PGE$_2$-induced CXCL12 Production and CXCR4 Expression Controls the Accumulation of Human MDSCs in Ovarian Cancer Environment

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Running title: PGE$_2$ controls CXCR4-driven accumulation of MDSCs

Keywords: PGE$_2$, CXCR4, CXCL12/SDF-1, migration, MDSCs

Financial support: This work was supported by grants from NIH (1PO1 CA132714) (P.K.) and by a UICC American Cancer Society Beginning Investigators Fellowship funded by the American Cancer Society (N.O.).

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Conflict of interest disclosure: The authors declare no competing financial interests.

Word count (excluding references): 3,317 (+208 Abstract); No. figures: 4; No. tables: 0; No. references: 39.
Abstract

Signals mediated by CXCL12 (SDF1) and its receptor CXCR4 are centrally involved in cancer progression, both directly by activating cancer cells and indirectly by inducing angiogenesis plus recruiting T regulatory and plasmacytoid dendritic immune cells. Here we show that in ascites isolated from ovarian cancer patients, both CXCL12 and CXCR4 are controlled by the tumor-associated inflammatory mediator prostaglandin E\textsubscript{2} (PGE\textsubscript{2}), which attracts myeloid-derived suppressor cells (MDSC) into the ascites microenvironment. In this setting, PGE\textsubscript{2} was essential both for expression of functional CXCR4 in cancer-associated MDSCs and for production of its ligand CXCL12. Frequencies of CD11b\textsuperscript{+}CD14\textsuperscript{+}CD33\textsuperscript{+}CXCR4\textsuperscript{+} MDSCs closely correlated with CXCL12 and PGE\textsubscript{2} levels in patient ascites. MDSCs migrated towards OvCa ascites in a CXCR4-dependent manner that required COX2 activity and autocrine PGE\textsubscript{2} production. Inhibition of COX2 or the PGE2 receptors EP2/EP4 in MDSCs suppressed expression of CXCR4 and MDSC responsiveness to CXCL12 or OvCa ascites. Similarly, COX2 inhibition also blocked CXCL12 production in the OvCa environment and its ability to attract MDSCs. Together, our findings elucidate a central role for PGE\textsubscript{2} in MDSC accumulation triggered by the CXCL12-CXCR4 pathway, providing a powerful rationale to target PGE\textsubscript{2} signaling in OvCa therapy.
Introduction

Stromal cell-derived factor-1 (SDF-1, now designated as CXCL12) is over-produced by stromal and tumor cells in different tumor microenvironments (1-4). CXCL12 exerts multiple tumor-promoting functions, either directly through its cognate receptor CXCR4 present on cancer cells enhancing tumor growth, migration and invasiveness, or indirectly by recruiting endothelial progenitors, needed for tumor angiogenesis (3, 5, 6). Moreover, regulatory T cells (T_{reg}) and plasmacytoid dendritic cells (pDC), which play a crucial role in immune evasion, are attracted to the tumor environment through CXCL12 (6-8). By recruiting and retaining these immunosuppressive cells, the tumor microenvironment limits the effectiveness of immune responses (9).

Myeloid-derived suppressor cells (MDSC) (10, 11) have emerged as critical elements of cancer-induced immune dysfunction. MDSCs perform their suppressive functions at the site of tumor growth (10) and represent a heterogeneous population of immature myeloid cells (iMC) involving immature precursors of macrophages, granulocytes, and dendritic cells (DC), capable of suppressing immune response \textit{in vitro} and \textit{in vivo} (12). MDSCs residing within the tumor environment are deficient in costimulatory-molecule expression, and inefficient in lymphoid homing (13). Instead, they abrogate adaptive immune responses to cancer cells, as shown in experimental animal models and human cancer patients (14).

High activity of the ovarian cancer (OvCa) microenvironment to attract other types of suppressive cells (6-8) suggests that, in addition to accelerated development of MDSCs in cancer setting, the predominance of MDSCs in OvCa microenvironment may result from their enhanced attraction and/or retention. While the molecular mechanisms that regulate the development and function of MDSCs in the cancer setting have been extensively studied,
particularly in the mouse system (13, 15-21), the mechanisms guiding MDSCs to human cancer environments remain poorly understood.

Guided by prior mouse studies showing the involvement of PGE$_2$ in the regulation of CXCL12 production in cancer-associated fibroblasts (22), and the ability of PGE$_2$-producing tumor cells to enhance CXCR4 expression on differentiating mouse MDSCs (23, 24), we tested the relevance of PGE$_2$ in the regulation of the CXCR4-CXCL12 interplay in human OvCa-infiltrating MDSCs. Our data demonstrate that the CXCR4-CXCL12 axis is the key pathway mediating the attraction of monocytic MDSC into the tumor environment of ovarian cancer patients, with PGE$_2$ responsible for the induction of both functional CXCR4 and CXCL12. The ability of COX2 inhibitors and PGE$_2$ receptor blockers to reverse the chemokine responsiveness of fully-developed MDSCs and to decrease the levels of CXCL12 produced in the OvCa environment provides a new tool to counteract immune suppression in therapeutic regimens aimed at restoring immune surveillance in cancer patients.
Materials and methods

Patients. Human OvCa ascites and sera were obtained from previously untreated patients with advanced epithelial OvCa in stage III or IV, after obtaining written informed consent. The nature and possible consequences of the studies were explained. All specimens were provided under the protocols approved by the University of Pittsburgh Institutional Review Board (IRB0406147) or the Roswell Park Cancer Institute Institutional Review Board (CIC02-15). Patients underwent a primary surgical debulking procedure for clinical staging. Fresh ascites were obtained intraoperatively, and blood draws were performed either immediately preoperatively or at the first postoperative visit prior to any adjuvant therapy.

Isolation of ovarian cancer ascites-infiltrating cells and MDSCs. Human OvCa ascites were collected aseptically and infiltrating primary cells harvested by centrifugation. CD11b+ MDSCs were obtained after centrifugation of ascites, followed by RBC lysis and positive magnetic selection of CD11b+ cells (CD11b EasySep Isolation kit; Stem Cell Tech, Vancouver, Canada). The isolated cells were CD11b+ >95% pure. Control CD11b+ cells were isolated from healthy donor buffy coats, using the same method.

Flow cytometry. Two- and three-color cell surface and intracellular immunostaining analysis was performed using Beckman Coulter Epics XL or Accuri flow-cytometer. OvCa-isolated cells were stained with the following antibodies CCR2-PE, CCR5-PE, CCR6-PE, CXCR1-PE, CXCR4-PE, CD11b-FITC, CD14-PE, CD33-APC, CD34-PE/Cy7 (BD and eBioscience, San Diego, CA). Rat IgG2α-PE, IgG1-FITC, IgG1-APC and IgG1-PE/Cy7 isotype controls, and rat IgG2α-FITC isotype control were from BD PharMingen.

Chemotaxis assay. Chemotaxis assays were performed as previously described (25). rhu-CXCL-12α (5-50 ng/ml; R&D) in IMDM+0.5% FBS or OvCa ascites were used as chemotaxis
media. When indicated, cells were pre-treated for 10 min with CXCR4 antagonist AMD3100 (1000 ng/mL; Sigma) and CCR5 antagonist vicriviroc (1000 ng/ml) before chemotaxis experiments to block the CXCR4 and CCR5-dependent chemotaxis. For desensitization, CXCL12 or CCL5 were added to the cells in the upper chamber 10 min before the chemotaxis experiment. The concentrations of the blocking agents used did not have any significant impact on the viability of cultured cells, as determined by the live cell counts.

**Isolation of peripheral blood naïve CD8⁺ T cell populations and CD3/CD28 in vitro effector generation.** Naïve CD8⁺CD45RA⁺CD45RO⁻ T cells were isolated from PBMCs by negative selection using the naïve CD8⁺ T cell enrichment cocktail (Stem Cell Tech), resulting in a uniform population of CD8⁺CD45RA⁺CD45RO⁻ cells. CD8⁺ T cells were stimulated with CD3/CD28 Dynabeads (5 µl/ml; Invitrogen Dynal AS, Oslo, Norway) in the presence or absence of OvCa ascites-isolated MDSCs. CFSE staining of CD8⁺ T cells (Invitrogen) was performed according to the manufacturer’s instructions. On day 4-5, expanded CD8⁺ T cells were analyzed for the expression of granzyme B expression and proliferation.

**ELISA.** OvCa ascites were collected into collection tubes, centrifuged at 2,000 rpm for 10 min and the supernatants were immediately stored at −80°C until use. OvCa ascites were used immediately after defrosting and were not subjected to further freeze-thaw cycles. OvCa ascites and conditioned media generated by culturing OvCa ascites-infiltrating primary cells for 48 h were analyzed for CXCL12/SDF-1 and CCL5 by indirect sandwich ELISA (R&D) and PGE₂ by competitive parameter immunoassay according to the manufacturer’s protocol (R&D).

**Taqman analysis of mRNA expression.** mRNA levels of CD11b, IL-10, IDO1, ARG1, NOS2, COX2, CXCR4, CXCL12/SDF-1 were analyzed in OvCa-ascites infiltrating primary cells vs. patient’s matched PBMCs and OvCa ascites-isolated CD11b⁺ cells vs. control CD11b⁺ cells.
(isolated from normal blood), either after their isolation or after the overnight incubation in the presence or absence of the COX2 inhibitor Celecoxib (20 μM), EP2 antagonist AH6809, EP4 antagonist AH23848 and EP3 antagonist L798106. The concentrations used did not have any significant impact on the viability of cultured cells, as determined by the live cell counts. Taqman analysis was performed as previously described (25) on the StepOne Plus System (Applied Biosystems). The expression of each gene was normalized to HPRT1 and expressed as fold increase ($2^{-\Delta CT}$), where $\Delta CT = CT_{(Target\ gene)} - CT_{(HPRT1)}$.

**Statistical analysis.** All data were evaluated using GraphPad Prism 5 software and analyzed using Student’s t test (two tailed), with $P < 0.05$ considered as significant ($P<0.05$ marked *; $P<0.01$ marked **; $P<0.001$ marked ***). A linear correlation between two continuous variables was tested with the $R^2$ coefficient of determination. When indicated, the data from multiple different patients and control donors are expressed as means and SD from N donors (see the N values in the figure legends). The data from representative experiments was obtained from triplicate cultures with cells from an individual donors. Each of such independent experiments was reproduced at least three times.
Results

High expression of CXCR4 on cancer-isolated monocytic MDSCs mediates their attraction to CXCL12-producing OvCa-infiltrating cells. CXCR4 is over-expressed on tumor-infiltrating suppressive pDCs (7), with high local levels of its ligand CXCL12 predicting reduced survival of OvCa patients (26). To address the relative role of CXCL12 in MDSC accumulation, we analyzed the expression of chemokine receptors on cancer-infiltrating MDSCs and their migratory responsiveness. Bulk tumor ascites contained high numbers of CD11b+ cells, mostly composed of monocytic MDSCs, as determined by their uniform expression of CD33 and CD34 (10, 14) (Fig. 1A). Such CD11b+CD33−CD34+ MDSCs lacked expression of the co-stimulatory molecules CD80 and CD83, with most cells expressing CD14, typical of the monocytic subset of MDSCs (10, 14, 18) (Fig. 1A and Supplementary Fig. S1). These cells expressed high levels of typical MDSC-associated suppressive factors (10, 14, 18), including arginase, IL-10, IDO1, IL-4Rα (CD124) and COX2 (Fig. 1B), and strongly suppressed CTL development (Fig. 1C). Such ascites-associated CD11b+ monocytic MDSCs demonstrated uniformly high expression of CXCR4 (Fig. 1D and E), when compared to blood-isolated monocytic cells (Figure 1E, bottom), in addition to lower levels of expression of CCR2, CCR5 and CXCR1.

In addition to the high expression of CXCR4 on isolated monocytic MDSCs (Fig. 1D and E), we observed that the numbers of MDSCs strongly correlated with the levels of CXCR4 ligand, CXCL12/SDF-1, in the tumor environment (Fig. 2A). The local gene expression of CXCL12 and secretion of CXCL12 protein was profoundly higher compared to levels in patient’s blood (Fig. 2B). In contrast, CCL5/Rantes was expressed at significantly lower levels (Fig. 2C), and did not correlate with the tumor infiltration of MDSCs (data not shown).
Moreover, migration of tumor-isolated MDSCs could be effectively suppressed by CXCR4 antagonist AMD3100 or by CXCL12 desensitization, but not with CCR5 antagonist vicriviroc or CCL5 desensitization (Fig. 2D). In accordance with these data, tumor-isolated MDSCs showed strong responsiveness to recombinant CXCL12 (Fig. 2E).

**Positive feedback between COX2 and PGE2 is responsible for both CXCR4 expression on monocytic MDSCs and the production of CXCL12 in OvCa.** Guided by the reported ability of PGE2, a factor implicated in CXCR4 induction in murine cells (23, 24), and the production of CXCL12 in mouse tumor-associated fibroblasts (22), we tested the potential role of PGE2 in the regulation of CXCL12 production and MDSC accumulation in OvCa patients. We observed a strong correlation between the expression of CD11b and COX2 expression (Fig. 3A) and between CXCL12 concentrations and the local production of PGE2 (Fig. 3B). In accordance with the potential causative role of PGE2 in the ovarian cancer-associated CXCL12 production, the expression and secretion of CXCL12 in OvCa ascites cells was inhibited by COX2 blockade (Fig. 4A and B), closely reflecting the degree of inhibition of COX2 expression and PGE2 release (Fig. 4A and B).

In accordance with the driving role of PGE2 in the CXCL12-mediated attraction of MDSCs to the tumor microenvironment, the ability of supernatants from 48 hour-cultured OvCa ascites cells to attract OvCa-isolated MDSCs to the OvCa environment was dependent on COX2 activity and was suppressed following COX2 inhibition during the generation of the OvCa cell-conditioned media (Fig. 4C). In accordance with the previous report showing that epithelial cells are the predominant source of CXCL12 in the OvCa environment (7), the COX2-dependent CXCL12 expression was particularly pronounced in total ascites cells, with only marginal levels expressed in the CD11b+ fraction (Fig. 4A).
Key role of PGE₂ - COX2 feedback in the persistence of CXCR4 expression by OvCa-associated monocytic MDSCs and production of CXCL12. Interestingly, overnight COX2 inhibition reduced not only CXCL12 and CXCR4 expression in OvCa ascites cells (Fig. 4A and D), but also the expression of endogenous COX2 in OvCa ascites primary cells and OvCa-isolated MDSCs (Fig. 4A), indicating the crucial role of an intact positive feedback loop between PGE₂ and COX2 in the persistence of CXCR4 expression on monocytic MDSCs and the production of CXCL12 in the OvCa environment. The expression of CXCR4 on OvCa-isolated MDSCs was also suppressed by EP2 and EP4 (but not EP3) blockade (Fig. 4D), indicating that the effects of endogenous PGE₂ on CXCR4 expression by MDSCs are mediated in part by EP2 and EP4.
Discussion

In the present study, we have identified the critical role of the CXCR4-CXCL12/SDF-1 migratory axis in the accumulation of immunosuppressive monocytic MDSCs in tumor microenvironment of OvCa patients. We have further demonstrated that the tumor-associated inflammatory mediator, PGE2, induces both CXCL12/SDF-1 chemokine production in the OvCa environment, as well as CXCR4 expression on MDSC precursors and their resulting responsiveness to CXCL12. Moreover, continued PGE2 signaling in fully-developed MDSCs isolated from OvCa patients is critically important for their continued expression of CXCR4 and responsiveness to CXCL12, promoting the attraction and retention of MDSCs in the tumor environment.

Correlation of OvCa-associated CXCL12 levels with the local PGE2 production and local infiltration of CD11b+CD14+CD33+ MDSCs substantiate the physiological role of PGE2 in controlling monocytic MDSC accumulation in human cancer. While the current data show that PGE2 is responsible for the induction of functional CXCR4 and CXCL12-mediated attraction of MDSCs, the current results also implicate that COX2- and PGE2- antagonism may help to overcome additional CXCL12-dependent mechanism of cancer progression, that include the direct impact of CXCL12 upon tumor growth and invasiveness (7, 26) as well as local accumulation of CXCR4-expressing suppressive pDCs and Tregs in cancer tissues and bone-marrow of cancer patients (7, 27).

In line with the possibility that local PGE2 gradient in the tumor-surrounding environment allows for the gradual recruitment of migrating MDSCs or MDSC-precursors via CXCL12-CXCR4, cancer cell inoculation has been shown to induce the appearance of VEGFR+CXCR4+CD11b+ cells in the blood of cancer-bearing mice (24). However, at present,
we cannot exclude the possibility that MDSCs or MDSC-precursors are attracted into tumor microenvironment via alternative pathway and tumor-produced PGE₂ induces CXCR4 expression and MDSC retention in the CXCL12-enriched tumor environment. While tumor cells themselves or tumor-associated stromal cells (e.g. fibroblasts (22), mesothelial cells (28) and vascular endothelial cells (29) may overproduce PGE₂ spontaneously (see Supplementary Fig. S2 for the variable levels of PGE₂ production by the non-MDSC component of OvCa ascites), an alternative possibility is that tumor cell-derived factors, e.g. mucins in the initial induction of the first wave of COX2 expression in infiltrating monocytic cells, as proposed in the colorectal cancer system (30).

OvCa-associated PGE₂ promotes the MDSC expression of COX2 (Fig. 4A), the key enzyme regulating PGE₂ synthesis (see Supplementary Fig. S2 for the consistently high levels of PGE₂ production by OvCa-isolated monocytic MDSCs), thereby creating a positive feedback loop where PGE₂ produced by MDSCs helps to maintain the CXCL12 responsiveness and local retention of CXCR4⁺ MDSCs. While this last mechanism can contribute to a vicious cycle amplifying the persistence of MDSCs within cancer settings, the requirement for continued production of PGE₂ in the accumulation of MDSCs allows for new modes of their pharmacologic modulation.

Since high level of CXCL12 in OvCa ascites represents a negative prognostic factor of OvCa patients (26), and PGE₂ also controls the production of CCL22 (25), another chemokine involved in recruiting CCR4⁺ Tregs and a negative prognostic factor in OvCa (4), our current data highlight the key role of PGE2 in the accumulation of multiple types of cancer-associated suppressive cells (4, 7, 27) and provide additional rationale for PGE₂ targeting in OvCa therapy.
In this last regard, we observed that pharmacological inhibition of PGE$_2$ production suppresses the production of CXCL12 in OvCa ascites cells (Fig. 4A and B). Moreover, even short-term exposure of the fully developed OvCa-isolated MDSCs to COX2 inhibitors (or EP2- and EP4-blockers) suppresses their expression of CXCR4 (Fig. 4A and D) and migratory responsiveness to recombinant CXCL12 (Fig. 4C).

Since overproduction of COX2 and PGE$_2$ is a hallmark of many tumor types (31-33), the presently-defined mechanism is likely applicable to the local accumulation of MDSCs observed in different cancer types (5, 9), as well as to additional (MDSC-independent) pathways of promoting tumor growth in different forms of cancer, with similarly wide therapeutic implications.

While chemokines play a crucial role in immune and inflammatory reactions, they have an equally important role in the development of a variety of cancers, being involved in cell transformation, survival, growth, metastasis, and tumor-associated angiogenesis (34). CXCL12 expressed by the tumor-associated fibroblasts promotes the progression of breast cancer by directly enhancing tumor growth and by recruiting endothelial progenitor cells that are required for tumor neoangiogenesis (3). COX2 and PGE$_2$ are also involved in tumor stroma formation by recruiting stromal fibroblasts via the CXCL12-CXCR4 axis (22), and mediating the angiogenic effects of bFGF and VEGF by enhancing CXCR4 expression in microvascular endothelial cells (35), and by inducing pro-angiogenic chemokines, CXCL5 (epithelial cell–derived neutrophil activator 78), CXCL8 (IL-8) and CXCL12 ((22, 36) and the current data).

In addition to the currently-demonstrated central role of PGE$_2$ in MDSC accumulation, PGE$_2$ has recently been shown to bias the chemokine production of DCs, abrogating their CXCL9-, CXCL10-, CXCL11-, CCL5- and CCL19-mediated ability to attract naïve, effector, and memory
T cells and NK cells (25, 37, 38). Instead, PGE₂ promotes the CCL22-driven interaction of DCs with undesirable T<sub>reg</sub> cells (25), known to be preferentially expanded and recruited to cancer tissues (8), where the levels of CCL22 and T<sub>reg</sub> infiltration have a strong negative prognostic value (4). The key role of PGE₂ in the induction of CCL22 and CXCL12, the chemokines attracting MDSCs, T<sub>regs</sub> and suppressive pDCs to different tumors (4, 7, 8, 27), and its ability to suppress the local influx of CTLs, Th1 and NK cells (25, 37, 38), suggests the possibility of targeting PGE₂ in order to correct the balance between the effector and suppressive cells at the tumor sites.

Overall, our current data helps to understand the biology of MDSCs arising in the cancer setting, pointing to the causative role of a tumor-associated inflammatory mediator, PGE₂, in one of the key aspects of monocytic MDSC biology, i.e. their accumulation. They also provide rationale for including inhibitors of PGE₂ synthesis and function to counteract the CXCL12-mediated immune-mediated and non-immune mechanism of tumor progression in the therapy of cancer patients.

Acknowledgements

The authors thank Drs. Michael Shurin, Shabaana Khader, Greg Lesinski, Anda Vlad, Julie Urban, and Jeffrey Wong for their critically reading of the manuscript.

Grant support

This work was supported by grants from NIH (1PO1 CA132714; P.K.) and by a UICC American Cancer Society Beginning Investigators Fellowship funded by the ACS (to N.O.).
Conflict-of-interest disclosure

The authors declare no competing financial interests.

References


Figure legends

Figure 1. CXCR4-CXCL12-mediated accumulation of monocytic MDSCs. MDSC-associated phenotype and functions of OvCa ascites-isolated CD11b+ cells express. (A) Characterization of OvCa-isolated CD11b+CD14+CD15-CD33+CD34+HLADRlowCD80-CD83- cells. High percentage of CD11b+ cells (8.9-50.0%, mean 24.2%, N=7) was present within the ascites total primary cell population. (B) Relative expression of IL10, ARG1, IDO1, IL4Rα, CXCR4 and COX2, in control and OvCa-isolated CD11b+ cells. (N=7, OvCa ascites-isolated; N=5, blood-isolated, control). Histograms present data of a single representative experiment with different donors as mean ± s.d. (C) Suppression of CFSE-labeled allogeneic naïve CD8+ T cell proliferation (CD3/CD28 stimulation (39)) in the presence or absence of OvCa–infiltrating primary cells or OvCa-isolated monocytic MDSCs (N=7). Percentages indicate the fraction of proliferating CD8+ cells. The gray squares represent the lymphocyte-specific gates, used to exclude (CFSE-unlabelled) MDSCs. (D, top) Uniform expression of CXCR4+ on CD11b+ MDSCs from ovarian cancer ascites. (D, bottom) CXCR4 is elevated on cancer-isolated CD11b+ monocytic cells (filled histogram) compared to control blood-isolated CD11b+ monocytic cells (unfilled, thick line). (B) CXCR4 expression in ovarian cancer-isolated MDSCs (CD11b+CD33+). In contrast, OvCa-isolated monocytic MDSCs (CD11b+CD33+) lack the expression of CCR6 and CCR7 receptors. (B, left) Representative data from one of seven different cancer patients is shown. (B, right) Cumulative data from seven different cancer patients, expressed as mean ± s.d. *P<0.05; **P<0.01; ***P<0.001.
Figure 2. CXCL12-driven accumulation of cancer-associated monocytic MDSCs. (A) Correlation between CXCL12 levels and frequency of CD11b⁺CD33⁺ cells in ovarian cancer ascites from different patients. The percentage of CD11b⁺CD33⁺ cells in ovarian cancer ascites was determined by flow cytometry analysis after staining of ovarian cancer ascites-infiltrating primary cells, harvested from fresh ascites by centrifugation (N=5 patients). The regression line and corresponding r² value is shown. (B) CXCL12 expression (left; N=19; vs. N=5) and protein levels (right; N=17; 1509 pg/ml vs. N=8; 193 pg/ml) in ovarian cancer ascites from different patients compared to cancer patient sera. (C) CXCL12 vs. CCL5 levels in ovarian cancer ascites from 17 different patients. (D) Specific migration of ovarian cancer-isolated MDSCs to ascites in the absence or presence of CXCR4 antagonist AMD3100, CCR5 antagonist vicriviroc, or CXCL12 and CCL5 desensitized. (E) Responsiveness of ovarian cancer-isolated MDSCs to rhCXCL12 (50ng/ml) and ovarian cancer ascites. All data (panels A-E) were confirmed in 3-7 independent experiments. The bars represent cumulative data from 3-7 different donors, expressed as mean ± s.d. *P<0.05; **P<0.01; ***P<0.001.

Figure 3. COX2/PGE₂ correlate with accumulation of cancer-associated monocytic MDSCs. (A) Correlation between CD11b (denoting the numbers of MDSCs) and COX2 relative expressions in cells isolated from OvCa patients (N=24). The regression line and corresponding r² value is shown. (B) Correlation between PGE₂ levels and SDF-1/CXCL12 concentrations produced in cancer environment (N=7 patients). The regression line and corresponding r² value is shown.

Figure 4. COX2/PGE₂ feedback in the cancer environment is responsible for the induction of CXCL12-driven accumulation of cancer-associated monocytic MDSCs. (A) Relative expression of COX2, CXCR4 and CXCL12 in OvCa-infiltrating primary cells (white bars) and OvCa–
isolated CD11b⁺ cells (black bars), compared to control blood-isolated CD11b⁺ cells, pre-
treated or not with Celecoxib (n.d.: not detectable). (B) CXCL12 and PGE₂ protein levels in
OvCa-infiltrating primary cell-conditioned media, obtained in the presence or absence of the
COX2 inhibitor celecoxib. (C) Migratory responsiveness to OvCa ascites in OvCa-isolated
MDSCs pre-treated or not with COX2 inhibitor (Celecoxib, 20 μM). (D) Relative expression of
CXCR4 in OvCa–isolated CD11b⁺ cells, pre-treated or not with Celecoxib, EP4 antagonist
AH23848, EP2 antagonist AH6809 and EP3 antagonist L798106. All data (panels A-D) were
reproduced in 3-7 independent experiments, using the cells from different donors. The
individual bars represent cumulative data from all donors, expressed as mean ± s.d. *P<0.05;
**P<0.01; ***P<0.001.
Figure 1
Figure 2
Figure 3

A

N = 24
p = 0.0282
r² = 0.2006

B

N = 7
p < 0.0001
r² = 0.9654
Figure 4

A

CXCL2/SDF-1

OvCa asc. cells (-)
OvCa asc. cells + Celecoxib
OvCa MDSC (-)
OvCa MDSC + Celecoxib
cont. CD11b+ cells (-)
cont. CD11b+ cells + Celecoxib

CXCR4

COX2

B

3,000

2,000

1,500

1,000

20.0

conc. COX2inh (μM)

C

2,000

1,500

1,000

500

(-) COX2inh

D

(-)

AH23848 (EP4)
AH6809 (EP2)
Celecoxib
L798106 (EP3)

CXCR4 mRNA