Tissue Factor as a Novel Target for Treatment of Breast Cancer

MARION COLE,^a MICHAEL BROMBERG^b^aDepartment of Medical Oncology, Fox Chase Cancer Center, Philadelphia, Pennsylvania, USA; ^bSection of Hematology, Department of Medicine, Sol Sherry Thrombosis Research Center, Temple University School of Medicine, Philadelphia, Pennsylvania, USA*Disclosures of potential conflicts of interest may be found at the end of this article.***Key Words.** Tissue factor • Breast cancer • Activated factor VII • Cell signaling**CME Learning Objectives**

Explain the process by which tissue factor (TF) initiates blood coagulation and is implicated in tumor progression.

Describe the proposed mechanisms of targeting TF in malignancy.

Discuss the applications of TF targets in developing new treatments for aggressive cancers including triple-negative breast cancer.

ABSTRACT

Tissue factor (TF), a 47-kDa transmembrane glycoprotein that initiates blood coagulation when complexed with factor VIIa (FVIIa), is expressed in several tumor types. TF has been shown to play a role in cell signaling, inflammation, angiogenesis, as well as tumor growth and metastasis. Activation of the TF signaling pathway has been implicated in mediating the function of many tumor cell types and has led to TF as a potential target in the treatment of several malignancies. Formation of the TF-FVIIa complex in breast cancer cells has been shown to exert an antiapoptotic effect and play a key role in tumor growth and metastasis. Breast cancer growth is suppressed by inhibi-

tion of TF-mediated PAR2 signaling, and deficiency in PAR2 delays spontaneous breast cancer development in mice. TF is expressed in triple-negative breast cancer (TNBC), an aggressive type of breast cancer in which there is currently a paucity of available targets. Various methods of targeting TF have been investigated and include immunoconjugates or icons, anti-TF antibodies, TF pathway inhibitors, targeted photodynamic therapy, and microRNAs. These investigations may give way to promising clinical therapies for breast cancer, especially in TNBC, for which there are relatively few effective treatment options. *The Oncologist* 2013;18:14–18

Implications for Practice: Tissue factor (TF), a kDa transmembrane glycoprotein that binds with factor VII during blood coagulation, has been expressed in many tumor types. It plays a role in tumor growth and metastasis, which has made it a potential target for disease intervention. One malignancy in which TF is frequently expressed, and for which it is a potential therapeutic target, is breast cancer—especially triple-negative breast cancer (TNBC). TF is highly expressed in aggressive breast cancers, and TNBC is an aggressive breast cancer that carries a poor prognosis. To date, few treatment options have been available for TNBC. Various methods of targeting TF have been investigated, including using anti-TF antibodies, immunoconjugates or icons, targeted photodynamic therapy, TF pathway inhibitors, and microRNAs. Each has had some success in experimental trials and is described in detail. Targeting TF is likely to lead to useful clinical applications in breast cancer, especially TNBC and other malignancies.

INTRODUCTION

Tissue factor (TF) is a 47-kDa transmembrane glycoprotein that initiates blood coagulation when complexed with its cofactor, factor VIIa (FVIIa). The TF molecule consists of a 219-amino-acid extracellular domain, a 23-amino-acid transmembrane domain, and a 21-amino-acid cytoplasmic domain [1]. The extracellular domain of TF is required for procoagulant function [2]. This domain consists of two fibronectin type III-like domains that resemble several growth factor and cytokine receptors [3]. The cytoplasmic domain of TF, which is not required

for procoagulant function, contains three serine residues that can be phosphorylated [4] and have been implicated in cell signaling [4, 5]. TF is essential for normal hemostasis and embryonic development [6, 7]. In addition, TF is expressed in a variety of tumor cell types and has been linked to the pathogenesis of cancer [6].

Upon vessel injury, TF expressed in fibroblasts is exposed to the bloodstream. Blood coagulation is initiated when TF binds to the serine protease, FVIIa [1]. Formation of the TF-FVIIa complex

Correspondence: Marion Cole, M.D., Fox Chase Cancer Center, 333 Cottman Avenue, Philadelphia, PA 19111, USA. Telephone: 240-893-5139; Fax: 215-728-3639; E-mail: Marion.Cole@fccc.edu; or Michael Bromberg, M.D., Ph.D., Temple University Hospital, 3400 North Broad Street, Old Med Sch Bldg, Ste 300, Philadelphia, PA 19140, USA. Telephone: 215-707-6356; Fax: 215-707-2783; E-mail: mbromber@temple.edu Received July 28, 2012; accepted for publication October 12, 2012; first published online in *The Oncologist Express* on January 3, 2013. ©AlphaMed Press 1083-7159/2013/\$20.00/0 <http://dx.doi.org/10.1634/theoncologist.2012-0322>

leads to activation of factor X and factor IX that, in turn, generates activated factor X (FXa) and activated factor IX (FIXa), respectively. Generation of FXa leads to the conversion of prothrombin to thrombin. Thrombin subsequently cleaves soluble fibrinogen to form a fibrin clot [1]. In addition, there is a circulating pool of TF that contributes to clot propagation [8]. An alternatively spliced TF (asTF) protein has also been identified, which appears to be active in promoting tumor growth and angiogenesis, but its role in blood coagulation is still unclear [9, 10].

Abnormalities in the coagulation cascade leading to a hypercoagulable state are a well-known complication of malignancy. Trousseau syndrome, a migratory thrombophlebitis, is a common manifestation of the increased coagulability seen in patients with cancer and often precedes the diagnosis of a malignancy [11]. Many aspects of cancer contribute to hypercoagulability, including TF expression in tumor cells and upregulation of TF in vascular endothelium and monocytes by inflammatory cytokines, interleukin-1, and tumor necrosis factor- α [6]. In addition, extrinsic compression of blood vessels by tumors, impaired clearance of activated coagulation factors in the setting of hepatic disease, treatments of the cancer including medications (cytotoxic agents and angiogenesis inhibitors), surgery, and patient immobility contribute to the hypercoagulability in malignancy [6].

TF is expressed in many tumor cell types, including breast cancer, gliomas, lung cancer, colorectal cancer, pancreatic cancer, prostate cancer, ovarian cancer, renal cell cancer, and hepatocellular cancer [12]. Tumor cells that are poorly differentiated appear, in general, to have increased TF expression compared with those that are more differentiated [6]. Tumors associated with high bloodborne TF levels are associated with a greater risk of thromboembolic disease [13] and with decreased overall survival [14]. Elevated circulating TF levels result from tumor shedding of TF-bearing microparticles (MPs) through leaky tumor blood vessels, tumor-induced upregulation of TF expression in monocytes and endothelial cells, and upregulation of endothelial cell TF expression by chemotherapeutic agents [6].

TF has also been implicated in tumor progression. Increased TF expression in tumor cells promotes their hematogenous metastasis [15, 16]. In addition, fibrin formation as a result of activation of blood coagulation and platelets shields tumor cells from NK cells and immune surveillance [6]. Finally, the TF-mediated signaling pathway has been implicated in tumor growth. Formation of either TF-FVIIa-FXa complex or TF-FVIIa complex on tumor cells results in cellular signaling, which involves phosphorylation of a p44/42 mitogen-activated protein kinase, Akt/PKB, mammalian target of rapamycin, and p70 S6K1 via activation of G-protein coupled protease-activated receptors (PARs) [17–21]. The functional consequences of activation of these pathways result in enhanced tumor cell migration, inhibition of apoptosis, and promotion of cellular growth [17–21].

TISSUE FACTOR IN BREAST CANCER

Human breast cancer tissue and cell lines have been shown to express TF [12, 22–24]. TF is highly expressed on aggressive breast cancer cell lines, including MDA-MB-231 and HS578 [25, 26]. The MCF-7 breast cancer cell line, which has low endogenous TF expression, displays increased metastatic potential when transfected with cDNA encoding for human TF compared with the vector transfected control line in immuno-

compromised mice [27]. Tumor TF expression is also of prognostic significance because high expression is predictive of decreased overall survival in patients with breast cancer [14].

TF plays a role in breast cancer cell signaling and promotes tumor cell migration and inhibition of apoptosis [17, 20, 21]. The presence of TF on breast cancer tumor vasculature, but not on the vasculature of benign breast masses, has implicated TF in neoplastic angiogenesis [28]. The role of TF in breast tumor growth is further strengthened in studies with mouse models, which have shown that breast cancer growth is suppressed by inhibition of the TF-mediated PAR2 signaling and that deficiency in PAR2 delays spontaneous breast cancer development in mice [29]. In addition, in patients with primary breast cancer, shorter recurrence-free survival correlated with tumors expressing phosphorylated TF (pTF) alone as well as coexpression of pTF and PAR-2. These results further support the role of TF-PAR2 signaling in breast cancer progression [30].

Several human breast cancer cell lines with the triple-negative breast cancer (TNBC) phenotype express TF, including MDA-MB-231, MDA-MB-468, and HCC-1806 [31]. TNBC, which lacks expression of receptors for estrogen (ER), progesterone (PR) and Her-2/*neu*, is an aggressive type of breast cancer that often affects younger women and has a poor

Tumors associated with high bloodborne TF levels are associated with a greater risk of thromboembolic disease and with decreased overall survival. Elevated circulating TF levels result from tumor shedding of TF-bearing microparticles (MPs) through leaky tumor blood vessels, tumor-induced upregulation of TF expression in monocytes and endothelial cells, and upregulation of endothelial cell TF expression by chemotherapeutic agents.

prognosis [32]. Preliminary data from our laboratory has also shown that TF is expressed in 80% (36/45) of TNBC patient biopsies (unpublished observations). Given the poor prognosis for TNBC and the paucity of targeted therapies for this type of breast cancer, targeting TF might be a promising therapeutic strategy.

Antibodies Against Tissue Factor

Formation of TF-FVIIa and TF-FVIIa-Xa complexes leads to activation of PAR2 and PAR1 signaling, which promote migration and proliferation of tumor cells. Anti-TF antibodies have been shown to inhibit metastases of human breast cancer cell lines in murine models [33, 34]. Two antibodies that block its coagulation function are the murine antihuman TF antibody TF8–5G9 and a humanized version of this antibody, CNTO 859 [33, 35]. CNTO-859 was shown to be highly effective in inhibiting metastatic spread of human MDA-MB-231 breast cancer cells to the lungs of SCID Beige mice, with a near complete reduction in lung metastases in the treated mice [35, 33].

Anti-TF antibodies that do not alter the procoagulant function of TF also result in inhibition of tumor progression [34]. Versteeg et al. [34] showed that the antitumor activity of the anti-TF antibody Mab-10H10, which does not inhibit coagulant activity but uncouples the interaction of TF to β 1 integrin,

results in decreased proliferation of MDA-MD-231 breast cancer cells. TF-dependent PAR2 signaling was also implicated in promoting tumor growth and shown to be inhibited by specific cleavage-blocking antibodies to PAR2. This effect was not seen with anti-PAR1 antibodies [34].

Although the approach involving antibodies against TF and TF-FVIIa complex appears promising, there may be associated risks. The most concerning risk is bleeding, which has been observed with some anticoagulants in patients with cancer [36]. Although a trial using TF pathway inhibitor, inactivated recombinant VIIa, in patients with acute lung injury was associated with a trend toward serious bleeding events [37], studies with anti-TF antibodies thus far have not shown a major incidence of bleeding. No serious bleeding events have been reported in clinical trials with Sunolch36 (also known as ALT-836), a monoclonal antibody against TF, in patients with coronary artery disease or acute lung injury [38, 39]. Currently, the risk of bleeding with anti-TF antibodies that inhibit coagulation is not yet known. Alternatively, anti-TF antibodies have been developed that specifically inhibit TF-FVIIa signaling without affecting the pro-coagulant function [34]. Taken together, these studies suggest that targeting either the procoagulant or signaling functions of TF might be useful therapeutic strategies in breast cancer.

Immunoconjugates (Icons)

Another therapeutic strategy that targets TF in tumors is immunotherapy using immunoconjugates (icons). Icons are chimeric molecules that involve a mutated factor VII (mfVII) targeting domain, which does not have coagulant function, and an Fc effector domain of IgG1 Fc (mfVII/Fc icon) [40]. Using one approach, the icon was encoded in a replication-incompetent adenoviral vector and then injected into the tumor cells, which then expressed the icon. In this study, Hu et al. [40] showed that the mutated factor VII domain of the icon binds with high affinity to TF and the Fc domain of the icon activates an immune cytolytic attack, which is mediated through natural killer cells as well as the complement pathway.

Icon therapy has been successful in mouse models of both melanoma and prostate cancer. In prostate cancer, icon injection into tumor cells resulted in inhibition of tumor growth and tumor regression in a SCID mouse model [40]. Another promising finding with regard to treatment of metastatic prostate tumors was that the mice that were injected with the icon showed regression of the tumor that was injected as well as the tumor that was not injected, suggesting that this therapy might be useful in disseminated tumors that might not be accessible for injection.

With regard to icon treatment safety, the prothrombin time (PT) was studied as a measurable parameter to assess for bleeding risk [40]. Bleeding risk was not readily detected in any of the examined organs, and the concentration of icon in the mice used to produce a tumor response was 1% of the minimum concentration required to prolong the PT [40]. The icon did not appear to bind to any of the normal organs in the mice [40].

More recently, investigators have used mutant factor VII (fVII) along with a photosensitizer to TF expressing tumor vasculature and tumors followed by laser irradiation. Hu et al. conjugated the photosensitizer verteporfin (VP) to mutant fVII for ligand-targeted photodynamic therapy [41, 42]. This

type of targeted photodynamic therapy induced a greater inhibition of breast cancer cell growth than nontargeted photodynamic therapy and may be a promising approach to chemotherapy-resistant breast cancer cells [43].

Although FVII is synthesized by the liver and released into the blood, recent studies have demonstrated that FVII is also produced ectopically by several cancer cell lines, including breast cancer [44–46]. Curcumin, a dietary compound, has been shown to inhibit constitutive ectopic expression through inhibition of p300/CBP activity. Icon therapy might also target ectopic factor VII as another approach to blocking the TF-FVIIa-PAR2 signaling pathway [46].

Tissue Factor Pathway Inhibitors

The TF pathway in blood coagulation is inhibited by TF pathway inhibitors (TFPIs). TFPI is expressed in breast, pancreatic, and colon cancer cell lines and may function to prevent auto-coagulation of vessels within the tumor [47]. Cells in tumor microvasculature endothelium produce TFPI-1; its main function is inhibition of TF-FVIIa-FXa complex. Inhibition of tumor growth, including both primary tumor as well as metastases, was demonstrated with the use of B16 melanoma mouse models treated with TFPI [47].

Other investigators have studied the effect of treating B16 mouse models as well as Lewis lung carcinoma mouse models with two small anticoagulants isolated from the hematophagous nematode *A. caninum*: rNAPc2 and rNAP5. Results from these studies showed that rNAPc2, which is directed against TF-FVIIa, inhibited tumor growth in both models, whereas rNAP5, which is directed against FXa, did not inhibit tumor growth. This finding suggested that the mechanism of tumor inhibition is specific to a pathway involving TF-FVIIa and not FXa. Furthermore, the function of TF-FVIIa in cancer angiogenesis and metastasis appears distinct from its function in coagulation. Other studies had shown a role for FXa in tumor metastases; however, the mechanism by which this takes place is thought to be by facilitating tumor cell seeding and not angiogenesis [47].

MicroRNA

One of the most recent therapeutic strategies targeting TF has used microRNA. In a recent study, microRNA-19 (miR-19) was shown to be instrumental in regulating TF expression in breast cancer cells at the posttranscriptional level [48]. In this study, breast cancer cell lines that were less aggressive, including

Using TF as a target might be useful in combating the most aggressive cancers, in which TF is frequently highly expressed. TF expression is observed in both human breast cell lines and tissues. Treating TNBCs, which has been difficult given their high grades, large tumor burdens, and historical lack of available therapeutic agents, may be a particularly useful clinical application for targeting TF.

MCF-7, T47D, and ZR-75–1, minimally expressed TF; however, in more aggressive breast cancer cell lines, including MDA-MB-231 and BT-20, TF was highly expressed. MicroRNAs are conserved molecules in organisms that regulate gene ex-

pression. MicroRNAs can bind to the 3' untranslated (UTR) region of the transcript and thus repress protein translation or make the mRNA unstable. In MCF-7 cells, translation of the 3' UTR region was found to be necessary for inhibition of TF expression. However, TF was expressed in the MCF-7 cells if the miR-19 binding site to 3' UTR was deleted. In addition, overexpression of miR-19 in MDA-MB-231 cells resulted in downregulation of TF expression. These findings indicate that altering microRNAs that regulate TF expression might be another novel strategy in breast cancer treatment.

CONCLUSIONS

TF is frequently expressed in a variety of human breast cancer types. Using TF as a target might be useful in combating the most aggressive cancers, in which TF is frequently highly expressed. TF expression is observed in both human breast cell lines and tissues. Treating TNBCs, which has been difficult given their high grades, large tumor burdens, and historical lack of available therapeutic agents, may be a particularly useful clinical application for targeting TF. Several different methods have been devised to target the TF pathway using anti-TF antibodies, icons, TFPIs, targeted photodynamic

therapy, and microRNAs. Each of these has had some success in recent experimental trials; however, potential side effects, such as bleeding and the specificity of targeted agents, remain to be fully clarified. A greater understanding of the mechanisms involved and the role of TF-mediated signaling in tumor growth and metastasis, as well as the TF pathway's overall interaction with other components of the immune response, will likely lead to the further development of TF as a novel target in breast cancer, as well as other malignancies in the future.

AUTHOR CONTRIBUTIONS

Conception and design: Marion Cole, Michael Bromberg

Manuscript writing: Marion Cole, Michael Bromberg

DISCLOSURES

The authors indicated no financial relationships.

Section Editors: Gabriel Hortobágyi: Antigen Express, Galena Biopharma, Novartis, Rockpointe (C/A), Novartis (RF), TaiVex (OI), and is a founder and member of the board of directors for Citizen's Oncology Foundation; Kathleen Pritchard: Novartis, Roche, AstraZeneca, Pfizer, Abraxis, Boehringer-Ingelheim, GlaxoSmithKline, Sanofi, Ortho-Biotech, YM Biosciences, Amgen, Bristol-Myers Squibb, Bayer Schering Pharma (C/A, H).

Reviewer "A": Johnson and Johnson (C/A)

Reviewer "B": None

(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board

REFERENCES

- Nemerson Y. Tissue factor and hemostasis. *Blood* 1988;71:1–8.
- Paborsky LR, Caras IW, Fisher KL et al. Lipid association, but not the transmembrane domain, is required for tissue factor activity. Substitution of the transmembrane domain with a phosphatidylinositol anchor. *J Biol Chem* 1991;266:21911–21916.
- Muller YA, Ultsch MH, de Vos AM. The crystal structure of the extracellular domain of human tissue factor refined to 1.7 Å resolution. *J Mol Biol* 1996;256:144–159.
- Zioncheck TF, Roy S, Vehar GA. The cytoplasmic domain of tissue factor is phosphorylated by a protein kinase C-dependent mechanism. *J Biol Chem* 1992;267:3561–3564.
- Belting M, Dorrell MI, Sandgren S et al. Regulation of angiogenesis by tissue factor cytoplasmic domain signaling. *Nat Med* 2004;10:502–509.
- Kasthuri RS, Taubman MB, Mackman N. Role of tissue factor in cancer. *J Clin Oncol* 2009;27:4834–4838.
- Konigsberg W, Kirchhofer D, Riederer MA et al. The TF:VIIa complex: Clinical significance, structure-function relationships and its role in signaling and metastasis. *Throm Haemost* 2001;86:757–771.
- Giesen PL, Rauch U, Bohrmann B et al. Blood-borne tissue factor: Another view of thrombosis. *Proc Natl Acad Sci U S A* 1999;96:2311–2315.
- Hobbs JE, Zakarija A, Cundiff DL et al. Alternatively spliced human tissue factor promotes tumor growth and angiogenesis in a pancreatic cancer tumor model. *Throm Res* 2007;120:S13–S21.
- Van den Berg YW, van den Hengel LG, Myers HR et al. Alternatively spliced tissue factor induces angiogenesis through integrin ligation. *Proc of the Natl Acad Sci U S A* 2009;106:19497–19502.
- Sack GH Jr, Levin J, Bell WR. Trousseau's syndrome and other manifestations of chronic coagulopathy in patients with neoplasms: Clinical, pathophysiologic, and therapeutic features. *Medicine* 1977;56:1–37.
- Callander NS, Varki N, Rao LV. Immunohistochemical identification of tissue factor in solid tumors. *Cancer* 1992;70:1194–1201.
- Khorana AA, Francis CW, Menzies KE et al. Plasma tissue factor may be predictive of venous thromboembolism in pancreatic cancer. *J Thromb Haemost* 2008;6:1983–1985.
- Ueno T, Toi M, Koike M et al. Tissue factor expression in breast cancer tissues: Its correlation with prognosis and plasma concentration. *Br J Cancer* 2000;83:164–170.
- Bromberg ME, Konigsberg WH, Madison JF et al. Tissue factor promotes melanoma metastasis by a pathway independent of blood coagulation. *Proc Natl Acad Sci U S A* 1995;92:8205–8209.
- Bromberg ME, Sundaram R, Homer RJ et al. Role of tissue factor in metastasis: functions of the cytoplasmic and extracellular domains of the molecule. *Throm Haemost* 1999;82:88–92.
- Jiang X, Bailly MA, Panetti TS et al. Formation of tissue factor-factor VIIa-factor Xa complex promotes cellular signaling and migration of human breast cancer cells. *J Thromb Haemost* 2004;2:93–101.
- Sorensen BB, Freskgard PO, Nielsen LS et al. Factor VIIa-induced p44/42 mitogen-activated protein kinase activation requires the proteolytic activity of factor VIIa and is independent of the tissue factor cytoplasmic domain. *J Biol Chem* 1999;274:21349–21354.
- Versteeg HH, Sorensen BB, Slofstra SH et al. VIIa/tissue factor interaction results in a tissue factor cytoplasmic domain-independent activation of protein synthesis, p70, and p90 S6 kinase phosphorylation. *J Biol Chem* 2002;277:27065–27072.
- Jiang X, Guo YL, Bromberg ME. Formation of tissue factor-factor VIIa-factor Xa complex prevents apoptosis in human breast cancer cells. *Thromb Haemost* 2006;96:196–201.
- Jiang X, Zhu S, Panetti TS et al. Formation of tissue factor-factor VIIa-factor Xa complex induces activation of the mTOR pathway which regulates migration of human breast cancer cells. *Throm Haemost* 2008;100:127–133.
- Sturm U, Luther T, Albrecht S et al. Immunohistological detection of tissue factor in normal and abnormal human mammary glands using monoclonal antibodies. *Virchows Arch A Path Anat and Histo-pathol* 1992;421:79–86.
- Muller M, Flossel C, Haase M et al. Cellular localization of tissue factor in human breast cancer cell lines. *Virchows Archiv B Cell Pathol Incl Mol Pathol* 1993;64:265–269.
- Vrana JA, Stang MT, Grande JP et al. Expression of tissue factor in tumor stroma correlates with progression to invasive human breast cancer: paracrine regulation by carcinoma cell-derived members of the transforming growth factor beta family. *Cancer Res* 1996;56:5063–5070.
- Schiemann S, Schwirzke M, Brunner N et al. Molecular analysis of two mammary carcinoma cell lines at the transcriptional level as a model system for progression of breast cancer. *Clin Exp Metastasis* 1998;16:129–139.
- Kirschmann DA, Seftor EA, Nieva DR et al. Differentially expressed genes associated with the metastatic phenotype in breast cancer. *Breast Cancer Res Treat* 1999;55:127–136.
- Hu TBM, Garen A et al. Metastasis is promoted by tissue factor gene transfection into MCF-7 breast cancer cells. *Proc Am Assoc Cancer Res* 1995;36:78.
- Cottrino J, Hair G, Kreutzer DL et al. In situ detection of tissue factor in vascular endothelial cells: Correlation with the malignant phenotype of human breast disease. *Nat Med* 1996;2:209–215.
- Ruf W, Yokota N, Schaffner F. Tissue factor in cancer progression and angiogenesis. *Throm Res* 2010;125:S36–S38.
- Ryden L, Grabau D, Schaffner F et al. Evidence for tissue factor phosphorylation and its correlation with protease-activated receptor expression and

the prognosis of primary breast cancer. *Int J Cancer* 2010;126:2330–2340.

31 Finn RS, Dering J, Ginther C et al. Dasatinib, an orally active small molecule inhibitor of both the src and abl kinases, selectively inhibits growth of basal-type/“triple negative” breast cancer cell lines growing in vitro. *Breast Cancer Res Treat* 2007;105:319–326.

32 Maegawa RO, Tang SC. Triple-negative breast cancer: Unique biology and its management. *Cancer Invest* 2010;28:878–883.

33 Ngo CV, Picha K, McCabe F et al. CNTO 859, a humanized anti-tissue factor monoclonal antibody, is a potent inhibitor of breast cancer metastasis and tumor growth in xenograft models. *Int J Cancer* 2007;120:1261–1267.

34 Versteeg HH, Schaffner F, Kerver M et al. Inhibition of tissue factor signaling suppresses tumor growth. *Blood* 2008;111:190–199.

35 Mueller BM, Reisfeld RA, Edgington TS et al. Expression of tissue factor by melanoma cells promotes efficient hematogenous metastasis. *Proc Natl Acad Sci U S A* 1992;89:11832–11836.

36 Zacharski LR, Henderson WG, Rickles FR et al. Effect of warfarin anticoagulation on survival in carcinoma of the lung, colon, head and neck, and prostate. Final report of VA Cooperative Study #75. *Cancer* 1984;53:2046–2052.

37 Vincent JL, Artigas A, Petersen LC et al. A multicenter, randomized, double-blind, placebo-controlled, dose-escalation trial assessing safety and efficacy of active site inactivated recombinant factor VIIa in subjects with acute lung injury or acute respiratory distress syndrome. *Crit Care Med* 2009;37:1874–1880.

38 Morrow DA, Murphy SA, McCabe CH et al. Potent inhibition of thrombin with a monoclonal antibody against tissue factor (Sunol-H36): Results of the PROXIMATE-TIMI 27 trial. *Eur Heart J* 2005;26:682–688.

39 Morris PE, Steingrub JS, Huang BY et al. A phase I study evaluating the pharmacokinetics, safety and tolerability of an antibody-based tissue factor antagonist in subjects with acute lung injury or acute respiratory distress syndrome. *BMC Pulm Med* 2012;12:5.

40 Hu Z, Garen A. Targeting tissue factor on tumor vascular endothelial cells and tumor cells for immunotherapy in mouse models of prostatic cancer. *Proc Natl Acad Sci U S A* 2001;98:12180–12185.

41 Hu Z, Rao B, Chen S et al. Targeting tissue factor on tumour cells and angiogenic vascular endothelial cells by factor VII-targeted verteporfin photodynamic therapy for breast cancer in vitro and in vivo in mice. *BMC Cancer* 2010;10:235.

42 Hu Z, Rao B, Chen S et al. Selective and effective killing of angiogenic vascular endothelial cells and

cancer cells by targeting tissue factor using a factor VII-targeted photodynamic therapy for breast cancer. *Breast Cancer Res Treat* 2011;126:589–600.

43 Duanmu J, Cheng J, Xu J et al. Effective treatment of chemoresistant breast cancer in vitro and in vivo by a factor VII-targeted photodynamic therapy. *Br J Cancer* 2011;104:1401–1409.

44 Koizume S, Jin M, Miyagi E et al. Activation of cancer cell migration and invasion by ectopic synthesis of coagulation factor VII. *Cancer Res* 2006;66:9453–9460.

45 Yokota N, Koizume S, Miyagi E et al. Self-production of tissue factor-coagulation factor VII complex by ovarian cancer cells. *Br J Cancer* 2009;101:2023–2029.

46 Koizume S, Yokota N, Miyagi E et al. Hepatocyte nuclear factor-4-independent synthesis of coagulation factor VII in breast cancer cells and its inhibition by targeting selective histone acetyltransferases. *Mol Cancer Res* 2009;7:1928–1936.

47 Hembrough TA, Swartz GM, Papathanassiou A et al. Tissue factor/factor VIIa inhibitors block angiogenesis and tumor growth through a nonhemostatic mechanism. *Cancer Res* 2003;63:2997–3000.

48 Zhang X, Yu H, Lou JR et al. MicroRNA-19 (miR-19) regulates tissue factor expression in breast cancer cells. *J Biol Chem* 2011;286:1429–1435.