The Role of Platelets in the Tumor Microenvironment

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Abstract

Platelets are small, circulating anuclear cells that have an important and well-defined role in hemostasis and wound healing. Known as the "band-aids of the blood," platelets rapidly activate, aggregate, and release a plethora of growth factors, cytokines, and other biological mediators at sites of vascular damage, thereby forming a clot. Compelling evidence has revealed that tumors can co-opt the normal functions of platelets to advance disease progression and metastasis. We now know that platelets are a key component of the tumor microenvironment and that they promote cancer progression in a myriad of ways. Results from in vitro and in vivo modeling have shown that platelets drive tumor cell invasion and epithelial-to-mesenchymal transition, promote angiogenesis, facilitate intravasation and extravasation of tumor cells, protect circulating tumor cells from shear forces and immune surveillance, and function as long-distance cargo carriers that transmit signals between primary tumors, metastases, and the bone marrow. Platelets have also been reported to have anti-cancer functions by supporting tumor blood vessel normalization and containing anti-angiogenic factors. Therefore, a key challenge to cancer research and treatment remains how to inhibit the pro-tumorigenic effects and/or promote the anti-tumor functions of platelets using conventional and new treatment regimes. In this chapter, we examine the current understanding of the role of platelets in cancer development and progression and explore platelet-targeted therapies as a novel and promising approach to cancer treatment.

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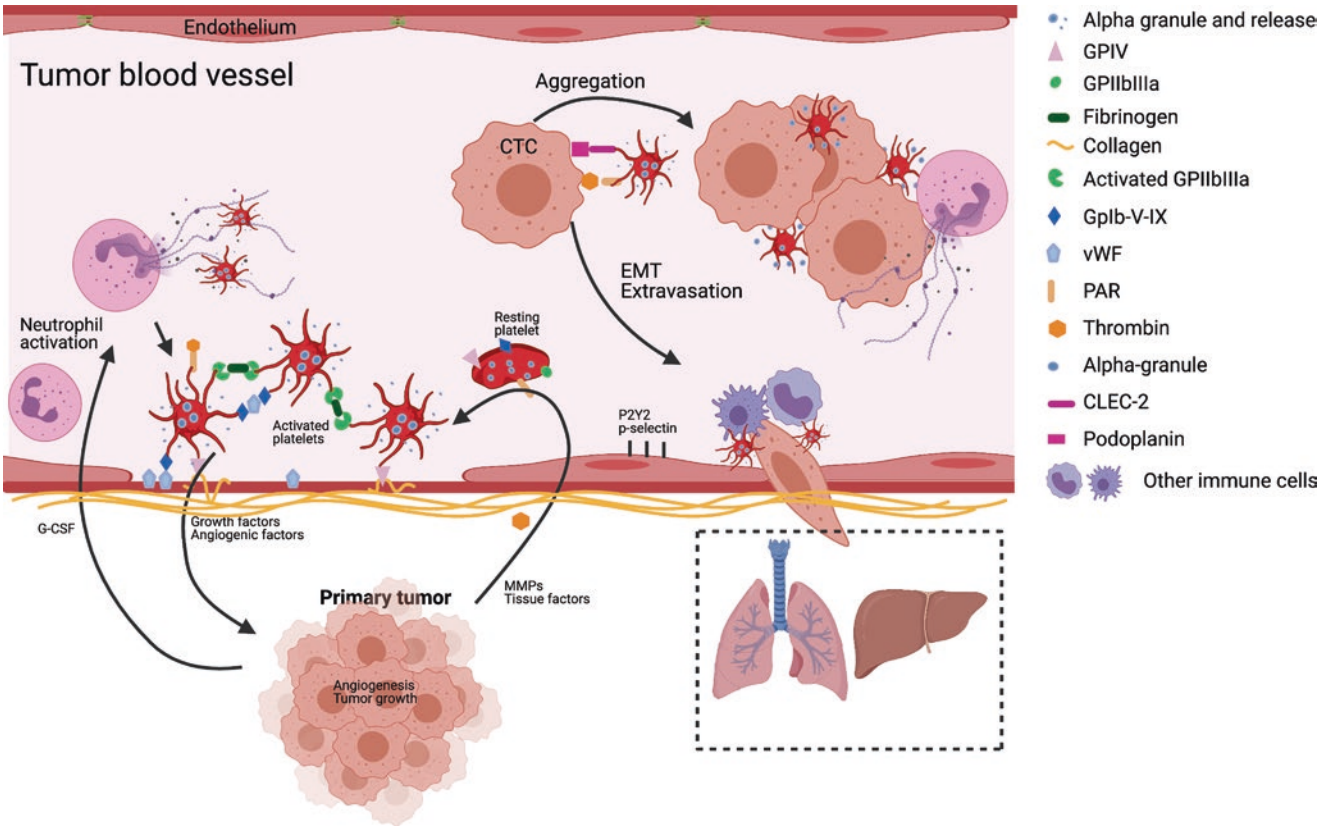

Mechanisms of platelet activation in cancer. Tumor endothelium is often damaged, or "leaky," leading to exposure of underlying collagens and extracellular matrix proteins, which engage glycoproteins on the cell surface of circulating platelets. Platelets activate, undergo a shape change, and release their granular contents. Fibrinogen bridges form between platelets to strengthen their aggregation. Tumors trigger platelet activation and aggregation through a variety of mechanisms, including directly releasing factors to activate platelets and releasing factors activating immune cells such as neutrophils to activate platelets. After tumor cells intravasate into the bloodstream, direct interactions between tumor cells and platelets via ligand/receptor pairing can also lead to platelet activation. Activated platelets can recruit immune cells to tumor clusters and induce tumor cells to undergo epithelial-to-mesenchymal transition, which can be important for tumor cell survival and extravasation. Adapted from "Blood Vessel (Straight, Light Background)," by BioRender.com (2021). Retrieved from https://app.biorender.com/biorender-templates

Take-Home Lessons

- Platelets play an important role in cancer, including the promotion of tumor angiogenesis, growth, invasion, and metastasis.
- Platelets interact with circulating tumor cells (CTCs) and protect CTCs from shear stress and immune surveillance.
- Platelets act as sponges to take up tumor-secreted factors and carry molecular signals to distant locations throughout the body.
- Anti-platelet therapies could have great potential in the treatment of cancer.

Platelets are best understood for their role in thrombosis and hemostasis. These tiny, anuclear circulating cells form clots at sites of vascular damage to initiate the wound healing process. However, we have also learned that platelets are a critical component of the tumor microenvironment (TME) and can profoundly affect tumor progression and metastasis. For example, platelets aid in disseminating tumor cells by protecting them from high shear forces and immune surveillance within the circulation. The resultant tumor cell-platelet aggregates facilitate embolization, promote the adhesion of tumor cells to the vascular endothelium, and release a variety of soluble factors that promote tumor growth and metastasis. Platelets are, by design, carriers of a myriad of cytokines and growth factors, many of which are known to affect disease

progression. Cytokines released from activated platelets not only impact the function of tumor cells but also affect other cells in the TME such as endothelial cells, fibroblasts, and immune cells [1–3]. In this chapter, we discuss what is known about the complex cross-talk that occurs between platelets, tumor cells, and other tissue cells in malignancy and highlight features of such communication that may be vulnerable to therapeutic intervention.

Platelet Function

Before exploring the role of platelets in cancer, it is beneficial to review normal physiological platelet function. Platelets are small (1–3 μm), discoid-shaped cell fragments that are released from progenitor cells called megakaryocytes in the bone marrow. Structurally, platelets are anuclear and contain three distinct types of granules: alpha-granules (the most abundant type), dense granules, and lysosomes [4]. Over 300 biologically active factors, including cytokines, adhesion molecules, and coagulation mediators are contained within alpha-granules, which can be selectively released upon platelet activation [5]. Although platelets do not have nuclei, they contain some cytosolic mRNA and translational machinery; hence, protein synthesis can occur to a limited extent [6]. The platelet surface is coated with glycoproteins, adhesion molecules, and signaling receptors, thus enabling them to interact with other cells and to become activated upon contact with agonists such as thrombin, collagen, ADP, thromboxane, and epinephrine [7].

Platelets are often thought of as the "band-aids of the blood" [8]; they prevent blood loss during injury by forming a clot at the site of vascular damage. Damage to the vascular wall causes exposure of subendothelial collagens and von Willebrand factor, which serve to attract circulating platelets by engaging their cell-surface glycoproteins, such as GPIbα, thereby leading to adhesion at the site of damage [9]. Local sources of collagen and thrombin at the wound site initiate platelet activation via GPVI and PAR receptors, respectively, causing platelets to undergo a drastic shape change and to release their granule contents [9]. GPIIbIIIa on the platelet surface is activated, causing fibrinogen binding and allowing for platelet aggregation and the formation of fibrinogen bridges that stabilize the clot [9]. Activated platelets release pro-coagulation factors and serve as a surface for clotting factors to assemble, further strengthening the platelet plug [7].

It is easy to imagine how activation of platelets at inappropriate times or locations could lead to adverse situations. Improper activation and aggregation can lead to the formation of blood clots while the release of growth factors and inflammatory cytokines from alpha-granules can promote atherosclerosis and tumor progression [10]. Indeed, pathological platelet function has been shown to occur in a variety of cancer types, and platelets are accepted as key players in a number of the processes underlying disease progression and metastasis.

Identifying a Role for Platelets in Cancer

A link between cancer and abnormal coagulation was first noted in the 1800s when Jean-Baptiste Bouillaud reported a case of deep vein thrombosis occurring in a cancer patient [11]. French physician Armand Trousseau is widely credited as the first person to definitively propose a link between cancer and hypercoagulability of the blood when he noted that patients with cancer were more likely to develop a blood clot than the general population and that blood clots could be predictive of an undiagnosed malignancy [12]. Platelets were specifically implicated in 1872 when a link between elevated platelet count and cancer was reported [13]. Levin and Conley published a detailed examination of thrombocytosis (elevated platelet count) and cancer in the 1960s, finding that thrombocytosis was present in 38% of patients with inoperable tumors [14]. Since then, thrombocytosis has been correlated with poor outcomes in a variety of solid tumor types including cancers of the breast, lung, ovary, colon, kidney, and brain [15–20]. Thrombocytosis is also associated with an increased risk of venous thromboembolism (VTE) in many cancer patients [21]. Cancer patients have a four- to sevenfold greater risk of developing a pulmonary embolism or a deep vein thrombosis than healthy individuals [22].

Is elevated platelet count merely coincidental or do platelets play a direct, active role in cancer progression? To answer that question, Gasic et al. depleted platelets from mice before injecting tumor cells in an experimental murine model of metastasis [23]. Depletion with neuraminidase or anti-platelet serum decreased metastasis, while the infusion of platelet-rich plasma reversed that effect, suggesting that platelets play an active role in cancer progression. Subsequent mouse studies have revealed that disruption of platelet function also reduces metastasis formation; a greater than 50% reduction in metastasis was seen in both GPVI and P-selectin knockout mice [24–27]. Interestingly, metastasis was reduced by 80% in a mouse model of gray platelet syndrome, a disorder in which platelets lack alpha-granules [28] These animal studies verified that platelet activation and alpha-granule release were involved in metastasis.

Taken together, the early observations in cancer patients and experimental mouse models suggested that platelets facilitate metastatic spread. Nevertheless, questions remain about how, mechanistically, platelets influence the metastatic process. Numerous research efforts have focused on answering that question and in this chapter, we highlight research demonstrating the role of platelets at every stage of cancer

progression and the metastatic cascade, from the primary tumor site to the tumor cell's journey through the circulation and finally during extravasation and metastatic seeding and growth [29].

Tumor Cell-Induced Platelet Activation and Aggregation

Normally, platelets are only activated at sites of vascular injury and remain inert (resting state) while in transient contact with healthy cells and tissues. However, tumors and their microenvironment are far from normal and have been described as "wounds that never heal" due to persistent inflammation and tissue remodeling [30]. The TME of most solid tumors is conducive to inappropriate platelet activation and thus co-opts platelet function for the tumor's benefit. Activated platelets have been observed within primary tumor tissue in pre-clinical breast cancer models and patient-derived xenografts [31]. Angiogenic vessels associated with tumors are often abnormal and leaky, with gaps between endothelial cells and areas of exposed collagen, allowing platelets entrance and access to tumors [31, 32]. Tumor cells can activate platelets by producing the potent activator, thrombin, and elevated thrombin levels have been observed within the TME of several types of cancer [33–35]. Tumor-derived cathepsin B, matrix metalloproteinase (MMP)-2 and MMP-14 have all been shown to activate platelets and tissue factor (TF) can also be aberrantly released from tumor cells, indirectly activating platelets through the initiation of the coagulation cascade [36, 37]. Direct contact between platelets and tumor cells can also lead to activation; for instance, tumor cell podoplanin or mucins can interact with and activate platelet CLEC-2 and P-selectin, respectively [38–41].

Interactions between platelets and tumor cells either at the primary tumor site or within the circulation often lead to a phenomenon called tumor cell-induced platelet aggregation (TCIPA). TCIPA occurs when tumor cells activate platelets, leading to activation and release of platelet-derived ADP and generation of thromboxane to further trigger aggregation [42–44]. In this process, fibrin is generated, thereby cross-linking tumor cells and platelets, while glycoproteins such as GPIIbIIIa strengthen the platelet–tumor cell aggregates through fibrinogen bridges [45]. Aggregates composed of platelets and tumor cells have been observed within the circulation since the 1970s [46, 47], and tumor cell lines of breast, colon, prostate, lung, and pancreatic origin, to list a few, have been shown to aggregate platelets in vitro [48–51]. These aggregates can be observed in the blood of patients and are implicated in tumor cell immune evasion and embolization [52].

In addition to activation by direct platelet–tumor cell interaction, tumor cells can induce long-range activation of distant platelets. For instance, tumor cells release TF-coated microparticles that can travel through the circulation and may be involved in cancer-associated VTE [53, 54]. Another mechanism of indirect platelet activation can occur when tumor cells secrete granulocyte colony-stimulating factor (G-CSF), causing circulating neutrophils to release platelet-activating neutrophil extracellular traps (NETS) [55, 56].

The cross-talk between platelets and tumor cells that mediates activation and aggregation is thought to be crucial for platelets to support tumor progression. Overall, tumor cells have a diverse arsenal of mechanisms to induce platelet activation, and the specific methods utilized by a particular tumor may depend on the cancer type, stage, or location. For instance, some glioblastoma and pancreatic cell lines release thrombin to induce TCIPA, while MCF-7 breast tumor cells can release MMP-2 or ADP to achieve TCIPA [42, 43, 57, 58]. But regardless of the specific mechanism, activation of platelets seems to be a common phenomenon in cancer progression. In the next sections, we will discuss in detail how activated platelets and platelet–tumor cells aggregates are thought to influence cancer progression.

Platelets in Tumor Growth and Invasion

Platelets are packed with a myriad of biologically active growth factors and cytokines that are critically important during wound healing but can be detrimental when co-opted by tumors. In vitro studies have shown that platelet-derived growth factor (PDGF) and platelet-activating factor (PAF) directly drive tumor cell proliferation [59, 60]. However, the evidence that platelets have a role in influencing the growth of primary tumor proliferation and growth in vivo is limited [59, 60]. A vast body of evidence both in vitro and in vivo suggests that, instead, platelets in the primary TME predominantly influence tumor progression by driving invasion [61, 62].

Platelets promote invasion through a variety of mechanisms. Epithelial-to-mesenchymal transition (EMT) is one process by which tumor cells become invasive. During EMT, tumor cells of epithelial origin lose their cell-to-cell adhesions and polarity, becoming more mobile and developing the characteristics and markers of mesenchymal cells. Platelets were shown to induce the expression of key EMT regulators such as twist, snail, slug, vimentin, and fibronectin while downregulating E-cadherin [63]. Findings from these studies also demonstrated that platelet-derived TGF-β1 drives EMT through activation of the TGF-β1 receptor and NF-κB signaling pathways in the tumor cells, with which they are in direct contact [63, 64]. Furthermore, conditional ablation of platelet TGF-β1 reduced metastasis in mice [63]. While TGF-β1 released from platelets has been identified as the main factor responsible for platelet-induced EMT, hepa-

tocyte growth factor (HGF) and PDGF may contribute to EMT as well [65]. Platelet-derived autotaxin has also been shown to directly induce breast tumor cell migration and invasion [66–68].

Another mechanism by which platelets promote tumor cell invasion is to alter the TME. Simply adding platelets or releasate from activated platelets increases migration and invasion of tumor cells in culture [62]. By releasing MMPs directly into the peritumoral space, platelets could break down the extracellular matrix to enable tumor cell migration [69]. Furthermore, platelets induce MMP expression in other components of the microenvironment including tumor cells and endothelial cells [70–72]. Stromal cells in the TMEs are also influenced by platelet-derived factors as indicated by studies showing that tumor-promoting cancer-associated fibroblasts proliferate and differentiate in response to signals from activated platelets [73, 74].

Platelets Promote Angiogenesis

Angiogenesis is critical for most solid tumors to survive and grow beyond a diameter of 1–2 mm [75]. A role for platelets in tumor angiogenesis was first proposed by Judah Folkman in 1998 and, indeed, platelets are now known to be intimately involved in the angiogenesis process [76]. Platelets are packed with various pro-angiogenic and anti-angiogenic regulators but the net effect of releasates from platelets activated by tumor cells, both in vitro and in vivo, tends to strongly promote endothelial capillary tube formation in vitro, indicating a pro-angiogenic effect [31, 77]. Over 80% of circulating VEGF, a potent pro-angiogenic mediator, is carried within the platelets of both healthy individuals and cancer patients, and VEGF levels within platelets correlate with disease progression [78–80]. In vivo models by depletion of platelets, showed decreased retinal neovascularization, corneal angiogenesis, and tumor angiogenesis [31, 69, 81].

Platelets package different angiogenic mediators into distinct alpha-granules that can be released differentially depending on the specific agonist bioavailability or receptor activation [77]. ADP activation leads to VEGF release and a pro-angiogenic releasate, while activation with thromboxane A_2 causes retention of VEGF and release of the anti-angiogenic protein endostatin, leading to a releasate with net anti-angiogenic effects [77]. Platelet activation via the thrombin receptor PAR1 mediates VEGF release, while stimulation of the PAR4 receptor leads to endostatin release and retention of VEGF [3, 82]. Those studies showed that platelets can make "choices" about which contents to package and release based on the stimulus they receive. Differential packaging of platelets is likely to occur during their production by megakaryocytes. In vitro studies show megakaryocytes can sort and package contents (e.g., bFGF, VEGF) into distinct alpha-granules that are differentially distributed into platelets [83]. On the contrary, other studies suggest the packaging of proteins with conflicting functions into the alpha-granules could be stochastic [84]. By mathematical analysis of the localization of 15 different human alpha-granule proteins with pro-angiogenic or anti-angiogenic function, a Gaussian distribution indicated random packaging of proteins to individual alpha-granules [84]. However, proteins could also be packaged into distinct zones or with different levels [84, 85], and can be released differentially upon activation by tumor cells to favor tumor angiogenesis.

The promotion of angiogenesis within a tumor by platelets could be due to the basal functions of platelets in physiology. Platelets are the "first responders" to a wound [86], and their degranulation releases factors that initiate clotting, angiogenesis, and immune cell recruitment to facilitate wound repair [87]. By promoting angiogenesis, platelets attempt to preserve the integrity of leaky blood vessels within tumors [81]. On the other hand, platelets could potentially prevent tumor progression by blood vessel normalization. Blood vessel normalization is a therapeutic strategy for cancer, given the synergist efficacy of both anti-VEGF therapy and chemotherapy through normalizing tumor blood vessel structure and maturation to facilitate the uniform administration of anti-cancer therapies to tumors [88]. Platelets may also have important role(s) in blood vessel normalization, where they have been reported to recruit and induce differentiation and maturation of endothelial progenitor cells [89]. Tumors from thrombocytopenic mice showed impaired vessel density and maturation [69]. Specifically, platelets seem to support pericyte coverage in angiogenic vessels and angiopoietin-1 and serotonin released from platelets may promote vessel maturation [69, 90] These data support a paradoxical anti-tumor function of platelets by establishing blood vessel homeostasis. It is possible that the initial purpose of platelet recruitment to the tumor is to repair the leaky tumor blood vessels; however, platelet activation by the TME induces pathological angiogenesis. Therefore, more research is needed to parse the specific signals, conditions, events, and intermediates that favor platelet-induced angiogenesis and vessel stabilization.

In addition to regulating blood vessel integrity, platelets can also act like sponge, taking up molecules from their environment. Angiogenic factors, including VEGF and basic fibroblast growth factor, released by primary tumor are taken up by platelets, stored, trafficked, and delivered to other locations such as distant metastatic sites [91–93]. A study using a murine model of luminal breast cancer demonstrated that platelets sequester angiogenic regulators from the site of an aggressively growing primary tumor and deliver them, via the circulation, to indolent tumors located at distant anatomi-

cal sites where these platelets contribute to growth and angiogenesis of the otherwise indolent tumor [31]. Platelet inhibition with aspirin prevented tumor progression, suggesting the potential role of platelets in delivering angiogenic signals from one tumor to the other [31]. Those studies highlight the potential for platelets to serve as long-haul cargo carriers, shuttling signals between distant sites as orchestrated by the tumor.

Mechanisms by which platelets endocytose proteins are active areas of investigation. Platelets from mice lacking expression of dynamin2, vesicle-associated membrane protein-3 or adenosine 5′-diphosphate–ribosylation factor 6 (Arf6) demonstrated decreased fibrinogen uptake, thus implicating those factors as critical for the platelet endocytic machinery [94–96]. Receptor-mediated endocytosis could be an important aspect of platelet function. Platelets from dynamin2 knockout mice showed not only decreased fibrinogen in their alpha-granules, but also dysfunctional responses to stimulation via GPVI [97]. Taken together, these findings imply endocytosis alters both the content and function of platelets. However, little is currently known about the mechanism(s) by which platelets endocytose proteins that favor tumor progression, leaving a critical gap in our knowledge. A deeper understanding of these processes should provide a source of potential therapeutic targets.

Overall, platelets contribute significantly to tumor angiogenesis via a number of mechanisms; they release potent pro-angiogenic factors upon stimulation by tumor cells, they mature and normalize unstable tumor-associated vessels, and they collect angiogenic mediators and deliver them to distant sites, propagating the angiogenic signal from the tumor. Angiogenic neovasculature not only nourishes the tumor but also provides a route for tumor cells to escape into circulation.

Platelet–Tumor Cell Interactions Within Blood Circulation

In order to metastasize, tumor cells need to enter either blood or lymphatic vessels, which serve as conduits for their transport to distant sites. Tumor cells in the blood are often referred to as circulating tumor cells (CTCs) and are a promising predictor of poor prognosis in the clinic. In a large global pooled analysis, breast cancer patients without detectable CTCs at baseline or at follow-up had significantly improved outcomes relative to patients who persistently tested positive for CTCs (47.05 months vs. 17.87 months, hazard ratio = 3.15, $p < 0.0001$) [98].

Hematogenous metastasis is thought to be an inefficient process [99] due to harsh shear stresses and constant immune surveillance faced by tumor cells. It is estimated that the vast majority of tumor cells are destroyed within hours of introduction into circulation, well before they can ever successfully form metastases [100, 101]. As previously discussed, contact between platelets and tumor cells causes heterotypic aggregates to form. Such aggregates can be readily identified in the circulation of cancer patients and in pre-clinical mouse models, they form within minutes of tumor cell introduction into the bloodstream [64]. The mechanical forces exerted on tumor cells in the blood are far greater than those experienced in the TME and are often enough to cause their destruction [102]. Platelets have been shown to provide protection from such forces by coating tumor cells, shielding them from shear stress modeled by plate viscometer [103].

By binding to tumor cells, platelets induce tumor cell alterations that favor their survival. As mentioned in the previous section, platelets can induce tumor cell EMT [63], which is important for gaining tumor cell stemness [104] to support their survival in the non-adherent environment of blood circulation and initiation of new tumor formation in the secondary site to form metastasis [105]. Platelet interactions have also been shown to induce tumor cell expression of immune regulatory factors, such as CCL2 [63], which recruits tumor-associated monocytes and macrophages to suppress immune surveillance in the blood and secondary sites [106]. CTC clearance from the circulation has been shown to be mediated by natural killer (NK) cells [107, 108]. Activated platelets express glucocorticoid-induced tumor necrosis factor receptor ligand (GITRL) on their surface, which binds to the GITR receptor on NKs, leading to inhibition of NK cell activity [109]. Platelets can also inhibit NK cells by downregulation the expression of the NKG2D cell-surface receptor, which is used by NK cells to identify and lyse tumor cells [110]. Furthermore, platelets can protect tumor cells from NK-mediated killing in the blood by transferring MHC class I molecules to the CTC surface [111]. Tumor cells may also avoid lysis in the blood by aberrantly expressing integrins normally found on platelets in a phenomenon known as platelet-mimicry [112, 113].

CTCs have also been found in the blood as clusters. Compared with single CTCs, clustered CTCs are more resistant to apoptosis, have more metastatic potential, and predict poorer prognosis in breast and prostate cancer patients [114]. One way CTC clusters have enhanced survival advantages is by forming homotypic attachments, thereby increasing their resistance to anoikis [115]. Other hypotheses include the notion that CTC clusters are less likely to experience excessive shear force and moreover, larger CTC clusters are more easily trapped in small capillaries of secondary sites [116]. Increased coagulation factors, such as platelet tissue factors F3, F5, and F12, have been found in CTC clusters compared with single CTCs [114], suggesting the platelets that bind to CTC clusters are more activated than those who bind to single CTCs. In a study that analyzed gene expression profiles

of CTC clusters and single CTCs, Gene Set Enrichment Analysis revealed that "Hallmark_Coagulation" was the most significantly enriched pathway in CTC clusters compared with single CTCs [117]. Nevertheless, the mechanism(s) underpinning the survival advantages of CTC clusters are still not completely resolved, and the contribution of platelets has yet to be investigated.

Extravasation

Tumors cells must find ways to successfully exit circulation to seed a new metastatic site. Immobile platelet–tumor cell aggregates have been observed in the microvasculature [118, 119] and it was historically assumed that this was a passive process with aggregates simply getting stuck within narrow vessels. We now know that arrest and extravasation are active processes and that platelets are key players in both of them. Platelet surface selectins mediate rolling along the endothelium slowing their velocity in circulation and allowing for further association with endothelial cells. P-selectin on activated platelets interacts with the endothelium while simultaneously mediating binding to tumor cells, thus tethering tumor cells to the endothelium [26, 40]. The importance of P-selectin in this process has been demonstrated in mice through the pharmacological blockade as well as genetic ablation of P-selectin [41]. Platelets can also bind CD97 (adhesion G protein-coupled receptor) on the tumor cell surface, which conducts bidirectional signaling to both platelets and tumor cells during extravasation [120]. The activation of platelets by tumor CD97 leads to platelet ATP release, which disrupts endothelial cell junctions by activating the endothelial $P2Y_2$ nucleotide receptor [121] and binding of platelets to tumor CD97 increases tumor cell invasiveness by Rho activation [120]. Other factors released by platelets including MMP-1, TGF-β, and ADAM12 also facilitate the breakdown of junctions between endothelial cells, allowing tumor cells to cross the now leaky endothelial barrier and enter the surrounding tissue parenchyma [119, 122].

Once disseminated tumor cells have arrived at new metastatic sites, activated platelets promote colonization, angiogenesis, and ship signals to and from distant sites. To quote Yan and Jurasz, "…perhaps a small revision is required to Paget's 'seed and soil' hypothesis of metastasis to include 'seed, soil, and fertilizer', in which platelets take on an unenviable but supportive role of 'fertilizer'" [123]. However, it remains unclear if platelets support tumor cells at secondary sites through the same mechanisms employed at the primary tumor and this question warrants further investigation.

Platelets Coordinate the Systemic Effects of Tumors

As discussed previously, tumors can activate, alter and use platelets to carry molecular signals to distant locations throughout the body, making platelets an integral part of the systemic communication and coordination that occurs in cancer [124–126]. Platelets can propagate messages that serve to mobilize bone marrow progenitors, alter the bone function and even prepare sites to accept future metastases. Tumors recruit bone marrow-derived cells (BMDCs) and endothelial progenitor cells to the TME. Stromal cell-derived factor 1 (SDF-1) and VEGF released from activated platelets have been implicated in mobilizing BMDCs and progenitor cells from the bone marrow [89, 127–129]. Platelets also appear to promote metastasis within the lung by recruiting pro-metastatic granulocytes to platelet–tumor cell aggregates during extravasation through the release of CXCL5 and CXCL7 [64]. Platelets have also been demonstrated to serve as long-range communicators between primary tumors, distant tumors, and the bone marrow, by cooperating with BMDCs to promote the vascularization of distant tumors [31].

Bone remodeling often occurs in the setting of metastatic disease and platelets may mediate this process as well. The presence of primary melanoma or prostate tumor increased bone formation in mice, while platelet depletion reversed this effect [130]. In these two cancer models, platelets traffic tumor-derived MMP-1 and TGF-β to the bone where they promote bone formation. Conversely, platelets are also capable of increasing bone resorption to facilitate bone metastases. In a pre-clinical breast cancer model, platelets promoted osteolytic bone loss by a complex mechanism in which lysophosphatidic acid (LPA) released from activated platelets drove IL (Interleukin)-6 and IL-8 secretion from tumor cells to stimulate bone-destroying osteoclasts [131]. Furthermore, platelets release autotaxin from their alpha-granules, a molecule that catalyzes the production of LPA and guides tumor cells to the bone by interacting with tumor cell αvβ3 integrins [130].

Platelets clearly help orchestrate the complex coordination of events that enable tumors to metastasize. More studies are required to parse the precise role of platelets in the spread of specific tumor types and in the homing of tumor cells to particular sites of metastasis. Additionally, it is necessary to confirm whether similar mechanisms are at play in human patients and, if so, determine whether they are vulnerable to therapeutic intervention (Fig. 15.1).

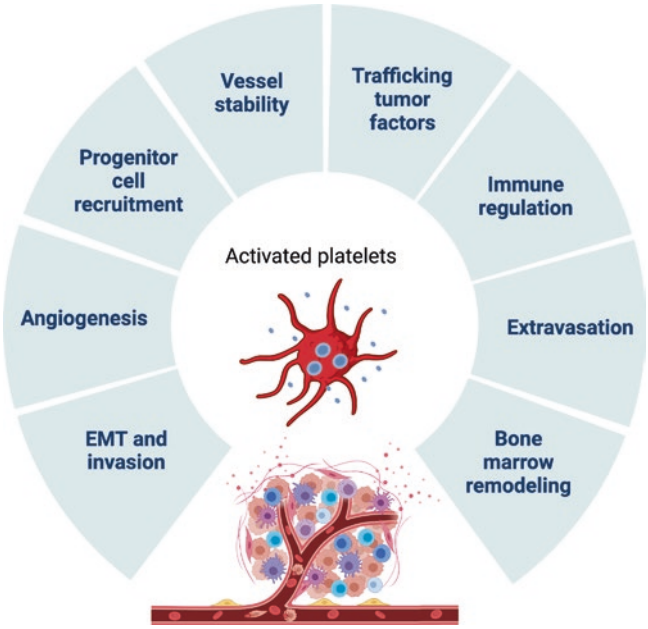

Fig. 15.1 Mechanisms by which platelets promote cancer. Platelets have been found to employ a wide variety of strategies to promote tumor progression and metastasis. Platelets can induce tumor cell EMT to drive tumor cell aggressiveness [63–68], promote angiogenesis [31, 69, 81], impact vessel stability facilitating circulating tumor cell extravasation [64, 120], remodel bone marrow [132–134], and recruit tumor-promoting progenitor cells [89]. In addition, platelets can carry and deliver signals between distant sites including primary and secondary tumors and bone marrow [124–126]. Adapted from "RNA Secondary Structures" and "Tumor microenvironment," by BioRender.com (2021). Retrieved from https://app.biorender.com/biorender-templates

Platelet Microparticles and the Tumor Microenvironment

Recently, interest in platelet-derived microparticles (PMPs) and their potential role in cancer has been growing. PMPs are shed from platelets following activation and consist of membrane-bound proteins and cytoplasmic components [135]. In vitro, PMPs have similar pro-angiogenic and pro-metastatic potential as platelets and increase endothelial cell migration and capillary tube formation as well as promote tumor cell MMP production and invasion through Matrigel [136–138]. PMPs may also transfer membrane receptors and adhesion molecules to the surface of tumor cells, conferring a more invasive phenotype. In vivo, Lewis lung carcinoma cells were more metastatic when coated with PMP prior to injection and, in an ischemia model, the introduction of PMPs increased angiogenesis [139, 140].

Overall, PMPs recapitulate many of the same metastatic and angiogenic effects that are observed with whole platelets. They may potentially provide a mechanism for tumor mimicry, with tumor cells incorporating platelet markers to their cell surface after fusion with PMPs. They may also serve as a way for activated, spent platelets to continue to play a role in the TME and should be considered when conceptualizing the complex crosstalk that occurs in cancer.

The Role of Platelets in Hematological Malignancies

So far most of our knowledge about the function of platelets in cancer comes from studies of solid tumors, particularly carcinomas. Relatively little is known about the role of platelets in hematological malignancies, and little can be extrapolated from work in solid tumors due to vast differences in the tumorigenesis processes and the TMEs. However, some studies can offer insights.

Unlike cell lines derived from solid tumors, many leukemia cells do not activate platelets and have been shown to inhibit the activation and aggregation of platelets [141–143]. Patients with leukemia often present with thrombocytopenia (low platelet count) and their platelets display lower numbers of dense granules [144, 145]. Conversely, a few AML and CML cell lines can activate and aggregate platelets, and the resulting releasate increases tumor cell proliferation and survival [146–148].

The role of platelets in multiple myeloma (MM) was also investigated. These patients often have elevated soluble P-selectin and thrombopoietin (TPO) levels and are at increased risk of developing VTEs [132–134]. Platelet activation is positively correlated with MM disease progression [149]. Platelets contribute to MM cell proliferation in vitro and tumor engraftment in bone marrow in vivo, potentially by stimulating tumor cell IL-1β production, as IL-1β deletion in tumor cells aborts any platelet-induced effects [149]. Other factors released by platelets that are known to support MM progression include but are not limited to IL-6, SDF-1, and insulin-like growth factor-1 [132–134].

Overall, the role of platelets in hematological malignancies is not well explored. Because thrombocytopenia is a feature of many hematological malignancies, platelets may not be as important as those involved with solid tumors. However, since platelets are shown to be altered in some hematological cancers, it would be wise to examine the interactions of these tumor cells with megakaryocytes, particularly in cancers such as multiple myeloma where the bone marrow serves as a TME. It may be that platelets serve different functions in the different types of hematological cancers and detailed investigations into each type could be beneficial.

Platelets Are Altered in Cancer Patients

Interestingly, platelets isolated from some cancer patients are fundamentally different from those of healthy individuals. Platelets from breast, prostate, lung, and colon cancer

patients often display higher baseline activation, suggesting they may be more reactive and have a lower threshold for activation [150–153]. Elevated platelet surface levels of the activation marker P-selectin as well as increased platelet-derived plasma markers such as CD40 ligand, β-thromboglobulin, and soluble P-selectin have been observed in patients with tumors, and these markers tend to correlate with disease progression and poor prognosis [154, 155]. Patients presenting with elevated soluble P-selectin are more than twice as likely to develop a VTE compared to cancer patients with low levels [155].

Platelet contents are also altered in patients; total numbers of alpha-granules are higher, and pro-tumorigenic factors such as VEGF are enriched in platelets from cancer patients compared to those from healthy donors [92, 156]. Platelets from cancer patients also contain altered mRNA transcripts [157] and can be used as a non-invasive liquid biopsy in aiding cancer detection [157–159]. For example, TIMP1 mRNA is upregulated in colorectal cancer patients compared with healthy controls or patients with inflammatory bowel diseases [160]. Tropomyosin 3 mRNA is significantly elevated in breast cancer patients and positively correlated with metastasis [161]. These transcripts could come from two sources: platelets take them up from plasma, which contains tumor cell releasates or tumor cells induce the production of alternative splice variants within platelets. Evidence suggests that mRNAs may be produced and packaged at higher levels at the megakaryocyte level in addition to being taken up from the tumor environment [162].

Thrombocytosis is associated with some specific types of cancer. Lung, colorectal and ovarian cancer are typically the most commonly diagnosed cancers in patients with thrombocytosis [163, 164], but breast cancer patients usually present with normal platelet counts [165]. However, the prognostic effect of thrombocytosis has been found in most types of cancer, including breast, ovarian, lung, and other types of cancers [166]. TPO is the dominant driver of megakaryocyte differentiation and maturation. Pre-clinical studies of ovarian cancer reveal that tumor-derived IL-6 drives TPO production in the liver, leading to a boost in platelet production by megakaryocytes in the bone marrow [167]. IL-6 levels in patients correlate with platelet count, and anti-IL-6 therapy reverses this trend [168, 169]. However, more studies are needed across all tumor types to determine if this mechanism is broadly responsible for tumor-associated thrombocytosis. Another hypothesis posits that tumor cells themselves provide a source of TPO, but this has only been observed in vitro [170]. Other mechanisms that have been proposed are based on reactive thrombocytosis observed in conditions of systemic inflammation. Pro-inflammatory cytokines such as G-CSF, GM-CSF, and IL-1α are often elevated during inflammation as well as cancer progression and have also been implicated in megakaryocyte maturation and platelet production [171–174].

Although an abundance of evidence proves that platelet function, contents, and numbers are altered in cancer patients, we have only begun to understand how that occurs. Studies are needed to elucidate the effect of tumors on megakaryocyte biology and the platelets that result. Understanding the mechanism(s) underlying cancer-associated thrombocytosis remains important, as therapies directed at this process could prevent tumors from generating platelets that favor their progression.

Anti-platelet Therapy and Cancer

Based on their multifaceted role in cancer, platelets are a very attractive therapeutic target. Disrupting the communication between platelets and tumor cells by targeting platelets or tumor cells could theoretically block mechanisms of invasion, EMT, angiogenesis, immunosurveillance escape, and activation of other host cells in the microenvironment and bone marrow to prevent metastasis. Platelet-mimicry, along with many shared surface markers between platelets and tumor cells, suggests that platelet-targeted drugs could also impact the tumor.

Pre-clinical data are quite promising and reveal that targeting several platelet receptors may be an effective approach for limiting cancer progression. Anti-platelet drugs that are currently available for the treatment of cardiovascular disease are now being explored as anti-tumor agents. For example, GPIIbIIIa blockers have been shown to inhibit lung metastasis in a murine model but have not yet been studied in patients with cancer [175]. Clopidogrel and ticagrelor, as P2Y12 antagonists, were used to treat cardiovascular disease, show anti-tumor properties in vivo and in vitro, and can prevent ovarian cancer growth and bone loss in mice [176, 177]. Anti-coagulants including fondaparinux and low molecular weight heparins (LMWH) inhibit tumor cell inducing platelet activation and attenuate the angiogenic potential of platelets in vitro [178]. These drugs make attractive candidates since they are often already given to cancer patients due to their tendency to develop clots. However, clinical data from the use of LMWH in cancer patients show mixed results [179–182]. Large-scale clinical trials are needed to access the efficacy of currently available anti-platelet drugs. What is more, some newly developed platelet-targeting drugs have shown promising efficacy in pre-clinical models of breast cancer. Antisense oligonucleotides that silence hepatic thrombopoietin gene (THPO-ASO) could reduce plasma TPO levels and decrease platelets count by targeting bone marrow megakaryocytes and treatment of THPO-ASO inhibits breast cancer progression in MMTV-

PyMT mouse model [183]. Anti-GPVI therapeutic antibody (JAQ1) has also been shown to reduce metastasis burden in mouse colon and breast cancer models [184]. These data suggest the importance of platelets in cancer progression and finding more efficient and safe methods to target platelets will further improve standard cancer treatment.

Aspirin is perhaps the most intriguing anti-platelet agent that has been studied to date, as aspirin is broadly used with minimal side effects. However, the function of aspirin in cancer treatment remains controversial, with varying efficacies across cancer types, patient cohorts, and study design. Some reports show a beneficial function of aspirin in decreasing cancer risk and death [185, 186]. A long-term epidemiological study revealed that individuals who take aspirin daily are less likely to be diagnosed with cancer and show improved survival if they do develop cancer [187]. Some studies indicate no benefit or even worse prognosis in cancer patients regularly taking aspirin [188–190]. Despite the contradicting results, aspirin is still being extensively studied in cancer research, as it can target both tumor cells and the TME [191]. Better understanding the mechanism of action in both platelets and tumor cells could lead to optimized usage of this drug in some specific types of cancer. The mechanism of action for aspirin's efficacy in cancer was originally thought to be due to decreased inflammation via cyclooxygenase (COX) inhibition. However, the low doses (below 162 mg/day) taken were not enough to prevent inflammation but do cause platelet inhibition through irreversible acetylation of COX-1. Subsequent studies also point to a platelet-based mechanism; platelet inhibition with aspirin diminishes platelet activation, protein release and their ability to induce angiogenesis [77]. Mouse models also confirm that platelet inhibition with aspirin decreases metastasis and improves outcomes [23, 31, 192]. The mechanism of aspirin in inhibiting metastasis has been investigated. Recent studies report that the inhibition of COX-1/thromboxane A_2 pathway of platelets by aspirin decreases platelet–tumor cell aggregation, endothelial activation, and tumor cell–endothelial cell adhesion, which decreased metastasis [193]. Another study shows that aspirin-treated platelets fail to induce IL-8 secretion from tumor cells [194]. Aspirin seems most effective in chemoprevention [195–197], but may also be beneficial if taken in combination with standard treatment to target the tumor and the environment at multiple angles. Further exploring the mechanisms by which aspirin inhibits the tumor promotional function of platelet is critical in the development of drugs that are more specific and efficacious than aspirin but work on the same principle.

Concluding Remarks/Summary

Platelets a now known to be key players in cancer progression and metastasis. These little cells supply the tumor with growth factors and mediators of invasion, provide potent pro-angiogenic regulators and help maintain tumor vessel integrity, protect circulating tumor cells from shear stress and immune attack, and help set up new metastatic niches. Platelets also serve as long-haul cargo carriers, delivering messages to and from the tumor in ways that allow cancer to progress. Such systemic changes also lead to alterations in platelet function, content and number. Overall, research into the role of platelets in cancer has rewarded us with an abundance of novel factors, receptors, and signaling pathways that could serve as powerful new biomarkers, potential therapeutic targets, or even novel drug delivery tools in the fight against cancer.

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