Epithelial Cancer Cell Migration: A Role for Chemokine Receptors?

Chris J. Scotton, Julia L. Wilson, David Milliken, Gordon Stamp, and Frances R. Balkwill

Imperial Cancer Research Fund Translational Oncology Laboratory, Barts and the Royal London School of Medicine and Dentistry, London EC1M 6BQ, United Kingdom [C. J. S., J. L. W., D. M., F. R. B.], and Department of Histopathology, Division of Investigative Sciences, Imperial College School of Medicine, Hammersmith Hospital Campus, London W12 ONN, United Kingdom [G. S.]

ABSTRACT

We investigated the possibility that chemokine gradients influence migration of human ovarian epithelial tumor cells. Of 14 chemokine receptors investigated, only CXCR4 was expressed on ovarian cancer cells. CXCR4 mRNA localized to a subpopulation of tumor cells in ovarian cancer biopsies. Ovarian cancer cell lines and cells freshly isolated from ascites expressed CXCR4 protein. The CXCR4 ligand, CXCL12, was found in ascites from 63 patients. CXCL12 elicited intracellular calcium flux and directed migration and changes in integrin expression in ovarian cancer cells. CXCR4 may influence cell migration in the peritoneum, a major route for ovarian cancer spread, and could be a therapeutic target.

INTRODUCTION

Chemokines are small, secreted peptides that control the migration of leukocytes, especially during immune and inflammatory reactions (1, 2). They are divided into two major subfamilies, CC and CXC, based on the position of their NH2-terminal cysteine residues, and bind to G protein-coupled receptors, whose two major subfamilies are designated CCR and CXCR. Chemokines are also produced by most, if not all, cancers. There is strong evidence that they are major determinants of the macrophage and lymphocyte infiltrate found in melanomas; in carcinomas of the ovary, breast, and cervix; and in sarcomas and gliomas (3–5). However, chemokines may play other roles in cancer. Some potenti available factors, whereas others can be angiostatic (1, 6). Alterations in the balance between these chemokines may contribute to the development of the tumor vasculature.

Using human ovarian cancer as an example, we have investigated another role for chemokines in cancer: the possibility that the malignant cells in epithelial tumors may use chemokine gradients as part of the process of metastatic spread. Only 1 of 14 chemokine receptors investigated was expressed on the ovarian tumor cells. We present evidence that interaction of this chemokine receptor, CXCR4, with its ligand, CXCL12, may influence the spread of epithelial ovarian cancer. This observation has implications for the development of new biological therapies for cancer.

MATERIALS AND METHODS

Samples. Human ovarian tumor biopsies were frozen in liquid nitrogen. Sections for in situ hybridization were mounted on glass slides coated with 3-aminopropyl-triethoxy-silane, air-dried, and stored at -70°C. Red cells were removed from ascites with ACK buffer (0.15 M NaCl, 10 mM KHCO3, 0.1 mM Na2EDTA, 1 mM MgCl2, and 10 mM CaCl2).

Cell Lines. Ovarian cancer cell lines PEO1 and PEO14 (from S. Langdon, Imperial Cancer Research Fund Oncology Unit, Edinburgh, United Kingdom), OVCAR-3 (purchased from American Type Culture Collection), and IGROV (from J. Bénard) were grown in RPMI 1640 supplemented with 10% FCS and 10 μg/ml insulin (for PEO1 and PEO14); SKOV-3 and CAOV-3 (purchased from American Type Culture Collection) were grown in DMEM supplemented with 10% FCS. Cell lines were cultured in pyrogen-free conditions. To allow recovery of cells after trypsinization, cells were cultured as single cell suspensions in Teflon-coated pots (Tuf Tainer, Perbio Science UK Limited) at 37°C and then washed. Fluorescence was measured in a PTI fluorometer (Perbio Science UK Limited), adsorbed to filter paper, and dried under vacuum. Autoradiography was performed using Kodak Biomax MS film with a Transcreen LE intensifying screen (Sigma Chemical Co.).

In Situ Hybridization. Antisense and sense riboprobes labeled with α-35S-labeled UTP were generated from 1100-bp fragments of CXCR4 cDNA cloned in pcDNA3 (Stratagene, Cambridge, United Kingdom) using Sp6 and T7 RNA polymerases (Promega Ltd., Southampton, United Kingdom). The cDNA was a gift from Antonio Sica (Mario Negri Institute, Milan, Italy). Antisense β-actin was used as a positive control in all experiments. In situ hybridization was carried out using the method described in Ref. 5. Image capture was performed using Image Grabber PCI (Neotec Ltd., London, United Kingdom).

mAbs and Flow Cytometry. PE-labeled mAbs against CXCR4 (12G5; R&D Systems, United Kingdom), FITC-labeled Her2/Neu (Neu 24.7; BD PharMingen), and isotype-matched labeled controls were used. Unconjugated β-actin was used as a positive control in all experiments. In situ hybridization was performed using Cellquest software (BD PharMingen).

Calcium Flux. Five × 103 cells/ml were incubated with 5 μM Fluo-3 (Molecular Probes, Cambridge Biosciences, Cambridge, United Kingdom) in HBSS plus 0.5% BSA, 1 mM CaCl2, 1 mM MgCl2, and 10 μM ZnCl2 for 30 min at 37°C and then washed. Fluorescence was measured in a P1T1 fluorometer (excitation wavelength, 485 nm; emission wavelength, 530 nm).

Migration. Chemotaxis was assayed using Falcon Transwells (24-well format, 8-μm pore; BD PharMingen). Media (0.5 ml) containing 5 × 105 cells were added to the upper chamber, and 0.5 ml of medium alone or media supplemented with CXCL12 were added to the lower chamber. After overnight inoculation at 37°C and 5% CO2, cells on the upper surface of the filter were removed using a cotton wool swab. Migrated cells on the lower surface were stained using DiffQuik (Dade Behring, Düdingen, Switzerland). For each transwell, the number of migrated cells in 10 medium-power fields (∼20) was counted.

ELISA for CXCL12 in Ascitic Fluid. Concentration of the chemokine CXCL12 (SDF-1α) in ascitic fluid was measured using Quantikine ELISA kits (R&D Systems, Abingdon, United Kingdom) according to the manufacturer’s protocol. The sensitivity of the assay was 18 pg/ml.

Received 3/15/01; accepted 5/9/01.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom requests for reprints should be addressed, at Imperial Cancer Research Fund Translational Oncology Laboratory, Barts and the Royal London School of Medicine and Dentistry, Charterhouse Square, London, EC1M 6BQ, United Kingdom. Phone: 44-0-20 7882-6106; Fax: 44-0-20 7882-6110; E-mail: f.balkwill@icrf.icnet.uk.

2 The abbreviations used are: RPA, RNase protection assay; mAb, monoclonal antibody; PE, phycoerythrin.
Chemokine receptor expression by RPA. The cells did not express CCR1, CCR2a, CCR2b, CCR3, CCR4, CCR5, CCR7, CCR8, CXCR1, CXCR2, CXCR3, CXCR5, or CXCER1. Two of the six cell lines (IGROV and CAOV-3) strongly expressed CXCR4 mRNA (Fig. 1A). This chemokine receptor was also expressed in biopsies from eight of 10 primary ovarian tumors and 19 of 20 samples of ovarian cancer ascites (Fig. 1C).

CXCR4 Localizes to Tumor and Stromal Cells in Tumor Biopsies. In situ hybridization localized CXCR4 mRNA to a proportion of neoplastic cells in 10 of 10 ovarian cancer biopsies studied (Fig. 2, A–C). Expression of CXCR4 mRNA was not uniform throughout the tumor. In five of the biopsies, between 5% and 20% of tumor cells were labeled, and in the other five biopsies, 1–5% of tumor cells were positive for CXCR4. There was no correlation between CXCR4 positivity and areas of necrosis or hot spots of angiogenesis. CXCR4 mRNA was also detected in mononuclear and endothelial cells. Tumors with a strong lymphoid infiltrate in the stroma showed highest mononuclear cell labeling for CXCR4 (Fig. 3, A and B). The presence and distribution of tumor-infiltrating lymphocytes were assessed by H&E staining and CD8 immunostaining, and the CXCR4 labeling pattern was clearly in excess of these populations in every sample. CD8 cells also showed a different distribution from CXCR4-labeled tumor cells. In addition, it was possible to distinguish between neoplastic and tumor-infiltrating lymphocyte cell nuclei. Some endothelial cells within the tumor were also labeled in each biopsy. A proportion of the tumor cells was positive at any one time. Alternatively, some cells may have acquired constitutive CXCR4 expression, or within stromal invaginations (Fig. 3, C and D). Endothelial cells in tissue adjacent to the tumors were positive for CXCR4.

CXCR4 Protein Can Be Detected on Ovarian Cancer Cells. As shown in Fig. 4A, CXCR4 protein could be detected on IGROV and CAOV-3 cells using flow cytometry. Two-color flow cytometric analysis of cells freshly isolated from ovarian cancer ascites revealed CXCR4 surface expression on cells that were also positive for the tumor marker HER2/neu (Fig. 4B).

The CXCR4 Receptor Is Functional on Ovarian Cancer Cells. CXCL12 (100 ng/ml) elicited an intracellular calcium flux in both IGROV and CAOV-3 cells (Fig. 4C). No release of intracellular calcium was detectable when cells were incubated with 10 ng/ml CXCL12. Both IGROV and CAOV-3 cells demonstrated chemotaxis toward CXCL12 (Fig. 4D).

Binding of CXCL12 to its receptor caused internalization of CXCR4 on IGROV cells after approximately 15 min, as determined by flow cytometry (Fig. 5A). The receptor was recycled to the cell surface within 30 min. CXCL12 induced significant migration of IGROV and CAOV-3 cells at concentrations of 100 ng/ml (IGROV, $P = 0.0001$; CAOV-3, $P = 0.0002$) and 300 ng/ml (IGROV, $P = 0.0001$; CAOV-3, $P = 0.0003$). Stimulation of CAOV-3 and IGROV cells with CXCL12 at 100 ng/ml for 24 h increased cell surface expression of the β1 integrin (Fig. 5B).

Ascitic Fluid from Ovarian Cancer Contains High Levels of CXCL12. CXCL12 levels were assayed by ELISA in 63 samples of ascitic fluid from patients with ovarian cancer. High concentrations of this chemokine (range, 613-9333 pg/ml; median, 6021 pg/ml) were detected in all of the samples.

**DISCUSSION**

We provide evidence that chemokine gradients may influence routes of ovarian tumor cell migration. Response to chemokine gradients may be an important component of the multistep process of metastasis of epithelial tumors. Binding of ligand to the only chemokine receptor that was reproducibly expressed by human ovarian cancer cells (CXCR4) induces actions that would permit a role in tumor cell migration.

Only a proportion (between 1% and 20%) of the tumor cells in the ovarian cancer biopsies expressed CXCR4 mRNA. Receptor levels could be regulated by cytokines in the tumor microenvironment. For example, in T cells, CXCR4 can be up-regulated by interleukin 4 (7). We have preliminary evidence that ovarian cancer cell CXCR4 protein is up-regulated by transforming growth factor β, a cytokine that is found in ovarian cancer, but down-regulated by IFN-γ, which is absent from this tumor microenvironment (8). This could explain why a proportion of the tumor cells was positive at any one time. Alternatively, some cells may have acquired constitutive CXCR4 expression during malignant progression. In contrast, all of the HER2/neu-positive tumor cells that had spread into ascites were CXCR4 positive.

The ability of the ovarian cancer cell lines to elicit an intracellular calcium flux and migrate in response to CXCL12 indicates the functionality of the CXCR4 cell surface receptors. It is possible that the high levels of CXCL12 found in ascites could create a chemokine gradient for migration of tumor cells into the peritoneum. Treatment of the ovarian cancer cell lines with CXCL12 significantly increased cell surface expression of the β1 integrin, which may affect peritoneal adhesion of cells. The significance of this remains to be determined.

---

**Fig. 1.** Chemokine receptor mRNA expression in ovarian cancer. mRNA expression was measured by RPA. GAPDH and L32 are housekeeping genes. A and B, chemokine receptor expression by ovarian cancer cell lines. C, chemokine receptor expression in primary solid tumor biopsies and cells isolated from ovarian cancer ascites.
However, previous studies have shown that CXCL12 induced adhesion of most circulating lymphocytes and CD34+ progenitor cells (9). Analysis of mRNA by RPA showed expression of other chemokine receptors in the tumor and ascites samples, which are a mixture of tumor cells, leukocytes, and connective tissue cells. In solid tumors we also detected expression of the CC chemokine receptor CCR1, but this localized to infiltrating leukocytes. More chemokine receptor mRNAs were expressed in cells from ascites, but apart from CXCR4,

Fig. 2. CXCR4 mRNA expression by epithelial tumor cells in biopsies of human ovarian cancer. A, variable degree of labeling of two tumor epithelial cell groups in a serous carcinoma separated by a cellular stroma that is largely unlabeled. B, grade 3 serous carcinoma with heterogeneous labeling of tumor epithelial cells and focal weak labeling of some cells in the stroma. C, grade 1 serous carcinoma with neoplastic epithelial cells clearly labeled for CXCR4.

Fig. 3. CXCR4 mRNA expression by tumor and stromal cells in biopsies of human ovarian cancer. A, H&E-stained section of grade 3 serous carcinoma with stromal lymphoid infiltrate. B, same serous carcinomas in A with CXCR4 labeling of tumor epithelial cells (left of field). The lymphoid cells within the stroma (right of field) show a denser pattern of label. C, endothelial cells in the papillary core of a grade 1 serous carcinoma (stained with H&E) are clearly labeled for CXCR4. D, there is focal weak labeling of occasional tumor cells. E, stromal endothelial cell labeling in a grade 2 serous carcinoma with equivocal epithelial cell labeling. F, tumor epithelial cells show a low index of CXCR4 labeling together with a linear pattern of CXCR4-labeled endothelial cells in the adjacent stroma.
Chemokines and Cancer Cell Migration

There is increasing evidence that the inflammatory cells, cytokines, and chemokines found in human tumors are more likely to contribute to tumor growth, progression, and immunosuppression than they are to mount an effective antitumor response (reviewed in Ref. 13). Over the past 10 years, study of the cytokine and chemokine network has led to the development of a range of antagonists for the treatment of inflammation and allergy. We suggest that such agents may also be of benefit in the treatment of malignant disease. The chemokine receptor CXCR4, which is now known to be functional on both breast and ovarian cancer cells, is of particular interest. It is the coreceptor for the macrophage trophic HIV virus (14), and CXCR4 antagonists are currently in Phase I clinical trial for the treatment of HIV/AIDS. In view of the expression of this receptor on both tumor and endothelial cells in human ovarian cancers, there is a rationale for the use of CXCR4 antagonists as part of a biological approach to the treatment of ovarian cancer. IGROV cells express functional CXCR4. These cells form i.p. tumors in nude mice and may provide a useful preclinical model to study the action of CXCR4 antagonists in tumor growth and spread (15).

ACKNOWLEDGMENTS

We thank Sue Hoare for performing the CXCL12 ELISA analysis and surgeons at the Department of Obstetrics and Gynecology, Guy’s and St Thomas’ Hospital for providing patient material.

REFERENCES


Epithelial Cancer Cell Migration: A Role for Chemokine Receptors?

Chris J. Scotton, Julia L. Wilson, David Milliken, et al.


Updated version  Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/61/13/4961

Cited articles  This article cites 15 articles, 7 of which you can access for free at:
http://cancerres.aacrjournals.org/content/61/13/4961.full.html#ref-list-1

Citing articles  This article has been cited by 51 HighWire-hosted articles. Access the articles at:
/content/61/13/4961.full.html#related-urls

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.