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

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Introduction

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 vivo. 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)

Oncogenic events drive fTF expression

fTF 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 fTF up-regulation.7 This is in agreement with the finding that in CRC patients mutations of K-ras, p53 are associated with fTF 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 fTF expression in cancer cells. EGFRvIII overexpression in glioma cells results in fTF 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 fTF expression.9 Moreover, endometrial cancer cell lines display enhanced fTF levels in an EGFr-dependent fashion,10 and inhibition of EGFR signaling diminishes fTF 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 fTF expression through both the scatter factor/hepatocyte growth factor (HGF) and as a result of a mutation in the c-MET oncogene, whereas fTF...
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 environment4 (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. PAR2s 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 binds 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, 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

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FXa, matrix metalloproteinase-1, and activated protein C. fTF/FVIIa, FXa, trypsin, and tryptase are able to activate PAR2, whereas PAR4 is activated by thrombin and plasmin. In mouse models, PAR3 has been found to serve as a cofactor for PAR4, but recent data suggest that human PAR3 may also be activated directly by thrombin. On activation, PARs couple to heterotrimeric G-proteins after which further signaling events are initiated.

Signaling of fTF/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. Activation of both the MAPK and PI3 kinase pathways contributes to a pro-malignant transcriptional program and stimulates onco- genic protein synthesis.

Covalent attachment of fatty acids, specifically palmitoylation, to the fTF cytoplasmic domain may regulate fTF activity by routing fTF to membrane compartments in which fTF signaling function is minimal. Indeed, palmitoylation of Cys 245 results in the enhanced localization of fTF into sphingolipid rafts of the cell membrane, which leads to impaired PAR2 signaling.

Efficient PAR2 signaling and Ser 253 phosphorylation of fTF depend on binding of fTF to β1-integrins. fTF/β1-integrin complex formation stimulates fTF-dependent PAR2 activation and facilitates breast cancer development by contributing to both tumor angiogenesis and growth. Reciprocally, fTF 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 fTF/FVIIa/PAR2 signaling. Ectopic production of FVII is regulated by epigenetic and hypoxia-driven processes in several solid tumor cell lines, whereas EGFR signaling in gliomas not only up-regulates TF expression, but also expression of FVII and PAR2, thus orchestrating the generation of a multitude of events that contribute to fTF/FVIIa/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 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.36,37 Since its discovery, the role of asTF in coagulation has been a matter of debate.39 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 αSβ1 and αVβ3 integrins on endothelial cells, thus acting as a pro-angiogenic stimulus. Integrin ligation by asTF activates a plethora of downstream signaling components, such as focal adhesion kinase, PI3K, MAPK, and Akt,3 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 vivo in cancer models.

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 result of enhanced tumor angiogenesis. Knockdown of fITF 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 fITF function has similar effects.42 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.30 Constitutive association of fITF with β1-integrin 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 polonya 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 (∆CT) of fITF inhibits VEGF production and tumor growth in a xenograft model44 and angiogenesis and tumor growth in the PyMT model, whereas combination of PAR2 deficiency and cytoplasmic tail deletion does not further decrease tumor growth.43 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 ∆CT mice, tumor grafts harboring fITF with an intact cytoplasmic tail showed significantly more tumor angiogenesis.27 Moreover, fITF cytoplasmic tail deletion in PyMT mice resulted in large-diameter vessels in late-stage tumors, whereas this effect was reversed in PAR2-deficient, fITF cytoplasmic tail-deleted mice. Thus, the fITF cytoplasmic tail appears to have opposing effects in tumor growth and the host angiogenic response, where the latter effect may be attributed to fITF/FVIIa/PAR2 signaling in the host macrophage compartment.

Further evidence for non-tumor cell fITF signaling in cancer comes from experiments that use spontaneously immortalized embryonic fibroblasts from TF wild-type (WT), TF-/-, and TF cytoplasmic tail deleted (TF∆CT) embroys. Primary tumor growth was similar after engraftment of WT, TF-/-, and TF∆CT, 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 fITF, this indicates that the contribution of host- and tumor-derived fITF to cancer progression is highly dependent on the cancer type. Furthermore, the role of fITF 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 fITF.46 fITF 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 fITF mutants with diminished fITF/FVIIa procoagulant activity or a deleted cytoplasmic tail, a decrease in metastatic load was seen, suggesting importance of both fITF/FVIIa procoagulant activity and cytoplasmic domain function.48,49 fITF 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 fITF coagulant function inhibited metastasis in a breast cancer xenograft model, whereas blockade of fITF signaling function was without effect.50 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, fITF 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.5 asTF is thought to augment angiogenesis by acting as an integrin-activating agent,6 but the exact mechanism remains unclear. Future studies with specific targeting of either asTF or fITF in constitutively asTF-expressing cancer cell lines will increase the knowledge on asTF in cancer biology.
In summary, evidence from experimental studies indicates a direct role for $\alpha$TF in cancer biology via PAR2 signaling in cooperation with integrins, leading to enhanced primary tumor growth. $\alpha$TF may have a distinct role from $\alpha$TF in primary tumor growth by integrin ligation, but this remains to be elucidated. The effects of $\alpha$TF on metastasis are a result of downstream coagulation activation as shown by the mammary metastasis model using 5G9; however, the role of $\alpha$TF in metastasis is not investigated yet. The role of the cytoplasmic domain of $\alpha$TF remains unclear, but the literature to date suggests different or even opposing roles for the $\alpha$TF 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 $\alpha$TF 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>40</td>
<td>100</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, $\alpha$TF, and $\alpha$TF mRNA levels are associated with advanced stage; low $\alpha$TF 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>$\alpha$TF and $\alpha$TF 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>$\alpha$TF, but not $\alpha$TF, 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>Colorectum</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>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>Kidney</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>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>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 fITF 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,67,72 and gliomas.78,79 Studies of the upstream oncogenic events that lead to enhanced fITF expression have been conducted in colorectal8 and lung cancer,13,14 and associations were identified between fITF 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 fITF expression is an independent predictor of poor overall or relapse-free survival,13,54,55,59-61,64,66 prostate cancer,71 and gliomas.79 Associations between fITF and VEGF expression are described in gastrointestinal cancers,59-61,64,66 prostate cancer,71 and gliomas.79 Associations between fITF and VEGF expression are described in breast and lung cancer,14,55 colorectal cancer,68 and prostate cancer,13,14,40,57 high clinical staging in lung,13,14,40,57 prostate,62,63 colorectal,65,66 and prostate cancer,13,14,40,57 and metastases in cancers of breast,53 lung,36 esophagus,59 gastric,60 hepatic,61 pancreatic,63 and colorectal77 tissues. Other studies, however, could not find such associations between fITF 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 fITF.

The fITF/VIIa/PAR2 axis is supposed to drive angiogenic events through enhanced production of angiogenic factors, such as VEGF. Associations have indeed been found between fITF expression and microvessel density in lung cancer,14 throughout all gastrointestinal cancers,59-61,64,66 prostate cancer,71 and gliomas.79 Associations between fITF 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 fITF when phosphorylated (pTF), and therefore only when involved in PAR2 signaling, was used to investigate whether the effects of fITF in cancer could be attributed to direct signaling effects of the fITF/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 aITF in relation to clinicopathologic characteristics has only been studied in non-small cell lung cancer, and these studies reveal a correlation between high aITF mRNA levels and advanced tumor stage, whereas low levels of aITF mRNA relate to less advanced stages of cancer progression.50 In another study, high aITF 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 fITF expression in cancer of other origins. Mouse studies indicate that fITF expression influences fibrosarcoma progression,41 and rat osteosarcoma cells display fITF-dependent coagulant activity,85 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 fITF. This is indeed the case in several leukemic cell lines, but the risk for thrombosis could not be attributed to enhanced fITF expression on tumor cells.84 Furthermore, fITF expression on circulating cells was negatively associated with bone marrow microvessel density.85 Because monocytic activation leads to bona fide expression of fITF, further research into monoblastic and monocytic leukemias is warranted as well as further assessment of fITF expression in bone marrow biopsies.

In summary, most human epithelial cancers are characterized by abundant levels of fITF. In keeping with the observations from experimental studies, fITF is likely to drive tumor angiogenesis, to enhance tumor growth, and to influence metastasis. 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 fITF and VEGF expression, tumor volume, microvessel density, and metastatic risk leading to diminished survival is evident, which is in concordance with findings in experimental studies. Limited data are available concerning the role of aITF in human malignancies because, at present, no studies have been performed on a large series of tumors. Future studies investigating fITF versus aITF 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. fITF expression is an important determinant of cancer progression, as well as a contributor to thrombosis susceptibility, and inhibiting fITF function may be a potential avenue for treating cancer and cancer-related thrombosis. Although studies investigating fITF-targeted cancer therapy remain sparse, some studies provide clues that fITF-directed treatment of cancer may indeed prove to be beneficial.

As proper PAR2 signaling relies on the formation of either the fITF/VIIa or fITF/VIIa/FXa complex, the effect of therapies lowering FVII and FX in cancer patients provided some insight in whether such indirect anti-fITF–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 fITF-dependent. Experimental work reveals that warfarin diminishes the metastatic potential, but this is seemingly independent of fITF.41 Similarly, heparins may affect cancer progression by modulating fITF-mediated signaling events, but at present it is unclear to what extent fITF-specific signaling events contribute to the possible effects of warfarin or heparin treatment on cancer.

Specific inhibition of fITF/VIIa/PAR2signaling with the fITF antibody 10H10 may have therapeutic potential while leaving the coagulant properties of fITF unaffectted.50 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 fITF, as RNAi has proven to be beneficial in mouse experiments.8 Nevertheless, 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 fITF-expressing tumors, remains uncertain.

Several studies on the efficacy of fITF targeting in cancer have been undertaken or are still ongoing. The nematode fITF/VIIa inhibitor
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 fITF targeting drugs are under investigation in clinical studies: ALT-836 (Altor Bioscience), a TF-inhibiting antibody, and PCI-27483 (Pharmacycics), 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 fITF in tumor biology, and the results from these studies will be helpful for deciding whether fITF targeting is a viable option for future treatment of cancer and cancer-related thrombosis.

Delivery of antitumor drugs to sites of enhanced TF expression

Taking advantage of enhanced tumoral fITF 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 fITF. A FVII/IgG Fc effector domain chimera induced long-lasting regression of both the injected tumor and tumors injected at distant sites, probably through mediating an NK cell–dependent cytotoxic antitumor response. FVII-bound photosensitizers have also shown positive results in fITF-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 fITF-bearing tumoral endothelium and cancer cells, even when tumors became chemoresistant.

Others have investigated the delivery of exogenous fITF to the tumor vasculature to specifically infarct tumor vessels. A conjugate containing the heparin binding domain of VEGF and truncated fITF induced specific coagulation in tumor vessels, whereas a conjugate of fITF 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 fITF-mediated approaches for targeting tumoricidal drugs or infarcting tumor vasculature may be hampered by off-target effects as well, as other parts of the vasculature may express fITF. Phototherapy is perhaps most promising in circumventing such unwanted effects because it onlyexerts 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 fITF 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 fITF expression on tumor cells are now well defined, and fITF has prominent effects on tumor growth via PAR2 signaling and integrin ligation, hereby influencing cell survival, cell motility, and the production of angiogenic factors. The importance of fITF in the progression of cancer is underscored by its abundant expression in human cancers from different origins. Furthermore, fITF has gained attention as a potential therapeutic target by harboring tumoricidal drugs to fITF-expressing cancer cells or via direct inhibition of its cellular effects. Despite this progress, questions remain, especially regarding the relative contribution of fITF and αsTF to cancer progression.

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Authorship

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