Coagulome and the tumor microenvironment: an actionable interplay

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Review

Coagulome and the tumor microenvironment: an actionable interplay

Antoine Galmiche [1,2,*] Janusz Rak,[3] Lubka T. Roumenina,[4] and Zuzana Saidak[1,2]

Human tumors often trigger a hypercoagulable state that promotes hemostatic complications, including venous thromboembolism. The recent application of systems biology to the study of the coagulome highlighted its link to shaping the tumor microenvironment (TME), both within and outside of the vascular space. Addressing this link provides the opportunity to revisit the significance of biomarkers of hemostasis and assess the communication between vasculature and tumor parenchyma, an important topic considering the advent of immune checkpoint inhibitors and vascular normalization strategies. Understanding how the coagulome and TME influence each other offers exciting new prospects for predicting hemostatic complications and boosting the effectiveness of cancer treatment.

Highlights

A hypercoagulable state established within the tumor accounts for the frequent hemostatic complications that occur in cancer patients, including venous thromboembolism.

The coagulation cascades exert multiple direct and indirect effects on cancer cells and the components of the tumor microenvironment, both within and outside of the vascular space.

The recent application of systems biology and single-cell approaches provides a comprehensive and deeper view of the coagulome and highlights its link to the tumor microenvironment.

The study of the coagulome provides unprecedented opportunities to explore the tumor microenvironment, to better predict the hemostatic complications, revisit the significance of clinical biomarkers, and it might ultimately enable us to steer the tumor immune response or vascular integrity.

Hemostatic complications in solid tumors and the rise of the coagulome

Cancer is associated with a wide spectrum of hemostatic complications, ranging from thrombotic events to hemorrhagic manifestations. **Venous thromboembolism** (**VTE**) (see Glossary) is a common manifestation of cancer-associated thrombosis (CAT) and a significant cause of cancer-related mortality [1,2]. Determinants of hemostatic complications in cancer are multifactorial: the patient's age, reduced mobility, different anticancer treatments (surgery or radiochemotherapy), as well as intrinsic biological characteristics of the tumor all contribute to the risk of VTE. Importantly, malignant brain tumors (glioblastoma multiforme, GBM), pancreatic, gastric, and lung cancers carry a much higher risk of VTE than other sites, suggesting disease specificity [3]. Addressing the status of coagulation within a given tumor is challenging in the clinical setting, but a number of biomarkers exist (Box 1) and drugs that specifically target coagulation effectors, such as **direct oral anticoagulants** (**DOACs**), are available for the treatment of thrombosis in cancer patients [1,2].

The coagulation system has traditionally been depicted as a cascade (Figure 1), but cancer adds an important new dimension to its regulation and analysis. Progress in descriptive analyses and systems biology has led to the emergence of the concept of the tumor **coagulome** [4,5], a cancer-driven network of molecular effectors favoring thrombosis or bleeding. **Tissue factor** (**TF**) is at the center of the present model of thrombosis: a highly regulated molecular pivot normally shielded from the circulation only to be exposed upon injury. In cancer, TF is often constitutively overexpressed and acts either locally or at a distance, carried on the surface of tumor-derived extracellular vesicles (EVs), sometimes also referred to as microparticles, and released into the circulation [3]. In tumor tissue, coagulation can be activated by the disruption of vessel walls, leading to hemorrhage and intravascular coagulation, or as a consequence of increased vascular permeability and plasma extravasation, leading to extravascular coagulation [6]. In addition, entry of metastatic cancer cells into the circulation, shedding of procoagulant EVs, and recruitment/activation of inflammatory cells (immunothrombosis) may further amplify these complex processes. A detailed overview of clinical aspects of CAT can be found in several excellent review articles [7–9].

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Box 1. Assessing coagulation and fibrinolysis in the clinical setting

Classical clotting assays, such as the prothrombin time and INR (international normalized ratio) reflect a large number of parameters, in addition to the ability of the tumor to generate thrombin, and are therefore of limited interest in a purely oncological perspective. Other biological markers more directly reflect thrombin generation, the rapid turnover of fibrin (D-dimers, product of the degradation of crosslinked fibrin), the formation of neutrophil extracellular traps (NETs), or even circulating EVs (Table I). Some of these parameters are incorporated into VTE risk scores, such as the Khorana score and its derivative, the Vienna Cancer and Thrombosis Study (CATS) score [1,2]. This process still faces considerable challenges, and the predictive performances of the current scores are far from complete [1,2].

Table I. Selected biomarkers of coagulation/fibrinolysis and their link to hemostasis

Marker	Link to coagulation
Thrombin generation measured with specific substrates	Thrombin activity
Thrombin–antithrombin (TAT) complexes	
Prothrombin 1+2 fragment (F1+2)	
TF^{+ve} microparticles	
D-dimers	Product of fibrin degradation
Soluble P selectin	Biomarker of platelet activation
Citrullinated histone H3	Biomarker of NETosis

In the tumor, procoagulant conditions resulting in microvascular thromboembolism favor the formation of a hypoxic/nutrient-poor milieu by establishing a physical barrier that restricts the access of tumor cells to blood nutrients [6]. Coagulation reduces perfusion by causing luminal occlusion of microvessels and a build-up of the **fibrin** meshwork that leads to water retention and high fluid pressure in the interstitial space [6]. Depending on the extent to which perfusion is reduced, tumor

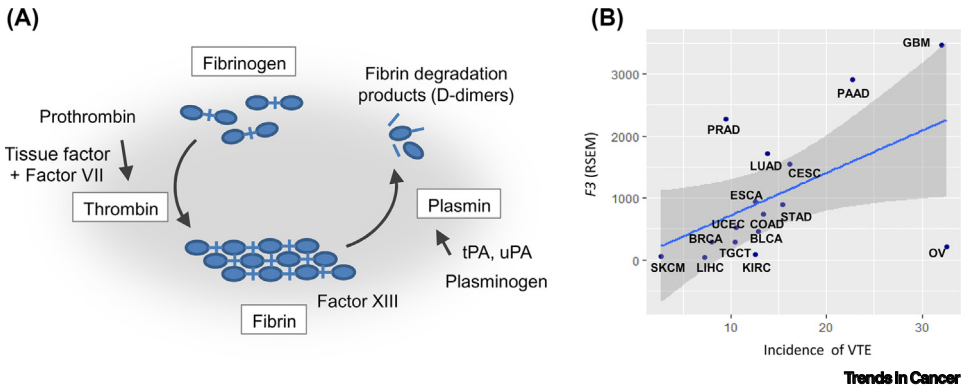

Figure 1. An overview of the coagulation and fibrinolysis cascades. (A) Coagulation is a highly coordinated process leading to fibrin polymerization and clotting. A key activator of coagulation is the tissue factor (TF, encoded by *F3*), expressed on the surface of cancer cells in a tumor-type and tumor-specific manner. TF forms a complex with Factor VII and activates the so-called extrinsic coagulation pathway. Conversely, the intrinsic pathway depends on Factor XII and is activated upon exposure of the subendothelium to blood. Both pathways lead to the activation of the serine protease thrombin (Factor IIa) that cleaves fibrinogen and produces fibrin. Fibrin monomers assemble into a high-order polymer that is stabilized upon crosslinking by Factor XIIIa. The fibrin network serves as a provisional matrix that can later be disassembled by plasmin. Both the tissue-type plasminogen activator (tPA) and the urokinase-type plasminogen activator (uPA) (encoded by the genes *PLAT* and *PLAU*, respectively) counterbalance the hemostatic effects of TF and thrombin by activating plasmin. (B) A correlation between the risk of venous thromboembolism (VTE, cumulative incidence for 1000 patients) and *F3* expression across common human tumors. The correlation is based on gene expression data retrieved from The Cancer Genome Atlas (TCGA) and the study of Saidak *et al.* [5]. PAAD, pancreatic adenocarcinoma; HNSC, head and neck squamous-cell carcinoma; PRAD, prostate adenocarcinoma; LUSC, lung squamous-cell carcinoma; LUAD, lung adenocarcinoma; COAD, colorectal adenocarcinoma; BRCA, breast carcinoma; KIRC, kidney renal clear-cell carcinoma; LIHC, liver hepatocellular carcinoma; GBM, glioblastoma multiforme.

Glossary

Coagulome: the multiple genes and proteins that collectively contribute to the equilibrium between coagulation and fibrinolysis. The study of the tumor coagulome and its regulation is amenable to systems biology approaches.

Complement: a system consisting of >30 proteins, overall organized as a cascade of proteases, with a key role in the defense against bacterial pathogens.

Direct oral anticoagulants (DOACs): DOACs represent a therapeutic alternative to low-molecular-weight heparins or oral vitamin K antagonists. Anticoagulants are used for the treatment or the prevention of cancer-associated thrombosis, typically after surgical intervention.

Fibrin: the cleavage product of fibrinogen, a serum protein produced by the liver. The cleavage of fibrinogen is catalyzed by activated thrombin and contributes to its polymerization into a scaffold specialized in tissue repair/remodeling. Fibrinolysis is the catalytic cleavage of fibrin and constitutes an important step for the replacement of fibrin by mature connective tissue during healing.

NETs (neutrophil-derived extracellular traps): NETs are web-like structures consisting of decondensed chromatin and proteases that are actively released by polymorphonuclear cells (PMNs). Besides their antimicrobial functions, NETs exert procoagulant and proinflammatory functions.

Peritoneal carcinomatosis: the intraperitoneal dissemination of a cancer. Intraperitoneal carcinomatosis is a late complication of several gastrointestinal and gynecological malignancies.

Thrombin: also known as factor II of the coagulation cascade, thrombin is a key protease that cleaves fibrinogen into fibrin, the final product of the coagulation cascade.

Tissue factor (TF): the product of the *F3* gene; it is a cell-surface glycoprotein that initiates the extrinsic blood coagulation cascades, triggered upon blood contact with extravascular tissues. TF is an important contributor to cancer-associated thrombosis.

Tumor microenvironment (TME): the non-malignant cells and structures (e.g., extracellular matrix) present within a tumor, including platelets and red

cells undergo a range of changes. Severe reduction in O_2/nutrient supply in the center of tumors often leads to cellular necrosis, while milder, adaptive responses are observed in other areas (hypoxia signaling). Fibrin deposition and crosslinking also determine the biophysical properties of the tumor tissue (solid stress, interstitial fluid pressure, tissue stiffness, and physical microarchitecture) [10]. A densely crosslinked fibrin meshwork may limit tumor progression by restricting the motility of cancer cells. Alternatively, raising the interstitial pressure can push cancer cells toward lymph nodes and promote their dissemination. Fibrin may also act as a regulatory matrix to support cancer cell survival, or regulate complex phenotypes, such as tumor dormancy [11]. Distinct topographical patterns of coagulation and fibrin deposition are found depending on the tumor type and stage. Fibrin may be deposited as a layer on the mesothelial surface in **peritoneal carcinomatosis** [12] or at sites of previous surgery, potentially explaining the preferential tendency of some cancer cells to implant at these sites [13]. Single-cell studies highlight the striking intratumoral heterogeneity of the tumor coagulome [14].

The coagulation system exerts some of its tumor-regulatory effects independently of fibrin polymerization [15]. **Thrombin** and other proteases of the coagulation cascade can directly induce cell signaling by interacting with the protease-activated receptors (PAR1–PAR4), a family of G-protein-coupled receptors (GPCRs) present on the surface of many cell types [16,17] (Box 2). Depending on the ligand and the precise cellular setting, PAR1 signaling can lead to different outcomes, for example cancer cell apoptosis [18], altered tumor cell differentiation [19], or enhanced stemness [20]. The overall effect of coagulation is complex and is tumor-model-dependent, as illustrated in a recent review of the effects of DOACs in tumor models [21]. While tumor regression or delayed progression is seen in some tumor models, in others DOACs promote tumor growth at implanted and metastatic sites [21]. In this article, we focus on the functional interplay between the coagulation system and the **tumor microenvironment** (**TME**), the complex web of cells and extracellular structures that surround cancer cells, whether at their origin or at the site of metastatic dissemination. In the next section, we examine how coagulation regulates the main cell types of the TME.

The TME as an effector of the coagulome

Platelets and red blood cells (RBCs)

The clotting process engages several cellular elements of the circulating blood and impacts both local and systemic aspects of cancer, including metastasis. In experimental models of hematogenous metastasis, coagulation supports the formation of circulating microemboli that contain cancer cells, platelets, and RBCs [22]. In late-stage renal-cell carcinoma (RCC), viable/proliferating cancer cells can be detected in venous thrombi that include platelets and RBCs [23]. A positive correlation between blood fibrinogen levels and the number of circulating tumor cells (CTCs) suggests that microemboli readily promote CTC survival [24]. Platelets can be activated by thrombin via the PAR receptors present on their surface, and their interaction with fibrin(ogen)

blood cells, polymorphonuclear cells, fibroblasts, vascular cells, and immune cells (monocytes-macrophages and lymphocytes). Some of the components play a supportive role in tumor growth, while others have antitumor effects (for example, immune cells). The volume of the TME may constitute as much as 30–40% of the tumor mass, especially in pancreatic, brain, and prostate cancer.

Venous thromboembolism (VTE): the formation of a thrombus (blood clot) within a vein. Venous thrombosis leads to vessel occlusion and is a source of potential severe complications, such as an embolism.

Box 2. Protease-activated receptors (PARs)

The protease-activated receptors (PAR1–PAR4) are a family of four GPCRs with a broad spectrum of expression across cell types and tissues [17]. Thrombin was the first protease found to activate PAR1, which was initially called the thrombin receptor. Since then, however, the PARs have been shown to be activated by a growing number of proteases [17]. PAR1 is encoded by the gene *F2R* and can, for example, be activated by thrombin, plasmin, elastase, and cathepsin G. PAR2 (*F2RL1*) is activated by trypsin, factor Va, factor Xa, elastase, and cathepsin G, while PAR3 (*F2RL2*) is activated by thrombin and PAR4 (*F2RL3*) is activated by plasmin. As such, the PARs are often described as sensors of coagulation proteases [17]. They have a common activation mechanism, with a proteolysis step that unmasks a tethered ligand. The cellular outcome of their activation is complex because they can transduce proinflammatory or mitogenic signals, both directly and indirectly in association with other receptors (for example through receptor tyrosine kinase signaling), and it depends on the cell type in which they are activated.

is at the heart of their vascular surveillance and local recruitment [25]. Activated platelets release procoagulant mediators, growth factors (platelet-derived growth factor, PDGF, and transforming growth factor-β, TGF-β), cytokines (Interleukin-1β, IL-1β), bioactive lipids, and ATP/ADP [22]. Platelet-derived EVs are produced by budding from their surface in response to contact with receptor agonists or shear stress [22]. MicroRNAs (miRNAs) are present within these EVs, potentially favoring horizontal RNA transfer between platelets and tumor cells [26].

The role of platelets in CAT is increasingly recognized, as is illustrated by the role played by podoplanin (PDPN), a platelet activator present on the surface of GBM cells or their EVs released into the circulation [27,28]. Platelets have long been recognized to promote the late stages of cancer progression [29]. They are able to shield cancer cells from innate immunity, while in circulation [22]. They also contribute to the vascular phase of cancer cell dissemination by favoring adhesion to the target endothelium [30], and they establish a permissive premetastatic TME at distant sites by recruiting monocytes/macrophages [30]. Platelets play a more complex role at earlier stages of tumor progression [22]. Platelet-derived EVs contribute to the vascular permeability observed in solid tumors and infiltrate their TME [26]. In colorectal tumors, extravasated platelets can form tight interactions with cancer cells [31], resulting in the production of chimeric EVs, the recruitment of tumoricidal macrophages, and decreased primary tumor growth [31]. Active malignancies impact the levels of platelets in the circulation, resulting in either their increase or thrombocytopenia [22], a notion that suggests the existence of multiple disease-specific regulatory circuits.

The extent to which RBCs are embedded within fibrin clots is variable [32]. Venous thrombi, formed inside vessels and in conditions of low shear stress, contain large numbers of RBCs. Specific molecular interactions involving Fas ligand/Fas receptor are created between RBCs and platelets that further increase thrombin generation [33]. RBCs also positively regulate inflammation in the TME. Their positive contribution to the recruitment of $CD45^{+ve}$ immune cells was recently shown in mice implanted with blood clots of different cell composition [34]. Senescent RBCs trapped within the TME represent a major source of iron delivered to tumor-associated macrophages (TAMs), favoring a tumoricidal (M1) phenotype [35]. Free hemoglobin and heme are also directly released into the extracellular space as a consequence of hemolysis and constitute danger-associated molecular patterns (DAMPs) able to interact with pattern-recognition receptors, such as the Toll-like receptors (TLRs). The contribution of RBCs is often underestimated in experimental studies exploring the TME. RBCs are important effectors of coagulation, and more studies are required to address their contribution to the TME.

Polymorphonuclear cells (PMNs)

Upon acute activation of coagulation, the thrombin–fibrin(ogen) axis initiates an inflammatory reaction and the recruitment of myeloid cells [36,37] (Figure 2). The tight coupling between coagulation and inflammation likely finds an explanation in the phylogeny of the defense against infections. During infection, coagulation represents a mechanism of early local containment, coupled to the subsequent inflammation-dependent elimination of the pathogens [38]. In inflamed tissues, coagulation favors vascular margination, endothelial adhesion, and transendothelial migration of PMNs. A close link between coagulation and the **complement** systems partially explains the coupling between coagulation and inflammation [39]. Thrombin can directly generate the inflammatory anaphylatoxins C3a and C5a through an unconventional pathway [39]. Complement has a complex, context-dependent role in cancer, promoting or suppressing tumor growth depending on the cancer type [40]. Little is known about the crosstalk between coagulation and complement in cancer, but the local production of C3a may recruit inflammatory cells, such as PMNs [41]. In a context of complement activation, these cells might further activate coagulation

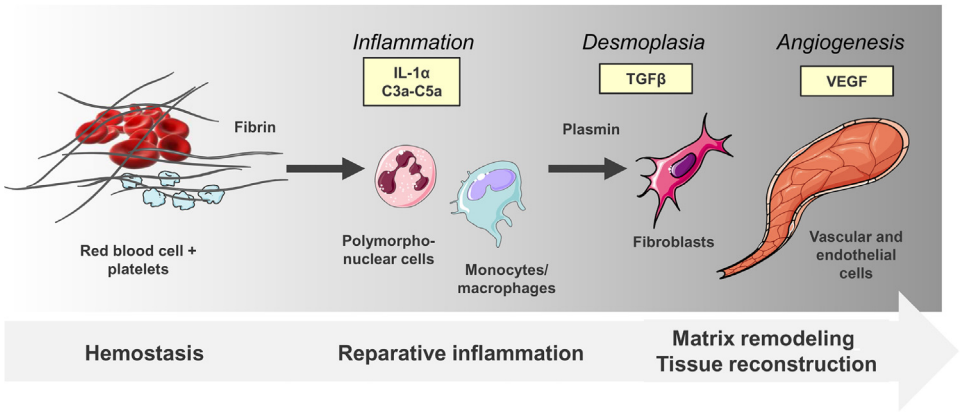

Figure 2. The main cellular events taking place during acute activation of coagulation. Thrombin and plasmin (ogen) have a key role in the instigation of an acute-phase response upon a broad range of types of tissue damage. The activation of coagulation initially ensures hemostasis and lesion containment. It also promotes the subsequent recruitment of myeloid cells (polymorphonuclear and monocyte–macrophage cells) that are key for reparative inflammation. Fibrinolysis, characterized by the activation of the protease plasmin, marks the last step, that is, matrix remodeling, essential for tissue reconstruction. The recruitment and activation of fibroblasts and vascular cells characterizes this late step. At each step, and above the indicated cell types, we indicate some of the key mediators that are targeted by coagulation (yellow boxes) and their possible contribution to key characteristics of the tumor microenvironment (TME).

[42]. In a murine model of skin squamous-cell carcinogenesis, plasmin also plays a role in the activation of the complement: urokinase-type plasminogen activator (uPA)$^{+ve}$ tumor macrophages cleave C5 and foster local inflammation [43]. The activation of IL-1α by thrombin is another mechanism that connects coagulation and the recruitment of leukocytes [44]. IL-1α is an essential inflammatory cytokine ubiquitously produced and maintained on the surface of eukaryotic cells as an inactive pro-protein (pro-IL-1α) [45]. By cleaving pro-IL-1α and releasing active IL-1α, thrombin recruits and activates PMNs, potentially contributing to the proinflammatory effect of coagulation [45]. Importantly, PMNs are also recognized for their ability to reinforce coagulation. Neutrophil-derived serine proteases (mainly elastase and cathepsin G) can cleave the tissue-factor pathway inhibitor (TFPI) [46]. Despite the strong reciprocal relationship established between coagulation and inflammation, little is known regarding this mutual regulation in cancer.

In late stages of breast cancer, during hematogenous dissemination, mixed cellular clusters of PMNs and CTCs are found in the circulating blood [47]. PMNs can undergo NETosis, a form of regulated cell death associated with the release of **NETs** (neutrophil-derived extracellular traps), that is, structures consisting of decondensed chromatin associated with histones and the PMN-specific serine proteases [46]. Beyond their classical function of trapping microbes, NETs might constitute a molecular platform for further activation of coagulation, and it has been suggested that they may stimulate tumor progression in various settings [48–50]. NETs are readily detectable in liver metastases of breast and colon tumors, and monitoring their levels in the serum of cancer patients might predict the risk of liver metastasis [50]. The negative prognostic value of a high neutrophil-to-lymphocyte (NLR) ratio highlights the systemic nature of alterations of PMN physiology in advanced tumor stages [51]. While proinflammatory cytokines (IL-1β and IL-6) exert a procoagulant effect in models of late-stage cancer [52,53], the extent to which NETosis is related to coagulation in late-stage cancer patients is still unclear [54,55].

PMNs may also contribute to early stages of tumor progression [51,56]. A pan-cancer transcriptomic study using The Cancer Genome Atlas (TCGA) identified the tumor-associated neutrophil

(TAN) infiltrate as a robust negative predictor of survival [57]. TANs can, however, have a dual action on tumors, either as a brake (N1 phenotype) or an accelerator of tumor progression (N2) [51]. A subset of myeloid-derived suppressor cells (MDSCs), present within tumors, emerges from an aberrant neutrophil maturation trajectory [58]. Elements of the coagulation system may regulate the TAN landscape. Thrombin was found to positively influence ovarian tumor infiltration by MDSCs [59], an effect that could be accounted for by the activation of PAR or via the complement (C3a) produced by cancer cells [41]. In a mouse model of intestinal carcinogenesis associated with chronic inflammation, coagulation was also found to induce a shift of PMNs toward a low density/N2 phenotype characterized by the expression of the matrix metalloproteinase-9 (MMP9) [42]. MMP9 degrades collagen and elastin and remodels the extracellular matrix (ECM) in addition to being able to activate TGF-β from its latent form [60]. Overall, the connection between coagulation and PMN/MDSC might therefore prevent anticancer immunity in cancer patients [61]. Addressing the many questions raised by the presence of subpopulations of PMNs and their products, such as NETs, promises to shed light on the mutual regulation of the cancer coagulome and the TME.

TAMs

TAMs represent an essential myeloid cell lineage recruited to the TME [62]. In most tumors, the presence of a dense TAM infiltrate is associated with poor prognosis [62]. TAMs are classically reported to be functionally polarized into two phenotypes: M1 (tumor suppressive, driven by IFN-γ (interferon-γ)) and M2 (tumor promoting, driven by IL-4 and IL-13) [62]. The simplified presentation of the two TAM subsets, however, under-represents the phenotypic heterogeneity of these cells [62]. During normal healing, macrophages remodel the fibrin polymer and eliminate dead cells (efferocytosis) [63]. During fibrinolysis, plasmin directly stimulates the chemotaxis and phagocytic behavior of TAMs [64,65].

In glioma, the recruitment of blood monocytes, and their differentiation into protumorigenic TAMs, might be linked to coagulation and vessel dysfunction [66]. TAMs undergo an M1-to-M2 switch during late stages of vascular dysfunction, coinciding with tumor vessel leakiness [66]. In turn, M2 TAMs contribute to the installation of dysmorphic vessels by supplying vascular endothelial growth factor (VEGF) [66]. Evidence of a direct effect of thrombin on TAM differentiation has been obtained in a model of ovarian cancer, in which exposing monocytes to thrombin induced NF-κB-driven transcription, elevated production of the proinflammatory cytokine IL-8, and accompanying M2 differentiation [67]. How thrombin favors M2 differentiation in this context is not clear, considering that an opposite PAR1-dependent pro-M1 effect is observed upon acute exposure of macrophages to thrombin *in vitro* [68]. In animal models, expression of TF by cancer cells was reported to promote monocyte recruitment, supporting the establishment of the premetastatic niche [69]. TAMs are not only a target, but also an important contributor to the tumor coagulome. Osteoclasts, a cell type that derives from monocytes, involved in physiological bone resorption, produce small quantities of prothrombin [70]. A recent study suggests that monocytic cells are a source of Factor X (previously thought to be generated exclusively in the liver), leading to cell-autonomous FXa-PAR2 signaling in these cells within the TME [71]. In a model of fibrosarcoma, targeted gene deletion of *F10* in the monocytic lineage reduced tumor infiltration with neutrophils/MDSC and increased the T cell infiltrate [71]. Monocytes also produce factor XIIIa in the TME of lung squamous-cell carcinoma [72].

Fibrinolysis is closely related to the TAM infiltrate, and this could account for the well-recognized prognostic value of uPA, uPAR (uPA-receptor), and PAI-1 (plasminogen activator inhibitor-1) expression in multiple cancer types [73,74]. In breast tumors, PAI-1 was found to recruit TAMs

through an interaction with the low density lipoprotein receptor-related protein 1 (LRP1) [75]. Autocrine production of IL-6 and STAT3 signaling is induced by PAI-1, favoring M2 polarization of TAMs [75]. In large transcriptomic analyses of human cancers, a clear positive correlation was found between *SERPINE1* (PAI-1) mRNA levels and the expression of the TAM M2 marker CD163 [5,75]. Overall, the coagulome appears to be strongly linked to the presence of TAMs and their phenotype within the TME.

Tumor-infiltrating lymphocytes (TILs)

An efficient cytotoxic T cell response directed against tumor cells is an element of favorable prognosis in many tumors [76–78]. Functional vessels are essential for T cells to reach the TME, but a reciprocal regulation of vessel physiology has also been observed: IFN-γ produced by T cells and acting on endothelial cells may contribute to tumor vessel normalization, for example, by ensuring vessel coverage with pericytes [79–81]. This crosstalk between vessels and T cells offers a rationale for combining immune checkpoint inhibitors (ICIs) with antiangiogenic strategies against various tumors [82] and could also be relevant in the regulation of coagulation within the TME. Indeed, dysfunctional tumor vessels and the ability of fibrin to install a physical barrier between cancer cells and immune cells limit the access of lymphocytes and their contact with cancer cells [83–85]. Furthermore, the high tumor interstitial pressure favored by coagulation reduces the lumen of tumor vessels and might aggravate the hypoxic/nutrient poor conditions, installing suboptimal conditions for T cell function [84].

Compared to the other cells of the TME, TILs express low levels of coagulome genes or their receptors, as shown in the single-cell analysis of mRNA levels of these genes in head and neck squamous-cell carcinoma (HNSC) (Figure 3) [86]. Indirect effects might therefore predominate over the direct regulation of TILs by coagulation products. Importantly, coagulation exerts immunomodulatory effects by changing the conditions of antigen presentation. Its impact on inflammation and MDSC differentiation might, for example, interfere with the stimulation of TILs [61]. TAM-autonomous production of FXa, leading to PAR2 signaling, promotes PD-L1 expression on the surface of these cells, skewing immune responses toward evasion [71]. In a model of fibrosarcoma, a targeted deletion of FXa in monocytic cells or treatment with the anti-Xa DOAC, rivaroxaban, led to increased tumor infiltration by T $CD8^+$ cells and better tumor control [71]. In pancreatic adenocarcinoma (PAAD), thrombin-PAR1 signaling in cancer cells potentially promotes an immunosuppressive microenvironment through the expression of genes, such as CSF2 and PTGS2 (encoding GM-CSF and the cyclooxygenase-2, respectively, that are key for the production of prostaglandins) [87,88]. Further studies are needed to examine these regulations in human tumors.

Cancer-associated fibroblasts (CAFs)

Fibroblasts are an important cellular population in the TME [89]. Despite the oversimplified presentation of their function as a source of ECM, CAFs include several cell lineages with distinct origins and plastic phenotypes [89]. These cells play different roles, with likely impact on the vasculature and coagulation system. A prominent desmoplastic reaction is found in PAAD and is thought to be produced by a subpopulation of CAFs that express α-smooth muscle actin (α-SMA) [90]. Other CAFs express IL-6 and exert specialized proinflammatory/immunosuppressive functions [90]. CAFs are a major source of TGF-β1, which favors the matrix-producing phenotype of CAFs [91]. In the TME, TGF-β might coordinate waves of profibrotic signaling and restrict immune effector cells from accessing the tumor [92,93]. While CAFs constitute the bulk of the cellular mass in pancreatic cancer, one of the most procoagulant tumor types, little is known about the relationship between CAFs and CAT in this setting.

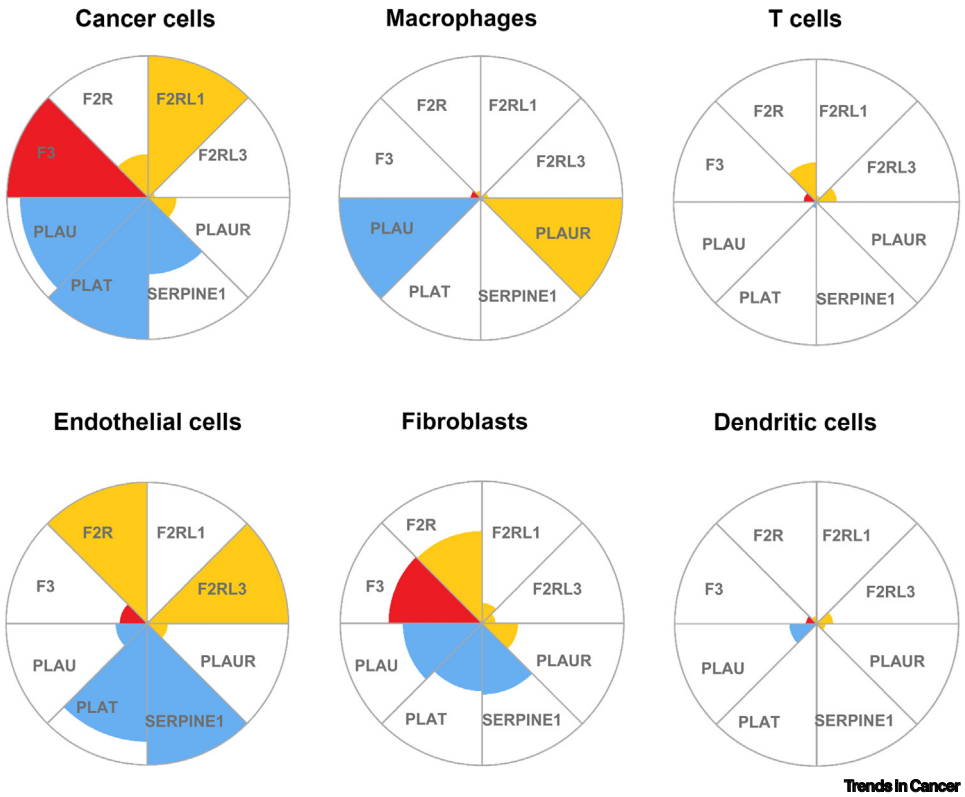

Figure 3. Cell-type-specific contribution to the coagulome in head and neck squamous-cell carcinoma (HNSC). HNSC is characterized by a relatively moderate risk of venous thromboembolism (VTE, possibly as a consequence of high fibrinolytic activity counterbalancing the high expression of tissue factor, TF). We present an analysis of the expression of the core coagulome genes (*F3*, *PLAU*, *PLAT*, *PLAUR*, *SERPINE1*, *F2R*) in the main cell types present within the tumor microenvironment (TME) of HNSC. The analysis is based on the results of the single-cell RNAseq analysis reported by Puram *et al.* [86]. The polar plots provide a comparison of the coagulome genes in cancer cells and five other cell types of the TME (macrophages, endothelial cells, fibroblasts, T cells, and dendritic cells). Note that procoagulant genes are shown in red (*F3,* encoding TF), actors of fibrinolysis in blue (*PLAU, PLAT, SERPINE1,* encoding uPA, tPA and PAI-1, respectively), and receptors in yellow (*F2R*, *F2RL1*, *F2RL3*, *PLAUR*, encoding PAR1, PAR2, PAR4, and uPAR, respectively).

In HNSC, CAFs express high levels of the core components of the coagulome and PAR1 [86] (Figure 3). This particularity defines CAFs as sensors of extravascular coagulation, especially in the context of chronic inflammation, prone to fibrosis and malignant transformation [94]. Liver fibrosis, an established precancerous state, highlights the role of PAR1 in collagen I deposition [95,96]. Thrombin can also stimulate the release of active TGF-β [97]. Indeed, TGF-β is produced and stored on the cell surface as a latent complex (latent TGF-β, LTGF-β) requiring proteolytic activation [97]. In a model of colorectal carcinogenesis, thrombin was found to control TGF-β release by cleaving GARP (glycoprotein A repetition predominant), a platelet receptor for LTGF-β [97]. In conditions of chronic inflammation, plasmin also activates TGF-β from its latent precursor [98]. The application of single-cell technologies is expanding our understanding of the variety of CAF phenotypes and it will be of the utmost importance to address the impact of coagulation on the different subpopulations of CAFs in human tumors.

Vascular cells/tumor endothelial cells (TECs)

Dysmorphic and leaky vessels are commonly seen in human tumors. In experimental glioma, longitudinal intravital imaging shows progressive vascular alterations, with an initial phase of

angiogenesis followed by reduced branching and vessel leakiness [66]. Vascular development, as well as angiogenesis, vascular aberrations, and permeability in cancer are strongly linked to the expression of VEGF [99]. It has been proposed that VEGF-driven vascular leakiness stimulates aspects of CAT and affects the TME [6]. Several studies have examined VEGF inhibition as a possible antitumor strategy [100]. VEGF targeting alone has, however, failed to show a significant increase in survival in most cancers [100]. Importantly, understanding of TEC biology has steadily increased over the past years, unveiling new players in angiogenesis and fundamental mechanisms that regulate tumor vessels [100]. Angiocrine signaling, that is, the production of active molecules by the tumor vasculature, is central in the perivascular niche [101,102]. For example, the Notch signaling pathway, which is composed of five ligands and four receptors (Notch 1–4), is a key actor in the perivascular niche [102]. A reciprocal interaction established between TECs (source of the Notch ligand Jag1) and cancer cells (source of VEGF-A) [103] might contribute to the formation of the perivascular niche and possibly perpetuate tumor vascular dysfunction.

The importance of coagulation as a promoter of angiogenesis has been suggested in early studies linking TF expression to the recruitment and proliferation of vascular cells [104–106]. Lymphangiogenesis is also stimulated by coagulation: thrombin cleaves and activates VEGF-C, the lymphangiogenic member of the VEGF family, after its release as an inactive precursor from the α-granules of platelets [107]. Thrombin also potentially regulates Notch signaling by cleaving the extracellular portion of Jag1 [108]. Importantly, single-cell analyses are expanding our understanding of tumor angiogenesis by unveiling TEC subpopulations with specific functions [109]. These include subpopulations specialized in immune regulation, characterized by gene expression programs similar to those detected in high endothelial venules (involved in leukocyte recruitment) [109]. Examination of the expression of PAR receptors and the effects of coagulation in each TEC subset with spatially resolved genomics/proteomics is eagerly awaited.

Thrombin may also activate vasculogenic mimicry, that is, the formation of vascular channels lined with non-endothelial cells [110]. Activation of PAR1 on the surface of non-small-cell lung cancer cells stimulates vasculogenic mimicry [110]. TEC stimulation by thrombin could also indirectly lead to vascular recruitment of pericytes, a cell type that contributes to vessel maturation [111,112]. Finally, the TME of lung tumors favors the remodeling of larger vessel walls, potentially contributing to pulmonary hypertension in these patients [113]. The recognized contribution of thrombin to the activation of vascular smooth muscle cell proliferation and their production of inflammatory cytokines (IL-6, CXCL8) could be essential in this situation [114]. The complex composition of tumor vessels calls for *in vivo* studies combining genome editing and advanced imaging technologies in order to precisely examine the role of coagulation in each cell type.

Toward a systems-level understanding of the tumor coagulome

While coagulation is well known to favor inflammation and the recruitment of myeloid cells in the context of acute healing (Figure 2) [37], much less is known regarding its role in the TME. Experimental studies in which cancer cells were mixed with blood clots suggest a comparable proinflammatory effect of coagulation in the TME [34]. Addressing the role of coagulation in real tumors is, however, much more complex. The microheterogeneity of the TME is a dynamic process resulting from the continuous interactions between the cells that constitute the tumor mass and their external cues [115]. Morphologic examination of most tumors suggests the simultaneous existence of cycles of coagulation and fibrinolysis that coexist in different spatially confined areas within each tumor [6]. The multiple interactions and feed-back loops that exist between cancer cells, inflammatory and vascular cells complicate the analysis of the regulation and the effects of coagulation in this setting and they confound biological and therapeutic predictions.

Meanwhile, clinical trials that have tested the possibility of applying thromboprophylaxis in ambulatory cancer patients have given disappointing results. This is, perhaps, not surprising as many of these efforts made the assumption that coagulation should act as a universal and reversible protumorigenic switch. The reality is more intricate. Low-molecular-weight heparins (LMWHs) are still the most widely used anticoagulants but their use in thromboprophylaxis did not extend the survival of lung cancer patients [116] despite a strong preclinical rationale showing their combined anticoagulant and anti-inflammatory effects in this context [117]. More recently, the introduction of DOACs brought a significant advancement toward more specific (targeted) thromboprophylaxis [118,119]. Randomized trials that have compared DOACs versus LMWHs in cancer patients at high risk of thrombosis have established the noninferior efficacy of DOACs against VTE – but also suggested that DOACs might potentially cause more systemic complications and bleeding in some situations [118]. These large well-conducted, prospective studies illustrate the delicate balance that exists in cancer patients, where physicians deal with two opposite complications, VTE versus bleeding. Importantly, DOACs target the core actors of blood coagulation and they are not directed against cancer-related/specific mechanisms that cause thrombosis. The intrinsically narrow therapeutic window of the currently approved anticoagulants may prevent attaining the expected biological effects. Moreover, noncanonical (noncoagulant) activities of coagulant proteins may not be responsive to treatments aimed at anticoagulation.

Genomic analyses that examine the tumor coagulome and address its correlation with the TME provide useful knowledge by unveiling the stunning diversity of the tumor coagulant landscape [5]. The 'core' coagulome of human tumors, that is, expression levels of the small set of genes experimentally proven to be key effectors of coagulation, greatly vary between tumor types and individual tumors [5]. This heterogeneity represents a tremendous obstacle to universal strategies of thromboprophylaxis. In some cases, such as in GBM, subsets of cancer cells representative of specific differentiation programs may accumulate multiple procoagulant activities not possessed by their counterparts within the same tumor [28]. Grasping this complex target, whether for the purpose of anticoagulation or to curtail coagulation-independent biological responses to the coagulome, requires a better understanding of the coagulome, that is, at the systems level. Importantly, as discussed in Box 3, currently only limited knowledge exists regarding the dynamic regulation of the tumor coagulome, an important requirement in order to design models that predict the risk of VTE and its biological parallels.

With technological progress in molecular and spatial profiling, an interesting perspective could emerge from a more precise delineation of the coagulome in individual tumors. The possibility

Box 3. Further exploring the regulation of the coagulome

Systems biology unveils the stunning diversity of the tumor coagulome and highlights its complex relationship with the various facets of tumor physiology. Mitogenic signaling and the presence of a stressful microenvironment, as is commonly found in human tumors, regulate the expression of *F3* [120,121]. Some of the somatic mutations that play a driver role in tumor progression, such as those found in MET or RAS, might directly regulate *F3* expression [122,123]. Genomic instability also directly regulates *F3* expression, as described by Bakhoum *et al.* who identified *F3* as a transcriptional target of NF-kB activated by the innate immune mechanisms sensing cytosolic DNA, cGAS-STING [124]. DNA methylation, an important epigenetic mark, modulates the expression of the coagulome [125]. DNA methylation marks are a stable determinant linked to cell differentiation and likely partially account for tissue- and cell-type-specific patterns of coagulome expression [5]. Cellular senescence, a complex and possibly heterogeneous cellular state characterized by proliferative arrest, could be associated with specific alterations of the coagulome. The induction of uPAR (encoded by the gene *PLAUR*) was recently identified as a dominant event in the secretome of cancer cells entering into senescence, either induced by oncogenic stress or cancer therapy [126]. Further adding to this complex regulation, the tumor coagulome may also respond to comorbidities, such as obesity [127,128], the presence of specific microorganisms [129,130], or even be linked to the age of the patient [131]. The convergence of these regulations defines the coagulome as a hub of tumor physiology, which opens up questions regarding its dynamic regulation over time.

of extending the number of genes with coagulation-related functions offers the opportunity to discover new tumor-specific regulators of the coagulome and its intersection with other dimensions of the TME [132]. Targetable nodes of prothrombic activity may emerge in specific disease states among multiple elements of the coagulome. For example, amidst other effectors, the glycoprotein podoplanin (PDPN) is expressed on the surface of GBM cells and released, via EVs, into the circulation. In this manner, PDPN contributes to platelet recruitment/activation in these tumors to an extent that may be more critical for the risk of VTE than the levels of TF and other potentially active factors [27,28]. Targeting PDPN might, at least in theory, provide more specific and potent anticoagulation in patients with GBM than the currently approved anticoagulants. Finally, the metastatic coagulome also remains poorly explored. Several coagulomes might coexist in an individual patient, depending for example on the location of metastases (see Outstanding questions).

Targeting the coagulome to normalize the TME?

Malignant tumors almost invariably present signs of chronic wound healing, as highlighted by Dvorak [6]. The activation of coagulation represents one of the most recurrent properties of human tumors, illustrating the concept that solid tumors are not only clonal expansions of malignant cells [6]. Targeting coagulation offers exciting perspectives for TME normalization, especially in the current era of increasing use of biotherapies directed against tumor vessels or immune checkpoint molecules [133]. Nevertheless, the past two decades of research on angiogenesis show that the intrinsic complexity of the TME is a formidable challenge, as illustrated by the failure of VEGF-targeting monotherapies to extend the survival of cancer patients and achieve long-lasting TME normalization [83]. The recent spectacular achievements obtained with ICIs in certain subsets of cancer patients are attracting attention to the essential role of the TME, as well as to the challenges involved in its targeting [134]. Indeed, achieving vascular normalization and turning the TME into a 'hot' immune microenvironment likely requires simultaneous reduction of extracellular matrix deposition, vascular decompression, vessel wall normalization, and endothelial differentiation toward a more immune 'friendly' phenotype [77,134]. Preventing the local activation of coagulation might be helpful in this setting.

Despite a promising biological rationale, a general synergy between anticoagulants and ICIs has not been observed, when all anticoagulants are considered under the current indications of ICIs (mostly melanoma and lung cancer) [135]. Importantly, some of the most procoagulant tumor types, such as GBM and PAAD, are notoriously refractory to ICIs and they were not included in these studies. It will be interesting to extend the study of the interaction between ICIs and anticoagulants to these tumor types – which are known to be at high risk of VTE. Metastatic tumors might also differ in their regulation of the TME. The hepatic tissue, which produces high concentrations of prothrombin, is known to favor metastatic infiltration with TAMs and limit T cell-dependent antitumor immunity [136]. Assessing the role of coagulation in the poor response of liver metastases to ICIs, and in general as a host tissue determinant of tumor immunity [137], seems worthwhile. Finally, two recent studies that have more precisely examined the type of anticoagulants used in cancer patients provide interesting hints and suggest that further investigations are warranted. In one retrospective study, the use of aspirin was associated with a significantly higher response rate of multiple tumor types to PD1-targeting [138]. Targeting platelets with aspirin might be a possible explanation for this observation, which remains at this stage preliminary [139]. A second, equally interesting study opens up the appealing possibility that the use of the oral Factor Xa-inhibitor, rivaroxaban, might be associated with higher response rates and increased progression-free survival in advanced melanoma treated with ICIs [140]. The possible TAM-autonomous production of FXa leading to PAR2 signaling and PD-L1 expression, that we mentioned earlier [71], offers a possible rationale for this observation and calls for a prospective evaluation of the interactions between FXa-inhibitors, such as rivaroxaban, and ICIs.

Concluding remarks

A biologically precise approach based on the identification of tumor subsets with defined molecular profiles/susceptibilities likely offers the best chance of translating the basic knowledge regarding the tumor coagulome into clinical advances. To achieve this goal, an important challenge ahead is to expand the use and interpretation of the biomarkers of the coagulome (Box 1). Interpreting biomarkers of coagulation and fibrinolysis is a complex issue in the clinical setting. The existence of a strong correlation between *F3* mRNA (TF) and the primary tumor type, as opposed to fibrinolysis which is more closely related to the cell composition of the TME [5], calls for a simultaneous exploration of the dynamics of coagulation and fibrinolysis [2]. A longitudinal assessment of D-dimers was recently reported to significantly improve the estimation of VTE risk in cancer patients [141]. Functional assays measuring thrombin generation were also applied for early diagnosis of postsurgical recurrence in breast cancer [142]. Translational studies modeling the coagulome and confronting it with markers of inflammation (circulating markers of PMN activation, cytokines, products of the complement, EVs etc.) and adaptive immunity in the TME are awaited. Conversely, we argue that innovative technical approaches, such as microfluidic systems and lab-on-chip devices, that are well suited for the reconstitution of complex biological interactions and model coagulation [143], will help to characterize the tumor coagulome and its interaction with the TME and might also provide potential biomarkers. These approaches will likely generate 'big data' and require intensive computational analysis, potentially involving machine learning instead of traditional linear analysis. Being able to more precisely decipher the mutual interplay and actionability of the nexus between the tumor coagulome and the TME offers not only medical but also new fundamental perspective for a better understanding of the regulation of both these systems and cancer as a systemic disease.

Outstanding questions

Can the knowledge on the coagulome gained through systems biology be translated into models that more accurately predict the risk of VTE? Is more personalized control of the coagulome achievable in the therapeutic context? Would it improve anticoagulation while reducing the associated risks (bleeding) in cancer patients?

How does the coagulome change during tumor progression? Does it, for example, change in different metastatic locations? Are the effects of morbidities, such as obesity or microbial dysbiosis, at least partially related to the regulation of the coagulome?

Does the coagulome account for some of the primary tissue-specific properties of tumors (for example their metastatic tropism or response to immune checkpoint inhibition)?

Can circulating biomarkers of hemostasis be revisited to explore the TME (vasculature functionality and immune status)? Could the coagulome be useful for clinically meaningful tumor stratification based on microenvironment subtypes? Would this help to predict which tumors are most likely to respond to immune checkpoint and/or angiogenesis inhibition?

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Declaration of interests

No interests are declared.

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