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REVIEW

Tissue factor signal transduction in angiogenesis

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Tissue factor (TF), a 47-kDa transmembrane glycoprotein, is a principal regulator of oncogenic neoangiogenesis and controls therefore the cancerous process. Although originally identified as a component of the coagulation cascade, it has become clear that TF functions as a cytokine-like receptor and this notion was confirmed by the discovery of coagulation-independent actions of TF (which include regulation of tumour growth, embryonic and oncogenic blood vessel formation as well as regulation of inflammation and sepsis). In accordance, TF-mediated signal transduction events are readily detected and the elucidation of the underlying molecular mechanisms has recently seen spectacular progress and it is now understood that the role of TF in angiogenesis is both coagulation-dependent and independent. The recent evidence for this emerging insight will be the subject of this review.

Introduction

Tissue factor, an unusual member of the cytokine receptor class II family

It has long been recognized that the interaction of tissue factor (TF) with factor VIIa (FVIIa) is of crucial importance for haemostasis (Figure 1). In addition, TF:FVIIa binding has important coagulation-independent functions, especially in embryonic angiogenesis, oncogenic angiogenesis, leukocyte diapedesis, inflammation and the progression of lethal *Escherichia coli* sepsis (reviewed in ref. 1). With the discovery of a variety of signal transduction events elicited in TF-expressing cells upon addition of FVIIa, there is now little doubt that TF:FVIIa complexation not only stimulates haemostasis but also alters cellular physiology of the TF-expressing cell, although the underlying molecular mechanism is controversial (1).

After its cloning in 1987, it was reported that the 295 amino acid TF polypeptide chain did not bear significant homology with other proteins. *In silico* studies, however, highlighted the high degree of structural similarity of TF with the super family of interferon receptors (IFNR)-α/β and γ (2). This notion was confirmed by the crystal structure of TF and it is often assumed that TF is derived from an ancestral gene that also gave rise to the cytokine receptor family. Indeed, recent *in silico* analysis revealed the presence of TF homologues in fish and insects, in the absence of interferon gamma receptors in these genomes, showing that TF is the most ancient member of the cytokine receptor class II family from which the interferon receptors are relatively novel branches (3).

TF, however, is unusual within the cytokine class II receptor family with respect to its short intracellular tail; its homology to other cytokine receptor family members is obscure at best. In addition, no evidence has been presented for the association of a signal transducing subunit to TF after its ligation, as is observed for all other cytokine receptor members. Importantly, the role of JAK (Just Another Kinase) activation, which mediates receptor phosphorylation and further signalling after cytokine receptor stimulation, has not been investigated, but obviously if JAK/STAT signalling after TF stimulation could be detected, this would provide dramatic proof that the sequence homology between TF and the other members of the cytokine class II family is of biological relevance for its pathophysiological effects.

Tissue factor, mouse genetics and angiogenesis

Early indications for a possible role of TF signal transduction in angiogenesis came from mouse genetics. TF / embryos do not survive beyond embryonic day 10.5, as a consequence of defects in establishing and/or maintaining vascular integrity in the developing embryo at a time when embryonic and extra-embryonic vasculatures are fusing, and blood circulation begins (4,5). Alternatively, in a different genetic background, TF deficiency causes abnormalities of vascular pericytes, resulting in defective yolk sac vessel development and subsequent embryo wasting (6). Importantly, genetic ablations of other elements of the coagulation cascade (e.g. factor X or fibrinogen) have the capacity to survive until partus and do display defects in vessel development. Hence, the genetic

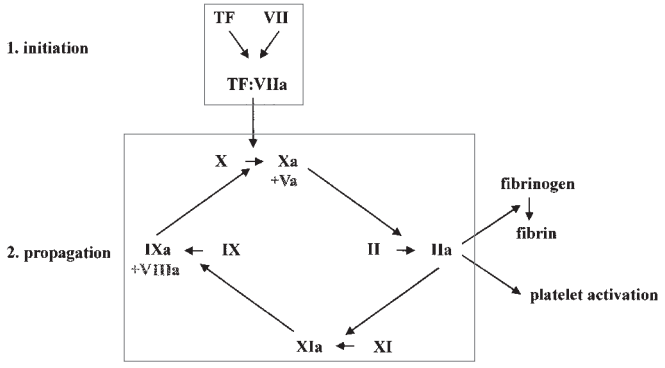

Fig. 1. Simplified model of the coagulation pathway. After rupture of the vessel wall, TF and FVII dimerize, after which the complex proteolytically cleaves FX to FXa. FXa action, which is catalysed by FVa, results in thrombin formation. Thrombin production results in fibrin deposition and activation of platelets. In addition, thrombin stimulates the subsequent formation of FXIa and FIXa. The latter two coagulation factors form a feedback loop; FIXa activity, stimulated by FVIIIa, leads to increased production of FXa, resulting in an even more abundant, thrombin-mediated deposition of fibrin.

Abbreviations: FVIIa, factor VIIa; TF, tissue factor.

evidence points towards a coagulation-independent function of TF in vascular development.

Very recently, the roles of the intracellular and extracellular domain of TF in development have been studied, using mice, harbouring TF with a targeted deletion of the 18 C-terminal intracellular amino acids (7). In contrast to complete TF deficient mice, both homozygous and heterozygous intracellular tail-deleted TF mice displayed normal embryonic development, survival, fertility and blood coagulation. Therefore, the TF intracellular tail does not appear to have any function in embryogenic angiogenesis, leaving the role of this domain in any physiological event quite unclear. Interestingly, strong sequence conservation between guinea pig, rabbit, cow and human intracellular TF exists, but the homology of the intracellular domain of these species with mouse and rat TF is weak. Thus, evolutionary pressure on the sequence of the intracellular domain exists in the former species but is absent in the latter. This may explain why murine genetics have proven unsuccessful in establishing a role for the intracellular domain of TF in angiogenesis (3).

FVII-knockout mice (factor VII is the natural ligand for TF) have been generated and develop normally throughout embryogenesis, but succumb to perinatal haemorrhage after birth (8). This seeming discrepancy between TF / and FVII / mice led to the conclusion that FVIIa is not involved in TF-dependent angiogenesis. However, using conditional FVII deficient mice, generated by targeted insertion of the tTA genetic switch to the FVII locus, it turned out that FVII is important in angiogenesis; crossing conditional knockout females with FVII+/ males enabled the generation of FVII / embryos in mothers that produce very low levels of FVII and therefore have markedly reduced potential for maternal transfer of FVII (9). Remarkably, such a cross produced no viable FVII / embryos beyond embryonic day 12.5, whereas younger embryos show malformations that resemble TF / embryos. Apparently, in the normal situation, maternal FVII rescues FVII / embryos and this implies that, FVII, like TF, plays an important role in angiogenesis.

Tissue factor and tumour angiogenesis

Clinical data clearly suggest that TF promotes tumour growth by enhancement of angiogenesis. In non-small-cell lung carcinomas (10) as well as in human prostate carcinomas (11), a significant relationship between TF expression and microvessel density exists. In addition, the significant correlation between TF and vascular endothelial growth factor (VEGF) expression in certain human tumours (10,12,13) implies that TF modifies the angiogenic properties of tumour cells by altering the production of growth regulatory molecules that act on vascular endothelial cells. Importantly, in a murine xenograft model, tumour cells transfected to overexpress TF grew more rapidly, and established larger and more vascularized tumours than control transfectants. Antisense TF transfectants grew the slowest and were the least vascularized. Anticoagulation of mice with warfarin did not alter the difference between these tumour lines (14). In agreement, using species-specific antibodies to TF, it was shown that TF was essential for melanoma metastasis in a comparable xenograft model, TF-inhibition resulting in significantly reduced numbers of tumour cells retained in the vasculature of the lungs (15). Finally, covalently inactivated FVIIa in these models has antimetastatic properties, emphasizing that proteolytic activity is necessary for the metastatic process (16). Thus, the role for TF in angiogenesis suggested by mouse genetics is supported by at least some clinical and experimental work and the notion that TF plays an important role in tumour vascularization has gained widespread acceptance.

TF signalling and angiogenesis

As expected from a member of the cytokine receptor class II family, TF:FVIIa interaction induces signal transduction (17,18), which has now been characterized in some detail and provides explanations as to how TF may stimulate angiogenesis. A prominent event in TF signalling is activation of p42/p44 MAP kinase (19), p38 MAP kinase (20) and c-Jun-terminal kinase (JNK) (20), which, in at least some cell types, form part of signalling cassettes, together with Src-like kinases and the lipid/protein kinase PI-3 kinase (21). The MAP kinase family has been increasingly associated with cell proliferation and soon the idea arose that FVIIa:TF might contribute to the process of angiogenesis simply by stimulating cell division. However, the mitogenic capacity of TF:FVIIa remains disputable; FVIIa:TF complex formation showed no proliferative effects in studies, using A14 fibroblasts (H.H.Versteeg, unpublished results), BHK cells transfected with TF and embryonic fibroblasts cells, although supra-physiological concentrations of FVIIa induced some proliferation in fetal lung fibroblasts (22–24).

A more likely mechanism involves the production of angiogenesis-stimulating proteins that is observed after FVIIa:TF interaction. Of special interest in this respect are the observations that TF:FVIIa interaction leads to RNA synthesis of *FGF-5*, *hbEGF*, *IL-1*β, *MIP2a*, *LIF* and *IL-8*, whereas in human WI-38 fibroblasts, *Cyr61* and *CTGF* transcripts show a TF:FVIIa-dependent upregulation (25,26). The heparin-binding EGF-like growth factor (hbEGF) is a well-established mediator of angiogenesis (27) whereas *Cyr61* and *CTGF* are associated with vasculogenesis (28). IL-1β stimulates production of VEGF in vascular smooth muscle cells and proximal tubular cells in kidneys (29,30) and IL-1 receptor antagonists inhibit angiogenesis in rats (31). The body of evidence for a role of IL-8 in angiogenesis is quite extensive; IL-8 serves as a mitogen and chemoattractant for vascular smooth muscle cells (32). Furthermore, human recombinant IL-8 is potently angiogenic in rat and rabbit cornea and induces proliferation and chemotaxis of human umbilical vein endothelial cells (33,34). However, neither IL-1β nor IL-8 knockout mice show severe abnormalities with respect to the vasculature, suggesting that although facilitating angiogenesis, IL-1β and IL-8 are not absolutely required (35,36). In addition, TF:FVIIa interaction leads to a substantial increase in cell protein synthesis (Figure 2), which might further facilitate production of these angiogenic factors (22). However, studies demonstrating that ectopic expression of these cytokines can overcome the need for TF during tumour angiogenesis are required to establish a causal relationship.

Some attention has recently been given to the notion that TF expression may facilitate the migratory events associated with blood vessel formation (37,38). Cell movement and chemotactic sensing heavily rely on cytoskeletal rearrangements, and recently TF has been shown to mediate cortical actin polymerization, lamellipodia, ruffles and filopodia (Figure 3) (21,39). On a molecular level, these phenomena are brought about by GTPases, functioning as molecular switches; and it was recently shown that upon stimulation of fibroblasts with FVIIa, these cells respond with activation of the small

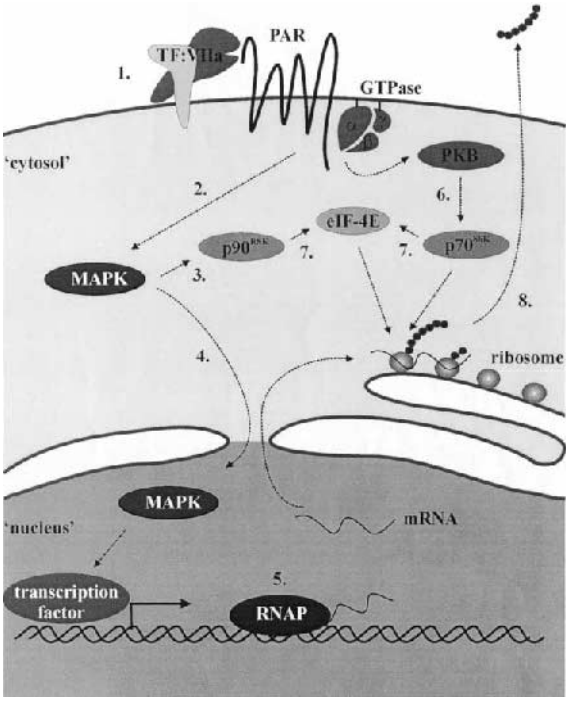

Fig. 2. Proposed and simplified model for FVIIa:TF-induced protein synthesis. FVIIa binds TF (1.) and proteolytically cleaves a protease-activated receptor, which in turn activates a heterotrimeric GTPase. Subsequently, the MAP kinase family members (P42/44, p38 and JNK) are activated via the small GTPase ras (2.). Upon activation, MAP kinase activates p90RSK (3.) and translocates to the nucleus (4.), where it activates transcription factors and subsequent RNA synthesis by RNA polymerase (RNAP) (5.). FVIIa:TF interaction also results in activation of PKB, and subsequently p70S6K (6.). The latter activates the ribosome by phosphorylation of the ribosomal protein S6. P90RSK and p70S6K activate the initiation factor eIF-4E (7.), which facilitates protein synthesis at the ribosome. The synthesized protein is either kept in the cytosol or transported to the plasma membrane, and secreted (8.).

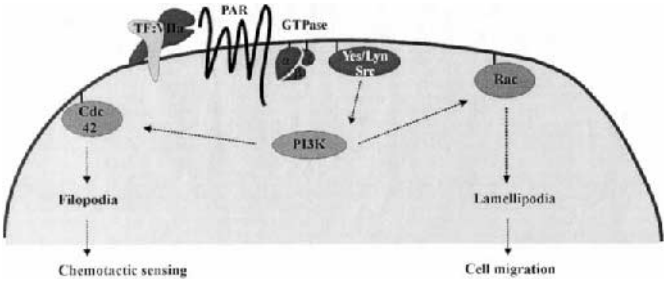

Fig. 3. FVIIa:TF-induced GTPase activation and subsequent reactions of the cell. After FVIIa:TF complex formation on fibroblasts, activation of Src-like kinases and PI3 kinase takes place. Subsequently, this signalling cassette activates the small G-proteins Rac and Cdc42, leading to actin rearrangements. These arrangements give rise to Cdc42-mediated filopodia, sensing the extracellular environment, and Rac-mediated lamellipodia, which indicate cell movement.

Rho-like GTPases Rac and Cdc42, leading to lamellipodia and filopodia, respectively (Figure 3) (21), but the importance of these events for angiogenesis remains poorly understood, prompting further research into this area.

Downstream coagulation factors and angiogenesis

Although genetic studies in mice do not support a role for downstream coagulation factors in TF-dependent vascular development, recent data indicate that the importance of these factors may have been underestimated hitherto. Specific inhibition of factor Xa by recombinant antistasin and tick anticoagulant peptide reduces factor Xa-induced SMC proliferation *in vitro* and *in vivo* after balloon angioplasty in a rabbit model of femoral atherosclerosis (40,41).

On a molecular level, FXa has recently been shown to induce some of the same pathways that FVIIa:TF utilize, among which the p42/p44, p38 and JNK MAP kinases and NF-κB-driven *Cyr61* and *CTGF* gene expression (20,26,42). More importantly, TF-induced thrombin production is widely seen as a major player in angiogenesis. Thrombin induces proliferation of human vascular endothelial cells and enhances incisional wound healing and neovascularization in normal rats (43). Furthermore, thrombin promotes endothelial cell alignment in Matrigel *in vitro* and angiogenesis *in vivo* (44), apparently by a mechanism independent of fibrin formation (45). The mechanism involved may be reminiscent to that employed by TF as thrombin stimulation release of the angiogenesis-promoting factors, including IL-1β, IL-6, Cyr61, CTGF and VEGF (26,46–49). Finally, TF-dependent fibrin generation is important for angiogenesis during wound healing and thus may also exert such a function in the cancerous process. Fibrin acts as a provisional scaffold during invasion of endothelial cells. Moreover, the fibrin structure determines the extent of neovascularization; *in vitro*, rigid, dense fibrin networks promote stable angiogenic structures, whereas a more malleable matrix induces a faster, but less stable ingrowth of capillary structures (50,51). Interestingly, *in vivo*, stabilization of these fibrin networks greatly improves wound healing, highlighting the role for fibrin in angiogenesis during tissue repair (52). Hence, it seems probable that downstream coagulation factors at least to some extent contribute to the TF-dependent angiogenesis.

Conclusions

Mouse genetics, expression data in human cancers, and rodent models of oncological disease support a role for TF in angiogenesis, suggesting that current efforts in targeting TF in cancerous disease are valid. With respect to the underlying mechanism, many questions remain unanswered, but a picture emerges in which TF acts as a receptor that is important for the generation of angiogenic factors, a process in which downstream coagulation factors may well participate. Better knowledge of TF signal transduction, however, may well turn out to be instrumental for devising rational therapy aimed at inhibiting TF-dependent tumour vascularization.

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