Suppression of chemotherapy-induced cytokine/lipid mediator surge and ovarian cancer by a dual COX-2/sEH inhibitor

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Although chemotherapy is a conventional cancer treatment, it may induce a protumorigenic microenvironment by triggering the release of proinflammatory mediators. In this study, we demonstrate that ovarian tumor cell debris generated by first-line platinum- and taxane-based chemotherapy accelerates tumor progression by stimulating a macrophage-derived "surge" of proinflammatory cytokines and bioactive lipids. Thus, targeting a single inflammatory mediator or pathway is unlikely to prevent therapy-induced tumor progression. Here, we show that combined pharmacological abrogation of the cyclooxygenase-2 (COX-2) and soluble epoxide hydrolase (sEH) pathways prevented the debris-induced surge of both cytokines and lipid mediators by macrophages. In animal models, the dual COX-2/sEH inhibitor PTUPB delayed the onset of debris-stimulated ovarian tumor growth and ascites leading to sustained survival over 120 days postinjection. Therefore, dual inhibition of COX-2/sEH may be an approach to suppress debris-stimulated ovarian tumor growth by preventing the therapy-induced surge of cytokines and lipid mediators.

debris | cyclooxygenase | soluble epoxide hydrolase | inflammation | oxylipins

Epithelial ovarian cancer is the fifth leading cause of cancer-related deaths in women (1). Tumor recurrence in ovarian cancer following front-line platinum- and taxane-based chemotherapy occurs in 70% of patients, resulting in poor 5-y survival rates (1). Although chemotherapy, targeted therapy, or irradiation are mainstays in cancer treatment, tumor cells killed by the treatment ("tumor cell debris") may play a central role in the tumor microenvironment to promote the growth of residual surviving cancer cells (2–6). Chemotherapy promotes tumorigenesis, angiogenesis, and metastasis via apoptotic tumor cell-induced macrophage chemotaxis and proinflammatory cytokines (7–10). Thus, cytotoxic cancer therapy is a double-edged sword; the very treatment meant to control cancer is also helping it survive and grow by inducing a protumorigenic microenvironment. Notably, a single dose of paclitaxel or carboplatin, the chemotherapeutic agents most commonly used in ovarian cancer, stimulates metastasis in mice (11). However, the mechanisms of chemotherapy-induced tumor growth remain poorly understood, providing a challenge for the development of effective treatments (12–16).

A detrimental consequence of chemotherapy is the induction of secreted protumorigenic factors, including inflammatory cytokines, chemokines, proangiogenic growth factors, and danger signals (e.g., alarmins), which collectively create a prometastatic environment (13, 14, 17). Moreover, endogenously produced bioactive lipid molecules, collectively known as eicosanoids, may also contribute to the therapy-induced prometastatic tumor microenvironment (18–20). Eicosanoids are derived from arachidonic acid and are pivotal regulators of inflammatory responses (21). Chemotherapy or irradiation stimulates the release of tumor-promoting lipid mediators, including prostaglandins, platelet-activating factor, sphingosine-1-phosphate (S1P), ceramide-1-phosphate (C1P), and lysophosphatidic acid into the tumor microenvironment (5, 19, 22, 23). Chemotherapy has been recently reported to stimulate the proliferation of ovarian cancer cells through a caspase-3–mediated arachidonic acid pathway (24). Moreover, in vitro studies suggest

Significance

Our study demonstrates that ovarian tumor cell debris generated by front-line chemotherapy promotes tumor growth by stimulating the release of proinflammatory cytokines and lipid mediators in the tumor microenvironment. Targeting the debris-mediated surge of protumorigenic factors provides a strategy for enhancing the efficacy of cytotoxic therapies. Here, we show that the dual cyclooxygenase-2 (COX-2) and soluble epoxide hydrolase (sEH) inhibitor PTUPB prevented the chemotherapy-induced cytokine/lipid surge and suppressed debris-stimulated ovarian tumor growth. Dual COX-2/sEH inhibition may have clinical implications for use in combination with cytotoxic cancer therapies to alleviate debris-mediated inflammation. Our results indicate that PTUPB may act as a "surge protector" against therapy-induced protumorigenic mediators to improve patient survival by preventing tumor recurrence.

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that apoptotic tumor cells activate macrophages to release proinflammatory bioactive lipids, such as S1P, and up-regulate cyclooxygenase-2 (COX-2), a key enzyme in the biosynthesis of prostaglandins (25). COX-2 is a potential therapeutic target in ovarian cancer as its expression is associated with chemoresistance and poor patient prognosis (26). Thus, chemotherapy-induced mediators, including cytokines and bioactive lipids, create a protumorigenic environment via multiple pathways.

While prostaglandins have been extensively studied in cancer, investigation of oxylipins derived from cytochrome P450 (CYP) enzymes has primarily focused on inflammation and cardiovascular functions (27). CYP-derived epoxyeicosatrienoic acids (EETs) stimulate the resolution of inflammation by promoting the clearance of cellular debris by local macrophages and activating antiinflammatory cytokine programs (28, 29). Clearance of tumor cell debris by macrophages has recently been shown to exhibit antitumor activity (2). EETs are rapidly metabolized in the body by soluble epoxide hydrolase (sEH) to their corresponding dihydroxyeicosatrienoic acids (DiHETEs) (30). Pharmacological inhibitors of sEH (sEHIs) stabilize EETs, promote the formation of proresolving mediators such as lipoxins (e.g., lipoxin A_4), and may counterregulate proinflammatory cytokines (31). In fact, abrogation of sEH suppresses chronic inflammatory bowel disease and inflammatory bowel disease-associated tumor formation in IL-10 knockout mice (32). Furthermore, inhibition of sEH reduces inflammation in multiple diseases, including atherosclerosis, abdominal aortic aneurysm, dyslipidemia, hypertension, and diabetes in various mouse models (30). Interestingly, sEHIs (e.g., *t*-AUCB) or sEH-null mice synergize with nonsteroidal antiinflammatory drugs (NSAIDs) and COX-2 selective inhibitors (e.g., celecoxib) to reduce inflammation (33, 34). Moreover, a dual COX-2/sEH inhibitor, 4-(5-phenyl-3-{3-[3-(4-trifluoromethyl-phenyl)-ureido]-propyl}-pyrazol-1-yl) benzenesulfonamide (PTUPB), is more potent in suppressing inflammatory pain and tumor growth than celecoxib, *t*-AUCB, or the combination of celecoxib and *t*-AUCB (35, 36). Strategies aimed at dampening the inflammatory response to tumor cell debris by blocking more than one pathway associated with production of proinflammatory mediators to prevent debris-stimulated tumor growth are poorly characterized.

Here, we show that a "surge" or series of proinflammatory cytokines and bioactive lipids induced by chemotherapy-generated tumor cell debris is suppressed by dual COX-2/sEH inhibition. Importantly, PTUPB inhibited debris-stimulated tumor growth in an ovarian cancer model, which resulted in sustained survival for over 120 d postinjection. To prevent tumor recurrence after therapy, it is critical to neutralize the inherent tumor-promoting activity of therapy-generated debris. Thus, dual inhibition of COX-2/sEH may be an approach in cancer therapy to suppress chemotherapy-induced proinflammatory mediators and debris-stimulated tumor growth.

Results

Chemotherapy-Generated Debris Stimulates the Rapid Onset of Ovarian Tumor Growth and Reduces Survival. To evaluate whether chemotherapy-generated debris is biologically relevant in ovarian cancer, we developed a debris-stimulated ovarian cancer model (Fig. 1). To confirm that cytotoxic platinum- or taxane-based chemotherapeutic agents used for treating ovarian cancer can generate "debris" or dead cells (apoptotic cells, necrotic cells, and cell fragments), we first treated murine (ID8) or human (OVCAR5) ovarian tumor cells with cisplatin, carboplatin, or paclitaxel. Cisplatin, carboplatin, or paclitaxel generated 67%, 78%, and 56% cell death in ID8 ovarian cells, respectively (*SI Appendix*, Fig. S1 *A–C*). Furthermore, carboplatin or paclitaxel also generated human OVCAR5 ovarian tumor cell debris by increasing the death rate three- to fourfold in the chemotherapy-treated cells compared with vehicle-treated controls (*SI Appendix*, Fig. S1 *D* and *E*). Thus, first-line cytotoxic platinum- and taxane-based chemotherapy generates debris in both murine and human ovarian tumor cell lines.

To assess the potential growth-stimulatory activity of such chemotherapy-generated tumor cell debris in vivo, tumor cells killed by chemotherapy in culture were collected and coinjected with living ovarian tumor cells into mice. In the widely used ID8 murine ovarian cancer model (37), cisplatin-generated ID8 debris coinjected with ID8 living cells markedly promoted intraperitoneal ovarian tumor growth and ascites, resulting in reduced survival of immunocompetent C57BL/6 mice (Fig. 1*A*). Paclitaxel-generated ID8 debris also stimulated growth of intraperitoneal and orthotopic ID8 tumors (Fig. 1 *B* and *C*). Thus, mice coinjected with chemotherapy-generated debris and living ovarian tumor cells exhibited markedly reduced survival and accelerated development of ascites compared with mice injected with living tumor cells alone. Remarkably, paclitaxel- or carboplatin-generated ID8 debris coinjected with ID8 living cells stimulated subcutaneous tumor growth in C57BL/6 mice (*SI Appendix*, Fig. S2*A*). The histologic findings of debris-stimulated tumors demonstrate tumor cells with numerous apoptotic cells and malignant cell debris compared with tumors generated by ID8 living cells alone (*SI Appendix*, Fig. S2*B*).

To demonstrate that stimulation of tumor growth by chemotherapy-generated debris was not specific to murine tumors, we developed a debris-stimulated human ovarian (OVCAR5) tumor model. Carboplatin- or paclitaxel-generated OVCAR5 debris coinjected

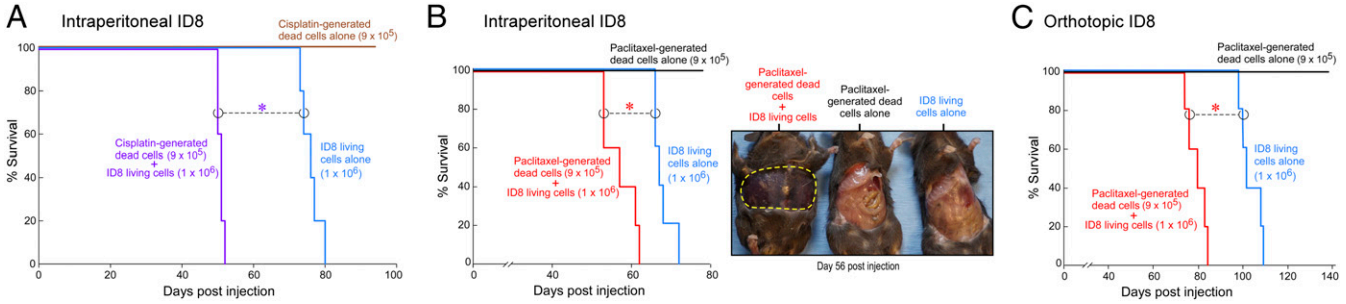

Fig. 1. Chemotherapy-generated ovarian tumor cell debris stimulates tumor growth and shortens survival. Percent survival of mice coinjected intraperitoneally with (*A*) cisplatin- or (*B*) paclitaxel-generated ID8 debris (9×10^5 dead cells) and ID8 living cells (1×10^6). $n = 5$ mice per group. Kaplan–Meier analysis indicated significantly shortened survival in mice coinjected with (*A*) cisplatin- (log-rank test = 9.65, $^*P = 0.0019$) or (*B*) paclitaxel- (log-rank test = 9.85, $^*P = 0.0017$) generated ID8 debris and ID8 living cells compared with ID8 living cells alone. Image shows representative mice on day 56 postinjection. Yellow dashed circle indicates ascites in mice coinjected with paclitaxel-generated debris and ID8 living cells. (*C*) Percent survival of mice coinjected orthotopically with paclitaxel-generated ID8 debris (9×10^5 dead cells) and ID8 living cells (1×10^6). $n = 5$ mice per group. Kaplan–Meier analysis indicated significantly shortened survival in mice coinjected with paclitaxel-generated ID8 debris and ID8 living cells compared with ID8 living cells alone (log-rank test = 6.00, $^*P = 0.014$).

intraperitoneally with OVCAR5 living cells led to a rapid onset of ascites and tumor growth reducing survival in immunocompromised (SCID) mice compared with living tumor cells alone (*SI Appendix*, Fig. S2*C*). Mice injected with chemotherapy-generated debris alone, without living cells, did not exhibit tumor growth or mortality, even at 94 d postinjection (Fig. 1 and *SI Appendix*, Fig. S2). Thus, cytotoxic chemotherapy can generate ovarian tumor cell debris that accelerates tumor growth in both murine and human ovarian tumor models, resulting in reduced survival.

Cytokine Surge Triggered by Debris-Stimulated Macrophages Is Prevented by Dual COX-2/sEH Inhibition. Proinflammatory cytokines released by macrophages in the tumor microenvironment exhibit protumorigenic activity (2, 38). We therefore assessed the release of cytokines by macrophages stimulated with paclitaxel- or carboplatin-generated ID8 debris. Chemotherapy-generated debris triggered the release of a series, or "surge," of proinflammatory cytokines, including TNF-α, MIP-2/CXCL2, MIP-1β/CCL4, CCL2/MCP-1, sICAM-1/CD54, and G-CSF, by RAW264.7 murine macrophages compared with macrophages not exposed to the debris (Fig. 2). Conditioned medium of the debris alone without macrophages contained scarce to undetectable levels of cytokines, thus the cytokines were macrophage-derived (Fig. 2*B*). To exclude that the debris-stimulated cytokine surge was specific to the RAW264.7 macrophage cell line, we performed cytokine array screening of conditioned medium from primary human monocyte-derived macrophages stimulated with paclitaxel-generated OVCAR5 debris. Indeed, chemotherapy-generated debris triggered the release of a surge of proinflammatory cytokines, including CCL2/MCP-1, MIP-1α/MIP-1β, CCL5/RANTES, CXCL1/GROα, and IL-8, by human monocyte-derived macrophages, compared with macrophages not exposed to the debris (*SI Appendix*, Fig. S3*A*).

Release of proinflammatory cytokines, such as CCL2/MCP-1, has been pharmacologically suppressed by dual inhibition of COX-2 and sEH with PTUPB in a kidney injury model (39). To evaluate whether combined COX-2/sEH inhibition can suppress the debris-stimulated cytokine surge by macrophages, we treated RAW264.7 macrophages with various concentrations of PTUPB before stimulation with chemotherapy-generated ID8 debris. Remarkably, PTUPB (5 μM) prevented the cytokine surge by macrophages stimulated with ID8 debris generated by either paclitaxel or carboplatin (Fig. 2). Moreover, PTUPB also suppressed an angiogenic cytokine surge by RAW264.7 macrophages stimulated with debris, including serpin E1/PAI-1, osteopontin, MMP9, and CCL2/MCP-1 (*SI Appendix*, Fig. S3 *B* and *C*). Additionally, PTUPB inhibited a debris-stimulated cytokine surge by primary murine peritoneal macrophages (*SI Appendix*, Fig. S3 *D* and *E*). PTUPB also suppressed debris-stimulated macrophage release of IL-1ra (Fig. 2 *A* and *C* and *SI Appendix*, Fig. S3*D*), consistent with the association of decreased IL-1ra levels and improved survival in ovarian cancer patients (40). Moreover, PUTPB did not induce cell death of RAW264.7 macrophages or ovarian epithelial cells (B/CMBA.Ov) (*SI Appendix*, Fig. S4 *A* and *B*). PTUPB also inhibited proliferation of ID8 tumor cells in vitro (*SI Appendix*, Fig. S4*C*). Thus, the protumorigenic and proangiogenic cytokine surge released by debris-stimulated macrophages was prevented by the dual COX-2/sEH inhibitor PTUPB.

Dual COX-2/sEH Inhibition Differentially Regulates the Release of Lipid Autacoid Mediators by Debris-Stimulated Macrophages. To determine whether debris triggers the release of bioactive lipid autacoids by macrophages, we performed LC-MS/MS–based oxylipin profiling on the conditioned medium of RAW264.7 macrophages stimulated with paclitaxel-generated ID8 debris. Indeed, the debris stimulated macrophages to release a surge of COX-derived lipid mediators, including $PGF_{2\alpha}$, PGD_2, and PGJ_2 (Fig. 3, orange bars) compared with macrophages not exposed to the debris (Fig. 3, yellow bars) or debris alone without macrophages (Fig. 3, blue bars). The dual COX-2/sEH inhibitor PTUPB suppressed this debris-stimulated surge of bioactive lipids released by the macrophages (Fig. 3, gray bars).

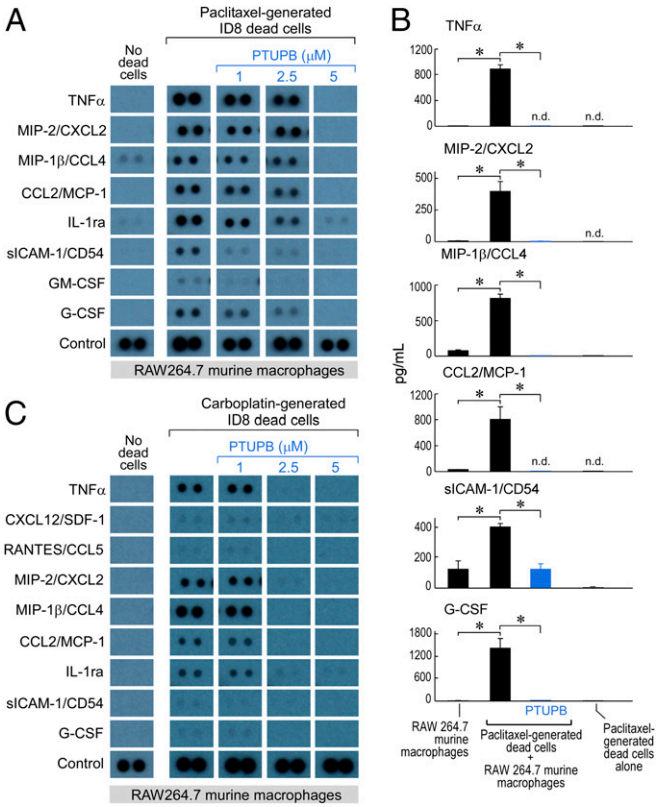

Fig. 2. Cytokine surge by debris-stimulated macrophages is prevented by the dual COX-2/sEH inhibitor PTUPB. (*A*) Cytokine array of conditioned medium from RAW264.7 murine macrophages treated with vehicle or PTUPB and stimulated with paclitaxel-generated ID8 debris. (*B*) ELISA quantification of proinflammatory cytokines released by RAW264.7 macrophages treated with vehicle (black bars) or PTUPB (5 μM) (blue bars) for 2 h and stimulated with paclitaxel-generated ID8 debris, or by paclitaxel-generated ID8 debris alone without macrophages. Data are presented as means (pg/mL) ± SEM $n = 7$–8/group. *$P < 0.05$ vs. RAW264.7 + paclitaxel-generated ID8 dead cells. n.d., not detectable. (*C*) Cytokine array of conditioned medium from RAW264.7 murine macrophages treated with vehicle or PTUPB for 2 h and stimulated with carboplatin-generated ID8 debris.

We next evaluated whether macrophages can reduce a series of lipids in response to chemotherapy-generated debris. LC-MS/MS–based oxylipin profiling of conditioned medium of the debris alone without macrophages (Fig. 3, blue bars) revealed levels of PGE_2, THF diol, 15-HETE, 11-HETE, and 5-HETE that were decreased when debris was added to macrophages (Fig. 3, orange bars). Interestingly, PTUPB neutralized the reduction of these lipids by debris-stimulated macrophages (Fig. 3, gray bars). Furthermore, PTUPB also suppressed the release of 15-oxoETE by macrophages in the presence of debris (Fig. 3, gray bars) compared with debris-stimulated macrophages without PTUPB (Fig. 3, orange bars) or macrophages not exposed to the debris (Fig. 3, yellow bars). In contrast, debris did not affect the release of TXB_2, 15d-PGJ_2, 12,13-DiHOME, 9,10-DiHOME, 12,13-EpOME, 9,10-EpOME, 9-oxoODE, 12-HETE, 13-HODE, 9-HODE, EKODE, 9,12,13-TriHOME, or 9,10,13-TriHOME by macrophages in the presence or absence of PTUPB (*SI Appendix*, Fig. S5). Taken together, these results suggest an active process in which dual COX-2/sEH inhibition differentially regulates the release of lipid autacoid mediators by macrophages stimulated with chemotherapy-generated tumor cell debris.

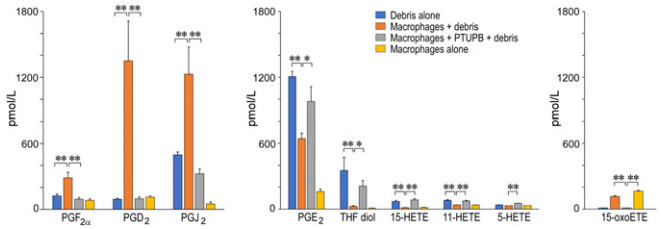

Fig. 3. Dual COX-2/sEH inhibition differentially regulates the release of lipid mediators by debris-stimulated macrophages. LC-MS/MS–based oxylipin analysis of conditioned medium from paclitaxel-generated ID8 debris alone without macrophages ("debris alone," blue bars) or from RAW264.7 macrophages stimulated with paclitaxel-generated ID8 debris (orange bars), PTUPB-treated macrophages (5 μM, 2 h) stimulated with paclitaxel-generated ID8 debris (gray bars), or macrophages not stimulated with debris ("macrophages alone," yellow bars). PTUPB inhibits the surge of $PGF_{2\alpha}$, PGD_2, and PGJ_2 (*Left*), while neutralizing the reduction of PGE_2, THF diol, 15-HETE, 11-HETE, and 5-HETE (*Center*) by debris-stimulated macrophages. PTUPB suppressed the release of 15-oxoETE by macrophages (*Right*). Data are presented as means (pmol/L) ± SEM $n = 10$ per group. $^*P < 0.05$ or $^{**}P < 0.01$ vs. macrophages + debris or macrophages alone.

Suppression of in Vivo Proinflammatory/Proangiogenic Cytokines and Debris-Stimulated Ovarian Tumor Growth via Dual COX-2/sEH Inhibition. We next assessed whether dual COX-2/sEH inhibition could suppress proinflammatory and proangiogenic cytokines in mice bearing intraperitoneal ovarian tumors (ID8). PTUPB reduced serum levels of CXCL13/BCA-1 and SDF-1/CXCL12 in mice administered systemic chemotherapy (*SI Appendix*, Fig. S6 *A* and *B*). In addition, PTUPB also inhibited proangiogenic factors serpin E1/PAI-1 and IGFBP1 in serum from mice intraperitoneally injected with ID8 (1×10^6 living cells) compared with control (Fig. 4*A*). Furthermore, ascites from PTUPB-treated mice bearing intraperitoneal ID8 tumors exhibited decreased levels of proinflammatory and proangiogenic cytokines, including PIGF-2, PTX-3, MMP9, CCL2/MCP-1, fractalkine/CX3CL1, and angiopoietin-1 compared with control on day 60 postinjection (Fig. 4*B* and *SI Appendix*, Fig. S6*C*).

To determine whether PTUPB could suppress debris-stimulated ovarian tumor growth, mice were coinjected intraperitoneally with paclitaxel- or carboplatin-generated ID8 debris and ID8 living cells. Remarkably, PTUPB induced sustained survival over 120 d postinjection in mice bearing debris-stimulated intraperitoneal ovarian tumors compared with control (Fig. 5 *A* and *B*). Moreover, PTUPB suppressed debris-stimulated orthotopic ovarian tumor growth and prolonged survival (Fig. 5*C*).

Discussion

Epithelial ovarian cancer is often clinically detected at a late stage in which cancer cells have already spread throughout the peritoneum (1). Most ovarian cancer patients who initially respond to surgery combined with platinum- and taxane-based chemotherapy, develop recurrent tumors within 1–5 y, leading to poor prognosis (1). While reducing tumor burden via killing cancer cells is considered a hallmark success of therapy, the resulting tumor cell debris promotes growth and metastasis of the surviving cancer cells (2). We show here that ovarian tumor cell debris generated by chemotherapy induces a surge of cytokines and lipid autacoid mediators that creates a protumorigenic microenvironment. Novel therapeutics that can be combined with conventional chemotherapy are required to ameliorate the underappreciated protumorigenic activity induced by the debris.

The ovarian tumor microenvironment is enriched in cytokines and bioactive lipids (41). High circulating levels of proinflammatory cytokines—such as IL-6, CCL2/MCP-1, and TNF-α—have been identified in ovarian cancer patients and play critical roles in the development and progression of the disease (42, 43). These cytokines perpetuate a vicious cycle by recruiting and activating cytokine-producing cells that further promote their protumorigenic activity. Recent studies show that cytokine antagonists may represent a possible strategy in the treatment of ovarian cancer. However, targeting a single cytokine/chemokine in platinum-resistant ovarian cancer (e.g., monoclonal antibodies against IL-6 or TNF-α) has had only transient and limited antitumor activity in patients (44, 45). Therefore, blocking only one or a few cytokines induced by cytotoxic therapy-generated debris may not prevent tumor recurrence.

In addition to cytokines, bioactive lipid mediators also play a role in treatment-induced tumor progression (19, 20). The second-line ovarian chemotherapeutic doxorubicin triggers the release of inflammatory prostaglandins and leukotrienes, causing an "eicosanoid storm" (46, 47). Here, we demonstrate via LC-MS/MS–based oxylipin profiling that PTUPB prevented a debris-induced surge of proinflammatory bioactive lipids—including $PGF_{2\alpha}$, PGD_2, and PGJ_2—by macrophages. PTUPB also neutralized the reduction of PGE_2, THF diol, 15-HETE, 11-HETE, and 5-HETE by debris-stimulated macrophages. Our findings are consistent with reports that PGE_2 promotes lipid mediator class switching to stimulate resolution (48) and that it can act as a damage-associated molecular pattern in therapy-induced dead tumor cells to regulate inflammation (49). In addition, while 15-HETE and 11-HETE exhibit protumorigenic activity in breast cancer, these lipids have been shown to possess antitumorigenic activity in various cancers, including ovarian cancer (41, 50). The COX-derived prostaglandins, LOX-derived HETEs, CYP450-derived DiHOMEs, and EpOMEs identified in the conditioned medium of the chemotherapy-generated tumor cell debris without macrophages indicate that the debris itself may be a source of tumor growth-stimulation.

Because the synthesis of oxylipins depend on common key enzymes, blockade of these biosynthetic enzymes may represent a promising therapeutic strategy. Targeting multiple inflammatory mediators simultaneously has long been a desired therapeutic strategy but is challenging to achieve with antibodies directed against individual cytokines. We demonstrate that an approach to suppress proinflammatory cytokines and bioactive lipids is the combined inhibition of COX-2 and sEH pathways. Dual COX-2/sEH inhibition with a single compound, PTUPB, has been reported to decrease inflammatory and oxidative stress markers in a kidney injury model (39). Inhibition of sEH by PTUPB blocks and even reverses the adverse toxicities caused by NSAIDs, such as gastrointestinal erosion in gastric ulcers (51) and may reduce cardiovascular risks associated with coxibs, such as celecoxib or

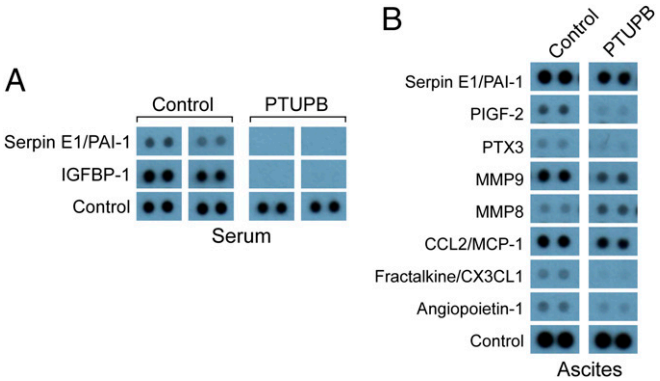

Fig. 4. PTUPB suppresses proangiogenic cytokines in vivo. Proangiogenic cytokine arrays of (*A*) serum or (*B*) ascites from control or PTUPB-treated mice intraperitoneally injected with ID8 (1×10^6 living cells). Serum and ascites was collected on day 60 postinjection.

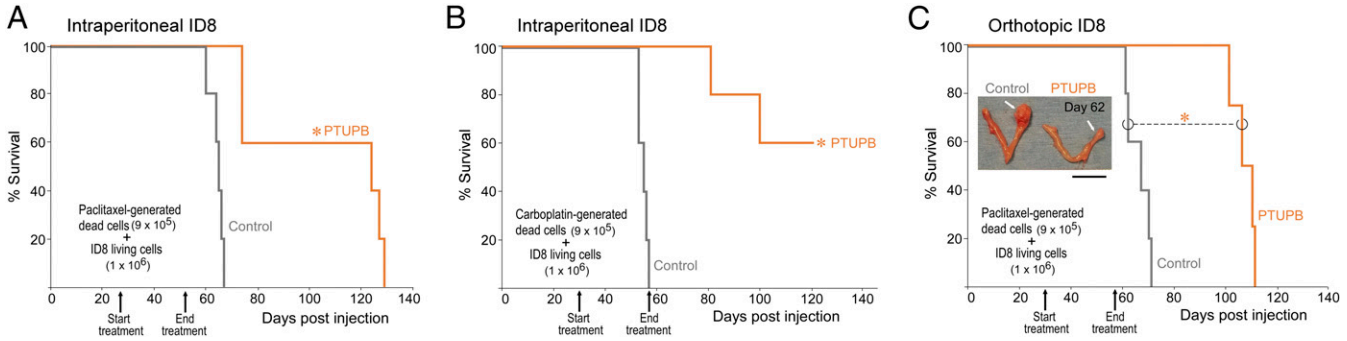

Fig. 5. Suppression of debris-stimulated ovarian tumor growth by PTUPB. Percent survival of mice coinjected intraperitoneally with (*A*) paclitaxel- or (*B*) carboplatin-generated ID8 debris (9×10^5 dead cells) and ID8 living cells (1×10^6), or (*C*) coinjected orthotopically with paclitaxel-generated ID8 debris (9×10^5 dead cells) and ID8 living cells (1×10^6). Image shows representative orthotopic tumors on day 62 postinjection. (Scale bar,1 cm.) Systemic treatment with PTUPB (30 mg/kg/d) or control initiated 4 wk postinjection. n = 4–5 mice per group. Kaplan–Meier analysis and log-rank testing indicated significantly prolonged survival in mice treated with PTUPB compared with control in the (*A*) paclitaxel-generated (log-rank test = 9.70, $^*P = 0.0018$) and (*B*) carboplatin-generated (log-rank test = 9.85, $^*P = 0.0017$) debris-stimulated intraperitoneal ovarian tumor models, as well as the (*C*) paclitaxel-generated debris-stimulated orthotopic ovarian tumor model (log-rank test = 7.91, $^*P = 0.005$).

rofecoxib (35). In this study, although PTUPB suppressed the release of critical protumorigenic cytokines in the debris-mediated surge (Fig. 2 and *SI Appendix*, Fig. S3 *B–E*), it did not entirely suppress the protumorigenic mediator osteopontin. This may be because macrophages endogenously express high levels of osteopontin (52). Osteopontin levels in patients can be modulated by chemotherapy and serve as a tumor marker with CA 125 for ovarian cancer (53, 54). In this study, PTUPB suppressed the release of osteopontin by macrophages exposed to ovarian tumor cell debris. PTUPB also enhances the antitumor activity of the chemotherapeutic agent cisplatin in nondebris-stimulated tumors (55). In addition, our findings complement previous studies demonstrating the ability of PTUPB to suppress primary tumor growth and metastasis with minimal toxicity (35, 56), because PTUPB prolonged survival in mice bearing debris-stimulated ovarian tumors and did not induce cell death of RAW264.7 macrophages or ovarian epithelial cells. The addition of PTUPB to existing therapy regimens could prolong survival by blocking the tumor-stimulatory activity of therapy-generated tumor cell debris. Thus, COX-2/sEH inhibition may represent a strategy to suppress tumor-associated inflammation via prevention of a surge of cytokines and lipid mediators, rather than targeting a single protumorigenic factor.

Dual COX-2/sEH inhibition could potentially have broad implications in alleviating the inflammatory, and in some cases fatal "cytokine storms" that can occur in various settings such, as in immunotherapy (57, 58), infectious diseases (e.g., Ebola, viral infections, or influenza), or immunological emergencies (e.g., graft-versus-host disease or autoimmune diseases) (57, 59). The complexity of the cytokine storm has limited the development of therapeutic strategies to inhibit the inflammatory response and clinical trials targeting specific cytokines have failed to date (59, 60). Here, our results indicate that the antitumor, antiangiogenic, and antiinflammatory activity of PTUPB may protect the body from a therapy-induced debris-mediated inflammatory response.

While the "cytokine and eicosanoid storm" is well characterized in infectious diseases (47, 57), the role for chemotherapy-induced cytokines and bioactive lipid mediators in cancer is underappreciated and poorly characterized. Ovarian cancer patients may benefit from the suppression of proinflammatory mediators associated with debris-stimulated tumor growth. Conquering debris-stimulated tumor progression is paramount to prevent tumor recurrence of treatment-resistant tumors. Thus, dual COX-2/sEH inhibition is a therapeutic modality that may complement cytotoxic cancer therapies by acting as a "surge protector" against the therapy-induced cytokine/lipid mediator surge to suppress debris-stimulated tumor growth.

Materials and Methods

Methods used for preparation of chemotherapy-generated tumor cell debris (2, 3), macrophage conditioned medium (2, 3), flow cytometry (2, 3), oxylipin profiling (LC-MS/MS) (61), and isolation of human monocyte-derived macrophages (2) were all previously described. MTT proliferation assays (Roche), cytokine arrays (R&D Systems), and ELISAs (R&D Systems) were performed according to provided recommended protocols. For isolation of primary murine peritoneal macrophages, female C57BL/6 mice were intraperitoneally injected with zymosan A (1 mg; Sigma Aldrich) and peritoneal macrophages were collected 72 h later by peritoneal lavage using sterile PBS. Full protocols are described in the *SI Appendix, Materials and Methods*.

In Vivo Studies. All animal studies were reviewed and approved by the Animal Care and Use Committee of Beth Israel Deaconess Medical Center. Ovarian tumor growth was monitored in 6-wk-old female C57BL/6 (The Jackson Laboratory) or SCID mice (Charles River). Systemic treatment with PTUPB (30 mg/kg/d) or control was administered via miniosmotic pumps (Alzet Inc.) implanted into the peritoneum of the mice 4 wk postinjection. Systemic chemotherapy with carboplatin (10 mg/kg every 3 d) or control initiated on day of tumor cell injection.

Statistics. Statistical analyses for in vitro studies were performed using Student's two-tailed unpaired t test. Data are represented as mean ± SEM with P values less than 0.05 considered statistically significant. The Kaplan–Meier product-limit method and log-rank testing were used to evaluate survival differences over time after the day of tumor cell injection between mice coinjected with tumor cell debris and living cells vs. living cells alone. Data are represented as percent survival with P values less than 0.01 considered statistically significant.

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