Coagulome and tumor microenvironment: impact of oncogenes, cellular heterogeneity and extracellular vesicles

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ABSTRACT

Cancer-associated thrombosis (CAT) results from the hemostatic system being dysregulated by the progression of cancer. Despite common clinical manifestations, the mechanisms of CAT may vary greatly because cancers develop along distinct biological trajectories that are imposed by the interaction between the tumor cell genome, the epigenome, the surrounding microenvironment, and the tissue of origin. The coagulome, or repertoire of coagulation effectors, expressed by stromal, inflammatory, and cancer cells at the tumor-vascular interface and systemically, reflects this biological variability. Complex landscapes of coagulant and non-coagulant cellular populations are revealed by single-cell RNA sequencing analyses conducted on unperturbed human cancer tissues. Additionally, through mediators of cell-cell interactions, soluble coagulants, and extracellular vesicles containing tissue factor, podoplanin, and other effectors, coagulomes are projected into the pericellular milieu and systemic circulation. As this complexity is currently outside of the clinical paradigm, one could argue that better CAT management could result from a more individualized analysis of coagulomes in cancer patients.

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Introduction: the vasculature as a gateway for systemic manifestations of cancer

Among the multiple complex facets of the tumor microenvironment, the vascular compartment plays a unique and integrative role.[1] The vasculature, including networks of blood vessels, lymphatics, lymph and the circulating blood, all shape the local tumor milieu and link the anatomically circumscribed cancer foci with the systemic circulation. This crucial connection is responsible for the widespread biological responses, comorbidities and, ultimately, for the metastatic progression of the disease.

Thus, tumor microcirculation plays both local and systemic roles in cancer. The local role of the tumor vasculature encompasses a plethora of perfusion-dependent and -independent processes. For example, the vasculature controls the behavior, metabolism and survival of cancer cells through the supply of blood enriched in oxygen, nutrients, regulatory plasma proteins, hormones and cells. Sustained blood flow through the tumor microcirculation regulates the influx of immune effectors, and drugs while mediating the removal of metabolites and shedding of tumor cells and their products into the general circulation.

Alteration within the blood vessel wall (endothelial cells, perivascular cells, extracellular matrix) across the tumor microvasculature enables the flux of fluids, molecules and cells between the circulating blood and the surrounding tissue. In this regard, cancer-related impact on vascular permeability and transmissivity may encompass processes such as regional modification of the blood-brain barrier, formation of the blood-tumor barrier,[2] different degrees of vascular leakiness, microhemorrhage, along with other structural and functional abnormalities triggered at the tumor-vascular interface.[3]

These crucial alterations occur in the course of events leading to formation, expansion and remodeling of the tumor micro-

circulation, including the onset of angiogenesis,[1] vascular cooption,[4] vascular dilatation (vasectasia),[5] lymphangiogenesis,[6] vasculogenic mimicry,[7] emergence of transient lymphoid structures,[8] and changes in immunoregulatory functions of endothelial cells,[9] among other effects.[10] These responses are increasingly well understood, well described, and, at least in some cases, have already served to identify therapeutic targets in cancer, as illustrated by the advent of antiangiogenic agents directed at the vascular endothelial growth factor pathway. Several of these agents have been approved for cancer treatment over the past two decades.[11-13]

Somewhat less explored are the perfusion-independent aspects of the tumor microcirculation, especially the potent secretory activity of endothelial cells (possibly also of pericytes, perivascular fibroblasts and myeloid cells).[14,15] Indeed, endothelial cell secretome has been described as an important regulatory force in mediating changes in the tissue and tumor microenvironments, impacting migratory behavior of cancer cells (possibly also other cells), their growth,[16] stemness and other responses.[17,18] This paracrine effect, initially described decades ago,[16,19] has more recently been brought to light in various biological contexts under the term of the 'angiocrine' regulation.[17,20,21]

Similarly, circulating blood components, such as red blood cells, leukocytes,[15] platelets,[22] coagulation proteases (*e.g.*, thrombin) and plasma proteins often play multiple roles, either related to their canonical homeostatic (and hemostatic) functions, or involving induction of cellular signaling responses across multiple organ sites, with consequences for cancer progression.[23]

As mentioned earlier, access to the vascular system enables the transition of a localized neoplastic growth to a complex, systemic disease. Indeed, even ostensibly non-metastatic cancers often elicit profound and morbid systemic effects on multiple organ systems. Some of the most striking examples of such 'remote' influences include functional alterations in the liver,[24] pancreas,[25] brain,[26] bone marrow and immune system,[27] as well as clinically overt paraneoplastic syndromes, such as cachexia,[28] or cancer-associated thrombosis (CAT).[29] These alterations may be further exacerbated in the course of a more advanced or metastatic disease. Conversely, the systemic effects of cancer progression mediated by the vasculature often precede and enable subsequent metastatic dissemination.[30-32] For example, the conditioning of distant organs by cancer-derived extracellular vesicles (EVs), cytokines and clotting factors leads to the formation of pre-metastatic niches that serve as sites of subsequent colonization by incoming cancer cells.[33-36]

Thus, cancers represent complex and highly interactive, multifactorial and multicellular processes that highjack, alter, and exploit elements of the circulation, including the hemostatic system, which becomes engulfed by, and alters, cancer progression. Amidst this complexity, the nexus between cancer and the coagulation system represents the focus of our remaining comments.

Cancer-associated thrombosis: implications for disease progression and heterogeneity

The formation of tumor-vascular interface represents a common feature of virtually all cancers, with implicit consequences for both blood vessels and blood.[3] Yet, the hemostatic consequences of this interaction are hardly straightforward, or uniform. Thus, in some cancers, the manifestations of CAT are relatively subtle, while in others the impact of the disease on the coagulation system may be more profound, morbid, and biologically, as well as clinically, manifest requiring prophylaxis and intervention.[37] In the latter case, the elevated hypercoagulability is often associated with heightened systemic risk for arterial and especially venous thromboembolism (VTE).[29] Moreover, in certain cancers, such as subsets of high-grade glioma, CAT may be associated with extensive microvascular thrombosis within the tumor mass) coupled with an impact on peripheral circulation in the form of dramatically heightened VTE risk.[38-41]

In its severe forms, CAT poses considerable clinical concerns due to morbidity associated with VTE, which may escalate to life-threatening pulmonary embolism.[29] In addition, the co-existing thrombosis leads to poor overall outcomes in cancer patients.[42] At the same time, the activated coagulation system and platelets often deploy disease-modifying mechanisms that may facilitate cancer progression and dissemination. For example, the formation of fibrin matrix and release of growth factors from activated platelets may facilitate tumor invasion, while activated sticky platelets in blood stream can coat extravasated cancer cells creating a shield for circulating cancer cells against immune effectors.[22,31,32,34] However, while thrombosis in cancer patients in its various forms has been recognized for over 150 years, the exact molecular chains of causation, mechanistic pathways leading to CAT and precise points at which clotting intersects with the biology of specific cancers still remain poorly defined.[37,43] It seems reasonable to suggest that CAT (or CATs) could become less intractable if a system of biologically based stratification could be developed and applied in a context-specific manner to defined populations of cancer patients.

Cancer coagulome: at the crossroads of thrombosis and biological regulation

Operationally, the upstream triggers of CAT implicitly lie within the molecular apparatus of cancer cells that evoke CAT, either directly or indirectly. Indeed, cancer progression may exert multiple indirect influences in the vascular system, leading to hemostatic perturbations. For example, the formation of aberrant and poorly perfused intratumoral vascular networks may lead to stasis and thereby promote microthrombosis. Moreover, the exposure to blood of procoagulant surfaces within perivascular tissues of the tumor bed may occur due to porosity and anatomical abnormalities of tumor blood vessels, resulting in the activation of the coagulation system. Similarly, the recruitment of procoagulant inflammatory cells, endothelial cell activation and other processes may compromise the anticoagulant functions of the vasculature.[37]

Cancer cells may also possess the molecular apparatus enabling them to interact with the hemostatic system directly. Some of the best-described effectors of such interactions include the expression by different tumor cell types of tissue factor (TF) podoplanin (PDPN), coagulation factor VII (FVII), prothrombin, or antifibrinolytic serpins, such as plasminogen activator inhibitor 1 (PAI-1).[44-51] To describe this cancer-associated molecular interface the term 'coagulome' has been coined previously, initially to capture the totality of relevant molecular features affected by disease progression (coagulation, fibrinolytic, and platelet regulating factors).[52] This term was later used to define the complex reper-

toire of putative regulators of clotting processes associated with cancer cells themselves,[53,54] or to characterize a wider procoagulant network of interactions involving multiple components of the disease, such as tumor cells, inflammatory cells, stroma, and blood elements, all of which may contribute to CAT in various ways and in different contexts.[55]

Defining cancer coagulome is important for at least three main reasons. First, the triggers of CAT could be markedly different than those leading to thrombosis in the course of other procoagulant conditions, such as major surgery, cardiovascular disease, or genetic thrombophilia. This is because cancer cells possess unique molecular makeup and functionalities incomparable to normal tissues. Second, different cancers exhibit vastly different VTE risks,[56,57] which suggests that different cancer-specific mechanisms of CAT may be operative between distinct diagnostic entities. It could also be argued that, although different cancers may carry comparable global VTE risks, they may differ in their abilities to activate specific prothrombotic pathways (*e.g.*, coagulation system or platelets) due to stark differences in their molecular profiles. Moreover, cancers originating from similar tissue sites may trigger vastly different CAT activating mechanisms. The cases in point are recent studies on high-grade glioma, where oncogenic mutations of the isocitrate dehydrogenase 1 and 2 (IDH1/2) genes had a protective effect against microthrombosis and VTE risk, while histologically similar IDH1/2 wild-type tumors, currently classified as proper glioblastomas (GBMs),[58] were associated with pronounced incidence of VTE, upward of 20%.[39,41] Interestingly, while the mechanistic basis of these differences remains to be conclusively elucidated, the IDH1/2-related changes in CAT correlate with the differential expression by cancer cells of at least two different prothrombotic effectors, such as TF and PDPN.[41,51]

Third, a better definition of cancer coagulome in specific disease contexts may enable a more targeted and personalized intervention, based on what can be gleaned from molecular causality and its impact on coagulome. For example, the identification of cancer-associated coagulant effectors (*e.g.*, TF), or mediators leading to activation of procoagulant inflammatory responses or platelets may enable directing anticoagulant therapy at upstream triggers of these events.[37,47,49,51] This could complement and improve the current paradigm built around therapies aiming at elements of the common coagulation pathway, such as factor Xa or thrombin, which are burdened with bleeding risks due to global perturbances in hemostatic requirements they induce.[59] Thus, molecular causation and composition of the cancer coagulome may have practical implications that are, perhaps, worthy of some consideration.

Oncogenic drivers of cancer coagulome: lessons from cancer genome and epigenome

While the impact of cancer progression on CAT may stem from multiple, sometimes non-specific, or indirect influences, cancer-specific factors are also clearly a play. For example, marked differences in VTE risk exist between different cancer types,[56] and along the path of cancer progression. In this sense, progression of pancreatic,[60] or colorectal cancer (CRC) has been linked to upregulation of TF by tumor cells,[61] and parallels corresponding increases in the VTE risk.[37] In patients with primary brain tumors, not only VTE but also microvascular thrombosis was found to correlate with the increasing tumor grade.[38] These and other examples illustrate the emerging interrelationship between biological properties of cancer cells and their ability to promote thrombosis.

At the root of progressive changes in the cancer cell phenotype are oncogenic events (mutations) affecting the cellular genome and epigenome, with a profound impact on the expression of multiple downstream genes.[62] It is, therefore, reasonable to suggest that oncogenic changes may influence cancer coagulome and have some bearing on VTE. This notion was originally proposed and later directly examined using experimental models of human and rodent cancer cell lines with precisely defined (or engineered) oncogenic alterations.[63] Some of these studies included a series of human isogenic CRC cell lines expressing either the wild-type KRAS gene, or its oncogenic mutant KRAS G13D allele, either in the presence or in the absence of TP53 tumor suppressor gene. Interestingly, this comparison revealed that more advanced mutational status correlates with increased cellular aggressiveness, higher expression of TF and greater release of TF-carrying procoagulant extracellular vesicles.[47] Similarly, the loss of PTEN tumor suppressor in the experimental glioma model resulted in the upregulation of TF,[64] while oncogenic MET receptor drove the upregulation of PAI-1 in a model of murine hepatoma.[49] In another study involving a series of isogenic human GBM cell lines, the enforced expression of oncogenic EGFRvIII stimulated the aggressive tumor phenotype *in vivo,* along with a dramatic upregulation of TF, FVII and thrombin receptor (PAR-1) by cancer cells.[65,66] Interestingly, in the same series of cell lines, the expression of platelet-activating PDPN ligand was down-regulated in concert with EGFRvIII expression by cancer cells. This may suggest that oncogenic events (such as EGFRvIII status) may control the switch between two qualitatively different pro-thrombotic cellular phenotypes/states (TF/coagulation-dependent and PDPN/platelet-dependent).[51]

In some of these experimental studies, the source of a systemic hypercoagulability readouts could be traced to the tumor microcirculation. For example, in mice harboring EGFRvIII-driven and TF-expressing GBM xenografts, the levels of D-dimer were predictably elevated in peripheral blood, but these readings were orders of magnitude higher within the tumor mass, compared to systemic circulation. These observations may indicate that, in this case, D-dimer could largely originate from the highly procoagulant tumor microenvironment rather than being generated systemically.[51] Whether this is generalizable, or not, the underlying processes were driven by the oncogenic mutation. Moreover, such a link between oncogenic events and procoagulant phenotypes of cancer cells has been repeatedly described in experimental studies employing different tumor models, as reviewed recently.[54]

In keeping with these findings the subsequent analyses of several clinical cohorts suggested that in cancer patients the incidence of VTE,[41,67-69] or upregulation of some of its effectors (*e.g.*, TF) may also be a function of oncogenic mutations.[70] For example, VTE was markedly more frequent in CRC patients with KRAS mutations relative to those whose tumors did not carry this genetic alteration.[67] In a large cohort of patients with different cancer types, mutations in STK11, KRAS, CTNNB1, KEAP1, CDKN2B, and MET were generally linked to the elevated VTE risk. Conversely, in the same cohort, certain onco-

genic mutations had protective effects leading to lower VTE risk, either in general (SETD2) or in specific tumor types (IDH1). In this regard, IDH1/2 status has been extensively validated as an element of the VTE risk prediction algorithm recently developed for high grade glioma.[39]

The impact of genomic mutations on the phenotype of cancer cells is not absolute, and it can be modulated by the cellular epigenome. This is in keeping with the role of chromatin structure, chemical modification, DNA methylation and other processes in the execution of the cellular genetic program. These effects underlie gene expression changes involved in normal cellular differentiation, adaptation, and plasticity, as well as their epigenetic aberrations driving malignant transformation.[71] Thus, while cancer cells may carry common genetic mutations, they may also respond to residual lineage-specific programs, or microenvironmental cues that could profoundly reshape their coagulome. This interplay is at the core of many aspects of cellular heterogeneity pervading cancer progression, including the formation of stem cell populations, progenitor cell pools and diversification of their progenies.[72] Indeed, gene promoter methylation, chromatin modifications and regulatory effects of non-coding RNAs, including microRNA, may mold the molecular repertoire of cancer cells including effectors of thrombosis, often acting in a cancer-specific manner.[54] For example, experimentation with *in vitro* model systems suggests that markers of cancer cell stemness may, in some cases converge with,[73] while in others diverge from,[74] effectors of the coagulation pathway, such as TF. In GBM-derived cell lines, EGFRvIII suppresses the expression of PDPN in a manner potentially involving the epigenetic modifier EZH2, while in patients with high-grade glioma expression of mutant IDH1, downregulates both TF and PDPN due to its global impact on gene methylation.[51,75] Likewise, specific microRNAs may control the levels of TF,[76] or impact other elements of the cancer coagulome.[37-77]

Cancer models and coagulome: advantages and possible pitfalls

It should be noted that while cancer cell lines and transgenic mouse models provide invaluable and well-controlled resources for studies on molecular causality impinging upon the regulation of cancer coagulome, they are often not identical to (or directly predictive of) their 'real life' counterparts in unperturbed human tumor microenvironments.[51] This important limitation is infrequently discussed in the literature and may be attributed to the genetic drift in long-term cultures, epigenetic modifications induced under *in vitro* conditions,[52] selection of cancer subclones, species-specific factors, changes imposed by experimental manipulation, and the absence of natural complexity and cellular diversification processes, which occur during natural cancer progression *in vivo*. It is surprising that more advanced and complex models of cancer, such as spheroids, tumor spheres, organoids, organs on chip or patient-derived orthotopic xenografts have scarcely been studied in terms of their ability to emulate CAT in cancer patients.[78,79] While greater investment in this regard could be valuable, the accurate recapitulation of the cancer-specific complexity of tumor cell 'communities', and dynamic aspects of the tumor-vascular interface may be difficult to achieve under purely experimental settings.

Cancer coagulome: lessons from single-cell RNA sequencing

One way to circumvent these limitations is to extract features of cancer coagulome and its upstream regulators directly from clinical cancer datasets increasingly available in the literature and achievable technologically. Such data often report on multiomic molecular profiles and single-cell sequencing (scRNAseq) results of cancer tissues that have never been subjected to experimental manipulations *in vitro*.[51,80] In particular, the advent of scRNAseq technology has fundamentally changed the outlook at the multicellular cancer 'architecture' and the dynamic of transitory phenotypic states of cancer cells as they interact with their microenvironment.[72,81,82] For example, in high-grade brain tumors, single-cell transcriptomes illuminated the fact that traditional distinctions between molecular subtypes of GBM, such as proneural, classical and mesenchymal disease,[58] are not reflective of the corresponding differences between seemingly phenotypically uniform cellular masses populating these tumors, as bulk RNA sequencing would seem to suggest.[83] Rather, these subtypes emerge as a function of complex equilibria that form between heterogeneous cancer cell subsets, among which the predominant population dictates the global molecular signature of the tumor as a whole.[81] The exact forces that control these cellular 'mosaics' are not entirely clear.[84] However, the phenotypic biases driving these brain cancer cell 'ecosystems' toward one equilibrium or another, appear to be imposed by prevalent oncogenic drivers, such as EGFR for astrocytic-type GBMs, or NF1 loss for mesenchymal tumors, which are also enriched for inflammatory stroma.[85]

These findings may potentially redefine the meaning of cellular coagulome in GBM and likely in other cancers, as well.[54] For example, the analysis of single-cell datasets suggested that transcripts for TF and PDPN may be expressed preferentially (though not exclusively) by specific cellular subpopulations, such as astrocytic or mesenchymal cancer cells, respectively (Figure 1).[51] Interestingly, progenitor GBM cells were relatively devoid of these pro-thrombotic effectors. Moreover, at the single-cell level, the impact of oncogenic drivers was more complex than could be inferred from cell culture studies. For example, a large proportion of EGFR expressing GBM cells did not express PDPN, which instead was enriched among EGFR non-expressing subsets of cancer cells. A fraction of cancer cells, however, expressed both TF and PDPN.[51] Thus, in complex cancers, such as GBM, tumor cells form coagulant mosaics, which contain subpopulations of highly coagulant cells interspersed with their counterparts expressing low (or no) apparent prothrombotic phenotypes.[51] How this coagulant heterogeneity impacts intra-tumoral microthrombosis, or projects its effects systemically, to trigger VTE is presently poorly understood.

Extracellular vesicles: emerging regulators of vascular responses and thrombosis in cancer

How could genetic and epigenetic alterations in cancer cells trigger thrombosis at remote organ sites and in anatomically distant, peripheral blood vessels? In this regard, several mutually non-exclusive scenarios could be considered. For example, systemic hypercoagulability originating from within the tumor

microcirculation may precipitate clotting processes at vulnerable sites, such as venous valves in lower limbs or in areas of vascular stasis.[86] Alternatively, cancer cells could trigger a systemic or peripheral hypercoagulable state through the release of circulating procoagulant mediators. In fact, several such cancer-related candidate mediators have been studied over the years, including enzymatic activities associated with cancer coagulant,[59] neutrophile extracellular traps (NETs),[87] or cancer-derived procoagulant microparticles,[46] more recently referred to as EVs.[88]

EVs and smaller membrane-less extracellular particles (EPs) (collectively referred to here as EVPs) represent an intriguing element in the cellular secretome with a possible role in thrombosis.[89] EVPs are highly heterogeneous due to diversity of biological processes leading to their formation. While small EVs (<100 nm) may originate from the cellular endosome (exosomes) and represent a part of the membrane protein recycling processes, other EVs originate at the cellular surface (ectosomes) following membrane blebbing, budding and protrusion. These EVs vary in size from ~100 nm (small microvesicles, ARMMs) to >2 μm in diameter (large oncosomes, migrasomes, exophers, apoptotic bodies) and in terms of molecular cargo, as well as function.[89,90] The biogenesis of EPs is currently unclear, but it leads to the formation of molecularly distinct particles, such as exomeres and supermeres, ranging in size from <50 nm to <35 nm respectively.[89] Different EVPs contain distinctive repertoires of proteins, lipids and nucleic acids and possess a remarkable ability to interact with biofluids and cells, whereupon they serve as hubs for macromolecular complexes, or as vehicles that transfer their cargo to cellular recipients, respectively. In the latter case EVP-cell interactions may elicit a range of biological responses, including changes in cellular phenotype.[91]

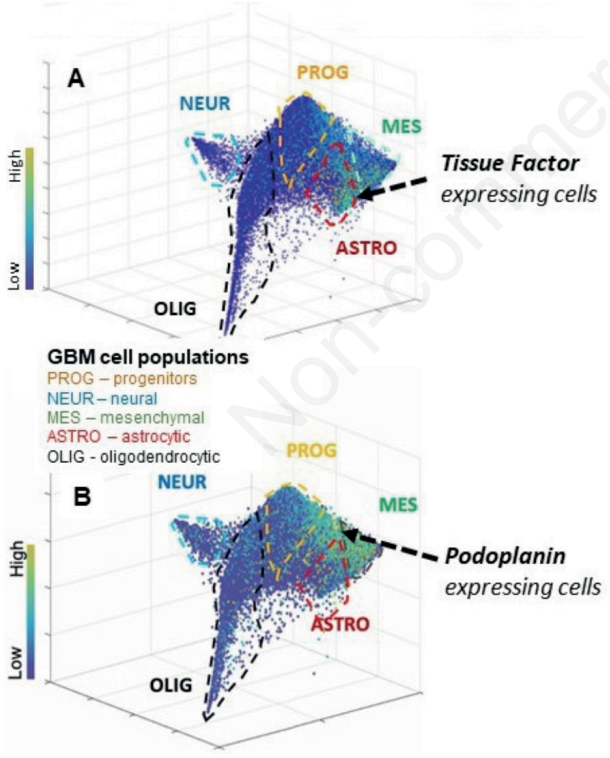

Figure 1. Heterogeneous cellular carriers of glioblastoma coagulome. Single-cell mRNA sequencing. Roadmap analysis of developmental programs expressed in glioblastoma cell subpopulations reveals cell subsets enriched in tissue factor (panel A; mostly astrocytic cells) or podoplanin (panel B; mostly mesenchymal cells). The plots were adapted with permission from N. Tawil Ph.D. Thesis (2021); analysis based on the pipeline described by Couturier *et al.*[82] and applied to coagulome.[51]

EVPs have long been known to carry potent vascular mediators.[92] While some of these molecules may directly interact with the hemostatic system,[46,93] others may exert their vascular effects through interaction with circulating cells, or the vascular wall, and by impacting angiogenesis, vascular permeability, inflammation and other processes.[17,94,95] TF, PDPN, phosphatidyl serine, mucins, inorganic polyphosphate are among the EVP-associated effectors found capable of impacting the hemostatic system under various pathological conditions, including cancer.[46,47,93,95-97]

There is mounting evidence that procoagulant EVPs may serve as an export mechanism for TF, PDPN and other effectors from cancer cells to their surroundings and to peripheral blood.[46,47,51,93,98] Particularly rich, in this regard, is the literature on TF-carrying, cancer-derived EVPs, which appear to possess the capacity to activate the coagulation cascade in several experimental systems, especially in models of pancreatic cancer, a tumor enriched in cellular TF.[98] Similarly, the release of TF-carrying EVPs has been documented in CRC,[47] GBM and other cancers.[93,99] However, the role of TF-EVPs in triggering and predicting VTE remains a subject of some debate, with some studies supporting,[99] and others questioning the role of this mechanism in the clinic.[100]

While the analysis of EVPs poses significant pre-analytical, technical and standardization challenges,[101] it is also possible that the cellular architecture of the respective cancers would need to be taken into consideration as a source of EVP cargo and variability. For example, in experimental models of GBM, the positivity of cancer cells for two or more putative prothrombotic effector molecules, such as TF and PDPN was paralleled by the release of EVs with the corresponding dual positivity (TF+/PDPN+; Figure 2). However, the same cells also exported EVs containing single, or none of these molecules. Since the cargo assembly during EV biogenesis is non-random, it is important to understand how these different, coagulant, or non-coagulant EVs, are formed and regulated.

Nonetheless, the enrichment in EVs carrying specific molecular cargo (TF or PDPN) was found to correlate with their potential to activate coagulation cascade and/or platelets in experimental settings.[51] As mentioned earlier, cancer cells positive for either PDPN, or TF, both, or none, are also readily detectable in scRNAseq datasets of human GBM.[51] It is therefore of considerable interest to determine whether VTE risk prediction that may be difficult to establish while monitoring TF-EVs alone, could be improved by analyzing EVs for multiple effectors, including through the use of technology platforms capable of generating multiplex data at the single EV resolution (Figure 2).[102] It is possible that comprehensive multidimensional molecular landscapes of coagulant EV subpopulations in cancer patients with the help of super-resolution technologies and machine learning may become diagnostically informative in the context of CAT.[102,104]

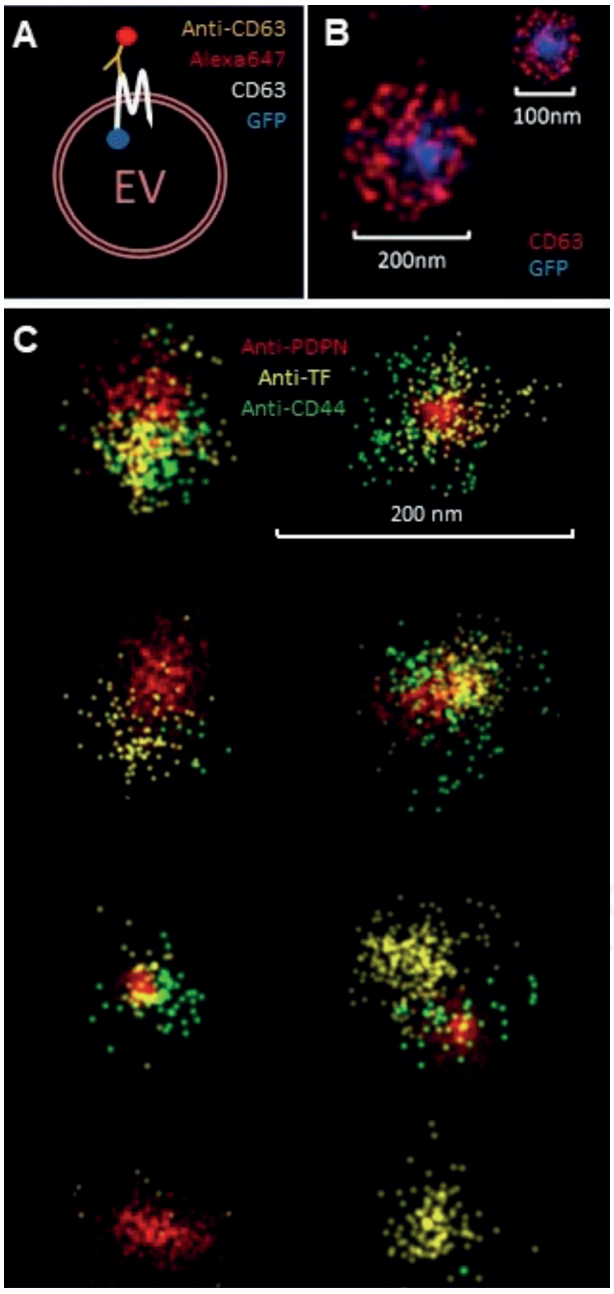

Figure 2. Structural complexity and coagulome of cancer-derived extracellular vesicles. A) Diagrammatic representation of extracellular vesicles from A431 epithelial cancer cells expressing CD63-GFP fusion protein; B) A431 extracellular vesicles imaged by ONI super-resolution microscope (dSTORM mode) for the expression of CD63 ectodomain (red) labeled with fluorescent antibody (Alexa 647) and for the intraluminal CD63-GFP tag (blue); C) heterogenous coagulant repertoires of individual extracellular vesicles derived from glioma stem cells-engineered to express podoplanin, and tissue factor, with endogenous CD44 expression (staining with fluorescent antibodies). All extracellular vesicles were imaged at the Centre for Applied Nanomedicine, RIMUHC (https://rimuhc.ca/research-initiatives/centre-for-applied-nanomedicine; with support from the ONI team and Mahsa Jalali). EVs, extracellular vesicles; GFP, green fluorescent protein; PDPN, podoplanin; TF, tissue factor.

Conclusions

While CAT may encompass all complexities of Virchow's triad, including unspecific and indirect influences, it is causally and molecularly triggered by the unique nature of the underlying neoplastic process. It may, therefore, be useful to consider (as one of the relevant factors) the drivers of cancer progression operating upstream of cancer coagulome, or of immediate clotting mechanisms. Both the biology of the underlying disease and the corresponding anticancer therapy may shape processes leading to VTE. Since these upstream effects are highly heterogeneous so could be the mechanisms triggering VTE, as well as its nature. Moreover, these may not be linear relationships. Rather, the consequences of oncogenic mutations may intersect with epigenetic alterations and interactions between cancer cells and their surroundings collectively impacting coagulome. Single cell profiling of cancers revealed that previously uncovered global properties of the tumor mass conceal more complex equilibria of cancer, stromal and inflammatory cells that underlie the malignant process and its vascular components. It is of interest to ask whether cellular landscapes of coagulant cancer types could help understand and address the VTE risks in individual cancer patients.

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