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# GENETIC PATHWAYS LINKING HEMOSTASIS AND CANCER

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ABSTRACT

Oncogenic events impact interactions of cancer cells with their surroundings. Amongst the most consequential, in this regard, is the influence on angiogenesis, inflammation and hemostasis. Indeed, mutant oncogenes (EGFR, HER2, RAS, MET, PML-RARα) are known to alter the expression of angiogenic and pro-inflammatory factors, as well as change the cancer cell coagulome, including the levels of tissue factor (TF) and other mediators (PAI-1, COX2). Accompanying losses of tumour suppressor genes (PTEN, p53), and changes in microRNA (miR-19b, miR-520) facilitate these effects. Transforming genes may also trigger ectopic production of coagulation factors (e.g. FVII) by cancer cells and their release and properties of procoagulant microparticles (MPs). By deregulating protease activated receptors (PAR1/2) oncogenes may also change tumour cell responses to coagulation factor signalling. These changes act in concert with microenvironmental factors (hypoxia), stress responses (therapy) and differentiation programs, including epithelial-to-mesechymal transitions (EMT) and through tumour initiating cell (TIC) compartment. In so doing, the coagulation system influences early (initiation, angiogenesis), intermediate (growth, invasion) and late stages (metastasis, relapse) of cancer progression. In fact, TF may act as a molecular switch that controls the transition between dormant, latent and progressive/metastatic disease. TIC-like cells may play a role in these effects, as they express TF and PAR-1/2, and respond to stimulation with their agonists. As major human malignancies (e.g. glioblastoma) are increasingly recognized to consist of a spectrum of molecularly distinct disease subtypes driven by specific genetic pathways, so too may their patterns of interaction differ with the coagulation system. A better understanding of these linkages may be a source of new diagnostic, prognostic and therapeutic opportunities.

## 1. INTRODUCTION – ONCOGENES AS DRIVERS OF VASCULAR ABERRATIONS IN CANCER.

Deregulation and mutations within the cellular genome are both common and causative among human malignancies [1]. While these changes have long been studied as a source of cell-autonomous (intrinsic) pathogenetic aberrations, their impact clearly extends beyond cellular boundaries. Indeed, processes of tumour stroma generation, inflammation and immunity, as well as changes in the local microenvironment, and a network of reciprocal interactions between cancer cells and various host cell compartments are now thought to be orchestrated by (and often specific to) genetic pathways of malignant progression. These effects are attributed to deregulation in production of growth factors, cytokines and other mediators by genetically transformed cancer cells [2], as well as their abnormal responses to the tumour microenvironment (hypoxia), along with other stressors and metabolic alterations. In the vast majority of human cancers the causation of these processes lies with genetic instability and the resulting cumulative impact of mutations affecting oncogenes and tumour suppressors [3;4].

The vascular system is affected by the genetic cancer progression at several pathogenetically significant levels. Thus, tumour microvasculature physically links cancer cells to various distant organ sites and systemic regulatory mechanisms. Blood and lymphatic vessels constitute an important conduit and interface through which cancer cell populations may emit and receive

metabolites, regulatory mediators, hormones, microparticles and cells. Indeed, tumour-vascular interactions define a number of critically important elements of the disease progression as a whole, including tumour initiation, dormancy, aggressiveness, invasion, distant metastasis, drug delivery, therapeutic responses and many others [5]. Consequently, tumour-induced pathological vessel growth and remodelling is viewed as an integral part of the neoplastic process [5], along with the related vascular-dependent comorbidities, especially the propensity for local and distant thrombosis, often referred to as Trousseau syndrome [6].

Genetic transformation affects the relationship between cancer cells and several interrelated compartments that control the vascular system, including bone marrow, peripheral vascular networks and the hemostatic system within. Indeed, cancer cells are capable of mobilizing bone marrow derived (BMDC) cellular populations to the sites of vascular growth, including regulatory and inflammatory cells, as well as progenitors with a potential to differentiate to myeloid or endothelial cells [7-9]. Tumour cells also interact with resident endothelial and mural cells of the vessel wall, which not only contribute structurally to the angiogenic growth, but also exhibit important paracrine (angiocrine) activities [10;11].

There is a well established link between oncogenic transformation and tumour angiogenesis [12;13]. While vascular response programs are evolutionarily conserved and serve to respond to the metabolic needs and stresses of growing, injured or hypoxic tissues [14;15], these effects are quantitatively and qualitatively altered in cancer. For example, isolated cancer cells exhibit strongly proangiogenic phenotypes as a function of their oncogenic status, even in normoxia [12;13]. Oncogenic mutations often mimic or exacerbate the effects exerted by 'unspecific' influences of the tumour microenvironment, such as hypoxia, metabolic stress or paracrine stimulation [12]. Some of the best known examples of mutant genes acting in this manner include both oncogenes: (RAS, SRC, MYC, EGFR, HER2, MET), and deficient tumour suppressors (VHL, p53, PTEN, p16) [12;13]. Some of the best described angiogenesis effectors acting downstream of these oncogenic pathways comprise vascular endothelial growth factor (VEGF), angiopoietins (Angs), interleukin 8 (IL-8), thrombospondin 1 (TSP-1) and several other vascular and inflammatory mediators 16;17. In addition, cancer cells exhibit unusual and genetically controlled proangiogenic properties, such as the ability to release phospholipids that regulate angiogenic properties of adjacent stromal cells18, and microparticles (exosomes) containing oncogenic and endothelial cell-stimulating cargo19-21. Moreover, in certain types of human malignancies (e.g. glioblastoma) tumour initiating cells (TICs) harbouring mutant oncogenes differentiate into endothelial-like cells and contribute to abnormal vessel wall properties (e.g. resistance to antiangiogenic drugs)22. It has been proposed 23 and later documented3;24;25 that oncogenic mutations also affect the 'liquid' compartment of the vasculature in cancer, namely the constituents of the hemostatic system of the circulating blood. In subsequent sections we will discuss this notion in more detail, and the emerging evidence to suggest that various perturbations within the coagulation system could be placed amongst the effector mechanisms, through which oncogenic mutations drive cancer progression, dissemination, morbidity and mortality.

## 2. CANCER RELATED DEREGULATION OF THE HEMOSTATIC SYSTEM

The reciprocal link between cancer progression and deregulation of the hemostatic system is well documented 26. Pioneering studies of Trousseau, Bouillaud and Virchow followed by over a century of investigations led to the notion that cancer often provokes overt thrombosis and bleeding, and may also cause pervasive, biologically significant, but often subclinical, coagulopathy 26-30. There are several measures of this interrelationship. For example, a nearly 3 fold increase in the risk of recurrent venous thrombembolism (VTE) is observed in patients following cancer diagnosis, and close to 20% VTE cases is attributed to an underlying cancer 26. While the absolute risk of VTE in cancer patients is only approximately 0.6%, this may raise several fold with advanced disease and particularly with certain types of therapy (surgery, radiation, hormonal therapy, chemotherapy, thalidomide)30-32. However, cancer types differ markedly in this regard, with the highest risks associated with some (pancreatic cancer, glioblastoma), but not other diseases (breast cancer, melanoma)33. The reasons for this difference are presently unclear, but suggest that coagulation perturbations are not 'unspecific'.

## 3. COMPLEXITY OF HEMOSTATIC ALTERATIONS IN CANCER

Is there one or many different cancer coagulopathies? How do they emerge and impact the disease, and what are the therapeutic implications and options? These questions remain largely unanswered in spite of the extensive coverage of clinical and mechanistic aspects of cancer coagulopathy in the recent literature 26;34-37. Therefore, it may be useful to highlight a few points relevant to this discussion and the related genetic underpinnings. First, the clinical manifestations of hemostatic perturbations in cancer are highly complex and heterogeneous when analysed in non-stratified patient cohorts. If the triggers of these perturbations lie in molecular pathogenesis (subtypes) of specific cancers, a more targeted (individualized) analysis of coagulopathy could be informative. Second, multiple hemostatic mechanisms have been implicated in cancer-related hemostatic aberrations, but their respective sources (cancer cells, host cells) and roles (in thrombosis, cancer progression) often remains unexplained or controversial. This includes studies implicating tissue factor (TF) 34;38;39, cancer procoagulant (CP) 40, thrombin 36, ectopically produced factor VII 41, prothrombin expression 42, changes in fibrin deposition and lysis 43;44, anomalies in PARs 34, deregulation of plasminogen activators and inhibitors (PAI-1) 45;46 involvement of activated protein C (APC) and its endothelial receptor (EPCR)47, release of procoagulant microparticles48 activation of platelets 49, extracellular DNA 50, as well as many other mechanisms. Linking these processes to their genetic triggers (if any) may facilitate more rational and effective therapies using both traditional (heparin) and new anticoagulants 29;51;52. Third, activation of the coagulation system influences cancer biology in many different ways that may depend on cancer genetics. For example, deposition of extracellular fibrin matrix may protect and stimulate cancer and stromal cells, shield metastatic emboli against immune attack, or trigger vascular occlusion leading to hypoxia and angiogenesis. These processes may have different meanings in molecularly distinct cancer contexts, e.g. due to alternative determinants of cell survival, immune evasion or metastasis. Fourth, in addition to clotting effects, coagulation factors affect intracellular signalling pathways in cancer, inflammatory, vascular and stromal cells. This is executed via activation of protease activated receptors (mainly PAR-1, PAR-2) and exposure to their agonistic coagulation proteases (VIIa, Xa, IIa). The resulting signals propagate through G proteins their downstream pathways

(MAPK, AKT, RHO, RAC) and changes in expression of several genes 53. Signals are also generated through interactions between TF and integrins, and by pathways involving beta-arrestin 34. It is noteworthy that oncogenic pathways may rewire cancer cell signalling circuitry and alter biological responses to coagulation-related stimuli54. Therefore, a better understanding of the link between molecular biology of specific cancers and molecular pathways of coagulation may render the complexity of this field more translucent.

## 4. PATHOGENETIC ROLE OF CANCER COAGULOPATHY

Hemostatic aberrations contribute to cancer morbidity, mortality and progression 26;52;55-57. Of the numerous indications to this effect, the most dramatic include the fact that thrombotic complications are the second most common cause of cancer related deaths, and coagulopathy tends to be increasingly prevalent with the extent of the disease, metastatic dissemination and the aggressiveness of therapy26. Elevated biomarkers of activated coagulation (D-dimers, TAT complexes), circulating procoagulant microparticles and clinical thrombosis are often associated with progressive disease and unfavourable outcomes58-62. On the other hand modest, but measurable survival benefits are associated with anticoagulation in cancer patients 28;63-65. There are great hopes that more targeted, biologically based, and context-oriented approaches to activated coagulation/inflammation state may have therapeutic value in cancer, especially as an adjunctive treatment. Selective inhibition of TF, VIIa, IIa, PARs and other targets has already been shown to modulate the disease pathogenesis and progression 3;26;34;66-68 combination therapy studies (including chemotherapy and antiangiogenic agents) are badly needed 32;69. Elevated TF and activated intratumoural coagulation have also been used to direct cytotoxic agents to cancer 70;71. Several additional observations may suggest that coagulation system biology may offer unexplored therapeutic opportunities.

*Thrombophilia may directly predispose to cancer.* A particularly striking example in this regard is related to the reported sharp (nearly 6 fold) increase in the risk of colorectal cancer incidence in patients with a preceding procoagulant syndrome due to factor V Leiden mutation, but not in the case of other defects, such as prothrombin G20210A, PAI-1 4G/5G, fibrinogen gamma 10034C>T , and factor XIII Val34Leu mutations 72. In agreement with this notion, well controlled experimental studies affirm a causative link between the activation of the coagulation system and cancer progression 26;34;73.

*Coagulation system affects early, intermediate and late stages of cancer progression.* It is increasingly clear that coagulation system activation impacts a wide range of processes, which are involved throughout cancer progression (cancer cell survival, invasion, angiogenesis, drug resistance, inflammation, immunity, and metastasis)74-76. Therefore, there is no reason to a priori assume that these influences will not apply to certain stages of malignancy, though their contribution may vary. The early involvement of coagulation system in cancer is illustrated by the notion that tumour initiation could be blocked by exposure to TF-neutralizing antibodies 77. Similar conclusion can be drawn from elegant experiments with a mouse model of chronic colitis, where protracted inflammation leads to the onset of multifocal intestinal cancer. In this setting, genetic targeting of molecular mediators of thrombin responses in the host, especially those with known

roles in signalling, coagulation and inflammation levels (PAR-1, Fibrinogen, αMβ2 integrin, respectively), markedly attenuated de novo tumorigenesis35. Early tumour onset and progression was also altered by PAR-2 deficiency in a model of spontaneous mouse breast cancer driven by the oncogenic polyoma middle T (PyMT) transgene76. Early and intermediate stages of cancer progression were found to be sensitive to the blockade of TF-dependent signalling, which resulted in reduced tumour growth and angiogenesis in models of colorectal, brain, and breast cancer3;66. Several studies investigated the late (metastatic) stages of cancer progression. This work implicated the involvement of TF, thrombin, PARs, fibrin, platelets and other effectors 66-69;78-82. While tumour cell-associated TF has emerged as the key trigger in many of these processes, host cell-associated TF was also found to play a role in some settings, such as mouse teratoma 83, and glioblastoma (Magnus, Hashemi & Rak – unpublished observation), but not necessarily in all other models 71;80;83. It should be noted that while these are remarkably diverse and often profound effects, different pathways and stages of cancer progression may differ in their dependence on specific coagulation effectors. For example, loss of p53 expression renders human colorectal cancer xenografts less responsive to pharmacological inhibition of the TF coagulation pathway than that of their p53-expressing counterparts (Magnus & Rak –unpublished observation).

*Impact of the coagulation system on cancer stem cells.* Whether the coagulation system is considered as having an early or late role in cancer, the very consequential targets of these effects could be tumour initiating cells (TICs)84. Such cells, also known as cancer stem cells (CSC), are primary carriers of the oncogenic transformation. Although often a minority within the tumour mass, TICs are believed to be responsible for a number of pivotal events in neoplasia, such as tumour initiation, dissemination, onset of metastatic growth, post-therapeutic recurrence, cessation of tumour dormancy and late relapse85. The pool of TICs appears to be controlled by the vascular microenvironment (perivascular niche)86, of which coagulation system was proposed to be an important component84. Indeed, we observed that TIC-like cells isolated from certain established cancer cell lines (A431) 77;87, or from spontaneous murine glioblastoma (mBTICs) express TF and PAR receptors, and respond to stimulation with VIIa and IIa. Moreover, these responses were influenced by oncogenic transformation, retention of stem cell properties and, at least in some cases, by mesenchymal transdifferentiation (EMT) of cancer cells, which is thought to induce stem cell-like properties 88 (Garnier, Bentley and Rak –unpublished observation).

*Transitions between dormant, latent and aggressive tumour states as a function of coagulation system activity.* Could procoagulant effects associated with the therapeutic injury (surgery, chemotherapy, anti-angiogenesis) offset the long term therapeutic benefits (e.g. overall survival) in cancer patients 89-91 and how? Indeed, even seemingly effective anti-cancer interventions invariably leave behind numerous viable cancer cells, which often remain permanently dormant but carry a silent tumourigenic potential92. There are indications that injury or other events leading to procoagulant/inflammatory responses could interrupt the state of tumour dormancy, and set off the tumour recurrence. In this regard, thrombin has been postulated to control several mechanisms relevant to tumour cell dormancy, such as angiogenesis, growth rate or interaction with platelets36;93. Moreover, recent evidence points to a role for TF as a switch that may promote transition from a dormant (non-tumourigenic) or latent (delayed-tumourigenic) state of cancer progression (Magnus & Rak – unpublished observations). Thus, the U373 human glioblastoma cell line expresses very low levels of TF and exhibits virtually no capacity to form tumours in mice after

either subcutaneous or intracranial inoculation 77, though the cells remain viable and arrested in a permanent dormant state (> 250 days). Interestingly, the enforced expression of biologically relevant levels of TF leads to aggressive tumour formation, albeit after a lengthy period of latent disease (Magnus & Rak – unpublished observation). Cancer cells isolated from such growths are permanently altered, and form aggressive tumours without extended latency. These changes are associated with unique gene expression signatures. Observations, such as these, suggest that the presence of TF in the tumour microenvironment may irreversibly alter the functional state and molecular evolution of tumour cells (e.g. through adaptation, mutation and/or selection), leading to cessation of dormancy and dynamic formation of growth-promoting functional units (Magnus & Rak – unpublished observation).

## 5. GENETIC PATHWAYS OF CANCER PROGRESSION AS TRIGGERS OF COAGULATION SYSTEM ACTIVATION.

Hemostatic perturbations in cancer are, at least in part, associated with pathways of genetic tumour progression. This link can be viewed as both direct and indirect in nature (Figure 1). The indirect effects include procoagulant or hemorrhagic alterations that result from ongoing angiogenesis, vascular permeability, structural and functional defects in the tumor microcirculation, inflammatory responses, and other events known to be regulated by molecular targets of oncogenic transformation (e.g. VEGF). In contrast, direct changes include the impact of oncogenic pathways on the cancer cell coagulome 94 (Figure 1). Indeed, cancer cells are intrinsically procoagulant, which is often attributed to high levels of TF expression26, a regulatory target of several oncogenic pathways. For the procoagulant phenotype of cancer cells to have an effect on the hemostatic system robust points of contact must be established between them. These include processes through which cancer cells encounter plasma proteins, including: (i) vascular hyperpermeability; (ii) microhaemorrhages in the tumour vascular bed 95; (iii) vascular invasion; (iv) intravasation of metastatic cancer cells 96; (v) endothelial-like transdifferentiation of cancer stem cells and their merger with the vessel wall22; (vi) vascular damage following anti-angiogenic therapy 32;94; and (vii) natural direct contact between cancer cells and blood in leukemia 97. Indeed, the coagulation system may be activated locally with systemic consequences, or the changes may spill over to the periphery with circulating microparticles, cancer cells and procoagulants 26;52;98. These processes cooperate with several types of oncogenic events that have been implicated in the procoagulant conversion of cancer cells.

*Oncogenes.* Some of the same signalling pathways that in normal cells regulate the expression of TF and other coagulation effectors are also frequent targets of gain-of-function mutations, or constitutive oncogenic activation in cancer cells. In this regard the emerging evidence points to procoagulant effects of several classes of oncogenes.

*Fusion oncoproteins.* In acute promyelocytic leukemia (APL) the PML-RARα oncoprotein represents an interesting case of genetic control of the cellular coagulome. This fusion product acts by blocking cellular differentiation (arguably increasing cellular stemness) and propels both growth and constitutive expression of TF by APL cells, in a manner that is reversible by all-trans retinoic acid (ATRA). Exposure of a APL-derived cell line to ATRA downregulates the expression of TF, while upregulating levels of anticoagulant thrombomodulin (TM) 99. This is consistent with the

ability of the PML-RARα to stimulate TF gene transcription through AP1 binding site 100 or via the GAGC motif in the promoter sequence101. Little is known about coagulation effects of other chimeric oncogenes, such as BCR-ABL, AML1-ETO, TMPRSS2-ERG, KIAA1549-BRAF and several others but they are to be expected 102.

*Oncogenic receptor tyrosine kinases.* Serum, growth factors and cytokines regulate TF (and other effectors) through activation of membrane receptors, of which several are tyrosine kinases (RTKs). The mutant, overexpressed or chronically stimulated RTKs are amongst the most frequent oncogenic drivers of cancer progression1, as well as notable regulators of the angiogenic and procoagulant phenotypes12. For example, human epithelial cancer cells (A431) harbouring amplification of the epidermal growth factor receptor (EGFR) express copious amounts of TF, in a manner that can be blocked by anti-EGFR antibodies and small molecule inhibitors 103, or stimulated by EGFR ligands (EGF, TGFα)77. These effects depend on the level of a scaffolding protein known as kinase suppressor of ras 1 (KSR1)104. The expression of the constitutively activated, ligand-independent EGFR (EGFRvIII) in human glioma cells leads to a dramatic increase of TF levels 77 along with the expression of FVII, PAR-1 and PAR-2 54. Of note are the observations suggesting that TF is able to transactivate EGFR 105 and that TF and EGFR signalling pathways cooperate in regulating gene expression54. EGFR-dependent TF upregulation was found to require AP-1-dependent transcription and correlated with overt procoagulant activity of cancer cells 73.

HER-2/neu exemplifies the ability of other members of the EGFR (ErbB/HER) family of RTKs, to regulate TF 104. HER-2 is amplified and overexpressed in one subtype of breast cancer, but may play a role in other malignancies as well (gastric and ovarian cancers), where it cooperates with EGFR and other RTKs (e.g. HER-3/4)106. In DAOY medulloblastoma cells, the MET receptor mediates upregulation of TF, which plays a role in the related prosurvival effects107. In a recent elegant study oncogenic MET was expressed in mouse hepatoma and shown to drive both tumourigenesis and a severe thrombo-haemorrhagic syndrome. The latter disorder was dependent on the marked upregulation of PAI-1 and COX-2 in cancer cells. Notable in this case is the overt link between oncogenic transformation and clinically apparent coagulopathy 25 EGFR and EGFRvIII also regulate PAI-1, urokinase plasminogen activator (uPA) and its receptor (uPAR), thereby impacting fibrinolysis, as well as the related cellular signals 108;109.

*Intracellular oncogenic kinases.* SRC and other intracellular kinases mediate many of the aforementioned regulatory effects of the RTKs, and may also be expressed as oncogenic mutants (e.g. v-src). Indeed, there is a complex regulatory link between oncogenic src and fibrinolytic activity of cancer cells, including the expression of uPA, uPAR and PAI-1 110;111. Similar relationships have also been observed in the case of other viral oncogenes (e.g. v-yes or v-ros) 112. Moreover, TF is also a regulatory target of the oncogenic v-src 113. Recent studies revealed that in essential thrombocytemia (ET), the proliferative behaviour of megakaryocytes driven by the underlying oncogenic mutation of the JAK2 kinase (JAK2 V617F) is associated with high levels of platelet TF, as well as by procoagulant and proinflammatory changes 114. Several other transforming kinases (ALK, ABL or AKT) with known roles in highly procoagulant human cancers remain unexplored with regards to their effects on the cellular coagulome.

*Transforming small GTP-ases.* RAS genes undergo oncogenic mutations in a particularly large proportion (up to 30%) of human cancers, and their protein products are almost uniformly activated in tumour cells downstream of numerous growth factor and stimulatory pathways1;115. They also epitomize the link between vascular events and malignancy, and exemplify the connections between intracellular signalling and the procoagulant phenotype 94. K-RAS mutations occur in 50-90% of pancreatic cancers, known for particularly high levels of TF and procoagulant complications60. Constitutively activated wild type RAS is also found in colorectal cancer (CRC), lung cancer (NSLC), leukemia (AML), glioblastoma (GBM) and in other cancers associated with high risk of thrombosis and known to express high levels of TF94;116. A more direct link has recently emerged from studies on the isogenic series of human CRC cell lines, where selective deletion of mutant K-RAS reduced the levels of TF, release of TF containing MPs, and procoagulant activity, as well as diminmished tumourigenic and angiogenic activity of CRC cells in vivo3. Oncogenic RAS also regulates the levels of PAR-1, uPA and possibly other hemostatic activities 94;111. It is possible that other transforming GTPases (RHO, RAC, RAL) may exhibit similar properties.

*Tumor suppressors.* Inactivation of tumour suppressor pathways is often a prerequisite of the overt oncogenic transformation 1. Several loss-of-function events have recently been linked to changes in the cancer cell coagulome.

*TP53.* Loss of p53 gene expression/function is very frequent in human cancer 1, and often affects both TF levels and procoagulant phenotype3;117. In human CRC cells targeting p53 gene in the presence of mutant K-RAS results in a marked elevation of the TF antigen and increase in procoagulant activity, along with greater release of TF-containing MPs into culture medium and to peripheral circulation of mice harbouring the corresponding xenografts3. In clinical samples mutations of p53 in the tumour tissue also correlate with the high expression of TF, especially in the case of brain (low grade glioma) 118 and lung cancers (NSCLC)117.

*APC.* Early loss of adenomatous polyposis coli (APC) gene function underlies the oncogenic activation of the Wnt pathway and progression of human CRC 1. Interestingly, some of these changes can be recapitulated in mice harbouring Min mutations of the APC ortholog, and render these mice prone to intestinal neoplasia. TF is upregulated in these lesions (Rozen – personal communication) and plays a significant role in the tumourigenic process, as indicated by diminution of polyposis in Min mice exposed to the TF/VIIa antagonist, rNAPc269.

*PTEN.* The activity of the PI3K/Akt pathway and the related oncogenic effects are largely controlled by the suppressor action of the PTEN phosphatase. The expression of PTEN is lost in a large percentage of brain tumours and in several other malignancies 1. TF and procoagulant activity are elevated in glioma cells upon loss of PTEN, especially in the context of hypoxia 24, or when combined with EGFR activation 73. However, PTEN loss may not be sufficient to drive TF levels in cancer cells. For example, in the indolent human glioma cell line (U373) the absence of PTEN coincides with exceedingly low TF-dependent procoagulant activity, which is dramatically elevated only after the expression of the mutant form of EGFR (EGFRvIII) 54. PTEN loss along with the loss of p53 expression also segregate with high TF levels in NSCLC117. These examples illustrate that it is the complexity of oncogenic pathways (context) rather than individual mutations that may control the procoagulant phenotype of cancer cells.

*Rb.* Retinoblastoma protein (pRb) is at the centre of the oncogenic circuitry which includes both tumour suppressors (Rb/p105, RBL1/p107, p16/Ink4A, p19/Arf) and oncogenes (cyclin D1, CDK4, E2F) involved in several human cancers1. In retinoblastoma cells expressing the mutant form of pRb, TF is expressed at high levels and acts as a stimulator of cell proliferation 119. However, in serum stimulated normal fibroblasts Rb family proteins (pRb, p107) were found to act as positive (rather than negative) TF regulators 120, suggesting that additional events may be operative in tumour cells.

*MicroRNA.* Several non-coding RNA species have recently been revealed as regulators of tumourigenesis. Of those the best characterized category are micro RNAs (miRs), each of which may control the expression of multiple target genes, by a combinatorial suppression of protein translation and mRNA stability. Several miRs have been identified as oncogenes and tumor suppressors, often depending on the tumour context 121. Notably, transcripts of several hemostatic proteins contain regulatory sites for miRs 122-124. Of those miR-20 and miR-19b have already been implicated in TF regulation, including in cancer cells 125;126. In TF-positive medulloblastoma cells where miR-520g becomes silenced through methylation, its enforced re-expression leads to a specific suppression of normally high TF levels (D'Asti & Rak –unpublished observation). Since miRs regulate several oncogenic pathways, their wide spread influence on the changes in the cancer coagulome is very likely and as yet unexplored.

## 6. COAGULOME IN THE EMERGING MOLECULAR SUBTYPES OF CANCER.

It is increasingly appreciated that cancer types traditionally defined by clinical and histological criteria do not reflect the biological complexity of these diseases. Indeed, the recent progress in biotechnology has enabled the subdivision of virtually all major cancers into molecular subtypes (effectively different diseases) characterized by gene expression signatures, methylation patterns, cytogenetic profiles, and miR expression, which ultimately define pathways of oncogenic causation 1;121;127. This is significant, as targeted agents have already been shown to possess disease subset-specificity (e.g. Herceptin in HER-2-positive breast cancer) and may ultimately be used in a patient-specific manner. It is possible, therefore, though this has not yet been studied, that the impact of this molecular diversity may include the cancer cell coagulome. This would impact the related risk assessment and pathogenesis of cancer-related thrombosis as well as the nature of biological effects associated with activation of the hemostatic system. Future studies in this regard are especially interesting in the case of glioblastoma, a highly procoagulant type of cancer, that has recently been subdivided into at least three different genetic pathways (primary, secondary and pediatric), and four distinct molecular subtypes (proneural, mesenchymal, classical and proliferative), each endowed with a unique repertoire of oncogenic alterations and somewhat different clinical course 127;128. One might postulate that each glioblastoma subtype may bear a different potential to interact with the coagulation system, and thus requires corresponding approaches to anticoagulation. This principle may also apply to emerging molecular subsets in other cancers.

## 7. SUMMARY

Oncogenic mutations represent irreversible and cancer-specific events that exert their effects at the intracellular, intercellular and systemic levels. Interactions between cancer cells and the coagulation system epitomize these properties, as cellular transformation changes both the procoagulant phenotype of cancer cells and their responses to coagulation system effectors. This is believed to have several biological consequences mentioned in this article (cytoprotection, angiogenesis, inflammation, metastasis and dormancy), and the axis composed of TF/VIIa, IIa and PARs is likely at the centre of this circuitry. Future studies will reveal whether the nature and contribution of these interactions to cancer progression differ between different cancers, their subtypes, and individual patients. Likewise the ability of the coagulation system to impact different subsets of TICs and their progeny (perhaps regulate their behaviour and ratio) would be of considerable interest. As individualized, molecularly-driven anticancer therapy is today closer to reality than ever before, so too could be the targeted management of coagulation system aberrations, if the molecular link between them and the underlying cancer is better understood.

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Conflict of Interest Statement:

The authors state that they have no conflict of interest with regards to this manuscript

**Figure 1. A link between oncogenic pathways and abnormal hemostasis in cancer.**

Oncogenes, tumour suppressors and microRNAs (miRs) act in concert with differentiation pathways and microenvironment to promote multifaceted changes in cancer cell phenotype. These changes may impact the hemostatic system in many different ways, including direct modulation of the cancer cell coagulome, shedding of procoagulant microparticles and release of soluble procoagulants (CP, TF, PAI-1). Several oncogene-driven processes may also exert indirect effects on hemostasis. These include: production of angiogenic factors (e.g. VEGF), which trigger formation of immature blood vessels permeable to coagulation factors; oncogene-regulated recruitment of procoagulant inflammatory cells, which contribute to metastasis and may also influence the properties of circulating tumours cells (CTCs); and tumour initiating cells (BTICs) that often masquerade as endothelial cells and may exhibit oncogene-regulated changes in their coagulome (see text for details).

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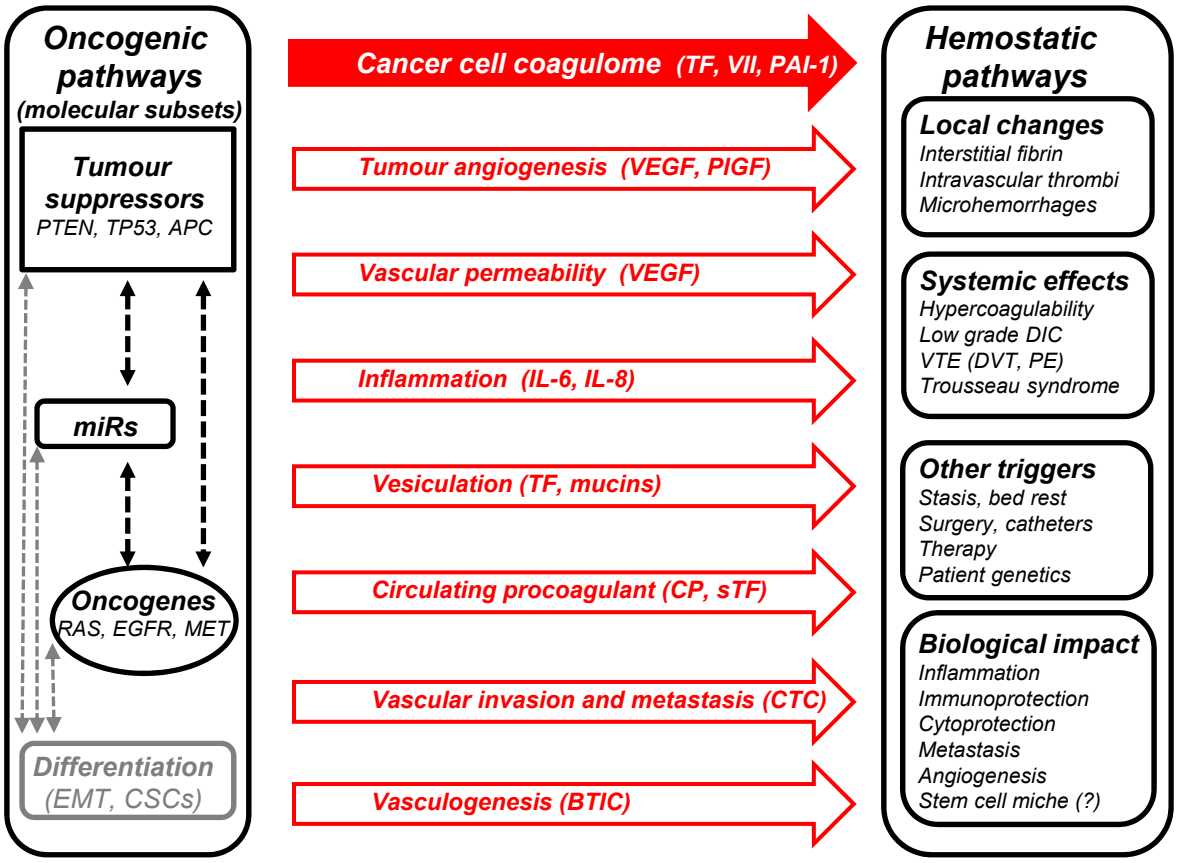
***A link between oncogenic pathways and hemostatic anomalies in cancer***




***Garnier et al Fig 1.***