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Tissue factor-PAR2 signaling crosstalk in cancer and angiogenesis

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Tissue factor (TF) has now well recognized roles as a signaling receptor involved in the regulation of angiogenesis, tumor growth, metastasis and inflammation. TF initiates coagulation, leading to thrombin signaling through G protein coupled, protease activated receptors (PAR) 1, 3, and 4.[1] However, the signaling activities of the TF-VIIa binary and the TF-VIIa-Xa ternary complex may occur prior to or even independently of generalized coagulation activation that lead to fibrin generation, platelet activation and thrombus formation.[2] The TF-VIIa complex activates PAR2 and the product of initiation of coagulation, Xa, while still assembled in the transient ternary TF-VIIa-Xa complex, signals through PAR1 or PAR2. Both upstream TF-specific and downstream thrombin–dependent coagulation signaling may thus contribute to cancer biology. The emerging evidence indicates that TF on tumor cells and host cells exerts non-overlapping biological effects through cell signaling pathways (Figure 1).

TF on tumor cells regulates the angiogenic balance of the tumor microenvironment[3] and this influence depends on factors that are specifically present only under *in vivo* conditions.[4] Upregulation of proangiogenic interleukin 8 by TF-VIIa signaling indicates direct TF-dependent PAR2 signaling as one possible pathway, but the finding that TF can regulate integrin function5 may further point to TF crosstalk in the interaction of tumor cells with the extracellular matrix of the tumor stroma. The TF-integrin crosstalk involves both, cytoplasmic and the extracellular domains. Three experimental approaches implicate the TF extracellular domain: (i) Cell spreading on anti-TF extracellular domain antibodies is blocked by anti-,1 antibody, (ii) purified TF extracellular domain supports adhesion of several integrins expressed in a heterologous CHO-cell background, and (iii) the spreading on anti-TF antibodies was not induced by certain antibodies. In particular, antibody 5G9 directed to the macromolecular substrate exosite on TF appeared to prevent integrin-interaction and cell spreading. The finding that the same antibody epitope is involved in integrin and substrate binding suggests that TF cannot interact with both at the same time. Thus, TF either triggers coagulation or regulates integrin function and TF's influence on cell migration is a truly non-coagulant function of TF.

The TF crosstalk with integrins does not prevent cell adhesion, rather it serves to regulate integrin-dependent migration. Specifically, TF inhibited the migration on laminin-5 that is dependent on activation of integrin $\alpha 3\beta 1$. This inhibition required TF cytoplasmic domain signaling and by mutagenesis we showed that the TF cytoplasmic domain suppressed integrin activation when it is not phosphorylated. TF phosphorylation is specifically induced by PAR2 signaling[6] and TF-VIIa mediated activation of PAR2 was sufficient to release integrin inhibition in a pathway that required phosphorylation of the TF cytoplasmic domain. Thus, TF-VIIa signaling may simultaneously counteract integrin suppression by phosphorylating the TF cytoplasmic domain and trigger locally pro-invasive PAR2 activation. Together, these pathways may promote local tumor expansion aided by proangiogenic growth factors.[5]

TF cytoplasmic domain signaling also plays an important role in tumor cell metastasis. Thrombin is well established as the central protease that drives tumor cell metastasis, initiating fibrin and platelet deposition. Platelets protect tumor cells from natural killer cell attack and secrete pro-angiogenic and tumor cell mitogenic substances from granules. Thrombin also acts on the tumor cells, changing their adhesive behavior and increases pulmonary metastasis. Thrombin-dependent PAR1 activation is central to these pathways and thrombin signaling is implicating in tumor cell survival upon implantation as well as the regulation of tumor cell motility. How-

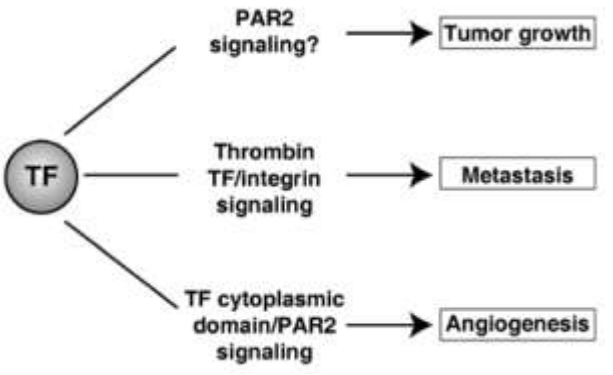

Figure 1. Cell signaling pathways initiated by TF in tumor biology.

ever, chemokinetic effects of thrombin on metastatic melanoma cells were not reproduced by stimulation with PAR1 agonist peptides. Intriguingly, the effect of thrombin on tumor cell migration was recapitulated by combined treatment with PAR1 and PAR2 agonist peptides.[7] PAR2 stimulation also enhanced metastasis, indicating that thrombin's prometatstatic effects on tumor cells may involve cross-activation of PAR2. The tethered ligand sequence of PAR1 is know to activate PAR2 as well and this cross-activation mechanism may lead to a unique signaling of PAR1 and PAR2 acting as homodimers.

Coagulation activation is critical for the early stages of metastasis and supports the tumor cell survival following initial arrest. Interestingly, integrin $\alpha 3\beta 1$ plays an important role in metastatic arrest to patches of laminin-5 exposed between endothelial cells in target organs.[22] These *in vivo* imaging studies of metastatic arrest indicate that spreading of the cells, rather than migration and extravasation across the endothelium is the major determinant for tumor cell survival during the thrombin-dependent stages of metastasis.[23] TF cytoplasmic domain signaling does not interfere with adhesion and spreading on laminin-5, but selectively suppressed cell motility dependent on activated $\alpha 3\beta 1$ integrin. Thus, the TF cytoplasmic domain may act synergistically with thrombin signaling by stabilizing the crucial spreading of tumor cells on patches of extracellular matrix exposed in target organs. However, thrombin signaling does not trigger TF cytoplasmic domain phosphorylation which is selectively induced by TF-dependent PAR2 signaling pathways. Signaling of TF associated proteases, by targeting the TF cytoplasmic domain, may thus regulate thrombin's prometastatic effects.

The abundant expression of TF in tumor cells has long distracted from potential roles of TF expressed by host cells present in the tumor stroma or by endothelial cells. We obtained evidence for a role of TF in angiogenesis in our studies with knock-in mice that lack the TF cytoplasmic domain ($TF^{\Delta CT}$ mice). Tumor angiogenesis was evaluated by transplanting syngeneic tumor to monitor subcutaneous tumor growth. Surprisingly, tumors grew ~2-fold faster in $TF^{\Delta CT}$ mice compared to wild-type controls. These results indicated that the TF cytoplasmic domain in host cells plays a negative regulatory role in tumor angiogenesis . In $TF^{\Delta CT}$ mice, tumors grew larger although injected tumor cells expressed high levels of TF, indicating that tumor cell expressed TF cannot trigger the same pathways as host TF. Indirectly, these data also argue that host cell TF regulated tumor expansion independent of local thrombin formation.

Aortas form $TF^{\Delta CT}$ mice also showed enhanced sprouting in an *ex vivo* angiogenesis assay. Enhanced sprouting in this assay required serum, and specific protease inhibitors showed that VIIa is the critical protease to drive angiogenesis from $TF^{\Delta CT}$ aortas. Purified VIIa enhanced angiogenesis only in the presence of platelet-derived growth factor (PDGF) BB, but not other pro-angiogenic growth factors, including VEGF-A, bFGF, or PDGF-AA. Deletion in of PAR2 in PAR2/$TF^{\Delta CT}$ double knock-out mice showed that PAR2 is required for enhanced angiogenesis. Importantly, PAR2 deletion *per se* did not affect angiogenesis and angiogenesis in the PAR2/$TF^{\Delta CT}$ double knock-out was similar to wild-type mice. One possible explanation for this finding is that the TF cytoplasmic domain exerts a potent negative regulatory control on PAR2 to prevent PAR2-dependent pro-angiogenic signaling. Developmental angiogenesis in the retina was also accelerated in $TF^{\Delta CT}$ mice, but not in PAR2/$TF^{\Delta CT}$ double knock-out mice. This shows that TF cytoplasmic domain signaling and PAR2 are connected *in vivo*.

Because PAR2 signaling leads to TF phosphorylation, we reasoned that in pathological angiogenesis hyperphosphorylation of TF may shut of the angiogenesis-suppressive functions of the TF cytoplasmic domain. Indeed, PAR2 expression was detected in the retina neovasculature of diabetic patients. Intriguingly, phosphorylated TF was associated with abnormal, proliferative neovasculature as well, whereas TF in normal vessel wall and neuronal tissue was not phosphorylated. Conceivably, TF may support physiological angiogenesis as well, but dephosphorylation of the TF cytoplasmic domain may serve as a break to arrest excessive neovascularization. However, it is also possible that an additional signal is required to turn on the otherwise silent TF-PAR2 signaling axis specifically in pathological neovascularization. Protein kinase pathways and modification of the TF cytoplasmic domain Cys residue which can be palmitoylated, are prime candidates for regulatory control of TF signaling. The TF cytoplasmic domains thus emerges a

key regulatory switch in metastasis and angiogenesis. How phosphorylation of TF influences TF-dependent PAR signaling will further clarify the role of TF in tumor biology in the future.

References

1. Coughlin SR. Thrombin signalling and protease-activated receptors. Nature 2000;407:258-64.
2. Ruf W, Dorfleutner A, Riewald M. Specificity of coagulation factor signaling. J Thromb Haemost 2003;1:1495-503.
3. Zhang Y, Deng Y, Luther T, Müller M, Ziegler R, Waldherr R, Stern DM, Nawroth PP. Tissue factor controls the balance of angiogenic and antiangiogenic properties of tumor cells in mice. J Clin Invest 1994;94:1320-7.
4. Yu JL, May L, Lhotak V, Shahrzad S, Shirasawa S, Weitz JI, Coomber BL, Mackman N, Rak JW. Oncogenic events regulate tissue factor expression in colorectal cancer cells: implications for tumor progression and angiogenesis. Blood 2004; 105:1734-41.
5. Hjortoe GM, Petersen LC, Albrektsen T, Sorensen BB, Norby PL, Mandal SK, Pendurthi UR, Rao LV. Tissue factor-factor VIIa specific up-regulation of IL-8 expression in MDA-MB-231 cells is mediated via PAR-2 and results in increased cell migration. Blood 2004; 103:3029-37.
6. Dorfleutner A, Hintermann E, Tarui T, Takada Y, Ruf W. Crosstalk of integrin α3,1 and tissue factor in cell migration. Mol Biol Cell 2004;15(10):4416-25.
7. Ahamed J, Ruf W. Protease-activated receptor 2-dependent phosphorylation of the tissue factor cytoplasmic domain. J Biol Chem 2004;279:23038-44.
8. Shi X, Gangadharan B, Brass LF, Ruf W, Mueller BM. Protease-activated receptor 1 (PAR1) and PAR2 contribute to tumor cell motility and metastasis. Mol Cancer Res 2004;2(7):395-402
9. Wang H, Fu W, Im JH, Zhou Z, Santoro SA, Iyer V, DiPersio CM, Yu QC, Quaranta V, Al Mehdi A, Muschel RJ. Tumor cell α3beta1 integrin and vascular laminin-5 mediate pulmonary arrest and metastasis. J Cell Biol 2004 March 15;164(6):935-41.
10. Belting M, Dorrell MI, Sandgren S, Aguilar E, Ahamed J, Dorfleutner A, Carmeliet P, Mueller BM, Friedlander M, Ruf W. Regulation of angiogenesis by tissue factor cytoplasmic domain signaling. Nature Med 2004;10:502-9.