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Oncogenic events regulating tissue factor expression

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A B S T R A C T

Tissue factor (TF) is the primary cellular initiator of blood coagulation and a modulator of angiogenesis and metastasis in cancer. Indeed, systemic hypercoagulability (Trousseau sign) in cancer patients and TF overexpression by cancer cells are both closely associated with disease progression, but their respective causes have long been elusive.

We have previously hypothesized[1] and recently demonstrated experimentally 2;3 that upregulation of TF expression in cancer is controlled in a qualitatively different manner than in other procoagulant processes, notably by cancer-associated oncogenic events, such as activation of K-*ras* and epidermal growth factor receptor (*EGFR*) or inactivation of *p53* tumor suppressor gene.

These respective transforming alterations exert their impact on both, cell-associated and soluble/circulating (microvesicle- associated) TF expression and activity. TF expression is also an important effector of the K-ras–dependent tumorigenic and angiogenic phenotype of colorectal cancer cells *in vivo.*

Thus, a causal link may exist between genetic tumor progression, angiogenesis and cancer coagulopathy[1;4;5,] and TF appears to be an important common denominator in these processes.[1;3;6-8]

Cancer is believed to arise and progress towards increasing malignancy as a result of cumulative genetic *hits* sustained by the tumor cell genome, often over a long period of time. Paradigmatic in this regard is the development of colorectal carcinoma (CRC), where sequential transition through clinical stages of the disease is paralleled by a series of relatively well-characterized alterations in the status of proto-oncogenes and tumor suppressor genes.[9] In this tumor type, activation of mutant K-*ras* and subsequent inactivation/loss of *p53* are key changes (Figure 1A), which drive many interrelated aspects of the malignant phenotype including aberrant mitogenesis and survival.[9]

Moreover, both of these genetic alterations are thought to contribute to proangiogenic properties of affected cancer cells[10] and metastasis.[9]

The involvement of the vascular system in malignancy encompasses not only angiogenesis, but also systemic hypercoagulability.[11] Cancer coagulopathy is often linked to upregulation of tissue factor (TF), the primary cellular initiator of the blood coagulation cascade.[12,6] Interaction between factor VIIa (fVIIa) and TF leads to activation of factor X (fXa) and generation of thrombin, with subsequent involvement of platelets and formation of a fibrin clot. Remarkably, as a member of the class II cytokine receptor family, TF is also capable of transducing intracellular signals and regulating gene expression.[6;8] In human cancer, TF positivity often correlates with clinical stage, histological grade, poor prognosis, expression of angiogenic factors and vascularity.[6;13.] In this context two main questions remain unanswered. First, what causes the upregulation of TF in certain cancer cells? Second, how are the consequences of TF expression related to the phenotypic changes induced by underlying 'cancer causing' genetic alterations?

Results

To address the aforementioned questions we first examined the impact of three different oncogenic changes on the expression of TF by cancer cells derived from three different human malignancies: colorectal carcinoma (CRC), squamous cell carcinoma (SCC), and astrocytoma/glioblastoma multiforme (GBM).

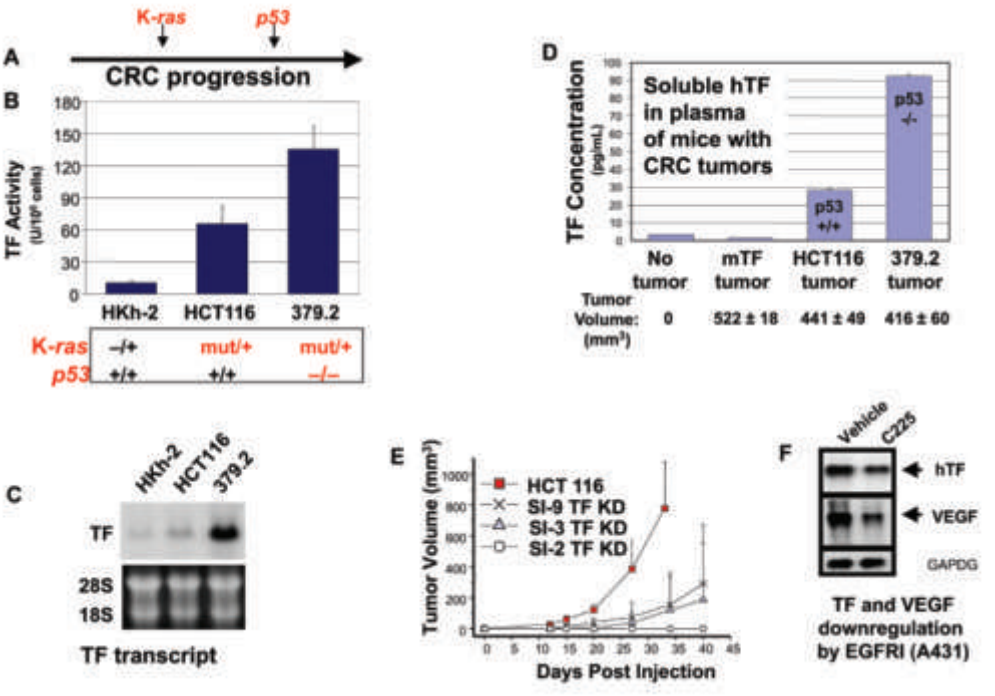

Figure 1. Oncogenic events and TF in cancer.

Upregulation of TF in SCC and GBM cells expressing oncogenic EGFR

Malignant properties of A431 SCC cells are almost entirely attributable to over-expression and constitutive activation of the EGFR. We observed that several different EGFR inhibitors (e.g. C225, AG1478) markedly reduced expression of TF mRNA and protein in these cells (Figure1F).[3] Conversely, expression of the activated/oncogenic EGFR (EGFRvIII) led to a dramatic increase in TF levels in U373 GBM cells.

Cumulative impact of K-ras and p53 on TF expression by human CRC cells

We observed that in two independently derived CRC cell lines, DLD-1 and HCT116, each of which harbors one mutant (and one wild-type) K-*ras* allele TF expression is elevated 2. To determine whether K-*ras* mutation is directly involved in this TF upregulation we examined several variants of the aforementioned cell lines (DKs-8, DKO-3 and HKh-2 cells, respectively), in which mutant K-*ras* alleles were genetically disrupted. In all cases, loss of oncogenic K-*ras* resulted in diminution of TF expression at multiple levels (procoagulant activity, immunoreactivity, protein and mRNA expression) (Figure 1BC). As CRC progression often involves activating K-*ras* mutations followed by inactivation of *p53*, we compared TF status in HCT116 cells (*p53*+/+) to their isogenic counterparts, in which this tumor suppressor gene was targeted by homologous recombination (379.2 cells, *p53*-/-). Again, we observed a marked increase in TF expression and activity upon removal of *p53*. Thus, alterations of both K-*ras* and *p53* cumulatively impact TF expression in human CRC cells in a manner that mirrors the gradual increase in TF levels observed clinically in this disease 2 (Figure 1 A-C).

Impact of oncogenic lesions on shedding TF-containing microvesicles into the culture medium and plasma

TF expression and activity was not only detected on the surface of human cancer cells, but also in the microvesicle fraction of cell-free, culture supernatant and in plasma of mice harbouring human tumor xenografts (Figure 1D).[2;4] Interestingly, the amounts of TF released in this manner corresponded to the oncogenic status of the respective CRC cells (e.g. K-ras^{wt}/*p53*^{wt} < K-ras^{mut}/*p53*^{wt} < K-ras^{mut}/*p53*^{mut} in CRC). Activation of EGFR in SSC and GBM tumors was also paralleled by release of TF containing microvesicles and could be reversed by pharmacological inhibitors of this oncogene (EGFRIs). Thus, we suggest that shedding of TF from cancer cells into the circulation and related procoagulant competence may be a function of genetic tumor progression. Oncogene-directed, anti-cancer therapeutics (e.g. EGFRIs) could therefore

act as *indirect anticoagulants* by modulating causes of cancer coagulopathy.[3]

The link between oncogene-driven TF upregulation, tumor aggressiveness and angiogenesis

Sorting of TF-negative HKh-2 cancer (CRC) cells for rare TF expressing cellular variants (revertants) revealed that such cells (unlike Hkh-2) are highly tumorigenic and possess newly acquired K-*ras* mutations. To determine whether TF is a marker or a necessary component of the K-*ras* driven aggressive and angiogenic phenotype, a selective TF-knock down (siRNA/KD) was achieved in a series of HCT116 sublines (SI-2, SI-3, and SI-9). Indeed, the latter cells manifested impaired tumorigenic and angiogenic properties *in vivo* (in SCID mice, but not *in vitro*), and exhibited sustained expression of angiogenesis inhibitors (thrombospondins 1 and 2)[2] (Figure 1E). These observations suggests that TF upregulation by oncogenic events may not only influence cancer-related coagulopathy, but also directly contribute to tumor growth, angiogenesis, metastasis and ultimately cancer related morbidity and mortality.

Conclusions

Our data suggest that TF is a target of at least three of the most common genetic alterations in human malignancy, namely *p53*, K-ras, and *EGFR*. This may suggest that such genetic alterations play a causative role in cancer coagulopathy.[2] More recently loss of PTEN tumor suppressor and exposure to hypoxia were also implicated in upregulation of TF by GBM cells.[7] Likewise, all-trans retinoic acid (ATRA) attenuates coagulopathy in patients with acute promyelocytic leukemia (APL), where ATRA acts essentially by blocking the oncogenic action of the PML/RARα gene product and TF expression by APL cells.[14] A recent elegant study by Boccaccio expands on the linkage between oncogenes and cancer coagulopathy by implicating *MET* oncogene as a regulator of PAI-1 and COX-2-related aberrations of hemostasis in mice harbouring liver tumors.[5] *We suggest that oncogene-directed (targeted) agents could at least in some cases ameliorate cancer-related coagulopathy. We also postulate that circulating TF (microparticles) could serve as surrogate marker of biological activity of such agents.*

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