

Tumor Hypoxia, the Physiological Link between Trousseau's Syndrome (Carcinoma-induced Coagulopathy) and Metastasis

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Introduction

Trousseau first reported over 100 years ago that cancer patients have an increased incidence of coagulopathies (1). Since Trousseau published his findings, thromboembolic disorders have been documented at elevated frequencies in patients with a wide variety of tumors such as lung, pancreas, stomach, and colon tumors (2). These clinical findings have been supported by laboratory studies that have identified altered levels of blood clotting factors in the serum of cancer patients. Indeed, the presence of an unexplained deep venous thrombosis can be clinical reason enough to screen for occult malignancy. Mechanistically, it has been proposed that normal host cells such as platelets, mononuclear phagocytes, and smooth muscle cells are responsible for activating procoagulant and angiogenic pathways (3). However, recent data derived from large "expression profiles" have generated insight into gene expression changes in solid tumors that indicate that tumor cells under microenvironmental stress can produce the same procoagulant and angiogenic factors that host cells secrete. Because studies reveal that solid tumors arising from a number of cell types express and secrete proteins involved in coagulation, it is unlikely that acquired genetic mutations in tumor-promoting genes alone are able to completely explain the clinical data. We propose that tumor-specific physiological changes, such as decreased oxygenation (tumor hypoxia), act to stimulate expression of blood clotting regulators independent of the cancer cell's origin. In this study, we discuss the supporting evidence for this new concept with regard to both coagulation and fibrinolysis in the vicinity of the tumor and through systemic circulation to distant sites.

The Contribution of Host Cells in Tumor-induced Coagulopathies

Manifestation of blood coagulopathies in cancer patients ranges from subclinical changes in clotting factors detected in laboratory tests to life-threatening thromboembolisms. This procoagulant state of cancer patients is thought to reflect the dysregulation of the coagulation and fibrinolytic activities of mononuclear phagocytes, platelets, and smooth muscle cells. Clearly, the involvement of these cells and their secreted procoagulant factors can contribute to the systemic hypercoagulopathies found in cancer patients. Evidence has also accumulated that many solid tumor cells themselves also possess procoagulant activity and can interact with host blood cells and the vascular endothelium (4). Thus, we propose that the tumor cell also contributes to the carcinoma-induced coagulopathy cascade. These new data direct our focus on the tumor cell as a critical mediator of the

procoagulant state, at least with regard to local changes in coagulation and fibrinolysis in the tumor. Data on whether tumor cells are able to invoke a systemic coagulopathy require more investigation and ultimately may require downstream simulation of cellular host cells and secreted coagulation factors. In fact, tumor cell- and host cell-induced coagulation and fibrinolysis may provide a synergistic means of inducing a hypercoagulable state in cancer patients. If solid tumor cells are able to initiate a hypercoagulable state, is the mechanism responsible for tumor cell-induced coagulation the accumulation of genetic alterations during tumor evolution, or is it a response to stress induced by the tumor microenvironment?

Evidence that Tumor Cell-induced Coagulopathy Is a Response to Hypoxia

Investigators have reported that stresses such as hypoxia (decreased oxygenation) increase the expression of genes involved in regulating coagulation (Table 1). These studies offer a physiological explanation for Trousseau's syndrome: tumor hypoxia induces the expression of genes to produce unregulated levels of blood clotting factors and their regulators. Implicit in this concept is the fact that changes in the oxygenation status of a solid tumor stimulate a wound healing response. For example, hypoxia has been shown to induce expression of genes encoding TF,² PAI-1, uPA, and uPAR, among others (Table 1). How and why hypoxia stimulates expression of these genes in a diverse group of tumors become more apparent when we examine the physiological conditions that lead to hypoxia in normal tissues during wound healing.

Wounding is a common physiological perturbation that leads to normal tissue hypoxia. A concerted response to hypoxia in the wounded tissue has evolved that stimulates the release of proteins involved in coagulation, fibrinolysis, and angiogenesis. Similarly, the same pathways that are initiated transiently by hypoxia in wounded tissues are initiated chronically in response to hypoxia in the tumor. In wounding, vascular damage leads to reduced blood flow and hypoperfusion. Vascular endothelial cell perturbation leads to exposure of subendothelial TF. When blood comes in contact with TF, the clotting cascade is initiated to form a thrombus to maintain hemostasis (Fig. 1). Because the primary goal after vascular injury is to maintain blood volume, the initial response to hypoxia is to induce the expression of procoagulant molecules such as TF and PAI-1.

Paradoxically, the result of this thrombus is to further compromise the damaged vessel's function, exacerbating blood stasis and local tissue hypoxia. Clearly, the clot does not block the vessel indefinitely because anoxic conditions would lead to tissue death. Instead, the associated tissue hypoxia transiently induces the expression of genes involved in the controlled dissolution of the clot. In the acute time

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² The abbreviations used are: TF, tissue factor; PAI, plasminogen activator inhibitor; uPA, urokinase-type plasminogen activator; uPAR, uPA receptor; LRP, LDL receptor-related protein; VEGF, vascular endothelial growth factor; PGF, placental growth factor.

Table 1 Hypoxia-regulated genes involved in coagulation/fibrinolysis and angiogenesis

Gene	Activity	Ref. no.
Tissue factor	Procoagulant/angiogenic	20 and 21
PAI-1	Procoagulant	15
uPA	Anticoagulant/adhesion	14
uPAR	Mitogen/adhesion	22
LRP	Regulator of uPAR/uPA	15
VEGF/VPF ^a	Angiogenesis/permeability/mitogen	23
Angiogenin	Angiogenesis	24
Angiopoeitin 2	Vascular remodeling	25
PGF (VEGF B)	Angiogenesis	26

^a VPF, vascular permeability factor.

frame, the result of this induced expression is to activate fibrinolysis and dissolve the clot, restoring vessel patency and oxygen delivery. Under normal physiological conditions after minor wounding, it is tissue hypoxia that drives expression of uPA and uPAR that leads to the controlled activation of the fibrinolytic cascade and timely reperfusion (Fig. 1). Both procoagulant and anticoagulant molecules are therefore necessary to regulate the fine balance between hemostasis and tissue perfusion.

In solid tumors, tissue hypoxia is the result of malformed vessels (5) but still induces the same molecules triggered by thrombus formation in normal tissues during wound healing. In this case, hypoxic tumor tissue induces the expression of regulators of coagulation/fibrinolysis in an attempt to restore perfusion. However, because the tumor tissue never becomes normoxic, a futile cycle of pro- and anticlotting factor production interacts with effector host cells to result in the systemic coagulopathies that are clinically observed in the patient. One explanation for the futile cycling is that the event-initiating hypoxia is not the transient formation of a thrombus, but the malformed vessels of the tumor. This chronic phenomenon had been observed and described, but it has only recently become apparent why tumors resemble "wounds that do not heal" (6).

Hypoxia Stimulates Angiogenesis to Increase Tumor Perfusion

Chronic tissue hypoxia has a second physiological function that is designed to lead to reperfusion: stimulating the angiogenic pathway. When normal tissue becomes hypoxic after severe wounding, if the recanalization of the damaged vessel is not successful in restoring normoxia, then a new vessel is needed. Chronic hypoxia leads to the induction of growth factors designed to generate new vessels to establish a collateral blood flow. For example, long-term hypoxia induces expression of endothelial cell growth factors such as VEGF/vascular permeability factor, PGF, angiogenin, and angiopoietin 2, all of which stimulate endothelial cell proliferation and/or migration (Table 1; Fig. 1). In addition to the requirement for increased numbers of endothelial cells, systematic proteolysis of the extracellular matrix is needed to allow the formation of capillary buds and their migration into the hypoxic tissue. With regard to this point, chronic hypoxia results in increased expression of collagenases and gelatinases (7) that are capable of degrading the extracellular matrix and allows endothelial cell migration (Table 1; Fig. 1). Additionally, uPA and uPAR have the ability to localize the general protease plasmin to the cells in the regions of hypoxia.

The combination of vascular permeability, capillary bud formation, and extracellular matrix degradation is necessary to generate a new vessel (Fig. 1). Because tumor neovascularization is often insufficient to satisfy the oxygen demands of the growing tumor, the resulting chronic hypoxia continues to induce the expression of genes involved in tissue remodeling and angiogenesis in both tumor and stromal tissue (8). Thus, the hypoxic tumor cells have an increased probability of being locally invasive through the degraded extracellular matrix and distantly metastatic through leaky new vessels.

Clinical Significance of Hypoxia-induced Procoagulant Gene Expression and Tumor Metastasis

Is there any relationship between tumor hypoxia, a systemic hypercoagulable state, and tumor metastasis? With the introduction of the

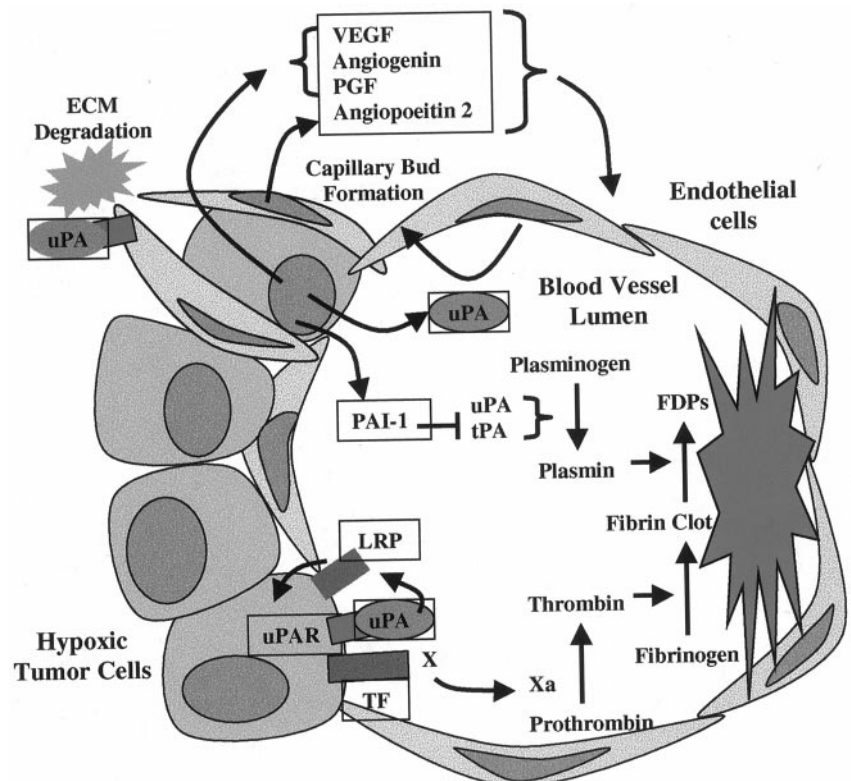


Fig. 1. Regulation of coagulation and metastasis by genes induced by hypoxia (see the text for details). Hypoxia-induced genes are boxed. ECM, extracellular matrix.

Table 2 Clinical studies correlating elevated hypoxia-induced factors to increased metastasis

Cancer site	Factor	Outcome identified	Ref. no.
Prostate	uPAR TF	Advanced disease, survival	27 and 28
Breast	uPAR PAI-1	Advanced disease, survival	29 and 30
Lung	PAI-1 TF	Metastasis	31 and 32
Colon	SuPAR ^a VEGF	Survival, disease progression	33–35
Stomach	Angiogenin uPAR PAI-1	Invasion, metastasis	36
Ovary	PAI-1 VEGF	Advanced disease	37 and 38
Bladder	Angiogenin	Invasion	39

^a SuPAR, soluble uPAR.

polarographic needle electrode, it is now possible for physicians to directly measure oxygen concentrations in the solid tumor. Recent investigations have demonstrated that solid tumors possess large regions with oxygen tension below 1–2 mm Hg. Several groups have used this technology to prospectively stratify patients based on tumor hypoxia and to correlate low tumor oxygen levels with increased local invasion, increased distant metastasis, and poor prognosis (9–11). However, none have related changes in tumor oxygenation and the secretion of coagulation factors and fibrinolytic enzymes.

Regulation of plasmin has been identified in a number of experimental models as important in determining the invasive nature of tumor cells. Tumor models have established a role for hypoxia-responsive uPA/uPAR expression in the binding of tumor cells to the extracellular matrix and the migration of the cells through that matrix (12, 13). This uPAR-dependent invasive activity can be enhanced by the treatment of cells with hypoxia (14). Modifying the signaling through uPAR by the hypoxia-responsive expression of LRP/ α_2 macroglobulin receptor (15) can alter the invasive activity (16), whereas the blocking uPAR signaling with an engineered construct can suppress tumorigenicity *in vivo* (17). The function of uPAR seems to be necessary for the intravasation of tumor cells through the target blood vessels (18). Such studies describing a role for uPAR/uPA/LRP in invasion offer a plausible explanation for the phenomenon first described by Young *et al.* (19) that pretreatment of tumor cells with hypoxia results in a significant increase in the frequency of metastatic lesions in lung invasion assays.

Because of the *in vitro* evidence supporting the importance of plasmin regulators uPAR/uPA/PAI-1 in regulating metastasis, several clinical studies have also tested connections between altered blood levels of clotting factors, metastasis, and tumor hypoxia. The clinical relationship between these factors has been most convincingly demonstrated in a large series of clinical studies that has identified circulating levels of TF, PAI-1, uPA, and uPAR as independent prognostic indicators of poor clinical outcome in cancer patients (Table 2). The increased levels of these procoagulant factors in the systemic circulation of cancer patients strongly suggests that hypoxia induces not only local coagulation and fibrinolysis but may also be strongly involved in systemic coagulopathies. In a summary of published studies (Table 2), patients with solid tumors of different histological origins exhibit increased levels of coagulation factors in their serum, which, *in vitro*, makes transformed cells prone to local invasion, distant metastasis, and decreased survival.

In summary, thromboembolic disorders and metastatic disease are often found together in cancer patients. This seemingly disparate consolidation of phenotypes is actually two manifestations of the expression of the same set of genes. The physiology of the solid tumor leads to the existence of chronic hypoxia, and it is the hypoxic

up-regulation of target genes that drives these processes. Thus, strategies that attempt to oxygenate a tumor through the delivery of erythropoietin that will make solid tumors more oxidic should be beneficial to anticancer therapies such as radiotherapy or chemotherapy and should also decrease metastatic spread of tumor cells induced by a low oxygen environment.

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