Tissue Factor-Dependent Signaling in Tumor Biology

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Initiation of coagulation by tissue factor (TF) has multiple links to tumor biology. At least three distinct TF-dependent pathways contribute to metastasis: (i) thrombin-dependent fibrin generation that facilitates tumor cell implantation [1], (ii) thrombin signaling that enhances metastasis by effects on tumor cells and the endothelium, and (iii) signaling of the TF cytoplasmic domain involving phosphorylation at Ser residues [2, 3]. TF is further expressed in angiogenic endothelial cells [4], but the specific functions of TF in angiogenesis are poorly defined by *in vivo* experiments. TF may contribute to angiogenesis by (i) generating a transitional fibrin matrix to support sprouting and/or sealing of hyperpermeable blood vessels; (ii) regulation of endothelial cell migration by cooperating with integrin signaling [5]; and (iii) protease-dependent cell signaling through protease-activated receptors (PARs).

Our recent work has focused on the role of PAR-dependent cell signaling in endothelial cells. PARs are activated by proteolytic cleavage, exposing a tethered ligand that initiates the G-protein-dependent signaling cascades [6]. The first cloned PAR, PAR1, is the prototypical receptor for thrombin. PAR1 is expressed by endothelial cells throughout the vascular bed. In embryonic development, PAR1 signaling has endothelial protective functions, and absence of PAR1 leads to partial embryonic lethality due to vascular failure [7]. We have shown that PAR1 is the signaling receptor not only for thrombin, but also for other proteases, i.e. activated protein C (APC) and Xa, of the pro- and anti-coagulant pathways [8–10] (fig. 1). Efficient signaling of the anticoagulant

pathway through PAR1 involves a receptor cascade in which thrombomodulin-bound thrombin activates protein C that is bound to the endothelial cell protein C receptor (EPCR) [11]. EPCR is a required co-receptor for activated protein C signaling through PAR1 in endothelial cells [8]. Because thrombin's effective concentration *in vivo* is tightly regulated by endothelial cell thrombomodulin, intravascular thrombin generation may result in direct or indirect endothelial cell signaling [12]. At low thrombin concentration, the thrombomodulin-protein C pathway may predominate with indirect thrombin signaling through EPCR-APC. *In vivo*, EPCR-APC signaling has been linked to protection of hypoxic ischemic brain injury and possibly in placenta development [13–15]. At higher concentrations, thrombin can directly cleave PAR1, resulting e.g. in platelet activation or other direct effects on tumor growth and metastasis. While thrombin is upstream of direct and indirect signaling responses, the involvement of additional receptors, such as thrombomodulin and EPCR, will alter the biological outcome of PAR1 activation. Thrombin generated during tumor development and angiogenesis may thus elicit distinct signaling responses, depending on the signaling route.

The initiation phase of the TF pathway is also closely linked to PAR signaling. Factor Xa not only activates PAR1, but also PAR2, while associated with the transient TF-VIIa-Xa initiation complex. The localization of proteolytic activity restricts the number of PARs that can be cleaved and thus constitutes one aspect of signaling specificity of the initiation reaction. In addition, PAR

Fig. 1. Schematic overview of how PAR signaling is linked to the pro- and anti-coagulant pathways. Within the vasculature, thrombin may signal directly, e.g. to activate platelets through PAR1 and PAR4, or indirectly, to protect the endothelium by activating the protein C pathway. In the anti-coagulant pathway, EPCR-bound activated protein C (APC) activates PAR1 on endothelial cells. Alternative substrates, such as fibrinogen, can compete with direct PAR1 cleavage by thrombin and thus contribute to threshold effects of direct versus indirect intravascular thrombin signaling. The TF initiation complex activates both PAR1 and PAR2. TF-VIIa can also activate PAR2.

signaling can be modulated by the TF cytoplasmic domain that serves as a docking site for the recruitment of adaptors and co-signaling molecules. We find that phosphorylation of the TF cytoplasmic domain is tightly regulated in endothelial cells. Phosphorylation of Ser²⁵⁸, rather than the protein kinase C (PKC) consensus site Ser²⁵³, influences the conformation of the TF cytoplasmic domain sufficiently to yield a phosphorylation-specific antibody that recognized the phosphorylated TF cytoplasmic domain. With this antibody, we characterized TF cytoplasmic domain phosphorylation in phorbol ester stimulated endothelial cells. Phorbol ester activates PKC, which is a known signaling intermediate downstream of PARs. Ser²⁵⁸ is primarily unphosphorylated in confluent endothelial cell monolayers and can be phosphorylated in the absence of the autologous TF transmembrane and extracellular domains. However, phosphorylation required maturation of TF in the Golgi-compartment, intact cholesterol-rich microdomains, and cell surface expression.

Thus, TF expressed on the surface of angiogenic endothelial cells is the target for outside in signaling that modulates the phosphorylation status of the cytoplasmic domain. Surprisingly, palmitoylation of the cytoplasmic Cys²⁴⁵ negatively regulates Ser²⁵⁸ phosphorylation, suggesting that targeting of the TF to specific membrane domain by post-translational modifications is an additional mechanism that controls TF-dependent cell signaling. Thus, emerging data suggest that TF-initiated coagulation pathways can influence angiogenesis and metastasis by cell signaling in the TF initiation phase and by generating thrombin with multiple cell signaling targets. Procoagulant and cell signaling functions of the TF coagulation pathway are difficult to separate experimentally. Because the cytoplasmic domain of TF is dispensable for the procoagulant function of TF [2], TF cytoplasmic domain deleted mice will be instrumental to specifically analyze the role of TF-dependent signaling in angiogenesis and tumorigenesis.

References

- 1 Palumbo JS, Kombrinck KW, Drew AF, Grimes TS, Kiser JH, Degen JL, Bugge TH: Fibrinogen is an important determinant of the metastatic potential of circulating tumor cells. *Blood* 2000;96:3302–3309.
- 2 Mueller BM, Ruf W: Requirement for binding of catalytically active factor VIIa in tissue factor dependent experimental metastasis. *J Clin Invest* 1998;101:1372–1378.
- 3 Bromberg ME, Sundaram R, Homer RJ, Garen A, Konigsberg WH: Role of tissue factor in metastasis: Functions of the cytoplasmic and extracellular domains of the molecule. *Thromb Haemost* 1999;82:88–92.
- 4 Contrino J, Hair G, Kreutzer DL, Rickles FR: In situ detection of tissue factor in vascular endothelial cells: Correlation with the malignant phenotype of human breast disease. *Nature Med* 1996;2:209–215.
- 5 Ott I, Fischer EG, Miyagi Y, Mueller BM, Ruf W: A role for tissue factor in cell adhesion and migration mediated by interaction with actin binding protein 280. *J Cell Biol* 1998;140:1241–1253.
- 6 Coughlin SR: Thrombin signalling and protease-activated receptors. *Nature* 2000;407:258–264.
- 7 Griffin CT, Srinivasan Y, Zheng Y-W, Huang W, Coughlin SR: A role for thrombin receptor signaling in endothelial cells during embryonic development. *Science* 2001;293:1666–1670.
- 8 Riewald M, Petrovan RJ, Donner A, Mueller BM, Ruf W: Activation of endothelial cell protease activated receptor 1 by the protein C pathway. *Science* 2002;296:1880–1882.
- 9 Riewald M, Kravchenko VV, Petrovan RJ, O'Brien PJ, Brass LF, Ulevitch RJ, Ruf W: Gene induction by coagulation factor Xa is mediated by activation of PAR-1. *Blood* 2001;97:3109–3116.
- 10 Riewald M, Ruf W: Mechanistic coupling of protease signaling and initiation of coagulation by tissue factor. *Proc Natl Acad Sci USA* 2001;98:7742–7747.
- 11 Esmon CT, Xu J, Gu JM, Qu D, Laszik Z, Ferrell G, Stearns-Kurosawa DJ, Kurosawa S, Taylor FB Jr, Esmon NL: Endothelial protein C receptor. *Thromb Haemost* 1999;82:251–258.
- 12 Ruf W, Dorfleutner A, Riewald M: Specificity of coagulation factor signaling. *J Thromb Haemost* 2003, in press.
- 13 Cheng T, Liu D, Griffin JH, Fernandez JA, Castellino F, Rosen ED, Fukudome K, Zlokovic BV: Activated protein C blocks p53-mediated apoptosis in ischemic human brain endothelium and is neuroprotective. *Nat Med* 2003;9:338–342.
- 14 Ruf W: PAR1 signaling: More good than harm? *Nature Med* 2003;9:258–260.
- 15 Isermann B, Sood R, Pawlinski R, Zogg M, Kalloway S, Degen JL, Mackman N, Weiler H: The thrombomodulin-protein C system is essential for the maintenance of pregnancy. *Nat Med* 2003;9:331–337.