Effect of Chemokine Receptors CXCR4 and CCR7 on the Metastatic Behavior of Human Colorectal Cancer

Carl C. Schimanski,[1] Stefan Schwald,[1] Nektaria Simiantonaki,[2] Caren Jayasinghe,[2] Ursula Gönner,[3] Vanessa Wilsberg,[1] Theodor Junginger,[3] Martin R. Berger,[4] Peter R. Galle,[1] and Markus Moehler[1]

[1]First Department of Internal Medicine and Institutes of [2]Pathology and [3]Surgery, Johannes Gutenberg University of Mainz, Mainz, Germany and [4]Unit of Toxicology and Chemotherapy, German Cancer Research Centre, Heidelberg, Germany

ABSTRACT

Purpose: **The expression of chemokine receptors CXCR4 and CCR7 has been associated with tumor dissemination and poor prognosis in a limited number of tumor entities. However, no data are currently available on the impact of chemokine receptor expression on disease progression and prognosis in human colorectal cancer.**

Experimental Design: **The expression of CXCR4 and CCR7 was evaluated in 96 patients with histologically confirmed colorectal cancers and in four colorectal cancer cell lines by immunohistochemical staining. Furthermore, cell migration assays were done with SW480, SW620, and LS174T cancer cells to confirm the effect of the CXCR4 ligand stromal cell–derived factor 1α on migration.**

Results: **Human colorectal cancer specimens and cell lines displayed a CXCR4 and CCR7 expression with variable intensities. Interestingly, strong expression of CXCR4, but not of CCR7, was significantly associated with higher Union International Contre Cancer stages 3/4 ($P = 0.0017$), lymph node metastasis ($P = 0.00375$), and distant metastasis ($P = 0.00003$) and further correlated with a reduced 3-year survival rate ($P = 0.1$). Strong CXCR4 and CCR7 expression positively correlated with the location of the primary tumor in the rectum ($P < 0.01$). Furthermore, activation of CXCR4-expressing cancer cells by stromal cell–derived factor 1α resulted in a significant increase of cell migration ($P < 0.014$).**

Conclusion: **Strong expression of CXCR4 by colorectal cancer cells is significantly associated with lymphatic and distant dissemination in patients with colorectal cancer as well as with cancer cell migration *in vitro*.**

Received 6/21/04; revised 11/4/04; accepted 12/13/04.
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.
Requests for reprints: Carl C. Schimanski, First Department of Internal Medicine, Johannes Gutenberg University of Mainz, Langenbeckstrasse 1, 55101 Mainz, Germany. Phone: 49-6131-177276; Fax: 49-6131-177276; E-mail: dr_schimanski@yahoo.de.
©2005 American Association for Cancer Research.

INTRODUCTION

Survival in colorectal cancer, ranging among the three most frequent malignancies in Western countries is delineated by local recurrence and lymphatic and hematogenous dissemination (1–3). Molecular determinants occurring during the development of sporadic colorectal cancer include mutations in certain tumor suppressor genes (*APC*, *DCC*, *Smad-2*, *Smad-4*, *p53*) and oncogenes (K-*ras*) that have been summarized in the adenoma-carcinoma sequence initially proposed by Fearon and Vogelstein (4), Cho and Vogelstein (5), and Vogelstein and Kinzler (6). However, because only 8% of colorectal cancers harbor concomitant mutations of *APC*, K-*ras*, and *p53* it seems very likely that additional pathogenic alterations are instrumental to mediate progression and metastasis of colorectal cancer (7).

Currently, tumor growth and metastatic dissemination are accepted as a result of a complex, dysregulated molecular machinery leading to several phenomena, such as the resistance of tumor cells to apoptosis, tumor cell migration, tumor cell invasion, and tumor cell immune escape mechanisms. Regarding these aspects, recent data suggest that chemokine receptors may direct lymphatic and hematogenous spreading and may additionally influence the sites of metastatic growth of different tumors (8). Originally, chemokines and their G protein–coupled receptors were reported to mediate different pro- and anti-inflammatory responses (9). The chemokine receptor CXCR4 was initially described to regulate the homing of lymphocytes in inflammatory tissues (10). Its natural ligand, the stromal cell-derived factor 1α (SDF-1α), is highly expressed in tissues of metastatic growth, such as lung, liver, and lymph nodes, and attracts lymphocytes to these organs (11). CCR7, the receptor for the chemokine CCL21, is expressed on naïve T cells, memory T cells, B cells, and mature dendritic cells and is considered to play an important role in lymphocyte cell trafficking and homing to lymph nodes (12, 13).

Most recently, these two chemokine receptors have shifted into focus as to their role in tumor spreading. High CXCR4 expression was associated with lymph node metastases in breast cancer and oral squamous cell carcinoma (14, 15). In patients with osteosarcoma, CXCR4 expression levels were positively correlated with detection of metastasis at the time of diagnosis but inversely with event-free survival, metastasis-free survival, and overall survival (16). CCR7 expression was positively correlated with lymphatic metastasis and poor prognosis in esophageal squamous cell carcinoma and gastric cancer (17, 18).

Supporting data from *in vitro* and murine tumor models underlined the key roles of both receptors for tumor cell malignancy. Stimulation of CCR7 by its ligand CCL21 induced migration and invasion of CCR7-expressing cancer cells (19). In line with this, inhibition of CXCR4 suppressed SDF-1–induced migration, invasion, and angiogenesis (20, 21). Furthermore,

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/11/5/1743/1963272/1743-1750.pdf by guest on 10 December 2023

down-regulation of CXCR4 in colorectal cancer cells led to decreased pulmonary metastasis in mice, both in number and in size (22).

However, no data are presently available on the expression of CXCR4 and CCR7 in human colorectal cancer and their impact on disease progression and prognosis. Therefore, we evaluated the expression of CXCR4 and CCR7 in colorectal cancer cell lines and specimens and correlated the results with the patients' clinicopathologic parameters and survival. Our results revealed that strong expression of CXCR4, but not of CCR7, significantly influenced lymphatic and hematogenous tumor dissemination and correlated with a decreased 3-year survival rate. Both receptors were further associated with the highly lymphotropic rectal tumor location. In addition, we analyzed the effect of SDF-1α on migration of SW480, SW620, and LS174T human colorectal cancer cells.

MATERIALS AND METHODS

Cell Culture. The human colorectal cancer cell lines SW480, SW620, LS174T, and HT29 were cultured in DMEM (Invitrogen, Carlsbad, CA) supplemented with 10% FCS, 100 units/mL penicillin, 100 μg/mL streptomycin (Cambrex, East Rutherford, NJ) and 1 mmol/L L-glutamine (Invitrogen).

RNA Isolation and Semiquantitative Reverse Transcription–PCR. RNA isolation was done using the RNeasy Kit according to the manufacturer's recommendations (Qiagen, Hilden, Germany). Gene transcription of actin, CXCR4 and CCR7 was analyzed by a two-step reverse transcription–PCR: reverse transcription was done with 2 μg of RNA (20 μL total volume; Omniscript RT Kit, Qiagen) according to the recommendations of the manufacturer. One microliter of the cDNA was used as a template for the specific PCR reactions. Primers applied were β-actin: forward 5′-TGA CGG GGT CAC CCA CAC TGT GCC CAT CTA-3′, reverse 5′-CTA GAA GCA TTT GCG GTG GAC GAC GGA GGG-3′ (661-bp fragment); CXCR4: forward 5′-TTC TAC CCC AAT GAC TTG TG-3′, reverse 5′-ATG TAG TAA GGC AGC CAA CA-3′ (206-bp fragment); CCR7: forward 5′-TCC TTC TCA TCA GCA AGC TGT C-3′, reverse 5′-GAG GCA GCC CAG GTC CTT GAA-3′ (529-bp fragment). For amplification a DNA Engine PTC200 (MJ Research, Watertown, MA) thermocycler was used. Cycling conditions of the respective PCRs were as follows: initial denaturation (4 minutes at 95°C) followed by the respective number of cycles (β-actin, 28; CXCR4 and CCR7, 32) of denaturation (1 minute at 94°C), annealing (1 minute; β-actin, 52°C; CXCR4, 56°C; CCR7, 62°C) and elongation (2 minutes at 72°C). After the last cycle a final extension (10 minutes at 72°C) was added and thereafter the samples were kept at 4°C. Fifteen microliters of the products were run on a 1.8% agarose gel, stained by ethidium bromide, and analyzed under UV light.

Western Blot Analysis. SW480, SW620, LS174T, and HT29 cells were washed with PBS and lysed in 0.5% NP40 solution. One hundred micrograms of protein were loaded on a 10% SDS-PAGE gel. The gel was transferred onto a polyvinylidene difluoride membrane after separation. The respective proteins were detected with anti-CXCR4 (1:500, CIO115, Capralogics, Hardwick, MA; 1:1000 donkey anti-goat IgG second antibody SC-2020, Santa Cruz Biotechnology, Santa Cruz, CA), anti-CCR7 (1:500, CIO131, Capralogics; 1:1000 donkey anti-goat IgG second antibody SC-2020 by Santa Cruz Biotechnology), and anti-actin (1:1000, A2066, Sigma, Munich, Germany; 1:1000 goat anti-rabbit IgG second antibody 170-6515 by Bio-Rad, Hercules, CA) and were visualized by ECL Western blotting analysis system (Amersham Biosciences, Piscataway, NJ).

Tissue Samples. Colorectal cancer tissue samples were obtained from 96 consecutive patients undergoing elective surgery for colorectal cancer at the University of Mainz between 1995 and 2001. The morphologic classification of the carcinomas was conducted according to WHO specifications. After resection, patients were followed up every 6 months. Patients with synchronic or metachronic metastasis underwent additional restaging every 3 months during chemotherapy.

Immunohistochemical Staining. The avidin-biotin complex method was used to detect the proteins CXCR4 and CCR7 (anti-CXCR4, dilution 1:300; anti-CCR7, dilution 1:250; both from Capralogics). Formalin-fixed and paraffin-embedded tissues were deparaffinized and subsequently microwaved in EDTA buffer. After preincubation with hydrogen peroxide, avidin/biotin blocking kit (Vector Laboratories Inc., Burlingame, CA), and rabbit serum (Vector Laboratories), the primary antibodies were applied for 1 hour at room temperature. After incubation with the secondary antibody (rabbit anti-goat biotinylated; dilution 1:200, Vector Laboratories), the avidin-biotin complex was added and the enzyme activity visualized with diaminobenzidine. Counterstaining was done with hematoxylin. For negative controls, only the secondary antibody was used. A negative control was done for every colorectal cancer sample (n = 96). For positive controls, formalin-fixed and paraffin-embedded tissue samples of the human spleen were applied.

Evaluation of Immunostaining. Immunostaining was evaluated by four authors independently (C.C.S., M.M., S.S., and N.S.), blinded to patient outcome and all clinicopathologic findings. The immunohistochemical staining was analyzed according to a scoring method that we have previously validated: the tumors were classified into four groups based on the staining intensity (0, absent; 1, weak; 2, intermediate; 3, strong staining). In the case of heterogeneous staining within the same sample, the respective higher score was chosen if >50% of cells revealed the higher staining intensity. If the evaluations did not agree the specimens were reevaluated and then classified according to the assessment given most frequently by the observers.

Cellular Migration Assays. Migration of SW480, SW620, and LS174T cancer cells was assayed with 24-well HTS FluoroBlok inserts in triplets (8-μm pore size; Becton Dickinson, Franklin Lakes, NJ). In brief, cells were serum starved for 24 hours before initiation of the assay. Cells (3×10^4) were resuspended in serum-free DMEM and added to the upper chamber. Consecutively, DMEM with 20% FCS and with or without 100 ng/mL SDF-1α was added to the lower chamber. Chambers were incubated for 24 hours at 37°C in a humid atmosphere of 5% CO_2. After incubation, the amount of migrated cells in the lower chamber was determined by luminescence assay according to the recommendations of the

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/11/5/1743/1963272/1743-1750.pdf by guest on 10 December 2023

manufacturer (Celltiter-Glo Cell Viability assay, Promega, Mannheim, Germany).

Statistics. The association of staining intensity with clinicopathologic patterns was assessed with χ^2 test and with unpaired Student t test, when appropriate. Differences in migration were evaluated with unpaired Student t test. Survival rates were visualized by applying Kaplan-Meier curves, and P values were determined by the log-rank test. $P < 0.05$ was considered significant and $P < 0.001$ highly significant in all statistical analyses.

RESULTS

CXCR4 and CCR7 Expression in Colorectal Carcinoma Cell Lines. CXCR4 expression and transcription of human colorectal cancer cell lines varied from strong (SW480, HT29) and intermediate (SW620) to weak (LS174T) as depicted by Fig. 1. Similarly, CCR7 expression and transcription varied from strong (SW480) to intermediate (LS174T, SW620, and HT29). CXCR4 and CCR7 immunostaining and Western blot analysis correlated with the respective transcription profile.

Tumor Characteristics and Patient Profiles. The selected group of patients represent the typical characteristics of colorectal cancer in industrialized countries, except for a lower percentage (16%; normal expectation, 50%) of rectal cancers (Table 1).

Immunohistochemical Staining of CXCR4 and CCR7. The staining for CXCR4 and CCR7 revealed predominantly a cytoplasmic, and in few specimens, an additional weak membranous location of CXCR4 and CCR7 (Fig. 2*A-H*). Homogenous cytoplasmic staining was found in 66% and 55% of all samples for CXCR4 and CCR7, respectively. A nuclear staining of CXCR4 or CCR7 was not observed. The respective expression rate for CXCR4 was 100% (96/96) and varied from weak (14%) to intermediate (28%) to strong (58%). A CCR7 expression was found in 98% (94/96) of all colorectal cancer specimens and varied from absent (2%), weak (27%), intermediate (44%) to strong (27%).

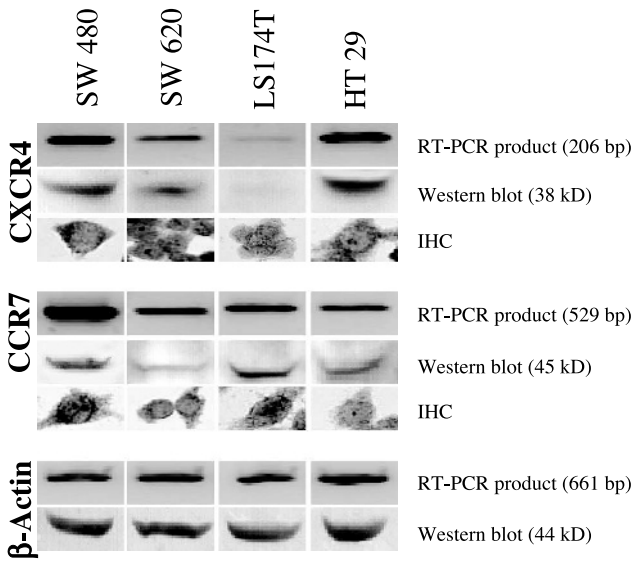

Fig. 1 Expression and transcription profile of CXCR4 and CCR7 in diverse human colorectal cancer cell lines. *IHC,* immunohistochemistry.

Negative controls of human colorectal cancer remained negative for every colorectal cancer sample ($n = 96$). Splenic lymphocytes revealed a strong CXCR4 and CCR7 expression matching human colorectal cancer tissue or cell lines with strong expression of CXCR4 or CCR7.

CXCR4 Expression in Colorectal Cancer. Strong CXCR4 expression tended to be associated with lower median age at diagnosis (62 versus 66 years; not significant (NS); Table 2) and with male gender (61% versus 42% females; NS; $P = 0.078$). Furthermore, a strong CXCR4 staining intensity was significantly correlated with rectal cancers (25% versus 0%; $P = 0.0006$). It is noteworthy that all rectal cancers (14/14) revealed a strong CXCR4 expression, whereas in colon cancers a strong CXCR4 expression was seen in only 51%. Concerning the Union International Contre Cancer (UICC) classification, a strong CXCR4 expression was significantly associated with UICC stages 3 and 4 ($P = 0.0012$) as compared with UICC stages 1 and 2, describing limited disease. Intensity of CXCR4 expression did not correlate with local progression of the primary tumor as indicated by the T status [tumor-node-metastasis (TNM) classification; $P = 0.86$]. However, a high expression was significantly associated with lymph node involvement (N status; $P = 0.0038$) and with distant metastases (M status; $P = 0.00003$). Evidently, CXCR4 expression did not have impact on the grading (G) or the resection status (R). Concerning survival, a strong CXCR4 expression was correlated with a decreased 3-year survival rate of 70%, as compared with 82% in the case of weak/intermediate CXCR4 expression. This difference is depicted in the respective survival plot of Fig. 3*A* (log-rank $P = 0.01$; rank sum, 0.078).

CCR7 Expression in Colorectal Cancer. Strong/intermediate CCR7 expression was associated with a lower median age at diagnosis of 62.5 years as compared with 65.5 years in cases with weak/absent expression (Table 3; NS). A strong/intermedia CCR7 staining intensity did significantly correlate with rectal cancers (21% versus 0% in cases with weak/absent CCR7 expression; $P = 0.0094$). However, in contrast to CXCR4, the CCR7 expression did not correlate with UICC or TNM classification. CCR7 expression was neither associated with gender, grading (G), nor with the resection status (R). An intermediate/strong CCR7 expression was correlated with a increased 3-year survival rate of 85% compared with 70% in the cases with absent/weak CCR7 expression, as depicted in the respective Kaplan-Meier plot of Fig. 3*B* (log-rank test: NS; $P = 0.34$).

Subgroup Analysis: CCR7 Expression in Rectal versus Colon Cancers. Intermediate/strong CCR7 expression was found in 54 (67%) colon and 14 (100%) rectal cancers (Table 4). In rectal as compared with colon cancers, an intermediate/strong CCR7 expression was significantly associated with higher UICC stages 3 and 4 (UICC classification; $P = 0.01$), dedifferentiation as indicated by grades 3 and 4 (grading; $P = 0.002$), lymph node (N status; $P = 0.03$) and distant metastases (M status; $P = 0.006$). No difference in T or R status was observed for this subgroup.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/11/5/1743/1963272/1743-1750.pdf by guest on 10 December 2023

Table 1 Patient and tumor characteristics

Patient characteristics	
Total number	96
Median age (y)	64
Gender	
Female	45 (47)
Male	51 (53)
Location	
Colon	82 (85)
Rectum	14 (15)
UICC	
1 + 2	46 (48)
3 + 4	50 (52)
T status	
1	4 (4)
2	16 (17)
3	65 (68)
4	11 (11)
N status	
0	48 (50)
1	18 (19)
2	30 (31)
M status	
0	50 (52)
1	46 (48)
Grading	
1	4 (4)
2	73 (76)
3	17 (18)
4	2 (2)
R status	
0	90 (94)
1	4 (4)
2	2 (2)
Median survival (d), not censored	1,325

NOTE. Values in parentheses are percentages.

Effect of SDF-1α on Migration of Colon Cancer Cells. The chemokine SDF-1α stimulated migration of SW480 (luminescence: 1795 ± 72 IU versus 905 ± 62 IU; $P < 0.001$) and SW620 (luminescence: 982 ± 68 IU versus 714 ± 23 IU; $P = 0.014$), but not of LS174T (luminescence: 340 ± 34 IU versus 336 ± 34 IU; NS) colorectal carcinoma cells (Fig. 4).

DISCUSSION

This is the first study analyzing the expression profiles of the chemokine receptors CXCR4 and CCR7 in a larger series of human colorectal cancer tissues and cell lines. We further determined whether CXCR4 and CCR7 expression influenced migration of cancer cells *in vitro* and the metastatic behavior of colorectal cancer and its prognosis in patients, as recently reported for breast cancer (14) and osteosarcoma (16).

The investigated human colorectal cancer cell lines revealed different intensities of cytoplasmatic CXCR4 and CCR7 expression. Whereas strong expression of CXCR4 was found in SW480 and HT29 cancer cells, other cell lines such as LS174T barely expressed CXCR4 at all. Similarly, varying intensities of CCR7 expression were detected in the respective cell lines.

Immunohistochemical staining of human colorectal cancer specimens displayed a cytoplasmic CXCR4 and CCR7 expression with variable intensities, matching the observations made in human colorectal cancer cell lines. A membranous localization of CXCR4 was observed in fewer cases, but an inducible translocation of CXCR4 from the cytoplasma to the membrane has been reported (23).

Interestingly, strong CXCR4 expression was significantly associated with rectal cancers, lymph node metastases, distant metastases, and higher UICC stages 3 and 4. Furthermore, a trend for male gender and older ages was observed in cases with intense CXCR4 expression. Thus, our results imply a substantial influence of CXCR4 on the lymphatic and hematogenous dissemination of colorectal cancer *in vivo* and are in line with recent publications reporting a similar effect of CXCR4 on disease dissemination in other tumor entities (16, 24, 25). CXCR4 expression has previously been associated with i.p. dissemination in ovarian cancer (24), intrapleural dissemination in non–small cell lung cancer (25), lymph node metastases in breast cancer (14), and oral squamous cell carcinoma (15). In osteosarcoma, the level of CXCR4 expression was inversely correlated with overall survival, event-free survival, and metastasis-free survival, but positively associated with detection of metastasis (16). CXCR4 expression was further up-regulated in glioblastoma and inhibition of this receptor was followed by arrest of tumor

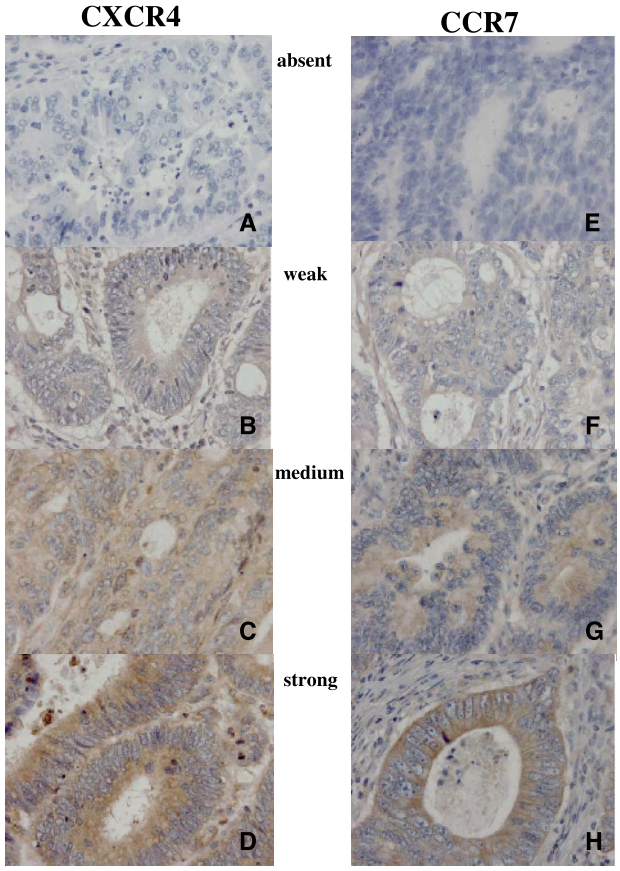

Fig. 2 Intensity of CXCR4 and CCR7 expression in different colorectal adenocarcinomas. *A* to *D*, cytoplasmic expression grades of CXCR4. *A,* no expression (negative control); *B,* weak expression; *C,* intermediate expression; *D,* strong expression. *E* to *H*, cytoplasmic expression grades of CCR7. *E,* no expression; *F,* weak expression; *G,* intermediate expression; *H,* strong expression.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/11/5/1743/1963272/1743-1750.pdf by guest on 10 December 2023

Table 2 Patient and tumor characteristics dependent on intensity of CXCR4 expression

	CXCR4 expression			
	Weak (1)	Intermediate (2)	Strong (3)	*P*
Total number (%)	13 (14)	27 (28)	56 (58)	
Median age (y)	66		62	NS
Gender				
Female	23		22	NS
Male	17		34	
Location				
Colon	40		42	$P = 0.0006$
Rectum	0		14	
UICC				
1 + 2	27		19	$P = 0.0012$
3 + 4	13		37	
T status				
1 + 2	8		12	NS
3 + 4	32		44	
N status				
0	27		21	$P = 0.00375$
+	13		35	
M status				
0	31		19	$P = 0.00003$
+	9		37	
Grading				
1 + 2	32		45	NS
3 + 4	8		11	
R status				
0	39		51	NS
+	1		5	
3-year survival (%)	82		70	NS ($P = 0.1$)

cell proliferation (26). Microarray analyses of cDNA revealed that CXCR4 overexpression also occurred in renal (27), esophageal (28), and pancreatic cancer (29), implying a relevant function of CXCR4 expression during carcinogenesis in these cancer entities. In contrast, a strong CXCR4 expression was associated with noninvasive gastric cancers but not with lymph node metastases (30). Similarly, strong CXCR4 expression was associated with a better outcome in early stages of non–small cell lung cancer but not with age, gender, or TNM stage (31). Because strong CXCR4 expression might rather occur in later tumor stages, concentration on early stages might have led to false-negative results. Nevertheless, the effect of a strong CXCR4 expression on disease dissemination might depend on the type and location of the primary cancer and most likely on other parameters, which have not been defined yet.

Diverse functional studies investigating the influence of CXCR4 expression and the activation by its ligand SDF-1α (CXCL12) have been done recently, revealing that CXCR4 is crucial for adhesion, migration, and invasion of CXCR4-expressing pancreatic, lung, and glioblastoma cancer cells (15, 20, 32, 33). To confirm the functionality of CXCR4 in colorectal cancer we did migration assays with SW480, SW620, and LS174T colorectal cancer cells. Migration of SW480 and SW620 cells intensively expressing CXCR4 was significantly stimulated by SDF-1α, implying an important role and intact function of CXCR4 during disease progression of colorectal cancer. In contrast, LS174T cells barely expressing CXCR4 revealed a weak basic migratory behavior that could not be stimulated by SDF-1α. These results indicate that the intensity of SDF-1α–induced cell migration *in vivo* correlates with cellular CXCR4 expression.

Previous publications further reported that CXCR4 activation induced β-integrin activation, which itself was critical to adhesion of CXCR4-activated melanoma cells to vascular cellular adhesion molecule and pulmonary endothelial cells (33, 34). *In vivo* induction of CXCR4 resulted in a dramatic increase of pulmonary metastases of melanoma cells that could be blocked by potent CXCR4 inhibitors (22). In another murine model, only CXCR4-expressing but not CXCR4-deficient CT-26 colon carcinoma cells did grow into macrometastases, but both cell types were able to colonize livers and lungs after injection (35).

The question arises why predominantly the lungs, liver, and lymph nodes are being target organs for metastases of colorectal cancer in humans. Because the filter function of these organs (36) does not solely explain the growth of metastases, other homing factors influencing selection of target organs have been proposed (37, 38). The differential expression of SDF-1α and vascular cellular adhesion molecule 1 by endothelial cells might explain at least one part of this process. Most endothelial cells express SDF-1α, but, presently, only pulmonary endothelial cells have been shown to coexpress vascular cellular adhesion molecule 1, thus mediating tumor cell/endothelial cell attachment (34). Even as endothelial cells of other target organs might similarly coexpress vascular cellular adhesion molecule 1, the SDF-1α expression mediating integrin activation seems to be highest in the typical homing organs adrenal glands, lungs, bone marrow, liver, and lymph nodes as compared with other nonhoming tissues, such as kidneys, heart, or even plasma (11, 15). Our results support the "homing" theory because CXCR4 expression co-mediates hematogenous dissemination of primary tumors to different organs through the chemotactic factors SDF-1 (37). The influence

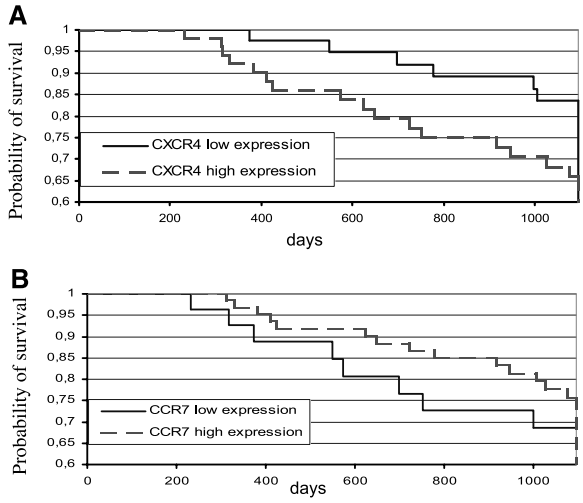

Fig. 3 The probability of survival of colorectal cancer patients is given in relation to time after surgery. *A,* patients with strong CXCR4 expression showed a reduced 3-year survival rate as compared with patients with an intermediate or weak CXCR4 expression (NS; $P = 0.1$). *B,* patients with an absent or weak CCR7 expression revealed increased 3-year survival rate as compared with patients with an intermediate or strong CCR7 expression (NS).

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/11/5/1743/1963272/1743-1750.pdf by guest on 10 December 2023

Table 3 Patient and tumor characteristics dependent on intensity of CCR7 expression

	CCR7 expression				
	None (0)	Weak (1)	Intermediate (2)	Strong (3)	*P*
Total number (%)	2 (2)	26 (27)	42 (44)	26 (27)	
Average age (y)	65.5		62.5		NS
Gender					
Female	15		30		NS
Male	13		38		
Location					
Colon	28		54		$P = 0.0094$
Rectum	0		14		
UICC					
1 + 2	16		30		NS
3 + 4	12		36		
T status					
1 + 4	3		17		NS
3 + 4	25		51		
N status					
0	16		32		NS
+	12		36		
M status					
0	18		32		NS
+	10		36		
Grading					
1 + 2	24		53		NS
3 + 4	4		15		
R status					
0	27		63		NS
+	1		5		
3-year survival (%)	85		70		NS

of CXCR4 on the lymphatic (as compared with the hematogenous) dissemination has not been investigated thus far, but SDF-1 is also highly expressed in lymph nodes and CXCR4 inhibition resulted in suppression of breast cancer lymph node metastases, implying similar pathways of lymphatic and hematogenous dissemination (39).

Thus far, first data implicated both external cellular factors such as hypoxia (Hif-1 pathway) and the activation of adenosine receptors as well as internal cellular alterations, such as the inactivation of the tumor suppressor protein *p53* and overexpression of *NFkB*, as important molecular regulators of the CXCR4 expression (23, 40–42). Loss of function mutations of the von Hippel-Lindau tumor suppressor gene *pVHL,* which normally degrades Hif-1, resulted in a strong CXCR4 expression and poor survival in renal cancer (43).

In contrast to CXCR4, high CCR7 expression did not correlate with gender, UICC stage, TNM classification, grading, resection status, or a reduced 3-year survival rate. Thus, our results did not confirm any influence of CCR7 on the dissemination of colon cancer as described for gastric cancer and esophageal squamous cell carcinoma (17, 18). In these tumor entities, high CCR7 expression was significantly associated with lymphatic invasion, lymph node metastases, and poor prognosis. It is noteworthy that the percentage of rectal tumors among all colorectal cancers was only 16% as compared with up to 50% in larger populations and was thus underrepresented. However, all 15 rectal cancers displayed an intermediate/strong CCR7 expression, which was significantly associated with progressed UICC stages, dedifferentiated tumors, lymph node metastases, and distant metastases as compared with colon tumors with the identical expression pattern. These data certainly justify a larger analysis of CCR7 expression in rectal cancers. The theory of CCR7–co-mediated mechanism of lymphatic dissemination is being supported by data, revealing that CCR7 expression of melanoma cells increased metastases formation in regional lymph nodes of mice (44). CCR7-mediated lymphatic dissemination has been compared with the homing of activated dendritic cells to CCL21-expressing lymph nodes via lymphatic vessels (18, 19, 45–47).

Very recently, certain chemokines and cytokines have been proposed to distinctly contribute to tumor growth, dissemination, and local immunosuppression/escape (48, 49). Our results, revealing a significant correlation between a strong CXCR4 expression and lymphatic and hematogenous tumor dissemination, support such a theory.

Table 4 Influence of location of primary on clinicopathologic parameters in cases with intermediate/strong CCR7 expression

	Colon	Rectum	*P*
Total number	54	14	
UICC			
1 + 2	28	2	$P = 0.01$
3 + 4	26	12	
T status			
1/2	15	2	NS
3/4	39	12	
N status			
0	29	3	$P = 0.03$
+	25	11	
M status			
0	30	2	$P = 0.0058$
1	24	12	
Grading			
1/2	9	8	$P = 0.002$
3/4	45	6	
R status			
0	50	13	NS
+	4	1	

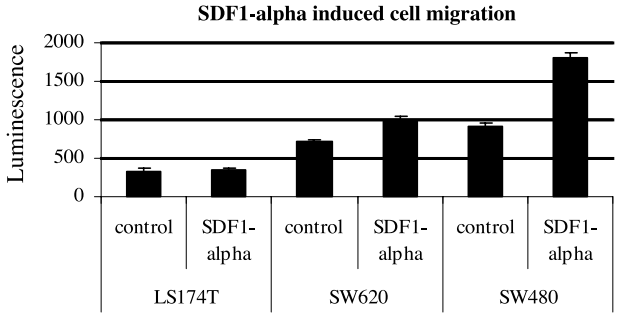

Fig. 4 Effect of SDF-1α on migration of different colorectal carcinoma cell lines. These assays confirm that migration of CXCR4-expressing colorectal cancer cell lines SW480 and SW620 is increased under SDF-1α (100 ng/mL) treatment. Migration of LS174T cells revealing only a weak CXCR4 expression is not stimulated by SDF-1α.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/11/5/1743/1963272/1743-1750.pdf by guest on 10 December 2023

CONCLUSION

Strong expression of CXCR4 by colorectal cancer cells is significantly associated with lymphatic and distant dissemination in patients with colorectal cancer as well as with a migratory phenotype *in vitro*. Thus, CXCR4 apparently plays an important role during colorectal cancer progression. Further efforts are necessary to evaluate the inhibition of metastatic growth by CXCR4 antagonists.

REFERENCES

1. Weir HK, Thun MJ, Hankey BF, et al. Annual report to the nation on the status of cancer, 1975-2000, featuring the uses of surveillance data for cancer prevention and control. J Natl Cancer Inst 2003; 95:1276–99.
2. Greenlee RT, Murray T, Bolden S, Wingo PA. Cancer statistics, 2000. CA Cancer J Clin 2000;50:7–33.
3. August DA, Ottow RT, Sugarbaker PH. Clinical perspective of human colorectal cancer metastasis. Cancer Metastasis Rev 1984;3:303–24.
4. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990;61:759–67.
5. Cho KR, Vogelstein B. Suppressor gene alterations in the colorectal adenoma-carcinoma sequence. J Cell Biochem Suppl 1992;16G:137–41.
6. Vogelstein B, Kinzler KW. The multistep nature of cancer. Trends Genet 1993;9:138–41.
7. Smith G, Carey FA, Beattie J, et al. Mutations in APC, Kirsten-ras, and p53-alternative genetic pathways to colorectal cancer. Proc Natl Acad Sci U S A 2002;99:9433–8.
8. Arya M, Patel HR, Williamson M. Chemokines: key players in cancer. Curr Med Res Opin 2003;19:557–64.
9. Zlotnik A, Yoshie O. Chemokines: a new classification system and their role in immunity. Immunity 2000;12:121–7.
10. Murdoch C. CXCR4: chemokine receptor extraordinaire. Immunol Rev 2000;177:175–84.
11. Phillips RJ, Burdick MD, Lutz M, Belperio JA, Keane MP, Strieter RM. The stromal derived factor-1/CXCL12-CXC chemokine receptor 4 biological axis in non-small cell lung cancer metastases. Am J Respir Crit Care Med 2003;167:1676–86.
12. Dieu MC, Vanbervliet B, Vicari A, et al. Selective recruitment of immature and mature dendritic cells by distinct chemokines expressed in different anatomic sites. J Exp Med 1998;188:373–86.
13. Hirao M, Onai N, Hiroishi K, et al. CC chemokine receptor-7 on dendritic cells is induced after interaction with apoptotic tumor cells: critical role in migration from the tumor site to draining lymph nodes. Cancer Res 2000;60:2209–17.
14. Kato M, Kitayama J, Kazama S, Nagawa H. Expression pattern of CXC chemokine receptor-4 is correlated with lymph node metastasis in human invasive ductal carcinoma. Breast Cancer Res 2003;5:R144–50.
15. Uchida D, Begum NM, Almofti A, et al. Possible role of stromal-cell-derived factor-1/CXCR4 signaling on lymph node metastasis of oral squamous cell carcinoma. Exp Cell Res 2003;290:289–302.
16. Larverdiere C, Hoang BH, Yang R, et al. CXCR4 expression in osteosarcoma correlates with metastatic behaviour and outcome in patients. Proc AACR 2004;45:abstract 1772.
17. Ding Y, Shimada Y, Maeda M, et al. Association of CC chemokine receptor 7 with lymph node metastasis of esophageal squamous cell carcinoma. Clin Cancer Res 2003;9:3406–12.
18. Mashino K, Sadanaga N, Yamaguchi H, et al. Expression of chemokine receptor CCR7 is associated with lymph node metastasis of gastric carcinoma. Cancer Res 2002;62:2937–41.
19. Saeki H, Moore AM, Brown MJ, Hwang ST. Cutting edge: secondary lymphoid-tissue chemokine (SLC) and CC chemokine receptor 7 (CCR7) participate in the emigration pathway of mature dendritic cells from the skin to regional lymph nodes. J Immunol 1999; 162:2472–75.
20. Mori T, Doi R, Koizumi M, et al. CXCR4 antagonist inhibits stromal cell-derived factor 1-induced migration and invasion of human pancreatic cancer. Mol Cancer Ther 2004;3:29–37.
21. Twitchell DD, London NR, Tomer DP, Tomer S, Murray BK, O'Neill KL. Tannic acid prevents angiogenesis *in vivo* by inhibiting CXCR4/SDF-1 α binding in breast cancer cells. Proc AACR 2004;45:abstract 51.
22. Murakami T, Maki W, Cardones AR, et al. Expression of CXC chemokine receptor-4 enhances the pulmonary metastatic potential of murine B16 melanoma cells. Cancer Res 2002;62:7328–34.
23. Richard CL, Tan EY, Blay J. Adenosin increases cell-surface CXCR4 expression on HT-29 human colorectal carcinoma cells. Proc AACR 2004;45:abstract 3330.
24. Bruneau DJ, Clark LJ, Jenson B, et al. CXCR4 expression in ovarian cancer is associated with peritoneal dissemination. Proc AACR 2004;45:abstract 1722.
25. Oonakahara KI, Matsuyama W, Higashimoto I, Kawabata M, Arimura K, Osame M. Stromal-derived factor-1α/CXCL12-CXCR 4 axis is involved in the dissemination of NSCLC cells into pleural space. Am J Respir Cell Mol Biol 2004;30:671–7.
26. Rubin JB, Kung AL, Klein RS, et al. A small-molecule antagonist of CXCR4 inhibits intracranial growth of primary brain tumors. Proc Natl Acad Sci U S A 2003;100:13513–8.
27. Schrader AJ, Lechner O, Templin M, et al. CXCR4/CXCL12 expression and signalling in kidney cancer. Br J Cancer 2002;86:1250–6.
28. Kawamata H, Furihata T, Omotehara F, et al. Identification of genes differentially expressed in a newly isolated human metastasizing esophageal cancer cell line, T.Tn-AT1, by cDNA microarray. Cancer Sci 2003;94:699–706.
29. Sato N, Fukushima N, Maitra A, et al. Gene expression profiling identifies genes associated with invasive intraductal papillary mucinous neoplasms of the pancreas. Am J Pathol 2004;164:903–14.
30. Kwak MK, Hur K, Kim WH, Lee HJ, Park DJ, Yang HK. Expression of chemokine receptors in human gastric cancer tissues. Proc AACR 2004;45:abstract 1900.
31. Spano JP, Andre F, Morat L, et al. Chemokine receptor CXCR4 and early-stage non-small cell lung cancer: pattern of expression and correlation with outcome. Ann Oncol 2004;15:613–7.
32. Barbero S, Bonavia R, Bajetto A, et al. Stromal cell-derived factor 1α stimulates human glioblastoma cell growth through the activation of both extracellular signal-regulated kinases 1/2 and Akt. Cancer Res 2003;63:1969–74.
33. Burger M, Glodek A, Hartmann T, et al. Functional expression of CXCR4 (CD184) on small-cell lung cancer cells mediates migration, integrin activation, and adhesion to stromal cells. Oncogene 2003;22:8093–101.
34. Cardones AR, Murakami T, Hwang ST. CXCR4 enhances adhesion of B16 tumor cells to endothelial cells *in vitro* and *in vivo* via β(1) integrin. Cancer Res 2003;63:6751–7.
35. Zeelenberg IS, Ruuls-Van Stalle L, Roos E. The chemokine receptor CXCR4 is required for outgrowth of colon carcinoma micrometastases. Cancer Res 2003;63:3833–9.
36. Weiss L, Grundmann E, Torhorst J, et al. Haematogenous metastatic patterns in colonic carcinoma: an analysis of 1541 necropsies. J Pathol 1986;150:195–203.
37. Stetler-Stevenson WG, Kleiner DE. Molecular biology of cancer: invasion and metastases. Philadelphia: Lippincott & Wilkins; 2001. p. 123–36.
38. Nicolson GL. Paracrine and autocrine growth mechanisms in tumor metastasis to specific sites with particular emphasis on brain and lung metastasis. Cancer Metastasis Rev 1993;12:325–43.
39. Muller A, Homey B, Soto H, et al. Involvement of chemokine receptors in breast cancer metastasis. Nature 2001;410:50–6.
40. Helbig G, Christopherson KW, Bhat-Nakshatri P, et al. NF-κB promotes breast cancer cell migration and metastasis by inducing the expression of the chemokine receptor CXCR4. J Biol Chem 2003;278:21631–8.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/11/5/1743/1963272/1743-1750.pdf by guest on 10 December 2023

41. Mehata SA, Christopherson KW, Broxmeyer HE, Kopelovich L, Goulet RJ, Nakshatri H. Understanding the metastatic switch in breast cancer: role of p53 on expression of CXCR4, a chemokine receptor involved in site-specific metastasis. Presented at the Annual Meeting of the AACR; 2004 Mar 27-31; Orlando, Florida.

42. Schioppa T, Uranchimeg B, Saccani A, et al. Regulation of the chemokine receptor CXCR4 by hypoxia. J Exp Med 2003;198: 1391–402.

43. Staller P, Sulitkova J, Lisztwan J, Moch H, Oakeley EJ, Krek W. Chemokine receptor CXCR4 downregulated by von Hippel-Lindau tumour suppressor pVHL. Nature 2003;425:307–11.

44. Wiley HE, Gonzalez EB, Maki W, Wu MT, Hwang ST. Expression of CC chemokine receptor-7 and regional lymph node metastasis of B16 murine melanoma. J Natl Cancer Inst 2001;93:1638–43.

45. Henning G, Ohl L, Junt T, et al. CC chemokine receptor 7-dependent and -independent pathways for lymphocyte homing: modulation by FTY720. J Exp Med 2001;194:1875–81.

46. Okada T, Ngo VN, Ekland EH, et al. Chemokine requirements for B cell entry to lymph nodes and Peyer's patches. J Exp Med 2002;196: 65–75.

47. Stein JV, Soriano SF, M'Rini C, et al. CCR7-mediated physiological lymphocyte homing involves activation of a tyrosine kinase pathway. Blood 2003;101:38–44.

48. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet 2001;357:539–45.

49. Brigati C, Noonan DM, Albini A, Benelli R. Tumors and inflammatory infiltrates: friends or foes? Clin Exp Metastasis 2002; 19:247–58.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/11/5/1743/1963272/1743-1750.pdf by guest on 10 December 2023