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

Yascha W. van den Berg,[1] Susanne Osanto,[2] Pieter H. Reitsma,[1] and Henri H. Versteeg[1,2]

Departments of [1]Thrombosis and Hemostasis and [2]Clinical Oncology, Einthoven Laboratory for Experimental Vascular Medicine, Leiden Univerisity Medical Center, Leiden, The Netherlands

It is now widely recognized that a strong correlation exists between cancer and aberrant hemostasis. Patients with various types of cancers, including pancreatic, colorectal, and gastric cancer, often develop thrombosis, a phenomenon commonly referred to as Trousseau 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. On complex formation with its ligand, coagulation factor VIIa, TF influences protease-activated receptor-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 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 summarize our current understanding of the role of TF in tumor growth and metastasis, and speculate on anticancer therapy by targeting TF. (*Blood*. 2012; 119(4):924-932)

Introduction

After Trousseau's description of thrombophlebitis as a complication of pancreatic cancer in the 19th century, the notion that increased expression of tissue factor (TF) underlies the relation between coagulation and cancer has become generally accepted. Full-length TF (flTF) is a 47-kDa membrane-bound glycoprotein that is present on subendothelial cells.[1] In the classic concept of coagulation, it is thought that endothelial disruption leads to exposure of flTF to the bloodstream. Exposed flTF can bind to its natural ligand factor VII (FVII), which then becomes activated FVII (FVIIa). The thus formed flTF/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, 2 essential components of a stable hemostatic plug.[1]

The primary function of subendothelial flTF is to serve as a hemostatic envelope surrounding the vasculature. However, under certain conditions, the expression of flTF is induced in monocytes and endothelial cells. flTF is also often expressed on cancer cells and the tumor vasculature,[2] and flTF-bearing microparticles can become shed by these cells.[3] These flTF-bearing microparticles are important contributors to the thrombotic phenotype in cancer patients.[3]

The flTF/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.[4] Furthermore, a soluble variant of flTF, known as alternatively spliced TF (asTF), stimulates angiogenesis independent of FVIIa.[5,6]

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 (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 MAPK and PI3K signaling pathways, thus leading to enhanced TF expression.[7] In vivo experiments confirmed that the K-*ras* and p53 mutations in CRC are indeed primarily responsible for flTF up-regulation.[7] This is in agreement with the finding that in CRC patients mutations of K-*ras*, p53 are associated with flTF expression in tumors.[8]

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, down-regulates EGFR-dependent flTF expression.[9] Moreover, endometrial cancer cell lines display enhanced flTF levels in an EGF-dependent fashion,[10] and inhibition of EGFR signaling diminishes flTF expression in vulva carcinoma cells constitutively expressing the EGFRvIII mutant.[11]

Recent studies in medulloblastoma cell lines indicate that Src family kinases stimulate an induction of flTF expression through both the scatter factor/hepatocyte growth factor (HGF) and as a result of a mutation in the c-MET oncogene, whereas flTF

Submitted May 31, 2011; accepted November 1, 2011. Prepublished online as *Blood* First Edition paper, November 7, 2011; DOI 10.1182/blood-2011-06-317685.

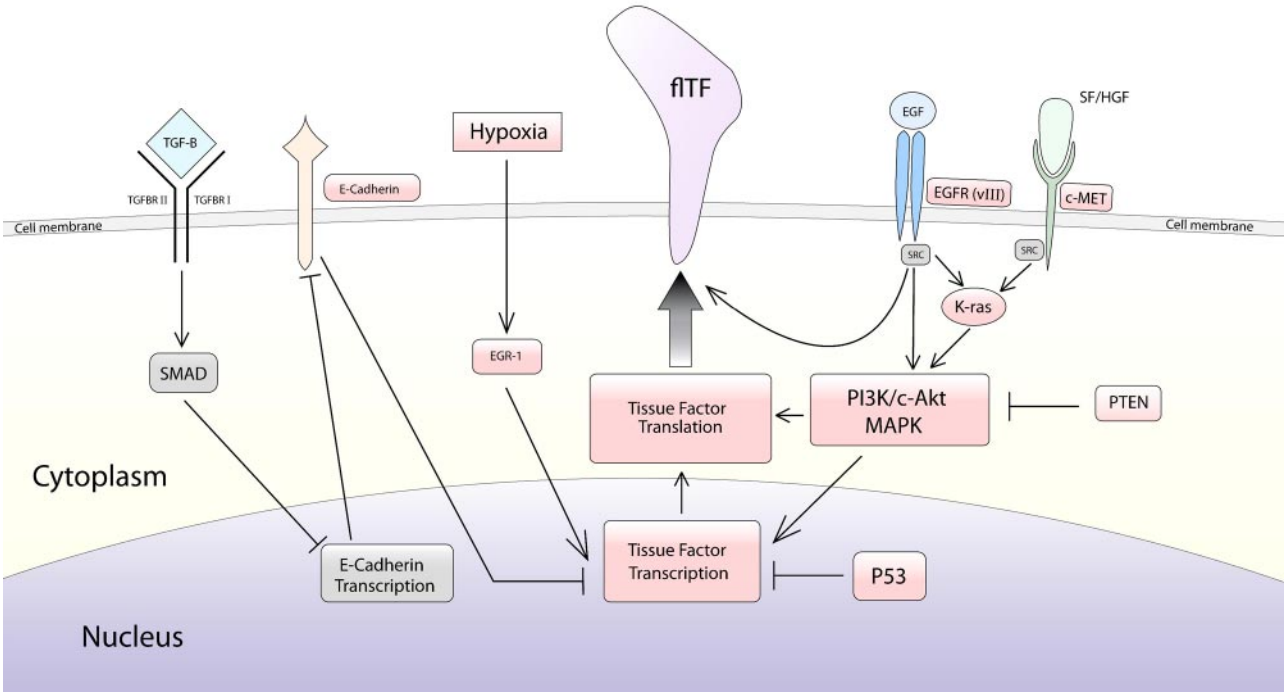

Figure 1. Defined oncogenic transformations drive TF expression in cancer. Defined pathways regulating expression of TF in cancer are as follows: (1) Epidermal-to-mesenchymal transformation and TGF-β signaling.[11,15] TGF-β indicates transforming growth factor-β; TGFBRI/II, transforming growth factor receptors I and II; and SMAD, contraction of "small" and "mothers against decapentaplegic." (2) Hypoxia-induced signaling.[19] EGR-1 indicates early growth response protein-1. (3) EGFR- and PTEN-dependent pathways.[9-11,13] EGF indicates epidermal growth factor; EGFR, epidermal growth factor receptor; and PTEN, phosphatase and tensin homolog. (4) Src-signaling pathways.[12] Src indicates Rous sarcoma oncogene cellular homolog[12]; the c-MET mutation leads to enhanced expression of HGFR (hepatocyte growth factor receptor). (5) Loss of K-ras and p53.[7,8,13,14] K-ras indicates V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; P53, protein 53; PI3, phosphatidylinositol-3′; and MAP, mitogen-activated protein.

expression via the HGF/c-MET axis elicits an antiapoptotic response and provides resistance to chemotherapeutical agents.[12]

Some of the in vitro findings described earlier in the Introduction 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 up-regulates flTF expression on tumor cells.[13,14]

In vivo experiments in a murine xenograft model with human vulva 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.[11]

TGF-β is an essential cytokine for EMT to occur and is coexpressed with flTF in tumor cells and tumor stromal cells,[15] suggesting the production of TGF-β production as a critical upstream event in up-regulation 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 have functional properties distinct from other cancer cell populations (eg, cancer stem cells may transdifferentiate to vascular cells).[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.[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.[18] Hypoxia-driven flTF expression is not dependent on hypoxia-activated factor-1α but rather on the early growth response gene-1.[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, which are required for a cell to adequately respond to its local environment[4] (Figure 2). Despite the structural homology between flTF and interferon receptors,[20] flTF/FVIIa signaling differs substantially from classic 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 4-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,

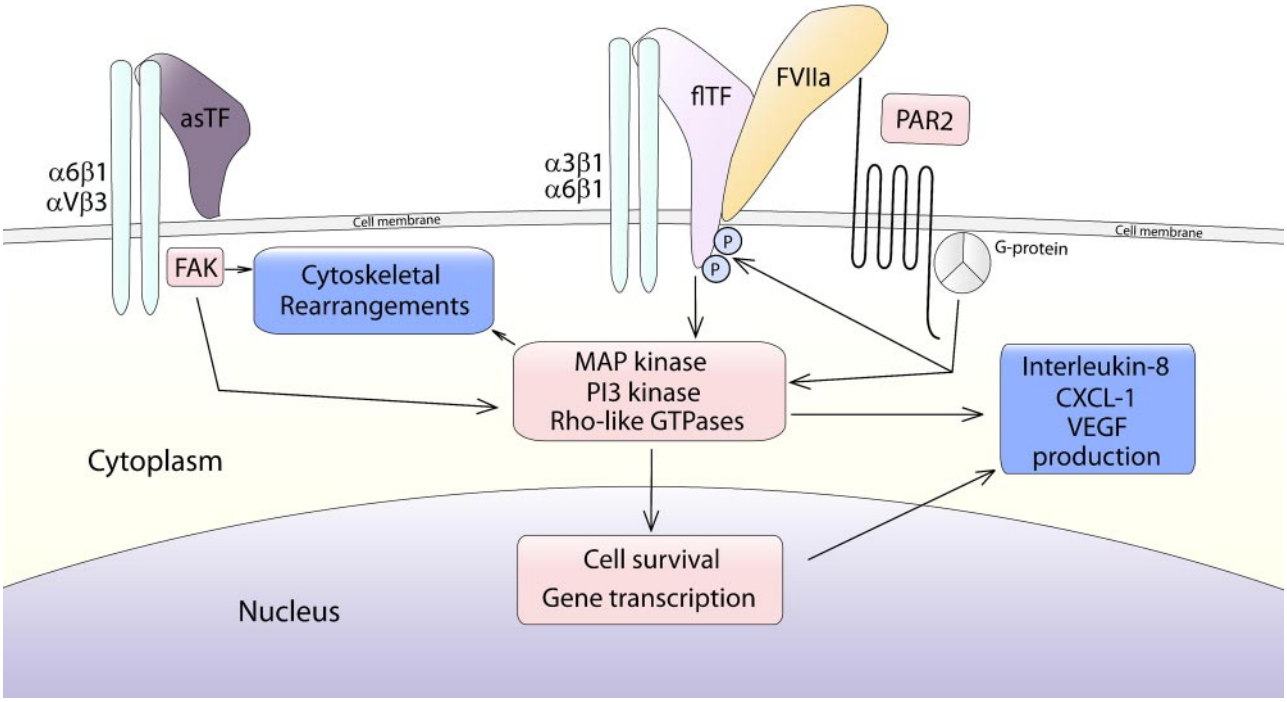

Figure 2. TF isoforms exert cellular effects via PAR2 and integrin ligation. The membrane-bound flTF/FVIIa complex signals via the G-coupled PAR2[4,24,25,30,31,43] when coupled to α6β1 or α3β1 integrins.[28-30] The phosphorylation status of the flTF cytoplasmic domain balances protease activated receptor (PAR2) signaling.[27,29,31] asTF ligates α6β1 and αVβ3 integrins, leading to signaling via focal adhesion kinases (FAK), independent of FVIIa and PAR2.[5] The resulting signaling pathways regulate cell survival and motility and induce release of angiogenic factors.[4,5,23,24,25,30] PI3 indicates phosphatidylinositol-3′; MAP, mitogen-activated protein; CXCL-1, chemokine ligand-1; and VEGF, vascular endothelial growth factor.

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.[4] In mouse models, PAR3 has been found to serve as a cofactor for PAR4,[21] but recent data suggest that human PAR3 may also be activated directly by thrombin.[22] On activation, PARs couple to heterotrimeric G-proteins after which further signaling events are initiated.[4]

Signaling of flTF/FVIIa via PAR2 elicits calcium transients and activation of the major members of the MAPK family, p44/42, p38, and 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.[4] Activation of both the MAPK and PI3 kinase pathways contributes to a pro-malignant transcriptional program and stimulates oncogenic protein synthesis.[4] flTF/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 immunologic modulators, such as GM-CSF (or CSF2) and M-CSF (or CSF1).[23-25] Although PAR1 signaling induces a similar series of proteins in breast cancer cells, the activation of the flTF/VIIa/PAR2 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 flTF/FVIIa complex may directly signal via the flTF 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 flTF/FVIIa is tightly regulated through posttranslational modification and protein interactions. Part of the early response in PAR2 signaling involves protein kinase C-α-mediated phosphorylation of Ser 253 in the flTF cytoplasmic domain, followed by proline-directed kinase-dependent phosphorylation of Ser 258. Genetic deletion of the cytoplasmic domain in mice results in a pro-angiogenic phenotype,[27] whereas 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 flTF cytoplasmic domain, may regulate flTF activity by routing flTF to membrane compartments in which flTF signaling function is minimal. Indeed, palmitoylation of Cys 245 results in the enhanced localization of flTF into sphingolipid rafts of the cell membrane, which leads to impaired PAR2 signaling.[29]

Efficient PAR2 signaling and Ser 253 phosphorylation of flTF depend on binding of flTF to β1-integrins.[28] flTF/β1-integrin complex formation stimulates flTF-dependent PAR2 activation and facilitates breast cancer development by contributing to both tumor angiogenesis and growth.[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.[32] Ectopic production of FVII is regulated by epigenetic and hypoxia-driven processes in several solid tumor cell lines,[33,34] whereas EGFR signaling in gliomas not only up-regulates TF expression, but also expression of FVII and PAR2,[35] thus orchestrating the generation of a multitude of events that contribute to flTF/VIIa/PAR2 signaling.

TF isoforms also elicit nonhemostatic cellular effects independent of PAR2 activation. A naturally occurring, soluble form of

TF has been characterized, which results from alternative splicing; whereas a 6-exon transcript encodes membrane-bound flTF, asTF mRNA is formed when exon 5 is skipped. This causes a shift in the reading frame; consequently, asTF contains a unique C-terminus and lacks a transmembrane region, rendering the protein soluble.[36,37] Since its discovery, the role of asTF in coagulation has been a matter of debate.[38] Increasing evidence supports a role for asTF in cancerous processes.[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 $\alpha 6\beta 1$ and $\alpha V\beta 3$ 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 PI3K, MAPK, and Akt,[5] although the relative contributions of these pathways to asTF-dependent angiogenesis are poorly understood.

In summary, 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 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. Knockdown 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] whereas pharmacologic 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 β1-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 promoter driven polyoma middle T antigen cassette (PyMT, leading to spontaneous breast tumors), attenuates tumor growth because of a delay in the angiogenic switch.[43] Similarly, genetic deletion of the cytoplasmic tail (ΔCT) of flTF inhibits VEGF production and tumor growth in a xenograft model[44] and angiogenesis and tumor growth in the PyMT model, whereas combination of PAR2 deficiency and cytoplasmic tail deletion does not further decrease tumor growth.[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.[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 use 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.[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.[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.[47] In studies that use cells expressing flTF mutants with diminished flTF/FVIIa 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.[48,49] 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.[30] Although thrombin is also reported to promote primary tumor growth through PAR1 activation, increasing evidence supports that its role in metastasis is more potent.[50] 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.[51] Further evidence for a prominent role of downstream coagulation activation in metastasis comes from experiments in genetically modified mice that 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.[52] 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 compared with asTF-negative cells on xenografting.[6] asTF is thought to augment angiogenesis by acting as an integrin-activating agent,[5] 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.

Table 1. Overview of studies on TF expression in human cancer

Type of cancer	Reference	No. of tumors	TF expression by IHC, no. (%)	Method	Main findings with respect to TF expression
Breast	53	115	93 (80.8)	IHC	TF expression is associated with well-differentiated epithelia and less lymph node metastases
	15	40	40 (100)	IHC	Increased TF intensity is found in infiltrative ductal carcinoma
	54	213	193 (90.6)	IHC	TF expression is associated with TF plasma levels and overall survival
	55	157	61 (31)	IHC	Phosphorylated TF is associated with diminished survival
Lung	56	55	46 (84)	IHC	TF expression is associated with metastasis
	40	21	NA	mRNA	TF isoforms are up-regulated in cancerous tissue, flTF, and asTF mRNA levels are associated with advanced stage; low asTF mRNA levels are associated with early stage
	12	8 (66.7)	IHC		
	11	NA	ELISA		
	13,14	64	NA	mRNA	TF expression is associated with staging, VEGF, and MVD; high TF mRNA levels are associated with poor survival
	64	47 (73.5)	IHC		
	30	NA	ELISA		
	57	39	22 (56)	IHC	TF expression is associated with staging, but not with survival
	80	57	NA	mRNA	flTF and asTF mRNA levels are associated with poor survival
Gastrointestinal					
Esophagus	58	36	NA	mRNA	flTF, but not asTF, mRNA levels are up-regulated in tumor tissue
	59	103	94 (91.3)	IHC	TF expression is associated with MVD, metastasis, and poor survival
Stomach	60	207	52 (25.1)	IHC	Intestinal-type cancer displayed enhanced TF expression and was associated with MVD, metastasis, and poor overall survival
Liver	61	58	58 (100)	IHC	TF is associated with MVD, metastasis, and poor overall survival
Pancreas	62	55	29 (52.7)	IHC	TF is associated with histologic grade and staging
	63	113	100 (88.5)	IHC	TF is associated with staging, metastasis, and overall survival
	64	240	211 (87.9)	IHC	TF is associated with MVD and thrombosis rate
Colorectum	65	79	46 (57)	IHC	TF is associated with staging and metastasis
	66	100	57 (57)	IHC	TF is associated with staging and MVD
	67	67	31 (46)	IHC	TF is associated with hepatic metastasis
	68	50	NA	ELISA	TF levels are associated with VEGF levels but not with clinicopathology
Urogenital tract					
	69*	29	NA	ELISA	In renal cell carcinoma, tumoral TF expression is lower than the surrounding parenchyma
	18	NA	mRNA		
Kidney	70	41	38 (88.3)	IHC	TF is associated with poor relapse-free and overall survival
Prostate	71	67	49 (73)	IHC	Tumoral TF is associated with VEGF and MVD
	72	73	55 (75.3)	IHC	Tumoral TF is associated with poor cancer-specific survival
	73	93	43 (47)	IHC	TF is associated with VEGF expression
	74	54	38 (70.4)	IHC	TF expression positively correlates with advanced stage and Gleason score
Bladder	75	218	142 (77.6)	IHC	TF expression confers a 3.15-fold increased risk for cancer-related death
Melanoma	77	86	83 (96.5)	IHC	TF does not associate with clinicopathology
	76	204	NA> 90%	IHC	TF is associated with Breslow thickness
Glioma	78	44	44 (100)	IHC	TF is associated with higher tumor grades
	79	29	19 (65.5)	IHF	TF is associated with higher tumor grades; TF is associated with MVD
Hematologic	85	93	NA	mRNA	TF mRNA in PBMC is not associated with MVD

TF indicates tissue factor; IHC, immunohistochemistry; NA, not applicable; MVD, microvessel density; and IHF, immunohistofluorescence.
*In this study, specimens from kidney, prostate, and bladder cancer were combined.

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 discuss whether the concepts described in the previous three sections, find support in studies that are primarily aimed at finding correlation between expression of TF isoforms and pathologic and clinical parameters. We will not discuss observational studies that examine flTF expression in human cancer without describing associations with clinical and pathologic parameters because of 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,[15,53-55] lung cancer,[13,14,40,56,57] gastrointestinal cancers,[8,58-68] urogenital cancers,[69-75] melanomas,[76,77] and gliomas.[78,79] Studies of the upstream oncogenic events that lead to enhanced flTF expression have been conducted in colorectal[8] and lung cancer,[13,14] 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 support the notion that flTF expression is an independent predictor of poor overall or relapse-free survival,[13,54,55,59-61,63,70,72,75,80] although some studies failed to find such a relation.[57,76,77] Furthermore, associations have been found with invasiveness in breast cancer[15] and melanomas,[15,76] high clinical staging in lung,[13,14,40,57] pancreas,[62,63] colorectal,[65,66] and prostate cancer,[74] and metastases in cancers of breast,[53] lung,[56] esophagus,[59] gastric,[60] hepatic,[61] pancreatic,[63] and colorectal[67] tissues. Other studies, however, could not find such associations between flTF expression and unfavorable pathologic and clinical parameters[54,55,68,76,77,81]; this may partially be because of differences in patient populations, population size, and detection techniques for flTF.

The flTF/VIIa/PAR2 axis is supposed to drive angiogenic events through enhanced production of angiogenic factors, such as VEGF. Associations have indeed been found between flTF expression and microvessel density in lung cancer,[14] throughout all gastrointestinal cancers,[59-61,64,66] prostate cancer,[71] and gliomas.[79] Associations between flTF and VEGF expression are described in breast and lung cancer,[14,55] colorectal cancer,[68] and prostate cancer,[71,73] which further strengthens the concept that TF expression promotes tumor angiogenesis. Furthermore, an antibody that only detects the cytoplasmic domain of flTF when phosphorylated (pTF), and therefore only when involved in PAR2 signaling, was used to investigate whether the effects of flTF in cancer could be attributed to direct signaling effects of the flTF/FVIIa/PAR2 axis. Indeed, expression of pTF strongly correlated with VEGF expression and survival in patients with tumors that were positive for pTF was diminished.[55]

To date, the expression of asTF in relation to clinicopathologic characteristics has only been studied in non-small cell lung cancer, and these studies reveal a correlation between high asTF mRNA levels and advanced tumor stage, whereas low levels of flTF mRNA relate to less advanced stages of cancer progression.[40] In another study, high asTF mRNA levels conferred an impaired survival to non-small cell lung cancer patients, but the relation with staging and tumor size could not be confirmed.[80]

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

In summary, most human epithelial cancers are characterized by abundant levels of flTF. In keeping with the observations from experimental studies, flTF is likely to drive tumor angiogenesis, to enhance tumor growth, and to influence metastasis. Because 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. Because 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 flTF 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 are available concerning the role of asTF in human malignancies because, at present, no studies have been performed on a 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, pharmacologic, 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 antineoplastic effect of vitamin K antagonists.[86,87] However, because of 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/PAR2signaling with the flTF antibody 10H10 may have therapeutic potential while leaving the coagulant properties of flTF unaffected.[30] 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.[7] Indeed, pharmacologic modalities are available for tumor-specific delivery of RNAi,[88] but again, the response to these antitumorigenic 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[89]; however, the company suspended the trial, so that it is unclear whether the inhibition of tumor growth found in mice[42] can be translated to humans. Currently, 2 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.[90] PCI-27483 at present is tested in a similar setup, but this study is limited to pancreatic cancer patients.[91] 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 a 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 because it leaves the coagulant properties of flTF unaffected, but whether abrogating flTF signaling may affect other physiologic 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 to 6 boundary offer opportunities for a specific blockade; however, the effect of interfering with asTF in cancers is still speculative.

Delivery of antitumor 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] probably through mediating an NK cell–dependent cytotoxic antitumor 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 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 $\alpha V\beta 3$ integrins and CD13, resulted in infarction of tumor vessels in mice, and in patients tumor perfusion decreased, whereas the compound was tolerated.[98,99]

Use of flTF-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 flTF. Phototherapy is perhaps most promising in circumventing such unwanted effects because it only exerts its effects by controlled exposure to laser light, which may be highly specific thanks to improving tumor imaging modalities.

In conclusion, during the last decades, it has become increasingly clear that flTF not only has a prominent role in the etiology of cancer-related thrombosis, but also that TF isoforms display nonhemostatic properties that are important in cancer progression. The oncogenic transformations leading to flTF expression on tumor cells are now well defined, and flTF 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 flTF in the progression of cancer is underscored by its abundant expression in human cancers from different origins. Furthermore, flTF has gained attention as a potential therapeutic target by harboring tumoricidal drugs to flTF-expressing cancer cells or via direct inhibition of its cellular effects. Despite this progress, questions remain, especially regarding the relative contribution of flTF and asTF to cancer progression.

Acknowledgments

The authors thank Chun Yu Wong for preparing the figures and Frits R. Rosendaal and Eric P. van der Veer for critically reading the manuscript.

Y.W.v.d.B. was supported by a Sanofi-Aventis/ISTH fellowship. H.H.V. was supported by The Netherlands Organization for Scientific Research (grant 17.106.329).

Authorship

Contribution: 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; and H.H.V. contributed to the design of the manuscript and wrote parts of the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Henri H. Versteeg, Department of Thrombosis and Hemostasis; Einthoven Laboratory for Experimental Vascular Medicine, C2-R, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, The Netherlands; e-mail: h.h.versteeg@lumc.nl.

References

1. Versteeg HH, Peppelenbosch MP, Spek CA. The pleiotropic effects of tissue factor: a possible role for factor VIIa-induced intracellular signalling? *Thromb Haemost.* 2001;86(6):1353-1359.
2. Contrino J, Hair G, Kreutzer DL, Rickles FR. In situ detection of tissue factor in vascular endothelial cells: correlation with the malignant phenotype of human breast disease. *Nat Med.* 1996;2(2):209-215.
3. Rak J. Microparticles in cancer. *Semin Thromb Hemost.* 2010;36(8):888-906.
4. Versteeg HH, Ruf W. Emerging insights in tissue factor-dependent signaling events. *Semin Thromb Hemost.* 2006;32(1):24-32.
5. van den Berg YW, van den Hengel LG, Myers HR, et al. Alternatively spliced tissue factor induces angiogenesis through integrin ligation. *Proc Natl Acad Sci U S A.* 2009;106(46):19497-19502.
6. Hobbs JE, Zakarija A, Cundiff DL, et al. Alternatively spliced human tissue factor promotes tumor growth and angiogenesis in a pancreatic cancer tumor model. *Thromb Res.* 2007;120(suppl 2):S13-S21.
7. Yu JL, May L, Lhotak V, et al. Oncogenic events regulate tissue factor expression in colorectal cancer cells: implications for tumor progression and angiogenesis. *Blood.* 2005;105(4):1734-1741.
8. Rao B, Gao Y, Huang J, et al. Mutations of p53 and K-ras correlate TF expression in human colorectal carcinomas: TF downregulation as a marker of poor prognosis. *Int J Colorectal Dis.* 2011;26(5):593-601.
9. Rong Y, Belozerov VE, Tucker-Burden C, et al. Epidermal growth factor receptor and PTEN

modulate tissue factor expression in glioblastoma through JunD/activator protein-1 transcriptional activity. *Cancer Res.* 2009;69(6):2540-2549.

10. Kato S, Pinto M, Carvajal A, et al. Tissue factor is regulated by epidermal growth factor in normal and malignant human endometrial epithelial cells. *Thromb Haemost.* 2005;94(2):444-453.
11. Milsom CC, Yu JL, Mackman N, et al. Tissue factor regulation by epidermal growth factor receptor and epithelial-to-mesenchymal transitions: effect on tumor initiation and angiogenesis. *Cancer Res.* 2008;68(24):10068-10076.
12. Provencal M, Berger-Thibault N, Labbe D, et al. Tissue factor mediates the HGF/Met-induced antiapoptotic pathway in DAOY medulloblastoma cells. *J Neurooncol.* 2010;97(3):365-372.
13. Regina S, Valentin JB, Lachot S, et al. Increased tissue factor expression is associated with reduced survival in non-small cell lung cancer and with mutations of TP53 and PTEN. *Clin Chem.* 2009;55(10):1834-1842.
14. Regina S, Rollin J, Blechet C, et al. Tissue factor expression in non-small cell lung cancer: relationship with vascular endothelial growth factor expression, microvascular density, and K-ras mutation. *J Thorac Oncol.* 2008;3(7):689-697.
15. Vrana JA, Stang MT, Grande JP, Getz MJ. Expression of tissue factor in tumor stroma correlates with progression to invasive human breast cancer: paracrine regulation by carcinoma cell-derived members of the transforming growth factor beta family. *Cancer Res.* 1996;56(21):5063-5070.
16. Wang R, Chadalavada K, Wilshire J, et al. Glioblastoma stem-like cells give rise to tumour endothelium. *Nature.* 2010;468(7325):829-833.
17. Milsom C, Anderson GM, Weitz JI, Rak J. Elevated tissue factor procoagulant activity in CD133-positive cancer cells. *J Thromb Haemost.* 2007;5(12):2550-2552.
18. Rong Y, Post DE, Pieper RO, et al. PTEN and hypoxia regulate tissue factor expression and plasma coagulation by glioblastoma. *Cancer Res.* 2005;65(4):1406-1413.
19. Rong Y, Hu F, Huang R, et al. Early growth response gene-1 regulates hypoxia-induced expression of tissue factor in glioblastoma multiforme through hypoxia-inducible factor-1-independent mechanisms. *Cancer Res.* 2006;66(14):7067-7074.
20. Bazan JF. Structural design and molecular evolution of a cytokine receptor superfamily. *Proc Natl Acad Sci U S A.* 1990;87(18):6934-6938.
21. Nakanishi-Matsui M, Zheng YW, Sulciner DJ, et al. PAR3 is a cofactor for PAR4 activation by thrombin. *Nature.* 2000;404(6778):609-613.
22. Ostrowska E, Reiser G. The protease-activated receptor-3 (PAR-3) can signal autonomously to induce interleukin-8 release. *Cell Mol Life Sci.* 2008;65(6):970-981.
23. Liu Y, Mueller BM. Protease-activated receptor-2 regulates vascular endothelial growth factor expression in MDA-MB-231 cells via MAPK pathways. *Biochem Biophys Res Commun.* 2006;344(4):1263-1270.
24. Hjortoe GM, Petersen LC, Albrektsen T, et al. Tissue factor-factor VIIa-specific up-regulation of IL-8 expression in MDA-MB-231 cells is mediated by PAR-2 and results in increased cell migration. *Blood.* 2004;103(8):3029-3037.
25. Albrektsen T, Sorensen BB, Hjorto GM, et al. Transcriptional program induced by factor VIIa-tissue factor, PAR1 and PAR2 in MDA-MB-231 cells. *J Thromb Haemost.* 2007;5(8):1588-1597.
26. Ott I, Weigand B, Michl R, et al. Tissue factor cytoplasmic domain stimulates migration by activation of the GTPase Rac1 and the mitogen-activated protein kinase p38. *Circulation.* 2005;111(3):349-355.
27. Belting M, Dorrell MI, Sandgren S, et al. Regulation of angiogenesis by tissue factor cytoplasmic domain signaling. *Nat Med.* 2004;10(5):502-509.
28. Dorfleutner A, Hintermann E, Tarui T, Takada Y, Ruf W. Cross-talk of integrin alpha3beta1 and tissue factor in cell migration. *Mol Biol Cell.* 2004;15(10):4416-4425.
29. Dorfleutner A, Ruf W. Regulation of tissue factor cytoplasmic domain phosphorylation by palmitoylation. *Blood.* 2003;102(12):3998-4005.
30. Versteeg HH, Schaffner F, Kerver M, et al. Inhibition of tissue factor signaling suppresses tumor growth. *Blood.* 2008;111(1):190-199.
31. Schaffner F, Versteeg HH, Schillert A, et al. Cooperation of tissue factor cytoplasmic domain and PAR2 signaling in breast cancer development. *Blood.* 2010;116(26):6106-6113.
32. Koizume S, Jin MS, Miyagi E, et al. Activation of cancer cell migration and invasion by ectopic synthesis of coagulation factor VII. *Cancer Res.* 2006;66(19):9453-9460.
33. Koizume S, Yokota N, Miyagi E, et al. Hepatocyte nuclear factor-4-independent synthesis of coagulation factor VII in breast cancer cells and its inhibition by targeting selective histone acetyltransferases. *Mol Cancer Res.* 2009;7(12):1928-1936.
34. Yokota N, Koizume S, Miyagi E, et al. Self-production of tissue factor-coagulation factor VII complex by ovarian cancer cells. *Br J Cancer.* 2009;101(12):2023-2029.
35. Magnus N, Garnier D, Rak J. Oncogenic epidermal growth factor receptor up-regulates multiple elements of the tissue factor signaling pathway in human glioma cells. *Blood.* 2010;116(5):815-818.
36. Guo W, Wang H, Zhao W, et al. Effect of all-trans retinoic acid and arsenic trioxide on tissue factor expression in acute promyelocytic leukemia cells. *Chin Med J.* 2001;114(1):30-34.
37. Bogdanov VY, Balasubramanian V, Hathcock J, et al. Alternatively spliced human tissue factor: a circulating, soluble, thrombogenic protein. *Nat Med.* 2003;9(4):458-462.
38. van den Berg YW, Versteeg HH. Alternatively spliced tissue factor: a crippled protein in coagulation or a key player in non-haemostatic processes? *Hämostaseologie.* 2010;30(3):144-149.
39. Rollin J, Regina S, Gruel Y. Tumour expression of alternatively spliced tissue factor is a prognostic marker in non-small cell lung cancer. *J Thromb Haemost.* 2010;8(3):607-610.
40. Goldin-Lang P, Tran QV, Fichtner I, et al. Tissue factor expression pattern in human non-small cell lung cancer tissues indicate increased blood thrombogenicity and tumor metastasis. *Oncol Rep.* 2008;20(1):123-128.
41. Zhang Y, Deng Y, Luther T, et al. Tissue factor controls the balance of angiogenic and antiangiogenic properties of tumor cells in mice. *J Clin Invest.* 1994;94(3):1320-1327.
42. Hembrough TA, Swartz GM, Papathanassiu A, et al. Tissue factor/factor VIIa inhibitors block angiogenesis and tumor growth through a nonhemostatic mechanism. *Cancer Res.* 2003;63(11):2997-3000.
43. Versteeg HH, Schaffner F, Kerver M, et al. Protease-activated receptor (PAR) 2, but not PAR1, signaling promotes the development of mammary adenocarcinoma in polyoma middle T mice. *Cancer Res.* 2008;68(17):7219-7227.
44. Abe K, Shoji M, Chen J, et al. Regulation of vascular endothelial growth factor production and angiogenesis by the cytoplasmic tail of tissue factor. *Proc Natl Acad Sci U S A.* 1999;96(15):8663-8668.
45. Yu J, May L, Milsom C, et al. Contribution of host-derived tissue factor to tumor neovascularization. *Arterioscler Thromb Vasc Biol.* 2008;28(11):1975-1981.
46. Palumbo JS, Talmage KE, Massari JV, et al. Tumor cell-associated tissue factor and circulating hemostatic factors cooperate to increase metastatic potential through natural killer cell-dependent and-independent mechanisms. *Blood.* 2007;110(1):133-141.
47. Mueller BM, Reisfeld RA, Edgington TS, Ruf W. Expression of tissue factor by melanoma cells promotes efficient hematogenous metastasis. *Proc Natl Acad Sci U S A.* 1992;89(24):11832-11836.
48. Bromberg ME, Konigsberg WH, Madison JF, Pawashe A, Garen A. Tissue factor promotes melanoma metastasis by a pathway independent of blood coagulation. *Proc Natl Acad Sci U S A.* 1995;92(18):8205-8209.
49. Mueller BM, Ruf W. Requirement for binding of catalytically active factor VIIa in tissue factor-dependent experimental metastasis. *J Clin Invest.* 1998;101(7):1372-1378.
50. Ruf W, Mueller BM. Thrombin generation and the pathogenesis of cancer. *Semin Thromb Hemost.* 2006;32(suppl 1):61-68.
51. Horowitz NA, Blevins EA, Miller WM, et al. Thrombomodulin is a determinant of metastasis through a mechanism linked to the thrombin binding domain but not the lectin-like domain. *Blood.* 2011;118(10):2889-2895.
52. Camerer E, Qazi AA, Duong DN, et al. Platelets, protease-activated receptors, and fibrinogen in hematogenous metastasis. *Blood.* 2004;104(2):397-401.
53. Sturm U, Luther T, Albrecht S, et al. Immunohistological detection of tissue factor in normal and abnormal human mammary glands using monoclonal antibodies. *Virchows Arch A Pathol Anat Histopathol.* 1992;421(2):79-86.
54. Ueno T, Toi M, Koike M, Nakamura S, Tominaga T. Tissue factor expression in breast cancer tissues: its correlation with prognosis and plasma concentration. *Br J Cancer.* 2000;83(2):164-170.
55. Ryden L, Grabau D, Schaffner F, et al. Evidence for tissue factor phosphorylation and its correlation with protease-activated receptor expression and the prognosis of primary breast cancer. *Int J Cancer.* 2010;126(10):2330-2340.
56. Sawada M, Miyake S, Ohdama S, et al. Expression of tissue factor in non-small-cell lung cancers and its relationship to metastasis. *Br J Cancer.* 1999;79(3):472-477.
57. De Meis E, Azambuja D, Ayres-Silva JP, et al. Increased expression of tissue factor and protease-activated receptor-1 does not correlate with thrombosis in human lung adenocarcinoma. *Braz J Med Biol Res.* 2010;43(4):403-408.
58. Ribeiro FS, Simao TA, Amoedo ND, et al. Evidence for increased expression of tissue factor and protease-activated receptor-1 in human esophageal cancer. *Oncol Rep.* 2009;21(6):1599-1604.
59. Chen L, Luo G, Tan Y, et al. Immunolocalisation of tissue factor in esophageal cancer is correlated with intratumoral angiogenesis and prognosis of the patient. *Acta Histochem.* 2010;112(3):233-239.
60. Yamashita H, Kitayama J, Ishikawa M, Nagawa H. Tissue factor expression is a clinical indicator of lymphatic metastasis and poor prognosis in gastric cancer with intestinal phenotype. *J Surg Oncol.* 2007;95(4):324-331.
61. Poon RT, Lau CP, Ho JW, et al. Tissue factor expression correlates with tumor angiogenesis and invasiveness in human hepatocellular carcinoma. *Clin Cancer Res.* 2003;9(14):5339-5345.
62. Kakkar AK, Lemoine NR, Scully MF, Tebbutt S, Williamson RC. Tissue factor expression correlates with histological grade in human pancreatic cancer. *Br J Surg.* 1995;82(8):1101-1104.
63. Nitori N, Ino Y, Nakanishi Y, et al. Prognostic significance of tissue factor in pancreatic ductal adenocarcinoma. *Clin Cancer Res.* 2005;11(7):2531-2539.
64. Khorana AA, Ahrendt SA, Ryan CK, et al. Tissue

factor expression, angiogenesis, and thrombosis in pancreatic cancer. *Clin Cancer Res.* 2007; 13(10):2870-2875.

65. Shigemori C, Wada H, Matsumoto K, et al. Tissue factor expression and metastatic potential of colorectal cancer. *Thromb Haemost.* 1998;80(6):894-898.
66. Nakasaki T, Wada H, Shigemori C, et al. Expression of tissue factor and vascular endothelial growth factor is associated with angiogenesis in colorectal cancer. *Am J Hematol.* 2002;69(4): 247-254.
67. Seto S, Onodera H, Kaido T, et al. Tissue factor expression in human colorectal carcinoma: correlation with hepatic metastasis and impact on prognosis. *Cancer*. 2000;88(2):295-301.
68. Altomare DF, Rotelli MT, Pentimone A, et al. Tissue factor and vascular endothelial growth factor expression in colorectal cancer: relation with cancer recurrence. *Colorectal Dis*. 2007;9(2):133-138.
69. Forster Y, Meye A, Albrecht S, et al. Tissue specific expression and serum levels of human tissue factor in patients with urological cancer. *Cancer Lett.* 2003;193(1):65-73.
70. Maciel EO, Carvalhal GF, da Silva VD, Batista EL Jr, Garicochea B. Increased tissue factor expression and poor nephroblastoma prognosis. *J Urol.* 2009;182(4):1594-1599.
71. Abdulkadir SA, Carvalhal GF, Kaleem Z, et al. Tissue factor expression and angiogenesis in human prostate carcinoma. *Hum Pathol.* 2000; 31(4):443-447.
72. Akashi T, Furuya Y, Ohta S, Fuse H. Tissue factor expression and prognosis in patients with metastatic prostate cancer. *Urology*. 2003;62(6):1078-1082.
73. Yao JL, Ryan CK, Francis CW, et al. Tissue factor and VEGF expression in prostate carcinoma: a tissue microarray study. *Cancer Invest*. 2009; 27(4):430-434.
74. Kaushal V, Mukunyadzi P, Siegel ER, et al. Expression of tissue factor in prostate cancer correlates with malignant phenotype. *Appl Immunohistochem Mol Morphol*. 2008;16(1):1-6.
75. Patry G, Hovington H, Larue H, et al. Tissue factor expression correlates with disease-specific survival in patients with node-negative muscle-invasive bladder cancer. *Int J Cancer*. 2008; 122(7):1592-1597.
76. Depasquale I, Thompson WD. Prognosis in human melanoma: PAR-1 expression is superior to other coagulation components and VEGF. *Histopathology*. 2008;52(4):500-509.
77. Kageshita T, Funasaka Y, Ichihashi M, et al. Tissue factor expression and serum level in patients with melanoma does not correlate with disease progression. *Pigment Cell Res.* 2001;14(3):195-200.
78. Hamada K, Kuratsu J, Saitoh Y, et al. Expression of tissue factor correlates with grade of malignancy in human glioma. *Cancer*. 1996;77(9): 1877-1883.
79. Guan M, Jin J, Su B, Liu WW, Lu Y. Tissue factor expression and angiogenesis in human glioma. *Clin Biochem.* 2002;35(4):321-325.
80. Rollin J, Regina S, Gruel Y. Tumor expression of alternatively spliced tissue factor is a prognostic marker in non-small cell lung cancer. *J Thromb Haemost*. 2010;8(3):607-610.
81. Kageshita T, Funasaka Y, Ichihashi M, et al. Differential expression of tissue factor and tissue factor pathway inhibitor in metastatic melanoma lesions. *Pigment Cell Res.* 2002;15(3):212-216.
82. Bledsoe JG, Slack SM. Tissue factor expression by rat osteosarcoma cells adherent to tissue culture polystyrene and selected orthopedic biomaterials. *J Biomater Sci Polym Ed.* 1998;9(12): 1305-1312.
83. Blom JW, Doggen CJ, Osanto S, Rosendaal FR. Malignancies, prothrombotic mutations, and the risk of venous thrombosis. *JAMA*. 2005;293(6): 715-722.
84. Negaard HF, Iversen PO, Ostenstad B, et al. Hypercoagulability in patients with haematological neoplasia: no apparent initiation by tissue factor. *Thromb Haemost.* 2008;99(6):1040-1048.
85. Negaard HF, Iversen N, Bowitz-Lothe IM, et al. Increased bone marrow microvascular density in haematological malignancies is associated with differential regulation of angiogenic factors. *Leukemia*. 2009;23(1):162-169.
86. Schulman S, Lindmarker P. Incidence of cancer after prophylaxis with warfarin against recurrent venous thromboembolism: duration of anticoagulation trial. *N Engl J Med.* 2000;342(26):1953-1958.
87. Pengo V, Noventa F, Denas G, et al. Long-term use of vitamin K antagonists and incidence of cancer: a population-based study. *Blood.* 2011; 117(5):1707-1709.
88. Stevenson M. Therapeutic potential of RNA interference. *N Engl J Med.* 2004;351(17):1772-1777.
89. Love TE. Safety study of recombinant NAPc2 to prevent tumor progression and metastases in metastatic colon cancer (www.clinicaltrials.gov; Bethesda, MD: National Library of Medicine). http://clinicaltrials.gov/ct2/show/study/NCT00443573?term=NAPC2&rank=1. Accessed September 14, 2011.
90. Wong HC. A study of ALT-836 in combination with gemcitabine for locally advanced or metastatic solid tumors (www.clinicaltrials.gov; Bethesda, MD: National Library of Medicine). http://clinicaltrials.gov/ct2/show/study/NCT01325558. Accessed September 14, 2011.
91. Hedrick E. Study of safety and tolerability of PCI-27483 in patients with pancreatic cancer patients receiving treatment with gemcitabine (www.clinicaltrials.gov; Bethesda, MD: National Library of Medicine). http://clinicaltrials.gov/ct2/show/study/NCT01020006?term=pharmacyclics&rank=1. Accessed September 14, 2011.
92. Hu Z, Garen A. Targeting tissue factor on tumor vascular endothelial cells and tumor cells for immunotherapy in mouse models of prostatic cancer. *Proc Natl Acad Sci U S A*. 2001;98(21): 12180-12185.
93. Cocco E, Hu Z, Richter CE, et al. hI-con1, a factor VII-IgGFc chimeric protein targeting tissue factor for immunotherapy of uterine serous papillary carcinoma. *Br J Cancer*. 2010;103(6):812-819.
94. Hu Z, Li J. Natural killer cells are crucial for the efficacy of Icon (factor VII/human IgG1 Fc) immunotherapy in human tongue cancer. *BMC Immunol*. 2010;11:49.
95. Hu Z, Rao B, Chen S, Duanmu J. Targeting tissue factor on tumour cells and angiogenic vascular endothelial cells by factor VII-targeted verteporfin photodynamic therapy for breast cancer in vitro and in vivo in mice. *BMC Cancer*. 2010;10:235.
96. Hu Z, Rao B, Chen S, Duanmu J. Selective and effective killing of angiogenic vascular endothelial cells and cancer cells by targeting tissue factor using a factor VII-targeted photodynamic therapy for breast cancer. *Breast Cancer Res Treat*. 2011; 126(3):589-600.
97. Duanmu J, Cheng J, Xu J, Booth CJ, Hu Z. Effective treatment of chemoresistant breast cancer in vitro and in vivo by a factor VII-targeted photodynamic therapy. *Br J Cancer*. 2011;104(9):1401-1409.
98. Kessler T, Bieker R, Padro T, et al. Inhibition of tumor growth by RGD peptide-directed delivery of truncated tissue factor to the tumor vasculature. *Clin Cancer Res.* 2005;11(17):6317-6324.
99. Bieker R, Kessler T, Schwoppe C, et al. Infarction of tumor vessels by NGR-peptide-directed targeting of tissue factor: experimental results and first-in-man experience. *Blood*. 2009;113(20):5019-5027.