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ROLE OF THE TISSUE FACTOR PATHWAY IN THE BIOLOGY OF TUMOUR INITIATING CELLS

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Abstract.

Oncogenic transformation and aberrant cellular differentiation are regarded as key processes leading to malignancy. They produce heterogenous cellular populations including subsets of tumour initiating cells (TICs), also known as cancer stem cells (CSCs). Intracellular events involved in these changes profoundly impact the extracellular and systemic constituents of cancer progression, including those dependent on the vascular system. This includes angiogenesis, vasculogenesis, activation of the coagulation system and formation of CSC-related and premetastatic niches. Tissue factor (TF) is a unique cell-associated receptor for coagulation factor VIIa, initiator of blood coagulation, and mediator of cellular signalling, all of which influence vascular homeostasis.

Our studies established a link between oncogenic events, angiogenesis and the elevated expression of TF in several types of cancer cells. The latter suggests that cancer coagulopathy and cellular events attributed to the coagulation system may have cancer-specific and genetic causes. Indeed, in human glioma cells, a transforming mutant of the epidermal growth factor receptor (EGFRvIII) triggers not only the expression of TF, but also of its ligand (factor VII) and protease activated receptors (PAR-1 and PAR-2). Consequently, tumour cells expressing EGFRvIII become hypersensitive to contact with blood borne proteases (VIIa, thrombin), which upregulate their production of angiogenic factors (VEGF and IL-8), and contribute to formation of the growth promoting microenvironment (niche). Moreover, TF overexpression accompanies features of cellular aggressiveness such as markers of CSCs (CD133), epithelial-to-mesenchymal transition (EMT) and expression of the angiogenic and prometastatic phenotype. Conversely, TF blocking antibodies inhibit tumour growth, angiogenesis, and especially tumour initiation upon injection of threshold numbers of tumourigenic cells. Likewise, TF depletion in the host compartment (e.g. in low-TF mice) perturbs tumour initiation.

These observations suggest that both cancer cells and their adjacent host stroma contribute TF activity to the tumour microenvironment. We postulate that the TF pathway may play an important role in formation of the vascular niche for tumour initiating CSCs, through its procoagulant and signalling effects. Therapeutic blockade of these mechanisms could hamper tumour initiation processes, which are dependent on CSCs and participate in tumour onset, recurrence, drug resistance and metastasis.

1. INTRODUCTION – THE INTERPLAY BETWEEN ONCOGENIC EVENTS AND CELLULAR INTERACTIONS DURING THE DEVELOPMENT OF MALIGNANT DISEASE

In their vast majority, human cancers contain distinct genetic abnormalities that are believed to play an initiating and/or promoting role at different stages of disease progression[1]. Based on their functional impact, these mutational lesions are classified as affecting either cancer susceptibility genes (triggers of genetic instability), tumour suppressor genes or oncogenes [1]. The latter two categories encode proteins that directly participate in various crucial cellular responses, in a manner that either inhibits or promotes the abnormal growth, respectively[1;2]. Consequently, cancer progression entails losses in function of tumor suppressors, coupled with non-redundant changes (mutations, amplification or overexpression) that activate cellular oncogenes [1]. These alterations (collectively referred to as oncogenic events) accumulate over time and cooperatively change the expression profiles of various downstream effector genes, whereby growth controls are released and the emerging transformed phenotype drives cells towards a selective growth/survival advantage over their normal non-transformed counterparts[2].

While genetic processes have traditionally been viewed as intrinsic to cancer cells and often cell-autonomous in nature, it is now recognized that their impact transcends cellular boundaries, and that oncogenic changes in certain tumour cells affects their adjacent cancer cells, normal host stromal cells, and impact various systemic controls of tissue homeostasis [3]. Notably, some of the most critical of these oncogen-driven intercellular (multicellular) interactions involve the various facets of the vascular system, as targets,

effectors or conduits of malignant growth and dissemination. These vascular effects may involve short, medium and long range interactions, all of which may engage not only endothelial, mural and inflammatory cells [4], but also the coagulation and fibrinolytic systems [3;5;6].

2. CONSTITUENTS OF THE VASCULAR CANCER STEM CELL NICHE

Genetic instability, oncogenic events and aborted cellular differentiation programs collectively lead to the emergence of heterogeneous populations of cancer cells[7]. This feature is a source of considerable conceptual and practical challenges, due to diversity and plasticity of such cellular pools with regards to their constituent phenotypes, ranges of tumourigenic aggressiveness, metastatic capacities, therapeutic resistance, angiogenic proficiency and other properties, which seem to remain in constant selective and adaptive flux [8].

The genesis of tumour cell heterogeneity is viewed as either stochastic or hierarchical in nature[9]. The latter concept implies that only a minority of cancer cells, termed cancer stem cells (CSCs), or tumour initiating cells (TICs) is responsible for initiation, recurrence and maintenance of the malignant growth as well as generation of the phenotypic diversity of the entire cellular population [10]. In contrast, tumourigenic properties are markedly reduced, or non-existent, in the progeny of TICs, i.e. the non-TICs, which often constitute the majority of cells in a given tumour[10]. In this context CSCs/TICs could be viewed as the primary targets of the cancer-causing oncogenic transformation, and are thought to either emerge from the genetically altered normal stem

cell compartment of the related tissue [11], or arise from more committed cells that assume CSC-like properties following their malignant conversion[9].

TICs/CSCs exhibit certain functional and phenotypic similarities to their corresponding tissue stem cells, including the expression of certain antigens (e.g. CD133, CD44, nestin, ABCG2), and some functional properties such as, high clonogenic capacity, formation of tumour spheres in defined medium, or the ability to differentiate to multiple lineages [10;12]. One aspect of the latter is associated with the process referred to as epithelial-to-mesenchymal transition (EMT), during which epithelial-derived cancer cells lose some of their lineage-specific properties (e.g. epithelial appearance or antigens) and assume properties of the mesenchyme (fibroblastic morphology, vimentin expression, motility), along with capacity to invade and metastasize [13]. It was noted recently that certain TICs exhibit properties of EMT[14] along with elevated angiogenic and, interestingly, also procoagulant activity[15].

The defining feature of TICs is their ability to efficiently initiate tumour growth *in vivo.* Indeed, while xenotransplantation into immunocompromised mice of large numbers of non-TICs either fails to trigger tumour formation, or does so with very low efficiency, even small numbers, or single TICs are sufficient to initiate malignant growth [16]. This feature may reflect a particular capacity of these cells to cause disease onset, recurrence or metastasis under natural settings [10;12;16-18]. While the nature, abundance and phenotypes of TICs are subjects of a considerable debate [19;20], the very existence of a differential tumourigenic potential amongst heterogeneous cancer cells is well

documented and highly intriguing. As mentioned earlier, this is manifested by experiments demonstrating differential tumour 'take' upon standard injection of distinct cancer cell subsets into immunocompromised (e.g. SCID, NOD-SCID or NOD-SCID-IL2Rg-/-) recipient mice [12;16;17;21]. A similar differential and organ-specific ability is observed in the process of metastasis which *de facto* explores the TIC-properties of cancer cells at secondary tissue sites [20;22;23].

A particularly intriguing feature of normal stem cells, as well as of their malignant counterparts (CSCs/TICs), especially in the central nervous system [24], is their close interrelationship with the vasculature [25]. This is manifested by the physical proximity of CSCs/TICs to tumour blood vessels [26], their elevated proangiogenic activity due to copious production of vascular endothelial growth factor (VEGF) [27] and possibly also other mediators [28], and their functional dependence on sustained angiogenesis and vascular access [29]. These observations led to the notion that CSCs/TICs exhibit their potential in a manner that is regulated my a unique perivascular micromilieu, often referred to as the *vascular cancer stem cell niche* [25].

Another type of a blood vessel-related niche for cancer cells was recently described in the process of metastasis, and termed a *pre-metastatic niche* [30]. In this case, seeding and growth of metastatically competent cancer cells at sites of their dissemination was found to be preceded by an influx into, and conditioning of these sites by bone marrow-derived cells harbouring type 1 VEGF receptor (VEGFR1/Flt1). This likely represents only one manifestation of pro-metastatic vascular changes (niches), to which many different

activities, cells and molecular mediators may contribute in various settings, and facilitate secondary growth of TICs at distant sites.

The relevant constituents of these various vascular niches are thought to include the supply of oxygen and nurtients, perivascular microenvironment and extracellular matrix, blood borne endocrine and regulatory factors and circulating cells. Notably, paracrine influences of endothelial cells ('angiocrine' mechanisms) markedly contribute to these perivascular niche effects [25]. Endothelial growth factors have long been recognized as affecting cancer cell behaviour [31;32], but only recently their role was implicated in the context of TICs/CSCs [25].

While the aforementioned elements of the vascular and pre/pro-metastatic CSC niches are increasingly well characterized, little (no) attention has been devoted to the possible contribution of the coagulation system in their formation[33]. This is surprising, as tissue factor (TF) expression and the activation of the clotting cascade have long been known to impact the efficiency of the metastasis process, and thereby implicitly the fate of TICs in it [34-37]. Indeed, a mounting body of observations suggest that TICs/CSCs may be affected by the coagulation-related mechanisms in several ways, many of which may converge upon TF and its effectors (Fig. 1).

3. TISSUE FACTOR AND PROTEASE ACTIVATED RECEPTORS AS ONCOGENIC TARGETS

Cancer patients are prone to thrombotic complications, which at least in part are related to tumour-dependent hypercoagulability [38-43]. In various clinical contexts this propensity is described as Trousseau's syndrome [40;44;45], or cancer coagulopathy [39], and linked to considerable morbidity, mortality and exacerbation of the underlying malignant process [42]. In addition to diverse 'unspecific' triggers of this condition (vascular permeability, stasis, bed rest, catheterization), cancer cells themselves frequently exhibit considerable clotting potential, along with their ability to release procoagulant mucins, proteases, inhibitors of fibrinolysis [38;46;47] as well as cell bound and soluble forms of TF [34;38;48;49].

As described extensively elsewhere, TF is a 47 kD transmembrane receptor that forms complexes with the circulating coagulation factor VII/VIIa (FVIIa) thereby creating conditions for the localized generation of the coagulation factor Xa (FXa) and thrombin (FIIa) activity. The resulting formation of fibrin clots and aggregates of activated platelets are not only hallmarks of physiological haemostasis, but also play a role in pathological states, such as thrombosis and coagulopathy [50].

Cancer growth may provoke unscheduled, protracted and exaggerated activation of the TF pathway, and through several different mechanisms[3;38]. These include: (i) accumulation of inflammatory and stromal cells with procoagulant properties within the tumour mass; (ii) abnormal expression of TF by the angiogenic or activated endothelial cells of the tumour vasculature[51]; (iii) structural anomalies, stasis and hyperpermeability of the tumour-related microcirculation and the resulting leakage of plasma (coagulation factors) into the perivascular space containing TF-expressing cells; (iv) entry of TF-

expressing cancer cells into the vascular lumen in the course of vascular invasion and metastasis[34;36;38]; (v) emission of TF-containing procoagulant microvesicles (microparticles) into the perivascular space and blood stream [49;52-55]. These and other TF-related processes may elicit systemic manifestations of cancer coagulopathy [38] and/or trigger thrombosis within the tumour vasculature, for instance as observed in glioblastoma [56].

TF-dependent activation of the coagulation cascade also influences cellular processes in a number of different ways. For instance, thromboembolic occlusions in the tumour vasculature may lead to hypoxia, which causes necrotic demise of some cancer cells, while stimulating treatment resistant, proinvasive and proangiogenic activities of others [56] (including CSCs[28]). Formation of the fibrin matrix and accumulation of growth factor (GF)-rich platelets may also affect these events (Fig. 1), including through stimulation of tumour growth, survival, differentiation and angiogenesis [39]. Fibrin mesh is often used to facilitate culture of cellular isolates[32], including endothelial, stromal and various progenitor cells, which may utilize this natural scaffold as their 'niche' and supportive of growth and differentiation processes [57;58].

The impact of the TF pathway on cancer cells may also be exerted in a more direct manner. Thus, the TF/VIIa complex is known to stimulate protease activated receptors (PARs), either directly (PAR-2), or via Xa (PAR-2, PAR-1) and IIa activity (PAR-1, PAR-3 and PAR-4) [59]. Additional pathways of TF-dependent signalling may also include interactions with integrins [60] and cellular chaperones (GRP78)[61], resulting in cellular

responses such as increased migration, survival and expression of the angiogenic phenotype [59]. This molecular apparatus effectively amounts into a blood sensing mechanism, by which normal cells coordinate their 'wounding' and repair-like responses. In cancer these responses may become distorted, at least in part due to exuberant levels of TF expressed by many types of tumour cells, for reasons that until recently have largely remained unknown [3].

In 2000 we postulated [62], and later demonstrated experimentally that the procoagulant conversion of cancer cells is directly linked to their oncogenic status, as both gain of function (EGFR, EGFRvIII, HER-2, K-ras) and loss of function (p53) mutations influence the levels of TF expressed by various types of tumour cells, including glioma, colorectal carcinoma, breast cancer and transformed epidermal cells[15;49;63;64]. These experiments have also implicated cellular transformation in production and active release of TF-containing microvesicles [5;15;49;65-67]. Indeed, other studies soon arrived at similar conclusions, by documenting deregulation of TF by oncogenic EGFR [48], MET [68] and PML-RARα [69]. In some instances mutant oncogenes, such as MET, precipitated an overt thrombohaemorrhagic syndrome in tumour bearing mice, coupled with deregulation of plasminogen activator inhibitor 1 (PAI-1) and cyclooxygenase 2 (COX-2), rather then TF [47]. This is striking as mice are generally unsusceptible to a clinically overt cancer coagulopathy[70]. Of note are studies suggesting that some of the genetic events occurring in various cancers, such as loss of PTEN in glioblastoma, may cooperate with hypoxia [71] and possibly with other extracellular influences to increase cellular levels of TF [72].

Some of the aforementioned studies also revealed that TF may act as an important effector of the oncogenic transformation [49]. Thus, both genetic and pharmacological blockade of this receptor was found to mitigate tumourigenic, angiogenic and metastatic properties of cancer cells harbouring mutant *ras* [36;49] and *EGFR* [15], a pattern likely applicable to other oncogenes as well[3;64].

On the other hand, the enforced expression of exogenous TF in indolent glioma cells was found to be insufficient to emulate the consequences of TF-inducing oncogenic influences exhibited by the constitutively active mutant of EGFR (EGFRvIII). Moreover, cells expressing the exogenous TF (TF-GBM) in the absence of EGFRvIII were similarly procoagulant to cells transfected with EGFRvIII (GBMvIII). In both cases this property was associated with a similar surface level of active TF, again expressed in an exogenous or endogenous manner, respectively. Interestingly, TF-GBM cells responded poorly to stimulation of intracellular signalling by addition of FVIIa and PAR activating peptides (PAR-APs), relative to their GBMvIII counterparts [64]. This was manifested by a disproportionate induction of angiogenic factors (Il-8) by these cells. Thus, GBMvIII produced markedly heightened levels of these factors in the presence of FVIIa and PAR-1/2-APs, and this was not the case following stimulation of their TF-GBM counterparts [64].

Likewise, the ability to form tumours by the respective cell types was different when TF was expressed alone or in the context of EGFRvIII. In both cases tumours did emerge after subcutaneous injection of tumour cells, while the non transfected and TF-negative

parental cells remained non-tumourigenic. However, the cells expressing TF exogenously, in the absence of EGFRvIII produced growth of much less pronounced aggressiveness, magnitude and rapidity than in the case of cells harbouring EGFRvIII and EGFRvIII-driven TF[64]. These results suggest that the coexpression of TF and EGFRvIII may be somehow required for efficient TF signalling, production of angiogenic factors and aggressive growth (beyond effects of EGFRvIII itself) [64].

These observations led us to examine the levels of other elements of the TF/PAR pathway, in EGFRvIII expressing and non-expressing glioma cells. We uncovered that oncogene-dependent transformation leads to coordinated upregulation of TF, PAR-1, PAR-2 and ectopic production of FVII [64]. As a result, the cells harbouring oncogenic EGFRvIII acquired not only the increased expression of TF, but also heightened sensitivity to TF stimulation, *via* PAR-1 and PAR-2 dependent pathways, followed by deregulation of their downstream genes involved in angiogenesis and other processes [64].

Collectively, these observations suggest that oncogenic events deregulate of the sensory mechanism, by which cells are programmed to respond to contact with blood, and blood borne regulatory activities (FVIIa, FXa, thrombin and other) [3]. Since oncogenic lesions are especially consequential when they affect CSCs/TICs, this raises the question as to how these changes in the coagulation system may affect their function and niche[33].

4. IMPACT OF CELL DIFFERENTIATION PATHWAYS ON TISSUE FACTOR EXPRESSION AND SIGNALLING

Stem cells give raise to their hierarchical progeny through processes of asymmetric cell division, lineage commitment and cellular differentiation, the remnants of which may also be observed in certain types of CSCs/TICs[10]. In fact the latter cells often retain the ability to enter multilineage differentiation, whereby they give rise to a heterogeneous spectrum of cells harbouring distinct lineage specific markers [10]. While TF and PARs are expressed during development [73-75] and tissue renewal, little is known about their control and function during the related stem cell commitment and differentiation processes [33]. However, a careful survey of gene expression databases may suggest such a possibility may exist [33]. Thus the responses to the TF/PAR stimulation in various cellular systems reveal changes in the expression of genes, some of which (Oct-4, Klf5) are involved in control cellular multipotentiality, differentiation, and lineage commitment (Table 1). It is of note that other elements of the coagulation system dependent signalling regulate various types of progenitor and stem cells in several different ways. This includes, for example, the effect of thrombin on smooth muscle cell [76], endothelial [77] and hematopoietic [78] progenitors. The functional consequence of such events in the contest of CSCs/TICs is a subject of our active investigation.

In addition, TF could also contribute to niche effects and emergence of the CSC/TIC population is several indirect ways. For instance, the proangiogenic responses due to TF-regulated expression of VEGF and other genes, could render an increase availablility of endothelial contact sites which were reported to facilitate homing of CSC/TIC or retention of their properties [18;24;26]. On the other hand, and somewhat paradoxically stem cells and CSCs preferentially retain their properties and phenotype under conditions of

hypoxia and under influence of hypoxia inducible factor (HIF) activated transcription of genes involved in multipotentiality, differentiation and 'stemness', such as Oct4, Notch and other [28;79]. Elevated expression of TF at the sites of CSCs/TICs, which are reported to be both perivascular and hypoxic, may provide an attractive, but still unproven explanation, at least in the context of glioblastoma. Thus, expression of TF in this highly angiogenic type of malignancy is linked to a simultaneous high rate of intravascular thrombosis and occlusion [56], followed by 'perivascular hypoxia', necrosis and formation of perinecrotic pseudopalisading cells, which may contain both invasive, TF expressing [56;80] and TIC-like cells [28].

5. CANCER CELL-ASSOCIATED TISSUE FACTOR AS A REGULATOR OF TUMOUR INITIATION AND GROWTH

One aspect of cancer-related abnormal differentiation that is relatively well studied in the context of cancer involves the aforementioned process of epithelial-to-mesenchymal transition (EMT) and its reversal, known as MET [13]. Notably, EMT is believed to represent a crucial step in the onset of a motile and metastatic phenotype in epithelial cancer cells, and a consequence of the cooperation between oncogenic, growth factor and differentiation pathways[13]. Interestingly, EMT was recently found to also be a part of the CSC/TIC phenotype [14], an observation consistent with a convergence between tumour initiating and metastatic properties [33].

Our recent studies suggest that TF expression is elevated in both, cancer cells harbouring markers of CSCs (e.g. CD133) [81] and those that display features of EMT [15]. Thus, human epidermal cancer cell lines (A431) driven by the oncogenic amplification of the EGFR can be induced *in vivo* and *in vitro* to give rise to a heterogeneous mixture of epidermoid (TF-low) and mesenchymal (TF-high) subpopulations [15]. The latter cells exhibit spindle morphology, express vimentin and are more procoagulant, metastatic, capable of releasing greater numbers of TF-containing microvesicles and more sensitive to TF-dependent VEGF upregulation then their epidermoid counterparts [15]. In addition, initiation of A431 tumour growth in immunodeficient mice by injection of threshold (low) numbers of cells is profoundly inhibited upon treatment of such mice with anti-TF antibodies, including those that exclusively block TF signalling (e.g. 10H10, data not shown). Collectively, these observations suggest that events involving CSC/TIC-like cells are affected by the status of TF and its effectors [33].

6. HOST CELL-ASSOCIATED TISSUE FACTOR AND TUMOUR INITIATION

While tumour cells in general, and CSCs/TICs in particular, are potentially important sources of TF within their perivascular niches, there is evidence that TF-deficient tumour cells may be capable of aggressive growth and tumour initiation[36;66;82]. This seemingly TF-independent tumourigenesis usually occurs in the context of experimental local injection of large (excessive) numbers of cancer cells, i.e. under conditions that mimic a post-initiation (late) phase of disease progression [33]. This notion is supported by the reported inability of TF-/- *ras*-transformed cancer cells to form experimental metastasis [36]. This is important since in metastasis assays, unlike in those involving subcutaneous

inoculation of large numbers of cancer cells, the resulting growth is inevitably driven by single (few) TIC-like cells trapped in the parenchyma, or vasculature of target distant organs, i.e. is clonal or oligoclonal rather then polyclonal in nature [33].

It is noteworthy that within the cancer stem niche host cells may, at least in theory, constitute a significant source of TF (Fig. 1). However, the role of this host-related TF in tumour formation has long remained unclear. A more extensive analysis of this question has become possible with the advent of mouse strains, in which TF levels could be lowered without causing the risk of bleeding or embryonic lethality. The examples of such strains include the low-TF mice in which the residual expression of TF amounts to 1% of wild type activity [83], and mice where TF can be conditionally expressed in a tissue specific manner[75]. In addition neutralising antibodies have been generated to distinguish between human (tumour) and mouse (host) TF effects [84].

Some of the new insights that have emerged from inclusion of these experimental tools revealed a distinct role of host TF in tumour formation. One line of this work involves embryonic stem (ES) cells which can serve as a model of tumourigenesis based exclusively cellular totipotentiality, aberrant differentiation and 'stemness', in the absence of any overt transforming genetic mutations[85]. Thus, ectopic (subcutaneous) injection of large numbers of ES cells gives rise to aggressive teratomas, the growth of which is dependent on host-mediated angiogenic responses [66;85]. To examine how this process is affected by the TF status we employed TF-proficient (TF+/-) and TF-deficient (TF-/-) ES cells and found they both form tumours in wild type immunodeficient (SCID)

mice [66]. TF-/+ ES cells also readily formed aggressive teratomas in hypomorphic low-TF/SCID mice. However, injection of TF-/- ES cells into such mice led to a complete arrest of tumour formation. This suggested that indicating that ES (stem) cells require a source of TF for expression of their tumourigenic properties (Fig. 2).

Interestingly, host-related TF seems to exert an influence on the functionality of the TIC niche that extends beyond the cumulative TF activity. For instance, while large numbers of various TF expressing cancer cells (LLC, B16F1, A431, U373vIII) readily form tumours in low-TF mice, this is not the case upon injection of some of the same cells at the limiting/threshold numbers (the lowest number of cells giving rise to 100% tumour take in wild type mice)[64;66]. These 'tumour initiation experiments' [15] suggest that the role of host cell-associated TF may extend beyond a simple quantitative addition of the procoagulant activity available at the tumour site, but instead has a more specific role in formation of the TIC niche [33]. One manifestation of this possibility is the altered distribution of alpha smooth muscle actin (αSMA)-positive perivascular cells in tumours growing in low-TF mice. This property is seemingly unrelated to the TF status of tumour cells themselves and its nature requires further study [66;86].

Taken together, the contribution of TF to the vaculat CSC niche could be at least fourfold: (i) the effects of TF upregulation by CSC themselves and the related pericellular coagulation, matrix formation and signalling; (ii) the contribution of TF-expressing CSC progeny (non-stem cells), which could be viewed as a 'CSC niche of cancer cells'; (iii)

the impact of host-dependent TF on the behaviour of CSCs; (iv) the long range influence of TF circulating in a form of microvesicles or oncosomes [5;33;49] (Fig. 1)

7. COULD TARGETING TISSUE FACTOR AND COAGULATION PROCESSES IMPACT CANCER STEM CELLS?

CSCs/TICs and their growth promoting/regulating niches are viewed as key to our understanding of several intractable problems associated with human cancers [10]. This is because they likely represent basic functional 'units' of several fundamentally important processes, including tumour initiation, drug resistance, disease recurrence, invasion, and metastasis [10;12;17;87]. If TF/coagulation pathway and other direct and indirect mechanisms affecting cellular properties play an even partial role in assembly, or functionality of these CSC 'units' this would offer both, a long sought after explanation of some of the linkages between coagulopathy and cancer progression, and also several valuable therapeutic opportunities. The latter prospect is illustrated by the impact of TF-directed antibodies [15;60;88], pharmacologicals [89;90] and genetic manipulation of either TF [66] or PAR status [60] on disease development in tumour bearing mice. Notably, at least some of these anti-tumour effects could be brought about through the impact on CSC niches. Moreover, these effects are achievable without anticoagulation (by modulating TF/PAR signalling), and therefore at no risk to the haemostatic safety ([60] Magnus and Rak – unpublished). Pursuing these questions further constitutes a promising and exciting direction for future translational studies.

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Figure Legends

Figure 1. Putative contribution of coagulation effectors to the vascular cancer stem cell niche. Indirect experimental evidence suggests that tumour initiating cells may be affected by their surrounding procoagulant environment and the related adhesive and growth stimulating influences. TF is postulated here to play a role in these niche effects. In this regard TF influence/activity could emanate from at least three different sources: (i) CSCs themselves – self assembly of the procoagulant CSC niche; (ii) non-CSC tumour cells – cancer cell-related CSC niche, and (iii) stromal, vascular and inflammatory cells – host-dependent procoagulant CSC niche (see text).

Figure 2. Impact of tumour and host tissue factor sources on the ability of embryonic stem cells to initiate the neoplastic growth. TF+/- and TF-/- ES cells exhibit aggressive properties upon subcutaneous inoculation in a manner dependent on the host TF status. TF-/- ES cells fail to form teratomas in low-TF/SCID mice [66](see text).

Table 2. The emerging linkages between tissue factor and coagulation system signalling and the regulatory events influencing various stem cell compartments

Impact on CSC/Niche		**Effects on TF or the Coagulation System**
Effect	***Marker***	***Observation***
Pluripotency Self-renewal	Nanog	Nanog overexpression in MSCs induces coagulation factor II (thrombin) receptor-like 2 upregulation [91]
	Oct-4	Overexpression of the transcription factor Oct-4 in MSCs induces tissue factor pathway inhibitor 2 upregulation[91]
	Klf5	FVIIa upregulates BTEB2/Klf5 expression in HaCaT human keratinocytes. Klf5 is involved in self-renewal of mouse ESCs [92-94]
	LIF	FVIIa stimulation upregulates LIF expression in HaCaT cell line; LIF is involved in stem cell self-renewal [95-97]
CSC markers	CD133	CD133-positive tumour cells express high level of TF, neutralization of TF activity inhibits the tumor growth [81]
EMT and Multi-lineage differentia-tion	E-cad. Vimentin Keratin	TF expression changes with induction of EMT and multilineage differentiation in human A431 cancer cells in vivo and in vitro[81]
Differentia-tion (NSCs)	Oct-2	FVIIa, PAR-1 or PAR-2 stimulation upregulates Oct-2, a regulator of neuronal differentiation [98;99]
Differentia-tion (HSCs)	M-CSF, GM-SCF	FVIIa stimulation induces M-CSF and GM-CSF expression, cytokines that control HSCs differentiation [100;98]
Differentia-tion (MSCs)	CCN1, CCN2	FVIIa stimulation induces CCN1/Cyr61 and CCN2/CTGF expression in MDA-MB-231 breast carcinoma cells. These genes are involved in Wnt-induced osteoblast differentiation of mesenchymal stem cells [98;100 101;102 103]
	Vimentin	TF downregulation by shRNA induces upregulation of vimentin, a marker of mesenchymal differentiation[104]

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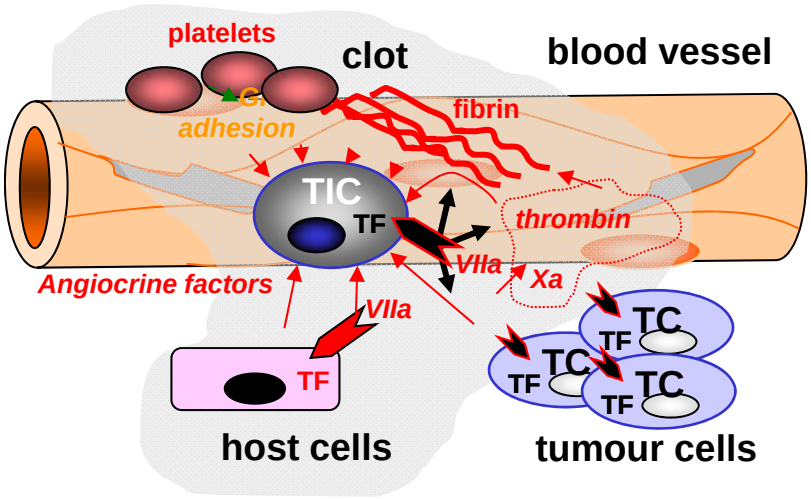
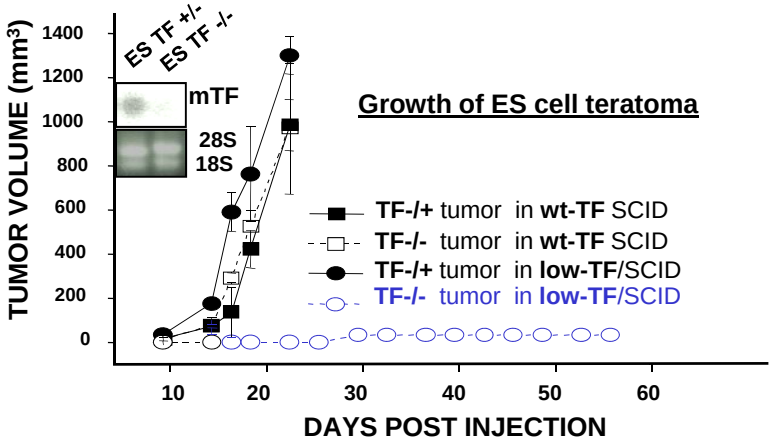
Tissue factor in the perivascular niche



Garnier et al Fig 1.

The contribution of tissue factor and coagulation system to the perivascular niche of tumour initiating cells (TICs)

TF-dependemt tumorigenesis driven by (embryonic) stem cells



Garnier et al Fig 2.

Impact of tumour and host tissue factor sources on the ability of embryonic stem cells to initiate the neoplastic growth.