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Contribution of Platelets to Tumor Metastasis

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Abstract

Extensive experimental evidence shows that platelets support tumor metastasis. The activation of platelets and the coagulation system play a critical role in the progression of cancer. Within the circulatory system, platelets guard tumor cells from immune elimination and promote their arrest at the endothelium, supporting the establishment of secondaruy lesions. These contributions of platelets to tumor cell survival and spread recognize platelets as a new avenue for therapy.

Cancer patients frequently present with signs of thrombosis, and these are most severe if the disease has progressed to a metastatic stage [1–3]. Severe forms of thrombosis include disseminated intravascular coagulation [G], migratory thrombophlebitis [G] and pulmonary embolism [G] as signs of aberrant platelet activation and aggregation [4]. However, even if thrombotic events are not detected, coagulation parameters are often elevated in cancer patients, and platelet turnover is generally enhanced. These clinical observations indicate a potential relationship between the blood coagulation system, platelet functions, and cancer spreading via the blood stream. To investigate if a functional relationship may exist between platelet activities and tumor progression, experimental models have been developed to understand how specific platelet functions might contribute to this process. Analytical animal models in which specific platelet functions were altered through drug treatment or controlled genetic ablation have yielded evidence that platelets can guard tumor cells from immune elimination within the circulatory system, promote tumor cell arrest within the vasculature and affect tumor cell survival, thereby supporting the establishment of secondary lesions.

In this Opinion article, we highlight evidence indicating that the activation of platelets and the coagulation system play a critical role in the metastatic progression of cancer. Insight from studies pointing to contributions of platelets to tumor cell survival and spreading support the notion that platelets are important, if not essential, in the development of metastases from the blood stream. The existing knowledge and further mechanistic studies could recognize platelets and their functions as a new avenue for anti-metastatic therapy.

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The metastatic process, platelets, and hemostasis

Tumor metastasis to distant organs depends on interactions between tumor cells and the host microenvironment within the circulation, lymphatic vessels and target tissues. This involves blood cells, components of the coagulation system, stromal cells, and the extracellular matrix. Cells within the blood stream that contribute to metastasis are endothelial cells, platelets, lymphocytes, macrophages, mast cells, fibroblasts, and bone marrow-derived progenitor cells [5]. The link between platelets and cancer was first documented in the mid 19th century by Trousseau who diagnosed himself and his patients with excessive blood clotting, caused by occult carcinoma that led to the inflammation of blood vessels [6]. He observed an association of thrombosis and cancer, and proposed that changes in the hemostatic system support the progression of the malignancy [7]. These initial findings suggest that tumors which are capable of activating platelets and forming clots are likely to spread and have a poor outcome. It is now well recognized that in cancer patients, tumor growth is accompanied by the development of an increased tendency toward blood clotting (hypercoagulable state), platelet abnormalities, and an increased risk of thromboembolic disease [G] [1, 4]. Moreover, thromboembolism is an early diagnostic feature [4] and a major cause of death [8]. Platelet activation is amplified in many cancers and found within the tumor vasculature [9–12]. Platelet counts commonly vary during cancer progression and may predict the clinical outcome. Thrombocytosis (high platelet count) associates with poor prognosis in colon, breast, lung, gastric, renal, cervical, pancreatic, endometrial, brain and ovarian cancers [9, 13–19]. Thrombocytopenia (low platelet count) also occurs, but is mostly associated with chemotherapy that suppresses platelet production in the bone marrow [20].

The basic biological functions of platelets in coagulation and hemostasis (Figure 1) are enacted during tumor metastasis. Platelets, or thrombocytes, are small, specialized blood cells released as anuclear cytoplasmic bodies from megakaryocytes in the bone marrow. One liter of blood contains about four hundred billion circulating platelets that are turned over every week. The main function of platelets is to halt hemorrhage after tissue trauma and vascular injury [21, 22]. This is initiated through platelet activation, which results in adhesion and release of a multitude of bioactive factors from platelet granules. The process leads to firm attachment of platelets to the injured vessel wall and platelet aggregation induced thrombus formation to seal the wound. Interaction between platelets and damaged endothelium or the subendothelial matrix initiates transient platelet adhesion. Binding of platelet receptors such as glycoprotein VI (GPVI) and glycoprotein Ibα (GPIbα) to exposed collagen and von Willebrand factor (VWF), respectively, leads to activation of platelet integrin αIIbβ3, also known as glycoprotein IIb/IIIa (GPIIb/IIIa), which enables firm adhesion and platelet aggregation. The serine protease thrombin, generated from its latent form in plasma, triggers a strong procoagulant response by fully activating platelets and converting fibrinogen into crosslinked fibrin. This maximizes local thrombus formation and release of platelet granules that contain active proteases, growth factors, matrix proteins and chemokines.

Components of the extrinsic hemostatic pathway, in particular coagulation factor VII (FVII) and tissue factor, are dramatically activated in cancer patients [23]. Tissue factor expressed by tumor cells interacts with coagulation factors VII (FVII) and coagulation factor X (FX), and

generates thrombin to enhance tumor progression [24, 25]. Thrombin not only leads to fibrin formation and activation of platelet receptors that promote thrombosis, but also supports tumor cell malignancy [26–28]. Thus, tissue factor and thrombin play critical roles in tumor growth, angiogenesis, and metastasis. These processes are reported in an extensive body of literature and were covered by other reviews [29–31].

A resurgence of inquiry in the field refined the original hypothesis of platelet involvement in tumor metastasis based on the use of transgenic animal models, and thereby tying in a broader knowledge and renewed appreciation of platelets as an important part of the host microenvironment [5, 32–36] (Table 1). Investigation led to a model of platelet-supported tumor metastasis where tumor cells enter the blood stream (intravasation), bind and activate platelets (cohesion) as well as leukocytes. Evidence is emerging that these host cells then assist tumor cell arrest at the vessel wall (adhesion), survival within the vasculature (immune evasion), enabling exit from the circulation (extravasation), and tumor cell survival and proliferation within target tissues of metastasis [8, 37–40]. In making this argument, we recognize that there are still many opportunities for research into the role of platelets and that, in many cases, discrete evidence is still needed. However based on reported results, we suggest that the hemostatic properties of platelets critically contribute to metastasis and should be considered in cancer treatment and targeted therapy.

Platelet role in angiogenesis and tumor vascular remodeling

Vascularization within a tumor is a portal through which tumor cells can enter the blood stream to disseminate. Platelets may stabilize vessel growth during tumor development and thus contribute to this process.

As tumors and metastases expand, they must recruit new blood vessels to provide nutrients and oxygen for further lesion growth. Blood vessel formation within tumors is called angiogenesis. Angiogenesis was originally hypothesized by Judah Folkman [41] as a necessary mechanism for tumor progression to prevent hypoxia-induced growth stagnation and necrosis. The finding that platelets release vascular endothelial growth factor (VEGF) upon activation was the first evidence that platelets promote angiogenesis [42]. Platelets were then formally hypothesized by Folkman and colleagues [43] to contribute to tumor angiogenesis. Even though platelets are poised to alter the tumor vasculature [9], initial evidence in mouse models remains equivocal. Platelets contain a multitude of factors that are released during platelet activation and which regulate the angiogenic process. These factors are either anti-angiogenic (angiopoietin −1 (ANGPT1, also known ANG-1), sphingosine 1 -phosphate (S1P), serotonin, thrombospondin −1 (THBS1)), or pro-angiogenic (VEGFA, epidermal growth factor (EGF), basic fibroblast growth factor (bFGF, also known as FGF2)), or function as cytokines (interleukin −1β (IL-1β), interleukin −8 (IL-8)) and proteases (matrix metalloproteinase −2 (MMP2), matrix metalloproteinase −9, (MMP9)) (Table 2) [44, 45]. Platelet activation can occur locally within the tumor vascular environment, as well as systemically as seen in patients with prostrate, breast, lung, colon or gastric cancer [8]. The leaky tumor vasculature may expose matrix proteins such as collagen, a potent activator of platelets. Tumor cells could act indirectly through binding of VWF to initiate platelet tethering, given that VEGF stimulates VWF secretion by endothelial cells [46]. Furthermore,

systemic deployment of C-X-C motif ligand 12 (CXCL12, also known as SDF-1) from platelets regulates neoangiogenesis through the recruitment of hematopoietic progenitors [47].

Due to the regulatory impact of platelets on vascular remodeling, they were surmised to help shape the tortuous vasculature within tumors. Platelets have been proposed to alter blood vessels through localized release of platelet factors [43]. Interestingly, platelets have compartmentalized pro- and anti-angiogenic α granules, which may be differentially released depending on the stimuli. For example, granules laden with VEGF are selectively released after ligation of protease-activated receptor PAR1, while granules containing endostatin are released upon PAR4 activation [48, 49]. These findings not only explain a pivotal role of platelets in regulating hemostasis, but also suggest that tumor cells could use platelets to balance an angiogenic milieu. In a similar way as platelet-derived LPA enhances bone metastatic growth and progression [50], platelet-released factors may play a role in tumor progression, and therefore warrant further investigation. The attenuated tumor growth observed in P-selectin-deficient mice [51] could affect metastasis based on the impaired recruitment of leukocytes, although the contributions of endothelial and platelet P-selectin are indiscriminate. In contrast, an initial study demonstrates that the transplantation of wild-type bone marrow into thrombospondin-1-deficient mice resulted in diminished tumor growth [52]. This implies that platelet delivery of an angiogenesis inhibitor may suppress tumor progression. Nonetheless, there is no change in tumor growth in mice deficient in fibrin, platelet activation or GPVI expression, and there are no overt vascular differences from the wild-type [53–55]. Fundamental questions of how platelets affect tumor angiogenesis, and in which way their pro- or anti-angiogenic capacities contribute to tumor progression remain to be investigated further.

A recent study provides evidence that platelets prevent hemorrhaging within a tumor [56], indicating that the hemostatic functions of platelets are likely critical for maintaining vascular integrity in tumors. Antibody-induced thrombocytopenia in mice was immediately followed by erupted hemorrhage within tumors, which resulted in reduced tumor cell proliferation and increased apoptosis [56]. The severe bleeding could be rescued by reconstitution with resting platelets, but not with platelets that were experimentally exhausted of their granule content after *ex vivo* thrombin activation. This indicates that secretion of platelet granules is crucial for platelet regulation of tumor vascular homeostasis. The resulting impact on tumor growth would be interesting to be studied further. It was proposed that platelets stabilize the tumor vasculature through anti-angiogenic factors such as ANGPT1, and serotonin, which presumably counteract VEGFA released from the tumor cells [56]. Another possibility is that platelets dampen inflammation and local tissue damage through modulation of leukocyte recruitment or cytokine release [57]. Therefore, tumors may develop a dependence on platelets for preventing hemorrhage during tumor growth and angiogenesis.

While platelet factors help to regulate tumor blood vessel integrity, adhesive platelet functions and thrombus formation, surprisingly, are apparently not required [58]. Mice deficient in either platelet GPIbα, VWF, P-selectin or integrin αIIbβ3 did not show tumor hemorrhaging. However, angiogenesis analyzed in the cornea of the eye clearly involved platelet adhesion to prevent hemorrhage, as seen in mice rendered thrombocytopenic or mice

lacking platelet receptor GPIbα [59]. Supporting this premise, previous *in vivo* imaging of platelets in tumors showed platelet rolling along the endothelium, but neither firm adhesion nor thrombus formation within the tumor microcirculation [60]. The slowed blood flow within tumors may allow for localized platelet granule release [43, 58] and not require involvement of receptors and ligands that mediate platelet adhesion under higher shear conditions. While platelets and their hemostatic functions impact vascular integrity and remodeling within tumors, there is no direct evidence that platelets also affect tumor cell intravasation and access to the circulation. As the molecular mechanisms though which platelets contribute to primary tumor growth and angiogenesis still need to be explored further, convincing evidence already indicates a critical role of platelets in promoting tumor cell metastasis from the blood stream (Figure 2).

Survival of tumor cells and immune evasion

The microenvironment within the blood stream is hostile for circulating tumor cells and their survival before extravasation is critical for metastasis. Antibody-induced [61] or genetic [62] depletion of platelets inhibits metastasis, whereas platelet reconstitution restores metastatic activity [63] as seen in several independent mouse models. A definitive study explored a model that lacks circulating platelets based on nuclear factor (erythroid-derived 2) (NFE2) knockout which interrupts platelet production through interfering with megakaryocyte maturation [62] (Table 1). Experimental metastasis is nearly completely inhibited in these mice. These results, together with reduced metastasis in PAR4- and fibrinogen-knockout mice, indicate that platelets and their activation play an essential role in hematogenous tumor cell spreading and involve thrombin-dependent and -independent mechanisms [62]. Platelets of *Par4*$^{-/-}$ mice are unable to aggregate in response to thrombin, but their thromboxane and GPVI receptors are not disturbed [61,64]. Treatment with thrombin inhibitor hiruidin further reduces metastasis in PAR4 null mice, implying a mechanism that involves other thrombin ligands such as fibrinogen [65], or PARs expressed by the tumor cells. In fact, host deficiencies in PAR1 and PAR2, where endothelial responses to coagulation proteases - but not those of the experimentally introduced tumor cells - are attenuated, did not provide protection against metastasis [62]. Thrombin activates PAR1 and PAR2 receptors expressed by host and tumor cells, and generally supports tumor growth, angiogenesis and metastasis [66, 67].

Mechanisms by which platelets assist hematogenous metastasis depend on the ability of platelets to act in concert with coagulation factors in protecting tumor cells within the blood stream. When proteases of the hierarchical coagulation cascade activate thrombin, this protein cleaves fibrinogen and induces fibrin formation. During this process, platelets crosslink through adhesion molecules, such as integrin αIIbβ3, which once activated binds soluble fibrinogen and then supports thrombus formation and platelet incorporation into fibrin clots. Newly activated platelets perpetuate thrombosis with discharge of additional fibrinogen, VWF, and fibronectin. Early observers showed that entanglements of platelets and fibrin surround tumor cells that are arrested within the vasculature [68–70]. Leukocytes and natural killer cells (NK cells) are found in close proximity of these metastasizing cells [71]. NK cells can disable blood borne cancer cells by direct contact and lysis. However, adhesion of platelets to tumor cells and their incorporation into platelet heteroaggregates may shield

the tumor cells from NK cell activity. This could explain why clearance of circulating tumor cells by NK cells is incomplete and allows cancer spreading from the blood stream [72]. Furthermore, incorporation of plasma fibronectin into fibrin clots enhances metastasis to the lungs, but less so to other sites, as seen in mice that were postnatally depleted of the plasma fibronectin gene in the liver [73].

The involvement of NK cells and hemostatic factors in metastasis was elegantly explored through development of novel mouse and cell models [74]. Platelets and tissue factor, and thus thrombin generation, were clearly identified as nodes in the impact that NK cells exert on the metastatic process. Fibrin deposition, induced by tumor cell-associated tissue factor, impedes tumor cell recognition by NK cells and thereby enhances metastasis through cloaking tumor cells within the circulation [53, 75]. This concept is based on studies of experimental and spontaneous metastasis in mice that lack Gαq, fibrinogen, or NK cells [51] (Table 1). Mice lacking the α subunit of the heterotrimeric guanine-nucleotide-binding protein G_q (Gαq), a protein critical for platelet activation, were mostly unable to support metastasis after tumor cell injection into the venous circulation. Spontaneous metastasis from primary subcutaneous tumors was also attenuated, while tumor growth itself was not affected. Similar results were seen in fibrinogen-deficient mice and in another study using mice treated with an inhibitor of platelet aggregation [53, 76]. In the presence of NK cells, platelet activation is required for tumor cell survival. Notably, the blocking of metastasis by Gαq or fibrinogen knockdown was abrogated when NK cells were immunologically or genetically depleted. This clearly indicates that the supportive effects of platelet activation and fibrinogen involve tumor cell shielding from NK cell function [51] (Figure 2).

The same group later recognized NK cell-independent mechanisms that determine early tumor cell survival in the target organ microvasculature. However, it was clearly documented that even NK-independent survival requires thrombin generation [53, 75]. In an elegant approach, tumor cells were generated from murine fibroblasts of tissue factor-deficient embryos by transformation with Ha-Ras. Tumor growth and metastasis of these cells was then studied in immuno competentmice lacking either tissue factor, Gαq, fibrinogen, NK cell function, or which were severely depleted of prothrombin [75]. Results from this model indicated that tumor associated tissue factor and distal components of the hemostatic system are important, but not critical, for initial tumor cell localization in the lung microvasculature. However, genetic reduction of prothrombin profoundly reduced the survival of the lodged tumor cells, and thereby determined early success of metastasis development [53, 75, 77]. In the presence of NK cells, platelet activation is required for tumor cell survival. Together, these findings suggest that the platelet-fibrin axis plays an important part in immune evasion. Platelets also contribute to NK cell-independent intravascular tumor cell survival. The exact mechanisms of how platelet activation and fibrin clotting protect from NK cells still need to be defined. Current hypotheses include that platelets provide a physical barrier to NK cell contact, or that platelets exert paracrine suppression of NK-mediated cytolytic activity [78]. A recent study illuminated one such mechanism by demonstrating that transforming growth factor-β (TGF-β) from platelets diminishes NK granule mobilization, cytotoxicity, and interferon-γ (IFN-γ) secretion [79]. Several other soluble mediators, including prostaglandin E_2 (PGE_2) that were suggested to participate, need to be studied for this capacity [78]. Overall, the ability of platelets to induce formation of heteroaggregates between tumor cells, platelets

and leukocytes may play an important part in determining tumor cell survival within the microvasculature of target organs of metastasis.

Tumor cell interaction with vascular cells

Transient interactions

The process of tumor cell arrest within the microvasculature of target organs is difficult to study because these adhesive events are rare in a living host. Therefore, tumor cell arrest *in vivo* cannot be analyzed as reliably and quantitatively as the attachment of circulating leukocytes during inflammation. Furthermore, *in vitro* models can mimic the complexity of the intravascular situation only in certain aspects. Nevertheless, studies focused on tumor cell interaction with vascular cells at early time points after introducing malignant cells into the circulation of drug treated or genetically altered animals have shed light on tumor cell arrest in target organ vessels [51, 80]. These approaches have also yielded information on adhesion receptors and ligands that mediate tumor cell anchorage at the endothelium and the following early events of metastatic colonization as summarized below.

Evidence suggests that rolling and tethering of circulating tumor cells to the vessel wall, and formation of heteroaggregates between tumor cells, platelets and leukocytes can be mediated by selectins, a family of transmembrane cell adhesion molecules. Selectins enable white blood cell trafficking by promoting their transient adhesion within the bloodstream during inflammation. Selectins are expressed by platelets, endothelial cells, and leukocytes, and metastasis studies indicate that platelets can use selectins to support initial transient tumor cell interaction with the endothelium in a manner similar to that observed during recruitment of leukocytes in inflammation [81] (Figure 2).

Selectins contain a conserved lectin domain that recognizes and binds glycoconjugates [G] on other cells [81]. All selectins recognize ligands that contain tetrasaccharide sialyl Lewis X/A antigens [82], and can require the clustering of selectin molecules for high affinity binding [81]. Some of these glycoconjugate ligands include P-selectin-glycoprotein ligand-1, mucins and CD44 variants, many of which are expressed by metastatic tumor cells [82]. There are at least three selectin types. L-selectin is constitutively expressed on leukocytes. E- and P-selectin are present on endothelial cells and platelets, respectively, but only when these are activated. Upon activation, P-selectin translocates to the cell surface from Weibel-Palade bodies [G] in endothelial cells, and from α granules in platelets.

Results from P-selectin knockout mice, established on an immunodeficient background, indicated that tumor growth and metastasis of colon carcinoma cells involves P-selectin function and platelets. This was clearly evident at early steps of tumor cell interaction with the pulmonary microvasculature during metastasis [51, 83]. P-selectin on endothelial cells was shown to contribute to experimental metastasis as well, as seen in irradiated wild-type mice after reconstitution with P-selectin-deficient bone marrow [84]. In this elegant study, intravascular tumor cell rolling and metastasis were attenuated, but not as strongly as in P-selectin null mice (Table 1). Thus, this model allowed to dissect contributions of P-selectin on endothelial cells and platelets [84]. Metastasis can also be attenuated by the anticoagulant heparin. In addition to inhibiting thrombin, heparin binding can mimic negatively charged

mucins at the tumor cell surface [71]. Thereby, heparin impedes tumor cell binding to P-selectin on platelets [29, 71, 85, 86]. P-selectin-dependent tumor cell tethering is mediated in part through binding to tumor cell mucins, which also induce platelet aggregation [51, 83]. These interactions were demonstrated using co-cultures of platelets, colon carcinoma mucins, and activated endothelium. The results may have relevance for human colon carcinomas that express mucin type P-selectin ligands. Non-mucin ligands of L- and P-selectins act synergistically to enhance metastasis through tumor cell binding to leukocytes and platelets [87]. In static co-cultures of tumor cells, platelets and leukocytes, selectin-dependent interaction between these cell types induces activation of endothelial cells to release C-C motif chemokine 5 (CCL5, also known as RANTES). *In vivo*, CCL5 likely expressed by activated endothelium recruits monocytes to further increase metastasis [88]. Therefore, these findings suggest that platelet-dependent activation of endothelial cells is required for monocytes to home to and promote development of the micrometastatic niche. Under shear stress [G] generated by blood flow, CD44 is a dominant ligand on colon cancer cells that mediates their binding to platelet P-selectin. This engagement can be competitively inhibited by fibrin, but not by fibrinogen [89]. Evidence supports that CD44-fibrin interaction helps to establish firm adhesion between platelets and tumor cells. Thus, a model emerges in which tumor cells co-opt platelets and leukocytes to form heteroaggregates, which may travel within the circulation and then tether and roll along activated areas of the endothelium in a P-selectin-dependent manner. The tethering process precedes higher affinity adhesion and tumor cell arrest [90] as a prerequisite for extravasation from the bloodstream.

Adhesion and selection of a metastatic phenotype

Adhesion receptors on platelets can crosslink with tumor cells and leukocytes to help establish firm tumor cell arrest within the vasculature. Platelets assist the attachment of tumor cells to endothelial cells and avidly bind exposed subendothelial matrix proteins. In this process, platelets promote selection of tumor cells with a metastatic phenotype (Figure 2).

The transition from selectin-dependent tumor cell rolling along the endothelium [90] to firm arrest is mediated by integrins. The main platelet integrin involved in this process is integrin αIIbβ3. In hemostasis, this receptor becomes activated in response to platelet agonist stimulation or platelet interaction with collagen at exposed sites of the subendothelial matrix. Activated integrin αIIbβ3 can bind soluble fibrinogen, which then forms inter-platelet bridges and leads to thrombus formation [91] (Figure 1). Fibrinogen also crosslinks tumor cells and platelets. Platelet integrin αIIbβ3 and tumor cell receptors, such as integrin αvβ3, mediate tumor cell-platelet cohesion that helps to overcome shear forces generated by blood flow, in lodging circulating tumor cells to the vessel wall [92]. Inhibitors of αIIbβ3 reduce tumor cell colonization e.g. of the lungs [63, 93]. This indicates that αIIbβ3 coordinates tumor cell-platelet cohesion and adhesion as important steps in metastasis. Tumor cell integrin αvβ3 then perpetuates adhesion, migration, and invasiveness in cooperation with platelets [92, 94]. For instance, knockout mice lacking the β3 integrin subunit gene have reduced bone metastases and bone destruction. This is because platelet integrin αIIbβ3 is required for platelet aggregation and critically contributes to homing of metastatic cells to the bone microenvironment, where osteoclast integrin αvβ3 mediates bone destruction in

growing metastatic lesions [95]. Direct evidence for platelet involvement in tumor cell lodging within target organ microvessels was obtained from lung preparations, isolated at early time points after tumor cell injection into the venous circulation and infusion with fluorescently labeled antibodies to identify the tumor cells, platelets and fibrin clots [80]. Tumor cells were found spread within pulmonary microvessels 2–6 hours post-injection, and they were associated with platelets and fibrin clots. Inhibition of clot formation with hirudin reduced tumor cell retention in the lung [80]. Platelets further support bone metastatic growth and cytokine-induced bone destruction by producing lysophosphatidic acid (LPA) in response to stimulation by tumor cells [50, 96]. Inhibition of platelet aggregation with Integrilin, an antagonist of platelet integrin αIIbβ3, blocks bone metastasis and thereby identifies platelets as a critical source of LPA in the tumor microenvironment [50, 96].

Generally, cell attachment to the vessel wall critically depends on the affinity state of integrin adhesion receptors [97]. This is well established for platelets and leukocytes [91]. The specialized functions of these cells in repair of vascular injury and inflammation require that the cells rapidly respond to local stimuli and engage in adhesive interactions that support their arrest at the vessel wall. Agonist stimulation leads to conformational changes of critical integrins, such as αIIbβ3 on platelets, and αMβ2 and α4β1 on leukocytes, and transition of the receptors into a high affinity state [98]. Once activated, the receptors can bind ligands from solution, leading to cell-cell cohesion and high affinity interactions with ligands or counter receptors on endothelial cells and proteins of the subendothelial matrix [99–102]. During metastasis, activation of platelets, leukocytes and potentially tumor cells can support their cohesion and firm arrest at the vascular endothelium, involving multiple receptors and ligands on each cell type. These include platelet integrin αIIbβ3, leukocyte β2 integrins and α4β1, tumor cell and endothelial cell β1 integrins and αvβ3, intercellular adhesion molecules ICAM, VCAM, as well as P-, L-, and E-selectins [82] (Figure 2).

Analyzing the adhesive behavior of blood borne tumor cells in a blood perfusion model [G] revealed that tumor cells can also express key integrins, such as αvβ3, in distinct states of activation [103]. Only the high affinity, activated form of αvβ3 on tumor cells mediates their interaction with platelets and supports tumor cell arrest during blood flow [103]. In this way, platelet-supported anchorage selects for a subpopulation of tumor cells that constitutively express the high affinity form of αvβ3 [104]. In subsequent steps of the metastatic cascade, this high affinity receptor further promotes tumor cell invasion by enhancing protease activation [104, 105, 106]. Furthermore, the high affinity form of tumor cell integrin αvβ3 stimulates tumor cell survival and proliferation in target organ tissues, as recently shown for breast cancer brain metastases. Activated αvβ3 elicits brain lesion growth through continuous, hypoxia-independent expression of tumor cell VEGF by inhibiting 4EBP1, a translational repressor known as eukaryotic translation initiation factor 4E-binding protein 1[107]. Thus, platelet-mediated selection of tumor cells expressing a high affinity receptor can identify a subset of tumor cells with an aggressive metastatic potential. Interestingly, some cancer patients apparently produce inhibitory antibodies against high affinity αvβ3 which inhibit tumor cell platelet interaction and target organ colonization from the blood stream [108]. These antibodies can also repress the growth of established, late stage metastases [109]. In the absence of β3 integrin expression, alternate mechanisms can drive tumor growth and progression. For example, mammary carcinoma development and spontaneous lung

metastasis were essentially unaffected in mice deficient for the β3 and β5 subunit genes or a combination of both, when the disease was driven by the epidermal growth factor receptor 2 (ERBB2, HER2/neu) oncogene[110].

Molecules mediating tumor cell migration and adhesion may also bind directly to counterreceptors on platelets. For example, at the leading edge of invasive cells, poliovirus receptor (also known as nectin-like molecule 5, NECL5) colocalizes with integrin αvβ3 [111]. The NECL5 receptor on colon cancer cells enhances experimental metastasis in a platelet-dependent manner. The process involves adhesion to a counter-receptor on platelets and thereby indirectly enables tumor cell attachment to the vascular endothelium. While intriguing, this mechanism awaits further investigation [111].

Involvement of the subendothelial matrix

Another important platelet adhesion receptor that potentially contributes to tumor progression is the GPIb-IX-V receptor complex. In hemostasis, this complex is central to thrombus formation primarily in the arterial circulation. The interaction between platelet GPIbα and VWF allows platelets to tether to the vascular endothelium, especially in the presence of high shear forces generated by arterial blood flow. This adhesion process enables platelets to recognize exposed sites of extracellular matrix, leading to platelet binding, activation, and thrombus formation [21]. The importance of the extracellular arrangement of GPIbα in cancer progression is highlighted in several studies [112, 113]. It was demonstrated that experimental metastasis of melanoma cells is reduced in immune competent mice lacking GPIbα [112]. In contrast, wild-type mice treated with monovalent antibody fragments directed against GPIbα showed enhanced metastasis of the same melanoma cell type. However, no underlying mechanism was identified [114]. Platelet GPIbα might be involved in tumor progression in a number of different ways. The receptor can bind thrombin and VWF. Which ligand is important, and which step of the metastatic cascade may involve GPIbα binding of either of these ligands still needs to be explored. For example, it was shown that VWF can actually inhibit experimental metastasis from the blood stream [115, 116] while thrombin clearly supports this process.

An important trigger of platelet interaction with the vessel wall is the exposure to subendothelial matrix proteins. When platelets encounter denuded sites of subendothelial matrix, they attach to these sites and become activated. During the metastatic process, microlesions in the endothelium could exist in the metastatic niche or be generated through leukocyte- or tumor cell-induced endothelial retraction [G]. Retraction exposes collagen, which is avidly recognized by platelet integrins, such as α2β1, as well as by the platelet glycoprotein receptor for collagen VI (GPVI) [21]. GPVI ligation causes platelet activation, aggregation and further amplifies platelet binding to collagen. Whether this mechanism contributes to tumor metastasis is still unclear, but tumor cell colonization of the lungs from the blood stream was found reduced in GPVI-deficient mice [55].

Extravasation

Platelets can modulate the permeability of the vasculature. Platelet-mediated enhancement of vascular permeability, for example, helps immune cells to colonize sites of inflammation.

It is possible that platelets might similarly assist tumor cells to exit the blood stream and enter target organs of metastasis. It is likely that such a process involves release of platelet granules and mircoparticles (Box 1).

Platelets and blood vessel permeability

While the profuse release of adhesive molecules, cytokines and mitogens from platelet granules apparently contributes to modulation of immune surveillance, it may also facilitate tumor cell extravasation, survival and growth, although this is mostly speculation for now. The integrity of the vascular endothelium within the microenvironment of attached tumor cell-platelet heteroaggregates may be directly, or indirectly, affected by growth factors released from platelet α granules, including platelet-derived growth factor (PDGF), TGF-β, EGF, insulin-like growth factor −1 (IGF-1), and VEGFA [44, 45]. Platelets also release the endothelial agonist S1P, a potent inhibitor of vascular leakage [117, 118]. In addition to S1P, platelets are major stores for LPA. Both are signaling molecules that affect cytoskeletal rearrangement, actin changes, cell-cell communication, and influence endothelial permeability. S1P decreases the permeability of endothelial cells, while LPA stabilizes the integrity of certain endothelial types but not in others [119, 120]. For instance, LPA notably induces permeability in brain microvascular endothelial cells [121]. Thus, platelets may affect blood vessel integrity and could impact tumor cell extravasation. Another potent bioactive factor released from platelets is serotonin. Platelets take up serotonin from plasma, store it in dense granules, and release it upon activation. This leads to modulation of vascular tone, inducing either vasoconstriction or vasodilation [122]. Introduction of tumor cells into the circulation of mice can increase plasma levels of serotonin significantly [123]. Blockade of serotonin receptors or calcium channels hinders experimental tumor metastasis, as seen in the liver [123, 124]. This exemplifies another means by which interference with platelet activation can augment metastasis. An additional potent vasoactive and inflammatory factor released by platelets is histamine. Histamine increases vascular permeability and thus augments leukocyte extravasation. Moreover, platelets release CCL5 which recruits monocytes and eosinophils and triggers further histamine release [125]. Together, the integration of circulating tumor cells into platelet-leukocyte heteroaggregates, arrested at the vascular endothelium, generates a rich microenvironment for the incorporated tumor cells that might enhance their extravasation and invasion of target tissues. These hypotheses have yet to be examined.

Therapeutic Implications

Physicians currently administer anticoagulants to prevent and treat venous thromboembolism in cancer patients [126]. Going beyond the use of anticoagulants for prophylaxis in paraneoplastic disease [G], these compounds need to be further investigated for the treatment of tumor progression. Low molecular weight heparin has potential to do both [29, 85, 127]. Antithrombotic therapies such as Aspirin, Warfarin and Cox inhibitors improve cancer patient survival, but not always. These agents have many targets, apart from tumor cells (platelets predominantly express Prostaglandin G/H synthase 1, also known as Cyclooxygenase-1 COX1) [85, 128, 129], and bleeding side effects may occur. Antiplatelet drugs reduce experimental metastasis in some animal models, but not in others [8].

Furthermore, the use of mouse models to assess platelet function in human cancer is limited due to species-specific differences. In particular, human platelets are primarily activated through the PAR1 thrombin receptor and coactivated by PAR4, whereas mouse platelets do not express PAR1 and are prominently activated via PAR4.

Platelets should be considered in the management and detection of human cancers [130–132]. The prometastatic properties of platelets raise questions of whether chemotherapy-induced thrombocytopenia and platelet restoration in these patients affect disease progression and outcome. Intriguingly, platelets specifically sequester regulators of angiogenesis (VEGF, platelet factor 4 (PF4), THBS1) released from tumors, as seen in mouse models [52, 133, 134]. Increased levels of these factors are detectable in platelets, but not plasma or serum, when dormant tumors are microscopic (<1 mm) [134]. Thus, tumor associated alterations of platelet granule contents could serve as valuable biomarkers for cancer progression.

Antiplatelet drugs used for the intervention of cardiovascular disease, such as integrin αIIbβ3 antagonists might be studied for their effects on tumor progression, metastasis and overall survival in clinical trials. Therapies targeting P-selectin [135], GPIb-IX-V, and GPVI could also be explored. Adverse effects due to normal platelet physiology may be overcome by targeting of pathological tumor cell-platelet interactions while maintaining normal platelet functions. So far, no specific platelet function targeted therapy has been included in standard cancer treatment. Furthermore, studies investigating possible links between heritable platelet dysfunctions in the human population and the incidence of metastatic disease have not been undertaken. The overall evidence from experimental models on contributions of platelets to cancer progression, however, predicts that a better understanding of the molecular mechanisms of platelet involvement will help identify promising new clinical approaches.

Perspectives

This Opinion article highlights evidence for a role of platelets in tumor metastasis. Metastasis is a complex process and platelets may contribute to its success at distinct stages of progression. Before the onset of tumor cell dissemination, platelets may affect the development and architecture of the tumor vasculature. The most profound influence of platelets on metastasis likely occurs when tumor cells have entered the blood stream. In that dynamic environment, platelet functions can affect the progression of hematogenous metastasis by complementing tumor cell adhesive properties, providing protection from immune elimination and, through their intricate interactions, select tumor cells with a metastatic phenotype. Future studies might show that the multitude of growth factors and cytokines released from platelet granules also directly impact tumor cell survival and proliferation, and the recruitment of inflammatory cells into the microenvironment of nascent metastatic lesions. Thus, investigation of platelets, their surface receptors, released products, and their critical influence on coagulation pathways, will help elucidate mechanisms of tumor metastasis, as well as vascular development and stability that support metastatic growth.

The changing requirements by disseminating tumor cells during metastasis provide challenging questions to address and understand the complexity of platelet involvement in different stages of cancer progression. Transgenic mouse models with defined aberrations in platelet and other host cell type functions will help to further elucidate critical molecular mechanisms. For example, one such model that has not been explored in the context metastasis is a model of Glanzmann's thrombasthenia [136]. This model recapitulates a human disease where platelets lack or have aberrant expression of integrin αIIbβ3. Models like this offer the opportunity to explore contributions of platelet receptors and functions that are key to platelet adhesion, aggregation and activation.

Important questions to be addressed are: Which stages of tumor progression are affected by platelet hemostatic functions? When are platelet adhesive functions critical to support or influence tumor progression? When and how is platelet involvement in inflammatory functions crucial? New concepts include the identification and properties of premetastatic niches, and how platelets might contribute to their development in distant organs. Platelet functions during the recruitment of bone marrow progentior cells may be involved [34, 47]. The development of megakaryocyte lineage-specific mouse models will help delineate platelet contributions, among those of other hematopoietic cells, during the progression of cancer and establishment of metastatic disease.

Supplementary Material

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Glossary

Disseminated intravascular coagulation:
pathological activation of coagulation (blood clotting) mechanisms that lead to formation of small blood clots in blood vessels throughout the body[4].

Migratory thrombophlebitis:
malignancy-associated hypercoagulable state leading to spontaneous platelet-rich clots in veins anywhere in the body that dynamically form, resolve and reappear[7].

Pulmonary embolism:
Blockage of the main artery of the lung or one of its branches by a platelet-rich clot that originated in the venous circulation and dislodged from where it initially formed[2].

Thromboembolic disease:
condition caused by traveling blood clots or emboli that occlude blood vessels; commonly manifesting as deep vein thrombosis or pulmonary embolism in cancer patients[2, 3].

Glycoconjugates:
carbohydrate-linked lipids and proteins, which include receptors and ligands that function as cell adhesion molecules[82].

Weibel-Palade bodies:

secretory granules of endothelial cells that particularly store and secrete multimerized von Willebrand Factor, and which translocate P-selectin to the membrane surface upon cell activation[154].

Shear stress:
the velocity of flowing blood is highest in the center of vessels and decreases toward the vessel wall, resulting in differential flow that generates a shearing force[21].

Blood perfusion model:
parallel plate flow chamber system in which adhesive interactions between blood borne tumor cells, blood cells, platelets and endothelial cells are monitored and measured by video microscopy[92].

Endothelial retraction:
disruption of adherence junctions of endothelial cells creates gaps in the endothelial monolayer and increases vascular permeability while exposing matrix proteins of the basement membrane[155].

Paraneoplastic disease:
disease induced as a result of tumor burden; generally caused by the release of tumor-derived hormones, peptides, or cytokines or by the misguided destruction of normal tissue by immune cells targeted against malignant cells[156, 157].

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Box 1

Platelet microparticles

When platelets are activated, they relinquish surface membrane particles (30–100 nm) and exosomes (100 nm-1 μm) derived from α granules fused with the plasma membrane. Both particle types contain parent platelet membrane proteins and lipids [137]. Platelet microparticles pack a repertoire of surface receptors including integrin αIIbβ3, the GPIbα-IX-V receptor complex, P-selectin, CXCR4, bioactive lipids such as LPA and S1P, cytoplasmic and secreted proteins. Furthermore, platelet microparticles provide a procoagulant membrane surface for the activation of thrombin [137]. Blood borne microparticles are also formed from endothelial cells, leukocytes and tumor cells, and contribute to many pathological disease states, especially cancer [138, 139]. The association of platelet microparticles with individual circulating tumor cells or with tumor cells in heteroaggregates could potentially add to their expression of adhesion molecules, stimulate the release of cytokines, and activate intracellular signaling pathways. As a consequence, downstream effects might alter the reactivity of blood borne tumor cells with the vasculature, stimulate adhesion and proliferation of cancer cells and impact metastasis. Preliminary studies demonstrate an increased invasiveness, migration, proliferation and experimental metastasis of tumor cells when incubated with platelet microparticles [140–144]. A mechanistic involvement of platelet microparticles in tumor progression has not been investigated in detail. However, high levels of these particles correlate with aggressive tumors and a poor clinical outcome [145–151].

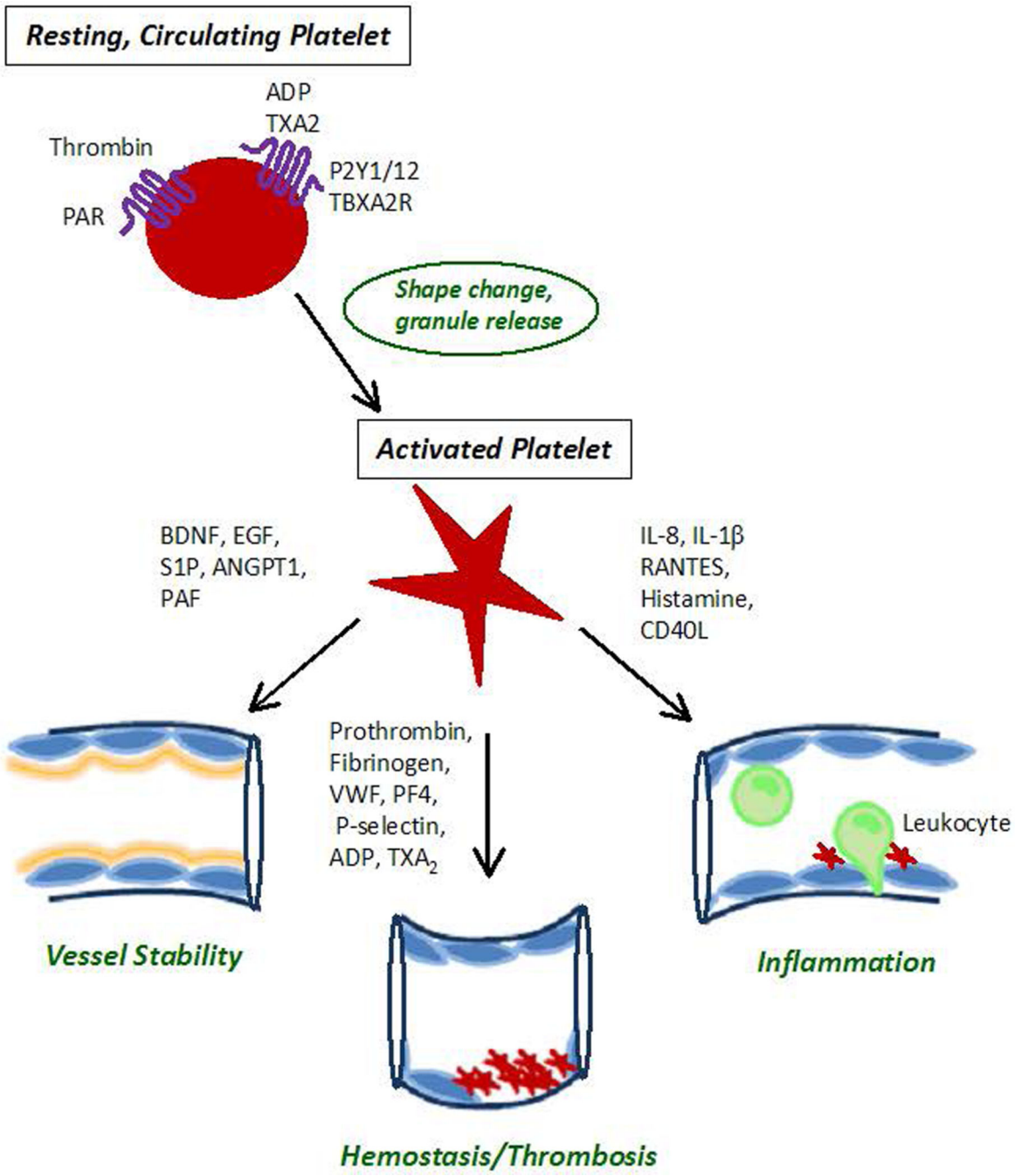

Figure 1. Platelet biology

During thrombus formation, thrombin activates G protein-linked protease-activated receptors (PARs) at the platelet surface, thereby triggering a flux in intracellular calcium and a decrease in cyclic adenosine monophosphate (cAMP). This allows for rapid reorganization of the platelet cytoskeleton and shape change, maximizing platelet adhesion during thrombus formation. Other platelet agonists include collagen of the subendothelial matrix exposed during vascular injury, adenosine diphosphate (ADP), and thromboxane A_2 (TXA_2). The latter bind G protein-coupled receptors P2Y and TXA_2R respectively, to

stimulate platelet degranulation and release of their contents as part of an amplification loop of platelet activation and aggregation. For instance, thrombosis is perpetuated by prothrombin, fibrinogen, von Willebrand factor (VWF), platelet factor 4 (PF4), P-selectin, TXA_2, and adenosine diphosphate (ADP) [21, 22]. Platelets are now also recognized as modulators of endothelial integrity and immune cell trafficking between the blood stream and sites of inflammation through their released factors [45, 152]. Platelets sustain vascular stability through maintenance of endothelial cell junctions via the release of brain-derived neutrophic factor (BDNF), epidermal growth factor (EGF), sphingosine 1 -phosphate (S1P), angiopoietin −1 (ANGPT1), and platelet-activating factor acetylhydrolase (PAF) [152]. Activated platelets dispense interleukin-8 (IL-8), IL-1β, C-C motif chemokine 5 (CCL5, also known as RANTES), CD40 ligand (CD40L) and histamine that stimulate endothelial cells to upregulate adhesion receptors and chemokine release to promote leukocyte recruitment and tissue infiltration during the inflammatory response [22, 153]. The potential role of platelets in cancer progression and target organ colonization by circulating tumor cells is summarized in this review.

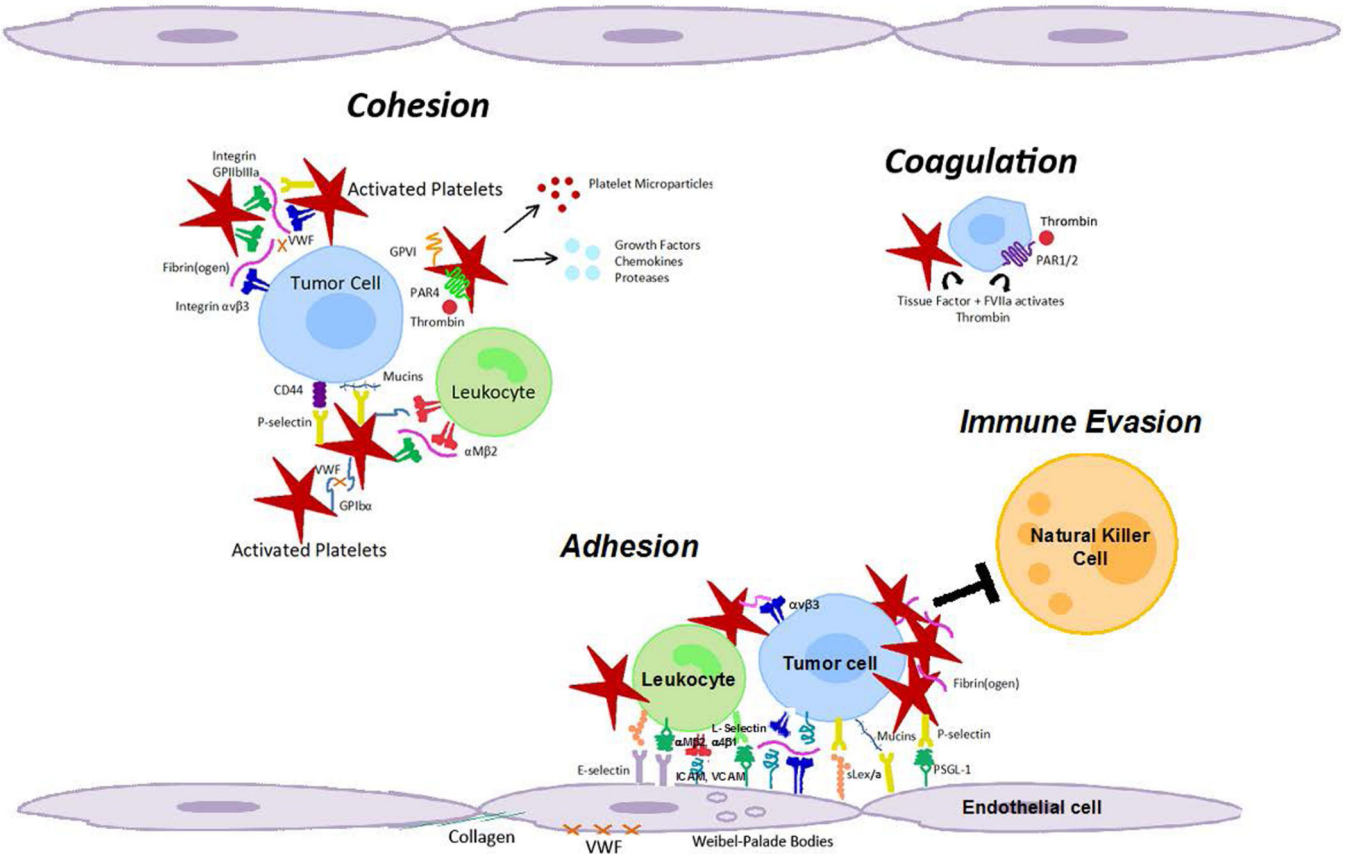

Figure 2. Molecular coordination between platelets and tumor cells supports metastasis from the blood stream.

As platelets become activated, they undergo a shape change, increase their adhesiveness, release granules and microparticles, and perpetuate cohesion of heteroaggregates containing tumor cells, platelets and leukocytes. Platelet granules contain growth factors, chemokines, and proteases. *Cohesion:* Circulating tumor cells can interact with activated platelets and leukocytes, and form heteroaggregates that support attachment to the endothelium and thereby contribute to metastasis. *Coagulation:* Platelet-initiated coagulation steps can activate receptors on tumor cells (e.g. PAR1/2) and promote release of tissue factor that further enhances procoagulant activity. *Adhesion:* Initial, transient adhesion involving selectins, mucins, CD44, and other sialyl Lewis X/A containing glycoconjugates is followed by firm attachment mediated by integrins and intercellular cell adhesion molecules. *Immune evasion:* Multivalent plasma proteins form intercellular bridges, and activated platelets and fibrin(ogen) protect tumor cells from natural killer cell lysis during hematogenous metastasis. Coincidently, tissue factor and FVIIa activate thrombin to cleave protease-activated receptors on platelets and tumor cells to promote invasion and metastasis. GP (Glycoprotein), PAR (Protease-activated receptor), ICAM (Intercellular adhesion molecule, ICAM-1), VCAM (Vascular cell adhesion molecule, VCAM-1), PSGL1 (P-selectin-glycoprotein ligand 1), sLex/a (Sialyl Lewis X/A antigens), VWF (Von Willebrand factor).

Table 1.

Key platelet molecules involved in tumor metastasis, and models used for their identification

Platelet or progenitor molecule	Mouse model Genotype Phenotype / background	Conclusion and implicated role in tumor metastasis
NF-E2	*NF-E2*−/− Complete platelet deficiency Immune deficient host	Platelets support experimental metastasis [60]
Gαq	*Gαq*−/− Defective platelet activation Immune competent host	Platelet activation protects tumor cells from natural killer cells; platelets support spontaneous metastasis [51]
PAR4	*Par4*−/− Impaired platelet activation Immune competent host	Thrombin-activated platelets enhance metastasis [60]
P-selectin	*P-selectin*−/− Immune competent host	Platelets cohere with colon cancer cells and leukocytes to enhance metastasis [49, 81]
GPIbα	*GpIbα*−/− Immune competent host	GPIbα supports experimental metastasis of melanoma cells [106]
GPVI	*GpVI*−/− Immune competent host	GPVI enhances experimental metastasis of melanoma and lewis lung carcinoma [53]
LPA	*Platelet depleted mice* LPA receptor overexpressing tumor cells	Platelet-derived LPA enhances bone metastasis and growth of breast cancer cells [48]
β_3 integrin	*Integrin* β_3 −/− Immune competent host	$\alpha IIb\beta_3$ on platelets supports bone metastasis [92]

Gαq (Guanine-nucleotide-binding protein G_q), GPIbα (Glycoprotein Ibα), GPVI (Glycoprotein VI) LPA (Lysophosphatidic acid), NFE2 (Nuclear factor (erythroid-derived 2)), Par4 (Protease-activated receptor 4)

Table 2.

Platelet surface molecules and released factors with known role in cancer

Name	Function	Name	Function
Tumor Promoting		***Tumor Promoting***	
Cell Surface		**α Granules**	
αIIbβ3	Firm platelet adhesion and aggregation; tumor cell metastasis [63]	CCL2	Macrophage chemokine; tumor cell invasion and tumor macrophage infiltration [158]
GPIbα	Platelet adhesion and recovery from vascular injury especially under high shear blood flow; tumor cell metastasis [112]	CCL3	Proinflammatory cytokine [153]
GPVI	Platelet adhesion, activation and aggregation; tumor cell metatasis [55]	CCL5	Chemokine that recruits leukocytes during inflammation; tumor progression [88]
P-selectin	Stabilize platelet aggregation and leukocyte arrest on endothelium; tumor growth and metastasis [51]	CCL7	Proinflammatory, macrophage chemokine [159]
PAR4	Thrombin receptor that activates platelets; enhances metastasis [62]	Gro-α	Neutrophil chemokine and monocyte arrest [160]
Gαq	α subunit of G protein mediates signaling during platelet a ctivation; enhances metastasis [53]	CXCL5	Proinflammatory cytokine [160]
α Granules		IL-8	Proinflammatory, leukocyte chemoattractant, angiogenesis [160]
Prothrombin	Activated by tissue factor to convert fibrinogen to fibrin, procoagulant, tumor progression [25]	CXCL12	Chemokine; tumor progression and metastasis [5]
SERPINE1	Inhibits plasminogen activation and fibrinolysis; tumor invasion and angiogenesis [161]	BDNF	Neuronal development *t* and function; *receptor Trkb* supports tumorigenesis and metastasis[162]
Plasminogen	Protease that breaks down fibrin in blood clots [161]	TP	Tumor progression and angiogenesis; chemotherapy activation [163]
uPA	Serine proteinase that converts plasminogen to plasmin [161]	**Dense Granules**	
EGF	Enhances cell growth, proliferation, differentiation and migration [9]	Serotonin	Platelet aggregation, vascular tone, and cardiac functions; tumor angiogenesis and metastasis [124]
TGF-β	Regulates inflammation; *both enhancing and inhibitory* roles in tumorigenesis and progression [164]	Histamine	Proinflammatory, increases vascular permeability [165]
PDGF	Enhances stromal cell survival, proliferation and migration, angiogenesis [9]	ATP	Triggers calcium influx and amplifies platelet aggregation [22]
bFGF	Mitogenic, tumor progression and angiogenesis [9]	ADP	Potent activator of platelets, adhesion and aggregation [22]
HGF	Tumor progression, metastasis, and angiogenesis [9]	IL-1β	Proinflammatory cytokine, induces angiogenesis [153]
IGF1	Mitogenic, tumor progression and angiogenesis [9]	VEGF	Regulates vascular permeability and growth, stimulates angiogenesis [43]
VEGF	Regulates vascular permeability and growth, stimulates angiogenesis [43]	***Tumor Inhibiting***	
ANGPT1	Maintains vascular integrity; tumor progression and angiogenesis [166]	**α Granules**	
MMP-1	Tissue remodeling and matrix degradation [106]	PF4	Promotes blood coagulation; inhibits tumor growth and angiogenesis [133]

Name	Function	Name	Function
MMP-2	Tissue remodeling, matrix degradation and tumor cell invasion [106]	TSP-1	Enhances platelet aggregation and inhibits angiogenesis [52]
MMP-9	Matrix degradation and tumor cell invasion, metastasis [104]	VWF	Matrix protein, forms multimers to aggregate platelets, binds GPIb-V-IX; inhibits tumor cell metastasis [115]
Fibrinogen	Coagulation, precursor of fibrin clots, matrix protein, platelet adhesion and aggregation; protects and adheres tumor cells in circulation [53]	PGK	Glycolytic enzyme; anti-angiogenic factor [167]
Fibronectin	Extracellular matrix protein, binds integrins, fibrin, and collagen; tumor cell invasion and metastasis [73]	TIMP	Inhibits matrix metalloproteinases, antiangiogenic [106]
PAF acetylhydrolase	Inflammation and hemostasis, lipid messenger [168]	Collagen α1(XVIII) chain isoform 1 precursor (Endostatin)	Angiogenesis inhibitor, derived from collagen type XVIII [43]
S1P	Vascular permeability, cell invasion and survival [169]	**Dense Granules**	
LPA	Cell proliferation, survival and migration [170]	TFPI	Serine protease inhibitor, inactivates TF-FXII coagulation [171]
P-selectin	Cell adhesion molecule, proinflammatory, marker of platelet activation; tumor progression [51]	PF4	Promotes blood coagulation; inhibits tumor growth and angiogenesis [133]

ADP (Adenosine diphosphate), ATP (Adenosine triphosphate), ANGPT1 (Angoipoietin-1), bFGF (Basic fibroblast growth factor), BDNF (Brain-derived neutrophic factor), CCL (C-C motif chemokine), CXCL5 (C-X-C motif chemokine), EGF (Epidermal growth factor), GPIbα (Glycoprotein Ibα), GPVI (Glycoprotein VI), Gro-α (Growth-regulated protein homolog), HGF (Hepatocyte growth factor), IGF1 (Insulin-like growth factor 1), IL(Interleukin), LPA (Lysophosphatidic acid), MMP-2 (Matrix metalloproteinase-2), PGK (Phosphoglycerate kinase), SERPINE1 (serpin peptidase inhibitor, also known as plasminogen activator inhibitor-1, PAI 1), PF4 (Platelet factor 4), PAF (Platelet-activating factor acetylhydrolase), PDGF (Platelet-derived growth factor), PGK (Phosphoglycerate kinase), S1P (Sphingosine 1 -phosphate), TFPI (Tissue factor pathway inhibitor), TIMP (Tissue inhibitor of metalloproteinases), TSP-1 (Thrombospondin-1), TP (Thymidine phosphorylase), TGF-β (Transforming growth factor-β), uPA (Urokinase plasminogen activator), VEGF (Vascular endothelial growth factor), VWF (von Willebrand factor). See [45] for additional information.