TISSUE FACTOR IN TUMOR PROGRESSION

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ABSTRACT

The linkage between activation of the coagulation system and cancer is well established, as is deregulation of tissue factor (TF) by cancer cells, their vascular stroma and cancer associated inflammatory cells. TF is no longer perceived as 'alternative' coagulation factor, but rather as a central trigger of the coagulation cascade and an important cell associated signalling receptor activated by factor VIIa, and interacting with several other regulatory entities, most notably protease activated receptors: PAR-1 and PAR-2. Preclinical studies revealed the role of oncogenic transformation and tumor microenvironment as TF regulators in cancer, along with the impact of this receptor on gene expression, tumor growth, metastasis, angiogenesis and, possibly, formation of the cancer stem cell niche. Increasing interest surrounds shedding of TF containing microvesicles (MVs) form cancer cells, their entry into the circulation and role in the intercellular transfer of TF activity, cancer coagulopathy and other processes. Recent data also suggest differential roles of cell autonomous versus global effects of TF in various settings. Questions are raised as to the consequences of TF expression by tumour cells themselves and by their associated host stroma. Progress in these areas may soon begin to impact clinical practice and, as such, raises several important questions: Can TF be exploited as a therapeutic target in cancer, where and when may this be safe and beneficial? Is expression of TF in various disease settings useful as a biomarker of cancer progression, or the associated hypercoagulability? What clinical questions related to TF are especially worthy of further exploration, at present and in the near future? Some of these developments and questions will be discussed in this article.

VASCULAR INVOLVEMENT IN CANCER PROGRESSION

Pathogenesis of human malignancies is tightly linked with the vascular system and at a number of 'strategically' important levels [1]. In this regard, two processes stand out as particularly ubiquitous and important, namely: formation of new blood vessels (tumour angiogenesis) and activation of the coagulation system (coagulopathy). In this article we will review evidence that these processes are not only tightly interconnected but also, at least to some degree, driven by common molecular mechanisms, of which tissue factor (TF) is an important paradigm, and one of the central effectors.

The linkage between tumour progression and the vascular system is sometimes described in terms of vascular 'supply' and 'demand', and is recognized as being applicable to both, solid tumours and haematopoietic malignancies. The central tenet in this context is that, almost invariably, tumour cells evoke growth of new blood vessels (angiogenesis), as well as invade, co-opt, or cause the remodelling, regression, or collapse of the pre-existing blood vessel networks in their vicinity [1-4]. Tumours also act systemically by releasing circulating angiogenesis regulators [1;5], which are either soluble or trapped as cargo of circulating cells, platelets [6], microvesicles (MVs) [7-9], or exosomes [10]. Another systemic effect is related to mobilization of various subsets of bone marrow-derived regulatory cells [4;11-13], including endothelial progenitors (EPCs, cells involved in vasculogenesis) [11;14].

These processes are essential for disease progression due to the reliance of cancer cells on metabolic exchange and oxygen [1], access to vascular and lymphatic channels, both necessary for tumour dissemination [1]. Tumour progression is also impacted by endothelial cell-derived growth

factors and proteolytic enzymes, plasma-derived proteases, extracellular matrices (ECMs) including provisional fibrin matrix, trophic growth factors and chemoattractants present in the vascular system, all of which affect (in a perfusion independent manner) processes of tumour initiation, growth, invasion and metastasis [15-17].

Tumour microcirculation also represents a unique conduit for delivery of systemically acting anti-cancer therapeutics, is an important factor in tumour responses to ionizing radiation [1], and a target of a novel class of anticancer therapeutics (antiangiogenic agents), several of which have already entered clinical practice in oncology [1;4]. Many of the presently approved agents (e.g. bevacizumab, sorafinib and sutent) are directed against 'generic' blood vessel stimulating pathways, notably vascular endothelial growth factor (VEGF) and its receptors [4;18;18-21]. However, intense efforts are underway to develop additional, new agents with more diverse spectrum of vascular activity, the latter potentially including some aspects of the coagulation system.

Effective targeting activated tumour blood vessels is possible because angiogenesis is a rare process in postnatal life, with the exception of tissue repair processes, exercising muscle, revascularization of ischemic organs and cyclical changes within the female reproductive system [1]. In addition, tumour blood vessels exhibit profound structural [22], cellular [23] and molecular alterations [24], which separate them qualitatively (or at least quantitatively) from their normal counterparts, thereby providing ample potential for selective targeting [4].

In the context of this discussion it is important to note that tumour blood vessels exhibit compromised ability to contain plasma proteins (are leaky), poorly sustain blood flow (are prone to stasis) and provide inadequate antithrombotic luminal surfaces (promote intravascular thrombosis) [3;25;26]. In addition to these abnormalities, which are inherent to the neoplastic growth pattern, it is worth considering that cancer is more prevalent in at the later stages of life, a circumstance that invokes blood vessel related comorbidites, such as: atherosclerosis, obesity and other factors, acting at both local and systemic levels [27]. These, changes may modulate the properties of tumour blood vessels, state of the haemostatic system and responses to various therapies, whether cancer, blood vessel, or thrombosis-directed [27]. Amidst a plethora of effects involved, those affecting the haemostatic and fibrinolytic systems, are of special interest, as historically they were the earliest to be recognized, and yet are perhaps still amongst the least understood [25;26;28-31].

HEMOSTATIC PERTURBATIONS ASSOCIATED WITH MALIGNANCY.

Cancer patients are at increased risk to develop thrombosis. While the expert description of the nature, mechanisms and implications of this propensity is offered in other articles included in this issue, it should be mentioned that manifestations of this condition can be rather diverse. Thus, cancer patients may exhibit venous thromboembolism (VTE), including deep vein thrombosis (DVT), pulmonary embolism (PE)[32], or syndromes that resemble low grade disseminated intravascular coagulation (DIC) [26], or manifest mainly laboratory alterations [26;31;33]. Still, up to 90% of patients with metastatic cancer are affected by some form of coagulopathy [34-36]. This is important for at least two main reasons: First, the associated risk of thrombosis often creates the need for thromboprophylaxis [37]. Second, as discussed below, the ongoing

coagulopathy may become a constituent (rather than side effect) of the ongoing malignant process, and hence appropriate anticoagulation of cancer patients may also have an impact on morbidity and survival, due to, or irrespectively of thrombotic complications [28;30;31;37-39].

What makes cancer coagulopathy particularly challenging is precisely the latter aspect. In this area further progress may ultimately depend on a better understanding of, not only what the appropriate level of anticoagulation might be (e.g. as measured by INR), but also what are the specific cancer-related mechanisms and causal factors that evoke coagulopathy in the first place. In this regard, all the elements of the Virchov's triad are believed to contribute in some fashion [26;34]. Those include the aforementioned structural and functional anomalies of the tumor vasculature, resulting in the exposure of pro-coagulant, extravascular and cellular surfaces, as well as stasis. In addition, external influences such as immobility and iatrogenic effects of surgery, central lines or anticancer medication, including novel targeted agents, may provoke pro-thrombotic conditions both locally and systemically [40]. Consequently several markers have been developed to monitor activation of the coagulation system in various cancer settings, including: D-dimers, thrombin-antithrombin complexes (TAT), prothrombin 1.2 fragments (F1.2), thrombin generation potential, platelet levels, PAI-1, and functional coagulation assays, all of which are mostly indirect and not particularly cancer-specific in nature [26;34;37;41-43]. Tissue factor (TF) has only sometimes been included in these studies [26;43], even though for several reasons its involvement and properties may deserve a greater attention.

TISSUE FACTOR

TF is a 47 kDa transmembrane cellular receptor for activated factor VII (FVIIa) [44]. This property defines TF as the principle regulator of the blood coagulation cascade [44]. Formation of the TF/VIIa complex [44] on cellular surfaces leads to proteolytic conversion of circulating factor X (FX) to an active form (FXa) (along with activation of FIX), which in turn activates small amounts of prothrombin (FII) to thrombin (FIIa) [44]. Thrombin generation is further amplified through the contribution of platelets and factors Va, VIIIa and IXa resulting in further burst of thrombin activity and a rapid generation of clots containing insoluble fibrin and platelets [44].

Once generated, TF/VIIa complexes, Xa and thrombin are able to interact with cells through protease-activated (G protein-coupled) receptors (PARs) [45;46]. In healthy tissues the scope of this activity is restricted by normally extravascular expression of TF. Hence, formation of TF/FVIIa complex can occur only under very special circumstances, including: penetrating vascular injury, blood extravasation, rupture or discontinuity of the endothelial lining, induction of TF expression on endothelial cells (e.g during angiogenesis), entry into the blood stream of a large number of TF expressing cells (e.g. inflammatory leukocytes, metastatic cancer cells or leukaemic blasts) [44] or TF-containing membrane microvesicles [32]. Moreover, the level of TF involvement in these settings is to some extent organ-specific [47].

In view of the central role of TF in the coagulation cascade it is intriguing that this receptor is consistently upregulated in a large spectrum of human malignancies (Table 1). This may suggest that TF is either an epiphenomenon associated with some common events occurring during the malignant process (i.e. potentially useful as a biomarker), or contributes to this process as its

integral part (i.e. putative therapeutic target), or both [48]. Indeed, the emerging literature supports these possibilities.

TISSUE FACTOR REGULATION IN CANCER CELLS AND STROMA

The elevated expression of TF in human malignancies may involve at least three different compartments: cancer cells, their adjacent stroma (blood vessels, fibroblastic and inflammatory cells) and the circulating blood [26;32;49]. In each of these instances the underlying mechanisms and pathogenetic consequences may differ considerably [7].

Cancer cells may exhibit TF levels that are up to 1000 fold above those of their normal counterparts. This upregulation is also observed in clinical samples of numerous types of human cancers (Table 1), with just a few exceptions (e.g. renal cancer). This consistency is sometimes attributed to various ubiquitous but unspecific, microenvironmental conditions, such as inflammation, thrombosis and hypoxia. However, since the increased TF expression by cancer cells is also observed *ex vivo,* it follows that the factors involved are likely intrinsic to the malignant process. Indeed, the evidence to this effect has recently emerged, including such processes as activation of oncogenic pathways, changes in the cellular differentiation status and, possibly, formation of the cancer stem cell (CSC) hierarchy [50].

UPREGULATION OF TISSUE FACTOR BY ONCOGENIC PATHWAYS

Oncogenic pathways are activated in virtually all types of cancer cells, predominantly by mutations that either activate specific oncogenes, inactivate their opposing tumour suppressors, or have a more wide spread effect on both, for instance through genetic hits affecting regulatory

sequences, such as micro RNA clusters [51]. It has recently been proposed that oncogenic changes may impact the level of TF and thereby affect cancer coagulopathy, angiogenesis and other vascular effects associated with cancer [52]. For this notion, there is already a growing body of experimental and early clinical evidence [7;53]. For instance, TF upregulation parallels the expression of several mutant oncogenes, including K-ras, EGFR, EGFRvIII, HER-2, PML-RARα and probably many others, and this is documented in cancer cells of colorectal, mammary gland, cutaneous, astrocytic and hematopietic origin [7;43;54]. Similarly, changes in TF could also be evoked by loss of function mutations of major tumour suppressor genes such as p53 and PTEN [7;53], in some instances acting in concert with influences of the tumour microenvironment (e.g. hypoxia)[53]. It could be speculated that, in addition to traditional signalling pathways, also other constituents of the malignant circuitry, such as changes in protein translation and the expression of regulatory micro RNA species [51] could influence TF levels in this context, both up and downstream of transforming oncogenes.

There is mounting evidence that TF may contribute to the tumour promoting action of oncogenic events, including through the impact on aggressive tumour growth, angiogenesis, metastasis and coagulopathy (described below). This is thought to be executed either by the canonical effect of TF on the coagulation system, or through TF-related coagulation-independent signals. In addition, oncogenic pathways stimulate the release of TF-containing microvesicles from cancer cells into the circulation [54;55], a process that may permit intercellular trafficking of this receptor, e.g. from cancer cell to tumour-associated endothelium [55]. This is likely a consequence of oncogene-dependent stimulation of the cellular vesiculation process [56], as well as the enrichment of microvesicles in TF content, e.g. due to TF upregulation in cancer cells [7]. In view of these

considerations, the levels of TF in the circulating blood could potentially have some utility as a disease biomarker, which could be reflective of several important parameters, such as tumour burden (global emission of tumour-derived TF), the extent of malignant transformation (TF expression at the cellular level), as well as the risk of cancer coagulopathy [50]. At least some of these possibilities are in keeping with the elevated content of TF-containing microparticles in blood of patients with advanced cancers [32]. In addition to microvesicles, circulating TF could also be found in a soluble form, notably as a cleavage product of the full length molecule, or a splice variant consisting of the soluble TF ectodomain [57-60]. Tumour cells may express soluble TF variants in various quantities [54;58-60], but the biological role of these isoforms remains to be more firmly established, both in terms of cancer related thrombosis and other biological effects attributed to TF (e.g. angiogenesis or metastasis) [61].

TISSUE FACTOR, CELL DIFFERENTIATION AND CANCER STEM CELL HIERARCHY

Tumour cells display various forms heterogeneity, which emerge either as a result of genetic diversification or aberrant differentiation. The latter may include epithelial-to-mesenchymal transition (EMT) and emergence of tumour initiating cells (TICs) also known as cancer stem cells (CSCs) [62;63]. In spite of the fact that EMT, CSCs and TF were implicated in several of the same processes their linkage has not been extensively studied, until recently [64-66].

EMT is a process whereby transformed cells of epithelial origin trans-differentiate and adopt, at least transiently, a more mesenchymal, invasive and motile phenotype, which is viewed as a prerequisite of tumour dissemination [62]. Recent preclinical studies indicated that tumour cells that constitutively express activated oncogenes may, in certain settings, also undergo

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multilineage differentiation and EMT-like processes, which, interestingly, may profoundly change their expression of TF [67]. For instance, experimental analysis of A431 carcinoma cells revealed their propensity to upregulate TF and responses to TF stimulation by FVIIa upon entry into the EMT pathway, either *in vitro* or *in vivo* [67]. In this manner TF could contribute to the processes of invasion and metastasis.

Properties of EMT along with several other markers are often attributed to a small subset of cancer cells known as CSCs [63;68]. These cells are viewed as original targets of the oncogenic transformation and the source of tumour growth initiation, repopulation and recurrence, therapeutic intractability and metastasis. The ability of CSCs to retain their properties and engage in these processes critically depends on their interactions with the compatible surroundings, usually referred to as cancer stem cell niche. Unlike CSCs, their progeny has limited proliferative potential, may enter various stages of multilineage differentiation and is inefficient at (or even incapable of) initiating tumour growth [63]. Interestingly, at least in some experimental systems, high levels of TF may become restricted to cells harbouring markers of CSCs, such as CD133 [69]. Indeed, there are experimental suggestions (which still need to be verified) that the abundance of these cells *in vivo* may be relatively low, resulting in the overall levels of TF that may be out of proportion to the functional significance of this receptor for tumour progression [67]. The latter is revealed by a significant delay in tumour initiation following injection of threshold (small) numbers of cells when the animals are exposed to anti-TF antibodies [67]. Thus, at least in some instances, TF expression by only a subset of cancer cells (rather then wide spread upregulation) may carry biological significance, e.g. at the earlier stages of disease progression. The mechanisms by which processes involved in EMT and CSC formation intersect with oncogenic

pathways and other stimuli, to impact the levels of TF in cancer cells, are presently unknown. This represents a challenge in translating these findings into prognostication and therapy, as does the present lack of understanding of the identity (e.g. that of CSCs) of cells that express TF in specific human tumours.

TUMOUR STROMA AS A SOURCE OF TISSUE FACTOR IN CANCER

Host cells that may be co-opted by, or infiltrate growing tumour masses represent a well-recognized source of TF expression in cancer. Thus, TF staining has been found on surfaces of endothelial cells associated with breast cancer [49], as well as on macrophages and myofibroblasts within the invasive edges of such tumours [70]. Moreover, macrophages positive for TF were also noted in patients with urinary tract malignancies [71], and in a subset of blood vessels associated with human glioma [69].

While some of the positivity for TF antigen may stem from the microvesicular transfer of this receptor from adjacent cancer cells [55], there is also evidence for the endogenous TF expression by the vascular tumour stroma and a functional role of this process in tumour pathogenesis. For instance, host (mouse) TF mRNA is readily detectable in xenotransplants of human tumour cells in immunodeficient mice (Magnus & Rak unpublished observation). In addition, selective removal of host TF expression and activity in such experimental systems leads to several biological consequences, such as growth diminution of certain types of TF-deficient tumours, remodelling of the tumour vasculature, or changes in toxic side effects of chemotherapeutic agents [55]. However, growth of TF-proficient tumours is usually (albeit not always) undiminished in mice with low levels of TF [55], and may even be accelerated in mice expressing TF mutant

lacking the intracellular C-terminal domain [72]. Thus, while host-related TF may have certain cell-autonomous functions in cancer, the available preclinical evidence suggests that its role is context-specific and, for the most part, less pronounced that that of TF expressed by cancer cells [55;73]. Still, the positivity for TF on the part of various stromal cell populations may herald their exposure to TF-inducing proinflammatory cytokines, hypoxia and other factors, or suggest their involvement in tumour invasion [70], angiogenesis [74], or coagulopathy [71]. While these possibilities are intriguing, they remain poorly understood at the moment.

THE FUNCTIONAL ROLE OF TISSUE FACTOR IN TUMOR PROGRESSION

There is mounting, albeit largely preclinical, evidence that TF contributes to tumour progression through both procoagulant and signalling mechanisms. For instance, blocking TF expression or activity in cancer cells using various approaches negatively impacted tumour growth, angiogenesis, expression of growth factors and metastasis in a number of animal studies [7;64-66;75-81]. Moreover, host related TF was targeted using genetic manipulations [55], antibodies [73] and specially designed, cytotoxic ligands, notably the agent known as Icon, which contains FVII as a recognition domain [82]. These efforts led to antitumor effects of varying magnitude [55], in some instances rather dramatic [82]. In some cases, selective genetic targeting of distinct TF domains, or usage of various anti-TF antibodies permitted separation of largely tumour-promoting, signalling effects of this receptor from its predominantly metastasis-promoting procoagulant effects [80].

While overwhelming majority of studies point to tumour promoting role of TF, there are also some intriguing and thought provoking exceptions. For instance, deletion of the *TF* gene in certain types of experimental cancer cells had no discernable impact on their localized tumour

forming capability [64;83], while instead such cells may have been metastasis-deficient in some studies [64]. One qualification that may potentially explain why TF may impact the entire tumour progression process in one case, and only metastasis in another, may be related to ways in which these respective processes are interrogated, and whether in the involvement of CSC-like cells is taken into consideration. Thus, metastasis occurs (and is studied) under (oligo) clonal conditions, where malignant growth has all the hallmarks of tumour initiation, albeit in the ectopic site. Studies, aimed at capturing this process often rely on injection of cancer cells into the circulation, which results in their dispersion and formation of secondary clonal nodules. This process [64], as well as injection of low numbers of cancer cells locally (to test initiation rate of primary tumour)[67], reflects the presence of CSCs and is usually highly sensitive to TF inhibition. This differs from the traditional induction of primary tumour growth that often involves local injection of large numbers of cancer cells, which rapidly initiate polyclonal expansion at the site of inoculation. In this case multiple CSCs and their progeny participate in the growth process and the effects of various TF-directed manipulations are also more variable and sometimes relatively weaker [67].

While the clinical parallels of these findings still need to be defined more clearly, the potentially relevant point is that CSC initiated tumour growth stage seems to be particularly responsive to TF inhibition [67]. While this could be attributed to a number of factors, one postulated possibility is that TF expressed by cancer, or stromal cells may contribute to formation of a provisional cancer stem cell niche, e.g. by recruitment of growth promoting activities of thrombin, platelets, and fibrin [69]. This microenvironment would foster survival, expansion, angiogenic activity and other disease promoting properties of these cells at the very early stages of tumour or metastasis

formation. Subsequent growth of the tumour mass and recruitment of other stromal element could complement or to some extent replace these TF effects, albeit in a context dependent manner. As the properties of CSCs and their niches are increasingly in focus of anticancer therapy efforts the potential link between CSCs and TF is, nevertheless, thought provoking.

Indeed, targeting TF in cancer emerges as an interesting therapeutic possibility. In this regard challenges include identification of the most suitable pharmacological means and suitable agents (either existing [84] or novel). Clearly, strongly anticoagulant TF antagonists may present a risk of bleeding [44] or be unable to affect important aspects of TF signaling [80]. Moreover, defining disease contexts in which such therapies could be effective requires further study. As in the case of any other cancer related effector molecule [85], the requirement for TF may change with tumour progression, e.g. due to redundant expression of other relevant molecular mediators e.g. procoagulant, or angiogenic activities. While conclusive answers to these questions remain presently elusive, their exploration may determine the prospects of adding TF antagonists to anticancer armamentarium and design of clinical trials in which to test their safety and efficacy.

SUMMARY

TF is frequently overexpressed in human tumours and exhibits dual, that is, signalling and procoagulant activity (Fig.1). Interestingly, in some cases tumour cells were found to ectopically produce the main TF ligand, factor VII, an observation that may suggest a cancer-specific 'autocrine' activation of the TF pathway [86]. Indeed, this and other aspects of TF biology could serve as a possible targets for both anticoagulant and anticancer therapies. As a vascular target, TF could be useful in guiding immune [82], or pharmacological [87] attacks on the tumour

microcirculation. On the other hand, the functional involvement of TF in tumour initiation, growth and angiogenesis, whenever documented, could rationalize development of TF directed agents capable of exerting anti-tumour effects, or blocking metastasis [80]. Encouraging recent experience with anti-tumour effects of heparinoids [37-39;88] may suggest that TF-directed agents could prove similarly or more effective. An exciting possibility is that some of these agents (e.g. signalling-specific antibodies) could be used at a minimal risk of compromised haemostasis [80]. The exploitation of these effects represents an attractive translational objective, albeit more fundamental research is still required. However, more extensive and disease-specific clinical analysis is warranted in the area of establishing whether tumour associated TF levels, as well as changes in circulating TF possess an independent prognostic and predictive (biomarker) utility [32;33]. Overall, several new findings related to TF create a fascinating prospect that this unique molecule could be explored in various ways to treat human cancer.

PRACTICE POINTS

- Deregulated haemostasis in cancer patients should be taken seriously, as it may herald disease progression, potentially out of proportion to the estimated increased risk for thrombosis or bleeding

- New anticancer agents that target oncognic and angiogenic pathways may produce changes in the cancer patient coagulome, especially in combination with other treatments, vascular comorbidities, aging and other factors that may be difficult to predict.

- Tissue factor is likely one of the main drivers of the deregulated coagulation in cancer patients.

RESEARCH AGENDA

- The precise mechanisms of tissue factor involvement in tumour growth, angiogenesis, metastasis and stem cell niche effects requires additional fundamental studies, especially in a disease specific settings.

- These studies should be coupled with parallel clinical research efforts.

- The linkage between tumor-associated and circulating tissue factor and progression of specific malignancies deserves further clinical studies to establish the rational basis for exploring the utility of this procoagulant receptor as a biomarker in prognostic and predictive settings.

- Careful evaluation of the available anti-tissue factor agents for their therapeutic promise in various cancer settings should precede consideration of future clinical trials.

- New anticancer agents should be tested more extensively for their impact on cancer-related coagulome, especially on tissue factor expression and activity.

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CONFLICT OF INTEREST STATEMENT

Janusz Rak was involved in consulting for Nuvelo Inc. in the area of TF targeting. Chloe Milsom, Nathalie Magnus and Joanne Yu claim no conflict of interest.

Table 1. Overexpression of Tissue Factor and Progression of Human Malignancies

Cancer	TF overexpression and correlates	Reference
Bladder	TF in urine of patients with bladder cancer	[89] Lwaleed et al 2000
	TF expression and bladder cancer progression	[90] Forster et al. 2003
	TF in bladder cancer N0 and patient survival	[91] Patry et al 2008
Brain	TF in high grade tumors and blood vessels	[92] Takano et al 2000
	TFPI and low TF in hemorrhagic GBM	[93] Takeshima et al 2000
	TF levels correlate with tumour progression	[94] Hamada et al. 2002
	TF staining increases with tumor grade	[95] Guan et al. 2002
	Proposed linkage between TF, vaso-occlusive events and pseudopalisades in GBM	[96] Brat & Van Meir 2004
Colon (CRC)	Increase in TF-containing microparticles in blood of cancer patient	[97] Hron et al. 2007
	TF and VEGF levels correlate in tumours	[98] Altomare et al. 2003

	TF levels correlate with angiogenesis, VEGF expression and disease progression	[99] Nakasaki et al. 2002
	TF levels correlate with poor prognosis and liver metastasis	[100] Seto et al. 2000
	TF overexpression correlates with increase in angiogenesis and metastasis	[101] Shigemori et al. 1998
	Increased TF levels in metastatic cell lines	[102] Kataoka et al 1998
	FVII-dependence of tumour-induced coagulation	[103] Szczepanski et al 1988
Gastric	TF levels correlates with venous invasion and lymphatic metastasis in intestinal-type tumours	[104] Yamashita et al. 2007
Leukemia	TF predominates in M3 and M4-M5 blasts	[105] Nadir et al 2005
	Increased TF levels in leukemic cells	[106] Nakasaki et al 2000
	TF overexpression in leukemic blasts	[107] Lopez-Pedrera et al. 1997
	Overexpression of TF in AML and ALL	[108] Tanaka et al 1993

	TF overexpression in AML	[109] Bauer et al 1989
Other Hematopoietic Malignancies	No increase in TF levels in hematopoietic malignancies	[110] Nygard et al 2008
	TF upregulation in paediatric lymphoma	[111] Semeraro et al 1994
Lung	Overexpression of full-length and variant TF (asHTF) in non-small cell lung cancer (NSCLC)	[112] Goldin-Lang et al 2008
	Hypercoagulability and disease progression in NSCLC in association with the presence of TF and asHTF in tumours and in circulation	[113] Rauch et al. 2005
	TF-containing microparticles in blood accompany Trousseau's syndrome	[114] Del Conde et al. 2007
	Microparticle-associated TF accompany DIC	[115] Langer et al. 2008
	Association of high TF expression with metastasis	[116] Sawada et al. 1999
	TF levels correlate with tumour angiogenesis	[117] Koomagi et al. 1998

Melanoma	Unchanged levels of TF with decrease in TFPI	[118] Kageshita et al. 2001
Ovary	Increase in TF expression and associated thrombosis	[119] Uno et al. 2007
	TF in serum is associated with poor prognosis	[120] Han et al. 2006
	Increased expression of TF and coagulation intermediates are detectable in ovarian cancer	[121] Zacharski et al. 1993
Pancreas	Circulating TF-containing microparticles parallel disseminated disease	[32] Tesselaar et al. 2007
	TF expression correlates with VEGF production, angiogenesis and thrombosis	[122] Khorana et al 2007
	Expression of cell-associated and soluble TF correlate with the onset of coagulation	[123] Haas et al 2006
	Involvement of TF in tumour invasiveness, metastasis and progression	[124] Nitori et al. 2005
	Increasing TF levels parallel tumour progression	[125] Kakkar et al. 1995

Liver	TF expression correlates with progression and angiogenesis in hepatocellular carcinoma	[126] Poon et al 2003
Prostate	TF levels correlate with Gleason score	[127] Kaushal et al 2008
	TF is present on prothrombotic microvesicles (prostasomes) from cancer patients and contributes to coagulopathy	[128] Babiker et al. 2007
	Plasma microparticle TF levels correlate with cancer coagulopathy and risk of recurrence	[129] Langer et al. 2007
	TF expression correlates with poor survival	[130] Akashi et al. 2003
	TF expression is associated with tumour angiogenesis and metastasis	[131] Ohta et al. 2002
	TF expression correlates with tumour progression and angiogenesis	[132] Abdulkadir et al. 2000

Abbreviations: TF – tissue factor; GBM – glioblastoma multiforme; VEGF – vascular endothelial growth factor; AML – acute myelogenous leukemia; ALL – acute lymphoblastic leukemia; NSCLC – non-small cell lung cancer; DIC – disseminated intravascular coagulation;

FIGURE LEGENDS

Figure 1. Upregulation of tissue factor as a constituent of cancer progression and coagulopathy – Possible implications. TF upregulation is largely driven by the oncogenic transformation process, in concert with, still poorly understood aberrant differentiation mechanisms. The latter may lead to formation of the cancer stem cell (CSC) pool and drive epithelial to mesenchymal transition (EMT), which is involved in initiation of tumour invasiveness and metastasis. Cancer cell-associated TF may contribute to the phenotype of CSCs, as well as to angiogenic, invasive and metastatic processes in tumours. Experimental targeting of this receptor interferes with these events (see text). Soluble forms of TF enters tumour microenvironment and blood as cargo of procoagulant microvesicles (MVs), also known as microparticles. TF-containing MVs along with cancer cells may trigger systemic coagulopathy and mediate the transfer of TF activity between various cells. The increasing understanding of these processes led to a suggestion that TF expression could be utilized to monitor both hypercoagulability and disease progression, as well serve as novel therapeutic targets in various settings of human malignancy (see text for details).

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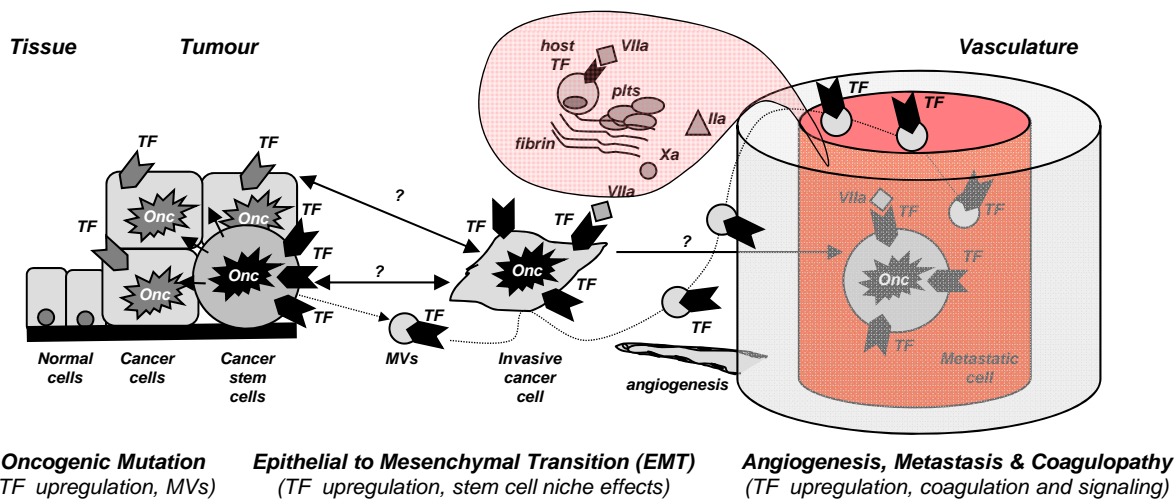
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Tissue   **Tumour**

Vasculature

host TF   VIIa

plts

fibrin

IIa

Xa

VIIa

TF

TF

TF

VIIa   TF   TF

Onc

TF

TF

Metastatic cell

TF

TF

TF

Onc

Onc

Onc

Onc

?

TF

TF

Onc

TF

TF

?

TF

?

MVs

TF

Normal cells   **Cancer cells**   **Cancer stem cells**

Invasive cancer cell

angiogenesis

Oncogenic Mutation
(TF upregulation, MVs)

Epithelial to Mesenchymal Transition (EMT)
(TF upregulation, stem cell niche effects)

Angiogenesis, Metastasis & Coagulopathy
(TF upregulation, coagulation and signaling)

Possible Clinical Implications:

Tumor-associated TF as a putative biomarker (in situ):   *(i) Coagulopathy*

(ii) Disease progression

Microvesicle (MV)-associated TF as a putative biomarker (blood assays): *(i) Coagulopathy*

(ii) Disease progression

Soluble (alternatively spliced) TF as a putative biomarker   *(i) Coagulopathy*

(ii) Disease progression (?)

Tissue factor as a putative therapeutic target: *(i) Indirect – anticoagulants*

(ii) Indirect – agents down-regulating TF expression (targeted anticancer drugs)

(iii) Direct – selective inhibitors of TF-dependent coagulation

(iv) Direct – inhibitors of TF-dependent signaling

(v) Direct – agents targeting both TF pro-coagulant and signaling activities

Rak et al Fig 1.
Upregulation of tissue factor as a constituent of cancer progression and coagulopathy – Possible implication