Paraneoplastic Thrombocytosis — the Secrets of Tumor Self-promotion

*Short Title:* Paraneoplastic Thrombocytosis

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Abstract

Paraneoplastic thrombocytosis is associated with many solid tumors and often correlates with reduced survival. Recent studies suggest that a pathogenic feedback loop may be operative between platelets and tumor cells, with reciprocal interactions between tumor growth/metastasis and thrombocytosis/platelet activation. Specific molecular pathways have been identified whereby tumors can stimulate platelet production and activation; while activated platelets can, in turn, promote tumor growth and metastasis. Taken together, these findings provide exciting new potential targets for therapeutic intervention.
Normally quiescent platelets in the circulation respond to localized breaches in vascular integrity by providing hemostasis with swiftness and precision. They do so by mobilizing their remarkable repertoire of capabilities to respond to injury: adhesion, activation, secretion, aggregation, recruitment, and wound healing. However, only in recent years have we begun to appreciate the functional versatility of platelets beyond their role in hemostasis; for example, in defending their human hosts against microbial invasion, participating in inflammatory and immune responses, and even contributing to development and regeneration of organs\(^1,2\). But now it is coming to light that a malignant tumor can use platelets to promote its growth and metastasis. In turn, the growing tumor can enhance the production and activation of platelet, thereby potentially creating a positive feedback loop to fuel the tumor’s growth.

**The hijacking of platelet functions by malignant tumors**

Tumor metastasis consists of tissue invasion by the tumor cells, bloodstream entry, an intravascular phase, extravasation of the tumor cells from the capillaries, and growth at a distant site. Tumor cells activate platelets and the metastatic potential of tumor cells correlates with their efficacy in inducing platelet aggregation\(^3,4\). Tumor cells generate thrombin, a potent platelet activator agonist, either (a) by direct contact with platelets or (b) indirectly by stimulating tissue factor-mediated activation of the coagulation system that generates thrombin within the tumor microenvironment\(^5\). Egan et al. showed that ovarian cancer-induced platelet activation is mediated by ADP released from tumor cells, and can be blocked by ADP receptor (P2Y\(_{12}\) and P2Y\(_{1}\)) antagonists\(^6\). More recently, Mitrungo et al. demonstrated that tumor cells could directly induce platelet activation and
secretion of dense granules containing adenine nucleotides via the platelet Fcγ receptor IIa (FcγRIIa)\textsuperscript{7}. Platelet activation by tumors throughout all phases of the metastatic cascade leads to the release of platelet-derived factors stored in their granules that then mediate the inflammatory, proliferative and pro-angiogenic activities of platelets to promote tumor growth, tissue invasion, and metastasis\textsuperscript{8,9}.

Conversely, platelets activated by tumor cells play major roles in aiding and abetting tumor progression. First, platelets can help tumor cells survive immune surveillance in the blood circulation. Activated platelets may act as protective ‘‘cloaks’’ for circulating tumor cells, shielding them from immune destruction by natural killer (NK) cells\textsuperscript{10,11}. This process is medicated by platelet-derived growth factor and TGFβ\textsuperscript{12-13}. Hematogenous dissemination of tumor cells can also be facilitated by “platelet mimicry”, in which tumor cells acquire a phenotype that closely resembles platelets and expresses platelet/megakaryocytic gene products like γIIb/β3, protease-activated receptors, and platelet endothelial cell adhesion molecule-1\textsuperscript{5,14}. Second, platelet-derived TGFβ stimulates the proliferation of ovarian cancer cells in vitro and in vivo\textsuperscript{15}, and promotes epithelial-to-mesenchymal transition in tumor metastasis\textsuperscript{16}. Third, platelets, leukocytes, and vascular endothelium may facilitate tumor cell extravasation and seeding through adhesion molecules P- and L-selectins, a hypothesis supported by the observation that tumor metastases are reduced in mice lacking them\textsuperscript{17-19}. More recently, Schumacher et al. showed that platelet dense granule-derived adenine nucleotides facilitate the transmigration of tumor cells across endothelium through activation of the endothelial ATP receptor P2Y\textsubscript{2}\textsuperscript{20}. Finally, for tumors to grow to sizes >2 mm they must establish
their own blood supply through angiogenesis, a process regulated by platelets and their alpha granules that contain both pro-angiogenic and anti-angiogenic proteins, including >80% of circulating vascular endothelial growth factor\(^2\). Contact with tumor cells activates platelets to preferentially release pro-angiogenic proteins. However, the mechanism of this selective release process through the possible organization of pro- and anti-angiogenic proteins into separate alpha granules or platelet populations remains ill-defined\(^2\).

**Mechanisms of paraneoplastic thrombocytosis**

Malignant tumors not only hijack platelet functions but they can also increase their production. During normal hematopoiesis, platelet production can be stimulated at different hierarchical levels primarily through thrombopoietin (TPO) and its receptor\(^2\). Inappropriately high levels of TPO generated by a variety of clinical disorders, like chronic inflammation and infection, lead to secondary (reactive) thrombocytosis of diverse etiologies. This is in contrast to the primary thrombocytosis of myeloproliferative neoplasms that is associated with normal or low TPO levels\(^2\). TPO usually is not overexpressed by solid tumors, but several other cytokines, including interleukin (IL)-1, IL-3, IL-6, IL-11, leukemia inhibitory factor, KitL, and oncostatin M, likely contribute to tumor-stimulated thrombopoiesis\(^2\). IL-6 in particular, acting as an autocrine growth factor, is overproduced in a variety of malignancies, including gastrointestinal, renal cell, prostate, epithelial ovarian, and lung cancer, as well as Kaposi’s sarcoma and glioblastoma multiforme\(^2\). The IL-6 effect is mediated through induction of TPO mRNA expression and protein production in the liver, and TPO neutralizing antibodies can
abrogate the paraneoplastic thrombocytosis\textsuperscript{25}. Importantly, increased serum levels of IL-6 correlate with platelet counts and anti-IL-6 antibody administration abolishes thrombocytosis in cancer patients\textsuperscript{25-29}. Clinically, thrombocytosis may precede the diagnosis of malignancy by months or even years\textsuperscript{30}, and accumulating evidence suggests that it is highly prevalent in many solid tumors at the time of diagnosis and correlates with significantly reduced survival and/or response to surgery and chemotherapy\textsuperscript{24}. Table 1 summarizes all retrospective, prospective, and meta-analytic studies of more than 300 patients that correlate thrombocytosis at the time of diagnosis with survival and treatment response in solid tumors.

In a landmark study of paraneoplastic thrombocytosis in patients with epithelial ovarian cancer, Stone et al. conducted parallel clinical and laboratory analyses of 619 women with newly diagnosed disease\textsuperscript{26}. More than 90\% of patients had high-grade serous or other epithelioid histology and stage 3–4 disease and 192 patients (31\%) had a platelet count above normal (> 450,000/mm\textsuperscript{3}). They found that patients with thrombocytosis had significantly higher levels of IL-6 and TPO than those without thrombocytosis, and they also had significantly shorter progression-free survival ($P<0.001$) and overall survival (2.62 vs. 4.65 years; $P<0.001$). Blocking IL-6 and TPO production with small interfering RNA led to normalization of platelet counts in an animal model of ovarian cancer, as did the clinical use of the anti–IL-6 antibody siltuximab in patients with ovarian cancer\textsuperscript{26}. Based on these studies, we propose a paracrine circuit, in which tumors produce cytokines such as IL-6 that induce thrombocytosis, and in turn, the increased number of platelets promote tumor growth and distant metastasis (Figure 1).
Targeting paraneoplastic thrombocytosis in anticancer therapy

Given the correlation between paraneoplastic thrombocytosis and reduced survival, as well as documented roles of platelets in tumor growth and metastasis, strategies aimed at blocking various steps in the platelet-facilitated tumorigenesis have been investigated in tumor cells and patients. A fundamental unanswered question, however, is whether an increase in platelet count is enough to fuel cancer progression. If this is the case, and the relationship between platelets and tumors was simply a quantitative one, strategies to reduce the platelet count safely and specifically might be salutary. However, it is also possible that malignant tumors can selectively stimulate the production of platelet populations that are selectively enriched in tumor-promoting properties (e.g. with disproportionately high granule content of pro-angiogenic proteins), or the induction of specific platelet properties conducive to tumor metastasis (e.g. by upregulation of platelet and endothelial adhesion molecules). These qualitative relationships would make the simple strategy of platelet cytoreduction less effective.

One approach is to reduce the production of IL-6 using anti-IL-6 antibody siltuximab, which not only decreases platelet counts in patients but also depletes levels of the growth factors produced by malignant tumors and platelets\textsuperscript{26,27}. In a mouse model of ovarian cancer, treatment with paclitaxel and siltuximab reduced tumor growth by over 90% compared with controls, significantly more than either agent alone\textsuperscript{26}. In a phase II trial of recurrent, platinum-resistant ovarian cancer patients, single agent siltuximab inhibited tumor growth in 8 out of 18 patients\textsuperscript{27}. Similar clinical studies in renal cell carcinoma and castration-resistant prostate cancer also demonstrated a beneficial effect\textsuperscript{28,29}. Another
approach is to inhibit platelet function to control tumor growth and metastasis. Clinical data evaluating the impact of antiplatelet agents including aspirin on cancer survival have begun to emerge\textsuperscript{5}. A recent post hoc analysis of large randomized cardiovascular prevention trials provided evidence for a reduced risk of cancer metastasis in patients taking aspirin at doses sufficient to inhibit platelet function\textsuperscript{31}. However, despite the evidence for a role of platelets in tumor metastasis, randomized clinical trials to prospectively test this hypothesis have been rare, mainly due to concerns that antiplatelet drugs may affect normal platelet function leading to bleeding complications\textsuperscript{8,9}. In addition, it has been recognized that unfractionated heparins and low molecular weight heparins can inhibit the interaction of selectins with their natural ligands\textsuperscript{32}. This leads to statistically significant improvements in overall survival of cancer patients especially those without metastatic disease who receive heparinoids\textsuperscript{33}. Interestingly, recent evidence suggests that these anticoagulants can also selectively inhibit platelet release of pro-angiogenic proteins and diminish platelet-mediated angiogenic response\textsuperscript{21}. Finally, blockade of platelet FcγRIIa and α\textsubscript{IIb}β\textsubscript{3} receptors would also be logical approaches\textsuperscript{7}, as well as blockade of the P2Y\textsubscript{2} receptor on endothelial cells or its downstream signaling pathway\textsuperscript{20}. However, these too will require rigorous preclinical testing, especially since prasugrel, a potent P2Y\textsubscript{12} receptor blocker used in the treatment of cardiovascular disease, was found to be associated with a higher rate of colonic malignancy in a large study\textsuperscript{34}. 
Conclusions

Significant progress has been made in unraveling the complex, reciprocal relationship between tumor progression and platelet function. It is likely that thrombocytosis is not simply an epiphenomenon of malignancy but rather a true paraneoplastic abnormality. In fact, paraneoplastic thrombocytosis appears to involve a “positive feedback loop”, whereby malignant tumors produce cytokines such as IL-6 that stimulate thrombocytosis while at the same time tumor cells themselves directly or indirectly activate platelets. In turn, increased numbers of activated platelets promote further tumor growth and metastasis, which leads to yet greater stimulation of platelet numbers and levels of activity (Figure 1). Interrupting this paracrine circuit with antiplatelet agents, heparinoids, or pharmacologic inhibition of IL-6 might prove beneficial for patients with solid tumors and thrombocytosis. However, it remains unclear whether the worse prognosis in these patients is due to thrombocytosis induced by IL-6 or due to IL-6 itself. This is particularly important as IL-6 has pleotropic effects in many tumors independent of thrombopoiesis.

Combining antiplatelet therapy with conventional antitumor therapy should be considered. The effect of existing antiplatelet drugs as adjuvants to conventional chemotherapeutics and hormonal therapies has been understudied. Lessons learned from the use of antiplatelet therapy in the treatment of cardiovascular disease, such as the concept of antiplatelet drug resistance, might have implication in their use in cancer treatment. In addition, more targeted antiplatelet therapy could be designed, based on our current understanding of how tumor cells specifically activate platelets and thereby recruit them to facilitate tumor progression and metastasis. Although not yet unequivocally proven, it is becoming increasingly evident that the activation of platelets
as well as their increased numbers (thrombocytosis) that are associated with a variety of solid tumors are not merely epiphenomena but rather intricately involved in the process of tumor progression.
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Author Contributions

All three authors made comparable contributions to planning, initial writing, editing, and figure design.

Conflict of Interest Disclosure

All three authors declare no competing financial interests.
References


11. Palumbo JS, Talmage KE, Massari JV, et al. Platelets and fibrin(ogen) increase


32. Stevenson JL, Varki A, Borsig L. Heparin attenuates metastasis mainly due to inhibition of P- and L-selectin, but non-anticoagulant heparins can have additional effects. *Thromb Res*. 2007;120(S2):S107-S111.


Table 1. Summary of studies with more than 300 patients correlating thrombocytosis with survival in solid tumors

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>N</th>
<th>Cutoff (mm$^3$)</th>
<th>Prevalence (n,% )</th>
<th>Study type</th>
<th>Survival results (thrombocytosis v. normal)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>398</td>
<td>400,000</td>
<td>86 (21.6%)</td>
<td>Retrospective</td>
<td>OS: HR=1.58 (p=0.006, mva)</td>
<td>Maraz A, et al. Anticancer Res. 2013</td>
</tr>
<tr>
<td></td>
<td>317</td>
<td>400,000</td>
<td>64 (20.2%)</td>
<td>Prospective</td>
<td>Time to Progression: (p=0.2, uva)</td>
<td>Kotsori AA, et al. Hosp Chron. 2006</td>
</tr>
<tr>
<td></td>
<td>611</td>
<td>400,000</td>
<td>98 (16%)</td>
<td>Retrospective</td>
<td>OS: HR=1.29 (p=0.0348, mva)</td>
<td>Aoe K, et al. Respiration. 2004</td>
</tr>
<tr>
<td></td>
<td>1115</td>
<td>400,000</td>
<td>358 (32.1%)</td>
<td>Retrospective</td>
<td>OS: HR=4.24 (p&lt;0.001, mva)</td>
<td>Pedersen LM, Milman N. Eur Respir J. 1996</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>336</td>
<td>400,000</td>
<td>184 (54.8%)</td>
<td>Retrospective</td>
<td>OS: HR=1.57 (p&lt;0.001, mva)</td>
<td>Herndon JE, et al. Chest. 1998</td>
</tr>
<tr>
<td>Breast</td>
<td>4300</td>
<td>400,000</td>
<td>161 (3.7%)</td>
<td>Retrospective</td>
<td>OS: HR=1.73 (p=0.0064, mva)</td>
<td>Taucher S, et al. Thromb Haemost. 2003</td>
</tr>
<tr>
<td>Gynecologic</td>
<td>578</td>
<td>450,000</td>
<td>129 (22.3%)</td>
<td>Retrospective</td>
<td>DFS: HR=1.38 (p=0.02, mva)</td>
<td>Allenworth SK, et al. Gynecol Oncol. 2013</td>
</tr>
<tr>
<td>Colorectal</td>
<td>453</td>
<td>300</td>
<td>226 (49.9%)</td>
<td>Retrospective</td>
<td>OS: HR=1.64 (p=0.039, mva)</td>
<td>Ishizuka M, et al. J Surg Oncol. 2012</td>
</tr>
<tr>
<td></td>
<td>636</td>
<td>370</td>
<td>77 (12.1%)</td>
<td>Retrospective</td>
<td>OS: HR=3.04 (p&lt;0.001, mva)</td>
<td>Sasaki K, et al. World J Surg. 2012</td>
</tr>
<tr>
<td></td>
<td>369</td>
<td>400,000</td>
<td>42 (11.4%)</td>
<td>Retrospective</td>
<td>OS: HR=2.48 (p=0.015, mva)</td>
<td>Ikeda M, et al. Ann Surg Oncol. 2002</td>
</tr>
<tr>
<td>Renal</td>
<td>804</td>
<td>450,000</td>
<td>63 (7.8%)</td>
<td>Retrospective</td>
<td>OS: OR=1.8 (p&lt;0.0001, mva)</td>
<td>Bensalah K, et al. J Urol. 2006</td>
</tr>
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OS: overall survival; DFS: disease free survival; HR: hazard ratio; RR: risk ratio; uva: univariate analysis; mva: multivariate analysis
Figure 1. Model of a “vicious cycle” of cooperation between platelets and tumors

Figure Legend

A. Tumors stimulate TPO production, usually indirectly by their elaboration of IL-6 or other cytokines. B. Bone marrow responds to TPO stimulation by increasing platelet production and release into the circulation. C. Circulating platelets are activated either by direct contact with tumor cells via tumor-activated Fcγ receptors, or indirectly through the generation of the potent platelet agonist thrombin in the tumor microenvironment (not shown). In turn, activated platelets that are attracted to the site of tumor stick to and provide a “protective cloak” for circulating tumor cells, shielding them from immune destruction by NK cells. (See ‘C enlarged’) D. Platelets further facilitate metastasis by augmenting circulating tumor cell extravasation. Platelets can also promote metastatic tumor growth by releasing pro-angiogenic proteins, including VEGF (not shown), thereby stimulating tumor angiogenesis. The positive feedback loop is completed as the platelet-assisted growing tumors secrete more TPO-stimulatory cytokines. (See ‘D enlarged’)}
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