The relationship between tissue factor and cancer progression: insights from bench and bedside

Running head: Non-hemostatic effects of tissue factor in cancer

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Abstract

It is now widely recognized that a strong correlation exists between cancer and aberrant hemostasis. Patients suffering from various types of cancers, including pancreatic, colorectal and gastric cancer, often develop thrombosis, a phenomenon commonly referred to as Trousseau’s syndrome. Reciprocally, components from the coagulation cascade also influence cancer progression. The primary initiator of coagulation, the transmembrane receptor tissue factor (TF), has gained considerable attention as a determinant of tumor progression. Upon complex formation with its ligand, coagulation factor (F)VIIa, TF influences Protease-Activated Receptor (PAR)-dependent tumor cell behavior, and regulates integrin function, which facilitate tumor angiogenesis both in vitro and in mouse models. Furthermore, evidence exists that an alternatively spliced isoform of TF (asTF) also affects tumor growth and tumor angiogenesis. In patient material, TF expression and TF cytoplasmic domain phosphorylation correlate with disease outcome in many, but not in all cancer subtypes, suggesting that TF-dependent signal transduction events are a potential target for therapeutic intervention in selected types of cancer. In this review, we will summarize our current understanding of the role of TF in tumor growth and metastasis, and speculate on anti-cancer therapy by targeting TF.
Introduction

After Trousseau’s description of thrombophlebitis as a complication of pancreatic cancer in the 19th century, the notion that increased expression of TF underlies the relation between coagulation and cancer has become generally accepted. Full-length TF (fITF) is a 47 kDa membrane-bound glycoprotein that is present on subendothelial cells. In the classic concept of coagulation, it is thought that endothelial disruption leads to exposure of fITF to the blood stream. Exposed fITF can bind to its natural ligand factor VII (FVII), which then becomes activated FVII (FVIIa). The thus formed fITF:FVIIa complex converts factor X (FX) to factor Xa (FXa) and FXa in turn activates prothrombin leading to formation of thrombin (factor IIa). Thrombin subsequently activates platelets and converts fibrinogen into fibrin, two essential components of a stable hemostatic plug.

The primary function of subendothelial fITF is to serve as a hemostatic envelope surrounding the vasculature. However, under certain conditions the expression of fITF is induced in monocytes and endothelial cells. fITF is also often expressed on cancer cells and the tumor vasculature, and fITF-bearing microparticles can become shed by these cells. These fITF-bearing microparticles are important contributors to the thrombotic phenotype in cancer patients.

The fITF:FVIIa complex also influences pathways that do not lead to blood coagulation, but rather activate cell-bound protease-activated receptors (PARs) that are of importance during the inflammatory and angiogenic response to injury. Furthermore, a soluble variant of fITF, known as alternatively spliced TF (asTF), stimulates angiogenesis independent of FVIIa.

In this review, we discuss the current knowledge of the role of the various TF isoforms in the modulation of cancer that comes from both experimental and patient-based studies.
Finally, we propose approaches for further clarifying the role of TF isoforms in cancer biology and its potential as a therapeutic target.

**Oncogenic events drive flTF expression**

flTF expression in cancer is the result of well-defined upstream events that occur during the process of oncogenic transformation (see figure 1). In colorectal cancer (CRC) mutations of both the K-ras proto-oncogene and p53, leading to loss of p53 function, result in a constitutive activation of the mitogen activated protein kinase (MAPK) and phosphatidylinositol-3’ kinase (PI3K) signaling pathways, thus leading to enhanced TF expression. *In vivo* experiments confirmed that the K-ras and p53 mutations in CRC are indeed primarily responsible for flTF upregulation. This is in agreement with the finding that in CRC patients mutations of K-ras, p53 are associated with flTF expression in tumors.

Amplification of epidermal growth factor receptor (EGFR) expression and a constitutively active mutant form of EGFRvIII have also been shown to modulate flTF expression in cancer cells. EGFRvIII overexpression in glioma cells results in flTF expression. Restoration of the tumor suppressor gene PTEN in these cells, which leads to inhibition of the PI3 kinase and MAPK pathways, downregulates EGFR-dependent flTF expression. Moreover, endometrial cancer cell lines display enhanced flTF levels in an EGFR-dependent fashion, and inhibition of EGFR signaling diminishes flTF expression in vulva carcinoma cells constitutively expressing the EGFRvIII mutant.

Recent studies in medullo-blastoma cell lines indicate that Src family kinases stimulate an induction of flTF expression through both the scatter factor/hepatocyte growth factor (SF/HGF) and as a result of a mutation in the c-MET oncogene, while flTF expression via the HGF:c-MET axis elicits an anti-apoptotic response and provides resistance to chemotherapeutical agents.
Some of the in vitro findings described above are supported in biopsies from a series of non-small cell lung cancer patients. In these samples, PTEN and p53 mutations were associated with flTF expression, suggesting that an accumulation of mutations in proto-oncogenes and tumor suppressor genes upregulates flTF expression on tumor cells\textsuperscript{13,14}.

\textit{In vivo} experiments in a murine xenograft model with human vulvar carcinoma cells show that epithelial-to-mesenchymal transition (EMT), and the concomitant inactivation of E-cadherin, result in further EGFR-induced flTF expression. These events lead to increased production of vascular endothelial growth factor (VEGF), thus enhancing the angiogenic potential of cancer cells\textsuperscript{11}.

Transforming growth factor-\(\beta\) (TGF-\(\beta\)) is an essential cytokine for EMT to occur, and is co-expressed with flTF in tumor cells and tumor stromal cells\textsuperscript{15}, suggesting the production of TGF-\(\beta\) production as a critical upstream event in upregulation of flTF in tumors. EMT also contributes to the generation of what are currently regarded cancer stem cells. Cancer stem cells form a subpopulation of tumor cells that fuels tumor growth, and has functional properties distinct from other cancer cell populations, e.g. cancer stem cells may transdifferentiate to vascular cells\textsuperscript{16}. Support for this notion comes from studies that show that CD133-positive cancer stem cells derived from a vulva carcinoma cell line, display enhanced flTF-dependent coagulant activity\textsuperscript{17}. Nevertheless, it remains unclear whether cancer stem cells derive their phenotype from expression of flTF or that expression of flTF is merely associated with the cancer stem cell phenotype.

Hypoxia may also modulate flTF expression by cancer cells. Analysis of human glioma specimens shows that flTF expression is highest in cells that surround sites of necrosis in hypoxic pseudopalisades\textsuperscript{18}. Hypoxia-driven flTF expression is not dependent on hypoxia-activated factor HIF1\(\alpha\), but rather on the early growth response gene-1\textsuperscript{19}.
Taken together, flTF expression is enhanced in tumors as a result of 1) well-defined mutated tumor suppressor genes and oncogenes, 2) EMT and 3) hypoxia.

**TF isoforms and their cellular effects on cancer**

Binding of FVIIa to flTF results in a series of signaling events that regulates a broad range of cellular responses such as: 1) gene transcription; 2) cell survival; and 3) cytoskeletal changes, that are required for a cell to adequately respond to its local environment (figure 2). Despite the structural homology between flTF and interferon receptors, flTF:FVIIa signaling differs substantially from classical interferon receptor signaling. Rather than actively recruiting the JAK/STAT complex to the intracellular domain of flTF, flTF:FVIIa typically triggers signaling via PAR2. PARs form a four-member family of 7-transmembrane domain cellular receptors that are activated by proteolytic cleavage of the extracellular amino terminus. This event leads to exposure of a tethered ligand that folds back to the second extracellular loop resulting in receptor activation. PAR1 is the archetypical thrombin receptor, but is also cleaved by other proteases such as plasmin, FXa, matrix metalloproteinase-1 and activated protein C. flTF:FVIIa, FXa, trypsin and tryptase are able to activate PAR2, whereas PAR4 is activated by thrombin and plasmin. In mouse models, PAR3 has been found to serve as a cofactor for PAR4, but recent data suggest that human PAR3 may also be activated directly by thrombin. Upon activation, PARs couple to heterotrimeric G-proteins after which further signaling events are initiated.

Signaling of flTF:FVIIa via PAR2 elicits calcium transients and activation of the major members of the MAPK family, p44/42, p38 and c-Jun N-terminal kinase (JNK). In addition, Src-like kinases, PI3 kinase, the Jak/STAT pathway and the Rho GTPases Rac1 and Cdc42 are activated, culminating in cell survival and cytoskeletal rearrangements. Activation of both the MAPK and PI3 kinase pathways contributes to a pro-malignant transcriptional
program and stimulates oncogenic protein synthesis\(^4\). \(\text{fITF:FVIIa}\)-mediated PAR2 activation also leads to the production of pro-angiogenic factors such as VEGF, Cyr61, VEGF-C, CTGF, CXCL1, and IL8, as well as of immunological modulators such as granulocyte-macrophage colony stimulating factor (GM-CSF or CSF2) and macrophage colony stimulating factor (M-CSF or CSF1)\(^23\text{-}25\). Although PAR1 signaling induces a similar series of proteins in breast cancer cells, the activation of the \(\text{fITF:FVIIa}\) axis appears to elicit a more efficient production of these angiogenesis and immune regulators \(^25\). The generation of these molecules can trigger angiogenesis in a paracrine fashion by targeting vascular cells. In addition to PAR2-dependent signaling, the \(\text{fITF:FVIIa}\) complex may directly signal via the \(\text{fITF}\) cytoplasmic tail through Rac1 and p38 by stimulating cytoplasmic tail-recruitment of the actin-binding protein 280 and potentially cytoskeletal remodeling\(^26\).

PAR2-mediated signaling via \(\text{fITF:FVIIa}\) is tightly regulated through post-translational modification and protein interactions. Part of the early response in PAR2 signaling involves protein kinase C (PKC)-\(\alpha\)-mediated phosphorylation of Ser 253 in the \(\text{fITF}\) cytoplasmic domain, followed by proline-directed kinase (PLK)-dependent phosphorylation of Ser 258. Genetic deletion of the cytoplasmic domain in mice results in a pro-angiogenic phenotype\(^27\), while complete abrogation of cytoplasmic domain phosphorylation inhibits PAR2-dependent cell migration in vitro. In contrast, phosphorylation of the cytoplasmic domain leads to more potent PAR2 signaling\(^28\).

Covalent attachment of fatty acids - specifically palmitoylation- to the \(\text{fITF}\) cytoplasmic domain, may regulate \(\text{fITF}\) activity by routing \(\text{fITF}\) to membrane compartments in which \(\text{fITF}\) signaling function is minimal. Indeed, palmitoylation of Cys 245 results in the enhanced localization of \(\text{fITF}\) into sphingolipid rafts of the cell membrane, which leads to impaired PAR2 signaling\(^29\).
Efficient PAR2 signaling and Ser 253 phosphorylation of flTF depends on binding of flTF to β1-integrins\textsuperscript{28}. flTF/β1-integrin complex formation stimulates flTF-dependent PAR2 activation and facilitates breast cancer development by contributing to both tumor angiogenesis and growth\textsuperscript{30;31}. Reciprocally, flTF positively regulates integrin function, thus contributing to the interaction between cells and the extracellular matrix environment.

Intriguingly, some tumors are known to produce FVII, thereby circumventing the requirement for FVII from the blood circulation for flTF:VIIa:PAR2 signaling\textsuperscript{32}. Ectopic production of FVII is regulated by epigenetic and hypoxia-driven processes in several solid tumor cell lines\textsuperscript{33;34}, whereas EGFR signaling in gliomas not only upregulates TF expression, but also expression of FVII and PAR2\textsuperscript{35}, thus orchestrating the generation of a multitude of events that contribute to flTF:VIIa:PAR2 signaling.

TF isoforms also elicit non-hemostatic cellular effects independent of PAR2 activation. A naturally occurring, soluble form of TF has been characterized which results from alternative splicing. Whereas a six exon transcript encodes membrane-bound flTF, asTF mRNA is formed when exon 5 is skipped. This causes a shift in the reading frame, and, consequently, asTF contains a unique C-terminus and lacks a transmembrane region, rendering the protein soluble\textsuperscript{36;37}. Since its discovery, the role of asTF in coagulation has been a matter of debate\textsuperscript{38}. Increasing evidence supports a role for asTF in cancerous processes\textsuperscript{5;6;39;40}. The affinity of asTF for FVII(a) is low, which is also reflected in an absence of asTF-dependent FVIIa signaling. On the other hand, asTF activates α6β1 and αVβ3 integrins on endothelial cells, thus acting as a pro-angiogenic stimulus. Integrin ligation by asTF activates a plethora of downstream signaling components such as focal adhesion kinase (FAK) PI3K, MAPK and Akt\textsuperscript{5}, although the relative contributions of these pathways to asTF-dependent angiogenesis are poorly understood.
In conclusion, TF isoforms, FVII, PAR2 and integrins have pleiotropic effects on cellular processes that are important in cancer biology at the level of cell survival, as well as the interaction of cells with their environment, in particular angiogenic events. The apparent lack of coagulant activity of asTF further underlines that the effects of TF isoforms can occur through coagulation-independent mechanisms. In the following paragraph, we will examine how these effects contribute to cancer progression in *in vivo* cancer models.

**TF isoforms in cancer: evidence from experimental studies**

Results from xenograft and syngeneic models in mice underline the role of flTF in primary tumor growth, tumor cell-host interactions and metastasis. Work over the past decade has indicated that flTF-driven primary tumor growth in murine models is the direct resultant of enhanced tumor angiogenesis. Knock-down of flTF in fibrosarcoma or CRC cells results in decreased angiogenesis through modulation of VEGF and thrombospondin levels and a concomitant decreased primary tumor growth in xenograft models\(^7\).\(^{41}\), while pharmacological blockade of flTF function has similar effects\(^42\). Notably, in many of these studies blockade of downstream coagulation factors was without effect, suggesting a role for flTF:PAR2-crosstalk in primary tumor growth. Indeed, antibodies that specifically block the signaling function of flTF (mAb-10H10) or PAR2, but not antibodies against the procoagulant function of flTF (mAb-5G9) or PAR-1, significantly inhibit tumor growth in breast cancer xenografts\(^30\). Constitutive association of flTF with \(\beta_1\)-type integrins facilitates the flTF:FVIIa:PAR2 axis in primary breast tumors. In support of a role for flTF-mediated PAR2 signaling, PAR2, but not PAR1 deficiency in mice that harbor a murine mammary tumor virus promotor driven polyoma middle T antigen cassette (PyMT, leading to spontaneous breast tumors), attenuates tumor growth due to a delay in the angiogenic switch\(^43\). Similarly, genetic deletion of the cytoplasmic tail (ACT) of flTF inhibits VEGF production and tumor growth in a xenograft
model\textsuperscript{44} and angiogenesis and tumor growth in the PyMT model, while combination of PAR2 deficiency and cytoplasmic tail deletion does not further decrease tumor growth\textsuperscript{31}. Thus, PAR2 and the flTF cytoplasmic tail have overlapping roles and are involved in extensive crosstalk in primary breast tumors.

In addition to flTF:FVIIa:PAR2 signaling in injected cancer cells, host flTF:FVIIa:PAR2 signaling appears to play a significant role. In ΔCT mice, tumor grafts harboring flTF with an intact cytoplasmic tail showed significantly more tumor angiogenesis\textsuperscript{27}. Moreover, flTF cytoplasmic tail deletion in PyMT mice resulted in large-diameter vessels in late-stage tumors, whereas this effect was reversed in PAR2-deficient, flTF cytoplasmic tail-deleted mice. Thus, the flTF cytoplasmic tail appears to have opposing effects in tumor growth and the host angiogenic response, where the latter effect may be attributed to flTF:FVIIa:PAR2 signaling in the host macrophage compartment.

Further evidence for non-tumor cell flTF signaling in cancer comes from experiments that employ spontaneously immortalized embryonic fibroblasts from TF wild-type (WT), TF-/- and TF cytoplasmic tail deleted (TFΔCT) embryos. Primary tumor growth was similar after engraftment of WT, TF-/- and TFΔCT, but after engraftment of TF-/- teratoma cells into mice expressing 1% of normal TF levels, teratoma growth was aborted\textsuperscript{45}. The used model, however, may not be valid because teratoma and cancer cell lines may make use of dissimilar cellular mechanisms when forming tumors. Taking into consideration that established melanoma and lung cancer cell lines are not impaired by a lack of host flTF, this indicates that the contribution of host- and tumor-derived flTF to cancer progression is highly dependent on the cancer type. Furthermore, the role of flTF in the response of the host immune system is partly understood, although natural killer cell activity-dependent mechanisms appear to cooperate with tumor cell-bound flTF\textsuperscript{46}. 
flTF facilitates outgrowth of metastases in murine models by inducing local proliferation and infiltration of metastatic cells rather than by influencing cell adhesion to metastatic sites. In studies that employ cells expressing flTF mutants with diminished flTF:VIIa proteolytic activity or a deleted cytoplasmic tail, a decrease in metastatic load was seen, suggesting importance of both flTF:FVIIa proteolytic activity and cytoplasmic domain function. flTF putatively influences metastatic outgrowth through similar mechanisms as in primary tumor growth, however, downstream coagulation activation is of greater importance during the stages when tumor cells are blood-borne and hatch to the endothelium during metastasis. This concept finds support in experiments where antibody blockade of flTF coagulant function inhibited metastasis in a breast cancer xenograft model, whereas blockade of flTF signaling function was without effect. Although thrombin is also reported to promote primary tumor growth through PAR1 activation, increasing evidence supports that its role in metastasis is more potent. This concept recently found even more support in a report on the pro-metastatic phenotype of mice with a thrombomodulin mutant with decreased affinity for thrombin. Further evidence for a prominent role of downstream coagulation activation in metastasis comes from experiments in genetically modified mice that either lack platelets, PAR4 or fibrinogen. Mice with either of these genetic modifications were protected from metastasis, which provides evidence that metastasis is facilitated by thrombin-activated platelets via PAR4. Thus, flTF on tumor cells initiates PAR2 dependent signaling with subsequent effects on tumor growth, and simultaneously induces thrombin generation that facilitates metastasis.

At present, mechanistic insight into the role of asTF in cancer biology is sparse. asTF-producing pancreatic cancer cells yield larger tumors in comparison with asTF negative cells upon xenografting. asTF is believed to augment angiogenesis by acting as an integrin-activating agent, but the exact mechanism remains unclear. Future studies with specific
targeting of either asTF or flTF in constitutively asTF-expressing cancer cell lines will increase the knowledge on asTF in cancer biology.

In summary, evidence from experimental studies indicates a direct role for flTF in cancer biology via PAR2 signaling in cooperation with integrins, leading to enhanced primary tumor growth. asTF may have a distinct role from flTF in primary tumor growth by integrin ligation, but this remains to be elucidated. The effects of flTF on metastasis are a result of downstream coagulation activation as shown by the mammary metastasis model using 5G9, however, the role of asTF in metastasis is not investigated yet. The role of the cytoplasmic domain of flTF remains unclear, but the literature to-date suggests different or even opposing roles for the flTF cytoplasmic domain in the host and tumor compartment.

TF isoforms in human cancer

In this section, we will discuss whether the concepts described above, find support in studies that are primarily aimed at finding correlation between expression of TF isoforms and pathological and clinical parameters. We will not discuss observational studies that examine flTF expression in human cancer without describing associations with clinical and pathological parameters due to space limitations. A comprehensive overview of the studies that we selected for this review is provided in table 1.

Ample evidence exists that flTF is abundantly expressed in a variety of solid tumors such as breast cancer, lung cancer, gastro-intestinal cancers, urogenital cancers, melanomas and gliomas. Studies of the upstream oncogenic events that lead to enhanced flTF expression have been conducted in colorectal and lung cancer, and associations were identified between flTF expression and p53 and K-ras mutations for both lung and colorectal cancers, and PTEN as well for lung cancer.
The majority of the cited studies supports the notion that fITF expression is an independent predictor of poor overall or relapse-free survival\textsuperscript{13,54;55;59-61;63;70;72;75;80}, although some studies failed to find such a relation\textsuperscript{57;76;77}. Furthermore, associations have been found with invasiveness in breast cancer\textsuperscript{15} and melanomas\textsuperscript{15;76}, high clinical staging in lung\textsuperscript{13;14;40;57}, pancreas\textsuperscript{62;63}, colorectal\textsuperscript{65;66} and prostate cancer\textsuperscript{74}, and metastases in cancers of breast\textsuperscript{53}, lung\textsuperscript{56}, esophagus\textsuperscript{59}, gastric\textsuperscript{60}, hepatic\textsuperscript{61}, pancreatic\textsuperscript{63} and colorectal\textsuperscript{67} tissues. Other studies, however, could not find such associations between fITF expression and unfavorable pathological and clinical parameters\textsuperscript{54;55;68;76;77;81}, this may partially be because of differences in patient populations, population size and detection techniques for fITF.

The fITF:FVIIa:PAR2 axis is supposed to drive angiogenic events through enhanced production of angiogenic factors such as VEGF. Associations have indeed been found between fITF expression and microvessel density in lung cancer\textsuperscript{14}, throughout all gastrointestinal cancers\textsuperscript{59-61;64;66}, prostate cancer\textsuperscript{71}, and gliomas\textsuperscript{70}. Associations between fITF and VEGF expression are described in breast and lung cancer\textsuperscript{14;55}, colorectal cancer\textsuperscript{68} and prostate cancer\textsuperscript{71;73}, which further strengthens the concept that TF expression promotes tumor angiogenesis. Furthermore, an antibody that only detects the cytoplasmic domain of fITF when phosphorylated (pTF) -and therefore only when involved in PAR2 signaling- was used to investigate whether the effects of fITF in cancer could be attributed to direct signaling effects of the fITF:FVIIa:PAR2 axis. Indeed, expression of pTF strongly correlated with VEGF expression and survival in patients with tumors that where positive for pTF was diminished\textsuperscript{55}.

To date the expression of asTF in relation to clinicopathological characteristics has only been studied in NSCLC and these studies reveal a correlation between high asTF mRNA levels and advanced tumor stage, whereas low levels of fITF mRNA relate to less advanced stages of cancer progression\textsuperscript{40}. In another study, high asTF mRNA levels conferred an
impaired survival to NSCLC patients, but the relation with staging and tumor size could not be confirmed\textsuperscript{80}.

Most of the aforementioned cancers are of epithelial origin, but this does not exclude a role for aberrant fITF expression in cancer of other origins. Mouse studies indicate that fITF expression influences fibrosarcoma progression\textsuperscript{41}, and rat osteosarcoma cells display fITF-dependent coagulant activity\textsuperscript{82}, but data on human sarcomas is lacking. Epidemiological evidence indicates that patients with hematological malignancies carry a high thrombotic risk\textsuperscript{83}, which suggests that circulating cancer cells may bear fITF. This indeed is the case in several leukemic cell lines but the risk for thrombosis could not be attributed to enhanced fITF expression on tumor cells\textsuperscript{84}. Furthermore, fITF expression on circulating cells was negatively associated with bone marrow microvessel density\textsuperscript{85}. Since monocyte activation leads to bona fide expression of fITF, further research into monoblastic and monocytic leukemias is warranted as well as further assessment of fITF expression in bone marrow biopsies.

In conclusion, most human epithelial cancers are characterized by abundant levels of fITF. In keeping with the observations from experimental studies, fITF is likely to drive tumor angiogenesis, to enhance tumor growth, and to influence metastasis. Since experimental studies indicate that PAR2 signaling acts in an early phase of tumor angiogenesis, the so-called angiogenic switch, the observations from experimental models may possibly not directly translate to human cancer with respect to clinical associations. This is because most cancers at the time of diagnosis have already passed the angiogenic switch. Since improvement of screening protocols will enable the detection of impalpable tumors, studies in smaller tumors may lead to a better understanding of TF isoforms in early tumorigenesis. Nevertheless, in most cancers a clear association between fITF and VEGF expression, tumor volume, microvessel density and metastatic risk leading to diminished survival is evident, which is in concordance with findings in experimental studies. Limited data is available
concerning the role of asTF in human malignancies since—at present—no studies have been performed on large series of tumors. Future studies investigating flTF versus asTF at the protein level may improve our understanding of the relative contribution of each TF isoforms to cancer biology.

**Targeting TF isoforms in the treatment of cancer**

Aside from surgical, pharmacological and radiotherapeutic treatments for cancer, a variety of new drugs are in development specifically targeting key signaling pathways and angiogenic processes. flTF expression is an important determinant of cancer progression, as well as a contributor to thrombosis susceptibility, and inhibiting flTF function may be a potential avenue for treating cancer and cancer-related thrombosis. Although studies investigating flTF-targeted cancer therapy remain sparse, some studies provide clues that flTF-directed treatment of cancer may indeed prove to be beneficial.

As proper PAR2 signaling relies on the formation of either the flTF:FVIIa or flTF:FVIIa:FXa complex, the effect of therapies lowering FVII and FX in cancer patients provided some insight in whether such indirect anti-flTF-signaling therapy has therapeutic potential. Cancer incidence has been investigated in vitamin K antagonist users which showed an anti-neoplastic effect of vitamin K antagonists\(^{86,87}\). However, due to the multiple targets of vitamin K antagonists, it is unclear whether these effects are solely flTF-dependent. Experimental work reveals that warfarin diminishes the metastatic potential, but this is seemingly independent of flTF\(^{41}\). Similarly, heparins may affect cancer progression by modulating flTF-mediated signaling events, but at present it is unclear to what extent flTF-specific signaling events contribute to the possible effects of warfarin or heparin treatment on cancer.
Specific inhibition of flTF:FVII:PAR2 signaling with the flTF antibody 10H10 may have therapeutic potential, while leaving the coagulant properties of flTF unaffected. As 10H10 was only investigated in early stages of tumorigenesis, more research is necessary to study its effects after the angiogenic switch has taken place. Another approach could be the use of RNA interference to target tumor flTF, as RNAi has proven to be beneficial in mouse experiments. Indeed, pharmacological modalities are available for tumor-specific delivery of RNAi, but again, the response to these anti-tumorigenic therapies in murine models of early tumorigenesis, and its translation to human flTF-expressing tumors, remains uncertain.

Several studies on the efficacy of flTF-targeting in cancer have been undertaken or are still ongoing. The nematode flTF:VIIa inhibitor recombinant NAPc2 has been studied in colorectal cancer, however the company suspended the trial, so that it is unclear whether the inhibition of tumor growth found in mice can be translated to humans. Currently, two other potential flTF-targeting drugs are under investigation in clinical studies, ALT-836 (Altor Bioscience ©), a TF-inhibiting antibody, and PCI-27483 (Pharmacyclics ©), a small FVIIa inhibiting molecule. The efficacy of ALT-836 is currently investigated in solid tumors in combination with gemcitabine. PCI-27483 at present is tested in a similar set-up, but this study is limited to pancreatic cancer patients. Both studies aim to target both the coagulant and signaling effects of flTF in tumor biology and the results from these studies will be helpful for deciding whether flTF-targeting is viable option for future treatment of cancer and cancer-related thrombosis.

Despite promising results, many questions remain before flTF-targeted therapies will become available for clinical application. For example, it is unclear what the effect on hemostasis will be in a patient population already displaying a severely unbalanced coagulation, although no bleeding effects have been reported in mouse studies. The 10H10 antibody may be attractive, since it leaves the coagulant properties of flTF unaffected, but
whether abrogating flTF signaling may affect other physiological processes is unclear. Moreover, the coagulant properties of flTF promote cancer progression through enhancing the metastatic potential. Therefore, targeting the coagulant function flTF may be necessary to really provide new therapeutic means. In contrast to flTF, asTF has no proven function in physiology yet, and a role for asTF in cancer biology is becoming more evident. This apparent cancer-specificity puts asTF forward as a new cancer target. Specific antibodies to the unique C-terminus of RNAi to the exon 4-6 boundary offer opportunities for a specific blockade, however, the effect of interfering with asTF in cancers is still speculative.

**Delivery of anti-tumor drugs to sites of enhanced TF expression**

Taking advantage of enhanced tumoral flTF expression to deliver tumoricidal drugs has shown promise. To this end, parts of FVII and tumoricidal compounds were combined into chimeric proteins that are capable of binding flTF. A FVII:IgG Fc effector domain chimera induced long-lasting regression of both the injected tumor and tumors injected at distant sites\(^92\), likely through mediating a NK cell dependent cytotoxic anti-tumor response\(^93,94\). FVII-bound photosensitizers have also shown positive results in flTF-targeted tumor therapy. Laser light triggers the photosensitizer that converts tissue oxygen into reactive oxygen species. In *in vivo* breast cancer models, photodynamic therapy indeed was able to target flTF-bearing tumoral endothelium and cancer cells, even when tumors became chemoresistant\(^95-97\).

Others have investigated the delivery of exogenous flTF to the tumor vasculature in order to specifically infarct tumor vessels. A conjugate containing the heparin binding domain of VEGF and truncated flTF induced specific coagulation in tumor vessels, whereas a conjugate of flTF with RGD and NRG peptides, targeting \(\alpha V\beta3\) integrins and CD13, resulted
in infarction of tumor vessels in mice, and in patients tumor perfusion decreased, whereas the compound was tolerated\textsuperscript{98,99}.

Use of fITF-mediated approaches for targeting tumoricidal drugs or infarcting tumor vasculature, may be hampered by off-target effects as well, as other parts of the vasculature may express fITF. Phototherapy is perhaps most promising in circumventing such unwanted effects, since it only exerts its effects by controlled exposure to laser light, which may be highly specific thanks to improving tumor imaging modalities.

Conclusions

During the last decades, it has become increasingly clear that fITF not only has a prominent role in the etiology of cancer-related thrombosis, but also that TF isoforms display non-hemostatic properties that are important in cancer progression. The oncogenic transformations leading to fITF expression on tumor cells are now well defined and fITF has prominent effects on tumor growth via PAR2 signaling and integrin ligation, hereby influencing cell survival, cell motility and the production of angiogenic factors. The importance of fITF in the progression of cancer is underscored by its abundant expression in human cancers from different origins. Furthermore, fITF has gained attention as a potential therapeutic target by harboring tumoricidal drugs to fITF-expressing cancer cells or via direct inhibition of its cellular effects. Despite this progress, questions remain, especially regarding the relative contribution of fITF and asTF to cancer progression.

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Authorship and conflict of interest

Y.W.v.d.B. performed literature searches, contributed to the design of the manuscript, and wrote the manuscript; S.O. and P.H.R. contributed to the design of the manuscript and edited the manuscript; H.H.V. contributed to the design of the manuscript and wrote parts of the manuscript.

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Table 1. Studies on TF expression in human cancer

<table>
<thead>
<tr>
<th>Type of cancer</th>
<th>Source</th>
<th>No. of tumors</th>
<th>TF Expression by IHC, No. (%)</th>
<th>Method</th>
<th>Main findings with respect to TF expression</th>
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<td>Breast cancer</td>
<td>Sturm 1992(^{53})</td>
<td>115</td>
<td>93 (80.8)</td>
<td>IHC</td>
<td>TF expression associates with well-differentiated epithelia and less lymph node metastases</td>
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<td>Vrana 1996(^{15})</td>
<td>40</td>
<td>40 (100)</td>
<td>IHC</td>
<td>Increased TF intensity is found in infiltrative ductal carcinoma</td>
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<td></td>
<td>Ueno 2000(^{54})</td>
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<td>193 (90.6)</td>
<td>IHC</td>
<td>TF expression is associated with TF plasma levels and overall survival</td>
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<td>Rýden 2010(^{55})</td>
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<td>61 (31)</td>
<td>IHC</td>
<td>Phosphorylated TF is associated with diminished survival</td>
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<td>Lung cancer</td>
<td>Sawada 1999(^{56})</td>
<td>55</td>
<td>46 (84)</td>
<td>IHC</td>
<td>TF expression is associated with metastasis</td>
</tr>
<tr>
<td></td>
<td>Goldin-Lang 2008(^{40})</td>
<td>21</td>
<td>NA</td>
<td>mRNA</td>
<td>TF isoforms are upregulated in cancerous tissue, IITF and asTF mRNA are associated with advanced stage. Low asTF mRNA levels are associated with early stage</td>
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<td></td>
<td></td>
<td>12</td>
<td>8 (66.7)</td>
<td>IHC</td>
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<td></td>
<td></td>
<td>11</td>
<td>NA</td>
<td>ELISA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Regina 2008(^{13,14})</td>
<td>64</td>
<td>NA</td>
<td>mRNA</td>
<td>TF expression is associated with staging, VEGF and MVD. High TF mRNA are associated with poor survival</td>
</tr>
<tr>
<td></td>
<td></td>
<td>64</td>
<td>47 (73.5)</td>
<td>IHC</td>
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<tr>
<td></td>
<td></td>
<td>30</td>
<td>NA</td>
<td>ELISA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>De Meis 2010(^{57})</td>
<td>39</td>
<td>22 (56)</td>
<td>IHC</td>
<td>TF expression is associated with staging, but not with survival</td>
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<td>Rollin 2010(^{40})</td>
<td>57</td>
<td>NA</td>
<td>mRNA</td>
<td>IITF and asTF mRNA levels are associated with poor survival</td>
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<tr>
<td>Gastrointestinal cancers</td>
<td>Esophagus</td>
<td>Ribeiro 2009(^{58})</td>
<td>36</td>
<td>NA</td>
<td>mRNA</td>
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<td></td>
<td>Chen 2010(^{59})</td>
<td>103</td>
<td>94 (91.3)</td>
<td>IHC</td>
<td>TF expression is associated with MVD, metastasis, and poor survival</td>
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<td></td>
<td>Yamashita 2007(^{60})</td>
<td>207</td>
<td>52 (25.1)</td>
<td>IHC</td>
<td>Intestinal-type cancer displayed enhanced TF expression and associate with MVD, metastasis, and poor overall survival</td>
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<td>Poon 2003(^{61})</td>
<td>58</td>
<td>58 (100)</td>
<td>IHC</td>
<td>TF associates with MVD, metastasis and poor overall survival</td>
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<td></td>
<td>Kakkar 1996(^{62})</td>
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<td>29 (52.7)</td>
<td>IHC</td>
<td>TF associates with histological grade and staging</td>
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<td>Nitori 2005(^{63})</td>
<td>113</td>
<td>100 (88.5)</td>
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<td>TF associates with staging, metastasis and overall survival</td>
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<td>Khorana 2007(^{64})</td>
<td>240</td>
<td>211 (87.9)</td>
<td>IHC</td>
<td>TF associates with MVD and thrombosis rate</td>
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<td>Shigemori 1998(^{56})</td>
<td>79</td>
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<td>IHC</td>
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<td>Nakasaki 2002(^{65})</td>
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<td>IHC</td>
<td>TF associates with staging and MVD</td>
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<td>31 (46)</td>
<td>IHC</td>
<td>TF associates with hepatic metastasis</td>
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<td>Altomare 2007(^{66})</td>
<td>50</td>
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<td>ELISA</td>
<td>TF levels associate with VEGF levels but not to clinicopathology</td>
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<td>Urogenital tract cancers</td>
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<td>Study</td>
<td>Tumor Type</td>
<td>TF Expression</td>
<td>Method</td>
<td>Findings</td>
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<td>Förster 2003</td>
<td>Renal cell carcinoma</td>
<td>Tumoral TF expression lower than surrounding parenchyma</td>
<td>ELISA</td>
<td>In renal cell carcinoma, tumoral TF expression is lower than the surrounding parenchyma.</td>
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<td>29 NA</td>
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<td></td>
<td></td>
<td>18 NA</td>
<td>mRNA</td>
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<tr>
<td>Prostate</td>
<td>Maciel 2009</td>
<td>TF associates with poor relapse-free and overall survival</td>
<td>IHC</td>
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<td></td>
<td></td>
<td>41 38 (88.3)</td>
<td>IHC</td>
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<td>Prostate</td>
<td>Abdulkadir 2000</td>
<td>Tumoral TF associates with VEGF and MVD</td>
<td>IHC</td>
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<td></td>
<td>67 49 (73)</td>
<td>IHC</td>
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<td>Prostate</td>
<td>Akashi 2003</td>
<td>Tumoral TF associates with poor cancer-specific survival</td>
<td>IHC</td>
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<td></td>
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<td>73 55 (75.3)</td>
<td>IHC</td>
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<td>Prostate</td>
<td>Yao 2009</td>
<td>TF associates with VEGF expression</td>
<td>IHC</td>
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<td></td>
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<td>93 43 (47)</td>
<td>IHC</td>
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<td>Prostate</td>
<td>Kaushal 2008</td>
<td>TF expression positively correlates with advanced stage and Gleason score</td>
<td>IHC</td>
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<td>IHC</td>
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<td>Bladder</td>
<td>Patry 2008</td>
<td>TF expression confers a 3.15-fold increased risk for cancer-related death</td>
<td>IHC</td>
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<td></td>
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<td>218 142 (77.6)</td>
<td>IHC</td>
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<td>Melanoma</td>
<td>Kageshita 2001</td>
<td>TF does not associate with clinicopathology</td>
<td>IHC</td>
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<td></td>
<td></td>
<td>86 83 (96.5)</td>
<td>IHC</td>
<td></td>
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<td>Melanoma</td>
<td>Depasquale 2008</td>
<td>TF associates with Breslow thickness</td>
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<td>204 NA &gt;90%</td>
<td>IHC</td>
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<td>Glioma</td>
<td>Hamada 1996</td>
<td>TF associates with higher tumor grades</td>
<td>IHC</td>
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<td></td>
<td></td>
<td>44 44 (100)</td>
<td>IHC</td>
<td></td>
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<tr>
<td>Glioma</td>
<td>Guan 2002</td>
<td>TF associates with higher tumor grades; TF associates with MVD</td>
<td>IHF</td>
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<td></td>
<td></td>
<td>29 19 (65.5)</td>
<td>IHF</td>
<td></td>
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<td>Hematological cancers</td>
<td>Negaard 2009</td>
<td>TF mRNA in PBMNC is not associated with MVD</td>
<td>mRNA</td>
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<tr>
<td></td>
<td></td>
<td>93 NA</td>
<td>mRNA</td>
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</table>

*In this study specimens from kidney, prostate and bladder cancer were combined; NA not applicable.*
Figure Legends

Figure 1. Defined oncogenic transformations drive TF expression in cancer

Defined pathways regulate expression of Tissue Factor (TF) in cancer. 1) Epidermal-to-mesenchymal transformation and TFG-β signaling\textsuperscript{11:15}. TGF-β, transforming growth factor-β; TGFBR1/II, transforming growth factor receptors I and II; SMAD, contraction of “small” and “mothers against decapentaplegic”; 2) Hypoxia-induced signaling\textsuperscript{19}. EGR-1, Early growth response protein-1; 3) EGFR- and PTEN-dependent pathways\textsuperscript{9:11:13}. EGF, Epidermal Growth Factor; EGFR, Epidermal Growth Factor Receptor; PTEN, phosphatase and tensin homolog 4) Src-signaling pathways\textsuperscript{12}. Src, Rous sarcoma oncogene cellular homolog (reference 12), SF/HGF, Scatter Factor/Hepatocyte Growth Factor; the c-MET mutation leads to enhanced expression of HGFR, hepatocyte growth factor receptor; 5) Loss of K-ras and p53\textsuperscript{7:8,13,14}. K-ras, V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; P53, protein 53. PI3, Phosphatidylinositol-3’; MAP, Mitogen-Activated Protein.

Figure 2. TF isoforms exert cellular effects via PAR2 and integrin ligation

The membrane-bound full-length tissue factor (flTF):factor VIIa (FVIIa) complex signals via the G-coupled PAR2\textsuperscript{4,24,25,30,31,43} when coupled to α6β1 or α3β1 integrins\textsuperscript{28,29,30}. The phosphorylation status of the flTF cytoplasmic domain balances protease activated receptor (PAR2) signaling\textsuperscript{27,29,31}. Alternatively spliced tissue factor (asTF) ligates α6β1 and αVβ3 integrins leading to signaling via Focal Adhesion Kinases (FAK), independent on FVIIa and PAR2\textsuperscript{5}. The resulting signaling pathways regulate cell survival and motility, and induce release of angiogenic\textsuperscript{4,5,23,24,25,30}. PI3, Phosphatidylinositol-3’; MAP, Mitogen-Activated Protein; CXCL-1, Chemokine ligand-1; VEGF, Vascular Endothelial Growth Factor.
Table 1. Overview of studies on TF expression in human cancer

Cancer type, source, number of studies tumors, TF as detected by immunohistochemistry, detection methods, and the study’s main findings regarding TF are listed. IHC, immunohistochemistry; IHF, immunohistofluorescence; MVD, microvessel density; TF, Tissue Factor; asTF, Alternatively Spliced Tissue Factor.
The relationship between tissue factor and cancer progression: insights from bench and bedside

Yascha W. van den Berg, Susanne Osanto, Pieter H. Reitsma and Henri H. Versteeg