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CXCR4/SDF-1α-mediated Chemotaxis in an *In Vivo* Model of Metastatic Esophageal Carcinoma

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Abstract. *Background/Aim: The chemokine receptor CXCR4 and its ligand (stromal cell-derived factor-1alpha; SDF-1α) play an important role in tumor cell chemotaxis and metastatic homing of esophageal carcinoma. Several methods are available to examine tumor cell migration in vitro. However, in vivo chemotaxis is subject to complex tumor-host interactions. The aim of this study was to establish an in vivo model of chemotaxis for esophageal carcinoma that allows the examination of tumor cell migration and metastatic homing in the complex microenvironment. Materials and Methods: CXCR4 expression of OE19 adenocarcinoma cells was determined by immunostaining in an orthotopic esophageal model. SDF-1α-mediated migration of cells was examined in vitro. An in vivo model of chemotaxis and metastasis was established by subcutaneous injection of OE19 cells into NMRI/nu mice and by daily stimulation with SDF-1α. Results: CXCR4 is expressed in the primary tumor and in the metastatic tissue. CXCR4-positive OE19 cells are susceptible to SDF-1α-mediated migration. The novel in vivo model leads to developement of metastases in liver, lung, peritoneum and retroperitoneum after stimulation with SDF-1α but not with PBS, and revealed an SDF-1α dose-dependent migratory effect. Conclusion: As metastasis is still the leading cause of tumor-related death, it is essential to investigate the complex tumor-host interactions involved in metastatic homing. We established an in vivo model of chemotaxis and metastasis for esophageal carcinoma, which allows investigation and inhibition of CXCR4/SDF-1α-mediated cell survival and proliferation, chemotaxis and homing, adhesion, and tumor angiogenesis.*

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Interactions between CXC chemokine receptor 4 (CXCR4) and stromal cell-derived factor-1alpha (SDF-1α) have been shown to play key roles in cancer cell survival, proliferation, chemotaxis, homing, adhesion, tumor angiogenesis, and resistance to conventional and targeted therapies (1). Given its extensive involvement in cancer progression, the CXCR4-SDF-1α axis has been considered to be a therapeutic target. Several inhibitors that block this signalling cascade are undergoing clinical trials (1). CXCR4, the G-protein-coupled receptor of CXCL12/SDF-1α, mediates a wide range of physiological and pathological processes, including the targeted metastasis of cancer cells. Chemokines such as SDF-1α are released in high amounts by certain organs such as lung, bone and liver (Figure 1A). The attraction of SDF-1α and CXCR4 causes breast cancer cells to migrate into other organs, where cancer cells proliferate and form metastastic tumors.

CXCR4 has also been suggested to play an essential role in tumor cell homing of esophageal carcinoma to lymph nodes and bone marrow (2), as well as of other tumor entities (3, 4). Expression of CXCR4 correlates significantly with overall and tumor-specific survival in esophageal carcinoma and is associated with a poor prognosis (2). Esophageal cancer is a highly aggressive neoplasm with a poor prognosis and a strong tendency for invasion and metastasis (5), and is one of the leading causes of cancer-related deaths world-wide (6, 7). In spite of multimodal therapy (surgery/combined radio/chemotherapy), curative treatment is rare, especially in advanced stages with lymph node or distant organ metastasis, where surgical resection is not an option (8). The key to developing novel treatment strategies is to understand the pathways of metastasis. Although the exact biological functions of CXCR4 in tumor dissemination of esophageal carcinoma are still poorly understood, inhibition of esophageal cancer metastasis by CXCR4 antagonists is an interesting therapeutic option.

Several *in vitro* assays and animal models have been established to facilitate examination of metastatic processes such as tumor cell migration and homing. We previously established an orthotopic esophageal carcinoma model (9-11). The orthotopic model exhibits the patterns of clinical local and metastatic behavior occurring in human esophageal carcinoma. There is also a model for investigating peritoneal carcinomatosis of esophageal carcinoma *in vivo* (12).

Several *in vitro* assays exist to investigate migration *in vitro*. Most popular are net migration assays of large cell populations, such as the filter assays (Figure 1B). They are useful for identifying chemoattractant molecules, but give no direct information about how these molecules influence the speed and direction of cell movement (13). There are also visual assays including measurements of orientation in gradients and time-lapse filming and polarization assays. Assays that simulate the situation in living tissues are becoming more popular and include migration through collagen, and fibrin gels or through monolayers of vascular endothelium (13). However, the *in vivo* environment is far more complex in comparison to conventional cell assay chambers (14).

The aim of this study was to establish an *in vivo* chemotaxis model for esophageal carcinoma that allows the examination of metastatic tumor cell migration and homing in the complex *in vivo* microenvironment.

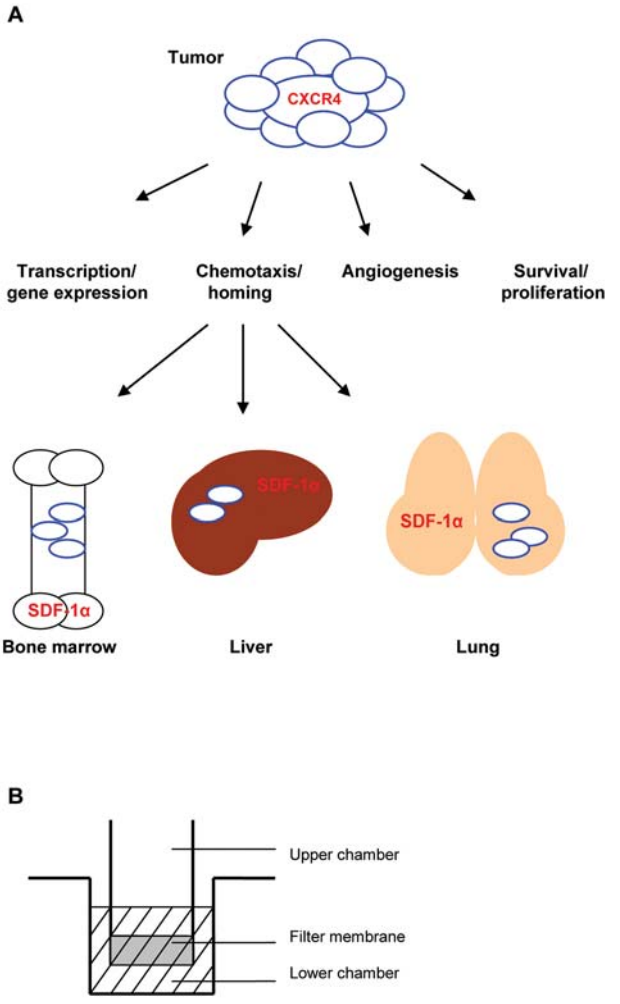

Figure 1. *A: In vivo migration of tumor cells. Tumor cell chemotaxis and metastatic homing of CXC chemokine receptor 4 (CXCR4)-positive tumor cells is mediated by stromal-cell derived factor-1alpha (SDF-1α) expression in destination organs e.g. bone marrow, liver and lung. B: In vitro chemotaxis: Boyden chamber principle. A cell suspension is placed in the upper chamber, while the medium containing the chemoattractant is placed in the lower chamber. Migratory cells that are attracted by the specific chemoattractant pass through the filter membrane.*

Materials and Methods

In vitro chemotaxis assay. The human cell line OE19 (European Collection of Cell Cultures (ECACC), Health Protection Agency, Wiltshire, UK) was cultured in RPMI 1560 medium (Biochrome KG, Berlin, Germany) as previously described (9, 12). Tumor cell migration through a microporous membrane was assessed based on the Boyden chamber principle. Cells were incubated with culture medium as the control for 90 min, and then plated onto the top chamber. Culture medium containing 100, 500 and 1000 ng/ml of recombinant human SDF-1α (R&D Systems, Minneapolis, MN, USA) was added to the lower chamber. The plate was incubated at 37˚C, with 5% CO_2 for 18 h. The migrated cells were then stained using 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI; Sigma-Aldrich, Munich, Germany) and were counted under a fluorescence microscope (Carl Zeiss, Jena, Germany).

In vivo chemotaxis model and evaluation of metastases. An orthotopic implantation model was established using the OE19 cell line, as previously described (9, 12, 15). All animal procedures were performed in accordance with a protocol approved by the Behörde für Wissenschaft und Gesundheit (Freie und Hansestadt Hamburg, Germany). NMRI/nu (U.S. Naval Medical Research Institute) mice were obtained from Charles River Deutschland (Sulzfeld, Germany) at 10 weeks of age. Eighteen NMRI/nu mice were injected subcutaneously 7×10^6 with OE19 cells in 200 μl PBS suspension. Starting the following day mice received a daily intraperitoneal injection of either Dulbecco's phosphate-buffered saline (DPBS; Gibco/ Invitrogen, Paisley, UK) (group 1), 100 ng SDF-1α (group 2) or 500 ng SDF-1α (group 3) for four weeks. At the end of this course mice were sacrificed and liver, lung, peritoneum and retroperitoneum were sampled. Total RNA was isolated from the samples by an RNA isolation kit (Qiagen, Hilden, Germany) and were reverse transcripted by the high capacity cDNA reverse transcritpion kit (Applied Biosystems, Darmstadt, Germany). Metastatic spread to the organs was detected by mRNA expression of the human polymerase (RNA) II polypeptide 2 *POL2* by real-time polymerase chain reaction (PCR) analysis. Results were normalised using 18S RNA expression of the tissue samples. PCR primers (TaqMan Gene Expression Assay-POL2 (made to order)), TaqMan Gene Expression Assay 18S Hs99999901_s1) and TaqMan Universal PCR Mastermix were ordered from Applied Biosystems. Expression in micrometastases is presented as cycle threshhold (ct) values.

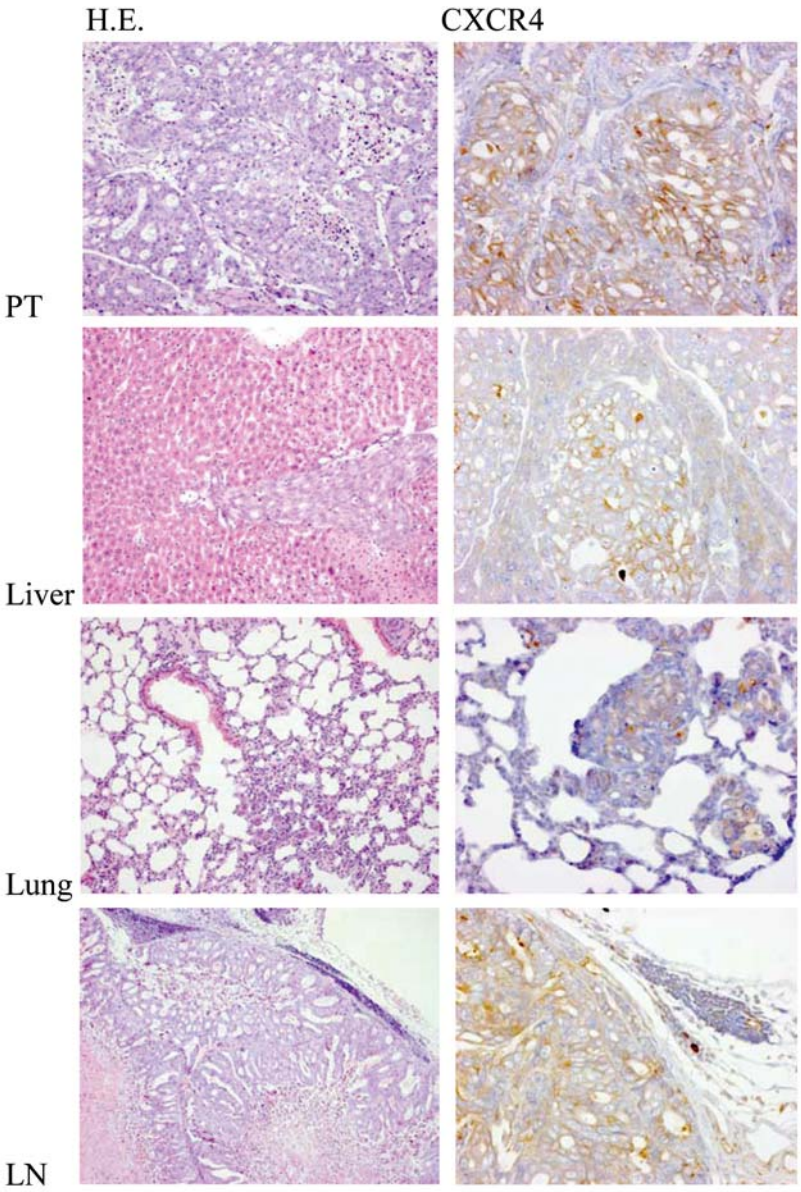

Figure 2. *Metastatic potential and CXCR4 expression of OE19 cells. Representative images of hematoxylin eosin (H.E.) and CXC chemokine receptor 4 (CXCR4) immunostaining of primary tumor (PT), liver, lung and lymph node (LN) tissue from an animal with orthotopically implanted esophageal tumor (magnification ×100). CXCR4 expression is observed in all tumor-bearing tissues.*

Immunostaining of metastases in an orthotopic model of esophageal carcinoma. The CXCR4 staining was performed using the primary anti-human CXCR4 monoclonal antibody [immunoglobulin G subclass 2a (IgG2a), clone 12G5; R&D Systems] at a dilution of 1:100, overnight at 4˚C. An irrelevant murine IgG1 monoclonal antibody (MOPC21; Sigma, Deisenhofen, Germany) was used as a negative control. The antibody reaction was developed with the Cell and Tissue Staining Kit, using the HRP-AEC-System, from R&D Systems (Minneapolis, MN, USA). Sections were counterstained with Mayer's hematoxylin solution (Merck, Darmstadt, Germany). Tumor tissue was identified by hematoxylin eosin (HE) staining.

Results

CXCR4 expression in primary tumor and metastases, SDF-1α-dependent migration. The OE19 cell line has been previously used in an orthotopic implantation model of esophageal carcinoma (9). High metastatic rates of up to 100% to liver, lung and lymph nodes were found in this model. As CXCR4 expression has been shown to affect metastatic homing of esophageal carcinoma cells to the lymph node and bone marrow (2), we now evaluated CXCR4

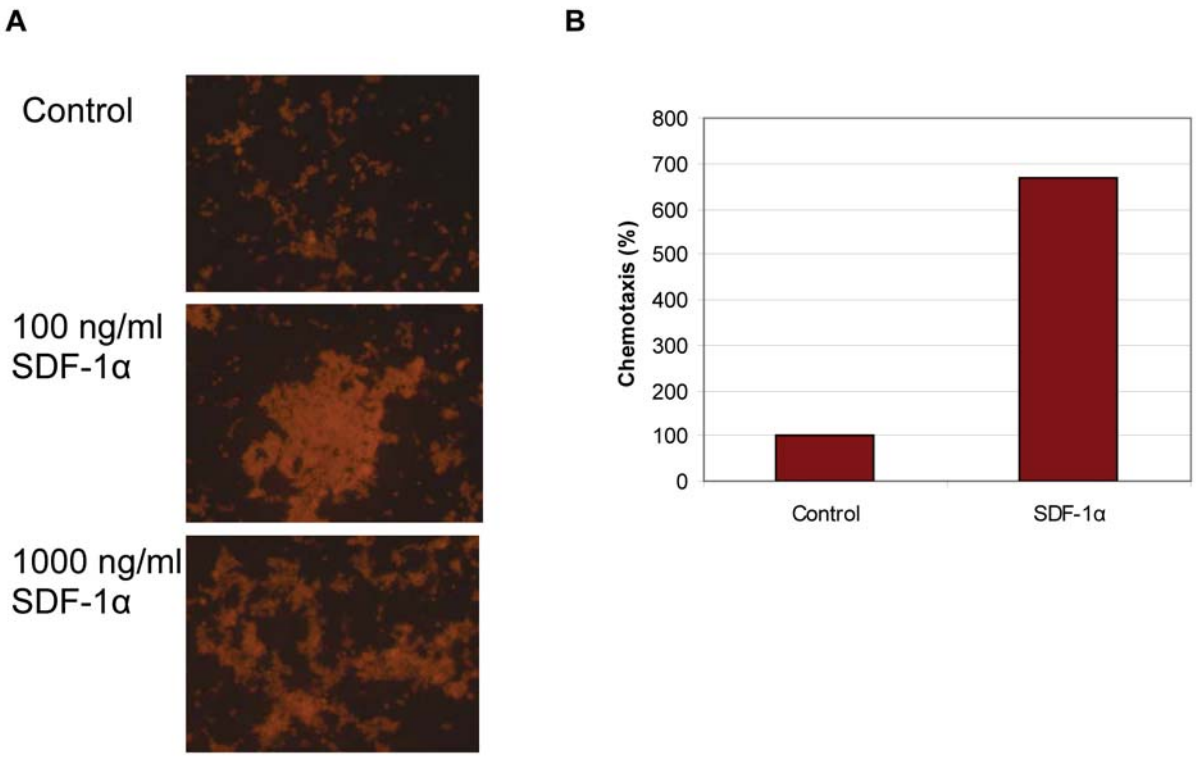

Figure 3. *Tumor cell migration in Boyden Chamber. A: Microscopic evaluation of the Boyden chamber-based migration assay shows a dose-dependent effect of stromal-cell derived factor-1alpha (SDF-1α)-stimulated chemotaxis on OE19 cells. B: A relevant migratory effect of SDF-1α-mediated migration on CXCR4-positive cells is observed at 250 ng/ml compared to unstimulated cells.*

expression in the primary tumor, lung, liver and lymph node metastases of this model by immunostaining. The cell line, the primary tumors and the metastases in lung, liver and lymph nodes exhibited overexpression of CXCR4 (Figure 2).

In order to achieve cell migration, CXCR4-positive cells need to respond to the stimulus of their ligand and chemoattractant SDF-1α. To verify whether the migration of OE19 cells is dependent on SDF-1α, firstly, *in vitro* studies were conducted. The migration assay based on the Boyden chamber principle showed a SDF-1α dose-dependent effect on cell migration of OE19 cells (Figure 3A). Relevant chemotaxis of OE19 cells was, thus, induced by SDF-1α (Figure 3B).

SDF-1α-mediated in vivo chemotaxis and model of esophageal carcinoma metastasis. In vitro migration assays are highly experimental and controlled assays without the effects of the surrounding host environment. To further investigate the migratory behaviour of metastatic cells *in vivo* a novel model was established, that provides the influences of tumor-host interaction on migration (Figure 4A). OE19 cells were injected into the flanks of NMRI/nu mice that were then randomized into three groups and treated for the course of four weeks with DPBS and SDF-1α injections of 100 ng/ml and 500 ng/ml, respectively.

At the termination of this *in vivo* experiment, relevant tissues (lung, liver, peritoneum, and retroperitoneum) were sampled and examined for metastatic spread by mRNA expression of *POL2,* by real-time PCR analysis which was normalized by *18S* expression. For each analysis, intraperitoneal tumor tissue was induced by intraperitoneal injection into a control mouse and was used as positive control. Cycle threshhold (ct) values were used for graphic depiction of results. Figure 4B shows relevant dose-dependent effects of SDF-1α injection. Especially in lung and the peritoneum, higher levels of human, thus metastatic, cells were found after daily injection of 500 ng/ml SDF-1α compared to mice injected with DPBS, and tumor tissue controls.

With this novel model, using the subcutaneous compartment as origin and the internal organs as destination compartments, we were able to show the SDF-1α-mediated migratory effect of CXCR4-positive OE19 cells. Moreover, we specifically stimulated metastatic homing to liver, lung, peritoneum and retroperitoneal tissue, thus for the first time establishing an *in vivo* model of chemotaxis as well as a model of SDF-1α-based metastasis for esophageal carcinoma.

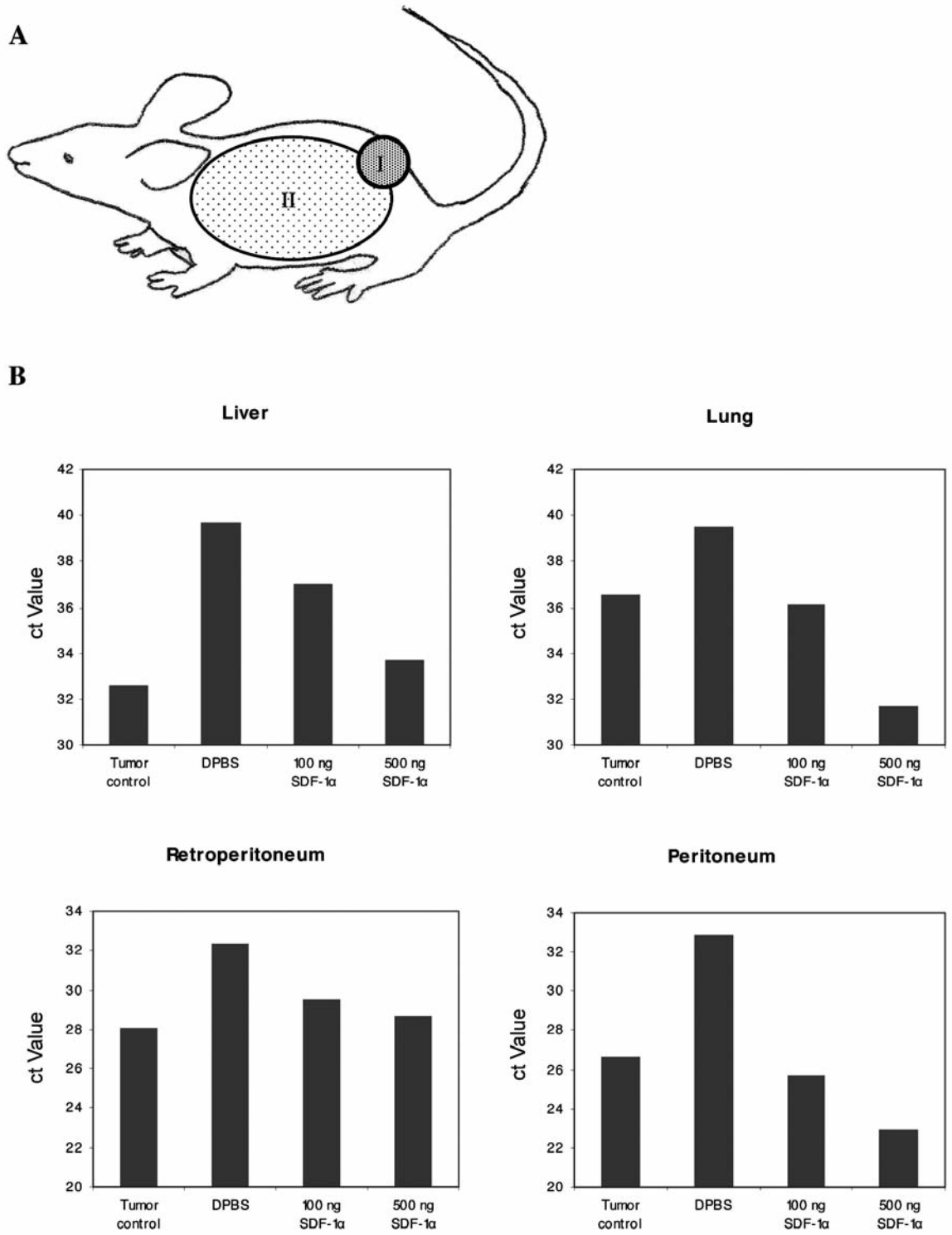

Figure 4. *Cell migration in vivo. A: Compartment-mimicking principle of an in vivo model of chemotaxis. Tumor cells are injected into the flank of the animal (I, compartment or origin). Metastases are monitored in the abdominal and thoracic tissues (II, destination compartments: lung, liver, peritoneum, retroperitoneum) after intraperitoneal stimulation. B: Metastatic spread to liver, lung, peritoneum and retroperitoneal tissue is analyzed by real-time polymerase chain reaction (PCR), according to the level of human polymerase (RNA) II polypeptide 2 (POL2). Means of cycle threshhold (ct) values were used for graphic depiction of results. There is a relevant effect of stromal-cell derived factor-1alpha (SDF-1α) stimulation (100 and 500 ng) on all organs compared to the sham-treated group (DPBS). Detection of human, and thus metastatic, cells was even greater in lung and peritoneum in the group treated with 500 ng SDF-1α, compared to the positive tumor control, indicating a strong metastatic effect.*

Discussion

Treatment of esophageal carcinoma is still limited due to the disease stage at initial diagnosis and surgical resectability. Surgical resectability of esophageal carcinoma is most often limited not only by a local tumor spread, but also by distant metastasis to parenchymatous organs, or peritoneal spread (12). Metastasis is still the leading cause of tumor-related death and morbidity. Therefore, it is essential to understand the complex pathophysiological pathways and processes leading to metastatic spread (12). These pathways of malignant tumor disease are complex and still poorly understood (16-20). At present there is no effective radio/chemotherapeutic or biological treatment for metastatic esophageal cancer.

It has been shown that the chemokine receptor CXCR4 is implicated in metastatic homing of esophageal carcinoma to lymph nodes and bone marrow (2). In the present study, we showed that CXCR4 is expressed in metastatic tissues of primary CXCR4-positive tumor cells in the orthotopic esophageal model. This indicates the importance of this receptor also in the metastatic homing to lung, liver and lymph nodes in esophageal carcinoma. CXCR4-mediated metastatic spread is dependent on its ligand, the chemokine SDF-1α. Here we show that the esophageal adenocarcinoma OE19 cells are susceptible to dose-dependent SDF-1α-mediated migration *in vitro*. Although the *in vitro* assay results show that SDF-1α is a strong chemoattractant for CXCR4-positive cells, these assays give a limited view of cell migration. *In vivo* tumor cell migration is dependent on several factors influencing the complex tumor-host interaction. Elucidation of these mechanisms is important for further understanding of metastatic homing. In this study, we developed a model of *in vivo* chemotaxis for esophageal carcinoma that allows the examination of metastatic tumor cell migration and homing in the complex *in vivo* microenvironment.

CXCR4 and SDF-1α play key roles in cancer cell survival, proliferation, chemotaxis, homing to specific metastatic sites, adhesion, tumor angiogenesis and resistance to conventional and targeted therapies (1, 21, 22). There is experimental evidence that the higher-order multimerization of CXCR4 enables it to effectively sense, with increased avidity, the chemotaxis-inducing ligand in the microenvironment (23).

An involvement of the CXCR4/SDF-1α-mediated migration has been shown for several physiological and pathological processes, including brain injury/tumor (24), spinal cord neural development (25), colorectal cancer (26), lung metastasis in breast cancer (22, 27), lung cancer (28-30), prostate cancer metastasis (31), ovarian cancer (27), cervical cancer (27), non-Hodgkins lymphoma (32), and acute myelogenous leukemia (33).

In esophageal carcinoma, CXCR4 has been shown to play an essential role in tumor cell homing to lymph nodes and bone marrow (2). Expression of CXCR4 significantly correlates with overall and tumor-specific survival in esophageal adenocarcinoma and squamous cell carcinoma, and is associated with a poor prognosis (2). Thus, the expression of CXCR4 is of major relevance for both histological entities, squamous cell carcinoma and adenocarcinoma of the esophagus (34). There are several studies on squamous cell carcinoma to support this claime (35-38).

Given its extensive involvement in cancer progression, the CXCR4-SDF-1α axis has been considered to be a therapeutic target. Several inhibitors blocking its signaling cascade are in clinical trials (1). Preclinical and clinical anti-CXCR4 agents (*e.g.* AMD3100, NOX-A12, CCX2066, CTCE9908) are currently available therapeutic options, targeting the CXCR4-SDF-1α and CXCR7-SDF-1α pathways (39).

Activation of the CXCR4-SDF1-α pathway leads to the following complementary actions: i) direct promotion of cancer cell survival, invasion, and the cancer stem and/or tumor-initiating cell phenotype; ii) recruiting of distal stroma (i.e. bone marrow-derived cells) to indirectly facilitate tumor recurrence and metastasis; and iii) promotion of angiogenesis directly or in a paracrine manner (33, 39).

To investigate tumor cell migration *in vitro,* several methods exist. *In vitro* migration assays, implicating either gradients or cell surface-bound forms of SDF-1α, are easy to perform and provide vital information regarding cell motility (40). Net migration, such as the filter assay is the most popular and is useful for identifying chemoattractant molecules, but give no direct information about how these molecules influence the speed and direction of cell movement (13). Visual assays, including measurements of orientation in gradients and time-lapse filming give detailed information about cell paths, and the polarization assay is useful for visual screening. Assays that are closer to simulating the situation in living tissues include migration through collagen or fibrin gels or through monolayers of vascular endothelium (13). Sharp growth factor gradients can be used for local activation of cell surface receptors and are suitable for rapid biological assays such as those of Ca(2+) transients (41). Novel chemotaxis techniques include microfluidic platforms, computer controlled flow devices and cell tracking software (14). Assays under fluid flow which using biochips have provided data that highlight the importance of shear stress on cell attachment and migration towards a chemokine gradient.

However, the *in vivo* environment is far more complex in comparison to conventional cell assay chambers (14). For example, there is evidence that the dependence of breast cancer cells on growth factor signaling, points to the importance of autocrine and paracrine factors in determining the migratory response of the cells using a microfluidic chemotaxis chamber (42). There is also evidence that stromal

fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1α secretion (43). This was determined by using a co-implantation tumor xenograft model that demonstrated that carcinoma-associated fibroblasts extracted from human breast carcinomas promote, in large part through the secretion of SDF-1α, the growth of breast carcinoma cells significantly more than normal mammary fibroblasts derived from the same patients (43).

Therefore, it is essential to have methods that allow the investigation of the complex tumor/host interaction that leads to metastatic homing. In this study we developed a novel *in vivo* model, which represents an experimental model of metastasis based on SDF-1α-mediated chemotaxis for esophageal carcinoma. This *in vivo* model allows for the investigation of CXCR4-SDF-1α-mediated cancer cell survival, proliferation, chemotaxis, homing, adhesion, and tumor angiogenesis, as well as exploration of novel biological and targeted therapeutics.

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References

1 Woodard LE and Nimmagadda S: CXCR4-based imaging agents. J Nucl Med *52*: 1665-1669, 2011.
2 Kaifi JT, Yekebas EF, Schurr P, Obonyo D, Wachowiak R, Busch P, Heinecke A, Pantel K and Izbicki JR: Tumor-cell homing to lymph nodes and bone marrow and CXCR4 expression in esophageal cancer. J Natl Cancer Inst *97*: 1840-1847, 2005.
3 Liotta LA: An attractive force in metastasis. Nature *410*: 24-25, 2001.
4 Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verastegui E and Zlotnik A: Involvement of chemokine receptors in breast cancer metastasis. Nature *410*: 50-56, 2001.
5 Lu CL, Ji Y, Ge D, Guo J and Ding JY: The expression of CXCR4 and its relationship with matrix metalloproteinase-9/vascular endothelial growth factor in esophageal squamous cell cancer. Dis Esophagus *24*: 283-290, 2011.
6 Malthaner R, Wong RK and Spithoff K: Preoperative or postoperative therapy for resectable oesophageal cancer: an updated practice guideline. Clin Oncol (R Coll Radiol) *22*: 250-256, 2010.
7 Wong R and Malthaner R: Esophageal cancer: A systematic review. Curr Probl Cancer *24*: 297-373, 2000.
8 Izbicki JR, Hosch SB, Pichlmeier U, Rehders A, Busch C, Niendorf A, Passlick B, Broelsch CE and Pantel K: Prognostic value of immunohistochemically identifiable tumor cells in lymph nodes of patients with completely resected esophageal cancer. N Engl J Med *337*: 1188-1194, 1997.
9 Gros SJ, Kurschat N, Dohrmann T, Reichelt U, Dancau AM, Peldschus K, Adam G, Hoffman RM, Izbicki JR and Kaifi JT: Effective therapeutic targeting of the overexpressed HER-2 receptor in a highly metastatic orthotopic model of esophageal carcinoma. Mol Cancer Ther *9*: 2037-2045, 2010.
10 Gros SJ, Dohrmann T, Peldschus K, Schurr PG, Kaifi JT, Kalinina T, Reichelt U, Mann O, Strate TG, Adam G, Hoffman RM and Izbicki JR: Complementary use of fluorescence and magnetic resonance imaging of metastatic esophageal cancer in a novel orthotopic mouse model. Int J Cancer *126*: 2671-2681, 2010.
11 Gros SJ: Orthotopic models of esophageal carcinoma and their use in drug discovery. Curr Protoc Pharmacol Chapter *14*: Unit14, 2011.
12 Gros SJ, Dohrmann T, Rawnaq T, Kurschat N, Bouvet M, Wessels J, Hoffmann RM, Izbicki JR and Kaifi JT: Orthotopic fluorescent peritoneal carcinomatosis model of esophageal cancer. Anticancer Res *30*: 3933-3938, 2010.
13 Wilkinson PC: Assays of leukocyte locomotion and chemotaxis. J Immunol Methods *216*: 139-153, 1998.
14 Toetsch S, Olwell P, Prina-Mello A and Volkov Y: The evolution of chemotaxis assays from static models to physiologically relevant platforms. Integr Biol (Camb) *1*: 170-181, 2009.
15 Gros SJ, Dohrmann T, Peldschus K, Schurr PG, Kaifi JT, Kalinina T, Reichelt U, Mann O, Strate TG, Adam G, Hoffman RM and Izbicki JR: Complementary use of fluorescence and magnetic resonance imaging of metastatic esophageal cancer in a novel orthotopic mouse model. Int J Cancer *126*: 2671-2681, 2010.
16 Izbicki JR, Passlick B, Hosch SB, Kubuschock B, Schneider C, Busch C, Knoefel WT, Thetter O and Pantel K: Mode of spread in the early phase of lymphatic metastasis in non-small-cell lung cancer: significance of nodal micrometastasis. J Thorac Cardiovasc Surg *112*: 623-630, 1996.
17 Izbicki JR, Pantel K, and Hosch SB: Micrometastasis in solid epithelial tumors: impact on surgical oncology. Surgery *131*: 1-5, 2002.
18 Koenig AM, Prenzel KL, Bogoevski D, Yekebas EF, Bubenheim M, Faithova L, Vashist YK, Gawad KA, Baldus SE, Pantel K, Schneider PM, Holscher AH and Izbicki JR: Strong impact of micrometastatic tumor cell load in patients with esophageal carcinoma. Ann Surg Oncol *16*: 454-462, 2009.
19 Pantel K and Riethmuller G: Micrometastasis detection and treatment with monoclonal antibodies. Curr Top Microbiol Immunol *213(Pt 3)*: 1-18, 1996.
20 Pantel K and Brakenhoff RH: Dissecting the metastatic cascade. Nat Rev Cancer *4*: 448-456, 2004.
21 Hannelien V, Karel G, Jo VD and Sofie S: The role of CXC chemokines in the transition of chronic inflammation to esophageal and gastric cancer. Biochim Biophys Acta *1825*: 117-129, 2012.
22 Helbig G, Christopherson KW, Bhat-Nakshatri P, Kumar S, Kishimoto H, Miller KD, Broxmeyer HE and Nakshatri H: NF-kappaB promotes breast cancer cell migration and metastasis by inducing the expression of the chemokine receptor CXCR4. J Biol Chem *278*: 21631-21638, 2003.
23 Hamatake M, Aoki T, Futahashi Y, Urano E, Yamamoto N and Komano J: Ligand-independent higher-order multimerization of CXCR4, a G-protein-coupled chemokine receptor involved in targeted metastasis. Cancer Sci *100*: 95-102, 2009.
24 van der Meulen AA, Biber K, Lukovac S, Balasubramaniyan V, den Dunnen WF, Boddeke HW and Mooij JJ: The role of CXC chemokine ligand (CXCL)12-CXC chemokine receptor (CXCR)4 signalling in the migration of neural stem cells towards a brain tumour. Neuropathol Appl Neurobiol *35*: 579-591, 2009.

25 Luo Y, Cai J, Xue H, Miura T and Rao MS: Functional SDF1 alpha/CXCR4 signaling in the developing spinal cord. J Neurochem *93*: 452-462, 2005.
26 Li JK, Yu L, Shen Y, Zhou LS, Wang YC and Zhang JH: Inhibition of CXCR4 activity with AMD3100 decreases invasion of human colorectal cancer cells *in vitro*. World J Gastroenterol *14*: 2308-2313, 2008.
27 Jaszczynska-Nowinka K and Markowska A: New cytokine: Stromal derived factor-1. Eur J Gynaecol Oncol *30*: 124-127, 2009.
28 Burger JA and Stewart DJ: CXCR4 chemokine receptor antagonists: Perspectives in SCLC. Expert Opin Investig Drugs *18*: 481-490, 2009.
29 Burger JA, Stewart DJ, Wald O and Peled A: Potential of CXCR4 antagonists for the treatment of metastatic lung cancer. Expert Rev Anticancer Ther *11*: 621-630, 2011.
30 Gangadhar T, Nandi S, and Salgia R: The role of chemokine receptor CXCR4 in lung cancer. Cancer Biol Ther *9*: 409-416, 2010.
31 Arya M, Patel HR, McGurk C, Tatoud R, Klocker H, Masters J and Williamson M: The importance of the CXCL12-CXCR4 chemokine ligand-receptor interaction in prostate cancer metastasis. J Exp Ther Oncol *4*: 291-303, 2004.
32 Zhang C, Cui GH, Liu F, Wu QL and Chen Y: Inhibitory effect of triptolide on lymph node metastasis in patients with non-Hodgkin lymphoma by regulating SDF-1/CXCR4 axis *in vitro*. Acta Pharmacol Sin *27*: 1438-1446, 2006.
33 Burger JA and Kipps TJ: CXCR4: a key receptor in the crosstalk between tumor cells and their microenvironment. Blood *107*: 1761-1767, 2006.
34 Gockel I, Schimanski CC, Heinrich C, Wehler T, Frerichs K, Drescher D, von LC, Domeyer M, Biesterfeld S, Galle PR, Junginger T and Moehler M: Expression of chemokine receptor CXCR4 in esophageal squamous cell and adenocarcinoma. BMC Cancer *6*: 290, 2006.
35 Sasaki K, Natsugoe S, Ishigami S, Matsumoto M, Okumura H, Setoyama T, Uchikado Y, Kita Y, Tamotsu K, Sakurai T, Owaki T and Aikou T: Expression of CXCL12 and its receptor CXCR4 correlates with lymph node metastasis in submucosal esophageal cancer. J Surg Oncol *97*: 433-438, 2008.
36 Sasaki K, Natsugoe S, Ishigami S, Matsumoto M, Okumura H, Setoyama T, Uchikado Y, Kita Y, Tamotsu K, Hanazono K, Owaki T and Aikou T: Expression of CXCL12 and its receptor CXCR4 in esophageal squamous cell carcinoma. Oncol Rep *21*: 65-71, 2009.
37 Schimanski CC, Galle PR, and Moehler M: Chemokine receptor CXCR4 - prognostic factor for gastrointestinal tumors. World J Gastroenterol *14*: 4721-4724, 2008.
38 Wang DF, Lou N, Zeng CG, Zhang X and Chen FJ: Expression of CXCL12/CXCR4 and its correlation to prognosis in esophageal squamous cell carcinoma. Ai Zheng *28*: 154-158, 2009.
39 Duda DG, Kozin SV, Kirkpatrick ND, Xu L, Fukumura D and Jain RK: CXCL12 (SDF1alpha)-CXCR4/CXCR7 pathway inhibition: An emerging sensitizer for anticancer therapies? Clin Cancer Res *17*: 2074-2080, 2011.
40 Vagima Y, Lapid K, Kollet O, Goichberg P, Alon R and Lapidot T: Pathways implicated in stem cell migration: The SDF-1/CXCR4 axis. Methods Mol Biol *750*: 277-289, 2011.
41 Yen CC and Soon LL: Simulating sharp gradients for short-term, Ca(2+) transients and long-term chemotaxis in cancer cells. Technol Cancer Res Treat *8*: 241-247, 2009.
42 Mosadegh B, Saadi W, Wang SJ and Jeon NL: Epidermal growth factor promotes breast cancer cell chemotaxis in CXCL12 gradients. Biotechnol Bioeng *100*: 1205-1213, 2008.
43 Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R, Carey VJ, Richardson AL and Weinberg RA: Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell *121*: 335-348, 2005.

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