Cancer induces a hypercoagulable state, and patients with cancer who suffer a thrombotic event have a worse prognosis than those who do not. Recurrent pathologic thrombosis in patients with cancer are clinically managed with anticoagulant medications; however, anticoagulant prophylaxis is not routinely prescribed owing to a complex variety of patient and diagnosis-related factors. Early identification of patients at risk for cancer-associated thrombosis would allow for personalization of anticoagulant prophylaxis and likely reduce morbidity and mortality for many cancers. The environment in which a thrombosis develops in a patient with cancer is complex and unique from patients without cancer, which creates therapeutic challenges but may also provide targets for the development of clinical assays in this context. Circulating tumor cells (CTCs) may play a role in the association between cancer and thrombosis. Cancer metastasis, the leading cause of cancer-related deaths, is facilitated by the hematogenous spread of CTCs, and CTCs accompany metastatic disease across all major types of carcinomas. The role of CTCs in the pathogenesis of thrombosis has not been studied due to the previous difficulty in identifying these rare cells, but the interaction between these circulating cells and the coagulation system is an area of study that demands attention. The development of CTC detection platforms presents a new tool by which to characterize the role for CTCs in cancer-related hypercoagulability. In addition, this area of study presents a new avenue for assessing the risk of cancer-associated thrombosis and represents a potential tool for predicting which patients may benefit from anticoagulant prophylaxis. In this review, we will discuss the evidence in support of CTC induced hypercoagulability, and highlight areas where CTC-detection platforms may provide prognostic insight into the risk of developing thrombosis for patients with cancer.

Keywords: circulating tumor cells, thrombosis, tissue factor, metastasis, blood
Therefore, efforts to identify patients with cancer who will develop venous thrombosis need to achieve better specificity by including additional factors, such as circulating tumor cell (CTC) count, which may have a role in developing cancer-related thrombosis.

**CTC—BLOOD PROTEIN INTERACTIONS**

Tissue factor (TF) is the physiological initiator of coagulation and is essential for hemostasis. TF is a transmembrane glycoprotein and requires phospholipids in order to be procoagulant (Nemer et al., 1988). TF-expressing cells are not typically exposed to the blood. Only upon injury, when the architecture of the blood vessel is disrupted, does the blood gain exposure to extravascular TF-expressing cells. Therefore, in hemostasis, the activation of coagulation is essentially localized to sites of hemorrhage.

In the context of metastatic cancer, CTCs intravasate into the vasculature in order to reach distant locations and establish metastatic foci. CTCs accompany metastatic disease across all major types of carcinomas (Allard et al., 2004). Tumors have been shown to express TF in vivo and TF expression has been shown to correlate with metastatic potential (Zacharski et al., 1983, 1986; Lee et al., 2011; Liu et al., 2011; Ma et al., 2011; Tornoe et al., 2011; Xu et al., 2011; Gil-Bernabe et al., 2012). Therefore, the process of hematogenous metastasis may present TF-expressing tumor cells to blood in the absence of a blood vessel injury. Whether metastasizing cancer cells are involved in the development of thrombosis has not been established. In vitro, cancer cells added to blood or plasma promote coagulation in a TF- and phosphatidylserine (PS)-dependent manner, and the coagulation kinetics are strongly dependent upon the number of cancer cells tested (Bermier-Lang et al., 2011; Tornoe et al., 2011; Yats et al., 2011; Welsh et al., 2012). However, extrapolation of in vitro coagulation assays to in vivo phenomenon is not straightforward. Cancer cell lines may not reproduce CTC procoagulant phenotypes, and extrapolation of in vitro coagulation kinetics to physiological scenarios is complicated by dynamic physiological environments seen in vivo. Along these lines, blood flow is a strong determinant of procoagulant activity (Gormolly et al., 1988), yet a myoarteriosus tumor cell has dynamic temporal and spatial relationships with the blood which are unique from TF-bearing cells exposed at the site of a blood vessel injury. For instance, an intravasating or extravasating tumor cell is stationary relative to the blood flow, and thereby would experience rapid changes in coagulation kinetics due to the blood flow-mediated transport of coagulation factors to the stationary cell. In contrast, CTCs in the bloodstream experience very little relative blood flow, as these cells are transported by viscous forces within the flowing blood. The resulting coagulation kinetics for CTCs is therefore reliant upon diffusion of coagulation factors to/from the cell surface; thus, the coagulation kinetics for CTCs is diffusion-limited.

The extent to which spatial and temporal relationships affect procoagulant activity of CTCs is a topic of current investigation. In this issue, Lee et al. (2011) model the generation and coalescence of thrombin by procoagulant CTCs within the circulation and predict local thrombin concentration gradients surrounding the CTCs to have complex relationships with cell counts and distributions within the vasculature. This work suggests that procoagulant CTC counts strongly determine local thrombin concentrations, which would likely be diagnostic for risk of developing thrombosis.

Cell-mediated coagulation requires the binding of coagulation factors from solution, followed by the assembly of enzyme complexes on the cell surface. This assembly is facilitated by the exposure of PS. The exposure of PS and subsequent assembly of enzyme complexes on the cell surface may be rate-limiting for the cell’s procoagulant activity. In vitro, the procoagulant activity of several cancer cell lines has been shown to correlate with the extent of PS exposure, more so than the relative expression of TF, supporting the notion that facilitation of enzyme complex assembly is the rate-determining mechanism for cancer cell-mediated coagulation (Barrowcliffe et al., 2002; Pickering et al., 2004). Further, space limitations of the PS-regions can severely reduce coagulation kinetics, indicating a role for quantification of procoagulant surface area to assess the procoagulant activity of a cell (Haynes et al., 2012). This limitation suggests that the procoagulant phenotype of CTCs may be dependent upon the physical parameters (size and surface area) of CTCs. In this issue, Phillips et al. (2012a,b) demonstrate methods to utilize light microscopy to quantify the physical parameters of volume, mass, surface area, and density of CTCs, providing a novel technique to assess CTC heterogeneity in cancer-associated hypercoagulability.

Characterizing the role for procoagulant CTCs to initiate coagulation requires a method to functionally probe the ability of CTC to mediate coagulation. Surface expression of TF could be determined through immunofluorescent labeling methods, but these approaches do not capture the activity of TF. The ability of TF to initiate coagulation is dependent upon the cell membrane environment, specifically the exposure of PS, as well as an “active” or “decrypted” form of TF in order to facilitate coagulation. In this issue, Tornoe et al. (2012) describe an approach utilizing fluorescently labeled coagulation factors to characterize the procoagulant nature of CTCs. This approach has potential to functionally characterize the ability of CTCs to bind coagulation factors, which is crucial for their ability to facilitate coagulation. This functional labeling lends itself to current CTC detection platforms (Kracavcic et al., 2004; Hieb et al., 2006), providing a novel method with which CTC platforms can provide novel insight into cancer-related thrombosis.

**CTC—PLATELET INTERACTIONS**

It has been shown that platelets, the primary mediators of hemostasis, play a key role in mediating hematogenous metastasis (Gasic et al., 1968, 1973). In vitro, tumor cells bind platelets under shear stress (McCarty et al., 2000, 2002) and cause platelet aggregation, and this ability correlates with metastatic potential in vivo (Karpukin et al., 1988; Amirkhosravi et al., 2003; Palmamo et al., 2005; Erpenbeck et al., 2010). The mechanisms by which platelet interactions confer metastatic potential upon CTCs are complex. Platelets can deter NK cell destruction of CTCs in the blood (Karpukin et al., 1988; Im et al., 2004; Kopp et al., 2009). Releasates from activated platelets include growth factors such as platelet-derived growth factor, vascular endothelial growth factor, and transforming growth factor beta, and may support tumor growth and promote the establishment of metastatic tumor...
While the extreme rarity of CTCs in human solid tumors has prevented their characterization, leukemias, which are composed of circulating cancer cells, or “liquid tumors,” supply vast numbers of cancer cells to the bloodstream, making the potential for metastatic disease straightforward. Thrombosis is commonly seen with specific leukemia subtypes, and surface expression of TF has been identified on some leukemic cells associated with coagulation (Xu, Stefano et al., 2005; Falanga et al., 2008; Liu et al., 2008; Falanga and Marchetti, 2009; Ku et al., 2009; Muul and Krc, 2010). However, conversely to solid tumors and certain leukemia subtypes, hemorrhage is more common in most acute leukemias as compared to thrombosis (Barbui et al., 1998; Barbui and Falanga, 2001; Falanga and Barbui, 2001; Falanga and Räckles, 2003). Suggested mechanisms by which leukemias induce hemorrhage include bone marrow crowding and suppression of platelet production leading to thrombocytopenia. Acute myelogenous leukemia French-American-British subtype M3 or acute promyelocytic leukemia (APL), is known to surface-express TF as well as Annexin II (Menell et al., 1999), and is associated with the highest risk of thrombosis and bleeding amongst all leukemia subtypes. TF activity has been demonstrated from APL cells in vitro, suggesting that these cells are procoagulant. However, rather than developing focal thromboses, patients with APL present with a diffuse coagulopathy that consumes the coagulation factors within the blood leaving the patient unable to maintain hemostasis, resulting in a hemorrhagic phenotype. The surface expression of Annexin II has been shown to correlate with incidence of hemorrhage, and an Annexin II-antagonist reduces hemorrhages (Menell et al., 1999). Therefore, in certain conditions, leukemia cells may initiate and propagate coagulation, while simultaneously facilitating anticoagulation, with the net coagulant effect resulting in consumption of coagulation factors until the patient is severely deficient and deposed toward clinically severe hemorrhage.

**REFERENCES**

Akl, E. A., Labedi, N., Barbui, M., Torremato, I., Spurti, F., Maiti, P., and Schummann, H. (2011a). Antiocoagulation for the long-term treatment of venous thromboembolism in patients with cancer. Cochrane Database Syst. Rev. 6, CD006650.

Akl, E. A., Venneti, S. R., Gunukula, S., Yousouf, V. E., Barbui, M., Torremato, I., Spurti, F., and Schummann, H. (2011b). Oral anticoagulation in patients with cancer who have no therapeutic or prophylactic indication for anticoagulation. Cochrane Database Syst. Rev. 12, CD006646.

Akard, W. J., Mattoo, J., Raybott, M., Connolly, M. C., Rao, C., Tobbie, A. G., Uhl, J. W., and Terreskin, L. W. (2004). Tumor cells circulate in the peripheral blood of all major cancer sites but not in healthy subjects or patients with normal-disease conditions. Clin. Cancer Res. 10, 6897–6904.

Akiyama, A., Marutomo, A., Nose, M., Yamasaki, T., and Francis, J. L. (2000). Inhibition of tumor cell-induced platelet aggregation and lung metastasis by the oral GP IIb/IIIa antagonist E454. Thromb. Haemost. 90, 549–554.

Assmann, B. K., Komorova, A., Meyers, C. A., Müller, D. M., and Spern, M. B. (1993). Transforming growth factor-beta in human platelets: Identification of a major storage site, purification, and characterization. J. Biol. Chem. 268, 7325–7330.

Barbui, T., and Falanga, A. (2001). Thrombosis and cancer. Semin. Thromb. Haemost. 27, 595–604.

Barbui, T., Foci, G., and Falanga, A. (1998). The impact of all-trans-retinoic acid on the coagulopathy of acute promyelocytic leukemia. Blood 91, 5009–512.

Barrow, J. A., Gruelley, G., Wiedewitsch, E., Nyeon, O., and Linet, M. (1998). Venous thromboembolism and cancer. Lancet 351, 1077–1080.

Barrowcliffe, T. W., Fabrizio, P., Jardi, M., Canollin, J., Rahalreme, M., and Fekah, J. (2002). Procoagulant activity of T lymphoblastoid cells due to exposure of negatively charged phospholipid. Thromb. Haemost. 87, 442–449.

Beyer-Lang, M. A., Akan, J. E., Torremato, I., Patil, I. A., Rock, P. E., Graber, A., and McCarty, O. J. (2011). Promotion of experimental thrombus formation by the procoagulant activity of breast cancer cells. Physiol. Biol. 8, 015014.
in colorectal cancer via the downregulation of MMPs and the induction of autophagy and apoptosis. Cancer Biol. Ther. 12, 896-907.

Tormoen, G. W., Cianchetti, F., Bock, P., and McCarty, O. J. (2012). Development of coagulation factor probes for the identification of procoagulant circulating tumor cells. Front. Oncol. 2:110. doi: 10.3389/fonc.2012.00110

Tormoen, G. W., Rugonyi, S., Gruber, A., and McCarty, O. J. (2011). The role of carrier number on the procoagulant activity of tissue factor in blood and plasma. Phys. Biol. 8, 046005.

Trousseau, A. (1865). “Phlegmasia alba dolens,” in Clinique Médicale de l’Hôtel-Dieu de Paris, ed. A. Trousseau (Paris: The New Sydenham Society), 654–712.

Welsh, J., Smith, J. D., Yates, K. R., Greenman, J., Marrawis, A., and Madden, L. A. (2012). Tissue factor expression determines tumor cell coagulation kinetics. Int. J. Lab Hematol. 34, 396–402.

Xu, C., Gai, Q., Chen, W., Wu, L., Sun, W., Zhang, N., Xu, Q., Wang, J., and Fu, X. (2011). Small interference RNA targeting tissue factor inhibits human lung adenocarcinoma growth in vitro and in vivo. J. Exp. Clin. Cancer Res. 30, 65.

Yates, K. R., Welsh, J., Echrish, H. H., Greenman, J., Marrawis, A., and Madden, L. A. (2011). Pancreatic cancer cell and microparticle procoagulant surface characterization: involvement of membrane-expressed tissue factor, phosphatidylserine and phosphatidylethanolamine. Blood Coagul. Fibrose 22, 680–687.

Zacharski, L. R., Memoli, V. A., and Rrousseau, S. M. (1986). Coagulation cancer interactions in situ in renal cell carcinoma. Blood 68, 394–399.

Zacharski, L. K., Schneider, A. R., and Sonneman, G. D. (1983). Occurrence of fibrin and tissue factor antigen in human small cell carcinoma of the lung. Cancer Res. 43, 3963–3968.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 01 June 2012; accepted: 23 August 2012; published online: 10 September 2012.

Citation: Tormoen GW, Haley KM, Levine RL and McCarty OJT (2012) Do circulating tumor cells play a role in coagulation and thrombosis? Front. Oncol. 2:115. doi: 10.3389/fonc.2012.00115

This article was submitted to Frontiers in Cancer Molecular Targets and Therapeutics, a specialty of Frontiers in Oncology. Copyright © 2012 Tormoen, Haley, Levine and McCarty. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and subject to any copyright notices concerning any third-party graphics etc.