Oncogenes, Trousseau Syndrome, and Cancer-Related Changes in the Coagulome of Mice and Humans

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Abstract
Cancer is often associated with venous thrombosis, a phenomenon that was first described by Trousseau in 1865 (Trousseau syndrome). Recent studies have begun to explain how oncogenic events may deregulate the hemostatic system. For instance, activated oncogenes (K-ras, EGFR, PML-RARα, and MET) or inactivated tumor suppressors (e.g., 53 or PTEN) may increase the risk of thrombosis by inducing the expression of tissue factor, a potent procoagulant molecule, and plasminogen activator inhibitor-1, a fibrinolysis inhibitor. In a more complex clinical reality, transforming genes may often act in concert with numerous epigenetic factors, including hypoxia, inflammation, anticancer therapy, contact between blood and metastatic cancer cells, and emission of procoagulant vesicles from tumors and their stroma into the circulation. To add to mechanistic insights gained from mouse models, which may not fully phenocopy human Trousseau syndrome, we suggest that valuable clues to progression and thrombosis risk may be obtained by monitoring multiple hemostatic variables in cancer patients (“coagulomics”).

The Elusive Nature of Cancer-Related Coagulopathy

Hemostatic perturbations associated with human cancer are common and diverse. In some instances, patients with overt or occult malignancy develop deep vein thrombosis (DVT) or pulmonary embolism, both manifestations of venous thrombembolism (VTE; ref. 1). In other cases, clinical findings may include features of disseminated intravascular coagulation (DIC), consumptive coagulopathy, or overt thrombomorrhagic syndrome, all of which are often collectively referred to as cancer coagulopathy. This condition is sometimes equated with Trousseau syndrome to recognize the seminal contribution of Armand Trousseau who in 1865 described one aspect of cancer coagulopathy, which he had termed phlegmasia alba dolens (2, 3). Indeed, abnormalities in coagulation variables accompany up to 90% of all metastatic cancers and include both clinical and laboratory findings [e.g., circulating D-dimers, thrombin-antithrombin complexes, tissue factor (TF), and other changes; ref. 3].

The mechanism of Trousseau syndrome likely includes all aspects of Virchow’s triad (i.e., elements of stasis, vascular trauma/pathology, and the hypercoagulability of blood itself; refs. 4, 5). For instance, TF expression by tumor cells themselves may activate the coagulation system in cancer patients through direct contact with coagulation proteases in blood plasma, e.g., via porous walls of intratumoral capillaries, by way of invasion of tumor cells into the vascular lumen, or through entry of metastatic cancer cells into the systemic circulation (3). In addition to local changes, tumor masses may release soluble procoagulants [e.g., cancer procoagulant (CP)], TF-containing microvesicles, procoagulant mucins, and inhibitors of fibrinolysis [e.g., plasminogen activator inhibitor-1 (PAI-1)] that would deregulate the cancer patient’s hemostatic system (1, 3, 6). We will refer to all the proteins involved in the hemostatic system as the coagulome (Fig. 1).

The Effect of Oncogenic Mutations in Cancer Cells on Trousseau Syndrome

Clinical progression of human malignancies can usually be traced to the accumulation of oncogenic defects in the genome of cancer cells. A parallel between these changes and a deterioration of the hemostatic system in cancer patients led to the suggestion that activation of oncogenic pathways may result in direct and/or indirect perturbations of the coagulome and ultimately in the development of Trousseau syndrome (7, 8).

The indirect ("nonspecific") effect of oncogenic events on Trousseau syndrome could occur through the known consequences of increasing tumor aggressiveness, such as vascular invasion, metastasis, hemorrhage, vascular permeability, and angiogenesis. For instance, frequent and oncogene-dependent up-regulation of vascular endothelial growth factor (VEGF) in cancer cells (8) could elicit vascular growth, remodeling, hyperpermeability, and TF expression by endothelial cells (9).

A recent series of experimental and clinical studies with molecularly well-defined types of cancer cells revealed a direct role of oncogenic events in the perturbations of the hemostatic system that are relevant for Trousseau syndrome (7). These observations include the negative effect of an epidermal growth factor receptor antagonist (e.g., C225 antibody) on the expression of TF in cancer cells (8). In addition, TF expression, procoagulant activity, and proangiogenic activity were all found to be modulated by oncogenic mutations of K-ras oncogene and p53 tumor suppressor gene in human colorectal cancer cells (10). In this setting, oncogenic lesions also provoked an increased emission of TF containing microvesicles into the circulation, a likely vehicle of systemic coagulopathy (10). These findings are consistent with the increases in TF expression observed at different Duke’s clinical stages of colorectal cancer (11). In human glioma cells, inactivation of PTEN tumor suppressor gene also resulted in up-regulation of TF, especially under hypoxic conditions (12). Moreover, patients with acute promyelocytic leukemia (APL), when treated with all-trans-retinoic acid to block the actions of the PML-RARα oncoprotein, exhibited lower TF expression and resolution of DIC symptoms (13).

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malignancy associated with highly symptomatic and lethal thrombohemorrhagic syndrome (14). This syndrome was found to be due to the up-regulation of PAI-1 and cyclooxygenase-2 (COX-2) because some of the related hemostatic abnormalities were corrected by treatment with the COX-2 inhibitor, rofecoxib, or the PAI-1 inhibitor, XR5118 (14). Collectively, these findings document a hitherto unappreciated link between genetic tumor progression and cancer coagulopathy, something that we have postulated earlier (7).

Do oncogenes drive cancer progression, at least in part, through eliciting coagulopathy? It is thought provoking that thromboprophylaxis with low molecular weight heparin (LMWH) resulted in tangible anticancer effect in several recent clinical trials encompassing a wide array of malignancies (4). This is consistent with the emerging realization that various proteins of the coagulation system play important roles in cellular processes of tumor growth, invasion, metastasis, and angiogenesis (15). For instance, TF may enhance tumor growth by increasing the local generation of coagulation proteases (4, 14). These proteases (FVIIa, FXa, and thrombin) activate intracellular signaling pathways in tumor cells by cleavage of protease activated receptors (PAR), particularly PAR-1 and PAR-2 (16). Finally, these changes may translate into expression of potent angiogenic factors (VEGF and interleukin-8), suppression of angiogenic inhibitors [thrombospondin (TSP)-1 and TSP-2], triggering prosurvival genes (Bcl-xl), and marked alteration of migratory and metastatic properties (reviewed in ref. 16). In this sense, TF acts as the primary ‘sensor’ translating the contact between cancer cells and plasma proteins into a profound phenotypic change. Thus, the effects of the coagulation system on cancer cells may ultimately stem from the ability of the latter to acquire highly procoagulant properties, a transition we argue to be a function of genetic tumor progression. As this contention is based on studies involving both cancer patients (13) and experimental mice (10, 14), it may be useful to ask to what extend the mechanisms of Trousseau syndrome are shared between the two species?

Cancer Coagulopathy in Men and Mice—Key Effectors, Manifestations, and Some Unanswered Questions

In spite of the operational similarity between various clinical and experimental models, in which constituents of cancer coagulopathy have been examined, numerous questions remain unanswered. For instance, it is unclear whether the same hemostatic effectors and
mechanisms are responsible for the coagulopathy associated with both mouse and human tumors. In this regard, the thrombohemorrhagic syndrome observed in mice harboring hepatomas driven by oncogenic MET (14) seems to be far more severe than what is typically observed in most human malignancies, especially at their early stages. In contrast, immunodeficient mice harboring highly thrombogenic, late-stage disease-derived human cancer xenografts, including those known for their expression of MET (e.g., MDA-MB-231), tend to manifest far more subtle and often undetectable changes in the hemostatic system. In spite of the high prevalence of coagulopathy in APL patients, thrombohemorrhagic syndromes have not been described in mouse models of APL, although such models are based on the expression of the same oncogene (PML-RARα). This discrepancy suggests that perhaps species-specific aspects of the coagulation and fibrinolytic systems (17) should be considered more seriously when extrapolating from mice to humans.

In spite of the relatively close homology between proteins of the murine and human hemostatic systems, they do not function in an identical manner (Table 1; ref. 18). For instance, mice are more resistant to lipopolysaccharide-induced activation of the coagulation system than humans. In addition, acute thrombosis often represents a lethal culmination of the atherosclerotic process in humans, whereas the same is rarely observed in corresponding mouse models of vascular disease, atherosclerotic plaque formation, and even plaque rupture (e.g., in ApoE−/− mice; ref. 21). Unlike humans, mice rarely (if ever) develop DVT in their extremities even when subjected to highly prothrombotic insults (e.g., as in mice with mutant form of the anticoagulant antithrombin; ref. 22). We have observed species-specific differences in the expression of the human and mouse TF gene. Moreover, although human TF efficiently activates murine extrinsic pathway, the opposite is not the case (18). Mouse platelets also differ from their human counterparts in terms of their number, size, half-life, and expression of PARs (17, 18). Interestingly, the levels of PAI-1 are 5- to 10-fold lower in mice than those of humans (18). This represents only a partial list of the most apparent differences between the two species (Table 1; refs. 17, 18).

Apart from species-specific features of the coagulation system itself (17), behavioral and iatrogenic factors may make it difficult to recapitulate a bona fide Trousseau syndrome in mice. Thus, cancer coagulopathy could be exacerbated by patient immobility, exposure to chemotherapeutic agents, indwelling venous catheters, and surgery. In contrast, mice usually remain very mobile, even if exposed to relatively high tumor burden and considerable therapeutic insults. They rarely receive treatment through i.v. lines and their procoagulant responses to therapies seem to be more subtle than those in humans, at least insofar as these questions have been studied. Hemostatic functions are also affected by circadian rhythm (23) and these effects are different between humans and mice, the latter being nocturnal. Some of these observations could perhaps explain why even pronounced prothrombotic side effects of novel anticancer drugs (e.g., SU5416 or thalidomide) have been largely overlooked during preclinical development of these agents (for review, see ref. 8).

The hemostatic mechanisms operating in different tissues appear to be different, at least with regard to the engagement of TF-dependent or TF-independent (i.e., extrinsic versus intrinsic) coagulation pathways (5). It follows that differences could also exist in procoagulant phenotypes of tumors derived from different organs, tissues, and cell types. It could also be expected that the site of cancer cell inoculation (e.g., orthotopic versus ectopic)
to generate tumor xenotransplants in immunodeficient mice, or sites of injection of an oncogenic adenovirus could affect the nature of the resulting hemostatic perturbations, as would different patterns of organ-specific metastasis. Interestingly, mice engineered to express low levels of TF (5) displayed tissue-specific hemostatic defects in their lungs, hearts, and uteri, but not in the liver. It is intriguing, in this context, that hepatomas induced in mice by transduction of the oncogenic MET led to severe but apparently TF-independent thrombohemorrhagic syndrome (14). In fact, the involvement of the liver during the neoplastic process is somewhat unique due to its role as the main source of the majority of coagulation factors. Thus, development of multifocal hepatic neoplasia or liver metastasis could affect systemic levels of clotting factors, whereas tumors growing in extraneoplastic tissues (lung, colon, breast, and brain) would not manifest this complication.

The role of TF in cancer is also associated with some paradoxes. For instance, TF expression by tumor cells, angiogenic blood vessels, and inflammatory infiltrates in cancer would be expected to induce thrombosis and occlusion of the related (intratumoral) microvasculature and macrovasculature. However, in spite of strong evidence for ongoing local coagulation (e.g., deposition of cross-linked fibrin in the tumor interstitium), high levels of TF, or detectable thrombin activity (3), tumor blood vessels largely remain patent and free of occlusive thrombi. Instead, symptoms of VTE are observed in remote organ locations and laboratory evidence of coagulopathy is present systemically (1). It is not clear why thrombotic events do not occur within the tumor mass, but concomitantly increased activity of the fibrinolytic system [e.g., plasminogen activators (both urokinase-type plasminogen activator and tissue-type plasminogen activator)] and plasmin may provide, at least, a partial explanation for this conundrum. Other factors of potential interest in this regard include de-encryption (activation) mechanisms of TF itself, local activities of physiologic anticoagulants, such as TF pathway inhibitor, thrombomodulin, antithrombin, and heparin cofactor II (1, 3, 5). Ultimately, a more comprehensive picture of cancer coagulopathy will likely emerge out of comprehensive profiling of the entire hemostatic system in individual patients (“coagulomics”), something that is critically dependent on further progress in technology involved in proteomics of blood plasma.

Concluding Thoughts

Oncogenic events have emerged recently as one of the putative causes of the procoagulant conversion of cancer cells and in the development of Trousseau syndrome in cancer patients (Fig. 1). Although this is a captivating notion, the practical implications of this new role of oncogenes remain unclear. Are the genetic features of a particular tumor (e.g., presence or absence of K-ras mutation) predictive of coagulopathy? Could agents directed against oncogenic proteins always reverse or attenuate prothrombotic complications? What is the net effect of such agents on the hemostatic system in various settings? What if on one hand they reduce expression of TF, PAI-1, and other procoagulant entities in live cells, whereas on the other hand, their action in vivo may increase clotting induced by apoptotic cancer cells? Would targeting COX-2 (by coxibs) be useful in treating Trousseau syndrome, although some of these agents are known to provoke cardiovascular complications? What could be the role of LMWH and other anticoagulants as adjuncts to both targeted and antiangiogenic therapies in various cancers? These and other questions remain to be addressed more conclusively. Still, the new linkage between Trousseau syndrome and cancer-specific oncogenic alterations may suggest that different (oncogene directed and coagulation directed) approaches need to be explored (perhaps combined) in this context for the purpose of both thromboprophylaxis and more effective anticancer treatment.

Acknowledgments

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