Differential Platelet Levels Affect Response to Taxane-Based Therapy in Ovarian Cancer

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Abstract

Purpose: We hypothesized that platelet levels during therapy could serve as a biomarker for response to therapy and that manipulation of platelet levels could impact responsiveness to chemotherapy.

Experimental Design: The medical records of patients with recurrent or progressive ovarian cancer were retrospectively queried for changes in platelet and CA-125 levels during primary therapy. In vitro coculture experiments and in vivo orthotopic models of human ovarian cancer in mice were used to test the effect of modulating platelet levels on tumor growth and responsiveness to docetaxel.

Results: Thrombocytosis at the diagnosis of ovarian cancer was correlated with decreased interval to progression (P = 0.05) and median overall survival (P = 0.007). Mean platelet levels corrected during primary therapy and rose at recurrence. Contrary to treatment-responsive patients, in a cohort of patients refractory to primary therapy, platelet levels did not normalize during therapy. In A2780, HeyA8, and SKOV3-ip1 ovarian cancer cell lines, platelet coculture protected against apoptosis (P < 0.05). In orthotopic models of human ovarian cancer, platelet depletion resulted in 70% reduced mean tumor weight (P < 0.05). Compared with mice treated with docetaxel, mice treated with both docetaxel and platelet-depleting antibody had a 62% decrease in mean tumor weight (P = 0.04). Platelet transfusion increased mean aggregate tumor weight 2.4-fold (P < 0.05), blocked the effect of docetaxel on tumor growth (P = 0.55) and decreased tumor cell apoptosis. Pretreatment aspiration of the platelets blocked the growth-promoting effects of transfusion.

Conclusions: Platelet-driven effects of chemotherapy response may explain clinical observations.

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Introduction

Thrombocytosis, defined as >450,000 cells/μL, is found in more than 30% of patients with epithelial ovarian cancer and is associated with decreased progression-free and overall survival (1). Paraneoplastic thrombocytosis, in addition to hypercalcemia, leukocytosis, and cachexia, has been shown to occur through the generation of IL6 (1, 2). IL6 expression correlates with ovarian cancer taxane sensitivity (3). Platelet transfusion leads to increased tumor cell proliferation (2). Recent clinical work supports the relationship between thrombocytosis and poor prognosis in ovarian cancer (4, 5).

The connection between platelets and metastasis is established (6–14). Platelets have been shown to mediate protection of micrometastases from NK cell–mediated clearance (15). Direct signaling between platelets and tumor cells contributes to the epithelial-to-mesenchymal transition (16). The role for platelets in metastasis has proven multifactorial, including platelet–tumor interactions involving multiple protein classes and functions (17–21).

Exposure of human adenocarcinoma cells to platelets increases survival, proliferation, and in vitro chemoresistance through the upregulation of antiapoptotic pathways, downregulation of proapoptotic pathways, promotion of DNA synthesis, increased cyclin expression, increased DNA repair protein expression, and increased MAPK expression (22). Induction of thrombocytopenia in a murine model of breast carcinoma results in greater taxane efficacy that correlates with increased vascular leakage at the tumor site (23). Platelets sequester and differentially release angiogenic and mitogenic mediators (24–28). Release of alpha-granule contents and platelet-driven neutrophil chemotaxis are variable based on pH, suggesting a complex regulatory function (29). Dense granules release agents known to modulate cell growth and migration (30).

Considering the growing evidence for correlation between platelet levels and clinical outcomes, we considered whether platelet levels could serve as a biomarker of treatment response. We investigated, using in vitro and preclinical in vivo models,
Platelet Levels Affect Response to Taxanes in Ovarian Cancer

Translational Relevance
Thrombocytosis is known to correlate with poor clinical outcomes in cancer and to be caused by tumor cells, and platelets are known to participate in metastasis. In this work, we show that in patients with recurrent ovarian cancer, elevated platelet counts at diagnosis correlated with decreased interval to progression and decreased overall survival. Changes in platelet counts during and after therapy may be a biomarker for response to that therapy and for recurrence. Platelets protect ovarian cancer cells from apoptosis in a manner not requiring a direct contact. Platelet transfusion results in increased tumor growth that can be at least partially blocked with aspirin. Furthermore, platelet transfusion decreases the efficacy of taxane-based chemotherapy, and platelet depletion increases the efficacy of the same therapy. These findings argue for reconsideration of the risk of platelet transfusion and thrombopoietin receptor agonists, as well as for consideration of antiplatelet reagents as chemosensitizers.

Materials and Methods

Approvals
Approval for relevant studies was obtained from the University of Texas at M.D. Anderson Cancer Center Institutional Review Board (IRB). All animal experiments were approved and supervised by the MDACC Institutional Animal Care and Use Committee.

Clinical analysis
Patients were retrospectively identified at the University of Texas at M.D. Anderson Cancer Center (MDACC), the University of Iowa, and the University of Virginia who were diagnosed with ovarian, primary peritoneal, or fallopian tube carcinoma. This database was partially overlapping with that reported by Stone and colleagues (1). Patients were excluded if they did not receive primary therapy or follow-up at the institution of record. To explicitly focus on patterns of recurrence and progression, patients were excluded who did not develop tumor recurrence or progression, patients whose recurrence was de novo for response to that therapy and for recurrence. Platelets protect ovarian cancer cells from apoptosis in a manner not requiring a direct contact. Platelet transfusion results in increased tumor growth that can be at least partially blocked with aspirin. Furthermore, platelet transfusion decreases the efficacy of taxane-based chemotherapy, and platelet depletion increases the efficacy of the same therapy. These findings argue for reconsideration of the risk of platelet transfusion and thrombopoietin receptor agonists, as well as for consideration of antiplatelet reagents as chemosensitizers.

whether modulation of platelet counts could influence response to chemotherapy, and whether such effects could be blocked to improve sensitivity to taxane-based chemotherapy.

Materials and Methods

Preclinical analysis
Docetaxel. Docetaxel (Sanofi-Aventis) is a commonly used taxane chemotherapy shown in phase III clinic trials to be equivalent to paclitaxel in the primary therapy of ovarian cancer (32). Docetaxel was obtained from surplus clinical samples from the clinical pharmacy associated with the University of Texas M.D. Anderson Cancer Center.

Cell lines and culture conditions. The derivation of the human ovarian cancer cell lines A2780, HeyA8, and SKOV3-IP1 are previously reported (33). Cell lines were obtained from the institutional Cell Line Core laboratory and per institutional policy (MD Anderson policy ACA#1044) cell line authentication was performed at least once per year. In this case, authentication was performed within 6 months of the work described. Authentication was performed by the short tandem repeat method using the Promega PowerPlex 16HS kit (Promega). Somatic mutations were detected using a Sequenom MALDI TOF MassArray system (Sequenom). Mycoplasma detection was performed using the MycoAlert Kit (Lonza). The cell lines were maintained in RPMI-1640 with 15% FBS. Cells lines were routinely genotyped to confirm identity and tested to confirm absence of mycoplasma. Cells were maintained at 37°C in a humidified incubator infused with 20% O2 and 5% CO2.

Platelet isolation for in vitro assays. Platelets were prepared for in vitro assays in a manner that would remove plasma contents and nucleated cells. Whole blood was drawn from the inferior vena cava of anesthetized nude mice into a syringe preloaded with 1:9 v/v 3.8% sodium citrate and mixed 1:1 v/v with tyrodes buffer lacking Mg2+ and Ca2+. Blood was centrifuged at 1,100 rpm for 3 minutes, twice, at room temperature. The platelet-rich plasma fraction was passed through a filtration column of Sepharose 2B beads (Sigma Aldrich) loaded into a siliconized glass column with a 10-μm nylon net filter (Millipore) and sepharose 2B beads previously washed in acetone 1:1 v/v, followed by 0.9% NaCl 1:1 v/v, and "Buffer 1" 1:1 v/v. Platelet-containing eluent was diluted 1:200 and platelets were counted with a hemocytometer by phase-contrast microscopy at 400× magnification.

In vitro assays. To examine potential effects of platelets on apoptosis and response to chemotherapy, we incubated cancer cells with platelets using a tissue coculture system and observed consistent protection against apoptosis. To assess the effect of platelets on apoptosis, cells were plated in 6-well plates at 50,000 cells per plate. At 50% confluence, media was changed to serum-free for 24 hours before starting treatment. After serum starvation, platelets were isolated and added to achieve a final number of 1× 108 platelets/mL. Docetaxel was dosed at 5 μmol/L based on previously published IC50 levels. Controls utilized an equivalent volume of the appropriate buffer. All treatments were performed in triplicate. After 72 hours of platelet and docetaxel exposure, apoptosis and cell viability were assessed using Annexin V and 7-amino-actinomycin-D staining (BD Pharmingen) by flow cytometry. Indirect contact with platelets was provided by the use of an intervening cell culture insert with 0.4-μm pores (BD Falcon).
Proliferation was measured by flow cytometry (Click-it EdU Kit; Invitrogen). For platelet fixation experiments, plasma-free platelets were incubated in 1% paraformaldehyde (2). To test the effect of aspirin in this system, a 325-mg tablet of aspirin was dissolved in deionized, distilled water and filter sterilized. Cancer cells were plated, plasma-free platelets were isolated and coincubated with aspirin 30 μmol/L and in vitro experiments were performed as described. Internal controls (n = 3) were performed for each experiment given the variability in baseline apoptosis and proliferation rates seen between experiments to avoid batch error.

Orthotopic model of ovarian cancer in nude mice. Female athymic nude (NCr-nu) were purchased from Taconic Farms, Inc. The development and characterization of the orthotopic mouse model of ovarian cancer has been previously described (34). SKOV3-IP1 (1 × 10⁶ cells/mouse), A2780 (1 × 10⁶ cells/mouse), or HeyA8 (0.25 × 10⁶ cells/mouse) were lifted with trypsin/EDTA, washed with PBS, and resuspended in 200 μL of Hank’s balanced salt solution (HBSS, Mediatech, Inc.) and were injected into the peritoneal cavity of female nude mice.

Platelet-depleting antibody. To deplete platelets in mice for in vivo experiments, we used a commercially available rat anti-mouse monoclonal antibody directed against mouse GP1b-alpha (CD42b, Emfret Analytics) that causes irreversible platelet depletions within 60 minutes of administration without inducing platelet activation. Dose kinetics are previously validated (1).

Thrombocytosis, thrombocytopenia, and effect on chemotherapy in vivo. The cell lines A2780 and SKOV3-IP1 were used in the orthotopic model of nude mice. The animals were injected with tumor on day 0 as described above. Starting on day 7, animals were randomized into the following groups: twice weekly tail vein injections of control IgG (0.5 mg/kg); two weekly control IgG via tail vein injection and weekly docetaxel 35 mg i.p.; platelet-depleting antibody (0.5 mg/kg) via tail vein injection twice weekly; platelet-depleting antibody plus docetaxel; tail vein transfusion of platelet rich plasma isolated from nude mice; platelet transfusion and docetaxel. Mice were treated until they became moribund and then sacrificed.

Aspirinization of platelets. Pharmacy grade aspirin was acquired, and a single 325-mg tablet was dissolved in 500 μmol/L sodium acetate (pH 5.6). This was added 1:10 v/v to platelet rich plasma and the combination was incubated at 37°C for 15 minutes. Incubation with an equivalent sodium acetate solution without aspirin was used for control.

Effect of aspirin on thrombocytosis and malignancy in vivo. Using the A2780 orthotopic model of ovarian cancer, mice were injected with tumor on day 0. On day 7, the animals were randomized into the following groups: untreated control; intraperitoneal aspirin 20 mg/kg twice per week; 500 μL of platelet rich plasma isolated from nude mice and incubated with sodium acetate for 15 minutes (as described above) via tail vein injection weekly; tail vein transfusion of platelet rich plasma that had been incubated for 15 minutes with a 50 μmol/L solution of aspirin in sodium acetate (as described above). Mice were treated until they became moribund and then sacrificed.

Cleaved caspase-3 immunohistochemistry. Immunohistochemistry for cleaved caspase-3 was used to measure apoptotic rates ex vivo in tumor using a rabbit polyclonal anti-human antibody to cleaved caspase-3 (BioCare Medical; #CP229B). Paraffin-embedded tumor sections were heated, deparaffinized, and antigen retrieval was performed by steaming, and endogenous peroxides were blocked with 3% hydrogen peroxide in methanol. Nonspecific proteins were blocked with 4% fish gelatin in PBS. Slides were incubated in primary antibody (1:100), and the secondary antibody (ready-to-use) was followed by streptavidin horseradish peroxidase (ready-to-use). Slides were quantified by counting the number of positively staining cells per 200× field.

Statistical analysis
A two-sided long-rank statistic was used to compare Kaplan–Meier survival curves. Variables estimated to have a normal distribution were compared using the Student t test using Excel (Microsoft). The F test was used to compare variances where indicated. A P of <0.05 was considered statistically significant. For mouse experiments, sample size was estimated utilizing a two-way ANOVA model. For an effect size of 0.65, a sample size of 10 mice per group was considered sufficient to provide 80% power for α = 0.05 anticipating less than 10 groups.

Results
Thrombocytosis is associated with resistance to chemotherapy
We first identified patients known to have recurrent or progressive epithelial ovarian cancer (n = 353) for whom adequate laboratory data before treatment, treatment data, and posttreatment follow-up data were available. Demographics (Supplementary Table S1) indicated a median age 61 years (range 31–88 years). Ninety percent had advanced stage (III or IV) and 89% had high-grade disease. For primary therapy, all patients underwent a combination of surgical cytoreduction (60% had “optimal” cytoreduction to <1 cm gross residual disease) and taxane-based chemotherapy, most commonly paclitaxel and carboplatin. In this population, in which all patients developed disease recurrence, the mean platelet level was 409,000/μL (range 134,000–1,122,000 cells/μL) at diagnosis. Thirty-two percent had a mean platelet level >450,000 cells/μL at the time of diagnosis. Even after patients without diagnosed recurrence were excluded, thrombocytosis at diagnosis was associated with worse median progression-free survival (12.9 vs. 14.7 months, P = 0.05; Fig. 1A) and median overall survival (16 vs. 20.8 months, P = 0.007; Fig. 1A).

A subgroup of 96 patients was identified whose available laboratory data were adequate to consider platelet and CA-125 trends through primary diagnosis, primary treatment, surveillance, and until the clinical diagnosis of recurrence (Fig. 1B, Supplementary Table S2). CA-125 is a standard tumor marker followed in ovarian cancer to track the efficacy of primary therapy and in surveillance for recurrence. In this group of patients, 86% of patients had a normal CA-125 level (<35 U/mL) at the conclusion of primary therapy. In contrast, all patients had a normal platelet count <450,000 cells/μL (mean 206,000 cells/μL) after primary therapy. At the clinical diagnosis of disease recurrence or progression, CA-125 was elevated in 75% of patients. In parallel, at the diagnosis of recurrence, mean platelet counts were found to be increased 57.8% to 262,000 cells/μL compared with nadir levels found after
primary therapy was completed (P < 0.001; Fig. 1B; Supplementary Table S2). Among patients with a CA-125 <35 U/mL at the time of recurrence, platelet levels were increased by 49% (mean increase 108,400 cells/μL, P < 0.01) at the diagnosis of recurrence compared with the conclusion of primary therapy.

Among patients with ovarian cancer, approximately 10% will not respond to primary therapy and are considered to have “refractory” disease. From the 96 patients with complete longitudinal data, 10 patients were identified who had disease refractory to primary treatment. Ten additional patients (matched for stage, grade, histology, and primary therapy) were identified for comparison who experienced a durable response to therapy lasting more than 6 months. In the subgroup of patients with a thrombocytosis at the time of diagnosis, all patients consistently achieved normal platelet counts during primary therapy (Fig. 1C). These data suggest a correlation between the normalization of platelet counts during primary therapy and disease response to that therapy.

Platelets mediate resistance against chemotherapy-induced apoptosis in vitro

Tissue coculture with platelets demonstrated consistent protection against apoptosis, both directly and indirectly, and with or without exposure to docetaxel. Platelet activation was evident by the aggregation of platelets within the initial hours of 37°C incubation. Direct incubation of the A2780, HeyA8, and SKOV3-ip1 cells with platelets in serum-free conditions reduced apoptosis by 46.7% (P = 0.002), 64.4% (P < 0.001), and 47.3% (P = 0.004), respectively (Fig. 2A). After incorporating docetaxel, direct incubation of the same cell lines with platelets reduced apoptosis by 20.4% (P = 0.004), 74.0% (P < 0.001), and 15.1% (P = 0.007), respectively (Fig. 2A). To consider whether direct contact between platelets and tumor cells was required to observe these changes in apoptotic rates, ovarian cancer cells were indirectly incubated with platelets across a barrier with 0.4-μm pores for 72 hours in a serum-free environment with or without docetaxel.

Figure 1.

A, patients with recurrent ovarian cancer (n = 341) were identified and stratified according to their platelet counts at diagnosis into two groups: those with thrombocytosis (>450,000 cells/μL) and those with normal platelet counts (<45,000 cells/μL). The patients with thrombocytosis at the time of diagnosis had a significantly shorter median interval to progression (12.9 vs. 14.7 months, P = 0.05). The patients with thrombocytosis at the time of diagnosis had a significantly shorter median overall survival (16 vs. 20.8 months, P = 0.007). B, patients with recurrent ovarian cancer for whom longitudinal data were available through treatment and surveillance (n = 96). The mean platelet level at diagnosis was 403,000 cells/μL. During primary therapy, the mean platelet nadir was 198,000 cells/μL. At the conclusion of therapy, the average platelet level was 221,000 cells/μL, and this remained stable in the surveillance period, with the mean nadir count of 166,000 cells/μL during this time. At recurrence, mean platelet counts increased 27% to 262,000 cells/μL (P < 0.001). Of the patients with available longitudinal data, the mean CA-125 level at diagnosis was 332 U/mL (normal <35 U/mL). Only 86% had a normal CA-125 level at the conclusion of primary therapy with a mean level of 63 U/mL, and the mean posttreatment nadir was 23 U/mL. At the clinical diagnosis of disease recurrence, CA-125 was elevated in 75% of patients, with a median of 229 U/mL. C, a subgroup of the patients with complete longitudinal data was identified who experienced progression of disease through frontline therapy (n = 10). These patients were matched to a cohort who experienced a durable response to therapy lasting more than 6 months. In the subgroup of patients with a durable response, only 50% had thrombocytosis (>450,000 cells/μL) at the time of diagnosis, and all patients in this subgroup achieved normal platelet counts during therapy. In the treatment-refractory cohort, all patients had thrombocytosis at the time of diagnosis, and platelet levels were more heterogeneous during primary therapy, with only 50% having normalized platelet counts by the completion of primary therapy.
5 nmol/L. Indirect incubation of A2780, HeyA8, SKOV3-ip1, and 2774 cells with platelets in serum-free conditions reduced apoptosis by 60.8% (P < 0.001), 80.7% (P = 0.001), 82.3% (P < 0.001), and 25.3% (P = 0.002), respectively (Fig. 2B). After incorporating docetaxel, direct incubation of the same cell lines with platelets reduced apoptosis by 17.4% (P < 0.001), 31.9% (P < 0.001).
transfusion of aspirinized platelets. Aspirin by itself had no significant effect on mean aggregate tumor weight at necropsy. Platelet transfusion increased mean aggregate tumor weight from 2.1 to 4.1 g (P = 0.009). Pretransfusion aspirinization of platelets abrogated the increased tumor growth (2.1 vs. 2.1 g, P = NS). B, immunohistochemistry for cleaved caspase-3 demonstrated reduction in apoptosis in tumors of mice receiving platelet transfusion from 28.1/hpf to 17.6/hpf (P = 0.009). Pretransfusion aspirinization of platelets partially abrogated the reduction of apoptosis by 22.2% (P = 0.11 compared with control). Intraperitoneal aspirin had no statistically significant effect on the rate of apoptosis.

Intraperitoneal aspirin therapy did not have any statistically significant effect on the rate of apoptosis.

To determine whether platelet activation was necessary for the apoptosis protection, the above experiments were repeated using platelets fixed with paraformaldehyde. Fixation of platelets abrogated the antiapoptotic effect (P = 0.28, Fig. 2C), suggesting that platelet activation is necessary for the antiapoptotic effects. Platelet coculture has previously been shown to increase tumor cell proliferation, which was abrogated by platelet fixation (2). Acknowledging that aspirin is a moderate inhibitor of platelet activation, aspirin pretreatment of platelets was utilized to block the proliferative effects of platelets in vitro. The cell line SKOV3-ip1 was cocultured with platelets with or without aspirin (30 μmol/L for 24 hours and evaluated by flow cytometry for EdU incorporation as a proxy for proliferation. As anticipated, platelet coculture increased proliferation by 56.5% (P = 0.004). Inclusion of aspirin abrogated the effect of platelet coculture (Fig. 2C).

Effects of platelets on tumor growth and response to chemotherapy in vivo

All ovarian cancer cell lines utilized here are known to cause increased platelet counts (1). To simulate the effects of excess platelet counts, allogeneic platelet transfusions were performed. Noting that platelet activation was apparently necessary for the antiapoptotic effects in vitro, we considered whether in vivo effects from platelet transfusion might be blocked by utilizing aspirin. Nude mice were given intraperitoneal injections of A2780 cells, and 7 days later, they were randomized to the following treatment groups (n = 10 mice/group): untreated control, intraperitoneal aspirin, platelet transfusion, and aspirinized platelet transfusion. Platelet transfusion resulted in a 1.9-fold increase in the aggregate mean tumor weight compared with control (P = 0.01; Fig. 3A).

Intraperitoneal aspirin therapy did not have any significant effect on aggregate tumor weight. In contrast, preaspirinization of the platelets blocked the progrowth effect of platelet transfusion (P = 0.01 compared with platelet transfusion; P = NS compared with control; Fig. 3A).

In resected tumor specimens, ex vivo immunohistochemistry demonstrated that platelet transfusion resulted in a 37% lower rate of apoptosis compared with control (P = 0.009; Fig. 3B). Aspirin delivered intraperitoneally did not significantly change the apoptotic rate in tumor (P = 0.86; Fig. 3B). In contrast, aspirinizing platelets before transfusion blocked the antiapoptotic effect of platelets on tumor (P = 0.11; Fig. 3B).

We next studied the effect of platelets on response to taxane-based chemotherapy in vivo by reducing platelet counts using an anti-platelet antibody (APA) that sequesters circulating platelets and has been previously validated in our laboratory (1). Seven days after intraperitoneal injection of A2780 cancer cells, mice were randomized to the following treatment groups: control IgG, APA, control IgG with docetaxel, or APA with docetaxel. After 5 weeks, mice treated with APA had 65% decrement in mean aggregate tumor weight compared with control (P = 0.008, Fig. 4A) that was similar to the 70% decrease that resulted from treatment with docetaxel (P = 0.004, Fig. 4A). There was no statistical difference between the APA treatment and docetaxel treatment (P = 0.35, Fig. 4A). In comparison, mice treated with both the APA and docetaxel had an additional 62% reduction in aggregate tumor weight compared with that achieved by docetaxel alone (P = 0.04, Fig. 4A).

To confirm this finding and consider the effect of platelet transfusion, nude mice were given intraperitoneal injections of SKOV3-ip1 cells, and after 7 days were randomized to the following treatment groups: control IgG, APA, twice weekly platelet transfusion, control IgG with docetaxel, APA with docetaxel, and platelet transfusion with docetaxel. Platelet depletion and docetaxel resulted in similar reductions in tumor size at necropsy (Fig. 4B). Mice given platelet transfusions had a 2.4-fold increase in mean aggregate tumor weight compared with controls (P = 0.01, Fig. 4B). Compared with mice treated with docetaxel, treatment with docetaxel and platelet transfusion resulted in a 4-fold increase in mean aggregate tumor weight.
Animals treated with APA had a 65% decrease in mean aggregate tumor weight compared with control ($P = 0.008$) that was similar to the 70% decrease that resulted from treatment with docetaxel ($P = 0.004$ compared with control). There was no statistical difference between the APA treatment and docetaxel treatment ($P = 0.35$). Mice treated with both APA and docetaxel had an additional 62% reduction in aggregate tumor weight compared with that achieved by docetaxel alone ($P = 0.04$). In vivo, SKOV3-ip1-bearing nude mice were treated with control IgG, APA, and docetaxel, and/or platelet transfusion. Platelet depletion with APA resulted in a 43% decrease in mean aggregate tumor weight of borderline significance ($P = 0.07$). Docetaxel resulted in a similar reduction in mean aggregate tumor weight (69%; $P = 0.006$). Mice given platelet transfusions had a 2.4-fold increase in mean aggregate tumor weight compared with control ($P = 0.07$). Compared with mice treated with docetaxel, mice treated with docetaxel and platelet transfusion had a 4-fold increase in mean aggregate tumor weight ($P = 0.004$). Mice given platelet transfusions and treated with docetaxel had a mean aggregate tumor weight similar to that of untreated controls ($P = 0.55$). Compared with mice treated with docetaxel, mice treated with APA and docetaxel had a 51% decrease in mean tumor weight ($P = 0.02$, Fig. 4B). In a confirmatory experiment using the SKOV3-ip1 model, the animals were randomized to control IgG, twice weekly platelet transfusion, or platelet transfusion with APA. Platelet transfusion resulted in a 70% increase in mean aggregate tumor weight ($P = 0.001$, Fig. 4C), whereas the combination of platelet transfusion with APA resulted in a nonsignificant 40% decrease in mean aggregate tumor weight compared with control ($P = 0.06$, Fig. 4C).

**Discussion**

It is increasingly recognized that there are multiple biological components that participate in a cooperative relationship between the host and tumor cells. Crosstalk between various cell types, including platelets, leukocytes, and endothelial cells, has been shown to participate in the epithelial-to-mesenchymal transition, metastasis, as well as arrest of tumor emboli with the establishment of the metastatic niche (16, 35). Platelets have been shown to sequester angiogenesis regulators in addition to other mitogens (24) and release these compounds from alpha-granules in a manner that modulates angiogenesis (27). There is evidence that exposure to anticoagulants decreases platelet release of VEGF, suggesting that anticoagulants may alter the potential of platelets to facilitate angiogenesis (36).

In a cohort of patients enriched for recurrence of disease, we found that elevated platelet counts correlated with a decreased interval to progression and decreased overall survival. Overall survival as a trial endpoint is influenced by therapeutic crossovers; therefore, it is notable that thrombocytosis correlates with worsened overall survival, suggesting that platelet effects may be agnostic to the types of therapy used. Furthermore, we demonstrated that platelet counts might be useful as a tumor marker, in parallel to CA-125 levels, to follow treatment response and follow surveillance for recurrence. These data were limited by provider variation in the frequency of both CA-125 and CBC checks. Standardization as well as prospective analysis could allow the development of prospective algorithms to test for the predictive value of platelet response as a biomarker for tumor response.

In breast cancer models, chemotherapy was found to be more effective in the context of thrombocytopenia, and the effect was attributed to intratumoral hemorrhage facilitated by leukocytes and deficiency in $\beta$-2 or $\beta$-3 integrins (26, 37). On the basis of our observation in patients with ovarian cancer that elevated platelet counts are associated with higher rates of relapse and lower rates of response to chemotherapy, we hypothesized and confirmed that platelets might confer resistance to apoptosis, including that induced by taxane chemotherapy. Coincubation of cancer cells with platelets resulted in platelet aggregation, and blockade of platelet activation abrogated these effects. Aspirin at least partially blocked the increased tumor cell proliferation attributed to platelet coculture.

A series of meta-analyses of randomized and case–control studies have indicated a significantly reduced risk of malignancy in individuals treated with low-dose aspirin (38–41). In our model, platelet transfusion resulted in accelerated tumor growth that was partially blocked by pretreatment of the platelets with aspirin; however, intraperitoneal administration of aspirin did not have a clear effect. Aspirin is a moderate inhibitor of platelet activation and aggregation, and it is known that other activating stimuli (e.g., shear force, catecholamines, thrombin, and ADP) are capable of activating platelets despite aspirinization through non–thromboxane-dependent mechanisms (42). The intraperitoneal aspirin dose utilized here may not have been adequate to overcome these mechanisms.

The potential impact of platelet transfusion on cancer progression or survival has not been well studied. Concern has been
identified that erythropoiesis-simulating agents are associated with tumor progression and decreased survival (43–45). In this context, some centers are exploring the effects of agents such as romiplostim (a thrombopoietin receptor agonist) to maintain platelet counts >100 × 10^9/L in patients being treated with cytotoxic chemotherapy. Limited data report a 15% DVT rate and are not adequate to consider impact on progression and/or survival (46). Our model would suggest that care should be taken when platelet transfusions or thrombopoietin receptor agonists are considered in cancer patients.

We further demonstrated that reduction of platelet counts in vivo reduced tumor growth to the same extent as chemotherapy, and platelet transfusion strongly counteracted the antitumor effect of chemotherapy. Thrombocytopenia is a common toxicity of frontline chemotherapy, and clinical trials will decline to enroll, delay therapy, or remove patients from protocols based on persistent platelet levels less than 10 × 10^11 cells/µL (47). The effect of relative thrombocytopenia and platelet transfusion on the response to chemotherapies needs to be investigated in a larger number of patients in a controlled setting. If our results are confirmed, the risks of platelet transfusion in a patient population may be greater than previously thought. Furthermore, relative thrombocytopenia may be of therapeutic benefit, and within carefully defined safety parameters, the use of antiplatelet reagents may be considered as chemo-sensitizers.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions

References


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