Chapter 4 - Tissue factor-integrin interactions in cancer and thrombosis: every Jack has his Jill

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Summary

Tissue Factor (TF) is a 47kDa membrane protein that initiates coagulation by binding to FVII(a) and FX(a) and is a risk factor for thrombosis in many disease states. In addition to its coagulant activity, TF also influences cancer progression by triggering signaling effects via a group of G-protein coupled receptors named Protease Activated Receptors (PARs). TF localizes to cytoskeletal structures in migrating cells, influences cytoskeleton reorganization and promotes migration. Recently, integrins, important mediators of cell motility, have emerged as important binding partners for TF and influence both TF coagulant and PAR-2-dependent signaling functions. Direct binding of TF to integrins also impacts processes such as cell migration and signaling independent of PAR-2. A recently discovered alternatively spliced, soluble Tissue Factor isoform also ligates integrins to augment angiogenesis, thus fueling cancer progression. To date, the literature describes a complex interplay between different integrin subunits and distinct TF isoforms but our understanding of TF-integrin bidirectional regulation remains clouded. In this review, we aim to summarize the existing knowledge on integrins-TF interaction and speculate on its relevance to physiology and pathology.

Introduction

The blood coagulation system is a complex mechanism enabling an organism to control excessive blood loss after vascular damage. The 47 kDa transmembrane protein Tissue Factor (TF) is the initiator of the coagulation cascade; loss of the endothelial barrier results in exposure of TF to the circulation, resulting in binding and activation of blood-borne zymogen FVII. The active TF-FVII complex then converts FX into FXa, leading to thrombin formation and a fibrin network which in turn seals the site of bleeding [1]. Most TF resides at the cell surface in an inactive, cryptic state and it is believed that TF inactivation results from low local exposure of pro-coagulant phosphatidylserine (PS) [2]. However, increases in cell surface-exposed PS do not completely explain TF activation, as blocking surface PS does not block TF function in all cell models, and Protein Disulfide Isomerase/redox-dependent oxidation of TF has been proposed as an alternative model underlying TF activation/inactivation [3]. Blood coagulation is normally tightly regulated; consequently, deregulation of this system can lead to a variety of pathological events. It has now been established that cancer development leads to an increased risk of thrombosis, and conversely, excessive activation of blood coagulation profoundly influences cancer progression [4, 5]. TF levels are frequently upregulated in many cancers and are further enhanced in metastatic cells [6-8] through a concerted action of oncogenes and

inactivation of tumor suppressors. In colorectal carcinoma, disruption of oncogenic K-ras downregulates TF levels while loss of the tumor suppressor p53 increases TF levels [9]. In glioblastoma cells downregulation of the tumor suppressor PTEN and constitutive activation of EGFR has a similar effect [10]. Finally, TF is overexpressed in hypoxic tumors [11] and TGFβ upregulation by breast cancer cells leads to increased levels of TF on stromal fibroblasts [12].
TF can also trigger signaling events that influence cancer cell behavior. On the one hand, TF in complex with FVIIa activates a member of G-protein coupled receptors named Protease Activated Receptor (PAR-2) [13], while on the other, TF regulates cell adhesion and migration in PAR-2-dependent and -independent manners. Interestingly, both signaling and migration appear to be critically dependent on integrins.

Overexpression of TF on cancer cells and subsequent shedding of TF-positive vesicles called microparticles, from cancer cells has also been associated with enhanced risks of venous thrombosis in cancer patients, a phenomenon referred to as Trousseau's syndrome. Emerging evidence suggests that integrins also play a critical role in regulation of TF coagulant and pro-thrombotic activity. In this review, we will summarize our current insights into the reciprocal interactions between TF and integrins, with a focus on TF-integrin signaling and procoagulant activity.

TF and actin dynamics

Cells are highly versatile units that respond to extracellular stimuli by spreading on extracellular matrices or initiating (non)-directional migration. Both processes critically rely on dynamic reorganization of the actin cytoskeleton and formation of focal adhesions. During processes such as migration a cell becomes polarized, with actin polymerization taking place at the leading edge, forming lamellipodia and filopodia, while the rear side of the cell retracts [14]. Of note, the leading edge of a migratory cell is rich in proteins that play a role in protrusion formation and adhesion [15] and interestingly, in breast cancer cells and invasive bladder carcinoma TF resides at lamellipodias, ruffled membrane areas and invasive edges of the cancer cells [16, 17], suggesting that TF may play a role in actin dynamics and cell migration. Similar localization patterns are observed in non-cancerous cells; in migrating smooth muscle cells TF also translocates to the leading edge [18].

Actin is the main component of cytoskeletal elements thus by polymerization and depolymerization, it effects cell polarity and morphology. The actin polymerization process is under the control of actin binding proteins [19]. Interestingly, yeast two hybrid screening experiments show that Actin Binding Protein (ABP)-280 is a binding partner for

TF [20]. Tissue Factor has two cytoplasmic serine residues (Ser253, Ser258); stimulated phosphorylation of Ser253 by protein kinase C (e.g. after cell exposure to phorbol myristate acetate; PMA) triggers the phosphorylation of Ser258 in a Proline-directed kinase-dependent fashion and recently p38α MAP kinase was identified as a proline-directed kinase capable of phosphorylating Ser258 [21, 22]. Binding of ABP-280 to TF is regulated by the phosphorylation status of the TF cytoplasmic tail (TF CT). Mutation of Ser residues into Asp, thus mimicking phosphorylation, increases interaction of TF with ABP-280 while mutating Ser to Ala, thus preventing phosphorylation, has an opposite effect [20]. In addition, TF and ABP-280 co-localize at the leading edge of the cell, indicating a role for TF in adhesion and migration [17] and, TF/ABP-280 complex disruption by transfecting cells with TF Ser253Ala, Ser258Ala chimeras reduces the spreading of J82 bladder carcinoma cells [20].

TF may also regulate actin dynamics by influencing activation and expression levels of proteins that are instrumental for actin cytoskeleton remodeling and migration, such as Rac and Cdc42. Binding of FVIIa to TF in fibroblasts induces Src/PI3-kinase dependent activation of Rac and Cdc42, filopodia and lamellipodia formation [23]. In J82 bladder carcinoma cells, treatment with FVIIai leads to an migratory phenotype, dependent on TF CT-induced Rac1 and p38 activation [24]. In addition, silencing of TF leads to downregulation of Cdc42, RhoA and Rac1 levels thereby inhibiting actin reorganization and cell migration [18].

TF-integrin complex formation

As mentioned above, in migratory cells TF localizes to leading edges, a subcellular domain that is also enriched in integrins, raising the interesting possibility that TF and integrins can functionally interact. Integrins are heterodimeric cell surface receptors composed of an α and β subunit: 18α and 8β monomers can complex with each to form 24 different types of integrin receptors that in turn can bind to distinct extracellular matrices (ECMs) such as laminin 5 and fibronectin [25]. Upon binding, focal adhesions are formed and kinases such as FAK, Src family kinases are recruited while the adaptor proteins talin, paxillin and vinculin connect the integrin cytoplasmic tails to actin filaments [26]. ECM-integrin interactions inform cells on the local milieu (e.g. local ECM deposition), eliciting intracellular pathways that lead to appropriate responses such as migration or apoptosis. Indeed, integrin ligation plays a crucial role in cell survival; cells that do not integrin-dependently adhere to ECM proceed to go into anoikis [27, 28] while it should be noted that TF:FVIIa complex and the downstream protease FXa, inhibit this process [29]. These receptors are also involved in processes that regulate cancer progression such as

proliferation [30], angiogenesis [31, 32] and metastasis [33, 34]. In our review we will not discuss the various roles of integrins in these processes due to limited space. Instead we refer the reader to reviews available on these subjects [35-37].

As the involvement of both TF and integrins in cancer progression has been described in the literature in detail, the question whether these proteins interact and reciprocally influence each other's function in the regulation of tumor progression is valid. Co-localization studies using fluorescence microscopy and co-immunoprecipitation experiments **(Fig.1 and Fig.2)** [38] demonstrate that TF interacts with integrin α/β dimers. Incubation of MCF-7 lysates with recombinant TF-coated agarose beads, results in β1 integrin co-precipitation, suggesting physical interactions between of TF and β1 integrins [39]. Yet, transfection of CHO cells with different integrins shows that TF does not physically interact with all integrin complexes; αvβ3, α9β1 and α5β1 but not αvβ5 expression, increases CHO cell binding to immobilized TF [25]. HaCaT keratinocytes bind to immobilized TF-antibodies and binding is sensitive to α3, α5 and β1 integrin blockade [25] while the natural ligand of TF, FVIIa, induces complex formation of TF and β1 integrin [38]. This interaction is seemingly independent of TF:FVIIa's ability to cleave PAR-2, as the use of active site-blocked FVIIa (FVIIai) or a PAR2-blocking antibody does not prevent TF-β1 integrin complexation [38]. Of note, two different TF-specific monoclonal antibodies, mAb 5G9 which blocks TF-dependent FXa generation and mAb 10H10 which blocks TF-dependent PAR-2 activation, have opposite effects on TF-integrin complexation: 5G9 potently stimulates while 10H10 downregulates complex formation. Interestingly, the complex-promoting effects of FVIIa and 5G9 are not observed in malignant breast cancer cells, while 10H10 disruption of TF/integrin binding by 10H10 is maintained. Moreover, in xenograft experiments, growth of the breast cancer cell line MDA-MB-231-mfp is hampered after co-injection of 10H10 with cancer cells orthotopically, but not by 5G9 [38]. Overall, this implies that breast cancer growth *in vivo* may be a downstream event resulting from TF-integrin complexation (see below).

An unsolved question remains regarding how TF associates with integrins, but recent data suggest that TF reduction and divalent cations are involved. Two cysteine residues (Cys186-Cys209) located in the extracellular domain of TF are crucial determinants of TF function. Oxidation of the disulfide bridge between these two residues induce a coagulant, oxidized pool of TF while reduction results in a coagulant-inactive TF form that facilitates FVIIa-dependent PAR-2 activation [40]. Indeed, TF mutants (both human TF and its murine homologues) lacking either or both cysteine residues show reduced affinities for FVII(a) and are deficient in FX binding, showing that lack of the allosteric disulfide critically affects TF function [41, 42]. Interestingly, TF/β1 integrin complexation occurs after cellular

stimulation with relatively high levels of FVIIa, a feature that is in line with the low affinity of FVIIa for reduced TF. Furthermore, as discussed below, integrin β1 function as a "co-factor" to reduced signaling TF, supporting the idea that TF should be reduced to bind β1 integrin.

Presence of divalent cations such as Ca2+ and/or Mg2+ appears to be required for complex formation, suggesting a non-covalent association [38, 39]. The membrane proximal region (amino acids 202-210 in mature TF) may be involved as peptides representing this region inhibit reverse endothelial cell migration of monocytes [43].

In conclusion, the nature of TF-integrin complexation remains unclear. We will now focus on the functional implications of TF-integrin complexes in TF signal transduction, cell behavior and prothrombotic activity.

Integrin dependent TF signaling

Cancer progression, at least in orthotopic breast cancer models, is critically dependent on TF signaling activity via PARs. FVIIa bound to TF forms a proteolytically active complex that can activate PAR-2; cleavage of the PAR-2 N-terminus results in formation of a new N-terminus that can bind to the second solvent-exposed loop of the receptor, in turn activating downstream signaling pathways. Among the four different family members of this receptor (PAR1-4), PAR-2 is the only one that is activated by the binary TF/VIIa complex, but not by thrombin [13].

Extensive crosstalk between PARs and the TF CT is crucial to TF signaling. In *in vivo* cancer models, efficient PAR-2-dependent tumor angiogenesis requires presence of the TF CT in cancer cells, while *ex vivo* aortic sprouting models –mimicking the host compartment- paradoxically show that the TF CT inhibits PAR-2 signaling. Thus, PAR-2 and the TF CT are involved in a complicated bidirectional crosstalk, with the TF CT supporting or inhibiting PAR-2 signaling depending on whether TF and PAR-2 are expressed in tumor cells or in non-malignant stromal and endothelial cells [44, 45].

Recent evidence shows that integrins facilitate TF-dependent PAR-2 activation and signaling. Support for this comes from studies showing that treatment of HaCaT cells with mAb 5G9 results in a subtle increase in β1 integrin binding to TF but does not have an effect on PAR signaling. In contrast, incubation of these cells with the TF/β1 integrin-disrupting mAb 10H10 blocks TF-dependent PAR-2 signaling **(Fig. 2)** [38]. This is in concordance with the fact that incubation with the β1 integrin inhibiting antibody AIIB2

results in diminished TF-dependent PAR-2 signaling [38]. Thus, binding of β1 integrin to TF may have a boosting effect on PAR signaling. It should be noted that 10H10 also inhibits tumor growth in murine xenografts, a feature that is believed to result from inhibition of TF/β1 complexation. Below, we will summarize our knowledge on the involvement of β1 integrin in TF-dependent signaling in migration, angiogenesis and proliferation.

TF integrin signaling in migration

Migration is crucial for invasion and metastasis and recent literature indicates that TF critically regulates cell motility. It is pertinent to note that TF could contribute to cell migration through activation of PARs, but may also potentiate migration in protease-independent manners. TF-dependent migration of SW620 colorectal cancer cells [46], MDA-MB-231 breast cancer cells [47], and glioma cells [48] appears to be dependent on FVIIa proteolytic activity and activation of functional PAR-2. Migration of MCF-7 cells increases after FVIIa exposure, but not after FVIIai treatment [17] suggesting involvement of PARs in this setting as well. Also TF-dependent migration of non-cancerous cells, such as vascular smooth muscle cells [49] often show requirement for PAR-2. In this context, in porcine cerebral microvascular endothelial cells TF:FVIIa upregulates RhoA and cortactin, proteins critically involved in migration, in a PAR-2 dependent manner. During PAR-2 activation, cortactin relocates to the cell periphery and assists in lammelipodia formation. Fibroblasts show an increased chemotactic potential towards PDGF-BB upon treatment with FVIIa but not with FVIIai, suggesting that PAR-2 is also crucial to FVIIa-induced chemotaxis [50]. In a physiological setting, TF-PAR-2-dependent migration is likely to be involved in cutaneous wound healing [51].

In contrast to studies identifying a role for PAR-2 in TF-dependent signaling, a number of reports have also indicated that TF induces migration independent of PAR-2 activation. TF antibodies binding to the FVII(a) binding site induce migration of J82 bladder carcinoma cells [20] and this is dependent on α3, α5 and β1 integrins [25]. In the same cell type, migration toward immobilized fibronectin can be induced by FVIIai, which suggest that binding of FVII to TF in J82 cells is sufficient and proteolytic activity is not a prerequisite for migration [24]. In this setup, migration was dependent on the TF CT, activation of Rac and p38.

Interestingly, binding of 5G9, which promotes TF-integrin complex formation in HaCaT cells, also promotes migration of this cell type on laminin V in a α3β1-dependent manner. Also, in HaCaT cells and A7 cells transfected to express TF, 5G9 induces TF CT phosphorylation while phosphorylation is required for 5G9-dependent migration [25]. The

same study also showed that FVIIa - another inducer of TF/β1 complex formation- increased migration of A7 cells. However FVIIa did not elicit this effect in cells transfected to express TF CT phosphorylation-deficient mutants. Overall, it is reasonable to posit that PAR-2-independent migration is to some extent dependent on interaction of TF binding with integrins, under the control of FVIIa, and TF CT phosphorylation, potentially leading to a higher migratory capacity of cancer cells and metastasis. However, such a role for integrins in TF signaling has primarily been established *in vitro* and *in vivo* evidence for such a role is still lacking.

TF-dependent integrin signaling in angiogenesis and proliferation

Evidence that TF is critically involved in angiogenic repertoires comes from studies showing that TF null mice do not survive due to irregular formation of yolk sac vasculature, leading to bloodless yolk sacs and wasting of embryos [52, 53]. In *ex vivo* aortic sprouting experiments, absence of the TF CT augments vessel formation in a PAR-2 dependent manner [44]. *In vitro*, downregulation of TF in endothelial cells and vascular smooth muscle cells impedes the formation of tubules on matrigel. Silencing of TF in endothelial cells result in decreased activation of Akt and Raf/ERK and Ets-1 transcription factor, a critical intermediate in TF-dependent angiogenic processes *in vitro* [54]. Moreover, TF plays a role in vessel maturation by increasing levels of chemokine ligand-2 in endothelial cells thereby attracting vascular smooth muscle cells around the newly formed vessel [55]. Interestingly, these angiogenic processes were relatively independent of PAR2 function and it remains unknown whether and when TF signals through PAR2 to induce angiogenesis, but the experimental setting (*in vitro* vs *in vivo*) and pathological context (physiological angiogenesis vs tumor angiogenesis) may play a role.

Apart from its clear role in embryogenesis, TF signaling via PARs is also important for vessel formation in a cancer setting. Blockade of TF:FVIIa proteolytic function using a nematode anti-coagulant protein (rNAPc2) diminish tumor weight and vessel formation [56] and treatment of MDA-MB-231 cells with FVIIa or PAR-2 activaton peptides increase VEGF expression which is a key player in integrin signaling [57]. Similarly, TF-PAR-2 signaling *in vivo* produces VEGF and additional pro-angiogenic molecules such as IL-8 and CXCL-1. Importantly, in these models, the TF-β1 complex-disrupting antibody 10H10 decreases microvascular density in breast cancer xenografts and impairs IL-8 production, demonstrating that functional TF-β1 integrin coupling results in enhanced angiogenesis [38].

A TF isoform that results from alternative splicing and exclusion of exon 5 in the TF transcript yielding a soluble protein (alternatively spliced TF; asTF) [58], has recently also been implicated in angiogenesis. Unlike TF, asTF does not have procoagulant activity [59, 60] and is not involved in PAR activation [61]. Although TF and asTF have functionally different features, they appear to be involved in similar biological processes, albeit through different modes of action. In studies by Hobbs and colleagues, transfection of the pancreatic cancer cell line Mia-Paca-2 with asTF led to bigger and more vascularized tumors [62]. Nevertheless, it remained unclear whether asTF influences tumor growth resulting in (hypoxia-driven) angiogenesis, or whether asTF is a direct pro-angiogenic molecule. Our own studies revealed that asTF directly induces angiogenesis in matrigel plug assays and *ex vivo* aortic sprouting models **(Fig.3)**. Interestingly, proteolytically active FVII and PAR-2 were not required for asTF-dependent angiogenesis, but angiogenesis in this setting rather relies on integrin ligation. asTF ligates distinct integrin subsets to induce a repertoire of angiogenesis-related processes. asTF-dependent endothelial (tip) cell migration is dependent on αvβ3 and capillary formation takes place after asTF-α6β1 ligation [61]. Subsequent studies showed that murine asTF (masTF) is a functional homologue to human asTF (hasTF). Similar to what was observed for hasTF, masTF increased *ex vivo* sprout formation, endothelial cell migration and capillary formation, although masTF-dependent angiogenesis relies solely on β3 ligation, rather than on a combination of β1 and β3 [63].

TF-integrin interaction also influences cell proliferation. Human coronary artery cells treated with either recombinant TF or TF^{+} microparticles (see below) proliferated faster and TF-dependent proliferation was independent of FVIIa, as addition of recombinant FVIIa, and FXa- and FVII-blocking antibodies did not have any effects on proliferation. Rather, proliferation relied on TF binding to β1 integrin as functional blockade of TF-integrin complexation using a β1 integrin peptide diminished the proliferative phenotype [64]. This study again emphasizes that TF-integrin interaction might be a key player in both proliferative events and tumor angiogenesis in the absence of a functional TF-PAR-2 signaling axis. It should, however, be mentioned that an exogenous soluble artificial form of TF (sTF) induces a β1 integrin-dependent decrease, rather than increase, in proliferation rates in MCF-7 breast cancer cells [39]. Nevertheless, the effects of TF-β1 integrin ligation on proliferation may be cell type and integrin subset dependent.

TF-integrin interactions in thrombosis

A body of evidence suggests that TF is a major risk factor for thrombotic complications in different disease settings such as sepsis [65], cancer [66] and atherosclerosis [67]. In atherosclerotic plaques, TF is expressed by intraplaque monocytes/macrophages [68], and upon rupture of the vulnerable plaque TF is released into the bloodstream, and activates coagulation thus leading to thrombosis. Similarly, TF may play an important role in cancer-associated thrombosis, as TF is dramatically upregulated on cancer cells. However, it remains unclear how cell-exposed TF contributes to coagulation activation in disease. While TF is normally tethered to the cell surface, it may localize to the blood on the surface of submicron vesicles that are shed from the surface of intravascular cells, such as platelets, endothelial cells, and leukocytes, but also from tumor cells [69]. These vesicles that arise from blebbing of the cell are called microparticles (MPs) and their size ranges from 50-1000 nm. Several studies have found that plasma MP-TF concentration or MP-TF procoagulant activity positively correlates with the risk of VTE and even with recurrent VTE. In a cancer setting, TF activity on microparticles is higher in patients with VTE compared to patients without VTE [70-74] and high MP-TF levels in plasma are predictive of VTE in prospective studies [75]. It is not entirely clear how TF^+ MPs could contribute to development of a thrombus, but experimental studies suggest that MPs can induce a procoagulant state by fusing with endothelial cells and platelets through binding of MP PSGL-1 to endothelial/platelet p-selectin [76, 77]. Indeed, work by Thomas et. al. shows that TF+ MPs decrease bleeding times and lead to arteriole occlusion in a vessel damage model, in a p-selectin-dependent manner [78]. Nevertheless, how TF shedding and regulation of TF activity on the surface of MPs is regulated remains somewhat elusive.

In macrophages and smooth muscle cells, ATP-induced P2X7 receptor activation results in prothrombotic MP release in a PDI-dependent manner. Importantly, these MPs were shown to carry both TF and β1 integrin. Shedding of these proteins on MP surfaces is readily inhibited by the use of free thiol blockers and inhibitor PDI antibodies, indicating the involvement of thiol-dependent pathways in TF and β1 shedding [79]. At the same time, this demonstrates that also in a prothrombotic setting, TF and β1 localize to similar subcellular domains and it is tempting to speculate that TF and β1 redox-dependently associate on MP surfaces. A recent study shows that not only do TF and β1 colocalize on MPs, disruption of TF/β1 complexes decrease β1 levels on endothelial cell-derived MPs, suggesting that transfer of β1 integrin to MPs is dependent on its binding to TF. Moreover, MP-exposed β1 integrin can act as an adhesion molecule promoting sustained MP binding to ECMs such as collagen and fibronectin and thereby promoting coagulant activity of MP-TF [80].

Integrins may also regulate TF coagulant activity in a more direct fashion. Evidence for this comes from our studies employing TF/β1 co-precipitations. FVIIa enhances TF-β1 integrin complexation 2-3 fold, while coagulant activity in these co-precipitates was unchanged. Also, complex formation required high (10 nM) concentrations of FVIIa, a feature that suggests the involvement of (reduced) coagulant-inactive TF. Additionally, cellular exposure to the TF coagulant function-blocking antibody 5G9, thus precluding FX binding to TF:FVIIa, increases TF binding to β1 integrin [38], suggesting that TF complexation with β1 only occurs in the absence of FX binding. It is now appreciated that coagulant-inactive, cryptic TF may not be explained by one model [40, 41, 81-83] rather, coagulant inactive TF may comprise a set of TF molecules that are kept inactive by low local PS exposure, PDI-dependent mechanisms and β1 integrin-dependent mechanisms. Nevertheless, to support such a theory, further studies on this subject are warranted.

Although asTF on itself does not harbour any coagulant activity, asTF-integrin interaction is believed to influence arterial thrombosis. Our recent work showed that asTF is expressed in intraplaque macrophages and asTF can functionally bind to β1 integrins to enhance expression of endothelial cell adhesion molecules, such as VCAM, ICAM and p-selectin [79]. Indeed, exposure of endothelial cells to asTF promotes adhesion of peripheral blood mononuclear cells (PBMCs) under orbital shear conditions and under laminar flow. Moreover, asTF potentiates PBMC migration through MVEC monolayers under MCP-1 gradient, showing that asTF-β1 interaction may promote atherosclerosis and plaque instability by facilitating adhesion receptor-dependent monocyte transmigration.

Conclusion

The current understanding of the interaction between Tissue Factor isoforms and integrins is that they may play a critical role in cancer progression and thrombotic complications, although the mechanistic evidence for this is still slim. They may affect each other's function reciprocally; TF can affect migration and adhesion of cells by binding to integrins, while binding of integrins to TF can influence TF-dependent PAR-2 signaling and coagulant function.

It is still unclear how important these interactions are in (patho)physiology as support for a link between TF/integrin interaction and pathological outcome in e.g. cancer patients is still missing. Furthermore, the emerging picture is complicated by the fact that TF complexation with different integrin subsets and the effect of different extracellular matrices on complex formation have not yet been extensively characterized.

Nevertheless, emergence of TF specific antibodies that differently modulate TF-integrin complexes and thus TF function may prime extensive research on the interaction of TF with integrins and its subsequent impact on cancerous and thrombotic disease.

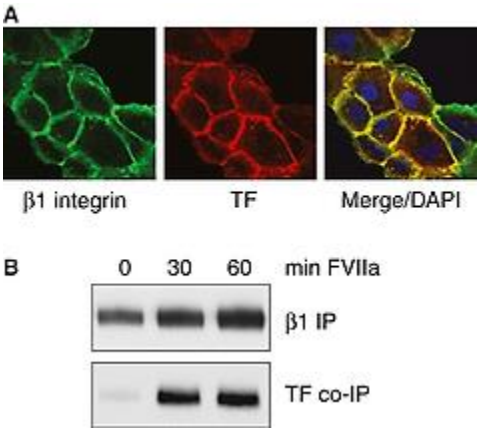

Fig.1 TF colocalizes and associates with β1 integrin in HaCaT cells. (A) β1 integrin and TF in HaCaT cells were stained using AIIB2 (anti-integrin β1) and 5G9 (anti-TF). Images were captured using confocal microscopy. (B) HaCaT cells were incubated with 10 nM FVIIa for the indicated times. β1 integrin was precipitated from lysates using anti-β1 antibody TS2/16. β1 and co-precipitated TF were assessed on Western Blot.

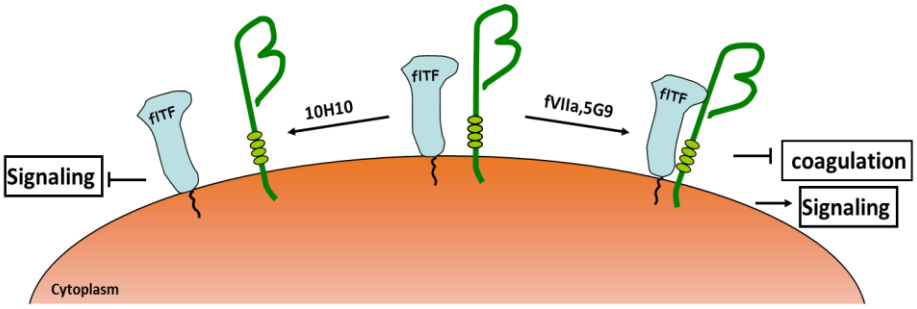

Fig.2 TF-β1 integrin complex formation on cells and functional implications. In this model TF associates with β1 integrin under basal circumstances. Stimulation with FVIIa or the TF mAb 5G9 further stimulate complex formation and may facilitate TF-dependent signaling, while inhibiting coagulation. Exposure to the TF mAb 10H10 disrupts TF-integrin complexes and inhibits TF-dependent signal transduction. Although TF-integrin complexes also contain α integrin subunits (α3 and α6), these are not shown to reduce complexity.

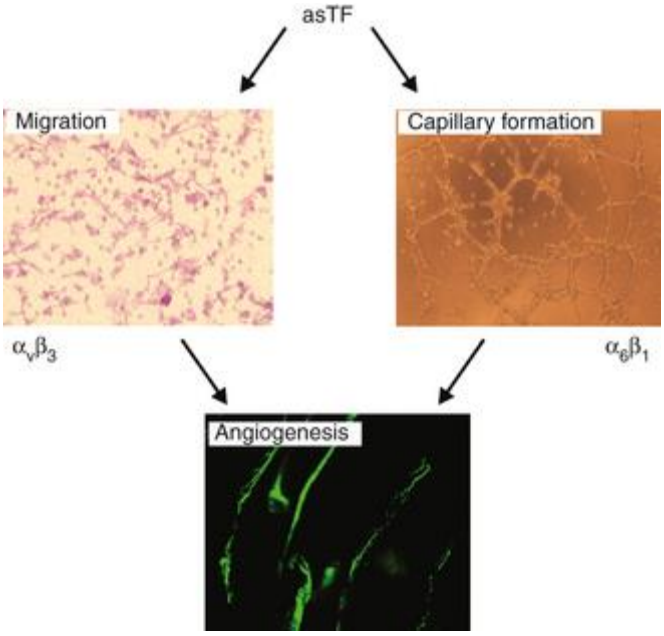

Fig.3 asTF-induced angiogenesis. asTF ligates endothelial αvβ3 integrins to induce (tip) cell migration as determined by transwells assays. In parallel, asTF facilitates capillary formation on a matrigel surface by binding to α6β1 integrin. Both processes are believed to be important to asTF-dependent angiogenesis, as determined in murine matrigel plug assays.

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