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Tissue Factor and Cancer: Regulation, Tumor Growth and Metastasis

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Abstract

There is a strong relationship between tissue factor (TF) and cancer. Many cancer cells express high levels of both full-length TF and alternatively-spliced (as) TF. TF expression in cancer is associated with poor prognosis. In this review, we summarize the regulation of TF expression in cancer cells and the roles of TF and asTF in tumor growth and metastasis. A variety of different signaling pathways, transcription factors and microRNAs regulate TF gene expression in cancer cells. The TF/factor VIIa complex enhances tumor growth by activating protease-activated receptor (PAR) 2 signaling and by increasing the expression of angiogenic factors, such as VEGF. AsTF increases tumor growth by enhancing integrin β1 signaling. TF and asTF also contribute to metastasis via multiple thrombin-dependent and independent mechanisms that include protecting tumor cells from natural killer cells. Finally, a novel anti-cancer therapy is using tumor TF as a target to deliver cytotoxic drugs to the tumor. TF may be useful in diagnosis, prognosis and treatment of cancer.

Keywords

Cancer; metastasis; thrombosis; tissue factor; tumor

Introduction

Full-length tissue factor (TF) is a transmembrane receptor and cofactor for factor (F)VII/FVIIa[1]. In addition to full-length TF, an alternative spliced (as) form of TF can be generated that lacks the transmembrane domain and is released from cells[2]. In contrast to TF, asTF has low procoagulant activity because it lacks the transmembrane domain[3,4].

TF is expressed by cells around blood vessels, such as adventitial fibroblasts, and body surfaces, such as epithelial cells, and plays a critical role in hemostasis[5]. TF also contributes

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Addendum

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to various forms of thrombosis[6]. Many cancers, particularly adenocarcinomas, express high levels of TF[7]. A high level of tumor TF expression is associated with poor prognosis in many types of cancers, including breast, prostate, colorectal and pancreatic cancer[7–15]. The TF/factor (F)VIIa complex and downstream coagulation proteases, such as FXa and thrombin, activate protease-activated receptors (PARs) on a variety of cells[16]. This signaling requires the procoagulant activity of the extracellular domain of TF but not its cytoplasmic domain[17]. In addition, the TF cytoplasmic domain contributes to signaling via interaction with integrins[17].

In this review, we summarize the current knowledge on the regulation of TF in cancer cells and the contributions of TF to tumor growth and metastasis (Figure 1).

Regulation of Tissue Factor Expression in Cancer Cells

Tumor cells have genetic and epigenetic alterations that constitutively activate signaling pathways involved in tumorigenesis[18]. These pathways control cell growth, cell motility, cell metabolism, cell death and the tumor microenvironment, such as angiogenesis and inflammation[18–20].

Many studies have assessed the regulation of TF in different types of cancer, including brain[21–25], breast[26–31], and colorectal[32–34] cancer. These studies demonstrated that multiple signaling pathways, transcription factors and microRNAs (miRNAs) regulate TF gene expression in cancer cells (Tables 1 and 2) (Figure 2). For instance, the Raf-MEK-ERK signaling pathway and the transcription factors activator protein 1 (AP-1) and nuclear factor-κB (NF-κB) induce TF gene expression in the human breast cancer cell line MDA-MB-231[26]. Another study found that hepatocyte growth factor activation of the c-Met kinase pathway induced TF expression via Src family kinases in the human brain tumor cell line DAOY[22]. More recently, the mammalian target of rapamycin (mTOR) kinase pathway was shown to induce TF gene expression in the human pancreatic neuroendocrine tumor cell line BON[35].

Activation of the epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases, including EGFR and human epidermal growth factor receptor 2 (HER2), induces TF gene expression in glioma cells[23,36]. The EGFR ligands EGF and TGFα increased TF expression in the human endometrial adenocarcinoma cell line Ishikawa[37], and the human squamous cell carcinoma line A431[27,36]. In addition, expression of a constitutively activated EGFRvIII variant induced TF gene expression in the human glioma cell lines U373 and U87MG[23,36]. Furthermore, TF expression was reduced by the neutralizing anti-HER2 antibody trastuzumab in the breast cancer cell line SKBR3[27].

TF expression was increased in human colorectal cancer cell lines by activation of the KRAS oncoprotein and by inactivation of the p53 tumor suppressor[32]. These changes led to activation of the MEK/mitogen-activated protein kinase and phosphatidylinositol-3-kinase (PI3K) signaling pathways. In addition, a correlation between mutations of KRAS and p53, and TF expression was shown in patients with colorectal and lung cancer[33,38].

Rong and colleagues showed that hypoxia coupled with the loss of the tumor suppressor PTEN led to the induction of TF gene expression via the Akt-mTOR and RAS-MEK-ERK pathways in the human glioma cell line 23.11[21]. Furthermore, increased TF expression was observed in cells that form the hypoxic pseudopalisades surrounding necrotic areas in human glioblastoma specimens[21]. Another study showed that the transcription factors early growth response gene-1 and hypoxia-inducible factor-1α independently induce TF gene expression under hypoxic conditions in the human breast cancer cell lines MDA-MB-231 and MDA-MB-435[28].

Epithelial-mesenchymal transition (EMT) is a process whereby epithelial cells lose cell-cell adhesion and polarization and gain the mesenchymal traits of motility and invasion[39]. One study found that induction of an EMT-like phenotype in vitro induced TF expression in human squamous cell carcinoma A431 cells[40]. In addition, the EMT transcription factors Snail and zinc finger E-box-binding homeobox 1 (ZEB1) induced TF gene expression in the breast cancer cell line MDA-MB-468[29]. These studies indicated that several cancer-dependent phenotypes, such as oncogenic mutations, tumor hypoxia and EMT, induce TF expression in cancer cells.

Alterations in tumor cell metabolism also regulates TF expression. Isocitrate dehydrogenase 1 (IDH1) is a metabolic enzyme and the mutant form of IDH1 is associated with increased levels of 2-hydroxyglutarate[41]. Accumulation of 2-hydroxyglutarate competitively inhibits demethylation of α-ketoglutarate-dependent enzymes, such as TET2, resulting in hypermethylation of a select group of promoters[42]. Importantly, there was increased methylation of the TF promoter and decreased TF expression in gliomas expressing mutant *IDH1* compared with gliomas expressing wild-type *IDH1*[24].

Recent studies have demonstrated that miRNAs regulate TF expression in cancer cells. For instance, transfection of a miR-19a mimic reduced TF expression in the breast cancer cell line MDA-MB-231[30] and colorectal cancer cell lines[34]. Furthermore, Teruel and colleagues showed that miR-19b, miR-20a and miR-106b reduced TF expression by 20–60% in MDA-MB-231[31]. MiR-93 and miR-106b reduced TF expression in leiomyoma cell line TF324 and leiosarcoma cell line SKLM-S1[43]. However, there was no inverse correlation between TF gene expression and these miRNAs in samples from patients with leiomyoma[43]. Expression of miR-520g reduced TF expression in the human medulloblastoma tumor cell lines DAOY and UW228[25]. More importantly, oncogenic amplification of the chromosome 19 miRNA cluster, C19MC, which includes miR-520g, was associated with reduced TF expression in pediatric embryonal brain tumors, providing a link between oncomirs and TF expression[25].

TF also regulates miRNAs. D'Asti and colleagues showed that administration an anti-TF monoclonal antibody (clone CNTO2559) to mice led to upregulation of 20 miRs and downregulation of 55 miRs in MDA-MB-231 subcutaneous tumors[44]. This antibody selectively inhibits signaling but not coagulation. These TF-regulated miRs are associated with the regulation of pathways that are activated in cancer, such as ErbB and PI3K/Akt[44].

Glioblastoma multiforme (GBM) can be subdivided into 4 subtypes: proneural, neural, classic and mesenchymal[45]. One study found different levels of TF expression among

subtypes of GBM with the classic subtype and the proneural subtype exhibiting the highest and lowest levels of TF expression, respectively[45]. More recently, Tawil and colleague found that single cells from proneural and classical subtypes of GBM showed different levels of TF expression[46]. Importantly, TF expression reversed the dormant phenotype of the non-tumorigenic human GBM cell line U373 by driving permanent changes in the gene expression profile, DNA copy number and DNA methylation state[47]. These data indicate that TF expression affects tumor characteristics and malignancy in GBM.

Tissue factor and tumor growth

There are several different tumor mouse models that use immunodeficient or immunocompetent mice, different sites of tumor growth (orthotopic or subcutaneous) and different cancer types[48]. Orthotopic models are superior to subcutaneous models but may require reporters to measure tumor growth in internal organs such as the pancreas. Genetically engineered spontaneous tumor models are more clinically relevant but the appearance of tumors can be variable[48]. Cancer cell lines are often used but may not fully reproduce the pathophysiology of human tumors. The choice of cell line is also very important. For instance, the human breast cancer cell line MDA-MB-231 is widely used in TF studies. However, it should be noted that this cell line expresses much higher levels of TF than a large number of primary breast tumor samples of varying stages and grades[49]. We have observed a wide range of TF expression in human pancreatic cancer cell lines[50,51]. Similarly, pancreatic patient-derived xenografts (PDXs) express different levels of TF (Hisada and Mackman, unpublished data). PDXs are considered a superior model compared to cell line-derived xenografts because tumors of PDX maintain the pathological features[52,53], gene expression patterns[54] and single nucleotide polymorphisms[55] of primary tumors. However, PDXs are more difficult to maintain.

TF has been described as a strong tumor growth enhancer[56]. Studies have shown that TF expression from plasmid vectors introduced into the murine sarcoma cell line Meth-A and the TF-negative human pancreatic cancer cell line MIA PaCa-2 enhances tumor growth in mice[57,58]. Conversely, silencing TF expression in Meth-A cells and the human colorectal cancer cell line HCT-119 with siRNA was associated with reduced tumor growth in mice[32,57]. An ovarian cancer cell line was shown to express FVII[59]. In addition, coagulation factors, such as FX, can readily enter the tumor from the blood due to the leaky tumor vasculature. This suggests that the TF/FVIIa and TF/FVIIa/FXa complex can be assembled on the surface of tumors cells. Consistent with this notion, subcutaneous growth of murine melanoma B16 tumors was also inhibited by tissue factor pathway inhibitor (TFPI)[60]. Similarly, the endogenous inhibitor of the TF/FVIIa complex, and a nematode factor X-dependent inhibitor of TF/FVIIa called NAPc2 but not by the FXa inhibitor NAP5, inhibited the growth of B16 tumors[60]. NAPc2 also inhibited tumor growth of HCT-119 colorectal tumors in nude mice[61]. An antibody that inhibits TF/FVIIa signaling (Mab-10H10) but not an antibody that inhibits TF-dependent coagulation (Mab-5G9) inhibited tumor growth of the MDA-MB-231 tumors in severe combined immunodeficent (SCID) mice[62]. In contrast to these studies, the absence of TF did not affect the growth of murine tumor lines in mice that express the strong oncogenic driver Ha-Ras (C12V)[63]. These data indicate that the TF/

FVIIa complex contributes to growth of a variety of tumor types but not all tumor types in preclinical models.

TF appears to enhance tumor growth via several mechanisms, including increasing angiogenesis via expression of VEGF[32]. TF/FVIIa-dependent activation of PAR2 induced another angiogenic factor interleukin-8 in the MDA-MB-231 in vitro[64]. In addition, growth of mouse mammary tumors in the virus-polyoma middle T (MMTV-PyMT) model was reduced in PAR2 deficient mice but not in PAR1 deficient mice[65]. Interestingly, phosphorylation of the TF cytoplasmic domain was shown to contribute to TF/FVIIa-PAR2 signaling and tumor growth in the MMTV-PyMT mouse model[66]. Further, there was an association between phosphorylation of the TF cytoplasmic domain and PAR2 expression in patients with recurrent breast cancer[67]. Together, these studies suggest that TF/FVIIa-PAR2 signaling contributes to tumor growth in mouse models of breast cancer.

The MAB-10H10 antibody not only blocked TF/FVIIa-PAR2 signaling in MDA-MB-231 cells but also disrupted the association of TF with β1 integrin[62]. An integrin binding motif (KGE) in the FVIIa protease domain was identified that is required for binding of the TF/FVIIa complex with the active conformer of integrin β1[68]. A point mutation in this binding motif inhibited TF/FVIIa binding with integrin β1 and integrin signaling but not PAR2 signaling. This study indicates that there is crosstalk between the TF/FVIIa complex and integrin trafficking in cancer cells (Figure 3A).

One study investigated the contribution of host TF to tumor growth. Low levels of host TF did not affect subcutaneous growth of tumors of murine lung carcinoma cell line Lewis Lung Carcinoma (LLC) and murine melanoma cell line B16F1[69]. However, low levels of host TF was associated with reduced blood vessel size in the B16F1 tumors[69].

The role of tumor and host PAR1 in tumor growth is complex (Figure 3B). Tumor PAR1 has been shown to positively and negatively regulate tumor growth. In vitro growth of a variety of PAR1 expressing human colon cancer cell lines (HT29, CI.19A, LoVo, and HCT116) but not the PAR1-negative cell line LS174T was enhanced by either thrombin or a PAR1 agonist peptide[70]. In addition, silencing PAR1 expression with shRNA in the human malignant pleural mesothelioma cell line REN reduced growth in nude mice[71]. Other studies have examined the role of PAR1 in murine pancreatic cancer cell lines, including Panc02 and KPC lines derived from genetically engineered mice. Silencing of either TF or PAR1 in a KPC line derived from LSL-$KRAS^{G12D/+}$, LSL-$P53^{R172H/+}$, $Elas^{CreER/+}$ mice significantly reduced growth of subcutaneous and orthotopic tumors (Dr. Matthew Flick, Cincinnati Children's Hospital, personal communication). Surprisingly, another study found that reducing PAR1 expression in either Panc02 cells or KPC cells derived from LSL-$KRAS^{G12D/+}$, LSL-$P53^{R172H/+}$, $p48^{Cre}$ mice resulted in increased growth in mice[72]. At present, it is not clear why these two groups observe opposite results.

Similar to tumor PAR1, host PAR1 has been shown to positively and negatively regulate tumor growth. One study observed reduced growth of murine pancreatic Panc02 tumors in PAR1 deficient mice compared with to the growth of tumors in wild-type mice[73]. In contrast, Adams and colleagues showed that growth of prostate tumors in the transgenic

adenocarcinoma of the mouse prostate (TRAMP) mouse model and colon tumors in the adenomatous polyposis coli Min ($APC^{Min/+}$) mouse model was increased in PAR1 deficient mice[74]. Further studies are required to elucidate the roles of tumor and host PAR1 in tumor growth in different models.

Several studies have shown that asTF enhances tumor growth. One study showed that expression of asTF in human pancreatic cancer cell line MIA PaCa-2 enhanced tumor growth in nude mice[75]. Another study found that expression of asTF in the TF-negative human breast cancer cell line MCF7 enhanced cell growth in vitro, and this was inhibited by an anti-asTF antibody (clone Rb1)[76]. AsTF-dependent cell growth was inhibited by silencing β1 integrin gene expression in MCF7 cells[76]. Moreover, orthotopic growth of MCF7 tumors was increased in cells expressing asTF[76]. A recent study found an association between asTF-dependent gene expression and estrogen receptor (ER)-dependent gene expression profiles[77]. Furthermore, growth of MCF7 cells expressing asTF but not the parental MCF7 cells was significantly increased when cells were treated with an ER agonist, estradiol, both in vitro and in vivo (Non-Obese Diabetic (NOD) /SCID)[77]. In addition, asTF-ER-dependent growth of MCF7 was inhibited by silencing β1 integrin expression in vitro, which suggests that asTF requires β1 integrin to enhance tumor growth[77] (Figure 3C).

Similar to the studies with breast cancer, expression of asTF in the human pancreatic ductal adenocarcinoma cell line Pt45P1 enhanced orthotopic growth in nude mice[78]. Furthermore, growth of wild-type Pt45P1 tumors were inhibited when cancer cells were pre-incubated with an anti-asTF antibody (clone RabMab-1)[78]. RNA sequence analysis revealed that Pt45P1 expressing asTF showed significantly higher expression of genes associated with MAPK signaling than parental Pt45P1 cells[79] In addition, phosphorylation of Akt was observed in Pt45P1 cells expressing asTF[79]. These studies indicate that asTF contributes to growth of breast and pancreatic tumors in preclinical mouse models.

Preclinical studies have led to clinical trials that inhibit either the TF/FVIIa complex or downstream proteases. One trial investigated the anti-cancer effect of a small molecule FVIIa inhibitor (PCI-27483) combined with gemcitabine in patients with metastatic or locally advanced pancreatic cancer (ClinicalTrials.gov identifier: NCT01020006). In the trial 16 patients received gemcitabine and 18 patients received gemcitabine plus PCI-27483 (1.2 mg/kg BID). Patients receiving PCI-27483 had increased anemia, coagulopathy, gastric bleeding and upper gastrointestinal bleeding compared with the controls, which suggested that inhibition of the TF/FVIIa complex may increase the risk of hemorrhage. Another trial investigated if administration of the FXa inhibitor rivaroxaban before surgery or chemotherapy has an anti-cancer effect in patients with early stage breast cancer (EU Clinical Trials Register Eudract No: 2014-004909-33). At present, no results have been reported.

Tissue factor and metastasis

Metastasis involves cancer cells spreading from a primary site to a secondary site[80]. Treatment of metastatic tumors is much less successful compared with the treatment of localized tumors because metastasis is often a marker of advanced disease. Different cancers

exhibit an organ-specific pattern of metastasis. For instance, breast and prostate cancers often metastasize to bone[81]. One of the most popular models of metastasis is the experimental metastasis model (also known as the hematogenous metastasis model) where cancer cells are injected via the tail vein of mice and form tumors in the lung. There are also some “spontaneous metastasis” models where primary tumors in different organs spontaneously metastasize to other organs, such as the lung. Detection of metastasis is facilitated by expression of reporters in the cancer cells.

Clinical studies have shown that a high level of TF expression in tumors is associated with metastasis in a variety of cancer types, including colorectal cancer, gastric cancer, and pancreatic cancer[7,9,13,82].

One study used different antibodies to demonstrate a role for TF procoagulant activity (extracellular domain) but not TF signaling in metastasis of human melanoma cell lines M24 and C8161 cells into SCID mice[83]. Expression of TF in the TF-negative human melanoma cell line A7 increased clot formation and tumor cell survival in mice compared to the parental cells[84]. Another study found that injection of recombinant murine TFPI reduced metastasis of TF-expressing B16F10 cells[85]. Similarly, expression of TFPI in B16F10 cells reduced metastasis[84].

The role of the TF cytoplasmic domain in metastasis is controversial. An early study found that expression of different TF mutants in the low TF expressing human melanoma cell line YU-SIT1 showed a role for the TF cytoplasmic domain but not TF procoagulant activity in metastasis[86]. An independent study found both TF procoagulant activity and the cytoplasmic domain contributed to metastasis of Chinese hamster ovary cells in SCID mice[87]. However, two recent studies found no role of the TF cytoplasmic domain in metastasis[63,84]. One study generated three C57BL/6-derived tumor lines that either lacked TF, expressed wild-type murine TF or expressed a mutant TF lacking the cytoplasmic domain[63]. This study found that TF supports metastasis via its procoagulant activity and independently of the cytoplasmic domain[63]. Another study expressed either wild-type TF or a TF mutant lacking the cytoplasmic domain in the TF-negative human melanoma cell line A7 and found increased metastasis with both lines compared with the parental cells[84]. Finally, expression of TF in TF-negative PyMT breast cancer cells enhanced metastasis[88]. These data indicate that the procoagulant activity of the TF/FVIIa complex plays a role in metastasis in mice.

TF may enhance metastasis by increasing fibrin deposition or by facilitating generation of coagulation proteases that activate PARs (Figure 4). Many studies have investigated the role of the TF-thrombin-fibrin pathway in experimental metastasis. Palumbo and colleagues showed a significant reduction in experimental metastasis of murine melanoma B16-BL6 and LLC cells in fibrinogen-deficient mice compared with wild-type controls[89]. In addition, inhibition of thrombin with hirudin significantly reduced metastasis of B16-BL6 in both wild-type C57BL/6 and fibrinogen-deficient mice[89]. The same group showed that spontaneous metastasis from subcutaneous LLC tumors to the lung and regional lymph nodes was reduced in fibrinogen-deficient mice compared with wild-type controls[90].

One study examined the roles of platelets, PAR4 and fibrin(ogen) in experimental metastasis of B16-F10 cells in both syngeneic C57BL/6 and SCID mice. Metastasis was significantly reduced in mice with low platelets (NF-E2 deficient mice), in mice lacking PAR4, which is the main thrombin receptor on platelets in mice, and in fibrinogen-deficient mice[91]. Another study observed reduced metastasis of LLC and spontaneous metastasis from subcutaneous LLC tumors to lung in mice lacking Gα, which is required for platelet activation[92]. Interestingly, immunologic and genetic depletion of natural killer (NK) cells abolished the reduction of metastasis of LLC cells in Gα-deficient and fibrinogen-deficient mice, which suggest that fibrin and platelets impeded NK cell elimination of tumor cells[92]. A study with TF expressing C57BL/6-derived tumor cells demonstrated that TF contributes to metastasis by restricting NK cell-mediated clearance of micrometastases in a fibrin(ogen)-dependent and platelet-dependent manner[63]. In addition, TF plays a role in metastasis in a thrombin-dependent mechanism independent of NK cells[63]. A recent study demonstrated that the TF-thrombin pathway enhanced metastasis by recruiting macrophages[84]. Furthermore, macrophage function was essential for tumor cell survival independent of NK cells because protection was lost by either ablating CD11b-positive cells or by using Mac1 (αM/β2)-deficient mice (Figure 4).

TF-dependent generation of downstream coagulation proteases, such as thrombin, may enhance metastasis. Shi and colleagues found that thrombin but not a PAR1 agonist peptide increased the migration of M24 cells in vitro[93]. Interestingly, a combination of PAR1 and PAR2 agonist peptides reproduced the enhancement of migration observed with thrombin. PAR2 agonist peptide alone did not increase cell migration. Furthermore, stimulation of M24 cells with the PAR2 agonists trypsin or PAR2 agonist peptide increased metastasis of these cells in CB-17 SCID/Beige mice[93]. Another study analyzed the role of PAR1 in the metastasis of the human melanoma cells lines A375SM and C8161[94]. Silencing PAR1 in the tumor cells led to an increase in the expression of the tumor suppressor Maspin and decreased the metastasis[94]. These data indicate that tumor PAR1 and PAR2 contribute to metastasis.

Host PAR2 was shown to contribute to spontaneous metastasis in the MMTV-PyMT model but not experimental metastasis of murine melanoma cell line B16-F10[65,91]. Host PAR1 did not contribute to metastasis in either the spontaneous or the experimental metastasis (B16-F10 cells) models[65,91]. However, a study using hyperthrombotic mice found that deletion of PAR1 in both the tumor and the host but not either alone reduced metastasis of PyMT breast cancer cells[88]. These studies indicate that the roles of PAR1 and PAR2 in metastasis are complex.

A study by Unruh and colleagues showed that overexpressing of asTF in orthotopic pancreatic Pt45P1 tumors increased metastasis in nude mice compared with parental Pt45P1 tumors[79]. AsTF expression significantly increased gene expression of components of various oncogenic pathways, including MAPK signaling cascade, Rho signaling, cell migration, and EMT[79]. In contrast, expression of asTF in TF-negative PyMT breast cancer cells did not affect metastasis[88]. These data suggest that asTF can activate pathways involved in metastasis.

Targeting tissue factor as a novel anti-cancer therapy

TF expression by tumors and possibly tumor-associated endothelial cells has been used as a target in a novel approach to anti-cancer therapy. The rationale for using TF as a tumor target is that it is expressed at high levels on many tumors but is not expressed within the vasculature. This should allow targeted deliver to the tumor. There are two strategies that have been employed: 1. target TF to induce infarction within the tumor; 2. target TF to deliver cytotoxic drugs to the tumor. However, targeting TF may increase the risk of bleeding because TF is essential for hemostasis. In addition, TF expression in tumors is variable and it is unclear if there is a minimal amount of TF that is required for these approaches to be effective.

One group developed two different bispecific antibodies where one arm of the antibody targets the tumor vasculature and the other binds a truncated form of TF (tTF)[95,96]. The antibody-tTF conjugate is referred to as a ‘coaguligand’. The tTF form has very low procoagulant activity in the circulation but its activity is increased when it is localized to a phospholipid surface and this allows local thrombosis of tumor vessels and infarction of the tumor[95,96]. One study showed that one coaguligand induced regression of murine neuroblastoma C1300 subcutaneous tumors in nude mice[95]. Another study targeting VCAM-1 expression on the tumor vasculature reduced growth of human Hodgkin’s L540 tumors in CB17 SCID mice[95,96]. Other studies used conjugates consisting of tTF fused to proteins that bind to the tumor vasculature[97,98]. A prostate specific membrane antigen (PSMA)-directed/tTF fusion protein reduced the size of human prostate LuCap subcutaneous tumors in WEHI nude mice[97]. In addition, a heparin-binding domain (HBDt)-directed/tTF fusion proteins inhibited growth of breast carcinoma N202 orthotopic tumors in mice[98]. However, this approach has not been used clinically possibly because of concerns about thrombosis associated with tTF.

Hu and colleagues were the first to develop immunoconjugates that use TF to target the Fc effector domain of IgG to tumors. The Fc domain induces a cytolytic immune response by NK cells and complement activation to kill the tumor[99–101]. A mutant version of FVII (mFVIIasm) was used that had a point mutation in the active site that dramatically reduced it procoagulant activity but did not affect its high-affinity binding to TF. Intravenous injection of adenoviral vectors expressing a conjugate expressing human Fc inhibited the growth of human melanoma TF2 subcutaneous tumors in SCID mice[99]. In addition, mFVIIasm-humanFc reduced the growth of human melanoma LXSN and human prostate C4–2 subcutaneous tumors, and experimental metastasis of TF2 subcutaneous tumors to lung in SCID mice[100,101]. Similarly, mFVIIasm-mouseFc reduced the growth of B16F10 and murine prostate RM-1 subcutaneous tumors and spontaneous metastasis of RM-1 subcutaneous tumors to bone in C57BL/6 mice[100,101].

More recently, antibody drug conjugates (ADCs) have been developed that target TF. One group developed an anti-TF antibody-conjugated epirubicin incorporated in micelle (NC6300). This conjugate reduced the growth of TF high human pancreatic BxPc-3 and TF high human gastric 44As3 subcutaneous tumors but not TF low human pancreatic SUIT2 subcutaneous tumors in nude mice[102]. In the second study, an anti-human TF antibody

(clone 1859) that does not inhibit TF activity was used because this would reduce the risk of bleeding. This anti-TF antibody-conjugate reduced the growth of BxPc-3 subcutaneous tumors but not SUIT2 subcutaneous tumors[103]. Three groups developed different anti-TF antibody conjugates with monomethyl auristatin E (MMAE)[104–106]. MMAE inhibits cell division of actively dividing cells by blocking the polymerization of tubulin and shows an anti-tumor effect[107]. Koga and colleagues showed that both anti-human TF antibody-MMAE and anti-mouse TF antibody-MMAE conjugates inhibited the growth of TF high BxPc-3 and TF low human pancreatic Capan1 subcutaneous tumors. ADCs were shown to accumulate into tumors both actively and passively[105]. In a second study, the same group showed that different anti-human TF antibody-MMAE conjugates inhibited growth of BxPc-3 subcutaneous tumors in nude mice[108]. A study by Zhang and colleagues showed that their anti-TF antibody-MMAE conjugate reduced growth of both BxPc-3 and human liver HCC1806 subcutaneous tumors in a dose-dependent manner[106]. Finally, Breij and colleagues showed their anti-human TF antibody-MMAE conjugate that uses a non-inhibitory anti-TF antibody called Tisotumab vedotin inhibited growth of TF high human pancreatic HPAF-II subcutaneous tumors in SCID mice[104]. Moreover, the authors showed that their anti-human TF antibody-MMAE conjugate significantly reduced growth of several types of subcutaneous PDX tumors in PDX, including pancreatic tumors[104].

There are 6 clinical trials that aimed at investigating the effect of Tisotumab vedotin in patients with different types of cancer, including colorectal, pancreatic, and lung cancer (ClinicalTrials.gov identifier: NCT03245736, NCT03485209, NCT03438396, NCT03657043, NCT02552121, NCT02001623). It will be interesting to see if the anti-tumorigenic activity of Tisotumab vedotin observed in mouse studies translates to the clinical setting.

Conclusions

Many basic studies have investigated the regulation of TF in cancer cells, the roles of TF and asTF in tumor growth and metastasis, and the effect of anti-TF antibody/drug conjugate on tumor growth. However, the results are highly dependent on the selection of cancer cells, mice, tumor models and one must be careful with making generalizations. Directly targeting TF with antibodies may cause an increase in bleeding. Thus, ADCs that target TF using a non-inhibitory antibody may be a safer approach to reduce tumor growth without increasing bleeding.

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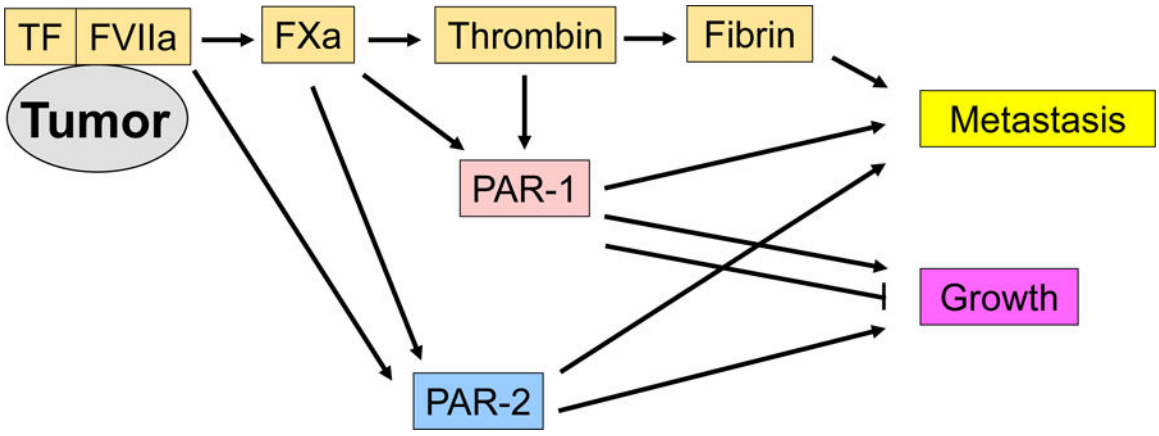

Figure 1.

Contributions of tumor tissue factor (TF) to tumor growth and metastasis. The tumor TF and factor (F) VIIa complex contributes to tumor growth and metastasis by activating coagulation cascade generating fibrin and by directly and indirectly activating protease activated receptors (PARs). There are negative and positive roles of PAR-1 in tumor growth.

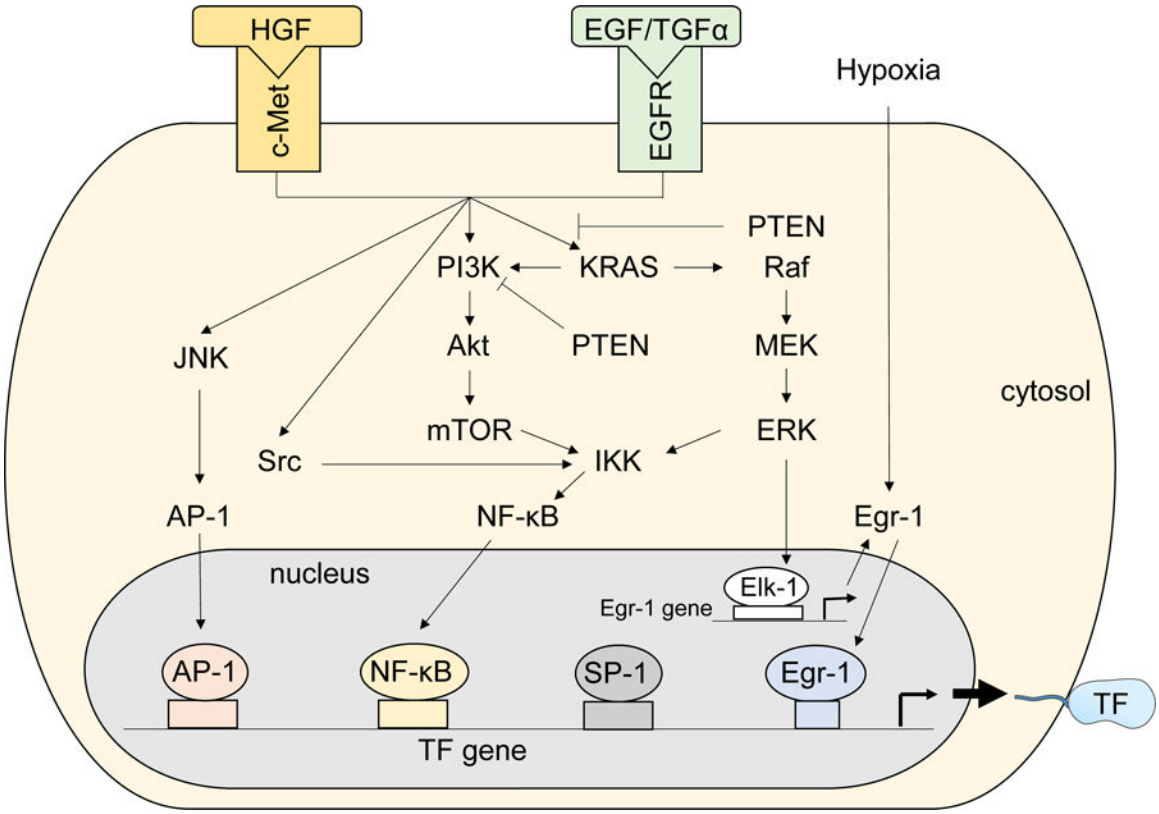

Figure 2.

Proposed regulation of tissue factor (TF) expression in cancer cells. Hepatocyte growth factor (HGF)/c-Met and EGFR pathways activate multiple kinase pathways, including c-Jun N-terminal kinase (JNK), Src, phosphatidylinositol-3 kinase (PI3k)/Akt/mammalian target of rapamycin (mTOR), and KRAS/Raf/MEK/ERK. These kinase pathways enhance TF gene expression by expressing transcription factors, such as activator protein-1 (AP-1), nuclear factor-κB (NF-κB), and early growth response protein-1 (Egr-1). TF protein was modified from Servier Medical Art, licensed under Creative Common Attribution 3.0 Unported License. (http://www.servier.fr/servier-medical-art)

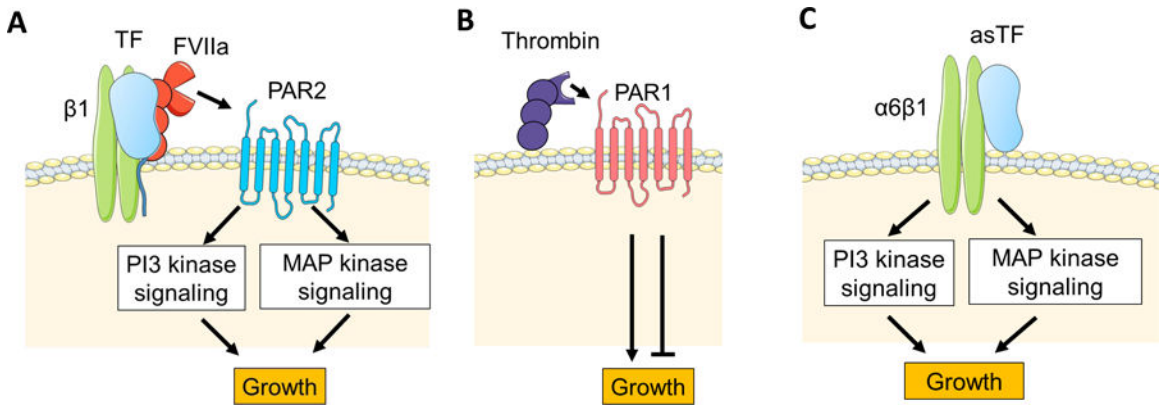

Figure 3.

Proposed mechanisms of tumor tissue factor (TF) and thrombin-dependent tumor growth. (A) The full-length TF/factor (F) VIIa complex binds to integrin β1 and the TF/FVIIa/integrin β1 complex activate protease activated receptor (PAR)2. PAR2 activates phosphatidylinositol- 3-kinase (PI3 kinase) and mitogen activated protease (MAP) kinase signalings resulting in tumor growth. (B) Thrombin activates PAR1 that has both positive and negative role in tumor growth. (C) Alternative spliced (as) TF is released from tumor cells and binds to integrin α6β1. This asTF/integrin α6β1complex contributes to tumor growth via PI3 and MAP kinases. Cells and proteins were modified from Servier Medical Art, licensed under Creative Common Attribution 3.0 Unported License. (http://www.servier.fr/servier-medical-art)

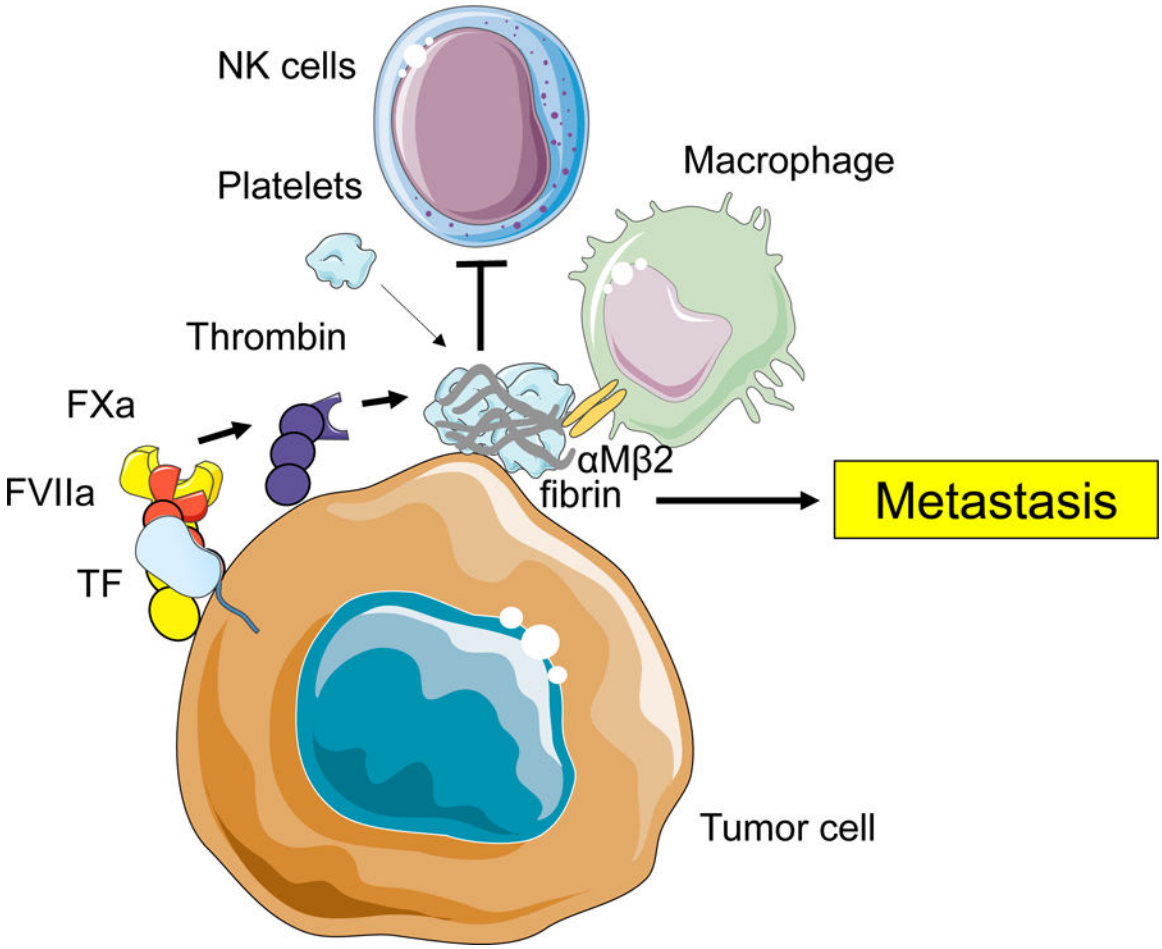

Figure 4.

Proposed mechanisms of tumor tissue factor (TF)-dependent metastasis. The full-length TF/factor (F)VIIa/FXa complex generates thrombin that activates platelets and generate fibrin. These activated platelets and fibrin inhibit the function of natural killer (NK) cells and attract monocytes/macrophage that helps establishment of premetastatic niche and tumor cell survival in metastatic niche. Cells and proteins were modified from Servier Medical Art, licensed under Creative Common Attribution 3.0 Unported License. (http://www.servier.fr/servier-medical-art)

Table 1

Tissue factor expression regulators and pathways in human cancer

Regulator	Pathway	Type of human cancer (cell line)	Refs
PI3K	PI3K/AKT/mTOR	glioma (23.11, U87MG)	21, 23
Akt	PI3K/AKT/mTOR	glioma (U87MG)	23
mTOR	PI3K/AKT/mTOR	glioma (23.11, U87MG), pancreatic (BON)	21, 23, 35
KRAS	RAS/MAP kinase	colorectal (379.2), colorectal and NSCLC primary tumor	32, 33, 38
Raf	RAS/MAP kinase	breast (MDA-MB-231)	26
MEK	RAS/MAP kinase	glioma (23.11), breast (MDA-MB-231)	21, 26
ERK	RAS/MAP kinase	glioma (U87MG), breast (MDA-MB-231)	23, 26
c-Met	MET receptor	brain (DAOY)	22
HGF	MET receptor	brain (DAOY)	22
Src	kinase	brain (DAOY)	22
EGF	EGFR family tyrosine kinase	breast (MDA-MB-468), endometrial (Ishikawa)	29, 37
TGFα	EGFR family tyrosine kinase	squamous cell carcinoma (A431)	36
EGFR	EGFR family tyrosine kinase	squamous cell carcinoma (A431), glioma (U87MG)	23, 27, 36
EGFRvIII	EGFR family tyrosine kinase	glioma (U373, U87MG)	23, 36
HER2	EGFR family tyrosine kinase	breast (SKBR3)	27
KSR1	EGFR family tyrosine kinase	squamous cell carcinoma (A431)	27
JNK1	JNK pathway	glioma (U87MG)	23
AP-1	transcription factor	glioma (U87MG), breast (MDA-MB-231)	23, 26
Egr-1	transcription factor	glioma (U87MG), breast (MDA-MB-231, MDA-MB-435)	23, 28
NF-kB	transcription factor	glioma (U87MG), breast (MDA-MB-231)	23, 26
SP-1	transcription factor	glioma (U87MG)	23
HIF-1α	transcription factor	breast (MDA-MB-231, MDA-MB-435)	28
p53	transcription factor	colorectal (379.2)	32, 33
Snail	transcription factor	breast (MDA-MB-468)	29
ZEB1	transcription factor	breast (MDA-MB-468)	29
PTEN	phosphatase	glioma (23.11, U87MG)	21, 23
IDH1	metabolic enzyme	primary glioma	24

PI3K: phosphatidylinositol-3 kinase; mTOR: mammalian target of rapamycin; HGF: hepatocyte growth factor; EGF: epidermal growth factor; TGFα: transforming growth factor alpha; EGFR: epidermal growth factor receptor; HER2: human epidermal growth factor receptor 2; KSR1: kinase suppressor of Ras 1; JNK1: c-Jun N-terminal kinase 1; AP-1: activator protein-1; Egr-1: early growth response protein-1; NF-κB: Nuclear factor-κB; HIF-1α: hypoxia induced factor-1α; PTEN: phosphatase and tensin homolog; IDH1: isocitrate dehydrogenase 1

Table 2

MicroRNAs regulating tissue factor expression in human cancer

miRNA	Type of human cancer (cell line)	Refs
miR-19a	breast (MCF7, MDA-MB-231), colorectal (LoVo, DLD1, HT29, SW 480), early stage colorectal primary tumor	30, 34
miR-19b	breast (MDA-MB-231)	31
miR-20a	breast (MDA-MB-231)	31
miR-93	leiomyosarcoma (SKLMS-1), primary cells from leiomyoma tumor	43
miR-106b	breast (MDA-MB-231), leiomyosarcoma (SKLMS-1), primary cells from leiomyoma tumor	31, 43
miR-520g	brain cancer (DAOY, UW228)	25