A Functional Role for Hemostasis in Early Cancer Development

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Abstract

Blood coagulation disorders have been known to be associated with cancer for many years. However, the mechanisms responsible for their relationship have not been understood. Recent work indicates that activation of the MET oncogene, which drives invasion and metastasis in cancer, can promote a cancer-associated thrombohemorrhagic syndrome that is mediated by transcriptional up-regulation of the procoagulation factors plasminogen activator inhibitor type-1 and cyclooxygenase-2. These findings reveal a long-sought mechanistic link between coagulation and cancer, highlighting a clinically important perspective on malignant invasion and metastasis. (Cancer Res 2005; 65(19): 8579-82)

Cancer and Coagulation

The deep relationship between hemostasis activation and neoplastic growth was documented initially in 1865, when the French clinician Armand Trousseau reported the association of migratory thrombophlebitis ("a condition of the blood that predisposes it to spontaneous coagulation") with occult malignancy (Trousseau's syndrome; ref. 1). Even today, with the enormous progress in early cancer detection, and the increased self-awareness of patients, an "idiopathic" (i.e., unexplained) venous thrombosis can be the first manifestation of a cancer that is otherwise asymptomatic. In the 1990s, large clinical studies convincingly showed that idiopathic venous thrombosis is associated with a significantly increased risk of malignancy (for a review, see ref. 2). These reports led to the conclusion that "either premalignant change promotes thrombosis, or cancer and thrombosis share common risk factors" (3). They also implied that early stages of cancer development could predispose the organism to blood hypercoagulation through mechanisms independent of those that are unleashed in advanced stages of cancer, where massive tissue damage can trigger hemostatic catastrophe.

The most common malignancies associated with thrombosis are derived from breast, colon, and lung, reflecting the prevalence of these tumors in the general population. However, when adjusted for disease prevalence, the cancers most significantly associated with thrombotic disorders are derived from pancreas, ovary, and brain (for a review, see ref. 4).

The idea that hemostasis activation could be advantageous for tumor growth and invasion had been conceived by Theodor Billroth, a contemporary of Trousseau who interpreted the pathologic finding of tumor cells embedded in a thrombus as evidence for the role of venous thrombosis in the metastatic process (5). Although the role of blood coagulation in tumorigenesis was mainly overlooked by tumor biologists in subsequent years, this area of inquiry was reinvigorated by H. Dvorak, (6). Specifically, Dvorak proposed that tumors build up their stroma by activating the host's wound-healing response, including blood clotting, which ultimately leads to fibrin deposition at the tumor site. Fibrin provides a quick-setting extracellular matrix that forms a provisional scaffold for newborn cancer cells, migrating inflammatory cells, and newly formed blood vasculature. Unlike in wounds, where the process of blood clotting terminates with tissue repair, tumor cells continuously activate deposition of the fibrin matrix, which is subsequently replaced by mature stroma. Thus, tumors can be conceived as "wounds that do not heal" (6). The relevance of this behavior for tumor expansion is further supported by findings indicating that coagulation factors are directly involved in the building and stabilization of new blood vessels. In fact, on one hand, angiogenic endothelial expresses tissue factor, which initiates the coagulation cascade. On the other hand, active coagulation factors, bound to endothelial surface receptors, elicit angiogenic signaling (for a review, see refs. 7, 8). In coupling coagulation to angiogenesis, a significant role is played by the thrombin receptor PAR-1, the genetic ablation of which prevents correct vascular development in mice (9).

The pathogenesis of the prothrombotic state in cancer is complex and likely multifactorial. It is generally thought that both the tumor, through production of procoagulant factors, and the host, through its inflammatory response, take part in the process (10). For the benefit of the tumor, activation of blood clotting should be confined to the neoplastic tissue itself, but sometimes the hemostatic imbalance extends beyond the primary site and produces systemic venous thromboses, which in a few instances clinically manifest as Trousseau's syndrome. The well-known fact that anticancer therapy itself can be thrombogenic suggests that cytotoxic agents cause the release of tumor-derived factors into the bloodstream, helping to convert a local into a systemic process.

In the last decade, research into the link between coagulation and cancer has been fostered by the goal of preventing the potentially lethal evolution of thrombosis (i.e., thromboembolism). Moreover, appreciation of the role of coagulation in tumor growth has raised the hope of identifying new targets for the treatment of cancer itself. The procoagulant activity of tumor cells has been correlated to the altered production of a number of molecules, including factors inducing platelet aggregation or directly involved in blood clotting: cytokines, which modulate inflammatory and endothelial responses, have also been involved (for review, see refs. 10, 11). As Trousseau's syndrome preferentially occurs in association with mucinous adenocarcinomas, the tumor-derived mucins have been proposed as systemic proaggregant factors (12).

Among procoagulant molecules expressed by tumors, a prominent role is played by tissue factor, a surface receptor and cofactor...
for activation of coagulation factor VII, the most common initiator of the serine-protease cascade leading to thrombin formation. Thrombin catalyzes conversion of circulating fibrinogen (possibly extravasated through leaky vessels into neoplastic tissues), into insoluble fibrin, which is then cross-linked (by coagulation factor XIII) to form the fibrin-gel matrix. Tissue factor is seldom expressed in normal epithelial tissue but is frequently expressed as a result of malignant transformation and, being an integral membrane protein, is a good candidate to explain local rather than systemic effects. The presence of tissue factor in neoplastic tissues has been associated with a proangiogenic phenotype, providing further circumstantial evidence for the ability of the coagulation system to regulate vessel formation (see ref. 11 and references therein). Cancer procoagulant is a cytosine protease capable of initiating the coagulation process as an alternative to the complex formed by tissue factor and coagulation factor VII. Cancer procoagulant is expressed in malignant and fetal tissues but not in normally differentiated tissue and is prominently involved in coagulation disorders associated with acute promyelocytic leukemia (reviewed in ref. 10).

Cancer cells are also known to promote coagulation through suppression of fibrinolytic activities. Fibrin is degraded by plasmin, which derives from plasminogen through the action of plasminogen activators. Plasminogen activator inhibitor type-1 (PAI-1) prevents generation of plasmin and thus ultimately promotes persistence and expansion of blood clots. Most interestingly, PAI-1 is a protein secreted in the blood, where it can sustain systemic prothrombotic effects (reviewed in ref. 13). This property is confirmed by the phenotype of PAI-1-transgenic mice, which exhibit venous occlusions (14), and by studies in humans that correlate high levels of PAI-1 in plasma with an increased risk of venous and artery occlusion (reviewed in ref. 15). PAI-1 is an attractive candidate to establish a functional link between hemostasis disturbance and tumor progression. In cancer patients, association of high levels of PAI-1 with poor prognosis has been extensively documented (16). PAI-1 is supposed to foster cancer onset and progression through multiple actions, although its role is counterintuitive, as it inhibits urokinase-type plasminogen activator, which promotes matrix degradation and cancer invasion (reviewed in ref. 13). In knock-out mice, the absence of PAI-1 prevents cancer invasion and vascularization (17), again supporting the role of blood coagulation in angiogenesis regulation (18).

**MET Activation Triggers a Thrombohemorrhagic Syndrome**

The MET oncogene encodes the tyrosine kinase receptor for hepatocyte growth factor (HGF), also known as scatter factor (SF). HGF/SF drives a genetic program of invasive growth that underlies tissue morphogenesis and repair in physiologic conditions and invasion and metastasis in cancer (for a review, see ref. 19). Interestingly, HGF/SF shares a number of structural and functional properties with enzymes of the coagulation cascade. In fact, it belongs to the plasminogen family of proteases that contain “kringle” motifs and a serine protease-like domain. Moreover, like clotting factors, HGF/SF undergoes a proteolytic cleavage to become biologically competent. This processing event is done by enzymes in the coagulation cascade, including urokinase-type plasminogen activator, a factor XII-like protease, and factor XII itself. Activation of the HGF/SF receptor encoded by MET occurs in a wide array of human tumors, either as a consequence of germ line or sporadic mutations, or, more commonly, overexpression. Interestingly, pancreatic and ovarian cancers, which show MET overexpression most frequently (20, 21), are also among the tumors most commonly associated with Trousseau’s syndrome (see above). MET elevation is often driven by hypoxia, the oxygen deprivation state that tends to be found in inner tumor masses. The likely mechanism is mediated by the hypoxia-activated transcription factor HIF-1α, which positively regulates the MET promoter (22). Thus, under low oxygen concentrations, the invasive growth response elicited by HGF/SF in cells is amplified.

Recently, we set up a genetically modified mouse in which the activated MET oncogene was targeted to the mouse liver (23). A unique aspect of this model was that lentiviral vectors were used, enabling tissue-specific targeting of the oncogene to rare cells within an otherwise normal tissue (for a review, see ref. 24). We found that these mice develop both a tumorigenic process and a hemostatic disturbance that is reminiscent of Trousseau’s syndrome (23). This linkage allowed us to investigate the genetic mechanisms that link activation of the MET signaling pathway to coagulation disorders in cancer and to investigate the effects of pharmacologic interference with these mechanisms.

After MET transduction into mouse liver, we detected the development of foci of hepatocyte clonal expansion, which slowly progressed towards malignancy. Surprisingly, we also observed the occurrence of a thrombohemorrhagic syndrome articulated in two phases. The first phase was thrombotic and preceded the onset of liver foci. This phase was characterized by the appearance of venous thromboses and by hyperactivation of the coagulation system. The second phase was characterized by a hemostasis disturbance that evolved into a hemorrhagic diathesis, displaying the clinical features of chronic disseminated intravascular coagulopathy. This phase was characterized by exhaustion of the hyperactivated hemostatic system, leading to the production of lethal hemorrhages in the mice. To investigate the mechanistic link between oncogene activation and hemostasis disorders, we studied the gene expression profile of cells transduced with the activated MET oncogene. Many of the 71 hemostasis-associated genes represented on the microarray were up-regulated; however, two genes in this group, PAI-1 and cyclooxygenase-2 (COX-2), were strongly induced (more strongly than any other gene in the whole set of 12,000 genes analyzed). As discussed above, PAI-1 is a circulating protein that inhibits clot degradation thus promoting systemic intravascular coagulation. COX-2 encodes an inducible form of prostaglandin synthase that catalyses the synthesis of arachidonic acid derivatives (prostaglandin G/H). These compounds are then transformed, by tissue-specific isomerases, into biologically active prostanoids, including prostacyclins and thromboxane, which modulate platelet functions (for a review, see ref. 25). Like PAI-1, COX-2 seems an attractive candidate to establish a functional link between hemostasis disturbance and tumor progression. Although the net outcome of COX-2 activity on the hemostatic balance is still controversial (26–28), COX-2 is renowned as a critical gene for cancer onset and progression. Indeed, COX-2 inhibition by rofecoxib or other nonsteroid anti-inflammatory drugs proved to be effective in preventing colorectal cancer, both in mouse models and human epidemiologic studies (for a review, see ref. 26). Like in the case of PAI-1, the pathogenic role of COX-2 has been linked mainly to
modulating angiogenesis (26), again linking blood clotting regulation to vessel formation.

In our model, we verified that PAI-1 is expressed in vivo by MET-transduced hepatocytes, and, most importantly, that PAI-1 is released in the blood serum, early after oncogene transduction, in coincidence with the appearance of venous thrombosis. The same is true for COX-2, the downstream catabolites of which are found in the urine of MET-transduced mice. We assessed the causal role of PAI-1 and COX-2 in unleashing and sustaining the thrombohemorrhagic syndrome, through administration of specific inhibitors of COX-2 (rofecoxib) or PAI-1 (XR5118; ref. 23 and references therein). These inhibitors hampered the evolution of disseminated coagulopathy and prolonged mouse survival. As PAI-1 and COX-2 were the two genes most strongly induced by activated MET, we hypothesize that these genes are critical not only for sustaining the thrombohemorrhagic phenotype but also for promoting tumor progression. Indeed, treatment with COX-2 inhibitors induced necrosis and regression of preneoplastic nodules in MET-transduced mice (23).

Our studies assign an important new role to the MET oncogene in driving a genetic program that not only transforms cells but also directs “landscaping events” linked to hemostasis that may be critical for the execution of invasive growth. A model summarizing how this process may work is presented in Fig. 1. In this process, deposition of a fibrin-gel provisional matrix forms a critical foundation from which cancer cells can draw support from a nurturing inflammatory and proangiogenic environment. Many if not all tumors may share a similar link between the hemostasis-associated deposition of fibrin. In some tumors, a systemic extension of this process that is unleashed may form the basis for the development of Trousseau’s syndrome. Further investigations of the role of oncogene signaling in this process may shed light on how such an extension may occur, which since the 19th century has been known to be a potential clinical correlate of occult malignancy.

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