Tissue Factor and Cancer Stem Cells
Is There a Linkage?

Chloe Milsom, Nathalie Magnus, Brian Meehan, Khalid Al-Nedawi, Delphine Garnier, Janusz Rak

Abstract—A common feature in the progression of multiple human malignancies is the protracted deregulation of the coagulation system, often referred to as cancer coagulopathy. Indeed, cancer cells and their vascular stroma often exhibit procoagulant properties, of which deregulation of tissue factor (TF) expression is a notable, although not the sole example. These changes can be traced to oncogenic influences affecting epidermal growth factor receptor (EGFR), EGFRvIII, K-ras, p53, PTEN, and probably many other proto-oncogenes and tumor suppressors in tumor parenchyma. Cancer stem cells (CSCs)/tumor initiating cells (TICs) are thought to represent the primary target and the main cellular effector through which oncogenic mutations exert their tumor-inducing effects. In so doing, CSCs/TICs depend on interactions with the tumor vasculature, which forms supportive niches for their clonal growth. We postulate that TF contributes to these interactions (directly or indirectly) through procoagulant and signaling effects, the latter executed in concert with juxtaposed protease activated receptors (mainly PAR-1 and PAR-2). TF/PAR system acts as a “blood sensing” mechanism, whereby cancer cells, including CSCs/TICs, may respond to plasma proteases (Factors VIIa, Xa, and IIa) and their related microenvironmental changes (fibrin deposition, activation of platelets). A growing body of still largely circumstantial evidence suggests that these events may contribute to the CSC/TIC niche, which could influence tumor initiation, metastasis, recurrence, and therapeutic intractability. Indeed, certain types of cancer cells harboring markers of CSCs (CD133) exhibit elevated TF expression and loss of tumor suppressors (Table 1).

Tissue Factor and Cancer Progression
Chronic activation of the coagulation system is among the most consistent correlates of advanced malignancy, and its prothrombotic consequences are the second leading cause of cancer related deaths (reviewed in1–4). Indeed, such procoagulant conditions and migratory occlusive thrombophlebitis may sometimes be detected before cancer diagnosis, in which case they are referred to as Trousseau syndrome.5 Although the nature of cancer coagulopathy in various settings is likely heterogeneous and complex in nature, it includes at least three classes of influences, namely: (1) nonspecific procoagulant effects of the disease and treatment, such as immobility, central venous catheters, medication, and consequences of surgery; (2) procoagulant effects indirectly related to cancer, including: tumor related vascular anomalies, leaky vessels, entry of metastatic cancer cells into the circulation, the impact of tumor-related angiogenic factors (eg, VEGF), as well as the effects of anticancer and antiangiogenic therapies; (3) cancer specific perturbations in the expression of hemostatic effector genes and deregulation of their activity. The latter occurs downstream of cancer-causing transforming alterations4,6 and is epitomized by deregulation of tissue factor (TF) by activation of oncoproteins and loss of tumor suppressors (Table 1).

Tissue factor (TF) is a 47-kDa transmembrane cellular receptor for the blood-borne protease, coagulation factor VIIa (FVIIa).7,8 As TF is normally absent in the vascular lumen, this interaction can only occur on vascular injury, abnormal TF expression on endothelial cells (eg, during angiogenesis), or after entry into the blood stream of a large number of TF-expressing material, such as TF-expressing cells and TF-bearing microvesicles.8–11 Formation of the TF/VIIa complex8,10 triggers proteolytic conversion of circulating factor X (FX) to an active form (FXa; along with activation of FIX). FXa activates small amounts of prothrombin (FII) to thrombin (FIIa).12 A subsequent burst of thrombin activity is facilitated by platelets and factors Va, VIIIa, and IXa leading to conversion of soluble fibrinogen into insoluble fibrin,12 activation of protease-activated (G protein–coupled) receptors (PARs), followed by further recruitment

Received April 29, 2009; revision accepted July 7, 2009.
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Arterioscler Thromb Vasc Biol is available at http://atvb.ahajournals.org
DOI: 10.1161/ATVBAHA.108.177444
of platelets and rapid clot formation. Notably, these events trigger changes in the intracellular signaling, mainly activation of PAR1 by factors Xa and IIa, and of PAR2 by the TF/VIIa complex. The resulting reprogramming of gene expression leads to modulation of growth, survival, migratory, and proangiogenic phenotype of TF expressing cells, including cancer cells and their related stroma (activated endothelial cells, fibroblasts, inflammatory cells).

Although the extent to which these events may be involved in different disease sites may vary considerably, several recent translational studies provide evidence as to the link between the activation of the coagulation system in general and TF expression in particular and survival of patients with pancreatic and ovarian cancer, in the latter case as a function of symptomatic pulmonary thromboembolism. In some of these instances TF was coexpressed with markers of angiogenesis (VEGF), suggesting a role beyond coagulation. Indeed, in several oncogene-driven tumors, TF overexpression appears to be required for efficient tumor growth, angiogenesis, and distant metastasis. This may suggest that this unique receptor (TF) is an integral part of the oncogenic circuitry that drives tumor progression at both the local and systemic level.

Tumor Cell–Derived Microvesicles

Oncogenic transformation of cancer cells not only triggers overexpression of TF on the cellular surface but also may drive emission of soluble forms of this receptor, either as a splice variant containing the TF ectodomain, or perhaps more commonly, as cargo of membrane microvesicles. However, the tumor-related TF appears to play a dominant role in most cases, as 4 different TF-expressing cell lines released ample quantities of TF-containing microvesicles, which could be incorporated into the membranes of adjacent endothelial cells causing their procoagulant conversion (Magnus, Al-Nedawi, and Rak, unpublished data, 2009).

Notably, not only the expression of TF but also production of microvesicles is controlled by transforming signaling pathways, as glioma cells harboring mutant EGFRVIII produce more microvesicular material than their indolent counterparts lacking this oncogene. Interestingly, these tumor-derived microvesicles contain not only TF but also active oncproteins (EGFRVIII and EGFR), and serve as a mechanism by which these proteins can be transferred between cells. Uptake of such oncogene-containing microvesicles (oncosomes) can lead to reprogramming of gene expression profiles in target cells, including changes in expression of growth and angiogenesis-related genes (eg, VEGF).

## Distinct Roles of Tissue Factor–Expressing Host and Cancer Cells

TF is expressed both by cancer cells and their related vascular tumor stroma, which in many instances includes inflammatory cells, such as macrophages. Although it seems reasonable to assume that this pattern may simply produce a cumulative procoagulant effect, recent studies point to not one but several different scenarios of tumor and host TF involvement. These studies were possible because of the availability of specific anti-human TF and anti-mouse TF antibodies and a unique strain of mice harboring a severely hypomorphic but functional human TF minigene (low-TF mice). The extremely low-TF expression (1%) in these mice is sufficient to circumvent the lethality of the germline TF gene disruption, but reveals the consequence of TF withdrawal from the host compartment in various cancer settings.

These studies revealed that TF-deficient ES cells form aggressive teratomas in TF proficient mice but fail to do so in low-TF mice. This may suggest that various pools of TF act cumulatively to promote tumor growth, or that TF can be shared between tumor and host cell populations via exchange of microvesicles. However, the tumor-related TF appears to play a dominant role in most cases, as 4 different TF-expressing
Tumor Cell Heterogeneity and Cancer Stem Cells

Among the many levels of heterogeneity that are recognized in cancer, the rapid diversification of tumor cell subpopulations within a given lesion is viewed as a major source of biological and therapeutic challenges.4,5 Such heterogeneity is viewed as either stochastic or hierarchical in nature, each associated with different implications.42 In particular, the notion of hierarchical cancer cell heterogeneity implies that only a subset (minority) of cancer cells is responsible for initiation, recurrence, and maintenance of the malignant growth, whereas these properties are markedly reduced, or nonexistent, in their progeny (the majority of cancer cells). The former cells, often referred to as tumor initiating cells (TICs), or cancer stem cells (CSCs),43 may either emerge from the stem cell compartment of the related tissue,44 or their phenotype could be reminiscent of such cells because of events secondary to the malignant transformation of more differentiated (transitory) cells.45 It is also possible that cellular interactions45 or extracellular influences could modulate cellular “stemness”46 and tumor-initiating capability of cancer cells.

TICs/CSCs exhibit certain similarities to tissue stem cells, for instance, by expressing certain common molecular markers (eg, CD133, CD44, nestin, ABCG2), and certain biological characteristics in vitro (high clonogenic capacity, formation of tumor spheres in defined medium), and in vivo (radiation/drug resistance). Some of these features define their functional “stemness,” which is associated with self-renewal capacity (formation of CSCs from a CSC), the ability to undergo asymmetrical cell division (dividing into a CSC and a committed progeny), capacity to differentiate into multiple lineages, low rate of mitogenesis, and expression of certain stem cell–related genes (eg, Oct-4, Nanog, Sox2; see Table 2).42,43,47,48 In some cases CSCs/TICs were found to exhibit a more aggressive and motile behavior and features consistent with epithelial-to-mesenchymal transition (EMT).49 The latter term refers to the ability of cancer cells of epithelial origin (carcinomas) to rapidly assume morphological and molecular features characteristic of the mesenchymal lineage (eg, spindle shaped appearance, loss of E-cadherin, expression of vimentin, and other changes), along with more motile and invasive properties.50 Collectively, this phenotype is thought to be required for the ability of individual CSCs/TICs to initiate primary tumor growth and cause disease recurrence or metastasis.48,51 Indeed, the most striking experimental criterion that distinguishes these cells from their progeny is the ability to produce tumor growth in mice on injection of very low cell numbers (sometimes single cells; ie, under conditions where the remaining cells are essentially nontumorigenic).48,52

Vascular Cancer Stem Cell Niche

It is increasingly clear that, like stem cells, the properties of TICs/CSCs are dependent on their cellular environment, including extracellular matrix (ECM), growth factors (GFs), neighboring cells, a source of numerous juxtacrine, paracrine, endocrine, and adhesive influences. Indeed, these properties, collectively referred to as a cancer stem cell niche (CSCN), likely play an important role in regulation of the tumor and metastasis initiating potential of these cells. Interestingly, there is growing evidence that various facets of the vascular system contribute to, modify, or indeed, constitute the CSCNs in certain disease settings.53 For instance, CSCs sometimes reside in direct proximity of tumor blood vessels,54 produce elevated amounts of angiogenic growth factors, such as VEGF,55 and become depleted after antiangiogenic therapy of experimental tumors.56 In this context it is interesting to ask whether another vascular aspect of cancer progression—activation of the coagulation system—may be involved in these interactions.5,6,14

Cancer Stem Cell Compartment and the Tissue Factor Pathway

The TF/PAR system represents a unique point of molecular convergence between coagulation, angiogenesis, and cancer progression.57 Therefore, it is surprising that these effectors have hardly been considered in terms of their possible impact on the CSC/TSC compartment and the CSC niche.58 This is in spite of the documented involvement of TF, PARs, thrombin (IIa), fibrin, platelets, and other related constituents in events that are otherwise known to engage CSCs/TICs.6,58 These include the impact of the coagulation system on tumor growth,23,59 cessation of tumor dormancy,60 cancer cell migration/invasion,61 angiogenesis,3,34,62 and especially on metastasis, where single cancer cells de facto “initiate” tumor growth in distant organ sites, and do so in a manner profoundly influenced by TF and the coagulation system.25,27,63

It is, therefore, reasonable to ask whether TICs/CSCs themselves, or elements of their niches (eg, stromal, endothelial, or other tumor and tumor-associated cells) or cells involved in formation of premetastatic niches in distant organs (eg, bone marrow—derived cells), express TF and PAR receptors and are able to respond to their respective agonists and regulators.6 In this regard, the contact between CSCs/TICs and blood-borne effectors of the coagulation system would seem almost inevitable because of the vascular proximity of these cells,54 but also extravascular leakage of plasma proteases and coagulation factors from the hyperpermeable tumor microcirculation (eg, VIIa), deposition of coagulation end products (fibrin) in the tumor parenchyma,64 and because of a direct entry of metastatic CSCs/TICs into the systemic circulation.26

The nature of such CSC/TIC interactions with the coagulation system are presently unknown but could include adhesive contacts with fibrin matrix, activation of cell-associated PAR-1 by soluble coagulation factors: Ila and Xa, activation of PAR-2 by the TF/VIIa complex, exposure of TICs/CSCs to platelet-related GFs, as well as the impact of circulating procoagulant
and nonprocoagulant microvesicles.\textsuperscript{25,35} Moreover, these effects could be modulated by pericellular activation of the fibrinolytic system and other regulatory processes (eg, the activated protein C and antithrombin pathways),\textsuperscript{15} none of which has thus far attracted any significant experimental attention.\textsuperscript{58}

In keeping with this notion a survey of published gene expression databases and cellular responses to various manipulations reveal a potential linkage between coagulation effectors and stem cell properties. This includes a direct regulation of common stem cell genes by coagulation factors, and conversely the alterations in the expression of TF and PARs as a result of cell fate decisions, differentiation, and other processes involving various types of normal and transformed stem cells, including CSC-like cells (Table 2). Though intriguing, these linkages are largely circumstantial, and the general understanding of the role of the TF/PAR pathway in cellular stemness is presently lacking.

### Table 2. Potential Linkages Between Cellular Stemness and the Effectors of Coagulation

<table>
<thead>
<tr>
<th>Stem Cells Properties and Genes Involved</th>
<th>Link With Coagulation</th>
<th>References</th>
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<td>Pluripotency and self-renewal</td>
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<tr>
<td>Nanog</td>
<td>Nanog overexpression in MSCs induces coagulation factor II (thrombin) receptor-like 2 upregulation</td>
<td>Liu et al\textsuperscript{83}</td>
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<td>Oct-4</td>
<td>Overexpression of the transcription factor Oct-4 in MSCs induces tissue factor pathway inhibitor 2 upregulation</td>
<td>Liu et al\textsuperscript{83}</td>
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<td>Klf5</td>
<td>FVIIa upregulates BTEB2/Klf5 expression in HaCaT human keratinocytes. Klf5 is involved in self-renewal of mouse ESCs\textsuperscript{84-86}</td>
<td>Camerer et al\textsuperscript{87}</td>
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<td>LIF</td>
<td>FVIIa stimulation upregulates LIF expression in HaCaT cell line; LIF is involved in stem cell self-renewal\textsuperscript{88,89}</td>
<td>Camerer et al\textsuperscript{87}</td>
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<td>Surface markers</td>
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<td>CD133</td>
<td>CD133-positive tumour cells express high level of TF, neutralization of TF activity inhibits the tumor growth</td>
<td>Milsom et al\textsuperscript{58}</td>
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<td>EMT and multilineage differentiation</td>
<td>TF expression changes with induction of EMT and multilineage differentiation in human A431 cancer cells in vivo and in vitro</td>
<td>Milsom et al\textsuperscript{58}</td>
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<tr>
<td>Oct-2</td>
<td>FVIIa, PAR-1 or PAR-2 stimulation upregulates Oct-2, a regulator of neuronal differentiation\textsuperscript{90}</td>
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<td>Differentiation of NSCs</td>
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<td>Keratin</td>
<td>TF expression changes with induction of EMT and multilineage differentiation in human A431 cancer cells in vivo and in vitro</td>
<td>Milsom et al\textsuperscript{58}</td>
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<tr>
<td>M-CSF, GM-CSF</td>
<td>FVIIa stimulation induces M-CSF and GM-CSF expression, cytokines that control HSCs differentiation</td>
<td>Petersen\textsuperscript{91}</td>
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<td>CCN1, CCN2</td>
<td>FVIIa stimulation induces CCN1/Cyr61 and CCN2/CTGF expression in MDA-MB-231 breast carcinoma cells. These genes are involved in Wnt-induced osteoblast differentiation of mesenchymal stem cells\textsuperscript{92,93}</td>
<td>Petersen\textsuperscript{91}</td>
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<tr>
<td>Vimentin</td>
<td>TF downregulation by shRNA induces upregulation of vimentin, a marker of mesenchymal differentiation</td>
<td>Wang et al\textsuperscript{95}</td>
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</table>

MSCs indicates mesenchymal stem cells; HSCs, hematopoietic stem cells; PAR-1/2, protease-activated receptor 1/2; NSCs, neural stem cells; ESCs, embryonic stem cells; TF, tissue factor.

The Possible Role of Tissue Factor in the CSC Compartment

To bridge the existing gap, we undertook a series of studies aiming to address the following set of questions: (1) Do CSC-like cells express TF, and under what conditions? (2) If so, does TF expression lead to responsiveness of these cells to VIIa, or to changes in their behavior? (3) Do TF blocking agents impede the events ascribed to CSCs/TICs, for instance tumor initiation on injection of threshold numbers of cancer cells? The experiments completed thus far suggest that, indeed, CSC-like cells seem to use the TF/PAR system, albeit in a complex and often context-dependent manner. The latter is exemplified by several findings: First, we found that not all tumorigenic cells with properties of CSCs/TICs express TF on their surfaces. Notably, the aforementioned experiments involving TF\textsuperscript{-/-} ES cells and their derived teratomas suggest that these multipotential but nontransformed stem cells do not require TF expression on their surfaces for TF expression to be secreted into the extracellular environment. However, this process remains TF-dependent, in that it requires the expression of TF within the host (CSC) niche.\textsuperscript{25} Thus, during this form of tumorigenesis the involvement of TF may not be cell-autonomous in nature (at least as it relates to ES/CSCs). This is to say that not in all instances CSCs themselves would require TF expression and intracellular signaling for TF to have an effect on their growth as tumors. Instead, such cells could, for example, rely on local thrombin generation owing to TF present on adjacent cells, or could use intercellular exchange of TF via
microvesicles, or use other mechanisms of TF “sharing” that remain to be established.

Second, at least certain types of human CSC/TIC-like cells may express increased levels of TF and depend on this property for tumor initiation. Thus, unlike in the case of ES cells, the vast majority of human tumors combine the effect of oncogenic mutations and features of cellular “stemness.” Indeed, it is thought-provoking that oncogenic events seem to preferentially affect the CSC/TIC compartment, and at the same time, are known to upregulate TF and PARs (Magnus and Rak, unpublished data). This is particularly obvious in the case of A431 human squamous cell carcinoma cells. This epidermoid cell line established from a spontaneous vulvar carcinoma exhibits strongly epithelial characteristics (cobble stone morphology, expression of E-cadherin) and a relatively simple mechanism of cellular transformation driven by amplification and overexpression of the EGFR gene, which also results in a marked increase in levels of TF on the cancer cell surface. Interestingly, we observed that a small subset of these cells is positive for CD133, and on their immunomagnetic enrichment, CD133+ cells (but not their mixed/parental, or CD133− counterparts) form tumor spheres in growth factor-supplemented media (Figure 1), as might be expected for cells with properties of CSCs/TICs. Moreover, the CD133− fraction of A431 cells exhibit 5 to 6 times higher levels of TF antigen and TF procoagulant activity (TF-PCA) relative to their CD133− counterparts. Although both of these cellular subsets expressed TF and were tumorigenic in mice, enrichment in CD133− cells in the inoculum led to a more rapid tumor formation (Figure 1; Milsom and Rak, unpublished data). Collectively, this may suggest that, at least in this context, high TF expression cosegregates with, and possibly contributes to, the CSC/TIC-like phenotype.

It should be mentioned here that the expression of CD133 (or any other molecular marker of CSCs), while potentially useful, informative, widely accepted, and ultimately unavoidable in the CSC analysis, is not tantamount to a positive, exclusive, and unequivocal identification of these cells in the mixed population. Similar reservations may potentially apply also to surrogate CSC assays in vitro, such as formation of tumor spheres, as in the A431 model, the absence of such phenotype in both CD133− and unsorted populations did not preclude tumor formation, an implicit evidence for the presence of TICs. Indeed, isolation of 2 CD133+ cancer cell subsets either with or without characteristics of CSCs has recently been reported in human glioma. Moreover, neuronal stem cells devoid of CD133 expression have also been characterized, findings that raise a cautionary note with regards to using single determinants.

However, the most reliable and meaningful characteristic of CSCs is their ability to initiate tumor growth, and in this regard our data suggest that TF may play an important functional role. In this regard has been the analysis of A431 tumors in vivo. Thus, in spite of their positivity for both EGFR and TF under various cell culture conditions, A431 cells injected into immunodeficient mice rapidly undergo a startling phenotypic diversification. This is manifested by changes in morphology and in the emergence of distinct cellular subsets expressing either high or low levels of TF, and mutually exclusive patterns of lineage markers, indicative of either epithelial (cytokeratin positive, keratin pearls), or mesenchymal (vimentin positive) differentiation. This spontaneous multilineage differentiation of a priori cytokeratin and vimentin negative parental A431 cells occurs in the absence of any significant changes in EGFR expression but results in an overall

![Figure 1. Cancer cells harboring stem cell markers express elevated tissue factor activity. The EGFR-driven A431 human squamous cell carcinoma cell line can be enriched for rare CD133-expressing cells by immunomagnetic sorting and the resulting cells (but not their CD133− counterparts or the unsorted population) form tumor spheres in growth factor supplemented serum free media. Both of these properties are hallmarks of the cancer stem cells/tumor initiating cell (CSC/TIC) compartment and coincide with 5 to 6 times higher levels of TF procoagulant activity (TF-PCA), relative to CD133− cells. Although both fractions of A431 cells form tumors in mice, enrichment for CD133− cells in the inoculum from 0.5% (originally found) to 50% leads to accelerated tumor initiation (30 to 50 days after inoculation), but the growth rate in both groups becomes similar once the tumor “take” has occurred (days 50 to 70 after inoculation). These preliminary observations suggest that high TF expression cosegregates with, and possibly contributes to, the CSC/TIC-like phenotype, at least in the context of A431 cells (adapted from Milsom et al).](http://atvb.ahajournals.org/)}
reduction in TF levels within the tumor, likely a consequence of the reduced number of cells expressing high levels of this receptor29 (Figure 2).

Some of these changes could also be recapitulated in vitro. For instance, when A431 cells are cultured at high confluence or in the presence of interferon gamma they acquire more epithelial features (cytokeratin expression), which is associated with a decrease in TF expression (Milsom and Rak, unpublished data, ●●). Conversely, disruption of cellular contacts between these cells (eg, with the anti–E-cadherin antibody [SHE78-7]) leads to expression of vimentin, epithelial-to-mesenchymal transition in vitro, increase in production of VEGF, and metastatic capacity (CSC/TIC-like features) as well as marked upregulation of TF.29 These observations suggest that the levels of TF are modulated by differentiation pathways, as CSC-like A431 cells assume properties of various lineages. The global downregulation of TF in A431 tumors is consistent with the notion that TICs/CSCs expressing high levels of this receptor often (though not always) become a minority amid their more mitogenic or differentiated progeny.43

Figure 2. Growth of cancer cells as tumors in vivo provokes multilineage differentiation and phenotypic heterogeneity. A431 cells in cell culture are uniformly positive for both EGFR and TF expression. However, injection of A431 cells into immunodeficient mice reveals a rapid phenotypic diversification with changes in morphology and TF expression. Within A431 tumors, distinct cellular subsets expressing either high or low levels of TF emerge along with a mutually exclusive patterns of lineage markers. The latter are indicative of either epithelial (cytokeratin positive) or mesenchymal (vimentin positive) differentiation, either of which is absent in A431

Figure 3. Putative cancer stem cell (CSC) niche effects exerted by different tumor-associated tissue factor pools. TF could act as an organizer of the CSC niche and regulator of tumor initiation by evoking procoagulant and signaling effects, and via at least 3 different scenarios: (1) CSCs could overexpress TF and use the resulting pericellular enzymatic activities and intracellular signals to modulate their tumor initiating activity, survival, and stemness. It is noteworthy that cells expressing CSC markers (eg, CD133) are not tantamount to CSC in a functional sense (see text). (2) CSC niche may be formed by TF-expressing cancer cells that themselves are unable to drive tumor initiation events but may generate procoagulant environment involved in these processes. (3) CSC niche effects could be exerted by host stromal cells, vascular and inflammatory cells (macrophages) expressing TF and PARs, the actions of which may be capable of influencing CSCs indirectly, through procoagulant events, changes in gene expression, and other interactions.
If TF was, indeed, associated with CSCs/TICs and played a meaningful role in their behavior, it might be expected that agents directed against this receptor should interfere with tumor initiation. In this regard, it is possible to establish a lower injection threshold of A431 cells, such that tumors occur with 100% frequency and mice still succumb to their disease within 3 to 4 weeks. Remarkably, we observed that this process can be largely aborted by a short 7-day treatment with injections of a neutralizing monoclonal antihuman TF antibody (CNTO859) given only within the first week after cancer cell inoculation. In those experiments, tumor growth (initiation) was completely ablated/halted for more than 6 weeks, and this occurred in the absence of any additional antibody injections. Eventually, A431 tumors did emerge with the usual aggressiveness, an observation suggesting that tumor-initiating A431 cells were blocked in their growth but not eliminated.29 Because the CNTO859 antibody blocks TF-dependent coagulation, but also (to some degree) TF signaling, one or both of these activities are likely essential for 431 tumor initiation.29 The exact nature of these effects remains unclear, but it could be related to formation of a vascular CSC/TIC niche, as exposure to CNTO859 also causes a decrease in VEGF production and reduction in microvascular density of A431 tumors.29

It is noteworthy that the dependence on TF expression is primarily observed in studies involving (oligo)clonal growth of cancer cells in vivo (where tumor growth is initiated by a limited number of cancer cells). This may occur either on local (eg, subcutaneous) injection of subthreshold numbers of cancer cells (ie, the smallest numbers of cancer cells that still give rise to 100% tumor take), and thereby could be informative in terms of the presence of a rate-limiting number of CSCs/TICs in the population as cells from which primary tumors could originate (“a stem cell experiment”).29 Another type of experiment designed to test “tumor initiation” events can be based on cancer cell injection into the systemic circulation, throughout which CSCs become dispersed to form distant “experimental metastases,”26 which are of clonal (single cell) origin.72

In contrast, far less dramatic antitumor effects are observed in the context of experiments aimed at rapid induction of polyclonal tumor growth (excess of CSCs/TICs), as is the case when massive numbers of cells are injected locally to rapidly produce “primary tumors,” or large tumors become established at the time of treatment.23,24,26,29,37,73 Once again, these discrepancies may suggest that the TF pathway plays an as yet uncharacterized but particularly sensitive role during events dependent on CSC/TIC-like cells. If so, local invasion of individual CSCs (eg, during intracranial dissemination of brain tumors), systemic metastasis, tumor recurrence postcytoreductive therapy, cessation of dormancy of occult, or minimal residual disease, or disease control in settings of adjuvant therapy could be of particular interest as contexts amenable to targeting TF (and possibly also PARs and other coagulation effectors).

Summary

TF pathway, including TF, PARs, their agonists and effectors, has traditionally been considered almost exclusively in the context of hemostasis and thrombosis,15 with somewhat more recent interest in their role in cellular signaling, angiogenesis and cancer.4,14,74 The latter is a particularly diverse cluster of disease states, each driven by a spectrum of distinct biological mechanisms,75 of which formation and progressive evolution of the CSC/TIC compartment is a vital part.43 The definition and identity of these cells remains to be understood in more detail, including the relationship between the expression of various markers (eg, CD133, tumor sphere formation) and the true tumor-initiating capacity, properties that are not necessarily tantamount to one another.71 Moreover, it is unclear how different CSC/TIC subpopulations coexist in various cancers71,76 and whether their properties are cell-autonomous.48 Still, the paradigm of hierarchical tumor cell heterogeneity represents a powerful organizing principle in cancer related studies73 and their possible linkage to TF is of considerable interest.

It is argued in this article that there are reasons to believe that TF, PARs, thrombin, and other coagulation-related mechanisms may affect CSCs/TICs and their niches, but this involvement is likely disease- and stage-specific. Nonetheless, the possibility of interfering with CSCs/TICs by modulating the activity of TF and PARs is intriguing and worthy of further analysis, especially in view of a large library of the available TF directed agents and anticoagulants, developed for cardiovascular indications, but with some already described antitumor activities.4,77 Those latter properties could be explored more fully if their impact (if any) on the CSC compartment was better understood.4,77,79 For this to be possible several outstanding questions require further clarification.

Do all, some, or none of the CSCs/TICs express TF and PARs, especially PAR-1 and PAR-2? What is the role of these receptors in formation of the cancer stem cell niche? Does the coagulation system act as a regulator of tumor initiation, or CSC/TIC stemness, and if so, is this a permanent, transient, or marginal role? Are these effects predominantly dictated by TF signaling, coagulation, or both? Which cancers (if any) are especially susceptible to these effects, at what stages of their progression? Could the existing agents (eg, anti-TF antibodies), if used properly, improve the control of CSCs/TICs in cancer patients? Could new and better drugs (eg, inhibitors of TF signaling) be developed for that purpose? Indeed, these and other questions warrant further extensive investigation and may pave the way to exciting future studies.

Acknowledgments

We thank our colleagues, especially: Drs Nigel Mackman, Jeff Weitz, Petr Klement, Abhijit Guha, and G. Mark Anderson for their help, feedback, and support, and our families, especially: Anna and Danuta Rak for their inexhaustible patience.

Sources of Funding

This work was supported by operating grants to J.R. from the Canadian Cancer Society (CCS) and Team Grant from Canadian Institutes of Health Research. J.R. is a recipient of the Jack Cole Chair in Pediatric Oncology at McGill University. Infrastructure funds were contributed by Fonds de la recherche en sante du Quebec (FRSQ).

Disclosures

J.R. consulted on the subject of tissue factor targeting in cancer, and received honoraria from: Tanox, Nuvelo, Bristol-Meyr Squibb, Genmab, research grants from Othera Inc and reagents from Centocor.
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Arterioscler Thromb Vasc Biol. published online July 23, 2009;
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272
Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the
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