Chapter 12
The Role of Platelets in the Tumor Microenvironment

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Abstract Platelets are small, anuclear cells found in the circulation that have an important and well-defined role in hemostasis and wound healing. Known as the "band-aids of the blood," these cells rapidly activate, aggregate, and release a potent milieu of growth factors, cytokines, and other biological mediators at the site of vascular damage, forming a clot. Compelling evidence has revealed that tumors can co-opt the normal functions of platelets in order to advance tumor progression and metastasis. Indeed, we now know that platelets are a key component of the tumor microenvironment and that they promote cancer progression in a myriad of ways; platelets drive tumor cell invasion and epithelial to mesenchymal transition, they promote angiogenesis, they facilitate intravasation and extravasation of tumor cells, they protect disseminated tumor cells from shear forces and immune surveillance within the circulation, and they function as long-distance cargo carriers that transmit signals between primary tumors, metastases, and the bone marrow. In this chapter, we will examine the current body of evidence on the role of platelets in cancer along with the underlying mechanisms and explore platelet-targeted therapies as a novel and promising approach to cancer treatment.

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L.A. Akslen, R.S. Watnick (eds.), *Biomarkers of the Tumor Microenvironment*,
DOI 10.1007/978-3-319-39147-2_12

Keywords Platelet • Angiogenesis • EMT • Alpha-granule • Tumor cell-induced platelet aggregation • VEGF • Endostatin • P-selectin • VTE • Thrombocytosis Thrombocytopenia

Platelets are best known 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 now also know that platelets are a critical component of the tumor microenvironment (TME) and can profoundly affect tumor progression and metastasis. For example, platelets aid disseminating tumor cells by protecting them from high shear forces and immune surveillance within the circulation, by forming tumor cell-platelet aggregates that facilitate embolization, by promoting adhesion of tumor cells to the vascular endothelium, and by releasing 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 other cells in the TME such as endothelial cells, fibroblasts, and immune cells [1–3]. In this chapter, we will discuss what is known about the complex cross talk between platelets, tumor cells, and other host cells that occurs in malignancy and highlight features of this 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. Their concentration in the blood is between 1.5 and 3.5 × 10^8/μL in healthy individuals, and they have a half-life in circulation of only 7–10 days [4]. Structurally, platelets are anuclear and contain three distinct types of granules; alpha-granules, dense granules, and lysosomes. Each platelet contains ~50–80 alpha-granules, thus making them the most abundant type of platelet granule [5]. Over 300 biologically active factors, including cytokines, adhesion molecules, and coagulation mediators (e.g., VEGF, PF4, P-selectin, and fibrinogen), are contained within alpha-granules, which can be selectively released upon platelet activation [6]. Dense granules contain a variety of signaling intermediates such as serotonin, ATP, and ADP that can enhance platelet activation [7, 8]. Lysosomal granules are packaged with proteases and glycosideases [9]. Although they do not have nuclei, platelets contain some mRNA and translational machinery that are present in the cytosol; hence, protein synthesis can occur to a limited extent [10]. 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"; they prevent blood loss during injury by forming a clot at the site of vascular damage. By design, they must be able to remain inactive, or resting, under normal conditions but be able to quickly activate and form a clot upon contact with damaged endothelium within the high shear, high-pressure conditions of the circulation. Damage to the vascular wall causes exposure of subendothelial collagen and von Willebrand factor, which serve to attract circulating platelets by engaging glycoproteins on the platelet cell surface, such as GPIbα, thereby leading to adhesion at the site of damage [11]. 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 [11]. GPIIbIIIa on the platelet surface is activated by fibrinogen allowing for platelet aggregation and the formation of fibrinogen bridges that stabilize the clot [11]. Activated platelets release pro-coagulation factors and serve as a surface for clotting factors to assemble, further strengthening the platelet plug [7] (Fig. 12.1a).

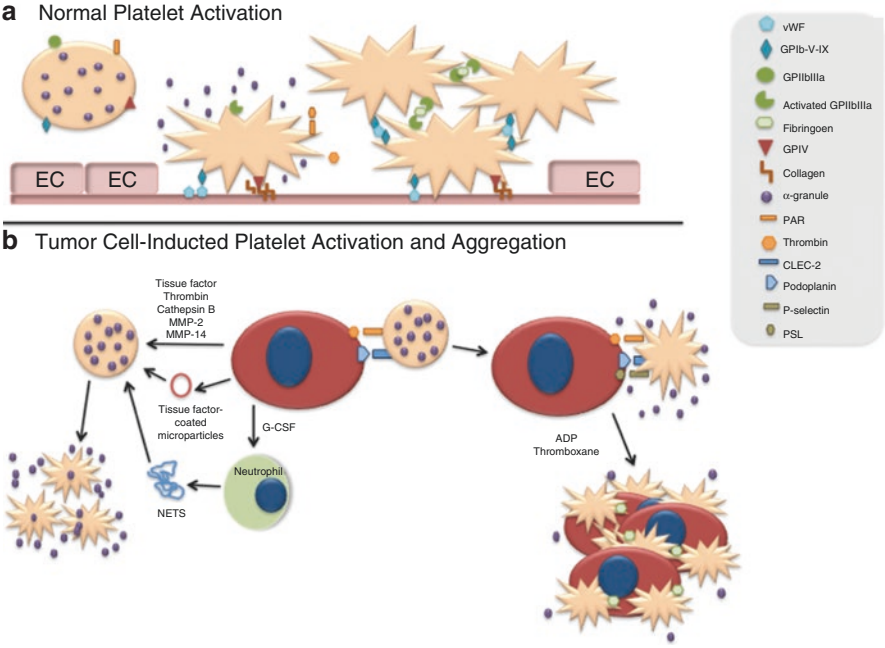

Fig. 12.1 Mechanisms of platelet activation. Under normal, physiological conditions, platelets rapidly form a clot upon contact with a site of vascular damage (**a**). Damage to the endothelium leads to exposure of underlying extracellular matrix proteins which engage glycoproteins on the cell surface of circulating platelets. Platelets activate, undergo a shape change and release their granular contents. Fibrinogen bridges form between platelets to strengthen aggregates. Granule contents increase platelet activation, trigger coagulation, and participate in the wound healing process (**a**). Tumors trigger platelet activation and aggregation through a variety of direct and indirect mechanism (**b**). Direct contact with tumor cells can cause platelet activation through a variety of ligand/receptors pairs. Tumor cell-secreted factors or microparticles also stimulate platelet activation while tumor cell-activated neutrophils can release platelet-activating NETS (**b**)

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 release of growth factors and inflammatory cytokines from alpha-granules can promote atherosclerosis and tumor progression [12]. 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 associated with cancer [13]. French physician Armand Trousseau is widely credited as the first to definitively propose a link between cancer and hypercoagulabilty 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 [14]. Platelets were specifically implicated in cancer in 1872 when a link between elevated platelet count and malignancy was reported [15]. 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 [16]. 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 [17–22]. Thrombocytosis is also associated with increased risk of venous thromboembolism (VTE) in many cancer patients [23]. Cancer patients have a four to sevenfold greater risk of developing a pulmonary embolism or a deep vein thrombosis compared to healthy individuals [24].

Is elevated platelet count merely coincidental, or do platelets play a direct, active role in cancer progression? To answer this, Gasic et al. depleted platelets from mice prior to injecting tumor cells in an experimental murine model of metastasis [25]. Depletion with neurominidase or antiplatelet serum decreased metastasis, while infusion of platelet-rich plasma reversed this effect, suggesting that platelets play an active role in cancer progression. Subsequent mouse studies show 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 [26–29]. Interestingly, metastasis was reduced by 80% in a mouse model of gray platelet syndrome, a disorder in which platelets lack alpha-granules [30]. These animal studies verified that platelet activation and alpha-granule release were involved in metastasis.

Taken together, observations in cancer patients along with experimental mouse models clearly demonstrate that platelets play a necessary role in metastatic spread. Nevertheless, questions remain about how, mechanistically, platelets influence the metastatic process. Numerous research efforts have focused on answering this question, and in this chapter we will examine research demonstrating the role of platelets

at every stage of cancer progression, from the primary tumor site to the tumor cell's journey through the circulation and finally during extravasation and metastatic growth.

Tumor Cell-Induced Platelet Activation and Aggregation

Normally, platelets are only activated at sites of vascular injury and remain inert (resting state) while in 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 [31]. The TME has developed a variety of ways to induce inappropriate platelet activation and co-opt platelet function for the tumor's benefit. In vivo, activated platelets have been observed within primary tumor tissue [32]. 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 [33]. 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 [34–36]. 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 initiation of the coagulation cascade [37, 38]. 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 [39–42].

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 [43–45]. 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 [46]. Aggregates composed of platelets and tumor cells have been observed within the circulation since the 1970s [47, 48], and tumor cell lines of breast, colon, prostate, lung, and pancreatic origin, to list a few, have been shown to aggregate platelets in vitro [49–52]. These aggregates can be observed in the blood of patients and are implicated in tumor cell immune evasion and embolization.

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 G-CSF, causing circulating neutrophils to release platelet-activating neutrophil extracellular DNA 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 [43, 44, 57, 58]. But regardless of the specific mechanism, activation of platelets seems to be a nearly universal phenomenon in cancer progression (Fig. 12.1b). 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. Activated platelets release cargo into the peritumoral space, thus impacting tumor growth, migration, and invasion. In vitro studies have shown that PDGF, thromboxane, and platelet-activating factor (PAF) directly drive tumor cell proliferation [59–61]. However, the evidence that platelets have a role in influencing primary tumor proliferation and growth in vivo is limited [59–61]. 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.

Platelets promote invasion through a variety of mechanisms. Epithelial to mesenchymal transition (EMT) is a critical process that tumor cells undergo in order to 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 induce expression of key EMT regulators such as twist, snail, slug, vimentin, and fibronectin, while downregulating E-cadherin [62]. Findings from these studies also demonstrated that platelet-derived TGF-β1 drives EMT though activation of the TGF-β1 receptor and NF-κB signaling pathways in the tumor cells, with which they are in direct contact [62, 63]. Furthermore, conditional ablation of platelet TGF-β1 reduced metastasis in mice [62]. While TGF-β1 released from platelets has been identified as the main factor responsible for platelet-induced EMT, hepatocyte growth factor (HGF) and platelet-derived growth factor (PDGF) may contribute to EMT as well [64]. Platelet-derived autotaxin has also recently been shown to directly induce breast tumor cell migration and invasion [65–67].

Another mechanism by which platelets promote tumor cell invasion is to alter the TME to favor migration and invasion. Simply adding live platelets or releasate from activated platelets significantly increases migration and invasion of tumor cells in culture [68]. By releasing MMPs directly into the peritumoral space, platelets break down the extracellular matrix to enable tumor cell migration. Furthermore, platelets induce MMP expression in other components of the microenvironment including tumor cells and endothelial cells [69–71]. Stromal cells in the TMEs are also influenced by platelet-derived factors as indicated by studies showing that tumor-

promoting cancer-associated fibroblasts (CAFs) proliferate and differentiate in response to signals from activated platelets [72, 73]. Finally, platelets are thought to facilitate tumor cell migration across the vascular endothelium by releasing factors that weaken junctions between vascular endothelial cells, thus allowing for tumor cells to escape into the circulation [74]. For example, results from studies using transendothelial migration assays demonstrated that platelet-derived ADP, TGF-β, and VEGF can all facilitate tumor cell migration across an endothelial barrier [65, 74, 75].

Platelets Promote Angiogenesis

Angiogenesis, the growth and expansion of the vasculature, is critical for most solid tumors to survive and grow beyond a diameter of 1–2 mm. 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 different pro- and anti-angiogenic regulators, but the net effect of releasates from tumor cell-activated platelets tends to strongly promote angiogenesis [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 vitro, platelets and platelet releasates increase endothelial cell proliferation, migration, and capillary tube formation [77, 81]. Use of in vivo angiogenesis assays has also confirmed that platelets are required for vessel development. To that end, platelet depletion was demonstrated to decrease retinal neovascularization, corneal angiogenesis, and tumor angiogenesis [32, 82, 83].

Platelets package different angiogenic mediators into distinct alpha-granules that can be released differentially depending on the specific agonist bioavailability or receptor activation. ADP activation leads to VEGF release and a pro-angiogenic releasate, while activation with thromboxane causes retention of VEGF and release of the anti-angiogenic protein endostatin, leading to a platelet releasate with net anti-angiogenic effects. 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, 84]. These studies show that platelets are able to make "choices" about which contents to release based on the stimulus they receive. Tumor cells are able to harness this differential release for their own benefit and mediate the preferential release of VEGF over endostatin, and releasates from tumor cell-activated platelets have a strong net pro-angiogenic activity [77]. Platelet inhibition with aspirin prevents MCF-7 breast tumor cell-induced VEGF release and, in fact, leads to a net anti-angiogenic effect of platelets [77]. Taken together, these studies provide strong evidence that platelets, upon activation by tumor cells, release potent pro-angiogenic mediators to provide the growing tumor with a blood supply.

Angiogenic vessels within the tumor tend to be immature and leaky, and platelets seem to preferentially adhere to angiogenic vessels over normal, mature vessels [85]. This differential adherence may be one way in which platelets are attracted to

and enter the TME. Once inside the TME, platelets not only drive angiogenesis but can also function to stabilize angiogenic vessels [86]. Specifically, platelets seem to support pericyte coverage in angiogenic vessels and angiopoietin-1, and serotonin released from platelets may promote vessel maturation [83, 87]. In this way, platelets normalize tumor vasculature and prevent intratumoral hemorrhage. While angiogenesis is primarily a pro-tumorigenic process, vessel stability is more complex and may have antitumor effects. Therefore, more research is needed to parse the specific signals, conditions, events, and intermediates that favor platelet-induced angiogenesis or vessel stabilization.

Platelets are also capable of taking up molecules from their environment. Angiogenic factors including VEGF and bFGF are taken up by platelets from the site of the tumor, stored, trafficked, and delivered to other locations such as distant metastatic sites [88–90]. A recent 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 anatomical sites where these platelets contribute to the growth and angiogenesis of the otherwise indolent tumor [32]. Platelet inhibition with aspirin prevented this effect, suggesting that platelets were, in fact, responsible for delivering angiogenic signals from one tumor to the other [32]. These studies highlight the potential for platelets to serve as long-haul cargo carriers, shuttling signals between distant sites as orchestrated by the tumor. However, little is currently known about the mechanism by which platelets endocytose proteins from their environment, 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 the circulation.

Platelet-Tumor Cell Interactions in the Circulation

The circulation is a very hazardous environment for newly disseminated tumor cells, one in which tumor cells face harsh shear stresses and constant immune surveillance. The vast majority of tumor cells are destroyed within hours of introduction into the circulation, well before they can ever successfully form metastases [91, 92]. As previously discussed, contact between platelets and tumor cells causes aggregates of the two cell types to form. These aggregates can be readily identified in the circulation of cancer patients and form within minutes of tumor cell introduction into the blood stream of mice, suggesting that tumor cells fair better in circulation when coated in platelets [63]. The mechanical forces exerted on tumor cells in the blood are far greater than what was experienced in the TME and is often enough to cause their destruction [93]. Platelets are naturally suited to thrive within the

vasculature and provide protection by coating tumor cells, shielding them from shear stress [94].

Circulating tumor cells are under constant assault from immune surveillance particularly from NK cells, and platelets play a crucial role in protecting them. Activated platelets express glucocorticoid-induced tumor necrosis factor receptor ligand (GITRL) on their surface, which binds to the GITR on NKs, leading to inhibition of NK cell activity [95]. Platelets can also inhibit NK cells by modulating expression of the NKG2D receptor on the NK cell surface [96]. NK cells use this receptor to identify and lyse tumor cells. TGF-β released from activated platelets can cause downregulation of NKG2D on the NK cell surface, making NKs less able to identify and destroy tumor cells [96]. Platelet-derived TGF-β also inhibits NK cell IFNγ production and stimulates the development of immune-dampening T-regulatory cells [97, 98]. Furthermore, platelets can protect tumor cells from NK destruction by transferring MHC class I molecules to the tumor cell surface [99]. Tumor cells may also avoid lysis in the blood by aberrantly expressing integrins normally found on platelets in a phenomenon known as platelet-mimicry [100, 101]. Although a great deal has been discovered regarding how platelets disrupt immune surveillance in the circulation, relatively little is known about how platelets interact with immune cells within the TME and if these mechanisms may also apply at these sites.

Extravasation

Tumors cells must find ways to successfully exit the circulation in order to seed a new metastatic site. Immobile platelet-tumor cell aggregates have been observed in the microvasculature [102, 103], 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 [28, 41]. The importance of P-selectin in this process has been demonstrated in mice through pharmacological blockade as well as genetic ablation of P-selectin [42]. Next, platelet integrins mediate arrest on the endothelium, while platelet-derived factors such as MMP-1, TGF-β, and ADAM12 break down junctions between endothelial cells, allowing tumor cells to cross the now leaky endothelial barrier and enter the surrounding tissue parenchyma [103, 104].

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 the unenviable but critical role of 'fertilizer'" [105]. 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 occur in cancer. Platelets can propagate messages that serve to mobilize bone marrow progenitors, alter bone function, and even prepare sites to accept future metastases. Tumors recruit bone marrow-derived cells (BMDCs) and endothelial progenitor cells to the TME. SDF-1, VEGF, and ANGPT-1 released from activated platelets have been implicated in mobilizing BMDCs and progenitor cells from the bone marrow [86, 106, 107]. 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 [63]. Kuznetsov et al. also demonstrated that platelets, acting as a long-range communication system between primary tumors, distant tumors, and the bone marrow, cooperate with BMDCs to promote vascularization of the distant tumors [32].

Bone remodeling often occurs in the setting of metastatic disease and platelets may mediate this process as well. The presence of a primary melanoma or prostate tumor increased bone formation in mice, while platelet depletion reversed this effect [108]. In these two models, platelets traffic tumor-derived MMP-1 and TFG-β to the bone where they promote bone formation. Conversely, platelets are also capable of increasing bone resorption to facilitate breast cancer metastasis to the bone. Boucharaba and colleagues demonstrated that platelets promote osteolytic bone loss by a complex mechanism in which lysophosphatidic acid (LPA) released from activated platelets drives IL-6 and IL-8 secretion from tumor cells [109]. These cytokines then stimulate bone-destroying osteoclasts. 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 [108].

Platelets clearly help orchestrate the complex coordination of events that allow 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 that these mechanisms occur in human patients and, if so, determine potential therapeutic interventions (Fig. 12.2).

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 or shear stress and consist of membrane-bound proteins and cytoplasmic components. In vitro, PMPs have similar pro-angiogenic and pro-metastatic potential as live platelets and increase EC migration and tube formation as well as promote tumor cell MMP production and invasion through matrigel [110–112]. 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

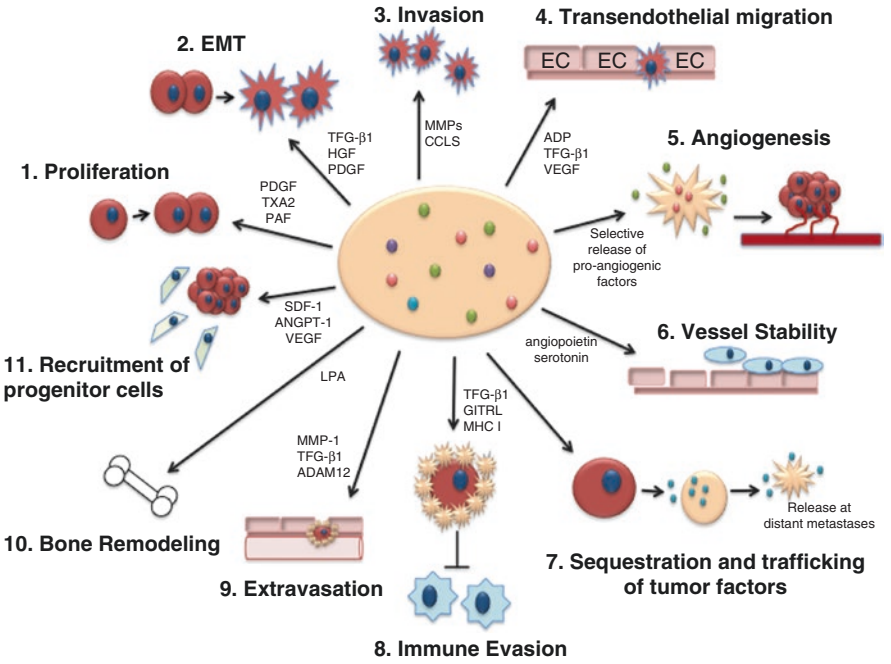

Fig. 12.2 Mechanisms by which platelets promote cancer. To date, platelets have been found to employ a wide variety of strategies to promote tumor progression and metastasis. These include direct effects on tumor cells including driving proliferation, invasion, and EMT [1–3]. Platelet-tumor cell cross talk also affects the vasculature in ways that promote angiogenesis, impact vessel stability, and facilitate intravasation and extravasation of tumor cells [4–6, 9]. Tumor cells instruct platelets to aid in immune evasion, remodeling of bone marrow, and recruitment of tumor-promoting progenitor cells [8, 10–11]. Additionally, platelets carry and deliver signals between distant sites including primary tumors, secondary tumors, and the bone marrow [7]. In this schematic, these mechanisms and any factors thought to be involved are highlighted

more metastatic when coated with PMP prior to injection, and, in an ischemia model, introduction of PMPs increased angiogenesis [113, 114].

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 cross talk 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, there are some studies that can offer insights.

Unlike cell lines derived from solid tumors, many leukemia cells do not activate platelets and have been shown to inhibit activation and aggregation of platelets [115–117]. Patients with leukemia often present with thrombocytopenia (low platelet count), and their platelets display lower numbers of dense granules [118, 119]. Conversely, a few AML and CML cell lines are able to activate and aggregate platelets, and the resulting releasate increases tumor cell proliferation and survival [120–122].

The role of platelets in multiple myeloma seems even more complicated. These patients tend to have thrombocytopenia but also have elevated soluble P-selectin and TPO levels and are at increased risk of developing VTEs [123–125]. Platelets contain and release factors known to support multiple myeloma progression such as IL-6, SDF-1, and IGF-1, suggesting that platelets are poised to play a role in multiple myeloma. However, studies have not yet been done to determine if these platelet-derived factors do, in fact, mediate the progression of this disease.

Overall, the role of platelets in hematological malignancies is not well explored. Because thrombocytopenia is a feature of many hematological malignancies, it stands to reason that the role of platelets may not be as important in that context as has been observed in solid tumors. However, since platelets are shown to be altered in 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 the 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 cancer patients are fundamentally different from those of healthy individuals. Platelets from breast, prostate, lung, and colon cancer patients display higher baseline activation, suggesting that they may be more reactive and have a lower threshold for activation than platelets from healthy donors [126–129]. Elevated surface levels of the activation marker P-selectin as well as increased platelet markers in the plasma such as CD40 ligand, β-thromboglobulin, and soluble P-selectin have been observed in cancer patients, and these markers tend to correlate with disease progression and poor prognosis [130, 131]. Patients presenting with elevated soluble P-selectin are more than twice as likely to develop a VTE compared to cancer patients with low levels [131].

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 [89, 132]. It was reported that platelets from cancer patients also contain altered mRNA transcripts [133]. These transcripts appear to come from two sources: platelets take them up from tumor cells, 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 [134].

Thrombocytosis is associated with nearly every type of solid tumor suggesting that the presence of a tumor likely influences platelet production. Thrombopoietin (TPO) is the dominant driver of megakaryocyte differentiation and maturation. Preclinical 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 [135]. IL-6 levels in patients correlate with platelet count, and anti-IL-6 therapy reverses this trend [136, 137]. 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 [138]. 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 [139–142].

Although an abundance of evidence proves that platelet function, contents, and numbers are altered in cancer patients, we have only begun to understand how this occurs. Studies are needed to elucidate the effect of tumors on megakaryocyte biology and the platelets that result. Understanding the mechanism or mechanisms underlying cancer-associated thrombocytosis remains crucial, as therapies directed at this process could prevent tumors from producing an ever-increasing army of platelets that can add fuel to the fire of tumor progression.

Antiplatelet 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 could theoretically block mechanisms of invasion, EMT, angiogenesis, metastasis, and activation of other host cells in the microenvironment and bone marrow. Platelet mimicry, along with many shared surface markers between platelets and tumor cells, suggests that platelet-targeted drugs could also impact the tumor as well.

Preclinical data is quite promising and reveals that targeting a number of different platelet receptors may be an effective approach for limiting cancer progression. Antiplatelet drugs that are currently available for the treatment of cardiovascular disease are now being explored as antitumor 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 [143]. Clopidogrel, a P2Y12 antagonist used to treat cardiovascular disease, shows antitumor properties in vitro and can prevent bone destruction and metastasis in mice [144]. Anticoagulants including fondaparinux and low molecular weight heparins (LMWH) inhibit tumor cell-induced platelet activation and attenuate the angiogenic potential of platelets in vitro [145]. 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 shows mixed results [146–149]. Large-scale clinical trials are needed to access the efficacy of currently available antiplatelet drugs.

Aspirin is perhaps the most intriguing antiplatelet agent that has been studied to date. A massive, long-term epidemiological study by Rothwell et al. revealed that individuals who take aspirin daily are less likely to be diagnosed with cancer and show improved survival if they do develop cancer [150]. The mechanism of action for aspirin's efficacy in cancer was originally thought to be due to decreased inflammation via COX inhibition. However, the doses taken were not high enough to prevent inflammation but do cause platelet inhibition. Subsequent studies also point to a platelet-based mechanism; platelet inhibition with aspirin diminishes platelet activation, protein release, and ability to induce angiogenesis [77]. Mouse models also confirm that platelet inhibition with aspirin decreases metastasis and improves outcomes [25, 32, 151]. Aspirin seems most effective in chemoprevention but may also be beneficial if taken as part of treatment [152–154]. The exact mechanism by which aspirin inhibits platelet function is not well understood, and answering this question could lead to the development of drugs that are more specific and efficacious than aspirin but work on the same principle (Fig. 12.3).

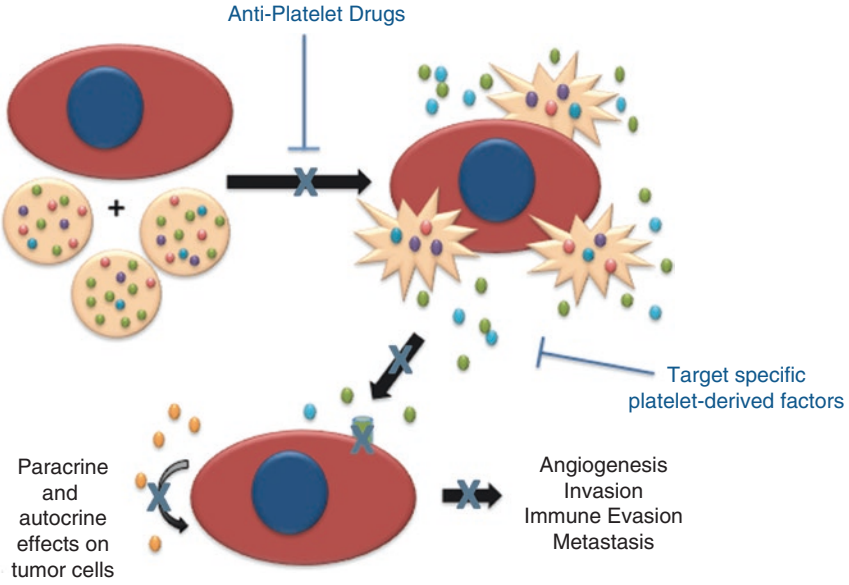

Fig. 12.3 Antiplatelet agents as novel cancer therapeutics. Because platelets play an active role in the progression and spread of many solid tumor types, they present an attractive target for therapy. Platelet inhibitors including aspirin, anticoagulants, and antiplatelet agents such as GPIIbIIIa blockers and P2Y12 antagonists may limit cancer progression by preventing tumor cell-induced platelet activation. Targeting specific platelet-derived factors and/or cognate receptors also present a currently underexplored approach to therapy that could prevent the tumor-promoting effects of platelets while sparing their important hemostatic and thrombotic functions

Conclusions

Platelets are 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 carries, delivering messages to and from the tumor in ways that allow the cancer to progress. These 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 or as potential therapeutic targets in the fight against cancer.

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