The platelet contribution to cancer progression

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Summary. Traditionally viewed as major cellular components in hemostasis and thrombosis, the contribution of platelets to the progression of cancer is an emerging area of research interest. Complex interactions between tumor cells and circulating platelets play an important role in cancer growth and dissemination, and a growing body of evidence supports a role for physiologic platelet receptors and platelet agonists in cancer metastases and angiogenesis. Platelets provide a procoagulant surface facilitating amplification of cancer-related coagulation, and can be recruited to shroud tumor cells, thereby shielding them from immune responses, and facilitate cancer growth and dissemination. Experimental blockade of key platelet receptors, such as GP1b-IX-V, GPIIbIIIa and GPVI, has been shown to attenuate metastases. Platelets are also recognized as dynamic reservoirs of proangiogenic and anti-angiogenic proteins that can be manipulated pharmacologically. A bidirectional relationship between platelets and tumors is also seen, with evidence of 'tumor conditioning' of platelets. The platelet as a reporter of malignancy and a targeted delivery system for anticancer therapy has also been proposed. The development of platelet inhibitors that influence malignancy progression and clinical testing of currently available antiplatelet drugs represents a promising area of targeted cancer therapy.

Keywords: angiogenesis, cancer, metastases, platelets, TCIPA.

Introduction

Tumor cells interact with all major components of the hemostatic system, including platelets. Platelets and platelet activation have been linked to key steps in cancer progression (summarized in Fig. 1). The contribution of platelets to malignancy progression has been suggested to be an organized process that underlies the pathobiology of cancer growth and dissemination rather than a simple epiphenomenon of neoplasia (reviewed in [1]). Here, we highlight current insights into how platelets contribute to cancer growth, maintenance and propagation and identify potential targets and directions for platelet-directed anticancer therapy in the future.

Platelet structure and function

Often numbering over 3–4 trillion in an individual patient with cancer, platelets represent the smallest circulating hematopoietic cells and are anucleate fragments formed from the cytoplasm of megakaryocytes. The platelet membrane consists of phospholipids and is covered with glycoproteins and integrins, which are essential for adhesion, aggregation and activation, the critical steps in platelet-mediated hemostasis. Important platelet membrane receptors include Glycoprotein Ib-IX-V (GPIb-IX-V), Glycoprotein VI (GPVI) and Glycoprotein IIb-IIIa (GPIIb-IIIa, also as integrin αIIbβ3), receptors that are essential for complete adhesion and aggregation [2,3]. Additional important receptors found on platelet membranes include the protease-activated receptors (PAR), PAR-1 and PAR-4, and the P2 receptors, P2Y1 and P2Y12, which principally mediate activation and aggregation [4]. Platelets also contain three types of granules: (i) dense granules containing platelet agonists such as serotonin and ADP that serve to amplify platelet activation, (ii) α granules containing proteins that enhance the activation process and participate in coagulation; and (iii) lysosomal granules containing glycosidases and proteases [5].

Many of the major structural components of platelets and platelet receptors that contribute to hemostasis have also been found to relate to malignancy progression (reviewed in Table 1). For example, in addition to coagulation-related proteins, platelets also store proteins within the alpha granule that can regulate angiogenesis and metastases [2,6]. Further, platelet receptors such as GPIIb/IIIa can mediate platelet angiogenic protein release in addition to their more traditional role in fibrinogen binding. At least one study has found ultrastructural changes in platelets from patients with lung cancer, including an increase in the number of platelet alpha granules [7]. Interestingly, these researchers also found that the number of alpha granules was associated with survival.

Functionally, platelets are complex cells capable of shape change, translational protein production, protein and metabolite release, cell-cell interactions and paracrine regulation. Most of these functions relate to the processes of platelet activation and aggregation that occur following exposure to
Platelets are involved in key steps of malignancy progression. In in vitro and in vivo murine models, a role for platelets has been demonstrated in tumor metastasis, tumor growth and angiogenesis. Our working understanding of the role of platelets in malignancy involves: (i) tumor cell-induced platelet aggregation can occur following tumor cell intravasation into the vasculature, thereby ‘protecting’ or ‘cloaking’ circulating tumor cells from physical clearance and immune surveillance, (ii) platelets facilitate tumor cell arrest within the vasculature, endothelial cell retraction and subsequent tissue invasion, (iii) platelets induce endothelial cell proliferation and new blood vessel formation, which are requisite for tumor-associated angiogenesis and growth and (iv) platelet-tumor and platelet-stromal interactions in the tumor microenvironment depend, in part, on platelet activation and platelet protein release, which contribute to the inflammatory response. Additional platelet-related proteins and metabolites that facilitate proteolysis and tissue remodelling also enhance tumor growth and metastasis (including bony metastases).

Tumor cell-induced platelet aggregation, activation and metastases

Platelets contribute to critical steps in cancer metastasis, including facilitating tumor cell migration, invasion [44–46] and arrest within the vasculature [47–49]. In cellular models of both breast cancer and ovarian cancer, invasiveness has increased following exposure to platelets [46,50]. In the latter, both activated platelet membranes and platelet releasate increased invasion. Platelet contents may be released into the peritumoral space following platelet activation and enhance tumor cell extravasation and metastases [51–55]. An important step in metastatic dissemination is the breakdown of vessel basement membrane. By releasing proteolytic enzymes such as gelatinase, heparanase and various matrix metalloproteinases (MMPs), activated platelets can directly degrade structural components, or alternatively, support this process by activating other proteinases and/or enabling tumor cells and endothelial cells to do the same [46,56–58]. Moreover, modulation of proteolytic activity is accomplished by growth factors released with decreased metastasis of TA3 ascites tumor cells. This antimetastatic effect was neutralized by infusion of platelet-rich plasma (PRP). Thrombocytopenia experimentally induced by a variety of mechanisms has also been associated with a reduction in the number of metastases in tumor transplant models [23,24].

Thrombocytosis is observed in 10–57% of patients with cancer, with the number varying based on cancer type [1]. The relationship between elevated platelet count and malignant tumors was initially reported by Reiss et al. in 1872 [25]. Subsequent studies have established this relationship for common cancers, including colorectal, lung and breast cancer, as well as gastric, renal and most urogenital malignancies [26–32]. Further, for the majority of malignancies, the extent of platelet count elevation is inversely correlated with survival, making thrombocytosis a marker of poor prognosis [26–32].

Insights into the mechanisms underlying the initial observations of thrombocytosis in malignancy have been forthcoming in more recent decades. Sierko & Wojtkiewicz [1] have recently summarized mechanisms underlying the humoral interaction between tumor cells, bone marrow endothelial cells (BMEC) and megakaryocytes. An important driver for thrombocytosis in malignancy is the secretion of tumor-derived cytokines such as IL-1, GM-CSF, G-CSF and IL-6, which stimulate thrombopoiesis through a thrombopoietin-dependent mechanism, influencing largely megakaryopoietic growth and differentiation [33–38]. Megakaryocytes have a similar ability to secrete inflammatory cytokines, which can in turn influence bone marrow endothelial cells to support megakaryocytic maturation [39,40]. VEGF and b-FGF are also released by megakaryocytes, and influence megakaryocytic maturation and transendothelial migration via an autocrine loop [41–43]. Although incompletely elucidated, the interactions between tumor cells, megakaryocytes and bone marrow endothelial cells appear to promote thrombopoiesis, and may influence tumor angiogenesis.

Early observations on platelets and cancer

Gasic et al., in 1968 [23], first described the association between platelet number and metastatic cancer potential. This group found neuraminidase-induced thrombocytopenia was associated with decreased metastasis of TA3 ascites tumor cells. This
by platelets, a topic recently reviewed by Sierko & Wojtukiewicz [1].

Tumor cells have the ability to aggregate platelets, a finding first reported in 1968, and referred to as tumor cell-induced platelet aggregation (TCIPA) [23]. It is now recognized that this aggregation correlates with the metastatic potential of cancer cells in both in vitro and in vivo models of experimental metastasis [59,60]. The mechanisms by which tumor cells induce platelet aggregation may differ by cancer type, but have in common the theme of conferring survival advantage. In turn, platelets can protect tumor cells in at least two ways: by coating them and thereby directly shielding them from physical stressors within the vasculature and by permitting evasion from the immune system's effector cells. For example, platelets have been shown to protect tumors from NK cells and TNF-α cytotoxicity [61,62]. Timar et al. [63] have raised the hypothesis that some malignant cells can acquire a platelet-like phenotype, with expression of similar adhesion molecules and receptors. This concept of 'platelet-mimicry' has been suggested to relate to the perceived lack of tumor-directed immune surveillance.

Table 1

<table>
<thead>
<tr>
<th>Platelet component</th>
<th>Principal role in thrombus formation</th>
<th>Role in malignancy</th>
<th>In vitro models</th>
<th>In vivo models</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPIIb/IIIa (αIβ3)</td>
<td>Activation allows fibrinogen binding and platelet plug reinforcement</td>
<td>Tumor cell and platelet interaction (via fibronectin, fibrinogen and VWF) demonstrated in numerous cell lines; inhibition decreases TCIPA and platelet-mediated angiogenic growth factor release</td>
<td>Decreased pulmonary metastasis following inhibition of receptor by antibody and receptor antagonists</td>
<td>[3,60,71,86–88,90,160,165,166]</td>
<td></td>
</tr>
<tr>
<td>GP Ib-IX-V</td>
<td>Binding of von Willebrand factor; anchors platelet to subendothelium</td>
<td>Limited data to suggest role in TCIPA; conflicting data on tumor cell-platelet interactions</td>
<td>Pulmonary metastasis decreased in mice lacking GPIb but increased when GPIb functionally inhibited by monovalent, monoclonal antibodies</td>
<td>[71,91,92]</td>
<td></td>
</tr>
<tr>
<td>GPVI</td>
<td>Platelet adhesion to collagen</td>
<td>Not studied to date</td>
<td>50% reduction in pulmonary metastases in GPVI-deficient mice</td>
<td>[93]</td>
<td></td>
</tr>
<tr>
<td>P-selectin</td>
<td>Mediates platelet-leukocyte tethering; triggers leukocyte activation</td>
<td>Facilitated interaction between tumor cells and endothelial cells via sialylated fucosylated carbohydrates</td>
<td>Deficiency or blockade of P-selectin inhibits the formation of melanoma metastases</td>
<td>[94–100]</td>
<td></td>
</tr>
<tr>
<td>P2Y receptors</td>
<td>ADP-mediated platelet aggregation</td>
<td>ADP-mediated VEGF release from platelets; ADP induced TCIPA</td>
<td>ADP depletion associated with reduced metastases</td>
<td>[67–71,124,133,163,167]</td>
<td></td>
</tr>
<tr>
<td>PAR receptors</td>
<td>Thrombin mediated platelet activation</td>
<td>Selective release of angiogenesis influencing proteins; induces TCIPA</td>
<td>Promote metastases</td>
<td>[11,122,123,168]</td>
<td></td>
</tr>
<tr>
<td>Alpha granules</td>
<td>Storage of proteins that enhance adhesive process: fibrinogen, VWF, MMP-II, P-selectin, factor V, PF-4, platelet activating factor</td>
<td>Uptake and storage of angiogenic proteins that are selectively packaged and released: VEGF, b-FGF, endostatin, angiostatin, TSP-1; storage and release of proteolytic enzymes and metastasis influencing proteins</td>
<td>Maintenance of intra-tumor vascular integrity</td>
<td>[6,117,120–122,126,141]</td>
<td></td>
</tr>
<tr>
<td>Platelet microparticles</td>
<td>Enhances thrombosis and secondary hemostasis</td>
<td>Increased tumor cell invasiveness, metastasis, MMP-2 up-regulation and angiogenesis; increased leukemia, prostate and breast cancer invasion/migration</td>
<td>Increased chemo-invasiveness and metastases formation in lung cancer models</td>
<td>[101–108]</td>
<td></td>
</tr>
</tbody>
</table>

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cell-induced platelet activation follows as their understanding is pivotal to the development of selective agents targeting the pharmacologic inhibition of these central pathways (see also [66] for extensive review).

Adenosine diphosphate (ADP) is contained in platelet dense granules and is considered a secondary mediator of aggregation. The major ADP receptors, P2Y1 and P2Y12, are both involved in platelet aggregation. Stimulation through these receptors also leads to shape change and thromboxane A2 generation by platelets [67]. ADP contributes to TCIPA induced by various tumor cell lines, including neuroblastoma, melanoma and breast carcinoma [68,69]. The P2Y12 receptor plays a central role in platelet activation and in TCIPA [70,71]. For example, by generating ADP, MCF-7 breast carcinoma cells activate and aggregate platelets via the P2Y12 receptor [71].

Thrombin has a multifaceted role in hemostasis and represents a key link between primary and secondary coagulation responses. Thrombin has also been linked to tumorigenesis and angiogenesis, with thrombin signaling being a major contributor to metastatic tumor dissemination [72]. Thrombin has been detected in situ in numerous tumor types, including small cell lung cancer, renal cell, melanoma and ovarian cancer [73–75]. Tumor-enhancing effects of thrombin include induction of TCIPA, increased tumor-cell adhesiveness, promigratory and chemotactic effects, and up-regulation of VEGF expression by tumor cells [76–79]. Importantly, thrombin is also the most potent platelet activator, and exerts its function via the platelet PAR receptors, PAR-1 and PAR-4. Secretion of ADP and thrombin by human tumor cells activates platelets and recruits them to participate in TCIPA [11]. Following thrombin-mediated platelet activation, up to 300 biologically active molecules can be released and deposited ad lib at sites of vascular injury, at the site of a wound or within the tumor and tumor vasculature [6].

Cathepsin B, cancer procoagulant factor and the matrix metalloproteinases (MMPs) are contributors to TCIPA. Cathepsin B and cancer procoagulant factor can induce platelet aggregation when released by tumor cells [80,81]. MMPs have demonstrated a similar ability to induce TCIPA in vitro [82]. MMPs can be released by both platelets and cancer cells in vivo (reviewed in [83]). Jurasz and colleagues have identified enhanced generation of MMP-2 as the potential cause of human platelet aggregability in the setting of metastatic prostate cancer [66].

Thromboxane A2 (TXA2) and its receptor (TX) also play integral roles in platelet-tumor aggregation. It has been shown that TX mediates platelet aggregation induced by murine and tumor cell lines [84]. TXA2 can be generated by platelets as a result of activation induced by other platelet agonists, an observation that highlights the complex and interrelated nature of platelet functional responses in the tumor.

Platelet adhesion receptors also play a critical role in tumor-platelet cross-talk and the process of hematogeneous metastasis (recently reviewed in [85]). The role of the GPIIb-IIIa receptor in TCIPA has been established for decades, and numerous metastatic models have highlighted the importance of this receptor in the tumor-platelet interaction model [86–89]. A recent role for the GPIIb/IIIa receptor in the release of proangiogenic proteins and fibrinogen has also been elucidated [87,90]. The involvement of the integrin receptor GPIIb/IIIa in tumor metastasis, on the other hand, has been more difficult to define [59,86,91,92]. Recently, the GPVI surface receptor, a member of the immunoglobulin superfamily, which principally binds collagen, has become a subject of active investigation. Importantly, a 50% reduction in experimental pulmonary metastases in GPVI-deficient mice was reported by Jain et al. [93]. Clinically, patients with GPVI deficiency exhibit a mild bleeding tendency, suggesting that this receptor could potentially be inhibited without major hemostatic consequence.

Finally, P-selectin is expressed on activated platelets and endothelial cells and has been identified as an important mediator of the interaction between these cells and the vessel wall [94]. This facilitated interaction also applies to tumor cells as P-selectin can bind to different tumor cell lines through binding of sialylated fucosylated carbohydrates [95,96]. In a similar manner, P-selectin appears to facilitate interactions between tumor cells and the surrounding endothelium, at least in the case of melanoma [97]. Deficiency or blockade of P-selectin has inhibited the formation of metastasis in various other experimental models [97,98]. This effect is most pronounced in mucin-producing cancers [99,100].

Platelet microparticles and malignancy

When platelets are activated or exposed to high shear stress, they release particles expressing membrane receptors and cytoplasmic constituents termed platelet microparticles (PMPs). A growing body of literature supports the direct involvement of PMPs in malignant cell proliferation and growth. PMPs have the ability to induce chemotaxis of many hematopoietic cells and increase their adhesive affinity to fibrinogen [101]. PMPs express multiple proteins and chemokine receptors, which can be transferred to surrounding cell membranes, including malignant cells, which then benefit from enhanced invasiveness [102–105].

In vitro, PMPs have been shown to induce proliferation and tube formation of human umbilical vein endothelial cells, to increase transmatrigel chemoinvasion of lung cancer cell lines, and to increase invasiveness of breast cancer cells [105]. In vivo, angiogenesis can be observed in the heart of ischemic rats when PMPs are injected into myocardium [106]. Injection of murine Lewis lung cancer cells coated with platelet PMPs was associated with significantly more metastatic lung disease [107]. Janowska-Wieczorek et al. [105] have recently demonstrated that PMPs promote adhesion of tumor cells to endothelium, induce chemotaxis and chemoinvasion, and up-regulate MMP production. MMP-2 up-regulation and increased malignant cell invasiveness have also recently been reported in prostate cancer [108]. PMPs appear to represent an important aspect of the functional interaction between tumors and platelets and may represent a novel treatment approach in the future.
The role of platelets in angiogenesis

Evidence supporting the link between platelets and angiogenesis has accumulated since Pinedo and Folkman first raised this hypothesis [109]. The growth of solid tumors and formation of metastases depend on the generation of neovessels, and it is recognized that tumor cells cannot grow beyond 2–3 mm in size without a new vascular network [110]. These vessels are needed not only to sustain and nourish the developing tumor cells, but also to allow delivery of proteases and cytokines that permit further invasion, extravasation and dissemination. This elaborate delivery and transportation system exists secondary to an altered balance between angiogenesis stimulators and inhibitors. These proteins are released by many components of the tumor microenvironment, including the tumor itself. This tumor microenvironment is comprised of stromal fibroblasts, resident macrophages and mast cells, mononuclear cells and platelets [111–115].

Platelets contain over 30 important angiogenesis regulating proteins. Platelets are now recognized as the major source of VEGF (a pro-angiogenic protein) in serum as the platelet pool comprises > 80% of total circulating VEGF in patients with cancer as well as healthy individuals [116,117]. Of interest is the observation that in some cancers, platelet-derived VEGF better predicts tumor progression than serum levels of VEGF [118]. Platelets also contain proteins that inhibit angiogenesis, including platelet factor-4 (PF-4), TSP-1 and endostatin [119,120].

Under normal physiologic conditions, platelets have been suggested to release angiogenic proteins to promote wound healing. These pro-angiogenic proteins are later counterbalanced by the release of angiogenic inhibitors from stromal cells and platelets, to stop uncontrolled growth in later stages of healing in non-malignant wounds [121]. These angiogenic mediators are packaged into distinct alpha granule populations, and selective release based on selective engagement of platelet receptors has been proposed [122]. Ma and colleagues first introduced the concept of differential release of platelet angiogenic proteins, by demonstrating that PAR-1 activation was associated with VEGF release and suppression of endostatin, while PAR-4 activation, conversely, stimulated endostatin release and suppressed release of VEGF [123]. These investigators subsequently treated rats with established gastric ulcers with an oral PAR-1 antagonist or vehicle. In this model, significant healing of ulcers did not occur in the rats treated with the PAR-1 antagonist [123].

Subsequently, the ADP receptors, P2Y1 and P2Y12, have been demonstrated to participate in the regulation of angiogenic protein release, though this pathway of platelet activation appears to release less VEGF than thrombin-mediated activation [124]. ADP-mediated platelet activation is associated with a net increase in the release of VEGF in healthy individuals, with no effect on endostatin release. This VEGF release can be abolished by selectively inhibiting the P2Y12 receptor [124].

The source and mechanism of platelet-derived angiogenesis proteins remain under active investigation in both healthy individuals and patients with cancer. Recent studies have offered insight. For example, in the circulation, platelets have been shown to uptake and store proteins that regulate angiogenesis [1,125,126]. In addition to protein uptake, Zaslavsky et al. [120] have recently demonstrated that the platelet source of TSP-1 is megakaryocyte derived, suggesting that enhanced production or endocytosis by marrow precursor cells may contribute to the platelet angiogenic protein content. Based on the findings that VEGF-A was regulated by Il-6 in a megakaryoblastic cell line, Salgado et al. [127] bring forward the hypothesis that higher VEGF levels in cancer patients may partly result from an IL-6 mediated up-regulation of the expression of VEGF-A in platelet precursors.

In vitro, proangiogenic effects of platelets were observed by Pipili-Synetos et al. [128], who noted that platelets stimulated endothelial cell proliferation and growth of capillary-like structures in Matrigel assays. An additional in vivo model of angiogenesis showed a reduction of retinal neovascularization in mice with induction of thrombocytopenia as well as inhibition of platelet aggregation by a highly specific alpha-IIbbeta3 receptor antagonist or aspirin [129]. This resulted in a 35–50% reduction of retinal neovascularization, further supporting the platelet contribution to angiogenesis [129]. Kisueka et al. also examined the role of platelets in four in vivo animal models of angiogenesis using both a cornea and Matrigel assay. They report that platelet-depleted mice experienced a significant reduction in corneal neovascularization and developed hemorrhage, and postulate that platelets support angiogenesis through release of growth factors and platelet-vessel wall interactions [130]. Brill has also demonstrated the role of platelet microparticles in models of angiogenesis [106].

Importantly, a clear understanding of the contribution of platelets specifically to tumor-associated angiogenesis remains under investigation. For example, while platelets enhance angiogenesis as in the examples above, platelet-endothelial interactions in tumor microvessels have been found to be reduced in murine models of tumor angiogenesis [131]. The platelet as a scavenger of VEGF and therefore a potent antiangiogenic cellular component of the tumor microvasculature could also be considered.

A complex and bidirectional relationship between tumor cells and platelets

There is growing evidence to suggest that the interplay between platelets and tumors is neither passive nor unidirectional (Fig. 2). Complex relationships between host, tumor and platelet within the cancer patient will need to be carefully delineated and significant research efforts are required if antiplatelet therapy is to be used successfully in the clinical setting. The platelet role in coagulation-mediated cancer progression, the platelet contribution to the tumor-stromal interaction and the contribution of platelets to inflammation and its subsequent role in malignancy progression are just several examples of these relationships [132]). Shared tumor cell
and platelet agonists and receptors offer both opportunity and potential obstacles for drug targeting. For example, drugs that inhibit the P2Y receptors on platelets may also interact with endothelial and cancer cell P2Y receptors and contribute to the overall impact of the drug [133–135]. The well-delineated role of thrombin signaling and activation of PARs found on malignant cells is another example of shared targets between tumor cells and platelets (reviewed in [136,137]). Some evidence suggests that platelets can be conditioned in vivo by tumor cells to deliver anti-angiogenic proteins [121,138]. In a murine model, Kerr et al. [138] have recently demonstrated that platelets preferentially store tumor-derived GM-CSF, TPO, TNF-α, TGF-β1 and especially MCP-1 over host-derived proteins. An emerging concept in the literature focuses on the platelet as a reporter of malignancy. For example, both platelet associated PF-4 and TSP-1 have been associated with early cancer growth and been proposed as biomarkers of early tumor progression [120,139]. Platelet granule proteins not only promote growth of tumor vessels, but prevent tumor hemorrhage, presumably by maintaining the integrity of the existing tumor vascular supply [140,141]. Though the precise mechanism underlying this phenomenon has not been fully elucidated, this appears to occur independently from thrombus formation. The prevention of tumor hemorrhage by platelets has more recently been found to relate, in part, to their ability to modulate vascular damage by tumor-infiltrating leukocytes [142]; an observation that further illustrates the complex tumor-stromal interaction, including the ability of platelet to influence inflammatory responses [140,143–145]. These observations suggest that the mechanism underlying the maintenance of neoplastic vessels by platelets may be distinct from that used for maintenance of host vessels, rendering pharmacologic inhibition of the former plausible. Selective platelet storage and release of stimulatory, inhibitory and regulatory proteins represents a novel conceptual framework to be explored in the understanding of tumor angiogenesis.

Antiplatelet therapy in the treatment of cancer

In 1989 and 1993, Dr Leo Zacharski and colleagues, writing for the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis Subcommittee on Hemostasis and Malignancy, published an update of clinical trials using antiplatelet therapy and anticoagulants in cancer [146,147]. At the time, over 20 studies, most of them pilot studies with 50 patients or fewer, were reported in the literature using an antiplatelet drug in the treatment (not prevention) of cancer. The majority of these studies focused on the drug dipyridamole in non-randomized studies, which reported variable response rates. An analogue of dipyridamole (RA-233, mopidamol) has also been studied in prospective randomized studies, with no survival benefit demonstrated in small cell and ovarian cancer but an approximate 100-day improvement in survival in non-small-cell lung cancer patients [148–150].

The remaining prospective studies using antiplatelet therapy focused on the use of aspirin in renal cell and small cell lung cancer and showed no effect [151]. Aspirin use has been most extensively studied in colorectal and breast cancer, with demonstrated efficacy in the colorectal cancer prevention setting [152]. Aspirin-mediated inhibition of platelet aggregation is well documented, and recently aspirin has also been shown to attenuate platelet protein release [153]. In vivo data suggesting a possible inhibitory role in the formation of metastasis were initially reported by Gasic et al. [154], who observed metastatic inhibition of MCA6 ascites sarcoma cells in mice, in the presence of aspirin. In a more recent publication, aspirin but not indomethacin suppressed the formation of lung metastasis in a metastatic hepato-cellular murine model [155]. Antimetastatic effects of aspirin, however, have not been seen consistently in all laboratory models.

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Clinical data evaluating the impact of aspirin therapy on cancer survival have begun to emerge. Fontaine et al. [156] have recently reported preliminary data suggesting that aspirin used in combination with the surgical treatment of non-small-cell lung cancer is associated with increased survival. Similarly, in a prospective observational study of women diagnosed with breast cancer, as reported in the Nurses’ Health Study, aspirin use was associated with decreased risk of breast cancer recurrence and death [157]. Additionally, aspirin use was found to decrease the proangiogenic effects of tamoxifen in patients with breast cancer [116]. Importantly, while we have recently reported on the use of aspirin therapy in women with breast cancer receiving tamoxifen therapy [116,159], there is a paucity of data to support the combination of antiplatelet therapy with existing tumor-targeted therapy.

Despite the limited number of prospective randomized trials, the laboratory data using antiplatelet therapy continue to accumulate. Early laboratory studies focused on prostacyclin and prostacyclin analogues, which have been previously reviewed [158]. In addition, blockade of the GPIIb/IIIa receptor using the monoclonal antibody 10E5, an inhibitor of human platelet GPIIb/IIIa, decreased lung colonization of cancer cells [160]. A challenging aspect of the administration of GPIIb/IIIa antagonists in the clinical setting has been the need for intravenous administration of these agents, which have recently reported preliminary data suggesting that aspirin has halted experimental metastasis formation in a murine model.

Table 2 Clinical outcomes associated with the use of platelet inhibitors in patients with cancer. Limited clinical data are available on the impact of platelet inhibitors on clinical outcomes in patients diagnosed with cancer. Murine model data are reviewed in the text and in Table 1

<table>
<thead>
<tr>
<th>Platelet inhibitor or modulator</th>
<th>Mechanism of platelet inhibition</th>
<th>Type of cancer(s) studied</th>
<th>Protocol designs</th>
<th>Observations in clinical studies</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>Inhibits platelet thromboxane production and platelet aggregation (anti-neoplastic effects of this drug are also anticipated to rely on COX-2 tissue and tumor inhibition)</td>
<td>Colon cancer</td>
<td>Double blind, randomized</td>
<td>No difference in overall survival</td>
<td>[169]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SCLC</td>
<td>Unblinded</td>
<td>No effect on survival</td>
<td>[170]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Renal cell carcinoma</td>
<td>Prospective randomized</td>
<td>No significant response or effect on survival</td>
<td>[151]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Breast cancer</td>
<td>Prospective observational study</td>
<td>Decreased recurrence and mortality from breast cancer</td>
<td>[157]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NSCLC (early stage)</td>
<td>Retrospective analysis</td>
<td>Increased survival post-resection</td>
<td>[171]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prostate cancer</td>
<td>Retrospective analysis</td>
<td>Improved PSA control in patients undergoing radiation</td>
<td>[172]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Breast cancer</td>
<td>Double blind</td>
<td>No significant response or improved survival</td>
<td>[173]</td>
</tr>
<tr>
<td>Benoral (aspirin-acetaminophen conjugate)</td>
<td>P2Y12 receptor antagonist; inhibits platelet aggregation induced by ADP</td>
<td>Prostate cancer</td>
<td>Retrospective analysis</td>
<td>Improved PSA control in patients undergoing radiation</td>
<td>[172]</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>Dipyridamole derivative; increase in platelet cyclic AMP; decreased platelet aggregation</td>
<td>Colon cancer</td>
<td>Double blind, randomized</td>
<td>No significant response</td>
<td>[149]</td>
</tr>
<tr>
<td>(Mopidamole)</td>
<td></td>
<td>NSCLC (early stage)</td>
<td>Double blind</td>
<td>Improvement in survival in limited stage/resected disease; no effect in disseminated disease</td>
<td>[149,150]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SCLC</td>
<td>Prospective randomized</td>
<td>No significant response</td>
<td>[150]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ovarian cancer</td>
<td>Prospective randomized trial</td>
<td>No effect on survival or recurrence</td>
<td>[148]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NSCLC (advanced stage)</td>
<td>Prospective randomized trial</td>
<td>No impact on survival or response</td>
<td>[174]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NSCLC (advanced stage)</td>
<td>Prospective non-randomized</td>
<td>No significant response compared with historical controls</td>
<td>[175]</td>
</tr>
</tbody>
</table>
model of lung cancer [87]. Integrin, a commercially available platelet-specific αIIbβ3 integrin antagonist, was administered to mice after establishment of bony metastases in a study by Boucharaba and colleagues, evaluating the role of platelet-derived lysosphosphatidic acid. This resulted in thrombocytopenia, decreased circulating Lpa plasma levels and a significant reduction in the number of osteolytic bony metastases [51].

Wenzel et al. have recently reported successful in vivo reduction of pulmonary metastases in a murine model of breast cancer using the platelet aggregation inhibitor cilostazol. By administrating liposomal cilostazol intravenously, they observed decreased ex vivo platelet aggregability and decreased platelet-tumor complex formation [161]. Similar results were obtained using liposomal dipymidole [162]. Few studies evaluating the common ADP receptor inhibitors, clopidogrel and ticlopidine, have been reported and they demonstrated limited success [163].

### Conclusion

Platelets play a multifaceted and important role in cancer biology (Table 3). The existing research suggests a compelling biological rationale for attempting to disrupt tumor-platelet cross-talk, with the goal of down-regulating tumor invasion, angiogenesis and spread. In the laboratory, platelet receptors, both constitutive and activation dependent, such as GP1b/IX/V, P-selectin and alphaIIb-beta3 integrin, can promote the progression and metastases of various tumor types and are obvious targets for further clinical study [164]. Additionally, control of the platelet reservoir of angiogenic proteins, which are both secreted and sequestered in a selective manner, represents an approach to angiogenic control within the tumor microenvironment.

The study of platelet inhibitors in the clinical setting will require a careful consideration of not only cancer type but stage of disease targeted. Importantly, appropriate trial endpoints must be chosen that are not by design predicated on direct and toxic tumor effects and secondary rapid cell kill and tumor shrinkage. A potential barrier that surrounds chronic administration of antiplatelet agents in the setting of active malignancy is directly related to the paramount role that platelets play in maintaining hemostasis. Currently available oral antiplatelet agents irreversibly inhibit their target, making the risk of bleeding more difficult to mitigate. Future work in the development of novel agents would ideally yield a molecule able to inhibit platelet-tumor interaction while maintaining sufficient platelet function to prevent bleeding. Potential new classes of agents include antibodies against P-selectin, platelet-specific oral integrin inhibitors, PAR-I antagonists and blockade of platelet-derived LPA.

How should we combine antiplatelet therapy with conventional cancer cell-directed therapy? Will other host factors that influence platelet activation, such as diabetes, be important in patient selection [135]? Existing antiplatelet drugs, such as aspirin and clopidogrel, remain understudied as adjuvants to conventional chemotherapeutic and hormonal therapies, particularly in animal models and the clinical setting. Increasing our translational database on the anticaner biology of antiplatelet strategies to include combination therapy and studies directed at prevention vs. low burden vs. high burden disease are imperative for the successful clinical translation of results. Importantly, we have learned much from the use of antiplatelet therapy in the treatment of cardiovascular disease, such as the concept of drug resistance. These considerations might be applied prospectively in oncologic studies. Future clinical trials formally addressing the role of antiplatelet therapy will need rigorous attention to patient selection, combination therapy with existing agents and trial endpoints but offer the hematologic community a significant opportunity to potentially improve cancer outcomes.

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**Table 3** Overview of important platelet-cancer cell interactions and their potential influence on cancer progression. A full discussion of these interactions is found in the text. These observations reflect in vitro and murine model data.

<table>
<thead>
<tr>
<th>Platelet-related mechanism</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet activation</td>
<td>Platelet activation enhances tumor cell-induced platelet aggregation, releases chemotactic cytokines, proteolytic enzymes and platelet microparticles that can support cancer growth and extravasation as well as angiogenesis</td>
</tr>
<tr>
<td>Increased in patients with cancer</td>
<td>Platelet activation provides a procoagulant surface to facilitate cancer-related coagulation</td>
</tr>
<tr>
<td>Facilitated by contact with tumor cells and tumor release/production of platelet agonists such as ADP and thrombin</td>
<td>Inhibition of key platelet activation and aggregation receptors decreases metastases</td>
</tr>
<tr>
<td>Tumor-cell-induced platelet aggregation (TCIPA)</td>
<td>Platelet aggregation correlates with metastatic potential in in vivo and in vitro models</td>
</tr>
<tr>
<td>Protection of tumor cells from environment</td>
<td>Platelets provide mechanical shielding from physical stressors</td>
</tr>
<tr>
<td>Production of platelet microparticles (PMPs)</td>
<td>Platelet-derived proteins down-regulate immune cells, thereby impairing their antitumor activity</td>
</tr>
<tr>
<td>Release of angiogenic proteins</td>
<td>Transfer of receptors to tumor cell membranes, which may increase invasiveness. May regulate MMP production and influence invasion</td>
</tr>
<tr>
<td>Prevention of tumor hemorrhage</td>
<td>Platelets contain pro- and anti-angiogenic proteins packaged into distinct alpha granules, which can be differentially released to support angiogenesis</td>
</tr>
<tr>
<td>Platelet-enhanced metastases</td>
<td>Platelets maintain tumor vascular integrity and reduce tumor hemorrhage</td>
</tr>
<tr>
<td></td>
<td>Platelets facilitate tumor cell migration and extravasation</td>
</tr>
</tbody>
</table>

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Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

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