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Mechanisms coupling thrombin to metastasis and tumorigenesis

Allison S. Remiker, Joseph S. Palumbo*

Cancer and Blood Diseases Institute, Cincinnati Children's Hospital Medical Center, and the University of Cincinnati College of Medicine, Cincinnati, OH, USA

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ABSTRACT

The association of malignancy and thrombophilia is bidirectional, as evidenced by four decades of studies in animal models showing that hemostatic system components support cancer progression. Consistent with this view, clinical studies have suggested that anticoagulants not only limit thromboembolic complications associated with cancer, but also improve survival by impeding cancer progression, and may even prevent the development of cancer. In order to fully capitalize on this association, a detailed understanding of the mechanisms coupling hemostatic factors to cancer pathogenesis is required. Multiple studies have shown that thrombin-mediated procoagulant functions strongly promote metastatic potential. In particular, the platelet/fibrin(ogen) axis has been shown to protect newly formed micrometastases from innate immune surveillance, contribute to creation of a metastatic niche by recruitment of prometastatic inflammatory cells, and promote the epithelial to mesenchymal transition of metastatic cells. Thrombin-mediated functions have also been shown to support tumor growth in some contexts, and have even been linked to tumorigenesis in the setting of inflammation-driven colon cancer. Here, local thrombin-mediated extravascular fibrin deposition, and specifically fibrin-$\alpha_M\beta_2$ integrin interaction, push intestinal inflammatory cells toward a pro-tumorigenic phenotype, resulting in the elaboration of key cytokines and growth factors that support the proliferation and survival of transformed intestinal epithelial cells. These studies reveal that hemostatic factors can serve as a bridge between pathological inflammation and the development of cancer. As a large proportion of cancers are caused by pathological inflammation, these studies suggest that therapies targeting the nexus between hemostasis and inflammation could be used to prevent cancer development.

1. Introduction

The association between malignancy and pathological hemostatic system activation is well-established. Malignancy leads to a hypercoagulable state that carries a significant risk of venous and arterial thromboembolism. In fact, cancer represents one of the single most significant known risk factors for venous thromboembolism (VTE), resulting in an overall increase in VTE risk of 4–7 fold [1–3], with some very aggressive cancers carrying a risk as high as 40–60 fold [2,4]. More than 4 decades of research using various animal models also supports the conclusion that the association between cancer and hemostatic system activation is *bidirectional*, whereby the pathological activation of hemostatic system components by malignant cells serves to drive key aspects of cancer progression, including tumor growth, invasion, stroma formation, and metastatic potential.

Multiple clinical trials have attempted to capitalize on the apparent role of the hemostatic system in cancer progression by exploring the potential efficacy of traditional anticoagulants to limit not only thromboembolic complications associated with cancer, but cancer progression itself [5–9]. Most studies have focused on low molecular weight heparins (LMWH), with the overall conclusion remaining somewhat controversial. LMW Heparins may have some benefit in limiting cancer progression, but this benefit appears to depend on the cancer type, cancer staging, as well as the specific formulation of LMWH [10]. Interpretation of the clinical data is complicated by the fact that many trials include patients with advanced disease, and the bleeding risks associated with anticoagulation in the setting of cancer limit the intensity of therapy. More recent studies have shown an association between long-term anticoagulation with warfarin and a decreased incidence of numerous types of cancer [11]. This finding suggests the tantalizing possibility that therapies targeting the hemostatic system could prevent the development of cancer, and implicates hemostatic system components in very early events important in tumorigenesis.

The anticoagulants used in all of these trials target thrombin generation, making bleeding risk a limiting factor. As thrombin has over a

* Corresponding author at: Cancer and Blood Diseases Institute, Division of Hematology, Cincinnati Children's Hospital Medical Center, 3333 Burnet Ave., Cincinnati, OH 45229-3026, USA.
E-mail address: joe.palumbo@cchmc.org (J.S. Palumbo).

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dozen recognized substrates, it is also conceivable that some thrombin functions could drive cancer progression, while others may actually limit it. Therefore, a mechanistic understanding of the role of hemostasis in cancer progression is needed if any real potential for limiting cancer progression through targeting hemostatic system components is to be realized. Analyses in gene-targeted mice with specific deletions and alterations in hemostatic system components continue to provide important insights into these mechanisms that will likely yield specific targeted therapies for impeding cancer progression that are far superior to standard anticoagulation.

1.1. Metastasis

A major focus of the work defining the role of the hemostatic system in cancer progression has been on understanding the role of hemostatic system components in metastasis. Cancer cells have the potential to activate hemostatic system components through numerous mechanisms. Malignant cells often express P- and E-selectins, providing tumor cells a means to bind platelets and the endothelium [3,10,12]. Tumor cells release naturally occurring polyphosphates (i.e., RNA and DNA) which can activate the intrinsic coagulation pathway [3,10,12]. Malignant cells also often express the primary cell-associated initiator of coagulation, tissue factor (TF), and can induce TF expression in stromal cells through secretion of inflammatory mediators [3,10,12]. While each of these mechanisms may contribute to metastatic potential, the most well-studied to date is TF. The view that TF expressed by malignant cells is a major determinant of metastatic potential is supported by numerous studies in which tumor cell-associated TF has been targeted by pharmacological or immunological inhibitors, silencers of TF expression, or complete genetic elimination [13–15]. Indeed, the crucial importance of tumor cell-associated TF to metastasis is evidenced by studies where the elimination of TF expression from malignant fibroblasts effectively eliminates metastatic potential [16].

TF plays a complex role in cancer progression that includes mechanisms coupled to thrombin generation, as well as signaling mechanisms independent of coagulation functions [17,18]. TF has been linked to regulation of signaling functions through mechanisms directly involving the TF cytoplasmic domain, as well as through regulation of signaling events coupled to protease activated receptor-2, and integrins [14,17,19]. These signaling mechanisms have been shown to support tumor cell invasion, tumor growth, and angiogenesis [14,17,19]. Certainly, the potential for TF-mediated signaling events to promote tumor invasion and angiogenesis would be expected to drive metastatic potential. Interestingly, the role of signaling functions related to TF in metastasis appears to be primarily through mechanisms that support intravasation (i.e., the capacity of tumor cells to enter the circulation) [19]. Once tumor cells have gained access to the circulation, TF continues to play a major role in metastasis, but through mechanisms coupled to procoagulant function [19].

The view that TF-mediated thrombin generation is a major determinant of metastatic potential is supported by numerous animal studies showing that specific thrombin inhibitors, or genetically-mediated decrease in prothrombin expression, significantly limit metastasis [12,20]. In fact, even a modest 50% diminution in prothrombin conferred by heterozygosity for a prothrombin null allele in mice dramatically limits metastatic potential [16]. Conversely, mice with a mutation in the native thrombomodulin gene that reduces thrombin binding potential and thrombin-mediated protein C activation (TM^{Pro}) have a profoundly prometastatic phenotype [21]. Together, these data support the view that a major mechanism coupling tumor cell-associated TF to metastasis is local thrombin generation, and subsequent thrombin-mediated procoagulant functions.

Thrombin is a multifunctional protease with over a dozen recognized substrates. Thrombin polymerizes fibrinogen to fibrin, triggers platelet activation, and activates the transglutaminase (factor XIII) that crosslinks fibrin. Thrombin also activates protein C, thrombin-activated fibrinolysis inhibitor, and three G protein-coupled protease activated receptors (PAR-1, -3, and -4) [22–28]. Considerable data has accumulated pointing to platelet functions as major determinants of metastatic potential, suggesting that thrombin-mediated platelet activation is at least one key mechanism coupling this central hemostatic protease to metastasis. The platelet/fibrin(ogen) axis has been shown to promote metastasis by impeding the clearance of newly formed micrometastases by natural killer (NK) cells [29]. The mechanisms by which the platelet/fibrin(ogen) axis mitigate NK cell-mediated killing remains to be fully defined. At least one potent platelet-derived inhibitor of NK cell function, TGF-β1, appears to play an important role in this process, but is likely not the entire explanation [30,31]. In addition to secreting inhibitors of NK cell function, platelet/fibrin microthrombi could prevent NK cell contact with tumor cells, essentially providing a protective "cloak" [32]. Additionally, platelet/fibrin derived signals could drive tumor cell extravasation, thereby protecting micrometastases from intravascular NK cell immunosurveillance mechanisms.

More recent studies have suggested that the prometastatic function of platelets is dependent on the capacity of platelets to contribute to the formation of a so-called "metastatic niche" through interactions with multiple cell types [33]. Platelet-derived cytokines and chemokines (e.g., CXCL5/7) recruit macrophages and neutrophils to early metastatic foci. Platelet-derived cytokines (e.g., TGF-β1) together with signals from recruited inflammatory cells are thought to synergistically promote an epithelial-to-mesenchymal transition (EMT) in metastatic tumor cells that supports cell motility, invasiveness (i.e., extravasation), survival and growth of the developing metastatic focus [33–36]. Platelet- and inflammatory cell-derived growth factors and cytokines are also thought to prime the endothelium for metastasis, resulting in the upregulation of adhesion molecules and increased endothelial permeability, thereby increasing the likelihood of tumor cell adhesion and extravasation [33,35,36].

It remains to be determined if the significant role of fibrin(ogen) in metastasis is limited to the local support of platelet aggregates associated with newly formed micrometastatic lesions, or if fibrin(ogen) plays a broader role in metastasis independent of platelet functions. In addition to binding the platelet integrin, $\alpha_{IIb}\beta_3$, fibrin(ogen) can regulate the function of numerous other cell types through interactions with multiple integrin and nonintegrin receptors (i.e., $\alpha_M\beta_2$, $\alpha_{IIb}\beta_3$, $\alpha_V\beta_3$, $\alpha_1\beta_5$, $\alpha_V\beta_1$, ICAM-1, cadherin, Toll-like receptors) [37–44]. Consistent with this, fibrin(ogen) has been shown to regulate important inflammatory functions independently of its role in platelet aggregation through direct interactions with innate immune cells [45,46]. While fibrin(ogen)-leukocyte interactions have been shown to play a major role in early steps involved in tumorigenesis in some contexts (see below), it is unclear if fibrin(ogen)-leukocyte interactions play a role in metastasis. In fact, the fundamental question of whether soluble fibrinogen or fibrin polymer is the instructive form of the molecule that supports metastasis remains to be determined. The availability of gene-targeted mice with specific structure/function alterations in fibrinogen will allow further dissection of the potential mechanisms coupling this protein to metastasis.

Although important mechanistic details remain to be defined, the data supporting a role for procoagulant functions in metastasis is quite strong. The importance of hemostatic system components in the early steps of metastasis formation may, at least in part, explain why anticoagulants appear to provide the strongest survival advantage to cancer patients with low stage disease. Unfortunately, the patients with the worst prognosis already have metastatic disease at diagnosis. Therefore, the potential to take therapeutic advantage of the mechanisms coupling hemostatic factors to cancer progression would require a detailed understanding of cancers where hemostatic system components promote tumor growth, or even tumorigenesis.