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Blood Coagulation and Blood Vessel Development: Is Tissue Factor the Missing Link?

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In this issue Arderiu and colleagues (1) investigate the role of tissue factor (TF) in angiogenesis using both in vitro and in vivo models. They find that TF expression in endothelial cells (ECs) stimulates the expression of chemokine ligand 2 (CCL2). This facilitates the recruitment of vascular smooth muscle cells (VSMCs) and the stabilization of EC-VSMC networks.

Judah Folkman, a pioneer in angiogenesis research, was intrigued by the connection between blood coagulation and blood vessel development and proposed that the two processes were intimately connected (2, 3). A summary of the key observations connecting TF and angiogenesis is shown in Figure 1. In 1994, Zhang and colleagues (4) were the first to show that TF expression by Meth-A sarcoma cells regulates their angiogenic activity in vivo. A High level of TF expression was associated with enhanced expression of the pro-angiogenic factor vascular endothelial cell growth factor (VEGF). Later, Yu and colleagues (5) demonstrated that antisense silencing of TF expression in a human colorectal cancer cell line reduced the growth of tumor cells in mice. A study with human MDA-MB-231 breast cancer cells revealed that the TF/FVIIa complex regulated the expression of interleukin 8 (IL-8), another angiogenic factor, via activation of protease-activated receptor 2 (PAR2) (6). Interestingly, the transcriptional program induced by activation of PAR2 was similar to that induced by activation of the thrombin receptor, PAR1, and included many angiogenic factors and chemokines (7). A summary of the proposed coagulation protease-PAR pathways that lead to the expression of angiogenic factors by tumors cells is shown in Figure 1A. One area of controversy is whether TF is expressed by ECs within tumors. One study (8) reported TF expression by ECs in invasive breast cancer but not by ECs of benign tumors. However, Luther and colleagues (9) did not observe TF expression by tumor ECs. Indeed, TF expression by ECs would be expected to induce clotting which would reduce rather than increase tumor growth. Host TF does appear to have a subtle contribution to angiogenesis in some tumors. For instance, B16F1 melanoma tumors grown in low TF mice had smaller vessels than tumors grown in mice with higher levels of TF (10). The host cell type that contributes to this phenomenon is unknown but could be macrophages, VSMCs or even ECs. The role of TF in tumor angiogenesis is summarized in two recent reviews (11, 12).

The next major discovery was that embryos lacking TF died at mid-gestation (13-15) (Figure 1B). One study concluded that yolk sac vessels of $TF^{-/-}$ embryos were more fragile due to a deficit in mesenchymal cells/pericyte accumulation (13). TF was found to be expressed by the visceral endoderm within the yolk sac (13). Intriguingly, the defect in the formation of the yolk sac vasculature observed in $TF^{-/-}$ embryos was remarkably similar to the defect observed in $PAR1^{-/-}$ embryos (16). PAR1 is not expressed on platelets in mice, which suggested that the defect in $TF^{-/-}$ embryos maybe due to a reduction in TF-dependent

thrombin generation and subsequent activation of PAR1 (13). Interestingly, PAR1 expression on ECs was able to rescue the defect in PAR1$^{-/-}$ embryos (17). The defect in the remodeling of the vascular plexus of this extra-embryonic tissue of TF$^{-/-}$ embryos has led some investigators to propose that TF is "essential" for angiogenesis in general. However, no blood vessel defects have been reported within the TF$^{-/-}$ embryos themselves or in the few TF$^{-/-}$ mice that have survived to wean (Mackman, unpublished data). Why does the TF-thrombin-PAR1 pathway contribute to the development of the yolk sac vasculature and not other vascular beds? One possibility is that a role of this pathway in physiological angiogenesis is only revealed in rapidly forming vasculature, such as the yolk sac (18, 19). TF may also play a role in the maintenance of vascular integrity in the placenta (20).

Other studies have suggested a role for the cytoplasmic domain of TF and an alternatively spliced version of TF (asTF) in angiogenesis. AsTF lacks the C-terminal region that includes the transmembrane and cytoplasmic domains (21). For instance, mice lacking the TF cytoplasmic domain have been reported to exhibit enhanced PAR2-dependent retinal angiogenesis (22). However, no defect in retinal angiogenesis was observed in mice that express very low levels of TF (Erlich and Mackman, unpublished data). In addition, there are no reported angiogenic defects in PAR2$^{-/-}$ mice. Therefore, at present, the role of the TF cytoplasmic domain in angiogenesis is uncertain. Interestingly, over-expression of asTF in a human pancreatic cell line increased the growth and microvascular density of tumors in mice (23). A further study found that that asTF enhanced angiogenesis ex vivo in a manner that was independent of either FVII or PAR2 (24). asTF was found to interact with the integrin αvβ3 to enhance EC migration and with α6β1 to increase the formation of capillaries in vitro (24) (Figure 1C). AsTF was also found to enhance angiogenesis in Matrigel plugs in mice (24). These studies suggest that asTF may play a role in angiogenesis, although it remains unclear how much asTF is expressed in vivo.

Arderiu and colleagues (1) modulated TF expression in human ECs and VSMCs and analyzed the ability of these cells to form capillary-like networks on 3 dimensional (3D) basement membrane (Matrigel) surfaces. Interestingly, TF mRNA was transiently expressed in the ECs. We have observed a similar transient expression of TF mRNA from ECs forming tubes within a 3D collagen matrix (Mackman and Davis, unpublished data). Gene silencing of TF was associated with a reduction in the formation of networks formed by ECs (1). Similar results were observed by silencing TF in VSMCs. Next, the authors examined networks formed by co-culture of ECs with VSMCs in vitro. A reduction of TF expression in either cell type inhibited network formation. Interestingly, TF expression has been shown to increase cell survival (25). Although the authors did not find any differences in apoptosis in ECs or VSMCs containing TF siRNA, a role of TF in cell survival in these experiments cannot be excluded. Lastly, a reduction in TF expression was associated with reduced "angiogenesis" in Matrigel plugs implanted into mice. However, it should be noted that many cell types, including tumors cells and VSMCs, align to form networks in matrigel and therefore one must be cautious in interpreting these structures as capillaries without demonstrating a lumen in the structures formed in vitro or the presence of blood in the structures formed in vivo (26). Thus, one of the complexities of the Matrigel system in vitro is that there is primarily cord-like cell alignment and minimal tube morphogenesis, and therefore, the data presented with respect to mural cell recruitment needs to be interpreted with caution since pericytes and VSMCs are known to recruit to EC tubes (rather than cords) in vivo (27). Other systems where pericyte recruitment to EC-lined tubes in 3D matrices in vitro has been investigated in more detail would represent better experimental approaches that could be used to confirm the findings presented in this study and to further assess the role of TF in these events (28,29). For example, previous work has demonstrated a role for EC-derived platelet-derived growth factor (PDGF)-BB and heparin-binding epidermal

growth factor (HB-EGF) in pericyte recruitment to EC-lined tubes in 3D collagen matrices (29).

What pathways are regulated by TF in ECs? Arderiu and colleagues (1) used an angiogenesis targeted microarray to compare transcripts in ECs containing either control or TF siRNA. Due to the previously reported association between TF and VEGF, one would have expected that TF silencing would reduce VEGF expression (4). However, VEGF expression was not reduced in the TF silenced cells. Instead, they found that expression of CCL2 (also known as monocyte chemotactic protein-1 [MCP-1]) was reduced in cells treated with TF siRNA. Further studies indicated that CCL2 acts as a chemoattractant for VSMCs by binding to the CCR2 receptor. Although silencing TF in VSMCs reduced network formation the TF-dependent pathway that function in VSMCs was not elucidated. Finally, Arderiu and colleagues (1) present data that ECs within atherosclerotic lesions express both TF and CCL2, although the resolution is low making it difficult to definitively conclude that ECs are the source of these proteins.

What regulates TF expression in ECs grown in 3D culture and how does it enhance CCL2 expression? At present, there is no information on how TF gene expression is regulated. Similarly, it is not known how TF expression increases the CCL2 expression. The fact that these changes are observed in cell culture suggests that this maybe a FVIIa and PAR2-independent pathway involving integrin $\alpha v\beta 3$. Finally, it should be noted that mice with a TF deficiency in either ECs or VSMCs (30,32) have no apparent defects in angiogenesis, which again indicates that TF is not “essential” for angiogenesis. Further studies are necessary to determine the role of full length TF, asTF and other coagulation proteins in different forms of angiogenesis.

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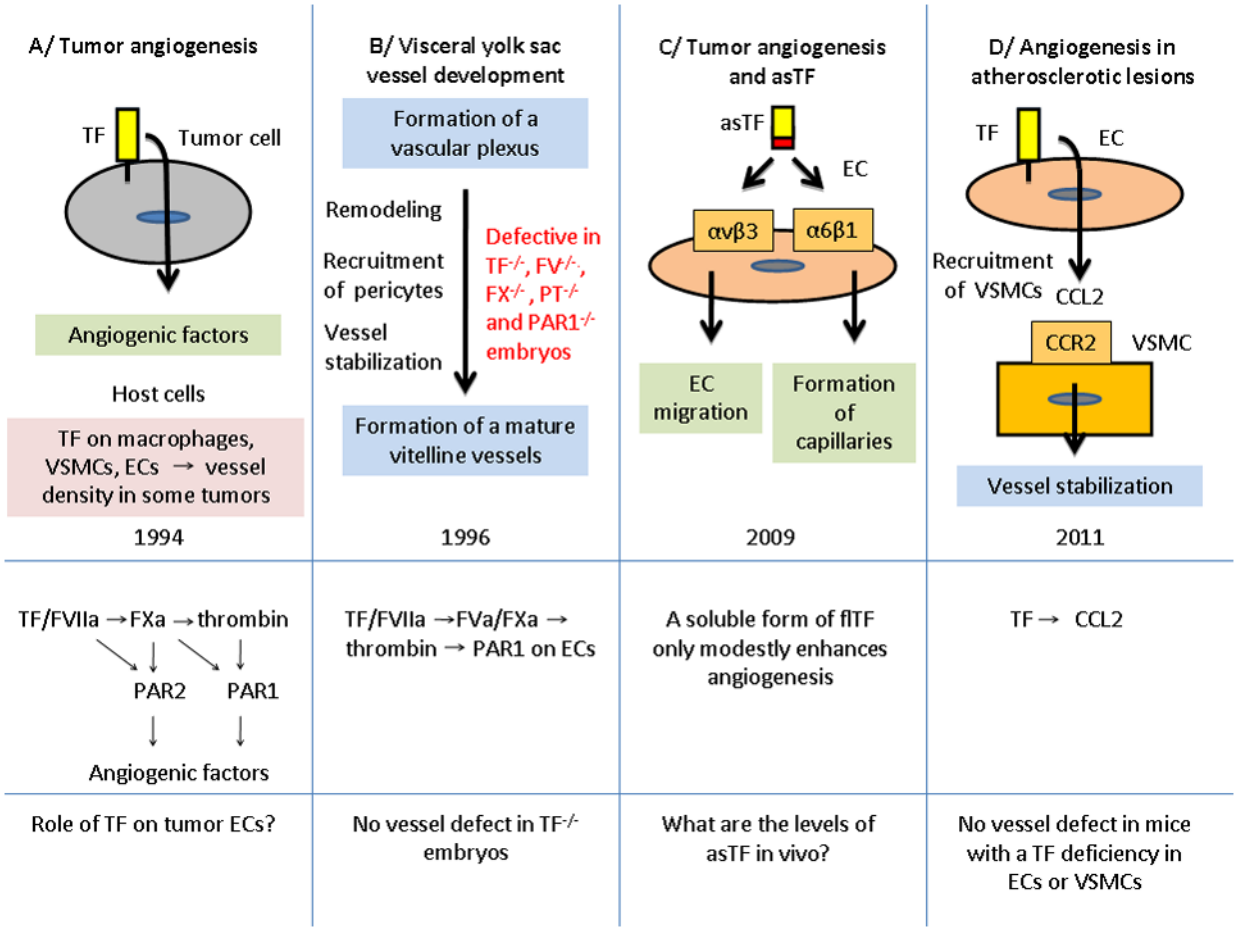

Figure 1. Roles of TF in angiogenesis

The figure shows a time line for studies on TF and angiogenesis. The different pathways that are thought to contribute to angiogenesis are shown below. PT, prothrombin.