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Single cell coagulomes as constituents of the oncogene-driven coagulant phenotype in brain tumours

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ABSTRACT

Molecular profiling of human cancers revealed a startling diversity in disease-causing mechanisms superseding histological and anatomical commonalities. The emerging molecular subtypes and disease entities are often driven by distinct oncogenic pathways and their effectors, including those acting extracellularly on the vascular and coagulation systems. Indeed, several oncogenic mutations such as those affecting protein-coding genes (RAS, EGFR, PTEN, TP53) and non-coding RNA (microRNA) regulate multiple effectors of the coagulation system (coagulome), including tissue factor, protease activated receptors, clotting factors, mediators of platelet function and fibrinolysis. This is exemplified by differential coagulome profiles in the molecular subtypes of glioblastoma, medulloblastoma and other human tumours. There is mounting clinical evidence that the mutational status of cancer driver genes such as KRAS or IDH1 may influence the risk of venous thromboembolism in patients with colorectal, lung or brain cancers. Notably, single cell sequencing in glioblastoma revealed a remarkable intra-tumoural heterogeneity of cancer cell populations with regard to their individual coagulomes, suggesting a combinatorial and dynamic nature of the global pro-thrombotic phenotype. We suggest that the cellular complexity of specific cancers may define their mechanisms of interactions with the coagulation system, and the risks of thrombosis. Thus, more biologically- based, disease-specific and personalized approaches may be needed to diagnose and manage cancer-related thrombosis.

1. Cancer-associated thrombosis

Cancer progression and therapy impact multiple facets of the vasculature, both locally and systemically. The resulting breakdown of the hemostatic integrity leads to a spectrum of pathological states often referred to as cancer-associated thrombosis (CAT), coagulopathy or Trousseau syndrome [1–5]. Indeed, hemorrhage, thrombosis and vaso-occlusion may occur both within the tumour capillary network and in peripheral blood vessels, leading to increased risk of the clinically manifest thromboembolism in the venous (VTE) or arterial macro-circulation (ATE) [1, 6]. This is important since thrombosis in cancer patients is associated with considerable morbidity and worsening of overall outcomes [7, 8], in the clinical reality where VTE and the resulting pulmonary embolism (PE) are frequently cited as the second leading cause of cancer-related deaths [9].

In addition to immediate clinical manifestations of thrombosis, activation of the coagulation system triggers more subtle, intracellular signalling mechanisms that may alter the biology of the underlying disease. Indeed, coagulation proteins influence the phenotype of cancer, stromal, inflammatory, immune and vascular cells as well as platelets, in ways that may contribute to tumour growth, angiogenesis, invasion, metastasis and altered therapeutic responses [10, 11]. These intracellular signals are mediated by transmembrane proteins that interact with coagulation and fibrinolytic effectors and include protease activated receptors (PARs), tissue factor (TF), thrombomodulin (TM), endothelial protein C receptor (EPCR), urokinase receptors (uPAR) and other 'sensors' of extravasated blood [12, 13]. While therapeutic interference with these processes may contribute to the benefits of thromboprophylaxis and anticoagulation [1, 14] the data are still incomplete or conflicting [15] and clinical needs remain unmet.

CAT has been often thought of as an unspecific aberration of the normal hemostatic machinery that can be reigned in by approaches that are proven to work under non-malignant hematological conditions. The core pathway of interest includes TF, a cell-associated receptor for coagulation factor VII/VIIa (FVII/VIIa) required to form a complex (TF/VIIa) capable of activating coagulation factor X (to Xa). In conjunction with formation of activated factor IXa the resulting generation of thrombin (IIa) leads to activation of platelets (via PARs) and conversion

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of plasma fibrinogen to an insoluble fibrin clot [16]. Clotting processes are also influenced by factors VIIIa and Va, the extrinsic coagulation pathway (XII, XI) and a number of regulatory feedbacks [17, 18] that control the magnitude and duration of the hemostatic response and, if altered, may contribute to pathology.

According to this paradigm diagnosis, prevention and treatment of CAT is predicated on the analysis of its immediate effectors, mostly elements of the aforementioned common coagulation pathway. The related medication inherently leads to weakening of the physiological hemostasis and the increased risk of bleeding, which ultimately limits the extent of such pharmacological intervention [1, 19]. Therefore, it is of great interest to identify more upstream and more cancer-specific causes (inducers and modulators) of coagulopathy and its elements (if any) that may be either non-essential for hemostasis [3] or unique to a particular neoplastic disease [5, 20].

2. Oncogenic pathways as putative triggers of CAT

While CAT undoubtedly arises amidst many complexities and stochastic circumstances, it is unlikely a random or unspecific process [21]. This contention is supported by evidence that specific cancer sites differ with regards to the incidence of thrombosis they may provoke, with pancreatic, brain, gastrointestinal, ovarian and hematological malignancies carrying the greatest VTE risk [22]. While certain tissue microenvironments (e.g. brain) may exhibit intrinsically high coagulant activity [5], distinct histological types of cancers arising from the same anatomical location may carry different likelihoods of CAT. For example, VTE occurs with very high frequency in high grade adult brain tumours, such as glioblastoma, but is far less prevalent in the case for low grade astrocytomas [23, 24], and relatively rare in non-astrocytic primary brain tumours. VTE risk may also be affected by regional differences in brain tissue [25] and by patient age, as signified by infrequent thrombosis associated with high grade astrocytomas in children [26]. Bleeding rather than thrombosis is also a feature associated with rare pediatric embryonal tumours with multilayered rosettes (ETMR) [27]. Furthermore, cancers with the same cellular origin may carry different coagulant properties as a function of their gene expression profiles and molecular subtypes [28–30] resulting from the diversity of pathways driving oncogenic transformation in specific disease contexts [31].

The links between genetic and epigenetic driver events in cancer cells and their coagulant consequences has been reviewed extensively elsewhere [5] and requires but a brief mention and handful of representative examples (Table 1). For instance, in human colorectal cancer (CRC) TF procoagulant activity, expression and release as extracellular vesicles (EVs) are all linked to progressive acquisition of transforming mutations of KRAS and TP53 genes [32]. Oncogenic MET driving mouse hepatoma leads to coincidental peripheral thromboembolism [33], while glioma cells defective for PTEN tumour suppressor are both more prothrombotic and more TF-positive than their wild type counterparts, especially under hypoxia [34]. In glioblastoma (GBM), the procoagulant phenotype and high expression levels of TF are associated with the classical and mesenchymal subtypes of the disease expressing high levels of growth factor receptors and wild type IDH1 gene, while TF levels are markedly lower in proneural GBM [28], and in conjunction with R132H mutation of IDH1 [30] (Fig. 1). Coagulome of pediatric medulloblastoma (MB) is also subtype-specific, with altered expression of TF, PAR-1, FX and other regulators in tumours carrying activated sonic hedgehog (SSH) or wingless (WNT) pathways, the latter driven by oncogenic mutation of beta-catenin (CTNNB1) [35]. Perturbations in coagulant properties of cancer cells were also linked with several other driver mutations, including protein-coding genes (SRC, EGFR, EGFRvIII, HER2) and non-coding RNA (miR520g, miR19b; Table 1) [5]. Conversely, inhibition of TF signalling in vivo caused widespread changes in the repertoire of tumour-related microRNA. Indeed, injections of the function blocking anti-human TF antibody that selectively suppresses TF signalling (but not TF clotting activity) into mice carrying human MDA-MB-231 breast cancer xenografts resulted in changes in the expression levels of some 75 microRNA species in the cancer cell population. This suggests a regulatory role of the TF pathway in mediating the microenvironmental control of the tumour microRNA network [36].

Table 1
Examples of oncogenic mutations implicated in coagulation and cancer.

Gene	Hemostatic effectors	References
Oncogenes		
K-Ras	TF, PAR-1, EVs	[20, 32, 57]
H-Ras	TFPI-2, uPA	[75–77]
Src	TF	[78]
EGFR	TF, EVs	[79–81]
EGFRvIII	TF, FVII, PAR-1, PAR-2, EVs	[50, 82, 83]
HER-2	TF	[84]
MET	COX-2, PAI-1, TF	[33, 85]
MET + SHH	PAR-1	[35]
PML-RARa	TF	[86]
Jak2V617F	platelet TF	[87]
Tumour suppressors		
TP53	TF, EVs	[32]
PTEN	TF	[34, 56, 88]
MicroRNA		
miR-520g	TF	[27]
miR-19b,miR-19a	TF	[89, 90]
miR-1258	Heparanase	[91]
miR-190a	PAR-1	[92]
miR-143/-145	PAI-1	[93]
miR-30b	PAI-1	[94]
miR-192	PAI-1	[95]
miR-143	COX-2	[96]

The mechanisms by which intracellular driver mutations or epigenetic alterations influence extracellular events surrounding CAT (and the tumour microenvironment in general) remain poorly characterized. The available evidence and inferences point to the role of both indirect and direct mechanisms. For example, the coagulation system may be affected indirectly by cancer-induced angiogenesis, inflammation and other processes triggered by the cancer cell secretome, which is known to be modulated by transforming genes [37]. For example, RAS-driven overexpression of the vascular endothelial growth factor (VEGF) [37] is responsible for fulminant angiogenesis and inflammatory cell recruitment likely to alter vascular permeability, endothelial gene expression, perfusion, interstitial tissue pressure [38] and other factors that may collectively contribute to thrombosis. RAS also directly controls production of inflammatory factors (G-CSF, IL-6, IL-8) [39] that may recruit granulocytes, capable of expressing or carrying TF (the mechanism remains somewhat controversial [40–47]), along with formation of DNA-containing neutrophil extracellular traps (NETs) endowed with procoagulant activity [48]. Oncogenes also influence formation and proteolysis of the extracellular matrix (ECM) [49] a process that may indirectly contribute to deregulation of angiogenesis, and facilitate activation of the extrinsic coagulation pathway [3]. These effects may be superimposed with hypoxia, necrosis, inflammation and effects of anticancer drugs and thereby overshadowed by the related complexities.

3. Oncogenic mutations as regulators of cancer cell coagulome

Oncogenic pathways also exert a more direct effect on a subset of genes that directly control clotting, fibrinolytic and regulatory effects of the coagulation system, and thus could be referred to as *coagulome* [28]. Indeed, malignant transformation leads to deregulated expression (e.g. overexpression) of cell associated key coagulation system effectors such as TF, PAR1, PAR2 [28,35] and to ectopic expression of coagulation proteases [50, 51]. For example, prothrombin (FII), FVII, FX and other