Targeting the Endothelium in Cancer – The Importance of the Interaction of Hemostatic Mechanisms and the Vascular Wall for Tumor Growth and Angiogenesis

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Patients who present with idiopathic venous thromboembolism (VTE) frequently harbor an occult cancer that does not become clinically evident until months or perhaps years later. Although Professor Armand Trousseau first documented this link between coagulation and malignancy in 1865 [1], the mechanisms underlying the association have only recently started to become more apparent. The key mediator of this link appears to be tissue factor (TF), the ubiquitous 47kd membrane protein receptor for factor VII (and factor VIIa) in the clotting cascade that is over-expressed in tumor cells, tumor-associated macrophages and tumor-associated endothelial cells. In addition to its role in the mediation of VTE in cancer, expression of TF by aberrant (angiogenic) endothelial cells may also be of critical importance both as a novel marker and as a regulator of tumor angiogenesis. Therefore, TF appears to be a useful target for so-called 'vascular targeting agents' [2], many of which will be discussed in this first session of the meeting.

TF may regulate tumor growth, diapedesis of tumor cells across endothelial barriers and tumor angiogenesis [3–5]. The function of TF in tumor angiogenesis, which is essential for tumor growth and metastasis, is mediated via both clotting-dependent and -independent pathways [3–7]. Other

key players downstream of TF activation that also induce angiogenesis include thrombin, protease-activated receptors (PARs) and fibrin. These pathways are schematically represented in figure 1 [4]. While angiogenesis is the central link between blood coagulation and tumorigenesis, coagulation products (via pathways other than those related to angiogenesis) may also promote tumor growth.

Clotting-dependent pathways of tumor angiogenesis likely involve activation of the TF receptor via ligand binding, followed by downstream production of thrombin and ensuing clot formation. Clotting-independent pathways appear to involve phosphorylation of the cytoplasmic domain of the TF receptor and subsequent downstream signaling events that occur independent of thrombin production or clot formation, and possibly even independent of ligand activation [5, 7] (fig. 2). The cytoplasmic tail of TF appears to regulate non-clotting-dependent mechanisms, including cytoskeletal reorganization, vascular remodeling, angiogenesis and cellular metastasis [6–9]. Following extracellular binding of the TF receptor, the actin-binding protein 280 (ABP-280) is recruited to the cytoplasmic tail where it participates in the assembly of actin filaments [8]. The carboxyl terminus of ABP-280 associates with the

Fig. 1. Coagulation cascade, angiogenesis and tumor growth and metastasis. The coagulation cascade is linked to tumor growth and metastasis via its induction of angiogenesis. Both clotting-dependent and -independent mechanisms induce angiogenesis. Tissue factor (TF) induces non-clotting-dependent mechanisms via phosphorylation of its cytoplasmic tail and subsequent signal transduction cascades. TF induces angiogenesis via clotting-dependent mechanisms by downstream generation of the serine protease thrombin. Thrombin also induces angiogenesis via clotting-independent and -dependent mechanisms. Clotting-independent mechanisms are thought to be mediated via proteolytic cleavage of the protease-activated receptors (PARs) and subsequent activation of G-protein-coupled signal transduction cascades that induce angiogenesis-related genes. Clotting-dependent mechanisms are mediated via fibrin deposition and platelet activation. Fibrin induces angiogenesis via clot formation. Cross-linked fibrin matrices provide provisional, pro-angiogenic matrices that facilitate endothelial infiltration and tubule formation. Degradation of the fibrin matrix exposes cryptic sites that facilitate endothelial cell adhesion and migration. By-products of fibrinolysis can also elicit pro-angiogenic effects. (Reproduced from [4] with permission of the publishers.)

cytoplasmic domain of TF, while its amino terminus interacts with the actin filaments. This association regulates mitogen activated protein (MAP) kinase signaling and phosphorylation of focal adhesion kinases (FAKs) that promote cell adhesion and migration. Mechanisms mediated

by the cytoplasmic tail of TF have been implicated in embryonic vessel development, tumor angiogenesis and metastasis [3–5, 8, 9].

Angiogenesis involves activation of endothelial cells, invasion of the endothelial cells through their basement membrane and migration to distal sites. A variety of studies have demonstrated that thrombin contributes to each of these events. Thrombin decreases adhesion of endothelial cells to basement membrane proteins via cAMP, making them more mobile. Thrombin also mobilizes adhesion molecules to the endothelial surface (e.g., P-selectin) that facilitate platelet and tumor cell adhesion [4, 5]. While not illustrated in figure 2, most of the cellular effects elicited by thrombin are mediated through the activation and subsequent signal transduction cascades of members of the protease-activated receptor (PAR) family, suggesting that proteolytic activity of thrombin is essential for the mediation of these events [4, 5]. However, clotting activation is not essential for thrombin to elicit these cellular effects of importance to tumor growth and metastasis.

The discussants in the opening session of the meeting will explore some of these pathways and provide an important ‘snapshot’ of an exciting new area of exploration for rational drug design – searching for new anticancer agents that can: (1) selectively inhibit the adhesive interactions between tumor cells and the endothelium; (2) target selectively tumor cells and tumor-associated blood vessels; (3) downregulate the increased expression of clotting proteins in cancer. Ultimately, it can be envisioned that combinations of novel anticoagulant drugs and anti-angiogenesis agents might be utilized to attack both the vascular proliferative component of cancer and the acquired thrombophilia.

Fig. 2. Tissue factor (TF) induces angiogenesis with both clotting-dependent and -independent pathways. Figure 2 illustrates the roles of tissue factor and fibrin in the hematogenous dissemination of malignant cells. The malignant cells from the primary tumor induces the sprouting of new vessels from a pre-existing vessel. Some of the more aggressive cells invade into the microvasculature and start metastasizing. A longitudinal cross-section of part of the blood vessel is illustrated, showing the elongated endothelial cells lining the vessel and components of the blood, including red blood cells (RBC), white blood cells (WBC), platelets and foreign tumor cells. The upregulated expression of TF on tumor cells and associated vascular endothelial cells promotes the formation of a thromboembolism of tumor cells, RBC, fibrin and platelets in the bloodstream.

I. Cross-section of the tumor vessel containing a thromboembolism. Circulating FVII is proteolytically activated to FVIIa when it binds to activated TF receptors on the endothelial cells (tumor cells or activated macrophages). The ligand-bound receptors initiate the coagulation cascade by activating factor X (Xa) and subsequently converting prothrombin (II) to thrombin (IIa) with the involvement of activated factor V (Va), phospholipid (PL) and calcium (Ca^{2+}). Thrombin cleaves soluble fibrinogen (FBG) to yield cross-linked fibrin deposits. Thrombin also activates platelets that contribute to

clot formation. Activated platelets release a number of pro- (e.g., VEGF) and anti-angiogenic factors (e.g., platelet factor 4) that contribute to tumor growth and angiogenesis. Fibrin induces the upregulation of TF on both endothelial and tumor cells, promoting the vicious cycle of clot formation and tumor growth. Fibrin also induces the secretion of interleukin-8 (IL-8), a pro-angiogenic factor. Thromboemboli protect tumor cells from destruction by both humoral and cellular immune mechanisms and also promote tumor growth and extravasation, leading to the formation of metastatic colonies. Analogous to the primary tumor, survival and growth of the metastatic tumor depends on the recruitment of new blood vessels.

II. Clotting-independent angiogenesis induced by TF. TF can promote the increased expression of the pro-angiogenic factor VEGF and the decreased expression of the anti-angiogenic peptide thrombospondin (TSP), independent of ligand binding. Secreted VEGF promotes the upregulation of TF on endothelial cells and also acts as a mitogen to the endothelial cells to induce angiogenesis. All of these proteins (both effector molecules and receptors) represent possible targets for novel anticancer drug discovery and combination chemotherapy may in the future incorporate one or more endothelial specific targets illustrated here. (Reproduced from [5] with permission of the publishers.)

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