Platelets

Linking Hemostasis and Cancer
Shashank Jain, John Harris, Jerry Ware

Abstract—Platelets are the main cellular component in blood responsible for maintaining the integrity of the cardiovascular system via hemostasis. Platelet dysfunction contributes to a wide range of obvious pathological conditions, such as bleeding or thrombosis, but normal platelet function is also linked to diseases not immediately associated with hemostasis or thrombosis, such as cancer. Since the description of Trousseau syndrome in 1865, various experimental and clinical studies have detailed the interaction of platelets with primary tumors and circulating metastatic tumor cells. Observations have suggested that platelets not only augment the growth of primary tumors via angiogenesis but endow tumor cells physical and mechanical support to evade the immune system and extravasate to secondary organs, the basis of metastatic disease. Many laboratory and animal studies have identified specific targets for antiplatelet therapy that may be advantageous as adjuncts to existing cancer treatments. In this review, we summarize important platelet properties that influence tumorigenesis, including primary tumor growth and metastasis at the molecular level. The studies provide a link between the well-studied paradigms of platelet hemostasis and tumorigenesis. (Arterioscler Thromb Vasc Biol. 2010;30:2362-2367.)

Key Words: angiogenesis ■ experimental metastasis ■ hemostasis ■ primary tumor ■ spontaneous metastasis

Despite major advancements in the basic biology of cancer and new therapeutic interventions, cancer still remains among the deadliest diseases of the modern age. Over the last few decades, advances in the field of basic and clinical sciences have led to the recognition of hemostatic and coagulation systems in the growth and spread of different cancers in mouse models, as well as in human patients. Various distinct proteins originally described to participate in hemostasis are now found to be involved in different steps of cancer progression (Figure 1). The key mechanisms whereby hemostatic and coagulation systems cooperate are (1) platelets along with coagulation factors interacting with tumor cells to make platelet–tumor cell emboli aiding tumor cell extravasation to the metastatic niche; (2) a platelet cloak around tumor cells protecting them from natural killer (NK) cell cytotoxic activity; and (3) platelets storing various growth factors, proteases, and small molecules that help in tumor growth, invasion, and angiogenesis. In this review, we discuss the role of various platelets factors in tumorigenesis via these mechanisms. We have included thrombin and fibrinogen, given their importance to the platelet response but recognize many other coagulation factors not discussed are also important for cancer.

Platelet Involvement in Tumor Cell Dissemination

In 1865, Armand Trousseau described some patients with unusual migratory thromboses. These patients developed visceral malignancy later. Now, Trousseau syndrome is explained as a thrombotic event preceding the diagnosis of an occult visceral malignancy and diagnosed from an initial intravascular coagulopathy, platelet-rich microthrombi, microangiopathic hemolytic anemia, or thromboembolic problems.1 For the homeostasis of the vasculature, it is crucial to maintain a normal platelet count in the blood. Experimental thrombocytopenia in mice induced by neuraminidase/anti-platelet serum resulted in a 50% reduction in experimental metastasis, and this could be reversed by transfusion of platelet-rich plasma transfusion.2 NF-E2 knockout mice (SCID background) with extreme thrombocytopenia have a significant reduction (94%) in metastatic burden in experimental metastasis models.3 Others have shown that intravenous injection of some tumor cells may cause significant thrombocytopenia (50% to 70%) in mice.4 Tumor cells that aggregate platelets in vitro produce more lung metastases than tumor cells lacking such ability, illustrating the platelet-activating potential of some tumor cells.5,6 Taken together, these seminal observations suggest a robust interaction between circulating platelets and tumor cells.

After activation, platelets release small vesicles, called microparticles or microvesicles. Platelet microparticles are small in size (0.05 to 1 μm) with a defined plasma membrane and express selected platelet membrane and cellular proteins.7 Lewis lung carcinoma cells treated with platelet-derived mi-
croparticles have increased metastatic potential in syngenic mouse models. Platelet microparticles increase invasive potential by increasing adhesion, proliferation, chemotaxis, and survival of breast cancer cell lines MDA-231 and BT-549 and the prostate cancer cell line CL-1. In the presence of microparticles, a number of cellular events have been documented, including upregulation of CXCR4, mitogen-activated protein kinase (MAPK) p42/44, matrix metalloproteinase (MMP)-2, and MMP-9, along with AKT phosphorylation. Like the platelet, the platelet microparticle facilitates tumorigenesis (Table).

**Platelet Receptors and Ligands Supporting Tumor Cell Growth and Survival**

Tumor cells contain various membrane receptors that can bind directly to platelets and mediate tumor cell–platelet binding and activate platelets (Figure 2). Flow cytometry, fluorescence microscopy, and intravital microscopy have revealed the presence of platelet–tumor cell aggregates in vitro and in vivo.

P-selectin is an adhesion receptor found in the α-granules of platelets and Weibel–Palade bodies of endothelial cells. After platelet activation, P-selectin appears on the platelet surface and aids the recruitment of other circulating platelets and leukocytes. Chondroitin sulfate glycosaminoglycans on the surface of human MDA-MET cells and murine 4T1 cells have been shown to bind selectively P-selectin. It has also been suggested that platelet P-selectin recognizes sulfated galactosylceramide SM2, SM3, and SM4 on MC-38 cells, and sulfatide removal results in inhibition of in vitro platelet P-selectin binding to MC-38 cells and reduced syngenic experimental metastasis in vivo. Experimental metastasis and subcutaneously implanted tumor growth was reported to be reduced in P-selectin–deficient mice and in an immunocompetent model with MC-38 colon carcinoma cells and B16 melanoma cells. Not only is the rate of tumor cell homing to lungs diminished in P-selectin–deficient mice, but tumor cells fail to make aggregates with platelets, resulting in a decreased number of metastatic nodules in the lungs of P-selectin–deficient mice.

A selectin ligand mimicry peptide, IELLQAR, has been found to have an inhibitory effect on B16-induced experimental metastasis. An inhibitor of sialyl Lewis X, such as AcGnG-NM, not only reduces binding of tumor cells to selectin coated surfaces, activated platelets, and tumor necrosis factor-α–activated endothelial cells but also diminishes experimental metastasis in SCID mice. Heparin inhibits the binding of P-selectin to its receptors and has been shown to inhibit experimental metastasis in syngenic mouse models. Human platelets also express MAPK p38α, a serine threonine kinase, and the expression of MAPK p38α is directly linked to platelet P-selectin expression. Mice lacking MAPK p38α are not viable, but heterozygous p38α+/− mice have reduced experimental metastasis, with no effect on primary tumor growth. More recently, a role for P-selectin using models of spontaneous tumor metastasis has been presented. Thus, through a wide array of studies, it can be concluded that P-selectin facilitates direct binding to tumor cells and augments tumor metastasis.

**Table. Hemostasis/Cancer Associations**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Origin</th>
<th>Primary Tumor</th>
<th>Metastasis</th>
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<tbody>
<tr>
<td>Platelet count</td>
<td>Platelet</td>
<td>↑</td>
<td></td>
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<tr>
<td>Platelet microparticles</td>
<td>Platelet</td>
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<tr>
<td>P-selectin</td>
<td>Platelet</td>
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<tr>
<td>MAPK p38α</td>
<td>Platelet</td>
<td>↑</td>
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<tr>
<td>GP Ibα</td>
<td>Platelet</td>
<td>No effect</td>
<td>↑</td>
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<tr>
<td>VWF</td>
<td>Platelet, EC</td>
<td>No effect</td>
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<tr>
<td>GPVI</td>
<td>Platelet</td>
<td>No effect</td>
<td>↑</td>
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<tr>
<td>GP Ib-IIIa</td>
<td>Platelet</td>
<td>↑</td>
<td>↑</td>
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<td>Fibrinogen</td>
<td>Platelet</td>
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<tr>
<td>PAR-1</td>
<td>Platelet, EC</td>
<td>↑</td>
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<tr>
<td>PAR-2</td>
<td>EC</td>
<td>↑</td>
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<tr>
<td>PAR-4</td>
<td>Platelet</td>
<td>↑</td>
<td>↑</td>
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<tr>
<td>Thrombin</td>
<td>EC, TC</td>
<td>↑</td>
<td>↑</td>
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<td>Chondroitin sulfate glycosaminoglycans</td>
<td>Tumor cells</td>
<td>↑</td>
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<tr>
<td>Sialyl Lewis X</td>
<td>Tumor cells</td>
<td>↑</td>
<td></td>
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<tr>
<td>Thrombospondin-1</td>
<td>Platelets</td>
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EC indicates endothelial cells; TC, tumor cells; ↑, stimulatory effect; ↓, inhibitory effect.
The platelet receptor glycoprotein (GP) Ib-IX supports adhesion of platelets on a compromised vascular wall and, as such, is a key initiator of the platelet paradigm in hemostasis.\(^2\) We reported that B16F10 mouse melanoma cell metastasis was reduced 15-fold in GP Ib-IX–deficient mouse colonies, suggesting an important role for adhesion in a syngenic mouse model. However, overexpression of the polyoma middle T antigen in mouse mammary tissue and lung metastasis were not affected by the absence of platelet GP Ib-IX in a model of spontaneous tumor formation (S.J. and J.W., unpublished observation, 2010). Confounding results have been described with the administration of the anti–GP Ib-IX antibodies and the opposite effect, namely increased colonization of the lung.\(^2\) Genetically absence of the platelet collagen receptor, GP VI, is also associated with a 50% reduction in experimental metastasis.\(^2\) However, primary tumor growth and angiogenesis was not altered in GP VI–deficient mice.\(^2\)

Although GP Ib-IX is widely considered to be a platelet-specific complex, several studies have suggested the expression of GP Ib-IX subunits by a variety of tumor cells.\(^2\) In examining the expression of the major subunit of the GP Ib-IX complex, the α-subunit of GP Ib, in lysates of commonly used human tumor cell lines, we have been unable to document the presence of GP Ibα (Figure 3). At this time, we conclude that the expression of GP Ib-IX by cancer cells is not a common mechanism contributing to tumor formation or metastasis.

Von Willebrand factor (VWF) is a key major ligand for the platelet GP Ib-IX complex. Lewis lung carcinoma and B16-B6–mediated experimental metastasis was increased 2- and 5-fold, respectively, in VWF-deficient mice.\(^3\) Lung colonization of tumor cells was increased 1 to 4 hours after injection in VWF-deficient mice, suggesting VWF may be responsible for tumor cell clearance in the circulation. VWF deficiency did not have any effect on the growth of primary tumors. However, it has also been reported that an anti-VWF antibody protects mice from experimental metastasis in mouse models.\(^3\) It is possible that in the absence of VWF, platelet GP Ib-IX availability is increased, resulting in increased experimental metastasis. More definitive experimental proof is required to test this possibility.

Integrin αIIbβ3 (GP IIb-IIIa) is the most abundant receptor on the platelet surface. It participates in hemostasis by bridging platelet/platelet interactions via the ligand, fibrinogen.\(^3\) GP IIb-IIIa inhibition by the monoclonal antibody 10E5 has been reported to diminish binding of CT26 and HCT28 cells to platelets in vitro.\(^3\) Integrin β3\(^+\) mice show a reduction in B16F10 melanoma-induced osteolytic experimental metastasis and reduced osteolytic bone invasion, both reversed by bone marrow transplantation of β3\(^+\)/marrow.\(^3\) Antibody inhibition of GP IIb-IIIa diminishes tumor cell adhesion on extracellular matrix under flow conditions, suggesting a role for GP IIb-IIIa in platelet–tumor cell emboli extravasation.\(^3\)  c7E3 (ReoPro) a mouse–human chimeric antibody for GP IIb-IIIa has antiangiogenic and antitumor properties in mouse models.\(^3\) A single treatment of this antibody in a xenograft model of SCID mice reduces experimental metastasis significantly. In addition, c7E3 also inhibits vascular endothelial growth factor (VEGF) secretion from
platelets in the presence of tumor cells. Taken together, these studies suggest the major platelet integrin receptor plays a significant role in tumorigenesis at several different mechanistic levels.

The major ligand of GP IIb-IIIa, fibrinogen, is also implicated in metastasis. As a central ligand supporting platelet–platelet interactions and as a key cleavage substrate for thrombin in coagulation, fibrinogen is essential in the well-characterized paradigm of hemoostasis and thrombosis. In the realm of tumor biology, fibrinogen supports the formation of platelet–fibrinogen–tumor cell emboli as tumor cells intravasate. Local deposition of fibrin and fibrin products have been found in solid tumors and reported to support angiogenesis. Experimental metastasis, spontaneous hematogenous metastasis, and lymphatic metastasis are significantly diminished in fibrinogen knockout mice.3,41,42 Thus, fibrinogen and fibrin participate in a variety of pathways contributing to tumor cell survival and growth.

Thrombin and Tumorigenesis

The role of thrombin in normal platelet function and in the pathways of blood coagulation highlight its importance as a central molecule linking the cellular (platelet) and biochemical (coagulation) paradigms of hemoostasis. Thrombin treatment of platelets facilitates platelet adhesion on tumor cells by 2- to 4-fold in various cancer cells (HM54, HCT8, CT26, and B16).45 Thrombin-activated tumor cells (CT26 and B16F10) show a 10- to 156-fold increase in experimental metastasis.46 Use of the thrombin agonist TRAP (thrombin receptor activation peptide) on CT26 or B16F10 cells also results in an increase in experimental metastasis.47 Thrombin has been found to break endothelial junctions and aid in VE-cadherin– and β-catenin–mediated angiogenesis and tumor growth.48 Thrombin also acts as a mitogenic agent for various mesenchymal tissues and cells by activating growth-stimulatory signals.49 Hirudin, a thrombin antagonist, has been found to diminish 4T1 mouse primary tumor growth and spontaneous tumor metastasis in the mouse.50

Thrombin also upregulates the expression of various growth factors. It induces the secretion/expression of MMP-1, MMP-2, VEGF, angiopoietin-2, CD31, and receptors KDR and CXCR2 from human umbilical vein endothelial cells (HUVECs) and angiopoietin-1 from platelets. Recently, it has been shown that thrombin upregulates secretion of GRO-α from MCF7 and HUVECs, and anti–GRO-α antibodies inhibit various angiogenic properties of MCF7 and HUVECs.57 Thrombin was also found to upregulate expression of TWIST (an angiogenesis and tumor growth promoting transcription factor) in tumor cells and endothelial cells.58 Interestingly, thrombin regulates proangiogenic and antiangiogenic factors differentially. In platelets, the protease-activated receptor (PAR)-4 agonist (ATPGFK) inhibits VEGF-A secretion while increasing endostatin secretion. The PAR-1 agonist (TFLLR) increases VEGF-A secretion and inhibits endostatin secretion.

Thrombin cleaves the platelet receptors PAR-1, PAR-3, and PAR-4 at their N-terminal end, which in turn activates G protein–mediated intracellular signaling. PAR1 expression has been directly correlated with the degree of invasiveness in primary breast tissue specimens. Overexpression of PAR-1 in B16 cells results in a 5-fold increase in experimental metastasis. It has also been reported that MMP-1 cleaves PAR-1 and enhances tumor growth and invasion of MDA-MB-231 cells in vivo. PAR-4–deficient mouse colonies (SCID and C57 background) display a significant reduction in B16F10 cell–induced experimental pulmonary metastasis. In a spontaneous tumor metastasis model, PAR-2 knockout mouse colonies have reduced mammary adenocarcinoma growth and associated spontaneous metastasis.

Platelets, NK Cells, and Tumorigenesis

As tumor cells intravasate to the circulation from a primary tumor, they interact with various components of the circulation system including platelets and immune cells. In mouse models of experimental metastasis, it has been found that most tumor cells entering the circulation do not survive, with ~0.01% colonizing the lung.51 NK cells are largely responsible for the elimination of cancer cells from the circulation. Experimental and genetic depletion of NK cells in mice causes a 2- to 5-fold increase in experimental metastasis.11,12 It has been proposed that platelets make a cloak around tumor cells and protect the tumor cell from NK cells.11 It addition, platelets and fibrinogen are linked to a significant reduction in the cytolytic activity of NK cells in vitro.11 NK cells express Mac-1 (integrin αMβ2), which has been shown to bind to platelet GP Iba. Whether a GP Iba–Mac-1–mediated platelet–NK cell interaction plays a role in regulating NK cell cytolytic activity for cancer cells remains to be examined. Taken together, these observations highlight the platelet and coagulation interplay in the NK cell response to tumor cells.

Platelets, Angiogenesis, and Tumorigenesis

In 1971, Judah Folkman proposed that tumor growth is dependent on angiogenesis. Platelets store various angiogenesis-regulating factors such as VEGF, platelet-derived growth factor, fibroblast growth factor, epidermal growth factor, hepatocyte growth factor, insulin-like growth factor, angiopoietin, lysosphosphatidic acid, sphingosine 1-phosphate, CD40 ligand, MMP-1, MMP-2, MMP-9, gelatinase A, and heparanase. Most of these angiogenic agents have been shown to participate in angiogenesis for tumor growth directly or indirectly. Platelets also contain antiangiogenic agents such as angiostatin, thrombospondin-1, platelet factor-4, endostatin, transforming growth factor β, and TIMP (tissue inhibitor of matrix metalloproteinases). Dissecting the relevance of pro- and antiangiogenic factors in the milieu of the platelet releasate represents a major challenge for the future.

Recently, it has been found that expression of a negative regulator of angiogenesis, platelet-derived thrombospondin-1, is increased in tumor-bearing mice after tumor resection. Primary tumor growth of Lewis lung carcinoma cells was significantly increased in thrombospondin-1–deficient mice, suggesting a role for angiogenesis in tumor growth. A chemically synthesized COOH-terminal peptide of platelet factor-4 (CXCL4L1) can inhibit angiogenesis and B16-induced melanoma growth in
vivo. Together, these results suggest there is a role for platelet-derived antiangiogenic factors and may represent new directions for future studies.

Conclusions

The role of platelets in hemostasis and thrombosis has been studied for several decades, with remarkable molecular insights defining the hemostasis or thrombosis paradigm. Indeed, many of the well-studied platelet receptors and pathways can influence other diseases. Obvious connections to tumor growth, angiogenesis, and metastasis have been described here. Thus, the potential for insights from one discipline to rapidly contribute to new understandings in a different discipline is exciting. Future studies will hopefully contribute to both disciplines ultimately leading to better prevention, diagnosis, and treatment of disease.

Sources of Funding

Supported by grants from the National Heart, Lung, and Blood Institute (HL50541) and the Department of Defense Breast Cancer Research Program.

Disclosures

None.

References


