Platelet-Based Drug Delivery for Cancer Applications

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Abstract

Platelets can be considered as the "guardian of hemostasis" where their main function is to maintain vascular integrity. In pathological conditions, the hemostatic role of platelets may be hijacked to stimulate disease progression. In 1865, Armand Trousseau was a pioneer in establishing the platelet-cancer metastasis relationship, which he eventually termed as Trousseau's Syndrome to describe the deregulation of the hemostasis-associated pathways induced by cancer progression (Varki, Blood. 110(6):1723–9, 2007). Since these early studies, there has been an increase in experimental evidence not only to elucidate the role of platelets in cancer metastasis but also to create novel cancer therapies by targeting the platelet's impact in metastasis. In this chapter, we discuss the contribution of platelets in facilitating tumor cell transit from the primary tumor to distant metastatic sites as well as novel cancer therapies based on platelet interactions.

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12.1 Cancer Metastasis

12.1.1 Primary tumor support

Cancer-associated thrombosis has exhibited multiple roles that not only promote cancer migration to distant organs but also support the stability and overgrowth of the primary tumor (Fig. 12.1). Of note, one report pointed out that the tumor microenvironment (TME) includes a significant number of platelets whose function is associated with the maintenance and support of the tumor mass expansion via secretion of platelet-derived microparticles (P-MPs) [2, 3]. Platelets are anucleated cells that serve as a storage for several P-MP types containing angiogenesis regulator factors, such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), matrix metalloproteinases (MMP), platelet factor-4 (PF4), plasminogen activator

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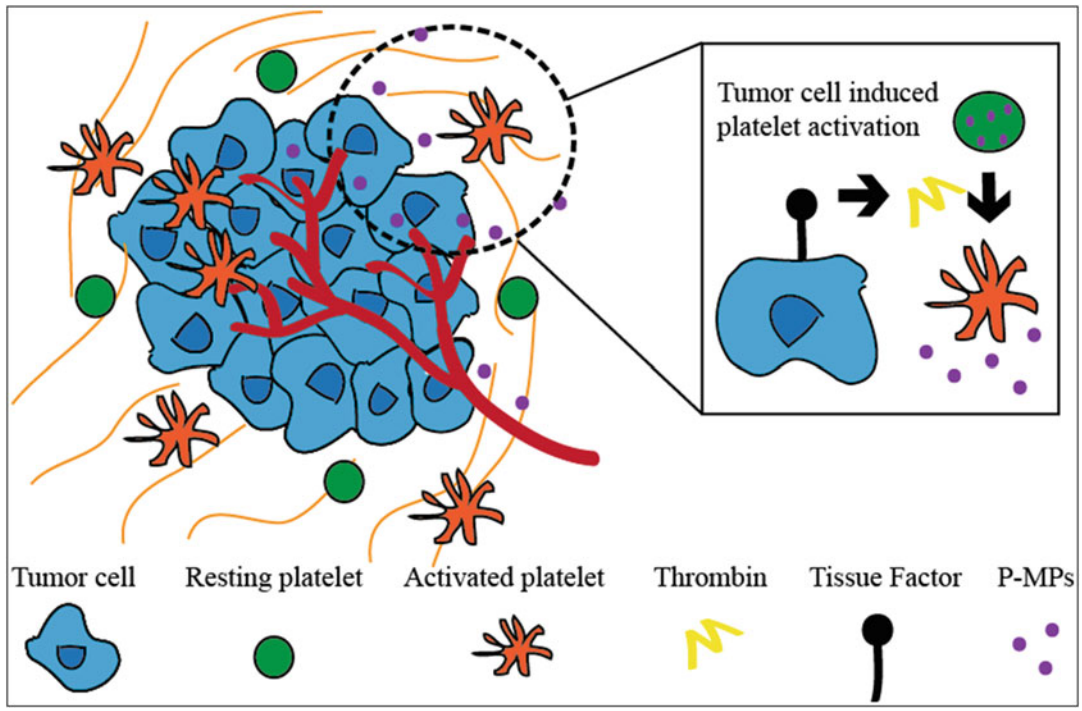

Fig. 12.1 Primary tumor mass expansion regulated by activated platelets

inhibitor-1, angiopoeitin-1, and transforming growth factor beta 1 (TGF-beta 1), among others [4].

Several studies have shown that P-MPs play important roles in promoting tumor cell proliferation, tumor vascular integrity, and cancer cell invasion. Once tumor cells are introduced, P-MPs induce an activation of MAPK and AKT signaling pathways to stimulate the protein overexpression required to self-sustain tumor cell proliferation. The P-MPs transport pro-angiogenic factors such as VEGF to induce abundant vascularization to supply nutrients and oxygen to support tumor overgrowth and stability [5, 6]. Studies have demonstrated that platelet depletion induces a substantial reduction in blood vessel density and coverage, leading to vascular leakage in the primary tumor where it is associated with tumor hemorrhage that initiates tumor hypoxia and necrosis [2, 7, 8]. Along with tumor overgrowth and stability, P-MPs induce a more invasive phenotype in malignant cells via secretion of matrix metalloproteinases and upregulation of their expression. Moreover, P-MPs can deliver adhesion molecules to tumor cells to provide the ability to bind to host cells, a key behavior in the metastatic cascade [5, 6].

P-MPs are considered a vital part of the TME, contributing to primary tumor engraftment and stability; however, their roles depend on the level of platelet activation. Strong evidence indicates that many tumor cells express tissue factor (TF) to trigger local thrombin synthesis in the TME, which binds to platelet receptors known as protease-activated receptors (PARs) to lead to activation of platelets [9]. TF expression in malignant cells is often correlated with two genetic modifications in carcinogenesis, namely, alteration in the k-*ras* oncogene and loss of tumor suppressor as *p53* [10]. Collectively, tumor cells express TF to generate thrombin as a paracrine signal, which triggers a strong positive feedback loop between tumor cells and platelets to self-promote tumor cell proliferation, invasion, and, eventually, metastasis.

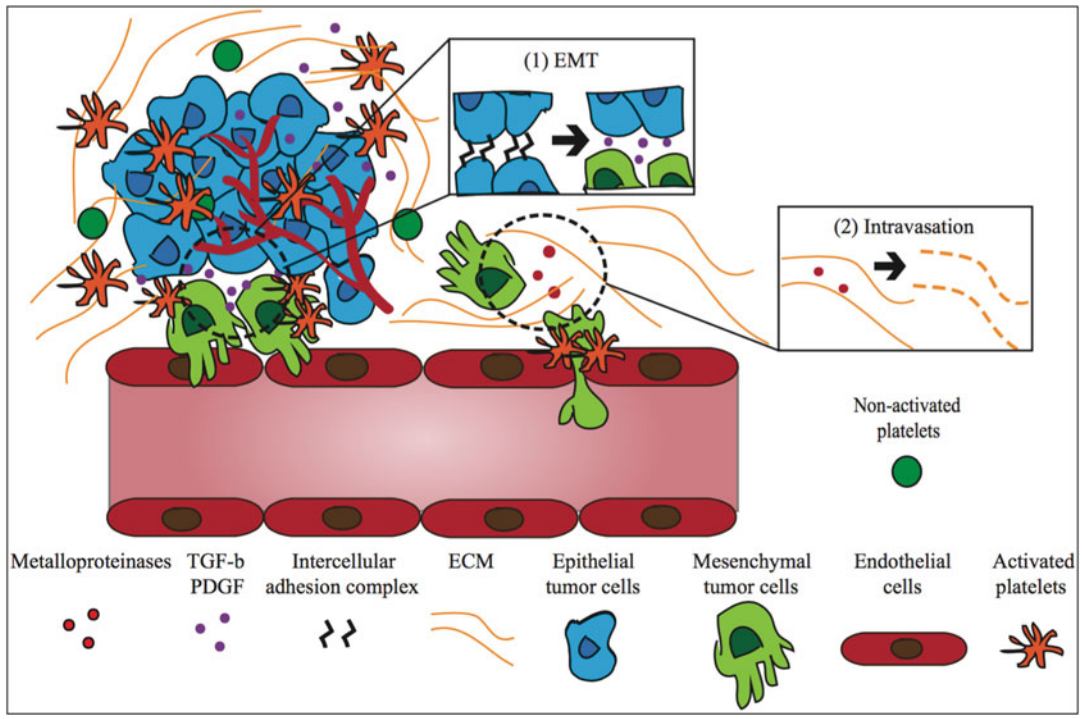

Fig. 12.2 Epithelial-mesenchymal transition affected by platelets

12.1.2 Epithelial-Mesenchymal Transition and Intravasation

Initially in metastasis, malignant cells go through a morphological change known as epithelial-mesenchymal transition (EMT) that produces an invasive phenotype in tumor cells. EMT is a reversible process that cells undergo through a combination of molecular signaling pathways, which leads to the loss of the intracellular adhesion complex. This allows tumor cells to become highly mobile to migrate and eventually invade the vasculature. As revealed by several studies, the principal role of platelets in early metastasis is associated with the secretion of growth factors, such as PDGF and TGF-beta, to trigger EMT activation in tumor cells (Fig. 12.2) [11]. Experimental data indicate that tumor cells pretreated with platelets show an upregulation of mesenchymal markers as well as a downregulation of epithelial markers [12]. Besides these platelet-derived factors, platelets can induce EMT activation in tumor cells via direct contact between platelet and tumor cells. A strong body of evidence supports the idea that platelet-tumor cell contacts are due to a variety of adhesion molecules present on the platelet membrane that use plasma proteins to mediate these interactions. For example, the $\alpha_v\beta_3$ integrin expressed in tumor cells can bind to GPIIb/IIIa platelet integrin via plasma proteins such as fibrinogen, fibronectin, and von Willebrand factor (vWF) [13, 14]. This interaction activates the $\alpha_v\beta_3$ integrin and triggers various signaling pathways to eventually stimulate the NF-kB pathway to induce the gene transcription required for EMT activation [12, 15]. While the TGF-β and NF-kB pathways are key mechanisms that induce EMT activation, they also induce MMP upregulation that is critical for tumor cell invasion due to enhanced extracellular matrix (ECM) degradation [12, 15, 16]. In summary, the secreted factors from platelets together with direct platelet-tumor cell contact can stimulate two different critical signaling pathways in an independent manner. The synergy of both pathways induces the EMT

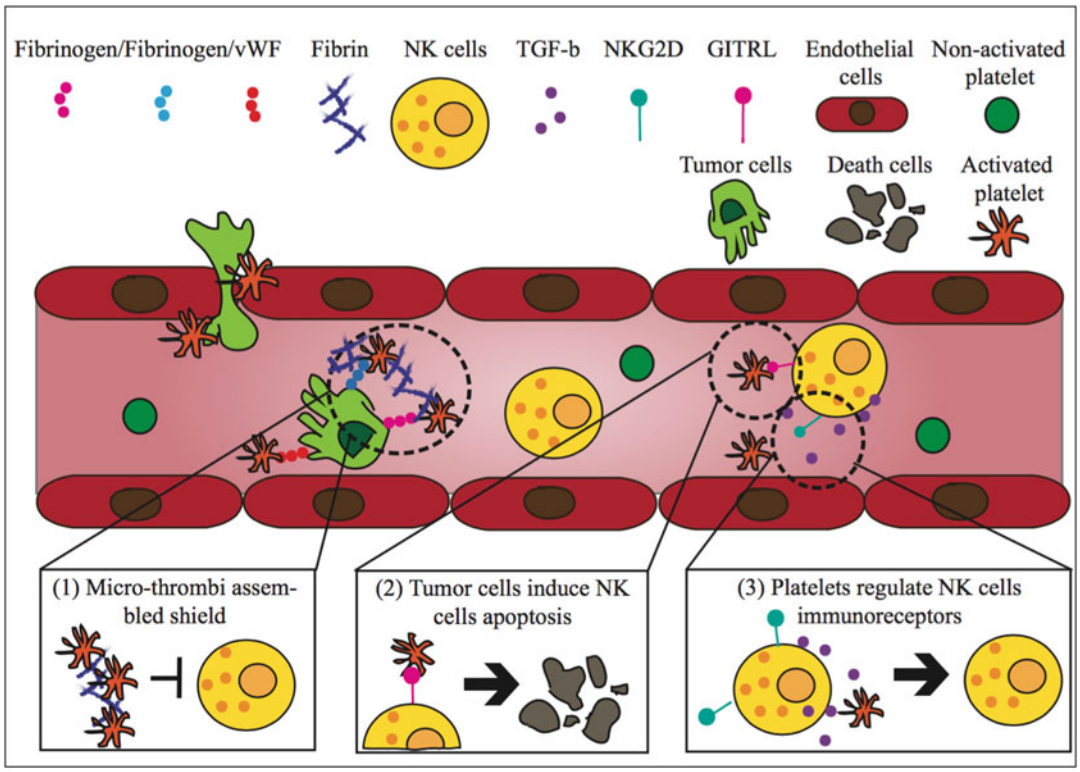

Fig. 12.3 Platelets protect tumor cells in the circulation

transformation in tumor cells and metalloproteinase expression to promote tumor cell invasion in the circulation, an early event in metastasis.

12.1.3 Protection Conferred to Circulating Tumor Cells

Once tumor cells enter the bloodstream, these "circulating tumor cells" (CTCs) travel through the vasculature and may arrive in a suitable microenvironment to form a secondary metastatic tumor. To reach distant organs, malignant cells must overcome several obstacles in the bloodstream such as mechanical stress and immune surveillance by natural killer (NK) cells. During intravasation, tumor cells become exposed to hemodynamic shear stresses from 0.5 dyn/cm^2 to 30 dyn/cm^2 and NK cell cytotoxicity, which can neutralize CTCs and eventually hinder metastasis [17, 18]. Accumulating experimental data strongly suggests that one role of pro-metastatic platelets is to protect CTCs from such stresses, facilitating malignant cell migration [19] (Fig. 12.3). Platelets protect CTCs by two mechanisms, which include a microthrombi-assembled shield and the downregulation of NK cell antitumor activity.

The microthrombi-assembled shield refers to platelets that adhere to CTCs in an envelope fashion, which, coupled with fibrin deposition, help to create a physical barrier that prevents direct contact, up to 80%, with NK cells and the mechanical influence of the bloodstream [20–24]. Besides the formation of a microthrombi cloak, platelets are able to impair the NK cell-regulated elimination of CTCs through platelet-derived and bound factors that can downregulate the activation of immunoreceptor expression and function.

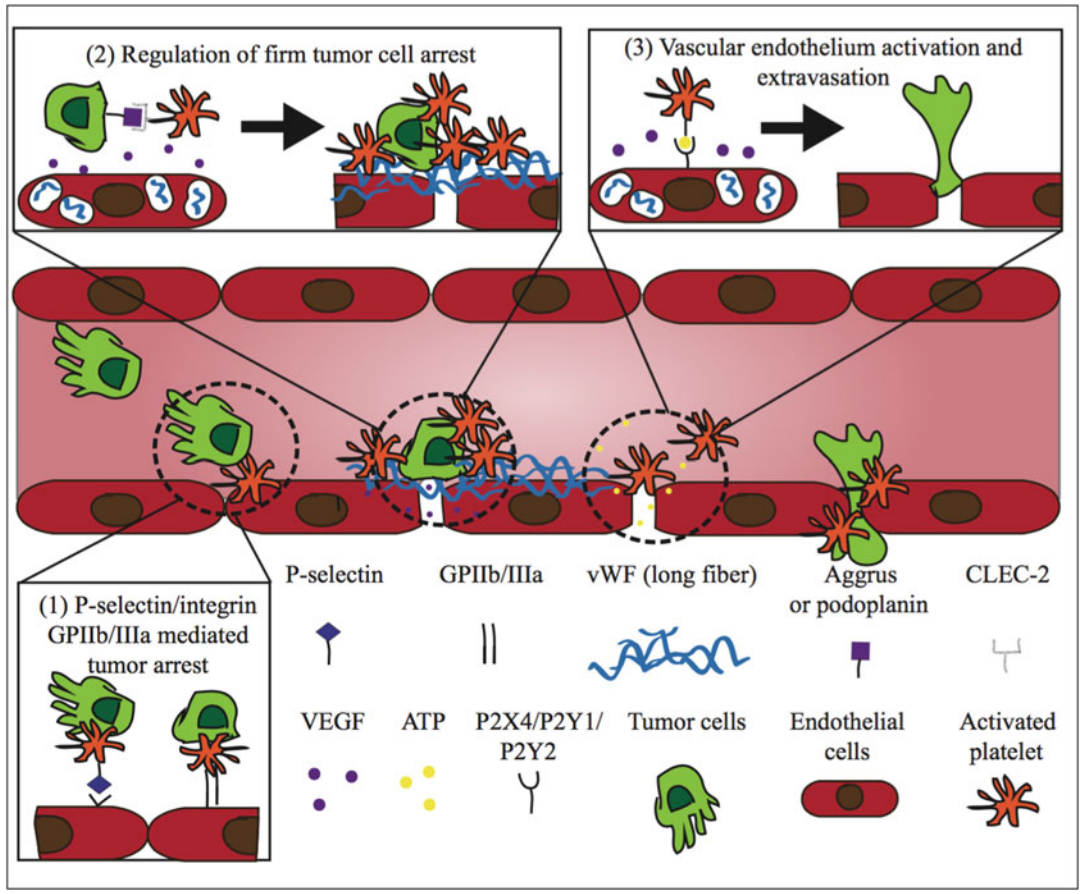

Fig. 12.4 Platelets facilitate tumor cell arrest and extravasation

Through platelet-tumor cell interactions in microthrombi, activated platelets secrete factors including TGF-β, which reduces the immunoreceptor expression of natural killer group 2 (NKG2D) that is critical for triggering NK cell antitumor effect [25, 26]. Similarly, platelets can express the glucocorticoid-induced TNF-related ligand (GITRL), which interacts with its GITRL receptor to inhibit NK cell antitumor activity by promoting apoptosis of NK cells [27, 28]. Altogether, cumulative evidence demonstrates that platelets impact CTC survival in the circulation through the interplay of coagulating proteins and platelet-derived and bound factors. Indeed, the principal pro-metastatic role of platelets is the protection of CTCs in the bloodstream since tumor cell survival is one of the most determinant factors that result in effective metastasis.

12.1.4 Circulating Tumor Cell Arrest and Extravasation

To facilitate tumor cell extravasation, CTCs can adhere to activated vascular endothelial cells and complete organ-specific transendothelial migration. Cumulative evidence suggests that platelets play a central role in mediating extravasation by facilitating the intercellular interactions between tumor and endothelial cells (Fig. 12.4). During initial arrest, malignant cells can tether over the vascular endothelium in a P-selectin-dependent mechanism enhanced by the platelet integrin GPIIb/IIIa. According to some previous studies, the integrin GPIIb/IIIa can immediately arrest tumor cells without previous tethering interactions [29, 30]. Platelet-tumor cell adhesion via integrin GPIIb/IIIa is due to the

expression by malignant cells of the counter-receptor GPIIb/IIIa which mediates cell-cell interactions via plasma proteins acting as a "molecular bridge" connecting both cells (see Sect. 12.1.2) [31–33]. Platelets contain other adhesion proteins that can also contribute to tumor cell-platelet-endothelial cell adhesion, such as glycoprotein Ibα and glycoprotein VI, which some studies have found relevant but not determinant in promoting tumor metastasis [34–36].

Once tumor cells arrive at the vascular endothelium, platelets can trigger two different mechanisms to maintain the cells' firm attachment until transendothelial migration is completed. These mechanisms include the activation of vascular endothelium and local platelet aggregation. The activation of vascular endothelium is mediated by tumor cell- and platelet-derived VEGF-A and induces the secretion of large fibers of vWF that form a mesh structure in the vascular wall to support tumor cell arrest [37]. Regarding platelet aggregation, tumor cells can express Aggrus protein that interacts with its counter-receptor, C-type lectin like receptor 2 (CLEC-2), in platelets to trigger a signaling pathway that induces platelet activation and aggregation [38, 39].

To complete transendothelial migration, malignant cells can elicit two molecular mechanisms using platelet-derived factors, VEGF, and adenosine nucleotides (ATP) [34, 35]. Several experimental studies have indicated that platelet- and tumor cell-derived VEGF activates the vascular endothelium, stimulating vascular permeability by relocating the main intercellular adhesion protein VE-cadherin in endothelial cells from the cell surface to the cytoplasmic compartment and creating gaps in the endothelium [40–42]. Recent studies have determined that platelet-derived ATP can bind to its receptors $P2X_4$, $P2Y_1$, and $P2Y_2$ on vascular endothelial cells to activate a signaling pathway that subsequently induces intracellular calcium increase and increases vascular permeability [43]. Overall, a substantial number of studies point out the significant role of platelets in mediating tumor cell arrest and the activation of vascular endothelium to promote cancer cell extravasation.

12.1.5 Colonization of the Secondary Metastatic Niche

At distant metastatic sites, disseminated tumor cells (DTCs) are not sufficient to stimulate self-seeding and proliferation to form a secondary tumor. Prior to development of a secondary tumor, a mature metastatic niche must be generated that is a suitable microenvironment and is promoted by host stromal cell recruitment and subsequent DTC seeding and proliferation (Fig. 12.5). Strong evidence suggests that metastatic niche formation is mediated by chemokines secreted by platelets and endothelial cells, such as stromal cell-derived factor (SDF-1), CXC motif chemokine ligand 5 and 7 (CXCL5/7), and CC chemokine ligand 5 (CCL5) [44–46]. The main host components recruited by these chemokines are bone marrow-derived cells that include hematopoietic progenitor cells ($VEGFR1^+$HPCs), granulocytes, and monocytes/microphages. $VEGFR1^+$HPCs are the first host cells recruited into the metastatic site and are crucial for triggering MMPs and VEGF-A production for revascularization of the tissue to create a pre-metastatic niche prior to DTC arrival [47]. To cause $VEGFR1^+$HPC recruitment, activated platelets close to the extravasation site secrete SDF-1 that interacts with its counter-receptor, CXCR4, in HPCs to trigger their migration [44–48]. Similarly, the activation of the SDF-1/CXCR4 pathway can mediate the expression of CXCR4 receptor following HPC migration [49].

Once DTCs arrive at the distant organ, granulocyte mobilization is necessary because these can provide MMPs that enhance DTC extravasation and homing in the metastatic niche. In this scenario, studies indicate that the secretion of CXCL5/7 from activated platelets generates a chemoattractant gradient that eventually induces granulocyte recruitment

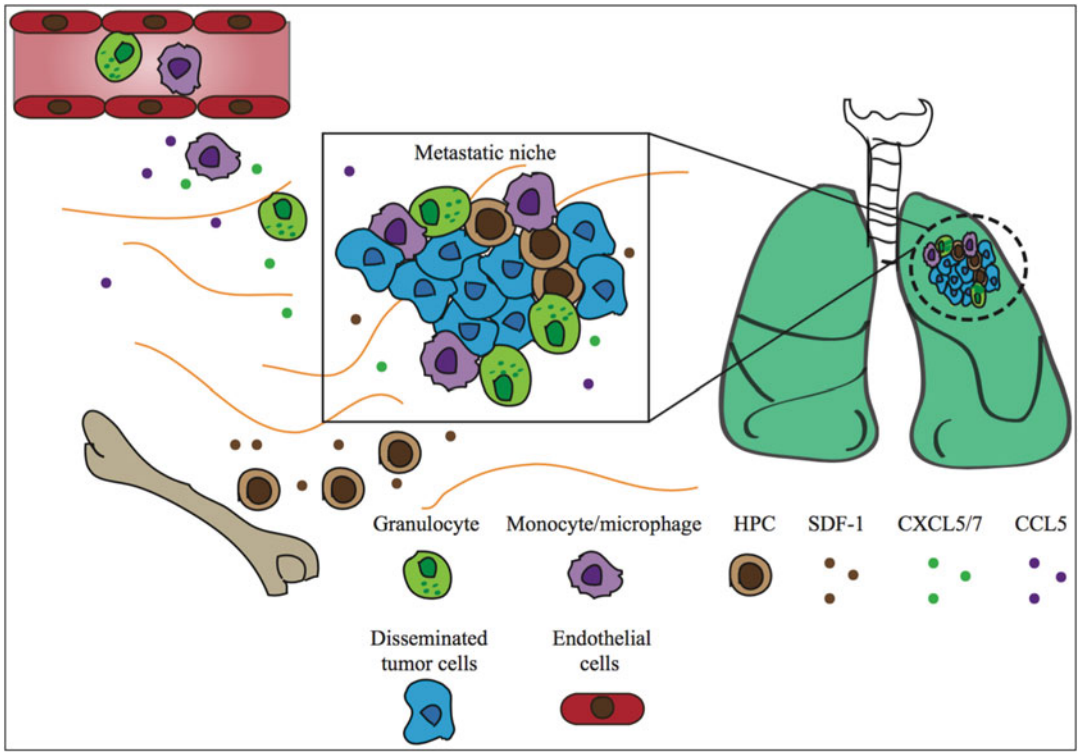

Fig. 12.5 Platelets recruit host cells to shape the metastatic niche

to platelet-tumor cell clusters to form a premature metastatic niche [45]. Along with granulocytes, monocyte/macrophage migration toward DTCs is necessary to guide the maturation of the metastatic niche. Monocyte/macrophage recruitment is crucial for triggering signaling pathways that promote the survival of DTCs escaping from immune surveillance. To achieve monocyte/macrophage recruitment, DTCs activate coagulation pathways to activate platelet aggregation, creating small thrombi that surround the DTCs and induce monocyte/macrophage migration [50]. DTC-leukocyte-platelet interactions in the clot mediated by P-selectin-dependent adhesion can trigger CCL5 secretion from the vascular endothelium to induce monocyte recruitment [46]. Altogether, platelets play a significant role in providing stimulating chemokine secretion as well as activation of chemokine secretion from other host cells to create a suitable microenvironment necessary for the promotion of metastatic tumor seeding and eventually tumor overgrowth.

12.2 Platelet-Based Cancer Therapy

12.2.1 Anticoagulants as Cancer Therapy

Cancer patients are more likely to develop venous thromboembolism (VTE) and have an increased risk of dying from thrombotic events than VTE patients without cancer. Increased procoagulant molecules such as tissue factor (TF) and cancer procoagulant (CP) during cancer development result in increased incidence of thrombosis and contributes to tumor growth and metastasis. The opposite also holds true in that patients with VTE have a fourfold increased risk in developing cancer after 1 year of diagnosis with VTE [51]. Given the link between coagulation and cancer, anticoagulants were studied for their antitumor benefits and to control thrombotic events in cancer patients.

Common anticoagulants examined for their effect on cancer are warfarin and low-molecular-weight heparin (LMWH). Warfarin works by interfering with vitamin K-dependent carboxylation of coagulating proteins such as prothrombin and factors VII, IX, and X [52]. One of the first clinical studies conducted to test the efficacy of warfarin in patients with various cancers found that warfarin treatment resulted in improved survival of patients with small cell lung cancer but had no effect on survival of patients with advanced non-small cell lung, prostate, colorectal, and head and neck cancer [53]. Additionally, a clinical trial involving fixed, low-dose administration of warfarin for stage IV breast cancer patients found no difference in survival between treatment groups [54]. However, a study comparing 6-week and 6-month treatments of warfarin in cancer patients found the effects of warfarin evident 2 years after treatment in patients with 6 months of treatment, and the antitumor activity was observed for 6 years [55]. Further research is required to understand how the stage of cancer in patients, administration of dose and timeline of treatment, as well as type of cancer can affect the antitumor activity of warfarin.

Like warfarin, LMWH works by enhancing the activity of antithrombin which inhibits thrombin as well as other factors such as factor IX, X, and XI. Additionally, heparin can bind to and inhibit platelet activity and can also inactivate factor II through binding to heparin cofactor II [56]. LMWH is considered the first line of therapy for cancer patients that are diagnosed with VTE. There are various LMWH derivatives that have been tested against cancer such as dalteparin, enoxaparin, as well as unfractionated heparin. Cancer patients treated with a form of LMWH, dalteparin, were found to have reduced recurrent thromboembolism compared to cancer patients treated with warfarin [57]. Another clinical study with patients diagnosed with breast, colorectal, ovarian, pancreatic, and other cancers found that treatments with LMWH showed no differences in survival after 1, 2, and 3 years post treatments. However, patients with good prognosis had significantly increased survival after 2 and 3 years of treatment compared to placebo [58]. Additionally, clinical trials testing LMWH in combination with chemotherapeutics in patients with advanced pancreatic cancer and small cell lung cancer also found improved overall survival in patients compared to chemotherapeutic alone [59, 60].

Other anticoagulants tested for antitumor activity include aspirin which is known to inhibit platelet activity. Aspirin has been shown to reduce the risk of adenocarcinoma and prevent metastasis in one clinical study [61]. Another study also showed a reduced reported diagnosis of cancer in diabetic patients treated with aspirin compared to those without [62]. However, whether the effects of aspirin on cancer progression are due to its platelet inhibition properties via inhibition of cycloogenase-1 (COX-1) pathway or anti-inflammatory affects via COX-2 inhibition have yet to be ascertained. Additional drugs include desirudin and argatroban, which are thrombin inhibitors; nonsteroid anti-inflammatory drugs such as ibuprofen; heparan sulfate mimetics such as M402; matrix metalloproteinase inhibitors which affect platelet activation; and pentasaccharide anticoagulants and direct factor X inhibitors which have recently been studied or considered for antitumor activity. Many researchers aim to improve pharmacokinetics, specificity, and delivery of anticoagulants through development of oral coagulants that target specific clotting factors on platelets. Some commercially available examples include thrombin inhibitor dabigatran and the factor-Xa inhibitors apixaban and rivaroxaban [63]. However, despite some of the positive results that show improved survival with the use of anticoagulants, recent studies show that a combination of anticoagulants with cancer therapies may contribute to potential drug interactions that result in gastrointestinal toxicity [64]. The heterogeneity of cancer and patient treatments prompts additional studies into the effects of anticoagulants.

12.2.2 Monoclonal Antibodies Against Platelet Proteins

Monoclonal antibodies (Mab) and inhibitors targeting platelet proteins provide another unique approach to targeting platelet function during cancer progression. Antibodies targeting platelet-derived growth factors (PDGF) are one of the emerging therapies for interrupting tumor progression. PDGF are mitogens released by platelets that promote cell growth and proliferation for various cells, such as mesenchymal cells, and have been found to promote angiogenesis and be overactive in cancer patients. To date, there are several inhibitors of PDGF, such as imatinib and sunitinib, which have been FDA approved to target the receptor kinases of PDGF for inhibiting leukemia and renal cancer progression [65]. However, as the PDGF receptor kinase inhibitors are not specific to PDGF kinases only, alternative options with increased specificity have been researched over the past few decades. Examples include Mab 6D11, CR002, and C3.1 which neutralize PDGF mitogenic activity and show specific targeting of PDGF ligands [66–68]. Other antibodies also target the receptor for PDGF including CDP860, IMC-2C5, and 3G3 [69–71]. IMC-2C5 did not exhibit any significant antitumor activity in murine xenograft models with the ovarian carcinoma cell line OVCAR-5 [69]. Monoclonal antibody 3G3, however, showed antitumor activity in mice xenograft models with glioblastoma and leiomyosarcoma compared to controls in one study [70]. Recently, the FDA-approved olaratumab, a monoclonal antibody targeting the PDGF receptor subunit, in combination with doxorubicin for treatment of soft tissue sarcoma shows significantly improved overall survival compared to doxorubicin alone.

Aside from PDGF, antibodies targeting platelet activity also exist to reduce tumor-associated thrombosis. Abciximab is one such antibody which targets the GPIIb/IIIa complex on the surface of platelets and has been shown to block platelet aggregation and secretion of angiogenic factors. Eptifibatide, XV454, and tirofiban also have similar functions as abciximab as they also target the GPIIb/IIIa glycoproteins and inhibit platelet activity, though the mechanisms of action vary with each antagonist [71]. The GPIIb/IIIa proteins are integrins that serve as receptors for binding to fibrinogen, fibrin, fibronectin, vitronectin, and von Willebrand factor [72]. When platelets are activated, these glycoproteins result in platelet aggregation as the platelets bind to fibrinogen and initiate clotting. The use of glycoprotein antagonists reduced platelet aggregation and tumor burden in mice and rats and have been shown to interrupt interactions in the tumor microenvironment [72–75].

Moreover, antibodies targeting other modalities of platelets such as fibronectin (antibody A3.3) and matrix metalloproteinases (such as MMP2) which are involved in platelet aggregation have been developed and have been shown to inhibit thrombin formation and aggregation [76, 77]. Antibodies targeting coagulation factors such as tissue factor, factor IX, and factor IXa are in development for both reducing thrombotic events and improving cancer outcome. One antibody developed against tissue factor is the recombinant mouse antibody D3H44, which effectively targets tissue factor and can be neutralized through the use of competing antibodies [78].

Antibodies targeting platelet proteins and functions continue to be characterized and discovered, though cost of production is one limitation to antibody commercialization. As the role of platelets in tumor progression is further elucidated, targeted cancer treatments involving the use of antibodies serve as attractive methods for future studies in achieving antitumor activity.

12.2.3 Drug Delivery Systems Using Platelets as Carrier

Though there are numerous drug delivery systems that target platelet-tumor microenvironment, exploiting platelets has also proven to be an effective method for targeting cancer progression. Platelets have been known to protect circulating tumor cells in the circulation by attaching to the surface of cells via GPIIb/IIIa and

other interactions and protecting the tumor cells from detection by surveilling immune cells [79]. Using this known property, some researchers have developed chemotherapeutic systems that specifically repurpose platelets as vehicles for delivery of cancer therapies. One such example involves loading isolated, inactivated platelets with doxorubicin, a chemotherapeutic, and testing the release of the drug from the platelets with the presence of activating agonists such as adenosine diphosphate (ADP). Results showed apoptotic effects on Ehrlich ascites carcinoma cells and human lung carcinoma cells [80]. Another example of using functionalized platelets as vehicles for tumor imaging and tumor targeting was to load kabiramide into platelets to prevent activation, and couple this with antitumor proteins such as transferrin to both target and image myeloma tumors [81]. This system showed successful loading and release of drugs as well as the accumulation of the loaded platelets to the site of myeloma xenotransplant in mice.

An alternative method that utilizes platelets as carriers involves attaching drugs to the surface of platelets rather than isolating and loading platelets. In one instance, fucoidan nanoparticles loaded with multiple drugs including a chemotherapeutic and imaging agent were targeted to P-selectin [82]. P-selectin is a natural adhesion receptor expressed by endothelial cells and activated platelets. The fucoidan-platelet system was able to deliver therapeutic locally, compared to delivery systems not targeted to P-selectin. Platelets have also been used as carriers for targeting antithrombotic agents to sites of thrombosis. Examples of this involve targeting liposomes with antithrombotic agents to platelets via GPIIb/IIIa and P-selectin interactions [83, 84]. This method provides the opportunity for direct treatment of thrombotic events through the release of drug agents and can be further tailored to deliver cancer drugs and reduce platelet shielding.

Platelet delivery systems provide many advantages compared to delivery systems utilizing traditional nanoparticle systems such as liposomes. Platelet-loaded systems can deliver more therapeutic drugs due to platelets having more volume and larger diameter than many nanoparticle vehicles. Additionally, platelets can remain up to 9 days in circulation before degradation, allowing for a longer therapeutic window compared to nanoparticle systems that may stay in circulation for 2–50 h. Lastly, targeting surface proteins of platelets or using platelets for delivery of therapeutics employs the natural interactions of platelets and cancer cells to interrupt cancer progression and metastasis. Properties of platelets such as deep tumor penetration and involvement in metastasis and angiogenesis allow for increased specificity in targeting the tumor environment that may not be achieved with simply relying on the enhanced permeability and retention effect. As the opportunities to utilize platelets for cancer therapy are explored, better treatment options to target tumors and tumor microenvironment may one day become alternatives to current cancer therapies.

12.2.4 Drug Delivery Systems Inspired by Platelets

Efforts to improve drug delivery for cancer treatment extend beyond the use of platelets for the delivery of cancer therapeutics. Emerging therapies have also explored the properties of platelets in the vasculature to develop mimetic carriers for the delivery of therapeutics. Several researchers have explored the use of platelet membranes for the delivery of therapeutics for either cancer treatment or wound healing. One such example that our group developed includes the use of silica particles that are coated with platelet membranes conjugated with tumor necrosis factor-related apoptosis-inducing ligands (TRAIL) to target circulating tumor cells. This approach targeted metastasizing cancer cells within their microenvironment and induced apoptosis in prostate and breast cancer cells while also localizing to cancer clusters [85]. In

a separate study, we also genetically engineered platelets to express TRAIL on their surface by a genetic modification in hematopoietic stem and progenitor cells. Approximately, 40% of the circulating platelets were expressing TRAIL, and it was shown that this could reduce the frequency of liver metastasis in an experimental metastasis model of prostate cancer [86].

A similar system involving platelet membrane-coated nanogel-based nanocarriers was used to deliver TRAIL and doxorubicin simultaneously and showed antitumor results indicating the flexibility of the delivery system [87]. One final example utilizing platelet membranes involved loading coated poly(lactic-co-glycolic acid) (PLGA) nanoparticles with docetaxel or vancomycin to target platelet-adhering pathogens as well as neointima growth [88]. This system could also be used as a cancer therapy as docetaxel is a common therapeutic used to treat various cancers such as breast and lung cancer. In all three cases, the use of platelet membrane resulted in increased therapeutic efficacy due to improved targeting and, in some cases, evasion of immune degradation of particles for increased circulation time.

Researchers have also explored platelet mimetic systems that use platelet ligand-receptor pathways to target metastasis. In one such realization, liposomal constructs were functionalized with either P-selectin or GPIIb/IIIa receptors and were used to target breast cancer cells for the delivery of antitumor therapeutics. This system mimics platelet interactions with cancer cells and binds to breast cells expressing platelet proteins to deliver therapy against metastasizing cells [89]. A recently developed method also explores platelet properties in creating nanoparticles, termed "platelet-like nanoparticles." These particles exhibit platelet functions by utilizing peptides for binding to collagen, von Willebrand factor, and integrin and recapitulate physical properties such as discoidal shape and flexibility of platelets to promote wound healing and reduce bleeding time [90]. Artificial platelets [90] and platelet-like particles [91] can be tailored to deliver therapeutics for cancer as the platforms become better characterized.

12.3 Biomechanical Properties

Cancer metastasis is a complex process that is regulated by genetic and biochemical alterations in cancer cells as well as in the microenvironment. However, there is emerging strong evidence that suggests mechanical signals as a critical factor in the cancer metastasis process [92, 93]. The platelet is a dynamic anucleated cell that promotes tumor growth and cancer cell migration via mechanotransduction.

The tumor microenvironment is stiffer compared with tissue in normal physiological conditions, due to three main factors: the dense ECM in the tumor stroma that is composed mostly of collagen and fibronectin; the solid stress caused by tumor cell proliferation; and the increase in interstitial fluid pressure (IFP) [94, 95]. Experimental data indicate an increase in platelet adhesion and activation in stiffer tissues compared to softer tissues. These studies demonstrate that platelets can sense the mechanical properties of the ECM substrate, with this being enough to induce and promote platelet activation and thrombi formation [96]. This suggests that tumor stiffness can induce platelet activation and recruitment into the TME to create a "wound healing" environment that is required to promote tumor cell growth and expansion as discussed above. Once platelets are activated in the intratumoral compartment, they release growth factors that promote aberrant blood vessel formation. Abnormal blood vasculature increases the hydrostatic pressure in the tumor, driving fluid into the interstitial compartment from the circulation and ultimately increasing the IFP [97].

Once tumor cells enter the circulation, platelets can adhere to tumor cells to form a microthrombi-assembled shield. In such a microthrombus structure, the platelet interaction force is between 1.50 and 2.61 nN, consistent with platelet activation thresholds [98]. However, tumor cell-platelet interaction force via integrin and plasma proteins such as fibrinogen and fibronectin is typically in the range of 80–120 pN [99–101]. Collectively, the intercellular interactions contained in this microthrombus structure are indeed sufficiently stable to

maintain this protective shield through the venous and arterial vasculature systems where the hemodynamic shear stress ranges from 0.5 dyn/cm^2 to 30 dyn/cm^2 [19]. Recently Jiang et al. [102] developed a microfluidic device to isolate CTCs based on a positive selection of platelet-shielded cells. Using this device, tumor cells may be isolated independently of epithelial vs. mesenchymal phenotype.

Platelets interacting with tumor cells can be found in various states of activation. However, when platelet-cloaked tumor cells are exposed to hemodynamic forces, shear stress activates the GPIIb/IIIa integrin to induce an outside-in signal and reorganize the cytoskeleton structure, maximizing the adhesive and procoagulant properties of the platelets [103]. During local platelet activation, proteins such as fibronectin and vitronectin are released and represent critical players in the regulation of $\alpha_v\beta_3$ integrin activation and clustering of tumor cells. The activation of this integrin is crucial to induce firm tumor cell arrest [104]. Overall, the platelet is a versatile cell that tumor cells hijack to promote tumor growth and cancer cell migration.

12.4 Biomechanics of Platelet-Cancer Therapies

Cancer cells in the circulation utilize cell adhesion markers on platelets such as integrins and selectins and structural proteins such as collagen for creating a protective "coat" to survive the high shear stress in blood and to migrate and form metastases. As such, targeting the biomechanical properties of platelets to affect the aggregation and adhesion of platelets with cancer cells is a growing area of interest in cancer therapeutics. Aspirin, aspirin-nicotinic acid, and heparin are a few examples of drugs that have been studied for the disruption of platelet-cancer interactions, termed tumor cell-induced platelet aggregation (TCIPA). In one study, aspirin was shown to affect collagen and adenosine phosphate interactions, while nicotinic acid inhibited the formation of tumor cell-platelet aggregates [105]. A recent study showed that heparin blocked initial P-selectin interactions of platelets with cancer cells in vitro and in vivo and showed potential for the inhibition of metastasis [106]. Inhibition of αvβ3 integrins through the use of antagonists showed similar outcomes by inhibiting the metastases of colon cancer while increasing survival in mice [107]. P-selectin and αvβ3 integrins have both been implicated in metastasis by promoting tumor cell tethering and adhesion under flow conditions and the formation of TCIPA [31, 108, 109]. The targeting of biomechanical properties shows promise in reducing the occurrence of metastasis, as platelet adhesion and aggregation promotes cancer cell protection and tethering within the vasculature for extravasation. However, different cancer types will exhibit different mechanisms of interaction with platelets and may not rely only on integrins or P-selectin for survival in circulation. Additionally, many studies do not recapitulate realistic blood flow conditions while studying effects of targeting aggregation or adhesion markers in TCIPA. As the mechanistic process of cancer metastasis is elucidated, the ability to interfere with tumor-platelet interactions may play a larger role in cancer therapies.

Biomimetic liposomes decorated with one or more molecules such as fibrinogen, collagen peptide, and von Willebrand-binding peptide have been investigated for applications in wound healing and hemostasis [110–112]. The functional properties of these nanoconstructs allowed for increased circulation times, aggregation with platelets, and specific targeting of vascular damage. The use of nanoparticles with platelet surface properties may serve to enhance alternatives to current nanoparticle delivery systems.

12.5 Conclusions

During cancer progression, tumor cells hijack host cells to support tumor growth and the migration of malignant cells. In this chapter, it was discussed how tumor cells benefit from platelet functions to support cancer progression. Platelets are involved not only in primary tumor growth and stability but also through cancer cell migration to distant organs. As platelets represent

a significant factor in cancer progression, some researchers have focused on developing novel cancer therapies by targeting platelets as well as its coagulation pathway. Monoclonal antibodies and anticoagulants against procoagulant factors have been studied as anticancer agents where their effects resulted in improved survival in cancer patients as stand-alone therapies or in combination with chemotherapy drugs. Alternatively, the use of platelets as drug delivery vehicles has been found to be effective in killing tumor cells and disrupting tumor cell-platelet interactions. In summary, platelets influence tumor cells in many ways and their interactions make platelets a potential carrier for novel anticancer drugs.

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