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Tissue Factor signaling: a multi-faceted function in biological processes

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1. ABSTRACT

Tissue factor (TF), originally discovered to initiate coagulation, is more recently recognized to be involved in other biological processes, such as migration and anti-apoptosis. TF-mediated signaling regulates gene expression and protein synthesis, leading to alterations in cellular behavior. The proteolytic activity of factor VIIa (FVIIa), beta-1 integrin interaction and protease-activated receptor (PAR) activation are some of the key events involved in TF signaling. Post-translational modifications of TF may regulate signaling but this remains elusive. *In vivo* studies have established that TF signaling severely contributes to processes like angiogenesis, cancer growth and inflammation. This review focuses on the mechanism underlying TF-mediated intracellular signaling with its related physiological and mainly pathological consequences.

2. INTRODUCTION

Tissue factor (TF), a 47 kDa transmembrane glycoprotein consisting of an extracellular, transmembrane and cytoplasmic domain, is the primary initiator of the coagulation cascade. TF is abundantly expressed at anatomical sites where the risk of bleeding is relatively high, like placenta, brain and lung tissues (1;2). Under quiescent conditions, TF is constitutively expressed on cells residing in subendothelial tissues including adventitial fibroblasts (3). Upon endothelial injury, perivascular TF becomes exposed to the blood stream and therefore is able to bind with high affinity to the circulating zymogen factor VII (FVII), which is activated to FVIIa. The TF:FVIIa complex then catalyzes the conversion of both inactive factor X (FX) and factor IX (FIX) into active FX (FXa) and FIX (FIXa) (4), leading to subsequent thrombin and fibrin

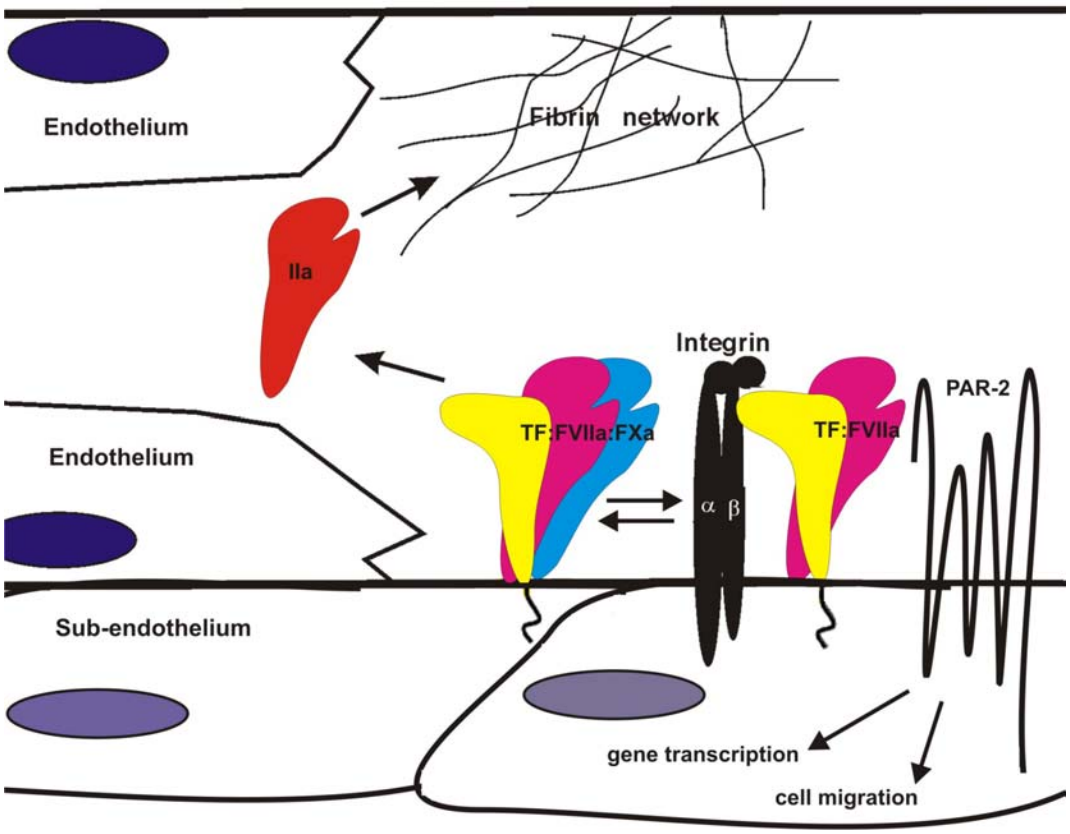

Figure 1. TF-dependent signaling. Upon vascular injury, leading to removal of the endothelium, TF expressed on subendothelial vascular smooth muscle cells and fibroblasts is exposed to the bloodstream, leading to subsequent FVIIa, FXa, thrombin (II) and fibrin formation. Simultaneously, TF:FVIIa cleaves PAR-2, leading to downstream cellular events such as cell migration and gene transcription. Association of TF with integrins facilitates this process.

generation as well as platelet activation, culminating in a hemostatic plug at the site of injury (Figure 1).

In addition to its prominent role in coagulation it has now been widely recognized that TF also influences non-hemostatic processes under physiological and pathological conditions. TF is critical for embryonic development, since TF-deficient mice die *in utero* due to abnormal vascular development and bleeding in the yolk sac (5-7). Furthermore, TF expression by cancer cells has been implicated in cancer-related thrombosis, but also in non-hemostatic tumor processes, including tumor growth, tumor angiogenesis and metastasis (8;9). Finally, the inflammatory response in several sepsis models is characterized by increased expression of TF in several organs and on monocytes circulating in plasma of patients (10-13); this upregulation of TF severely contributes to the inflammatory process. Importantly, TF shows similarities to the interferon receptors of the cytokine receptor class II family (14) and this has prompted researchers to probe the role of TF as a bona fide cellular receptor for its ligand FVIIa, in physiological and pathological processes. Here, we will review studies providing evidence for TF's role as a cellular receptor and its involvement in (patho)physiological events.

3. PARS

As discussed above, TF resembles a cytokine class II receptor (14) and initially it was believed that TF signaling occurred in a fashion similar to that evoked by e.g. interferon-gamma. However, in 2000, Camerer and colleagues showed that, rather than acting as a receptor itself, TF functions as a "platform" for FVIIa to activate protease-activated receptor (PAR)-2, and to a lesser extent PAR-1 in transfected cells (15) (figure 1), although this has never been shown in endogenous PAR-1 expressing cells. PARs belong to the G protein-coupled seven-transmembrane receptor family and are activated upon cleavage of the N-terminal domain. A tethered ligand domain is subsequently formed that binds to the secondary extracellular loop as part of the receptor, leading to the activation of heterotrimeric G-protein subunits. At present, four members of this receptor family are known: PAR-1, PAR-2, PAR-3 and PAR-4 (reviewed in (16)). PAR-1, or thrombin receptor, is the archetypical PAR and is activated by proteases such as thrombin, activated protein C (APC), FXa, plasmin and metalloproteases (17-19). Like PAR-1, PAR-3 and PAR-4 are readily activated by thrombin at higher concentrations than those needed for PAR-1 activation, although PAR-3 appears mostly to function as a

co-receptor for PAR-4 (20). Additionally, the thrombin-activated N-terminus of PAR-1 is able to transactivate PAR-2 (21). PAR-2 is unique with respect to its protease-specificity; it is the only PAR that is not cleaved by thrombin, but can be activated by proteases such as trypsin, mast cell tryptase, FVIIa and FXa (22). By forming a ternary complex with FVIIa and FXa, TF can target both endogenous PAR-1 and PAR-2 (15;23).

4. POTENTIAL MECHANISMS UNDERLYING TF SIGNALING

Like in coagulation, proteolytically active FVII similarly forms a complex with TF prior to inducing signaling, affecting gene expression and protein synthesis of cells. These events lead to the regulation of cellular processes such as migration and survival, which are important features for cells to participate in biological processes. TF may also initiate the signaling procedure via PAR-independent mechanisms. In this part of our review we will discuss the cellular consequences of TF:FVIIa signaling and the signal transduction pathways that underlie these events.

4.1. Calcium signaling

Extracellular stimuli induce calcium signaling via the activation of the phosphatidyl inositol (PI) hydrolysis pathway (reviewed in (24). In response to FVIIa exposure, several cell types expressing TF, such as MDCK cells, transfected COS-1 cells, human endothelial cells induced to express TF, and bladder carcinoma cell line J82, have been shown to elicit intracellular calcium release (25;26). Phospholipase C (PLC), a key player in PI hydrolysis, was identified to function as a downstream signaling mediator in TF:FVIIa-induced calcium response. Specifically PLC-beta is suggested to be activated, as inhibition of the upstream PLC-gamma-associated tyrosine kinases did not affect the FVIIa-induced calcium response (26). Following PLC activation, calcium release is directly induced by inositol triphosphate (IP3), whereas this release via diacylglycerol (DAG)-mediated protein kinase C (PKC) activation is an indirect effect (reviewed in (24)). FVIIa stimulation of TF-expressing baby hamster kidney cells (TF-BHKs) leads to PKC activation which was indicated to be an upstream signaling protein in mitogen-activated protein kinase (MAPK) signaling (27). However, it is pertinent to note that many TF-expressing cells do not show calcium transients in response to FVIIa (28). The nature of the mechanism governing TF:FVIIa-induced calcium signaling remains unclear, but differences in PAR-2 expression and TF cytoplasmic tail-dependent PAR-2 inhibition may play a role.

4.2. Gene expression

TF-dependent FVIIa signaling affects cellular behavior, amongst others, by influencing the transcriptional machinery. FVIIa stimulates the expression of poly(A)polymerase in fibroblasts, thus stimulating mRNA processing and stability (29). Moreover, in 2000, Camerer and colleagues showed that TF:FVIIa induces upregulation of a large set of mRNA species in immortalized HaCaT keratinocytes, including transcription regulators such as early growth factor-1 (egr-1), c-fos and c-myc, growth factors hbEGF, CTGF, and FGF-5 and cytokines such as IL-1beta, IL-8, LIF, and MIP2alpha (30). In addition, mRNA encoding proteins involved in cellular reorganization such as RhoE, uPAR, and collagenases 1 and 3, and miscellaneous proteins such as the prostaglandin E(2) receptor, PAI-2 and Jagged1 were upregulated. This pattern of mRNA transcription led the authors to conclude that TF:FVIIa primarily induces a wound healing program. Some of the upregulated genes in HaCaT cells could also be found upregulated in fibroblasts which supports the wound healing hypothesis (31). Interestingly, cancer cells also displayed an increased gene transcription upon TF:FVIIa stimulation, adding to the existing evidence that TF:FVIIa signaling influences tumor biology in a non-hemostatic manner (31-33).

Gene expression is largely under the control of the MAPK family, consisting of p44/42 MAP kinase, p38 MAPK and c-Jun-N-terminal kinase (JNK). Indeed, TF:FVIIa-induced signaling results in activation of one or several of these family members (34-36), and this has been shown to be a crucial step in TF:FVIIa-dependent gene transcription. The number and type of MAPK family members activated by TF:FVIIa is often cell type-dependent, and similarly TF-associated upregulation of genes expression is often dependent on different MAPK isoforms. For example, in HaCaT cells, IL-8 and LIF mRNA levels are increased as a result of p44/42 MAPK phosphorylation, whereas expression of CTGF depends solely on activation of the p38 MAPK pathway (30;37).

Another important event associated with TF signaling appears to be the activation of the janus kinase (JAK) pathway. In both BHK-TF and neuroblastoma cells, FVIIa induces phosphorylation of Jak2 and STAT5, which is dependent on activation of G_{12}/G_{13} subunits at least in BHK cells, leading to upregulation of Bcl family members (38;39). As will be discussed below, this event has major consequences for apoptosis and TF's ability to inhibit cell death.

4.3. Apoptosis inhibition

TF function is tightly interwoven with cancer biology and by inhibition of apoptosis in cancer cells, TF may influence cellular fate of tumor cells. PI3-kinase and its downstream target c-Akt/PKB are readily activated upon TF:FVIIa binding. The PI3-kinase/c-Akt pathway, as well as the p44/42 MAPK pathway, constitutes a potent inhibitory pathway to apoptosis. Indeed, upon stimulation of BHK-TF with FVIIa, starvation-induced caspase-3 activation and concomitant onset of apoptosis was largely reduced, which was inhibited by blockade of the p44/42 MAPK and the PI3-kinase/c-Akt pathways (40;41). Similarly, anoikis, i.e. lack-of-anchorage-induced cell death, can be inhibited by FVIIa-induced activation of these pathways (41). Although FVIIa by itself may induce cell survival, inclusion of FXa in the ternary complex, or additional activation of thrombin may lead to an even more pronounced inhibition of apoptosis (42). As discussed above, FVIIa enhanced survival of BHK-TF have also been shown to proceed via a heterotrimeric G-protein-dependent Jak/STAT5 pathway leading to upregulation of the anti-

apoptotic protein Bcl_{XL}(38), suggesting that TF activates a variety of signaling events to induce an anti-apoptotic effect.

In contrast to these studies, FVIIa-dependent signaling provokes a pro-apoptotic effect in HaCaT cells which was dependent on PAR-2-mediated phosphorylation of cAMP response-element binding protein (CREB). FXa also induces CREB activation in HaCaT cells but without any effect on apoptosis, whereas BHK-TF did respond with improved survival (43). The role of TF-mediated signaling in apoptosis is not univocal as both the TF binary and ternary complex may affect the cellular behavior differently, probably due to the induction of distinct signaling pathways investigated in various cell types. Despite the fact that diverse effects of TF on apoptosis hamper drawing a firm conclusion on how TF affects apoptosis, it is clear that TF:FVIIa signaling events greatly influence cellular survival.

4.4. Migration

Migration of cells is indispensable in both physiological and pathological processes such as wound healing and oncogenic progression. FVIIa enhances platelet-derived growth factor (PDGF)-BB-mediated migration of fibroblasts, activated monocytes, vascular smooth muscle cells (VSMCs) and TF-expressing aortic endothelial cells; FVIIa-induced migration in these cells could be blocked by inhibition of PAR-2, Src-family and PLC-dependent pathways, supporting the theory that TF:FVIIa complexation leads to activation of PAR-2 and subsequent activation of Src and PLC signaling (44). The effects on cellular migration may result from the activation of PDGF receptor-beta upon TF:FVIIa stimulation, suggesting a synergy of the TF:FVIIa complex with the PDGF receptor-beta. Other pathways have been described that may be involved in TF-associated migration such as upregulation of IL-8 and activation of the mTOR pathway (45;46). In addition, TF:FVIIa induces extension of filopodia and lammellipodia at the protruding edge of cells, which is a hallmark of migratory cells. These actin rearrangements are brought about by p38 MAPK-mediated activations of small Rho-family GTPases, such as Rac and Cdc42 (47).

TF also shows cross-talk with integrins such as integrin beta1. TF positively regulates integrin alpha3beta1-dependent cell migration on the extracellular matrix protein laminin-5, but only when the TF cytoplasmic domain is phosphorylated, suggesting that TF-PAR-2 interaction is involved in integrin function and subsequent cell migration (48). Indeed, PAR-2 deficiency results in impaired migration in response to FVIIa and FXa, suggesting the crucial role of PAR-2 in mediating migration (49). Interestingly, binding of FVIIa to TF promotes complex formation between TF and beta1 integrin on human keratinocytes, but this phenomenon was shown to be PAR-2 independent (32). It therefore remains elusive whether TF:integrin interaction is directly involved in migration. Rather, this FVIIa-dependent TF:integrin complex formation may serve to facilitate downstream TF:FVIIa:PAR-2 signaling influencing cell migration. These data indicate a role for TF in cell migration through interaction with FVIIa and integrin beta1 (figure 1).

4.5. PAR-independent signaling

The abovementioned signaling processes are generally thought to be mediated by PARs. However, recent data indicates that TF does not always require FVIIa to be functionally active in signaling. Exogenous recombinant TF alone was capable of inducing transcription of genes related to apoptosis, adhesion, migration and vessel growth in endothelial cells (50). The association of recombinant TF with beta1 integrins on the endothelial cell surface promotes proliferation through phosphorylation of MAPK (51). FVIIa binding to TF, independent of proteolytic activity, may also be sufficient to elicit activation of certain signaling pathways. Upon TF:FVIIa complexation the cytoplasmic domain appears to directly recruit signaling molecules. The TF cytoplasmic tail induced migration of the bladder carcinoma cell line J82 via Rac1 and p38 MAPK activation, however, direct evidence for Rac1 as an upstream signal molecule in p38 MAPK pathway in this context is lacking (52). The adhesive and migratory properties of J82 is affected by the cytoplasmic domain of TF in a phosphorylation-independent manner, possibly by interacting with and consequently recruiting the actin-binding protein ABP-280 to function as potential reorganizer of the cytoskeleton (53).

In 2003, an alternatively spliced soluble variant of TF, alternatively spliced TF (asTF) was discovered, which lacks both transmembrane and cytoplasmic domains and contains a unique C-terminal region. AsTF enhances endothelial adhesion and migration, however, not in a FVIIa:PAR-2-dependent manner, but rather as a ligand to alphaVbeta3-integrin to induce migration, and to alpha6beta1-integrin to form capillary structures (54). Integrin ligation by asTF resulted in a potent activation of focal adhesion kinase (FAK), p44/p42 MAPK, p38 MAPK and PI3-kinase, which were required for asTF-induced migration and capillary formation. Thus both membrane-bound and soluble asTF are able to signal independent of PAR activation.

4.6. Regulation of TF signaling

PAR-2-dependent TF:FVIIa signaling has been suggested to involve the redox status of the Cys^{186}-Cys^{209} disulfide bond in the extracellular domain of TF (55). Redox-dependent modulation of this allosteric disulfide by the oxidoreductase enzyme protein disulfide isomerase (PDI) yields two potential pools of TF: (1) a coagulant-active pool of TF with an intact disulfide bond that facilitates activation of PAR-1 and PAR-2 by the ternary TF:FVIIa:FXa complex and (2) a coagulant inactive pool of TF which facilitates FVIIa-dependent PAR-2 activation, although this mechanism remains a matter of debate (56). The functional implications of these two different signaling complexes are poorly defined at present. It is plausible that ternary complex signaling, resulting in both PAR-1 and PAR-2 activation, elicits different responses than that evoked by the PAR-2 activating binary TF:FVIIa complex.

Nevertheless, Petersen and others showed that at physiologically relevant concentrations of FVIIa alone, TF increased expression of IL-8, CXCL-1 and GM-CSF to a similar extent as that observed after ternary complex action in MDA-MB-231 breast cancer cells (57), suggesting that these two signaling complexes are more or less redundant. However, a recent report suggests that the endothelial protein C receptor (EPCR) is required for ternary complex, but not binary complex signaling (58). Thus, differences between ternary complex and binary complex signaling may not be characterized by the strength of the response, but rather the mere presence of EPCR on the TF-expressing cells.

In addition to redox regulation, TF signaling appears to be regulated by rafts, cholesterol-rich microdomains in the plasma membrane. Fractions of TF colocalize with PAR-2 in these lipid rafts and depletion of cholesterol reduces TF:FVIIa-dependent cleavage of PAR-2 (59). Strikingly, these data are in contrast with the concept that TF can be palmitoylated at the cytoplasmic Cys^{245} residue. Palmitoylated proteins tend to concentrate in lipid rafts (reviewed in (60)), whereas TF Cys^{245} mutant, which lacks the consensus palmitoylation sequence site, shows enhanced PAR-2 dependent TF phosphorylation (61;62). Therefore, these data seem to argue that signaling TF resides in a non-raft membrane environment.

Another post-translational modification that influences TF signaling is the stimulus-induced phosphorylation of cytoplasmic serine residues 253 and 258. PAR-2 agonists such as SLIGRL or FVIIa induce PKCalpha-dependent phosphorylation of Ser^{253}, followed by proline-directed kinase-mediated phosphorylation of Ser^{258} (61). Conversely, cytoplasmic tail phosphorylation appears to regulate PAR-2 signaling. Belting *et al.* showed that genetic deletion of the TF cytoplasmic tail in mice enhances angiogenesis *in vivo* and in *ex vivo* aortic sprouting experiments, suggesting that the unphosphorylated tail inhibits PAR-2 signaling (63). In support, Dorfleutner *et al.* demonstrated that the TF cytoplasmic tail inhibits PAR-2-dependent migration, but tail phoshorylation releases this inhibition (48). These observations indicate a bidirectional interaction between the cytoplasmic domain of TF and PAR-2 in regulating intracellular signaling.

Future experiments further investigating the involvement of TF in regulating cellular behavior, may increase the body of evidence for TF as a true player in the development of non-coagulant processes like angiogenesis, cancer and sepsis.

5. TF SIGNALING IN PHYSIOLOGICAL PROCESSES

Under physiological conditions, TF plays a prominent role in angiogenesis, which is a key event during development and wound healing. TF deficiency in mouse embryos leads to lethality early in development due to a defect in the yolk sac vasculature (5-7). Impaired maturation and stabilization of the vascular network is caused by the loss of pericyte recruitment, indicating a role for TF in regulating non-coagulant functions (5). Similarly, genetic deletion of the extracellular domain of TF, but not the cytoplasmic domain, resulted in increased embryonic mortality (64;65). The FVIIa binding site of TF, rather than the cytoplasmic tail, may therefore be involved in embryonic development, suggesting that the TF cytoplasmic tail is not strictly required for such function.

TF is expressed on VSMCs where it functions as a regulator for proliferation and migration, important aspects in vascular growth and angiogenesis (66). Specifically, the cytoplasmic domain of TF appears to regulate the proliferative and migratory behavior of VSMCs (67). Although both PAR-1 and PAR-2 are present on VSMCs *in vitro* and in the intimal lining of human coronary artery, solely PAR-2 is responsible for the TF:FVIIa induced migration of VSMCs (68). *Ex vivo* studies, by use of retinal vascularization and aortic sprouting models, pointed towards a negative regulation of PAR-2 signaling by the TF cytoplasmic domain (63). Thus, the contribution of TF signaling in the development of vascular structures is dependent on PAR-2.

Upon cutaneous injury, TF becomes exposed to the bloodstream where TF exerts its well-known role in initiating coagulation after binding FVII(a), which is indispensable for physiological hemostasis. On the other hand, the signaling function of TF is important in providing efficient repair mechanisms, which is supported by wound-healing experiments in FVII-deficient mice, as FVII-deficient mice showed a delay in wound healing apart from their hemostatic defects (69). TF:FVIIa complex-induced signaling in cultured keratinocytes results in transcription of genes encoding growth factors, cytokines and various other genes that may contribute to the repair process after local injury (30). The transcription factor egr-1 is indispensable in wound healing and has been shown to be regulated by TF:FVIIa signaling via PAR-2 (69).

Due to wounding, a repair mechanism is induced in which fibroblasts, constitutively expressing TF, participate to reorganize the extracellular matrix and to induce angiogenesis (70). The chemotactic factor PDGF-BB may attract fibroblasts to the site of injury and *in vitro* PDGF-BB was shown to be enhanced upon FVIIa-dependent PAR-2 activation downstream via Src and PLC pathways (44). Cultured human fibroblasts showed increased secretion of VEGF upon stimulation with the ternary TF:FVIIa:FX complex, which may promote the angiogenic process during wound healing (71). Angiogenic capillary sprouting supports the further wound repair process after invading into the ruptured extracellular matrix environment. Thus, TF promotes the healing of injured tissue by mediating repair processes.

6. TF SIGNALING IN CANCER-RELATED EVENTS

It is becoming increasingly clear that TF and cancer are interlinked. Cancer cells upregulate TF on their surface, leading to thrombotic complications, but additionally TF:FVIIa-dependent PAR-2 activation regulates tumor growth in a non-hemostatic manner. In this

part of our review we will discuss the involvement of TF signaling in cancer.

Aberrant TF expression in malignant tissues is observed in a variety of tumor specimens obtained from cancer patients (72). Increased expression of TF may be a consequence of oncogenic transformation leading to alterations in gene transcription. These oncogenes contribute to the progression of cancer due to loss of function in controlling cell proliferation, migration and survival, which results in a malignant phenotype and behavior of tumor cells. Mutating or deleting the proto-oncogene K-*ras* and disrupting the tumor suppressor gene p53 was shown to result in elevated expression levels of TF in colorectal carcinoma cells, and this TF upregulation was directly responsible for the increased tumor growth and angiogenesis in a mouse model (73). Human epithelial A431 carcinoma cells harboring oncogenic epidermal growth factor receptor (EGFR), due to gene amplification or mutation, upregulated TF expression which was similarly found to be involved in tumor growth, angiogenesis and in the onset of tumor development (74). These data show that a multitude of (proto)oncogenes lead to upregulated TF and consequently altered tumor growth. Apart from overexpression of TF, human glioma cells show simultaneous upregulation of PAR-1, PAR-2 and FVII through expression of an EGF receptor mutant (75).

TF:FVIIa-dependent signaling in gene expression and cancer cell motility has extensively been studied *in vitro* (45;49), however these data provided limited insight into the role of TF in cancer biology. *In vivo* studies have revealed that TF most likely influences tumor growth by activating the angiogenic switch. TF enhances angiogenesis in Meth-A sarcoma tumor cells *in vivo*, due to TF-mediated modulation of pro-angiogenic and anti-angiogenic factor transcription, such as VEGF and thrombospondin-1 (76). In addition, FVIIa-induced PAR-2-activation on TF-expressing breast cancer cells enhances transcription of the pro-angiogenic genes IL-8 and CXCL1 (33). Effective abrogation of tumorigenic events by targeting TF or the TF:FVIIa complex confirmed the importance of TF in tumor angiogenesis and progression, but failed to show whether TF:FVIIa-induced signaling lies at the basis of tumor progression (77-79). Nevertheless, a recent study made use of a TF coagulant activity inhibitory antibody (5G9) and a signaling inhibitory antibody (10H10) to establish the exact role of TF in tumor growth (32). Whereas 5G9 effectively reduced metastasis, but not primary tumor growth in mice, 10H10 potently suppressed primary tumor growth and tumor angiogenesis, but not metastasis. Apparently, TF signaling plays a pivotal role in the angiogenic switch whereas coagulation activation promotes metastasis. The inhibitory effect of 10H10 was shown to be due to interruption of the TF-beta1 integrin complex, as this complex promotes TF:FVIIa:PAR-2 signaling. Breast tumor growth was furthermore sensitive to PAR-2 but not PAR-1 blockade, providing strong evidence for the involvement of the TF:FVIIa:PAR-2 signaling module in breast cancer development (32).

The involvement of PAR-2-mediated signaling has been confirmed in mice harboring a genetic insertion of the polyoma middle T antigen (PyMT), resulting in spontaneously developing breast tumors. Tumor onset and further development of carcinomas was delayed in PyMT/PAR-2 -/- mice when compared to PyMT mice (80). Genetic deletion of the TF cytoplasmic tail in combination with PAR-2 deficiency resulted in similar reduced tumor volumes for both knock-out strains compared to controls, confirming that both TF and PAR-2 contribute to tumor growth (81). Further support for this functional overlap comes from studies showing that phosphorylated TF is present in human as well as in murine wild type breast tumors, but not in PAR-2 and TF cytoplasmic domain deficient tumors (82). Thus the TF cytoplasmic tail is associated with PAR-2 signaling in (early) breast cancer development *in vivo*. Colocalization of PAR-2 and phosphorylated TF in specimens of primary breast cancer patients correlated with poor prognosis, suggesting the clinical relevance of TF-induced PAR-2 signaling in human breast cancer (82).

In conclusion, TF signaling appears to influence primary tumor growth, but not metastasis, through pro-angiogenic gene expression and consequently induction of the angiogenic switch.

7. TF SIGNALING IN INFLAMMATORY EVENTS

During inflammation, monocytes differentiate into macrophages leading to increased TF expression, in turn eliciting inflammatory responses upon stimulation with FVIIa. Hence, macrophages potentially harbor a proinflammatory state by upregulation of cytokine transcription and release, which can be inhibited by *in vivo* blocking of TF (83;84). Also in healthy human volunteers, recombinant FVIIa enhances proinflammatory cytokine production, which is diminished after disrupting TF:FVIIa complex formation (85). This indicates a role for TF:FVIIa in augmenting inflammatory functions, probably via mononuclear cells.

The presence of hemostatic abnormalities, such as the imbalance between a procoagulant and anticoagulant state, is associated with sepsis. Upregulation of TF has been identified in lung, brain and kidney in rabbits systemically injected with LPS (13). Similarly, increased infiltration of TF-expressing inflammatory cells in lungs was determined in mice after receiving an *E.coli* injection in the peritoneum (86). Inhibition of TF by treating endotoxemic mice with an antibody directed against TF resulted in prolonged survival (87). TF-deficient mice showed attenuated coagulation, inflammation, and lethality after LPS challenge, for which mainly the hematopoietic cells were thought to be responsible (88). In addition, a role for TF:FVIIa signaling in sepsis has been suggested by a study in which *E.coli* infected baboons were treated with active-site blocked FVIIa, which resulted in decreased mortality, procoagulant activity and IL-6 and IL-8 plasma levels (89;90). In contrast, treating septic baboons with active-site inhibited FXa suppressed coagulation without promoting survival, which was probably due to the unaffected inflammatory responses (90). Although both complexes TF:FVIIa and TF:FVIIa:FXa are indispensable

in coagulation, TF:FVIIa rather than the ternary complex is involved in mediating inflammation. Studies on the involvement of the TF cytoplasmic domain in developing inflammatory responses in endotoxemic mice showed that lacking TF cytoplasmic domain reduced release of proinflammatory cytokines, leukocyte recruitment events and mortality rate (91). Based on these studies, TF seems to play a pivotal role in regulating the inflammatory response under septic conditions.

In addition to the up-regulation of TF upon inflammatory stimuli, PAR-2 expression may also be enhanced under inflammatory conditions. Under quiescent conditions, monocytes expressed very low levels of PAR-2, however, upon activation or monocyte differentiation into macrophages PAR-2 expression was elevated (92). Similarly, PAR-2 expression on endothelial cells *in vitro* or on coronary arteries was up-regulated after incubation with inflammatory agents (93;94) . Direct activation of monocyte-derived PAR-2 contributed to inflammation via production of IL-6, IL-8 and IL-1beta (92), additionally a similar effect was observed when stimulating VSMCs with FVIIa (25). *In vivo* studies revealed that PAR-2 activation contributed to rolling, migratory and adhesive properties of leukocytes which are important events during the early phase of the inflammatory response (95;96). These studies indicate that PAR-2 activation is involved in initiating the inflammatory reaction.

Further research into PAR signaling in relation to sepsis was performed with knock-out mice used in an experimental endotoxemic model. Genetic deletion of either the PAR-1 or PAR-2 gene did not result in altered mortality or inflammatory responses when compared with control mice (88;97). In a different approach, however, treatment of PAR-2 deficient mice with the specific thrombin-inhibitor hirudin, thus preventing thrombin-induced PAR-1 activation, decreased lethality and IL-6 levels when compared with hirudin- or saline-treated littermate controls (88). Camerer *et al.* showed in a similar experimental set-up that PAR-1 inhibition in PAR-2 knock-out mice or littermate controls did not affect survival, which could be explained by the sex differences of the mice used in both studies (97). Based on these observations, it is likely that both PAR-1 and PAR-2 contribute to inflammation in a sepsis model, however, more research is needed to uncover the exact interplay between PAR-1 and PAR-2 signaling in sepsis. Furthermore, direct evidence for a role of TF:FVIIa in activating PARs under these circumstances remains to be investigated.

8. PERSPECTIVE

In the last two decades, considerable evidence indicates the role of TF in several biological processes. Our knowledge about TF-mediated signaling and its regulation increases, however, this also raises more questions. TF signaling depends on a variety of factors, such as cell type, surrounding environment, the interaction with other proteases (FVIIa and FX) and receptors (PARs, integrin). In beneficial and pathologic conditions, these factors vary and may therefore affect TF signaling differently, which makes it a rather complex process. Thus, besides the knowledge that we have now, gaining more insight in unraveling the involvement of TF signaling under these circumstances remains of interest. The identification of the regulatory mechanisms for TF signaling is obscure and therefore need more attention. In addition, would the regulatory mechanism for TF signaling be different per disease and between distinct stages of disease?

Further research is needed with the focus to eventually develop effective treatment opportunities against the pathologic effects of TF. To develop a therapeutic approach in diseases like cancer and sepsis, it would be of considerable interest to investigate how to inhibit specifically TF signaling in these pathological processes without interfering with its coagulant function.

Gaining more insight into TF signaling in relation to its non-hemostatic functions is necessary in providing tools to specifically target TF and its signaling pathways in diseases.

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