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Reprogramming the immunosuppressive tumor microenvironment: exploiting angiogenesis and thrombosis to enhance immunotherapy

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This review focuses on the immunosuppressive effects of tumor angiogenesis and coagulation on the tumor microenvironment (TME). We summarize previous research efforts leveraging these observations and targeting these processes to enhance immunotherapy outcomes. Clinical trials have documented improved outcomes when combining anti-angiogenic agents and immunotherapy. However, their overall survival benefit over conventional therapy remains limited and certain tumors exhibit poor response to anti-angiogenic therapy. Additionally, whilst preclinical studies have shown several components of the tumor coagulome to curb effective anti-tumor immune responses, the clinical studies reporting combinations of anticoagulants with immunotherapies have demonstrated variable treatment outcomes. By reviewing the current state of the literature on this topic, we address the key questions and future directions in the field, the answers of which are crucial for developing effective strategies to reprogram the TME in order to further the field of cancer immunotherapy.

KEYWORDS

immunotherapy, tumor microenvironment, angiogenesis, thrombosis, vascular normalization, hypoxia, treatment resistance

1 Introduction

Immunotherapies have revolutionized cancer treatment; however, their efficacy remains limited to a certain select tumor types primarily due to tumor immune evasion mechanisms. A key pathway through which tumors evade the immune system is the reprogramming of cellular constituents of the tumor microenvironment (TME) towards an immunosuppressive phenotype. Thus, enhancing the effectiveness of immunotherapies by manipulating the TME is a major focus of current research.

The tumor vasculature is key in controlling immune cell infiltration into tumors. However, tumor blood vessels can be highly abnormal, characterized by tortuous, primitive, and leaky vessels with an erratic blood flow, impeding effective immune cell trafficking into tumors. Such an abnormal vasculature results in areas of the tumor not receiving adequate oxygen, leading to tumor hypoxia that has separate downstream immunosuppressive effects. From a therapeutic standpoint, anti-angiogenic drugs have been in the market for nearly 20 years and have been shown to enhance tumor blood flow by normalizing the tumor vasculature and mitigating the downstream immunosuppressive effects of neoplastic angiogenesis. In recent years, several clinical trials have evaluated the efficacy of combining anti-angiogenic agents with immune checkpoint inhibitors (ICIs), and these combination regimens are now approved for the treatment of lethal cancers such as renal cell carcinoma (RCC) and hepatocellular carcinoma (HCC).

Tumor coagulation, broadly known as cancer-associated thrombosis (CAT), manifested in the form of venous thromboembolisms (VTEs) is a frequent complication in cancer patients. Components within the TME involved in hemostasis, collectively termed the tumor coagulome, have recently been shown to reshape the TME, thereby modulating immunotherapy response. These findings have paved the way for studies aiming to enhance immunotherapy responses by administering concomitant anticoagulation.

In this review, we aim to provide a comprehensive analysis of these processes, shedding light on their roles in fostering an immunosuppressive TME and current challenges regarding their potential as therapeutic targets in clinical settings.

2 Tumor angiogenesis

2.1 The angiogenic shift in tumors

Tumors initially exist in an avascular stage (i.e., without blood vessels), which limits their growth and metastatic potential. The "angiogenic shift" is pivotal for tumor survival, marking their transition from an avascular state to a vascularized one. This shift involves significant adaptations in the TME to create a pro-angiogenic environment (1).

One prominent metabolic alteration observed in cancer cells is the upregulation of glycolysis even under well-oxygenated conditions, known as "aerobic glycolysis" or the "Warburg effect" (2). Hypoxia-inducible factors (HIFs), which are transcription factors activated in response to low oxygen levels, play a central role in this metabolic rewiring of cancer cells by inducing a state of "pseudohypoxia", redirecting cellular metabolism towards glycolysis (3, 4). This metabolic shift leads to the accumulation of lactate and tumor acidosis (5), exacerbated by poor tumor perfusion and the high metabolic demands of rapidly dividing cancer cells, which promote hypoxia and anaerobic glycolysis (6). In the hypoxic and acidotic TME, HIF-mediated gene expression changes enable tumor cells to survive in an otherwise inhospitable milieu (7).

HIFs are heterodimeric, composed of an alpha subunit (HIF-α) and a beta-subunit (HIF-β) (8). Activation of HIF-1α triggers the upregulation of pro-angiogenic mediators, including vascular endothelial growth factors (VEGF), platelet-derived growth factors (PDGF), and fibroblast growth factors (FGF) (9). Among these, VEGF plays a particularly crucial role in tumor angiogenesis (10–12).

2.2 Dysfunctional vessels in neoplastic angiogenesis

However, tumor angiogenesis often results in the formation of tortuous and leaky blood vessels with an erratic blood flow, leading to regions with poor blood flow and inadequate oxygenation (13). Tumor endothelial cells harbor numerous cytogenetic abnormalities, rendering them molecularly and morphologically unstable (14). VEGF signaling disrupts gap junctions between endothelial cells, increasing vascular permeability and interstitial hydrostatic pressure (15, 16). Furthermore, the detachment of pericytes from endothelial cells promotes vessel fragility and intra-tumoral hemorrhage (17–19). Additionally, the proteolytic degradation of the vascular basement membrane facilitates tumor cell intravasation and metastasis (20).

3 Angiogenesis reprograms the tumor microenvironment towards immunosuppression

In this section, we will delve into the multifaceted immunosuppressive effects of neoplastic angiogenesis on the TME, focusing on the direct effects of VEGF, tumor hypoxia, and acidosis.

3.1 Direct effects of angiogenic factors

VEGF downregulates the expression of leukocyte adhesion molecules such as ICAM-1 and VCAM-1 on endothelial cells, thereby inhibiting the infiltration of CD8$^+$ T-cells into tumors (21–23). Strategies aimed at vascular normalization, such as anti-VEGF medications or p21-activated kinase-4 (PAK4) inhibition, can restore the expression of adhesion molecules and enhance CD8$^+$ T-cell infiltration (24, 25). Endothelial cells express PD-L1 and Fas ligand, which suppress CD8$^+$ T-cell effector functions and promote T$_{regs}$-mediated immunosuppression (26, 27). Anlotinib, a VEGF receptor blocker, has been shown to downregulate endothelial PD-L1 and increase the ratio of CD8$^+$ T-cell infiltration to T$_{regs}$ (27). Additionally, VEGF promotes T-cell exhaustion, impairs dendritic cell (DC) maturation and function, recruits immunosuppressive cells like VEGR$^+$ T$_{regs}$, MDSCs, and pro-tumor M2 tumor-associated macrophages (TAMs), and contributes to an hypoxic and acidotic environment through the generation of dysfunctional vasculature (28–30).
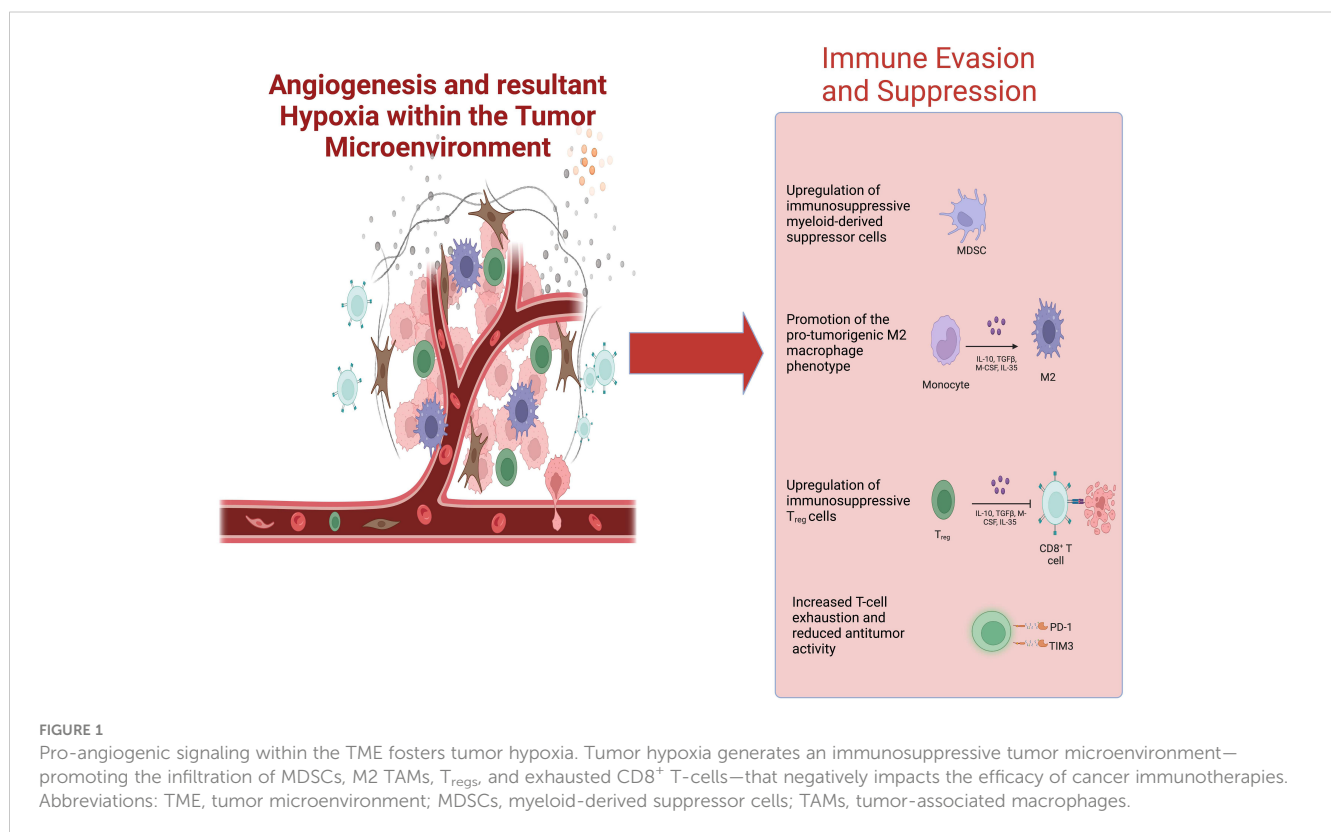
3.2 Tumor hypoxia

Hypoxia skews cells of the TME towards immunosuppressive phenotypes (Figure 1). Hypoxic regions within tumors serve as niches where immunosuppressive cells, such as myeloid-derived suppressor cells (MDSCs), M2 TAMs, exhausted CD8^{+} T-cells, and T_{regs}, preferentially accumulate (31). Pharmacologically inhibiting HIF-1/2 by 32-134D has been shown to downregulate genes involved in angiogenesis, glycolysis, and immune evasion. It also decreases the number of pro-tumorigenic M2 TAMs and MDSCs while increasing the infiltration of anti-tumor CD8^{+} cytotoxic T-cells and NK cells (32). Combining 32-134D with anti-PD-1 immune checkpoint inhibitors (ICIs) enhances therapy response (32). Additionally, inhibiting HIF-1α with echinomycin decreases PD-L1 expression on tumor cells, TAMs, and MDSCs when combined with anti-CTLA4 ICI therapy (33).Importantly, echinomycin augmented PD-L1 expression in normal tissues, promoting tolerance and protecting against immune-related adverse effects of ICIs (33).

TAMs play crucial roles in tumor growth, survival, and therapy resistance, which are directly correlated with HIF-1α- expression in these cells (34–36). Hypoxia upregulates the triggering receptor on myeloid cells-1 (TREM-1) receptor on TAMs, which skews naïve T-cells towards T_{regs} (37). Additionally, hypoxia induces an immunosuppressive M2 phenotype in TAMs by upregulating HIF-2α (38–40). If HIF-1α and HIF-2α are upregulated simultaneously in TAMs, whether one isoform predominates over the other, and the temporal dynamics based on type of hypoxia (intermittent vs continuous) or tumor stage remain unclear (41).

Tumor-associated neutrophils (TANs) have both pro-tumor and anti-tumor roles in cancer (42). Hypoxia prolongs neutrophil lifespan, enhances their degranulation function (43, 44), but attenuates the respiratory burst (45). Tumor hypoxia induces TAN recruitment through IL-8 and skews their phenotypes towards PMN-MDSCs, which suppress anti-tumor T-cell responses and promote tumor proliferation through neutrophil elastase (NE) (46–48). Reversing this phenotype by hyperoxia enhances anti-tumor immunity and tumor cell apoptosis (48).

VEGF impairs the differentiation of immature DCs into effective antigen-presenting mature DCs (49–51). Hypoxic regions of hepatocellular carcinoma harbor type-2 conventional dendritic cells (cDC2s) and immunosuppressive plasmacytoid dendritic cells, linked to the T_{regs} accumulation and CD8^{+} T-cell suppression (31, 52). T_{regs}, in turn, can downregulate surface HLA-DR expression on cDC2s, impairing their antigen-presenting function (53). Contrarily, hypoxia activates various anti-tumor functions in DCs such as pro-inflammatory cytokine secretion (54). cDC2s have also been shown to modulate tumor evasion from CD8^{+} T-cell cytotoxicity (55). Such data indicate that all-or-none approaches targeting dendritic cells may be unsuccessful and/or exert unwanted pro-tumorigenic side effects, highlighting the need for elucidating the extrinsic (environmental) and intrinsic regulators of DC plasticity (56).

FIGURE 1
Pro-angiogenic signaling within the TME fosters tumor hypoxia. Tumor hypoxia generates an immunosuppressive tumor microenvironment—promoting the infiltration of MDSCs, M2 TAMs, T_{regs}, and exhausted CD8^{+} T-cells—that negatively impacts the efficacy of cancer immunotherapies. Abbreviations: TME, tumor microenvironment; MDSCs, myeloid-derived suppressor cells; TAMs, tumor-associated macrophages.

The upregulation of HIF-1α in naïve T-cells favors T_{reg} differentiation (57) and indirectly augments T_{reg} recruitment through CCL28 and TGF-β production in the TME (58, 59). Persistent hypoxia and antigen stimulation drive CD8$^+$ T-cell exhaustion (60), with HIF-1α driving AMP production, which contributes to T-cell suppression and therapy resistance (60–62). However, conflicting data that observe HIF-1α and HIF-2α also support CD8$^+$ T-cell proliferation and anti-tumor activity (36, 63, 64). Different types of tumor hypoxia (continuous vs. intermittent) may have varying effects on HIF-1α and HIF-2α (65, 66). Future research is required to elucidate the major hypoxia-related signaling pathways driving T-cells into anti-tumor or immunosuppressive phenotypes, along with their associated cell surface receptors and extrinsic regulators in the TME.

Cancer-associated fibroblasts (CAFs) play diverse roles in tumor progression (see (67) for detailed review). Increased CAFs presence in esophageal cancer correlates with decreased CD8$^+$ cytotoxic T-cells and increased T_{regs} infiltration (68). CAFs secrete IL-6, which activates HIF-1α in tumor cells, augmenting their glucose uptake and glycolysis (69), which may stabilize T_{regs}. IL-6 also induces the differentiation of fibroblasts into CAFs and TAMs to adopt an M2 polarization (69). An anti-IL-6 antibody slows tumor growth by increasing CD8$^+$ T-cell infiltration and decreasing T_{regs} presence (68).

3.3 Tumor *acidosis*

Mechanistically, tumor acidosis induces an M2 phenotype on TAMs (70, 71), which release HMGB1 and arginase-1 that activate signaling pathways enhancing aggressive cancer phenotypes (72, 73). Tumor-derived lactic acid suppresses antigen-presenting functions of DCs to blunt T-cell activation (74), as well as directly attenuating CD8$^+$ T and NK cell effector functions (75–79). Increasing extracellular pH by administering bicarbonate slows tumor growth and increases the infiltration of anti-tumor CD8$^+$ T-cell s (80). Additionally, combining bicarbonate with anti-CTLA4 or anti-PD-L1 ICI improves therapy response (80).

The rapid consumption of glucose by tumor cells due to the Warburg effect limits glucose availability for CD4$^+$ and CD8$^+$ effector T-cells, favoring their suppression (81–87). This low glucose environment favors the functional stabilization of T_{regs} (88–91), as they can preferentially utilize fatty acids and lactate as metabolic substrates (92, 93). T_{regs} largely avoid glycolysis because high glucose concentrations in the TME and cellular uptake impair T_{reg} function (93). The T_{reg} avoidance of glucose is controlled by surface CTLA-4 and PD-1 (94, 95). However, chronic exposure to lactate, when studied apart from its acidic TME, increases the stemness of CD8$^+$ T-cells and augments anti-tumor CD8$^+$ immunity to suppress tumor growth (96, 97). Therefore, current research indicates that there exists a combinatorial influence of TCR signaling, hypoxia, low glucose, tumor acidosis, and lactate on the T-cell phenotype in the TME, with their combined effects potentially overshadowing lactate's anti-tumor effects.

4 The tumor coagulome and thrombosis

Venous thromboembolic events (VTEs) are a common complication in several cancer types and remain a leading cause mortality in these patients (98–100). This discussion focuses on key aspects of the tumor coagulome as a mediator of CAT.

4.1 Coagulation *cascade*

The extrinsic coagulation pathway is initiation by tissue factor (TF), a transmembrane protein usually expressed by perivascular cells that is normally shielded from circulation, only being exposed after blood vessel damage. Once exposed, TF binds and activates factor VII, leading to the cleavage and activation of factor X into factor Xa (fXa), ultimately generating thrombin.

TF is the most extensively studied pro-coagulant in cancer-associated VTEs (101–105). TF levels are elevated in cancer patients due to TF upregulation on the surface of cancer cells, promoting extravascular thrombosis. Tumor cells and non-tumor immune cells in the TME also secrete extracellular vesicles (EVs) expressing TF (TF$^+$-EVs), promoting intravascular thrombosis (106). TF-expressing tumor cells can also enter the circulation and induce CAT (107). TF upregulation in cancer is influenced by multiple factors, including genetic mutations, growth factors, inflammatory cytokines, and hypoxia (108). Beyond thrombosis, TF promotes cancer cell survival, proliferation, invasion, and metastasis (108). A recent study developed TF-chimeric antigen receptor natural killer (NK) cells that effectively target TF-overexpressing triple-negative breast cancer cells to decrease tumor growth without significant systemic adverse effects (109).

4.2 Platelets

Many cancer patients also display elevated serum levels of platelet-derived EVs and p-selectin in their serum, indicating systemic platelet activation. Tumor cell-induced platelet activation (TCIPA) involves multiple mechanisms (110–112). For example, the glycoprotein podoplanin (PDPN) expressed on the surface of many tumor cells binds the C-type lectin receptor-2 (CLEC-2) on the platelets leading to platelet aggregation and thrombus formation (113–115). Similarly, TF can directly activate platelets or facilitate tumor cell-platelet interactions (116, 117). EVs released by triple-negative breast cancer cells contain uPAR and PDGFRβ that can induce platelet aggregation (118). Platelet activation leads to degranulation and release of ADP and thromboxane A2, which can function in an autocrine manner to amplify platelet activation and aggregation (119). Other than CAT, platelets promote various other aspects of tumor progression, including sustained proliferative signaling, angiogenesis, epithelial-to-mesenchymal transition, immune evasion, and metastasis (120, 121).

4.3 Neutrophil extracellular traps

Neutrophils are known to produce neutrophil extracellular traps (NETs), which have been implicated in various pro-thrombotic diseases, including CAT (122). In cancer patients, NET markers such as extracellular DNA, myeloperoxidase (MPO), citrullinated histones, and NE are elevated and correlate positively with the incidence of VTEs (123–126). Moreover, NET components like DNA and cit-H3 are richly found in cancer-associated thrombi in mice and humans (125, 127–131). Circulating neutrophils retrieved from the blood of tumor-bearing mice or cancer patients are more prone to form NETs ex vivo (132).

Cancer cells create a systemic environment that promotes NETosis (132). Factors such as GM-CSF, IL-1β, CXCR1/CXCR2 agonists, cathepsin C, complement 5a, and EVs have been shown to promote NET formation in animal tumor models (133–139). Alternatively, tumors can stimulate NETosis through TCIPA, as activated platelets directly interact with neutrophils via p-selectin and high-mobility group box-1 (HMGB1), leading to NET production (140–142). Studies have shown that exogenous administration of Dnase-1, an enzyme that degrades NETs, or genetic deletion of peptidyl arginine deaminase-4 (PAD4) a protein essential for NETosis, significantly reduce thrombotic events and organ damage in mouse models of cancer (127, 128, 143).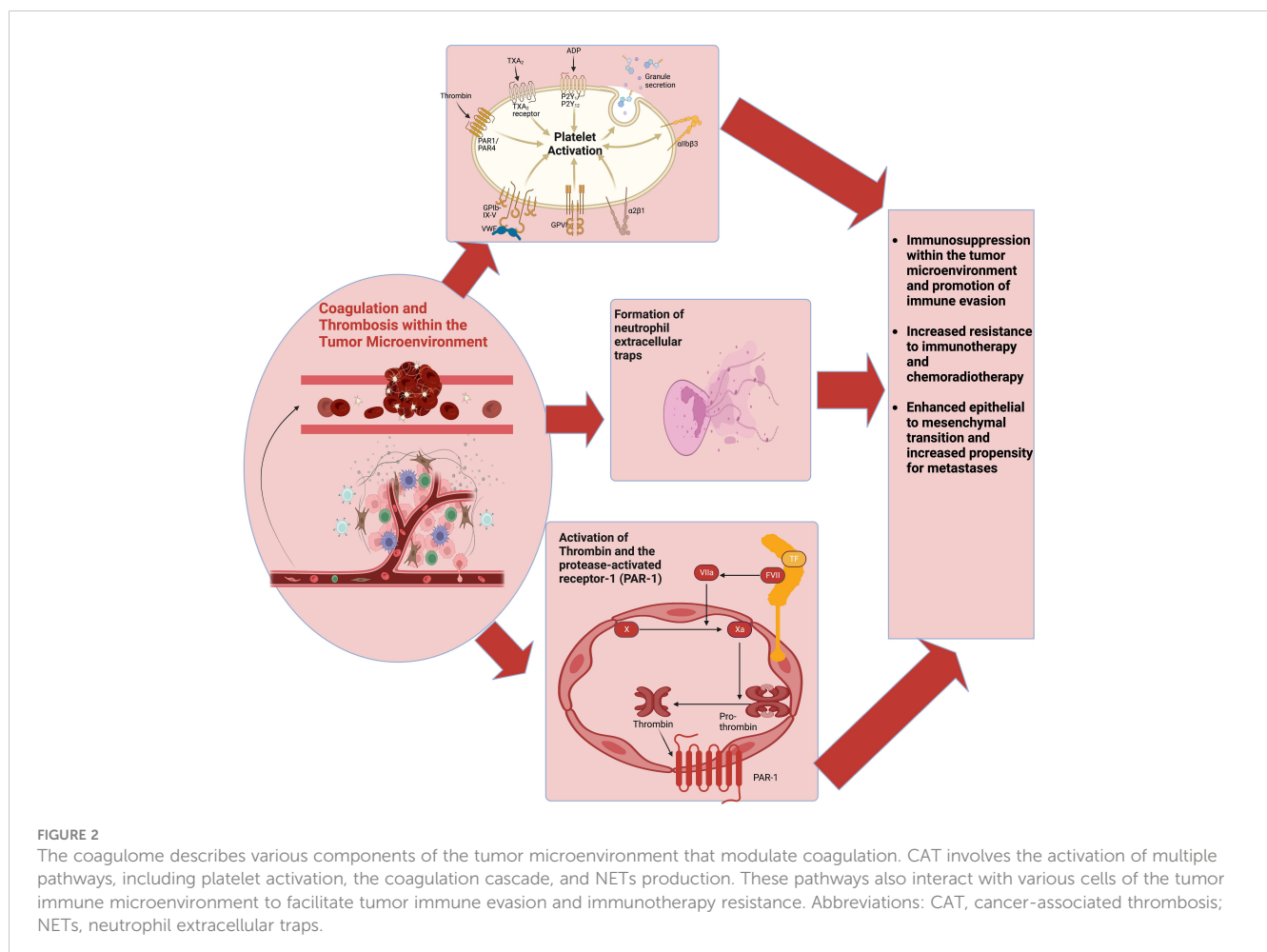 However, it is important to consider the risk of adverse events, such as infections, when using Dnase-1 therapy in immunosuppressed cancer patients (144).

5 The coagulome reprograms the tumor microenvironment towards immunosuppression

Preclinical studies have demonstrated that the tumor coagulome fosters an immunosuppressive TME (Figure 2).

5.1 Coagulation cascade

Within the TME, TAM-derived fXa promotes the expansion of MDSCs while inhibiting CD8$^+$ and NK cell functions, resulting in immunosuppression (145). Genetic depletion or pharmacologic inhibition of fXa using rivaroxaban enhances the anti-tumor response and improves the efficacy of ICIs in murine models of colorectal cancer and melanoma (145). Similarly, high levels of plasminogen activator inhibitor-1 (PAI-1) in murine lung carcinoma models increases increase the recruitment of TAMs and polarization towards an M2 phenotype whereas reducing PAI-1 levels decreases M2 TAMs and increases M1 TAMs (146).

FIGURE 2
The coagulome describes various components of the tumor microenvironment that modulate coagulation. CAT involves the activation of multiple pathways, including platelet activation, the coagulation cascade, and NETs production. These pathways also interact with various cells of the tumor immune microenvironment to facilitate tumor immune evasion and immunotherapy resistance. Abbreviations: CAT, cancer-associated thrombosis; NETs, neutrophil extracellular traps.

Thrombin and protease-activated receptor 1(PAR1) signaling has been shown to promote tumor growth, metastasis, and immune evasion in murine pancreatic adenocarcinoma (PDAC) models (147). Mechanistically, PAR1 signaling in PDAC cells downregulates their antigen-processing machinery, resulting in these cells being less efficiently recognized by anti-tumor immune responses, and upregulates immunoregulatory proteins like GM-CSF, involved in recruiting immunosuppressive M2 TAMs and MDSCs. Furthermore, PAR1-dependent tumor growth is mediated through the upregulation of cyclooxygenase-2 (COX-2) and GM-CSF, indicating that these mediators could be potential targets for therapies inhibiting PDAC growth (148).

However, thrombin has also been shown to induce effector T-cell activation, proliferation, and cytokine production in various non-neoplastic contexts (149–151). Furthermore, several mediators of the coagulation cascade besides thrombin also activate PARs, indicating that the outcomes of PAR signaling is multifactorial, depending on the specific ligands, PAR subtypes, and tumor context (152). For example, thrombin-PAR1 signaling does not promote tumor progression in transgenic adenocarcinoma mouse prostate (TRAMP) models, while protein C binding to PAR1 promotes tumor cell apoptosis and slows the progression of prostate and intestinal cancers (153). Based on these discrepancies, we advocate for caution when considering long-term thrombin or PAR1 inhibition as an anti-cancer strategy.

A recent study found that reducing prothrombin levels in mice before treatment with ICIs led to decreased CD8$^+$ T-cell infiltration and compromised anti-tumor immunity, resulting in a complete loss of therapeutic efficacy (154). Inoculating human CD8$^+$ T-cells with thrombin increased their activation, even cells lacking PAR1 and PAR2, indicating the presence of independent mechanisms for thrombin-induced CD8$^+$ T-cell activation. However, the study also revealed that PAR2 signaling suppresses T-cell activation and attenuates thrombin/PAR1-dependent activation of anti-tumor CD8$^+$ T-cells (154). Therefore, identifying the specific ligand-PAR receptor interactions that mediate beneficial or pathologic effects in different tumor types can aid the development of targeted therapies that avoid unintended pro-tumorigenic effects.

5.2 Platelets

Platelets play a significant role in promoting immunosuppressive T-cell phenotypes and impairing the cytotoxic function of NK cells (155). Platelets also promote the development of tumors such as colitis-associated cancer (CAC) by inducing polarization of myeloid cells to MDSCs and reducing the accumulation of CD8$^+$ T-cells in the colonic mucosa (156). Inhibiting platelet activation by clopidogrel decreases MDSCs and increases CD8$^+$ T-cell infiltration, thereby delaying CAC development (156).

In the circulation, platelets can surround tumor cells, forming tumor microthrombi that are protected from immune surveillance and anoikis (i.e., detachment-triggered apoptosis) (157–160). In the TME, thrombin cleaves glycoprotein A repetitions predominant (GARP) on the platelet surface, liberating surface-bound TGF-ß (161), which activates CAFs to lay down ECM that restricts CD8$^+$

T-cells to the periphery of the tumor (162). Platelet-derived TGF-ß also converts effector T-cells to T$_{regs}$ (163, 164). Preventing GARP cleavage, either by inhibiting thrombin or genetically deleting the GARP cleavage site through CRISPR/Cas9 technology, enhances CD8$^+$ T cell activation and survival (161).

Tumor-associated platelets also upregulate the surface molecule TLT-1, which promotes CD8$^+$ T-cell exhaustion by upregulating checkpoints PD-1 and TIM-3 (165). Furthermore, tumor cells can directly contact platelets in the TME and transfer PD-L1 to them (166, 167). Conversely, platelets can upregulate PD-L1 on ovarian cancer cells through physical contact and indirectly through TGF-β (168). High platelet PD-L1 expression is associated with an immunosuppressive TME by depleting effector T-cells, which lower OS and progression-free survival (PFS) in non-small cell lung cancer patients (NSCLC) (166). Serum levels of platelet PD-L1 might serve as a more accurate indicator of tumor PD-L1 burden and the likelihood of response to ICI therapy compared to the standard immunohistochemical-based quantification of PD-L1 on biopsy specimens (166). Interestingly, PD-L1-expressing platelets may partially explain the efficacy of ICIs even in PD-L1-negative tumors (169). Additionally, PD-L1 was found to activate platelets and amplify thrombosis (170).

5.3 Neutrophil extracellular *traps*

Studies have shown that a higher burden of TANs and NETs correlates with reduced T-cell infiltration in the TME, indicating their immunosuppressive effects (171–176). NETs induce exhausted states in CD8$^+$ T-cells in murine models (177), and PD-1 within NETs leads to the loss of T-cells by apoptosis (177, 178). Furthermore, NETs have been shown to promote the differentiation of helper T-cells into T$_{regs}$ in murine models of non-alcoholic steatohepatitis (NASH), facilitating the development of NASH-associated HCC (179). Reducing NETs by genetic PAD4-KO or Dnase-1 treatment in HCC mice reduces the activity of T$_{regs}$ and enhances anti-tumor NK cell responses (179).

Aside from their direct immunosuppressive effects, NETs also constitute physical barriers that restrict the access of CD8$^+$ T-cells and NK cell to tumors (139). In murine models of PDAC, neutrophil recruitment and NETosis induced by IL-17 protect tumor cells against cytotoxic CD8$^+$ T-cells, leading to reduced efficacy of immunotherapy (180). Moreover, in radiotherapy treatment for treating bladder cancer, the release of DAMPs such as HMGB1 within dead cell debris triggers NET production through binding TLR4 on the surface of TANs (181). These NETs then create a physical barrier between the tumor and infiltrating CD8$^+$ T-cells, reducing anti-tumor immunity (181).

Interestingly, NETs induced by intravesical BCG therapy contribute to anti-tumor immunity by recruiting anti-tumor T-cells and TAMs and enhance therapeutic efficacy of this treatment (182, 183). Tillack et al. showed that NETs primed T-cells by reducing their activation threshold, increasing T-cell responses against specific antigens or even suboptimal stimuli (184). These findings highlight the dual roles of NETs, which can either hinder or augment effective T-cell responses, which may depend on different

NET-inducing stimuli, different NET compositions, or perhaps the influence of other, yet undetermined factors in the TME that drive NETs to protective or pathologic functions depending on tumor stage and extent. Hence, exploring the mechanistic aspects of NETs beyond their mere production and physical presence in tumors is crucial. The extrinsic/environmental cues that regulate pro-tumorigenic and anti-tumor phenotypes of NETs remain unknown. Uncovering the specific pathological components of NETs will inform targeted therapeutic strategies mitigating their pathological functions while preserving their beneficial aspects.

6 Reprogramming the tumor microenvironment to enhance immunotherapy

6.1 Targeting angiogenesis to enhance immunotherapy

Anti-angiogenic therapies were originally developed to inhibit vascular formation, but they inadvertently caused excessive vessel pruning, tumor hypoxia, and decreased anti-tumor immune responses (185, 186). To overcome these limitations, Rakesh Jain proposed the concept of vascular normalization, which involves administering low-dose anti-angiogenic therapy to equilibrate angiogenic signaling within the TME (187). This approach would aim to achieve a state of vascular normalization, characterized by improved tumor blood flow and decreased hypoxia. Numerous studies since have reported findings consistent with the theory of vascular normalization, encapsulated within the idea of a 'normalization window' (188). The normalization window represents a brief period of time after administering anti-angiogenic therapy where the tumor vasculature is structurally normalized, during which administered therapeutics can achieve good infiltration into tumor sites (189).

The effectiveness of immunotherapies relies on the infiltration of T-cells into tumor sites in sufficient numbers (190). Tumors that are inflamed and have good immune cell infiltration, referred to as "hot" tumors, exhibit favorable responses to immunotherapies compared to "immune-desert" or "cold" tumors that lack inflammation (190, 191). A normal tumor vasculature, which is not leaky and exhibits a proper pattern of blood flow, is a prerequisite for effective T-cell trafficking into tumor sites.

Thus, vascular normalization with anti-angiogenic drugs can be an effective strategy to enhance therapy response by increasing T-cell infiltration into tumors. Additionally, anti-VEGF medications can counteract the direct immunosuppressive effects of VEGF on various cell types, as discussed earlier (29). Notably, ICIs and anti-angiogenic drugs can synergize in normalizing the tumor vasculature (192), as ICIs can independently improve vessel perfusion, evidenced by improved vessel morphology, increased pericyte coverage, and elevated vessel normalization markers (α-SMA and NG-2) in the TME (192, 193). Therefore, the possibility of combining immunotherapies and anti-angiogenic drugs has garnered significant research interest in recent years, aiming to enhance treatment responses and survival outcomes for cancer patients.

Preclinical studies have demonstrated the potential benefits of combining anti-angiogenic therapies with ICIs, showing increased T-cell infiltration, enhanced local anti-tumor immunity, and improved survival in murine models of different cancer types (194–197). Clinical trials evaluating the efficacy of ICI + anti-angiogenic regimens have shown superior outcomes in various cancers, based on which the FDA has approved the use of these combinations for the treatment of renal cell carcinoma, HCC, NSCLC, and endometrial carcinoma (198–201). We limited our discussion here because other extensive reviews have already been published on this topic (29, 196, 202).

However, combining ICIs and anti-angiogenic therapy has not been effective in highly desmoplastic tumors, such as cholangiocarcinoma, glioblastoma multiforme, and pancreatic adenocarcinoma (203). In these tumors, the dense stroma compresses tumor blood vessels, impeding perfusion and reducing the local delivery of these medications (204). The approach of "stromal normalization" aims to overcome this resistance by reducing stromal density. Angiotensin-converting enzyme (ACE) inhibitors (ACEi) and angiotensin-receptor blockers (ARBs) can inhibit CAFs and reduce ECM production, thus decreasing stromal density and contributing to stromal normalization (204, 205). Clinical studies have shown improved outcomes with anti-VEGF and adjuvant renin-angiotensin system (RAS) inhibitors across tumor types, including glioblastoma, renal cell carcinoma, hepatocellular carcinoma, and metastatic colorectal carcinoma (206–210). However, pancreatic ductal adenocarcinoma remains highly resistant to stromal normalization approaches (211, 212). Recently, a phase-II clinical trial found that adding losartan to chemoradiation therapy for locally advanced unresectable pancreatic cancer resulted in downstaging the tumor and a complete resection rate of 61% (213). This was recently shown to be due to losartan enhancing CD8$^+$ T-cell infiltration and decreasing T$_{regs}$ in the TME and reducing immunosuppressive FoxP3$^+$ cancer cells, thus enhancing anti-tumor immunity and tumor cell killing (214).

6.2 Targeting CAT to enhance immunotherapy

Another potential synergistic approach to reprogram the TME is combining ICIs with anticoagulants. Inhibiting TF has been shown to reduce tumor survival across many *in vitro* studies (215–217). Dabigatran, a thrombin inhibitor, can restrict tumor growth and modify the TME in favor of anti-tumor immunity (218). Rivaroxaban and low-molecular-weight heparin can limit tumor metastasis in mice fibrosarcoma models (145), with rivaroxaban additionally amplifying cytotoxic T-cell responses and stimulating antigen-presenting cells by modulating the FXa-PAR2 axis (145).

The higher risk of thrombotic events associated with ICIs is another compelling reason for combining anticoagulants with ICIs, albeit the mechanisms behind this association remain unclear A

retrospective study of 2854 patients receiving ICI found a four-fold increase in the risk of VTE after starting ICI therapy (219). Similarly, a large pharmacovigilance study identified a strong association between the use of ICI and thrombotic complications (220). Combining anticoagulants with immunotherapies could potentially mitigate this risk.

However, clinical evidence for the synergistic effects of ICIs and anticoagulants is conflicting. For instance, Nichetti et al. investigated the impact of the synergistic combination of anti-PD-L1 therapy and anti-platelet agents amongst NSCLC patients, concluding that this synergetic combination did not significantly improve PFS or OS in the multivariate analysis (221). A large study of 728 patients with advanced malignancies found no difference in OS and disease-free survival (DFS) when synergistically combining ICIs and various anticoagulants (including apixaban/rivaroxaban, dabigatran, heparin, and warfarin) (222). However, a recent retrospective study on 280 patients with advanced melanoma demonstrated that treatment with fXa inhibitors enhances the effects of ICIs and confers statistically significant superior PFS and OS (223).

In summary, while preclinical evidence suggests a crucial role for coagulation in fostering an immunosuppressive TME, clinical studies investigating the efficacy of combining anticoagulants and ICIs have yielded varying results. It is essential to recognize that the coagulome of malignant tumors differs significantly across tumor types (224). Interestingly, tumors with highly pro-coagulant properties, such as glioblastoma multiforme and pancreatic adenocarcinomas, are often resistant to ICIs (225). The complex interplay between the TME and coagulome needs further research to better appreciate the impact of targeting the coagulome on TME normalization. Studies into the therapeutically relevant variations in the coagulome among different tumor types are needed. Rigorous clinical trials encompassing different tumor subtypes are required to evaluate the impact of combining anticoagulants and ICIs on tumor progression and patient survival in order to substantiate the encouraging preclinical data.

7 Concluding remarks

Several factors need further exploration to improve the effectiveness of ICI + anti-angiogenic regimens, including the optimal dosing and duration of treatment for anti-angiogenic therapy across different tumor types, the underlying mechanisms driving therapeutic responses and resistance, the identification of predictive biomarkers enabling appropriate patient selection and effective therapy response monitoring, and optimizing drug delivery systems. An excellent recent review by Cao et al. covered in detail the applications of anti-angiogenic drugs in cancer and associated challenges (10). Despite the remarkable evolution of these drugs from bench to bedside, survival benefit compared to conventional

therapies remains incremental, hence the need for combinatorial approaches (10).

Despite tremendous basic science progress, clinical data on the efficacy of combinatorial approaches, particularly regarding anticoagulation and ICI therapy, are conflicting. The heterogeneity of the coagulome across various tumor types needs to be considered if anticoagulants and ICI combinations are to be furthered. Different components of the coagulome are related to different aspects of the tumor. For instance, levels of TF are closely related to the tumor type, whereas fibrinolysis is highly dependent on TME components (224). The spatial and temporal heterogeneity of the TME is also poorly understood, thereby contributing to discrepant findings attributing both pro-tumor and anti-tumor functions to various components in the TME. Such uncertainties confound translational efforts aimed at targeting these mediators, as there is a risk of inadvertently augmenting pro-tumorigenic processes. We find it likely that, given profound TME heterogeneity across tumor types, future studies will pave the way for a more personalized assessment of patient coagulation status, in line with the major trend of precision medicine in oncology practice.

Author contributions

AS conceptualized the manuscript. AS, MO, EA, AM, and EI prepared the initial draft and designed the figures. AS, KA, and AY reviewed the manuscript and prepared the final version. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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