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

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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 Vlla, 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 anticaner 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 (fITF) 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 fITF to the bloodstream. 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, 2 essential components of a stable hemostatic plug.1

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,2 and fITF-bearing microparticles can become shed by these cells.3 These fITF-bearing microparticles are important contributors to the thrombotic phenotype in cancer patients.3

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.4 Furthermore, a soluble variant of fITF, 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 fITF expression

fITF 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 fITF up-regulation. This is in agreement with the finding that in CRC patients mutations of K-ras, p53 are associated with fITF 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 fITF expression in cancer cells. EGFRvIII overexpression in glioma cells results in fITF 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 fITF expression.9 Moreover, endometrial cancer cell lines display enhanced fITF levels in an EGF-dependent fashion,10 and inhibition of EGFR signaling diminishes fITF 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 fITF expression through both the scatter factor/hepatocyte growth factor (HGF) and as a result of a mutation in the c-MET oncogene, whereas fITF...
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. 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 (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,
FXa, matrix metalloproteinase-1, and activated protein C. fITF/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

Signalizing of fITF/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 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 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 fITF/FVIIa/PAR2 axis appears to elicit a more efficient production of these angiogenic 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 fITF/FVIIa complex may directly signal via the 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 fITF/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 fITF 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 fITF cytoplasmic domain, may regulate fITF activity by routing fITF to membrane compartments in which fITF signaling function is minimal. Indeed, palmitoylation of Cys 245 results in the enhanced localization of fITF into sphingolipid rafts of the cell membrane, which leads to impaired PAR2 signaling.29

Efficient PAR2 signaling and Ser 253 phosphorylation of fITF depend on binding of fITF to β1-integrins.28 fITF/β1-integrin complex formation stimulates fITF-dependent PAR2 activation and facilitates breast cancer development by contributing to both tumor angiogenesis and growth.30,31 Reciprocally, fITF 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 fITF/FVIIa/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 fITF/FVIIa/PAR2 signaling.

fITF 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 fITF, 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. Since its discovery, the role of asTF in coagulation has been a matter of debate. Increasing evidence supports a role for asTF in cancerous processes, whereas the role of asTF in angiogenesis is 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 vivo.

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

Results from xenograft and syngeneic models in mice underline the role of fITF in primary tumor growth, tumor cell-host interactions, and metastasis. Work over the past decade has indicated that fITF-driven primary tumor growth in murine models is the direct resultant of enhanced tumor angiogenesis. Knockdown of fITF in fibrosarcoma or CRC cells resulted in decreased angiogenesis through modulation of VEGF and thrombospondin levels and a concomitant decreased primary tumor growth in xenograft models, whereas pharmacologic blockade of fITF function has similar effects. Notably, in many of these studies, blockade of downstream coagulation factors was without effect, suggesting a role for fITF/PAR2-crosstalk in primary tumor growth. Indeed, antibodies that specifically block the signaling function of fITF (mAb-10H10) or PAR2, but not antibodies against the procoagulant function of fITF (mAb-5G9) or PAR-1, significantly inhibit tumor growth in breast cancer xenografts. Constitutive association of fITF with β1 integrins facilitates the fITF/FVIIa/PAR2 axis in primary breast tumors. In support of a role for fITF-mediated PAR2 signaling, PAR2, but not PAR1, deficiency in mice that harbor a murine mammary tumor virus promoter driven polycyst middle T antigen cassette (PyMT, leading to spontaneous breast tumors), attenuates tumor growth because of a delay in the angiogenic switch. Similarly, genetic deletion of the cytoplasmic tail (ΔCT) of fITF inhibits VEGF production and tumor growth in a xenograft model and angiogenesis and tumor growth in the PyMT model, whereas combination of PAR2 deficiency and cytoplasmic tail deletion does not further decrease tumor growth. Thus, PAR2 and the fITF cytoplasmic tail have overlapping roles and are involved in extensive crosstalk in primary breast tumors.

In addition to fITF/FVIIa/PAR2 signaling in injected cancer cells, host fITF/FVIIa/PAR2 signaling appears to play a significant role. In ΔCT mice, tumor grafts harboring fITF with an intact cytoplasmic tail showed significantly more tumor angiogenesis.
In summary, evidence from experimental studies indicates a direct role for ITF in cancer biology via PAR2 signaling in cooperation with integrins, leading to enhanced primary tumor growth. αTf may have a distinct role from ITF in primary tumor growth by integrin ligation, but this remains to be elucidated. The effects of ITF on metastasis are a result of downstream coagulation activation as shown by the mammary metastasis model using 5G9; however, the role of αTf in metastasis is not investigated yet. The role of the cytoplasmic domain of ITF remains unclear, but the literature to date suggests different or even opposing roles for the ITF 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 ITF 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.

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

<table>
<thead>
<tr>
<th>Type of cancer</th>
<th>Reference</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>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>53</td>
<td>115</td>
<td>93 (80.8)</td>
<td>IHC</td>
<td>TF expression is associated with well-differentiated epithelia and less lymph node metastases</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>40</td>
<td>40 (100)</td>
<td>IHC</td>
<td>Increased TF intensity is found in infiltrative ductal carcinoma</td>
</tr>
<tr>
<td></td>
<td>54</td>
<td>213</td>
<td>193 (90.6)</td>
<td>IHC</td>
<td>TF expression is associated with TF plasma levels and overall survival</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>157</td>
<td>61 (31)</td>
<td>IHC</td>
<td>Phosphorylated TF is associated with diminished survival</td>
</tr>
<tr>
<td>Lung</td>
<td>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>40</td>
<td>21</td>
<td>NA</td>
<td>mRNA</td>
<td>TF isoforms are up-regulated in cancerous tissue; ITF, and αTf mRNA levels are associated with advanced stage; low αTf mRNA levels are associated with early stage</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>8 (66.7)</td>
<td>IHC</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>NA</td>
<td>ELISA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>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 levels are associated with poor survival</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>47 (73.5)</td>
<td>IHC</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>NA</td>
<td>ELISA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>57</td>
<td>39</td>
<td>22 (56)</td>
<td>IHC</td>
<td>TF expression is associated with staging, but not with survival</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>57</td>
<td>NA</td>
<td>mRNA</td>
<td>ITF and αTf mRNA levels are associated with poor survival</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esophagus</td>
<td>58</td>
<td>36</td>
<td>NA</td>
<td>mRNA</td>
<td>ITF, but not αTf, mRNA levels are up-regulated in tumor tissue</td>
</tr>
<tr>
<td></td>
<td>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>
</tr>
<tr>
<td>Stomach</td>
<td>60</td>
<td>207</td>
<td>52 (25.1)</td>
<td>IHC</td>
<td>Intestinal-type cancer displayed enhanced TF expression and was associated with MVD, metastasis, and poor overall survival</td>
</tr>
<tr>
<td>Liver</td>
<td>61</td>
<td>58</td>
<td>58 (100)</td>
<td>IHC</td>
<td>TF is associated with MVD, metastasis, and poor overall survival</td>
</tr>
<tr>
<td>Pancreas</td>
<td>62</td>
<td>55</td>
<td>29 (52.7)</td>
<td>IHC</td>
<td>TF is associated with histologic grade and staging</td>
</tr>
<tr>
<td></td>
<td>63</td>
<td>113</td>
<td>100 (88.5)</td>
<td>IHC</td>
<td>TF is associated with staging, metastasis, and overall survival</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>240</td>
<td>211 (87.9)</td>
<td>IHC</td>
<td>TF is associated with MVD and thrombosis rate</td>
</tr>
<tr>
<td>Colonrectum</td>
<td>65</td>
<td>79</td>
<td>46 (57)</td>
<td>IHC</td>
<td>TF is associated with staging and metastasis</td>
</tr>
<tr>
<td></td>
<td>66</td>
<td>100</td>
<td>57 (57)</td>
<td>IHC</td>
<td>TF is associated with staging and MVD</td>
</tr>
<tr>
<td></td>
<td>67</td>
<td>67</td>
<td>31 (46)</td>
<td>IHC</td>
<td>TF is associated with hepatic metastasis</td>
</tr>
<tr>
<td></td>
<td>68</td>
<td>50</td>
<td>NA</td>
<td>ELISA</td>
<td>TF levels are associated with VEGF levels but not with clinicopathology</td>
</tr>
<tr>
<td>Urogenital tract</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>69*</td>
<td>29</td>
<td>NA</td>
<td>ELISA</td>
<td>In renal cell carcinoma, tumoral TF expression is lower than the surrounding parenchyma</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>NA</td>
<td>mRNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostate</td>
<td>70</td>
<td>41</td>
<td>38 (88.3)</td>
<td>IHC</td>
<td>TF is associated with poor relapse-free and overall survival</td>
</tr>
<tr>
<td>Melanoma</td>
<td>71</td>
<td>67</td>
<td>49 (73)</td>
<td>IHC</td>
<td>Tumoral TF is associated with VEGF and MVD</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>73</td>
<td>55 (75.3)</td>
<td>IHC</td>
<td>Tumoral TF is associated with poor cancer-specific survival</td>
</tr>
<tr>
<td></td>
<td>73</td>
<td>93</td>
<td>43 (47)</td>
<td>IHC</td>
<td>TF is associated with VEGF expression</td>
</tr>
<tr>
<td></td>
<td>74</td>
<td>54</td>
<td>38 (70.4)</td>
<td>IHC</td>
<td>TF expression positively correlates with advanced stage and Gleason score</td>
</tr>
<tr>
<td>Bladder</td>
<td>75</td>
<td>218</td>
<td>142 (77.6)</td>
<td>IHC</td>
<td>TF expression confers a 3.15-fold increased risk for cancer-related death</td>
</tr>
<tr>
<td>Melanoma</td>
<td>77</td>
<td>86</td>
<td>83 (96.5)</td>
<td>IHC</td>
<td>TF does not associate with clinicopathology</td>
</tr>
<tr>
<td></td>
<td>76</td>
<td>204</td>
<td>NA &gt; 90%</td>
<td>IHC</td>
<td>TF is associated with Breslow thickness</td>
</tr>
<tr>
<td>Glioma</td>
<td>78</td>
<td>44</td>
<td>44 (100)</td>
<td>IHC</td>
<td>TF is associated with higher tumor grades</td>
</tr>
<tr>
<td></td>
<td>79</td>
<td>29</td>
<td>19 (65.5)</td>
<td>IHC</td>
<td>TF is associated with higher tumor grades; TF is associated with MVD</td>
</tr>
<tr>
<td>Hematologic</td>
<td>85</td>
<td>93</td>
<td>NA</td>
<td>mRNA</td>
<td>TF mRNA in PBMC is not associated with MVD</td>
</tr>
</tbody>
</table>

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.
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 colorectal8 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 cancer15 and melanomas,15,76 high clinical staging in lung,13,14,40,57 pancreas,62,63 colorectal,65,66 and prostate cancer,14 and metastases in cancers of breast,53 lung,26 esophagus,59 gastric,60 hepatic,61 pancreatic,63 and colorectal67 tissues. Other studies, however, could not find such associations between flTF expression and unfavorable pathologic and clinical parameters54,55,68,76,77,81; 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,65 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/VIIa/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.50 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,83 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 monocye 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-directed 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/VIIa or flTF/VIIa/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/VIIa/PAR2 signaling with the flTF antibody 10H10 may have therapeutic potential while leaving the coagulant properties of flTF unaffected.80 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.8 Indeed, pharmacologic modalities are available for tumor-specific delivery of RNAi,89 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; 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, 2 other potential ILT6 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 setup, but this study is limited to pancreatic cancer patients. Both studies aim to target both the coagulant and signaling effects of ILT6 in tumor biology, and the results from these studies will be helpful for deciding whether ILT6 targeting is a viable option for future treatment of cancer and cancer-related thrombosis.

Despite promising results, many questions remain before ILT6-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 ILT6 unaffected, but whether abrogating ILT6 signaling may affect other physiologic processes is unclear. Moreover, the coagulant properties of ILT6 promote cancer progression through enhancing the metastatic potential. Therefore, targeting the coagulant function ILT6 may be necessary to really provide new therapeutic means. In contrast to ILT6, 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 ILT6 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 ILT6. A FVII/IgG Fc effector domain chimera induced long-lasting repression of both the injected tumor and tumors injected at distant sites, probably through mediating an NK cell–dependent cytotoxic antitumor response. FVII-bound photosensizers have also shown positive results in ILT6-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 ILT6-bearing tumoral endothelium and cancer cells, even when tumors became chemoresistant.

Others have investigated the delivery of exogenous ILT6 to the tumor vasculature to specifically infarct tumor vessels. A conjugate containing the heparin binding domain of VEGF and truncated ILT6 induced specific coagulation in tumor vessels, whereas a conjugate of ILT6 with RGD and NRG peptides, targeting αVβ3 integrins and CD13, resulted in infarction of tumor vessels in mice, and in patients tumor perfusion decreased, whereas the compound was tolerated.

Use of ILT6-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 ILT6. 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 ILT6 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 ILT6 expression on tumor cells are now well defined, and ILT6 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 ILT6 in the progression of cancer is underscored by its abundant expression in human cancers from different origins. Furthermore, ILT6 has gained attention as a potential therapeutic target by harboring tumoricidal drugs to ILT6-expressing cancer cells or via direct inhibition of its cellular effects. Despite this progress, questions remain, especially regarding the relative contribution of ILT6 and asTF to cancer progression.

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