growth factor receptors can upregulate the entire complement of the upstream TF signaling complex consisting of TF, VIIa, PAR1 and PAR2 (17). The TF-VIIa complex activates tumor cell

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Correspondence to: Wolfram Ruf, M.D., Department of Immunology and Microbial Science, SP258, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, Tel: 858-784-2748, FAX: 858-784-8480, ruf@scripps.edu.

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PAR2 to influence important aspects of tumor progression, including survival, immune modulation and angiogenesis. The molecular pathways of constitutive and hypoxia-induced extrahepatic synthesis of FVIIa have been defined in considerable detail and identified a central role for the hypoxia induced factor (HIF) 2 α in the tumor cell autonomous synthesis of VIIa (18–20). Hypoxia-induced TF-VIIa-PAR2 signaling appears to be particularly important for glioblastoma progression (21–23). Glioblastoma cells furthermore release TF⁺ micro particles that elicit TF-VIIa signaling in trans by targeting PAR2 expressed by hypoxic endothelial cells (24), inducing specifically heparin binding epidermal growth factor (HB-EGF) that activates the MET receptor, previously shown to promote a prothrombotic state (25).

Breast cancer progression is also highly dependent on TF-VIIa-PAR2 signaling. TF-VIIa-PAR2 G protein-coupled receptor signaling induces pro-angiogenic factors, such as IL-8, CXCL1, or VEGF (8; 26–28) as well as growth factors for myeloid cells and macrophages (27). In clinical breast cancer biopsies, upregulation of PAR2 and TF was associated with a marked phosphorylation of the TF cytoplasmic domain (29) and only patients with phosphorylated TF had a cancer relapse in this small prospective study. The polyoma middle T (PyMT) oncogene-driven model of spontaneous breast cancer development mimics important aspects of human tumor progression and is dependent on the angiogenic switch regulated by components of the immune system (30). PAR2-deficiency results in delayed PyMT tumor development, low levels of the chemokine CXCL1 (KC) in the tumor stroma, and reduced counts of F4/80 positive macrophages in early tumors compared to wild-type mice (9). In this model, PAR2 signaling is required for tumor cell TF cytoplasmic domain phosphorylation *in vivo* (29) and, importantly, deletion of the TF cytoplasmic domain delayed tumor progression similar to PAR2-deficiency (6). Late stage tumors of TF cytoplasmic domain-deleted mice also displayed altered vessel architecture and reduced macrophage numbers in the tumor stroma (6). These genetic studies suggest the novel concept that TF with its cytoplasmic domain and PAR2 act together to promote pro-angiogenic and immune modulatory effects in tumor progression.

Proof of principle pharmacological inhibition experiments in xenograft models further substantiated the crucial role of tumor cell TF-VIIa-PAR2 signaling in tumor growth of breast cancer (8) and glioblastoma (22). These experiments were enabled by identification of a monoclonal antibody (10H10), which has no appreciable anticoagulant activity, but specifically inhibits TF-VIIa mediated PAR2 signaling (31), reduces pro-angiogenic IL-8 induction in breast cancer cells, and inhibits tumor growth and vessel density when co-injected during tumor inoculation into the orthotopic tumor microenvironment (8). These data confirmed that tumor cell TF-VIIa-PAR2 signaling is crucial for angiogenesis and suggested potential utility of targeting tumor cell pro-angiogenic signaling as an adjuvant cancer therapy.

TF also promotes angiogenesis independent of proteolytic cell signaling through a splice isoform, termed alternatively spliced TF (asTF). The alternative splicing event deletes exon 5 and thus eliminates substrate factor X binding and coagulant activity, but creates an alternative translation for the TF carboxyl terminus that renders asTF a soluble and secreted molecule. A binding site for integrins $\alpha v \beta 3$ and $\beta 1$ integrin heterodimers (32) is shared between full-length and asTF. asTF ligates integrins $\alpha 6 \beta 1$ and $\alpha v \beta 3$ to regulate endothelial cell migration, tube formation, and sprouting and has tumor promoting activities *in vivo* (7; 10). In addition, asTF regulates endothelial cell function and induces leukocyte recruitment through the upregulation of adhesion receptors (33; 34). It will be of interest to further define the relative contributions of full-length and asTF in regulating angiogenesis, immune cell function, and tumor progression in appropriate animal models *in vivo*.

Hematogenous metastasis depending on TF procoagulant function is modulated by a prothrombotic state

TF-initiated thrombin generation improves metastatic tumor cell homing and survival (12; 35) through fibrin formation and increased adhesion (36; 37), and platelet-dependent protection of tumor cells from natural killer cell attack (38–40). The platelet thrombin receptors glycoprotein (GP) Iba (41) and PAR4 (40) have been identified as thrombin targets in the metastatic process. In addition, the thrombin receptor PAR1 expressed by melanoma cells contributes to experimental metastasis (42–44), but spontaneous metastasis in oncogene-induced breast cancer in mice was not dependent on PAR1 (9), possibly indicating tumor-specific contributions of PAR1 to tumor progression.

Platelet-tumor cell interactions are an essential part of successful metastasis. Locally, platelets stimulate tumor growth and influence angiogenesis by releasing a variety of growth factors and chemokines stored in their granules (45; 46). Platelets also play a pivotal role in early stages of metastasis in which activated platelets release large amounts of TGF β that triggers TGF β /Smad and NF- κ B signaling pathways to promote epithelial to mesenchymal transition of cancer cells (47). The recruitment of platelets by tumor cells is increasingly recognized to initiate broad multicellular interactions within the metastatic niche. The aggregates of activated platelets and tumor cells lodged at the endothelium recruit leukocytes through tethering and adhesion events typically employed in inflammation. These interactions involve selectins, integrins, platelet receptors, and soluble and extracellular matrix proteins.

Experimental metastasis is significantly reduced in mice lacking P- or L-selectin (48; 49). Tumor cells express P-selectin glycoprotein (PSGL)-1 serves as counterligands for leukocyte selectins and together with mucins and CD44 tumor cell-platelet aggregates can trigger clot formation (3). Selectin-mediated platelet and leukocyte recruitment to tumor cells activates endothelial cells to release the C-C motif chemokine 5 (CCL5), which attracts monocytes to support metastasis (49). The important role of leukocyte recruitment to sites of metastasis is further demonstrated by the pro-metastatic effect of the chemokine CCL2 (MCP-1) that is synthesized by either stromal or tumor cells. CCL2 recruits inflammatory monocytes, which express the chemokine CCL2 receptor CCR2, as well as macrophages locally at sites of metastasizing tumor cells (50). These studies elucidated that tumor cell-initiated formation of micro-thrombi supports complex heterotypic cellular interactions contributing to successful metastatic tumor cell dissemination.

While the role of local or systemic coagulation activation in cancer is an important cause for cancer-associated thrombosis, an unexpected priming of metastatic niches by tumor-initiated coagulation was recently documented. Platelet thrombi recruit transiently monocyte and macrophage populations that are crucial for successful metastasis to the lung (51). Remarkably, in tumor bearing mice lung macrophages were expanded by tumor-derived thrombin and this priming markedly enhanced the metastatic success of injected tumor cells. Thus, the tumor induced prothrombotic state has an influence on the metastatic process. Additional experiments in clinically relevant prothrombotic animal models further expand the concept that the hemostatic system directly supports metastatic tumor progression.

Thrombin not only promotes clot formation, but is also crucial for the anticoagulant pathway upon binding to endothelial cell-expressed thrombomodulin (TM). TM-thrombin activates protein C (PC) in a process that is facilitated by binding of PC to the endothelial protein C receptor (EPCR). The anticoagulant system, specifically TM, PC, and EPCR, impair tumor metastasis (52–54). TM mutant mice (TM^{Pro}) have a single amino acid substitution (Glu⁴⁰⁴/Pro) and exhibit ~1000-fold decreased PC activation and ~100-fold reduced binding affinity

for thrombin (55). The resulting combined loss of local thrombin neutralization and diminished aPC generation models functional thrombomodulin deficiency that is clinically encountered under inflammatory conditions. TM^{Pro} mice display dramatically increased metastasis that is dependent on tumor cell TF, circulating prothrombin, as well as platelets (52).

Diminished activation of the anticoagulant pathway may contribute to the enhanced metastasis in TM^{Pro} mice. Accordingly, transgenic endothelial overexpression of EPCR or treatment with activated PC markedly reduce experimental metastasis in livers and lungs (54). Conversely, inhibition of endogenous PC increases metastasis (53). Consistent with local anticoagulant control of tumor-cell generated thrombin, mice carrying the activated PC-resistant factor V_{Leiden} mutation show increased hematogenous metastasis (56). Taken together, these studies suggest that thrombin neutralization by the endothelium and/or local aPC generation counteract tumor cell prometastatic ability, but the downstream targets of thrombin are incompletely defined.

Perspective

We have learned a great deal about the functions of the coagulation cascade, platelets and the fibrinolytic system in tumor progression and metastasis. However, new interactions are continuously emerging that shed light on novel molecular mechanisms that are employed by tumor cells to exploit functions of the hemostatic system. Emerging data show that EPCR is expressed by tumor cells (57–59) and contributes to survival of lung cancer metastases (60). I expect that further characterization of basic mechanisms and preclinical mouse models will continue to provide insights into potentially successful therapeutic avenues suitable to interrupt prometastatic and tumor promoting mechanisms of the coagulation cascade and its cellular receptors.

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