Expression of Interleukin 8 and Its Receptors in Human Colon Carcinoma Cells with Different Metastatic Potentials[1]

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ABSTRACT

Purpose: **In the present study, we examined the expression of a multifunctional cytokine, interleukin 8 (IL-8), and its receptors, CXCR1 and CXCR2, in human colon carcinoma cells with different metastatic potentials and determined their role in modulating phenotypes associated with metastasis.**

Experimental Design: **IL-8, CXCR1, and CXCR2 protein and mRNA expression were examined using ELISA, immunocytochemistry, and reverse transcription-PCR in human colon carcinoma cells with different metastatic potentials. IL-8-mediated proliferation, migration, and tumor-endothelial cell interaction were analyzed.**

Results: **IL-8 mRNA and protein expression was very low in Caco2 cells but elevated in KM12C cells and very high in KM12L4 cells, suggesting an association between the IL-8 production and metastatic potential. Similarly, CXCR1 and CXCR2 expression was lower in Caco2 cells than in low and high metastatic KM12C and KM12L4 cells. The recombinant human IL-8 enhanced the proliferation of colon carcinoma cells. Furthermore, proliferation of low and high metastatic cells expressing different levels of IL-8 was inhibited by neutralizing antibodies to IL-8, CXCR1, and CXCR2. We observed significant differences in the invasive potential of colon carcinoma cells expressing different levels of IL-8. In addition, we observed that IL-8 modulates adhesion of tumor cells to endothelial cells in an autocrine and paracrine manner.**

Conclusion: **Our present data suggest an association between constitutive expression of IL-8 and aggressiveness in human colon carcinoma cells and the possible role of IL-8 in modulating different metastatic phenotypes associated with progression and metastasis.**

INTRODUCTION

IL[3]-8 is a member of the chemokine superfamily of structurally and functionally related inflammatory cytokines that stimulate the migration of distinct sets of cells including neutrophils, monocytes, lymphocytes, and fibroblasts (1–4). CXCR1 and CXCR2, also named IL-8RA and IL-8RB, are receptors for IL-8. CXCR1 and CXCR2 both bind IL-8 with high affinity, but CXCR2 also binds to other CXC chemokines (5–9). Recent studies demonstrated that IL-8 regulates tumor cell growth and metastasis in melanoma (10), carcinoma cells of lung, stomach, pancreas, liver, gall bladder (11, 12), and prostate cancer (13). It also regulates angiogenesis in human bronchogenic carcinoma (14), squamous cell carcinoma (15), and non-small cell lung carcinoma in nude mice (16). Expression of CXCR1 and CXCR2 receptors was found in keratinocytes, fibroblasts, endothelial cells, melanoma, and colon cancer cells (9, 11, 17, 18). These receptors have also been implicated in angiogenic responses to IL-8 and neutrophil and lymphocyte migration (5, 7, 9, 17, 19).

Metastasis is a complex process influenced by genetic, biochemical, immunological, and biological changes and influenced by tumor-host interaction (20). Several growth factors and cancer-associated genes, such as epidermal growth factor, carcinoembryonic antigen, and type IV collagenase, are involved in colon carcinoma metastases (21). We have reported that IL-8 expression correlates with tumor growth and metastatic potential in melanoma cells (10, 22, 23). Recently, several studies report that IL-8 up-regulates inflammatory responses, tumor cell proliferation, and migration in colon epithelial cell lines (17, 24–26). However, whether the expression of IL-8 is related to the metastatic potential in colon carcinoma cells remains unclear.

The purpose of this study was to examine the expression of IL-8 and its receptors in colon carcinoma cells with different metastatic potentials and determine the role of IL-8 in modulating phenotypes associated with tumor progression and metastasis. We examined the expression of IL-8, CXCR1, and CXCR2 and their roles in proliferation of colon carcinoma cells with different metastatic potentials. Our data suggested that constitutive expression of IL-8 in colon carcinoma cells is associated with metastatic potential. Furthermore, these studies demonstrated that IL-8 might act as an autocrine/paracrine growth factor in colon cancer progression and metastasis.

MATERIALS AND METHODS

Cell Lines and Reagents. The human Caco2 cell line isolated from primary colonic tumor (27, 28) was obtained from

American Type Tissue Culture (ATCC, Manassas, VA). The heterogeneous low metastatic human KM12C cell line was originally established in culture from a Duke's stage B2 primary tumor surgical specimen (29, 30). The high metastatic KM12L4 cell line is a subclone derived from KM12C in nude mice (29). Human umbilical vein endothelial cells (HUVEC) were obtained from American Type Culture Collection. The cell lines were maintained in culture as an adherent monolayer in MEM supplemented with 10% fetal bovine serum, sodium pyruvate, nonessential amino acids, L-glutamine, 2-fold vitamin solution, and gentamicin (Life Technologies, Inc., Gaithersburg, MD). HUVECs were additionally supplemented with bFGF and endothelial cell growth factor. All cultures were free of *Mycoplasma* and pathogenic murine viruses. Cultures were maintained for no longer than 4 weeks after recovery from frozen stocks.

ELISA for Human IL-8. IL-8 levels in culture supernatants were determined using ELISA-paired antibodies purchased from Endogen, Inc. (Woburn, MA). This assay is a quantitative "sandwich" enzyme immunoassay. One hundred µl of the primary monoclonal antibody against IL-8 (2 µg/ml) were coated in Nunc Maxisorp plates in each well. After overnight incubation at 4°C, the plates were washed and blocked for 1 h with blocking buffer (4% BSA in PBS). After the plates were washed four times, 50 µl of culture supernatant or recombinant IL-8 protein at different concentrations (Endogen, Inc.) and 50 µl of biotinylated IL-8 antibody were added to each well. After 2 h of incubation, the plates were washed, and the immunoreactivity was determined using the avidin-HRP-TMB detection system (Dako Corp., Carpinteria, CA). The reactions were stopped by addition of 50 µl of 0.18 N H_2SO_4 and absorbance was determined using an ELISA microtiter plate reader (Bio-Tek Instruments, Inc., Winooski, VT) at 450 nm. A curve of the absorbance *versus* the concentration of IL-8 in the standard wells was plotted. By comparing the absorbance of the samples to the standard curve, we determined the concentration of IL-8 in the unknown samples.

Anaylsis by ICC. CXCR1 and CXCR2 expression by colon carcinoma cells with different metastatic potentials was analyzed by ICC. Briefly, cells (1×10^5) were plated into four-well chamber slides (Nunc, Inc., Naperville, IL). The cells were cultured for 72 h and used for immunostaining. Slides were washed twice with PBS and fixed with 4% glutaraldehyde for 10 min. After washing twice with PBS, cells were incubated with a blocking solution containing 5% normal horse serum in PBS for 20 min at room temperature. Excess blocking solution was drained, and samples were incubated with a 1:200 dilution of anti-CXCR1 or anti-CXCR2 antibodies (PharMingen, San Diego, CA) overnight at 4°C in a humidified chamber. The samples were then rinsed four times with PBS and incubated with a 1:500 dilution of biotinylated horse anti-mouse/rabbit IgG (Vector Laboratories, Burlingame, CA). The immunoreactivity was detected using the ABC Elite kit and 3,3'-diaminobenzidine substrate kit (Vector Laboratories) according to manufacturer's instructions. A reddish-brown precipitate in the cytoplasm indicated a positive reaction. Negative controls used all reagents except the primary antibody.

mRNA Analysis. Total cellular RNA was isolated from colon carcinoma cell lines (1×10^6 cells) using Trizol reagent (Life Technologies, Inc.). RT-PCR was performed as described earlier (31), and cDNAs were synthesized using total RNA (2

µg), oligo(dT)$_{12-18}$ primer, and superscript RT (Life Technologies, Inc.). Two µl of first-strand cDNA (1:10 dilutions) were amplified using PCR primer sets: IL-8 sense, ACA TAC TCC AAA CCT TTC CAC CC; IL-8 antisense, CAA CCC TCT GCA CCC AGT TTT C; CXCR1 sense, GAG CCC CGA ATC TGA CAT TA; CXCR1 antisense, GAG CCC CGA ATC TGA CAT TA; CXCR2 sense, ATT CTG GGC ATC CTT CAC AG; CXCR2 antisense, TGC ACT TAG GCA GGA GGT CT; β-actin sense, TGA AGT GTG ACG TGG ACA TC; and β-actin antisense, ACT CGT CAT ACT CCT GCT TG, and DNA thermal cycler (Perkin-Elmer, Foster City, CA) for different cycles. Each cycle set used a denaturing temperature (94°C) for 60 s, annealing temperatures (57°C) for 90 s, and extension temperature (72°C) for 90 s for a total of 35 cycles for β-actin and 40 cycles for the other genes. PCR fragments were separated on a 2% agarose gel containing ethidium bromide (0.5 µg/ml), visualized, and photographed, and relative intensity of the specific gene expression was determined using Alpha-Image Analysis system (Alpha Innotech, San Leandro, CA). Gene expression was expressed as expression index, the ratio of each signal to the signal from the housekeeping gene β-*actin*.

In Vitro **Proliferation Assay.** Colon carcinoma cells (5×10^3) were seeded into 38-mm^2 wells of 96-well flat-bottomed plates in quadruplicate and allowed to adhere overnight. The cultures were then washed and refed with medium alone (control) or medium containing different treatments for the duration of incubation. After treatment, proliferative activity was determined by the MTT assay (32) using a microtiter plate reader (Bio-Tek Instruments, Inc., Winooski, VT) at 570 nm. Growth stimulation/inhibition was calculated as the percentage of growth stimulation/inhibition: $[1 - (A/B)] \times 100$, where A is the absorbance of treated cells, and B is the absorbance in untreated control cells.

Migration Assay. Colon carcinoma cell migration in response to IL-8 was determined as described earlier (33, 34). Transwell chambers (6.5 mm; Corning Costar Corp., Cambridge, MA) with polycarbonate membrane containing 8.0 µm pores were coated with Matrigel (Collaborative Biomedical, Bedford, MA). Colon carcinoma cells (5×10^4) were plated onto Transwell chambers with medium (serum-free) alone or medium containing fibroblast cell conditioned medium (positive control) in duplicate and incubated at 37°C in 5% CO_2 incubator for 4 h. MTT was added, and cells were incubated for an additional 2 h. Cells from the top of the Transwell chambers were removed using cotton swab (residual cells). Cotton swabs containing residual cells and Transwell chamber (migrated cells) were placed in 24-well plates containing 400 µl of DMSO. After 1 h of gentle shaking, 100 µl of samples were removed, and absorbance was determined at 570 nm using an ELISA plate reader. The percentage of migratory activity was calculated as: percentage of migration $= A/[(A + B) \times 100$, where A is the absorbance of migrated cells, and B is the absorbance of residual cells.

Adhesion of Tumor Cells to Endothelial Cell Assay. ^{51}Cr-labeled tumor cells (1×10^4) were added to confluent HUVEC-coated wells (96-well plate) in quadruplicate and incubated with medium alone or medium containing different treatments for 2 h. Nonadherent cells were removed by washing twice with PBS and lysed with 1% SDS, and the residual
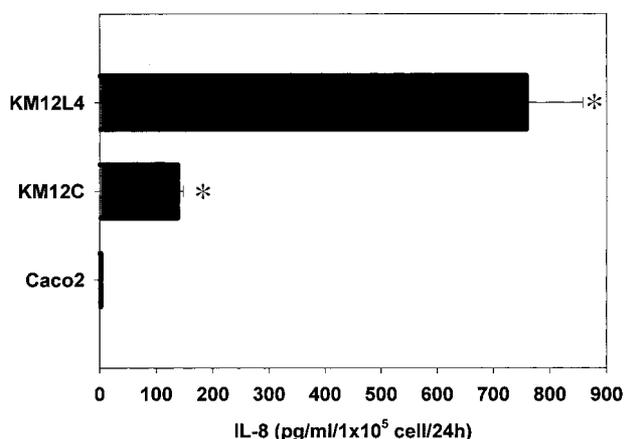
Fig. 1 Expression of IL-8 protein in human colon carcinoma cells with different metastatic potentials. Culture supernatants were harvested from 1×10^5 cells after 24 h of incubation and analyzed by ELISA. The values are means of triplicate cultures; *bars,* SE. This is a representative of two experiments done in triplicate. ∗, significantly different from Caco2 cells ($P < 0.05$).

radioactivity was analyzed using a gamma counter (Packard, Downers Grove, IL). The percent of binding is: $[(A/B) - 1] \times 100$, where A is the cpm of treated cells, and B is the cpm in untreated control cells.

Statistical Analysis. The significance of the data was determined by the Student's t test (two-tailed) using SPSS software (SPSS, Inc., Chicago, IL). $P < 0.05$ was deemed significant.

RESULTS

Expression of IL-8 in Colon Carcinoma Cells. In the first set of experiments, we analyzed the production of IL-8 protein in human colon carcinoma cells with different metastatic potentials. Cell-free culture supernatant was harvested from 1×10^5 cells with 24 h culture, and IL-8 protein level was determined by ELISA. Very low levels of IL-8 protein were detected in Caco2 cells (1.7 pg/ml; Fig. 1). High metastatic KM12L4 cells expressed the highest level of IL-8 protein (758 pg/ml), whereas low metastatic KM12C cells expressed lower levels of IL-8 (137 pg/ml; Fig. 1; $P < 0.05$).

Next, we analyzed the mRNA expression of IL-8 in nonmetastatic and low and high metastatic colon carcinoma cell lines by RT-PCR (Fig. 2A). Total cellular RNA was isolated from 1×10^6 cultured cells. The mRNA expression level of IL-8 was quantitated as an expression index (Fig. 2B). We did not observe IL-8 mRNA expression in Caco2 cells, whereas IL-8 mRNA levels were higher in KM12L4 than in KM12C cells. Different IL-8 protein levels and mRNA expression suggest that constitutive expression of IL-8 in human colon carcinoma cells correlates with metastatic potential.

Expression of CXCR1 and CXCR2 in Colon Carcinoma Cells. In the next set of experiments, we analyzed the expression of CXCR1 and CXCR2 by ICC (protein) and RT-PCR (mRNA). Colon carcinoma cells (1×10^5 cells/ml) were plated on four-well chamber slides. After 72 h culture, cells were fixed and immunostained with anti-CXCR1 or
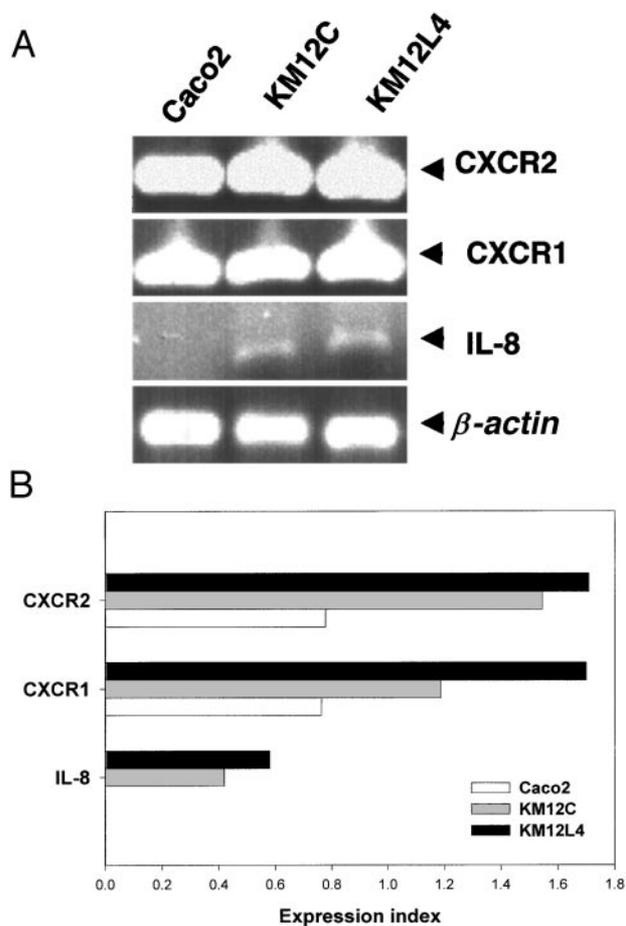

Fig. 2 Expression of IL-8, CXCR1, and CXCR2 mRNA in human colon carcinoma cells with different metastatic potentials. *A,* representative agarose-gel photograph showing IL-8, CXCR1, and CXCR2 specific mRNA transcripts amplified using RT-PCR. *B,* quantitative representation of the levels of IL-8, CXCR1, and CXCR2 mRNA. The values are presented as expression indices calculated by comparing the levels of intensity for the specific gene to the housekeeping gene β-*actin*. This is a representative of three experiments with similar results.

anti-CXCR2 antibodies. A heterogeneous pattern of CXCR1 as well as CXCR2 immunoreactivity was detected in the three colon carcinoma cell lines with different metastatic potentials (Fig. 3). Less than 10% of Caco2 cells showed CXCR1 or CXCR2 immunoreactivity. CXCR1 immunoreactivity was observed in 40% of KM12C and KM12L4 cells, whereas >60% of KM12C or KM12L4 cells bound to anti-CXCR2. Thus, expression of CXCR1 and CXCR2 was lower in Caco2 cells compared with KM12C and KM12L4 cells. We did not observe a difference in immunostaining intensity between KM12C (low metastatic) and KM12L4 (high metastatic) cells. RT-PCR analysis demonstrated that high metastatic KM12L4 cells expressed high levels of CXCR1 and CXCR2 mRNA compared with low metastatic or nonmetastatic KM12C and Caco2 cells (Fig. 2). These data suggest that colon carcinoma cells with different metastatic potentials differentially express receptors for IL-8.
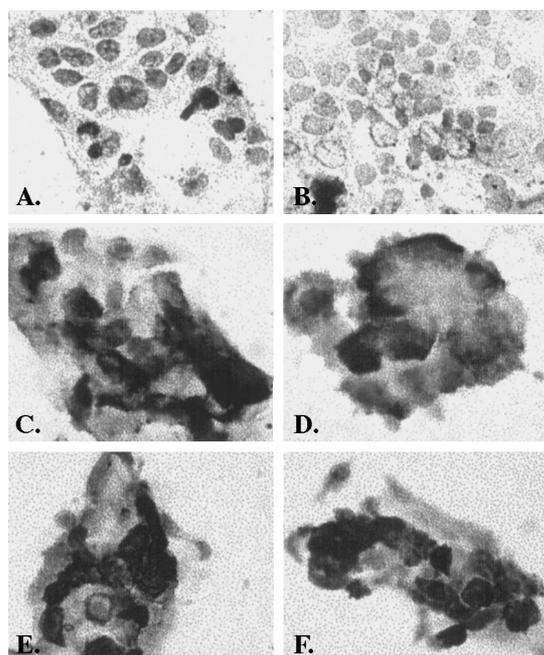
Fig. 3 Immunocytochemical staining of CXCR1 and CXCR2 in human colon carcinoma cells with different metastatic potentials. *A,* Caco2 cells with CXCR1 staining. *B,* Caco2 cells with CXCR2 staining. *C,* KM12C cells with CXCR1 staining. *D,* KM12C cells with CXCR2 staining. *E,* KM12L4 cells with CXCR1 staining. *F,* KM12L4 cells with CXCR2 staining. *A–F,* ×400.
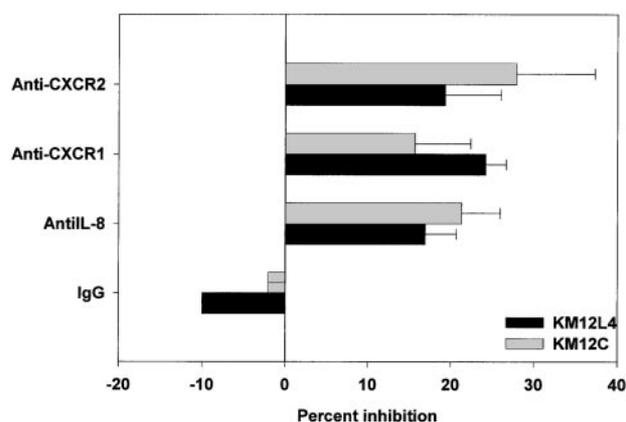

Fig. 4 Inhibition of IL-8-mediated proliferation of human colon carcinoma cells using neutralizing antibody to IL-8, CXCR1, and CXCR2. KM12C and KM12L4 cells (5×10^3) were cultured with medium alone or medium containing 0.1 μg/ml of control antibody of similar isotype, anti-IL-8, anti-CXCR1, or anti-CXCR2 for 72 h, and inhibition in cell proliferation was determined by MTT. The values represent the percentage of inhibition in cell proliferation of triplicate culture; *bars,* SE. This is a representative experiment of three done in triplicate.

Table 1 IL-8 induced stimulation of cell proliferation of human colon carcinoma cells with different metastatic potentials expressing different levels of IL-8

Caco2, KM12C, and KM12L4 cells expressing different levels of endogenous IL-8 were incubated with different concentrations of recombinant IL-8. The percentage of stimulation in cell proliferation by IL-8 was determined as described in "Materials and Methods." The values are mean % stimulation ± SE. This is representative of three experiments done in triplicate.

IL-8 (ng/ml)	% stimulation		
	Caco2	KM12C	KM12L4
0.0001	46.05 ± 13.89	22.74 ± 5.39	12.09 ± 3.86
0.001	34.19 ± 15.689	50.67 ± 5.35	17.16 ± 3.92
0.01	82.16 ± 16.73^a	40.95 ± 5.49^a	-0.99 ± 8.05
0.1	94.85 ± 19.21^a	55.49 ± 8.53^a	7.43 ± 7.12
1	105.22 ± 27.4	73.55 ± 21.69	5.23 ± 5.42
10	87.39 ± 16.02	48.87 ± 6.87^a	26.48 ± 2.05
100	100.98 ± 13.97^a	43.66 ± 9.43^a	7.82 ± 5.77

[a] Significantly different from KM12L4 cells expressing high levels of IL-8 at the corresponding concentrations of exogenously added recombinant IL-8 ($P < 0.05$).

Inhibition of IL-8-induced Proliferation by Antibody to IL-8, CXCR1, and CXCR2. To determine the function of the constitutive IL-8 expression in colon carcinoma cells, we examined whether autocrine IL-8 production and/or activity regulates colon cancer cell proliferation. KM12C (low IL-8 producer) and KM12L4 (high IL-8 producer) colon cancer cells (5000/well) were plated into 96-well plates in medium alone or medium containing neutralizing antibodies to IL-8, CXCR1, or CXCR2. Antibodies of the same isotype were used as a control. Colon carcinoma cell proliferation was inhibited by anti-IL-8, anti-CXCR1, or anti-CXCR2 in both KM12C and KM12L4 cells (Fig. 4). The percentage of inhibition ranged from 15.7 to 27.9% (Fig. 4). Effective inhibition of cell proliferation by antibodies to IL-8 and receptors CXCR1 and CXCR2 suggests that IL-8 might be an autocrine growth factor for human colon cancer cells.

Induction of Colon Carcinoma Cell Proliferation by IL-8. In the next set of experiments, we determined whether the exogenous addition of IL-8 regulates colon carcinoma cell proliferation. Caco2 (non-), KM12C (low), and KM12L4 (high) cells were incubated in medium alone or medium containing different concentrations of recombinant human IL-8. Caco2, KM12C, and KM12L4 cells treated with IL-8 showed increased cell proliferation (Table 1; Fig. 5). Stimulation of proliferation was higher in Caco2 (low IL-8 producer) or KM12C (low IL-8 producer) than in KM12L4 (high IL-8 producer) cells (Table 1). These data suggest that recombinant human IL-8 induced Caco2 and KM12C cell proliferation in a concentration-dependent

manner, and their response is inversely associated with levels of IL-8 produced by these cells.

Differential Migratory Potential of Colon Carcinoma Cells. We determined the putative role of IL-8 expression on the invasive potential of human colon carcinoma cells with different metastatic potentials. KM12C (low IL-8 expression) and KM12L4 (high IL-8 expression) were cultured in Matrigel-coated 8-μm-pore size Transwell chambers, and their migratory potential was determined. The percentage of migration was higher in KM12L4 cells (39.2 ± 6.7) as compared with KM12C cells (19.4 ± 3.5), suggesting an association between IL-8 expression and aggressiveness in colon carcinoma cells.
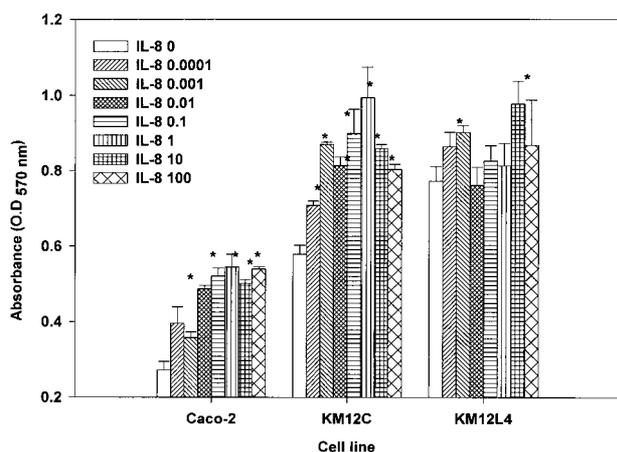
Fig. 5 Effect of exogenous IL-8 on colon carcinoma cells (5×10^3 cells/well in 96-well culture plates) with different metastatic potentials. Human colon carcinoma cells with different metastatic potentials and producing different levels of IL-8 were incubated with medium alone or medium containing different concentrations of recombinant human IL-8. After 72 h of incubation, cell growth stimulation by IL-8 was determined by the MTT assay. The values are absorbances in cell proliferation of triplicate culture; *bars,* SE. This is a representative experiment of three. ∗, significantly different from cells cultured with medium alone ($P < 0.05$).

Inhibition of Colon Carcinoma Cells Binding to Endothelial Cells. To determine the role of IL-8 in tumor-endothelial cell interaction, adhesion of colon carcinoma cells to HUVECs was examined. ^{51}Cr- labeled Caco2 and KM12C cells were plated on HUVEC-coated wells in the presence or absence of recombinant human IL-8. Treatment of cells with recombinant IL-8 enhanced colon carcinoma cells binding to HUVECs. The binding stimulation by IL-8 was >50% in low metastatic KM12C cells (Fig. 6*A*). In another set of experiments, we determined the autocrine role of IL-8 production in colon carcinoma-endothelial cell interaction by using high IL-8-producing KM12L4 cells and neutralizing antibodies against IL-8, CXCR1, and CXCR2 in different combinations. Binding of KM12L4 cells to HUVECs was inhibited by anti-IL-8, anti-CXCR1, and anti-CXCR2 alone or anti-IL-8 in combination with anti-CXCR1 and/or anti-CXCR2 (Fig. 6*B*). These results suggest a relationship between IL-8 and metastasis in colon carcinoma.

DISCUSSION

We observed a differential expression of IL-8 and its receptors, CXCR1 and CXCR2, in human colon carcinoma cells with different metastatic potentials. IL-8 protein and mRNA expression was significantly lower in nonmetastatic and low metastatic colon carcinoma cells than in high metastatic colon carcinoma cells, suggesting an association with metastatic potential. The expression of CXCR1 and CXCR2 protein and mRNA was lower in nonmetastatic Caco2 cells as compared with metastatic KM12C and KM12L4 cells. Exogenous recombinant IL-8 induced cell proliferation in colon carcinoma cells. Neutralizing anti-IL-8, anti-CXCR1, and anti-CXCR2 showed inhibition of cell proliferation in both KM12C and KM12L4
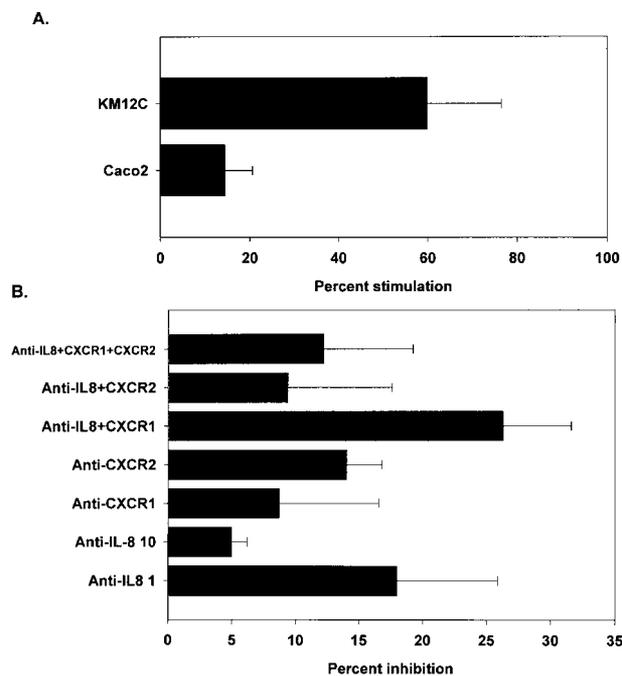

Fig. 6 IL-8-mediated regulation of human colon carcinoma cells binding to endothelial cells. ^{51}Cr-labeled Caco2, KM12C, and KM12L4 cells were plated in wells with confluent HUVEC and incubated with medium alone or medium containing recombinant human IL-8 (1 ng/ml), antibody (1 µg/ml) to IL-8, CXCR1, CXCR2 alone, or anti-IL-8 in combination with anti-CXCR1 or anti-CXCR2. *Bars,* SE. *A,* stimulation of colon carcinoma cell-HUVEC binding by IL-8. *B,* inhibition of colon carcinoma cell-endothelial cell binding by antibodies to IL-8, CXCR1, and CXCR2 in KM12L4 cells.

cells. Studies have reported that IL-8 regulates cell proliferation and angiogenesis in various tumors (10–18). Our data suggest that expression of IL-8 in colon carcinoma cells might play an important role in growth and metastasis. These data also confirm a recent study (23) that reports that IL-8 can be an autocrine factor, playing a role in colon carcinoma growth.

Primary malignant neoplasms consist of cells with different metastatic potentials expressing different levels of genes regulating metastatic phenotype (20, 35). KM12L4 is a high metastatic subclone from low metastatic KM12C cells. In the present study, we observed different IL-8 mRNA and protein expression among the three colon carcinoma cell lines with different metastatic potentials. Our data suggest that colon carcinoma cells with different metastatic potentials, constitutively expressing different levels of IL-8, respond differently to exogenous IL-8. Furthermore, neutralizing antibodies to IL-8 and its receptors inhibited the proliferation of colon carcinoma cells, suggesting that IL-8 may act as an autocrine growth factor and may contribute to metastatic potential in colon carcinoma cells.

IL-8 can bind two receptors, CXCR1 and CXCR2. CXCR1 only binds IL-8, whereas CXCR2 binds IL-8 and several other CXC chemokines (5–9). These receptors have been shown to play an important role in angiogenesis and tumor progression (3, 5, 7, 9, 36). In this study, different CXCR1 and CXCR2 mRNA and protein expression was observed in colon carcinoma cells in accordance with metastatic potential. In addition, antibodies to

CXCR1 and CXCR2 showed significant inhibition of cell proliferation in both KM12C and KM12L4 cells. These data suggest that inhibition of IL-8 production and/or activity can inhibit proliferation of colon carcinoma cells, and IL-8 may act as an autocrine or paracrine growth factor.

Tumor-endothelial cell interaction is important for tumor invasion and metastasis (37–39). A key event in cancer metastasis is transendothelial migration of tumor cells (40). This process involves multiple adhesive interactions between tumor cells and endothelial cells (35, 38). In the present study, we observed that recombinant human IL-8 enhanced adherence of colon carcinoma cells to endothelial cells. In addition, neutralization of IL-8 activity by antibodies to IL-8 and its receptors inhibited tumor cell adherence to endothelial cells in high metastatic KM12L4 cells producing higher levels of IL-8. These studies provide an additional evidence for the role of IL-8 in modulating phenotypes associated with metastasis. However, it is not clear whether IL-8-mediated regulation of adherence of colon carcinoma cells to endothelial cells requires a specific set of adhesion molecules. Studies are in progress to determine the role of adhesion molecules in IL-8-mediated tumor-endothelial cell interaction and transendothelial migration.

Our laboratory and others have demonstrated that IL-8 expression by malignant cells can induce the proliferation of tumor cells in an autocrine manner and induce angiogenesis, migration, and collagenase production, all of which are important steps in tumor growth and metastasis (23, 25, 41, 42). Several studies have reported that IL-8 regulates cell migration (25), proliferation, and angiogenesis in colon carcinoma cells (24, 25). Serum levels of IL-8 in colon cancer patients were significantly increased in patients with metastasis (43). The present study showed that constitutive expression of IL-8 in colon carcinoma cells correlated with different metastatic potentials.

In summary, we demonstrated a correlation between constitutive expression of IL-8 and its receptors in colon carcinoma cells with different metastatic potentials. Our data suggest that IL-8 may act as an autocrine/paracrine growth factor in colon carcinoma cells and modulate phenotypes associated with tumor growth and metastasis.

ACKNOWLEDGMENTS

REFERENCES

1. Matsushima, K., and Oppenheim, J. J. Novel of inflammatory cytokines inducible by IL-1 and TNF. Cytokine, *1:* 2–13, 1989.

2. Baggiolini, M., Dewald, B., and Moser, B. Human chemokines: an update. Annu. Rev. Immunol., *15:* 675–705, 1997.

3. Rossi, D., and Zlotnik, A. The biology of chemokines and their receptors. Annu. Rev. Immunol., *18:* 217–242, 2000.

4. Oppenheim, J. J., Zachariae, C. O., Mukaida, N., and Matsushima, K. Properties of the novel proinflammatory supergene "intercrine" cytokine family. Annu. Rev. Immunol., *9:* 617–648, 1991.

5. Holmes, W. E., Lee, J., Kuang, W. J., Rice, G. C., and Wood, W. I. Structure and functional expression of a human interleukin-8 receptor. Science (Wash. DC), *253:* 1278–1280, 1991.

6. Beckmann, M. P., Munger, W. E., Kozlosky, C., Vandenbos, T., Price, V., Lyman, S., Gerard, N. P., Gerard, C., and Cerretti, D. P. Molecular characterization of the interleukin-8 receptor. Biochem. Biophys. Res. Commun., *179:* 784–789, 1991.

7. Murphy, P. M., and Tiffany, H. L. Cloning of complementary DNA encoding a functional human interleukin-8 receptor. Science (Wash. DC), *253:* 1280–1283, 1991.

8. Cerretti, D. P., Kozlosky, C. J., Vanden Bos, T., Nelson, N., Gearing, D. P., and Beckmann, M. P. Molecular characterization of receptors for human interleukin-8, GRO/melanoma growth-stimulatory activity and neutrophil activating peptide-2. Mol. Immunol., *30:* 359–367, 1993.

9. Horuk, R., Chitnis, C. E., Darbonne, W. C., Colby, T. J., Rybicki, A., Hadley, T. J., and Miller, L. H. A receptor for malarial parasite *Plasmodium vivax*: the erythrocyte chemokine receptor. Science (Wash. DC), *261:* 1182–1184, 1993.

10. Singh, R. K., Gutman, M., Radinsky, R., Bucana, C. D., and Fidler, I. L. Expression of interleukin 8 correlates with the metastatic potential of human melanoma cells in nude mice. Cancer Res., *54:* 3242–3247, 1994.

11. Ishiko, T., Sakamoto, T., Yamashita, S. I., Masuda, Y., Kamohara, H., Mita, S., Hirashima, M., and Ogawa, M. Carcinoma cells express IL-8 and IL-8 receptor: their inhibition attenuates the growth of carcinoma cells. Int. J. Oncol., *6:* 119–122, 1995.

12. Kitadai, Y., Haruma, K., Sumii, K., Yamamoto, S., Ue, T., Yokozaki, H., Yasui, W., Ohmoto, Y., Kajiyama, G., Fidler, I. J., and Tahara, E. Expression of interleukin-8 correlates with vascularity in human gastric carcinomas. Am. J. Pathol., *152:* 93–100, 1998.

13. Inoue, K., Slaton, J. W., Eve, B. Y., Kim, S. J., Perrotte, P., Balbay, M. D., Yano, S., Bar-Eli, M., Radinsky, R., Pettaway, C. A., and Dinney, C. P. N. Interleukin-8 expression regulates tumorigenicity and metastases in androgen-independent prostate cancer. Clin. Cancer Res., *6:* 2104–2119, 2000.

14. Smith, D. R., Polverini, P. J., Kunkel, S. L., Orringer, M. B., Whyte, R. I., Burdick, M. D., Wilke, C. A., and Strieter, R. M. Inhibition of interleukin-8 attenuates angiogenesis in bronchogenic carcinoma. J. Exp. Med., *179:* 1409–1415, 1994.

15. Richards, B. L., Eisma, R. J., Spiro, J. D., Lindquist, R. L., and Kreutzer, D. L. Coexpression of interleukin-8 receptors in head and neck squamous cell carcinoma. Am. J. Surg., *174:* 507–512, 1997.

16. Arenberg, D. A., Kunkel, S. L., Polverini, P. J., Glass, M., Burdick, M. D., and Strieter, R. M. Inhibition of interleukin-8 reduces tumorigenesis of human non-small cell lung cancer in SCID mice. J. Clin. Investig., *97:* 2792–2802, 1996.

17. Moser, B., Barella, L., Mattei, S., Schumacher, C., Boulay, F., Colombo, M. P., and Baggiolini, M. Expression of transcripts for two interleukin 8 receptors in human phagocyte, lymphocytes and melanoma cells. Biochem. J., *294:* 285–292, 1993.

18. Yang, S. K., Eckmann, L., Panja, A., and Kagnoff, M. F. Differential and regulated expression of C-X-C, C-C, and C-chemokines by human colon epithelial cells. Gastroenterology, *113:* 1214–1223, 1997.

19. Peiper, S. C., Wang, Z. X., Neote, K., Martin, A. W., Showell, H. J., Conklyn, M. J., Ogborne, K., Hadley, T. J., Lu, Z. H., and Hesselgesser, J. The Duffy antigen/receptor for chemokines (DARC) is expressed in endothelials of Duffy negative individuals who lack the erythrocyte receptor. J. Exp. Med., *181:* 1311–1317, 1995.

20. Fidler, I. J. Critical factors in the biology of human cancer metastasis: twenty-eight G. H. A. Clowes memorial award lecture. Cancer Res., *50:* 6130–6138, 1990.

21. Singh, R. K., Tsan, R., and Radinsky, R. Influence of the host microenvironment on the clonal selection of human colon carcinoma cells during primary tumor growth and metastasis. Clin. Exp. Metastasis, *15:* 140–150, 1997.

22. Singh, R. K., Varney, M. L., Bucaca, C. D., and Johansson, S. L. Expression of interleukin-8 in primary and metastatic malignant melanoma of the skin. Melanoma Res., *9:* 383–387, 1999.

23. Singh, R. K., and Varney, M. L. IL-8 expression in malignant melanoma: implication in growth and metastasis. Histol. Histopathol., *15:* 843–849, 2000.

24. Brew, R., Southern, S. A., Flanagan, B. F., McDicken, I. W., and Christmas, S. E. Detection of interleukin-8 m RNA and protein in human colorectal carcinoma cells. Eur. J. Cancer, *12:* 2142–2147, 1999.

25. Brew, R., Erikson, J. S., West, D. C., Kinsella, A. R., Slavin, J., and Christmas, S. E. Interleukin-8 as an autocrine growth factor for human colon carcinoma cells *in vitro*. Cytokine, *12:* 78–85, 2000.

26. Wilson, A. J., Byron, K., and Gibson, P. R. Interleukin-8 stimulates the migration of human colonic epithelial cells *in vitro*. Clin Sci., *97:* 385–390, 1999.

27. Fogh, J., Fogh, J. M., and Orfeo, T. One hundred and twenty-seven cultured human tumor cell lines producing tumors in nude mice. J. Natl. Cancer Inst., *59:* 221–226, 1977.

28. De-Vires, J. E., Dinjens, W. N., De-Bruyne, G. K., Verspaget, H. W., vander Linden, E. P., de Bruine, A. P., Mareel, M. M., Bosman, F. T., and Ten-kate, J. *In vivo* and *in vitro* invasion in relation to phenotypic characteristics of human colorectal carcinoma cells. Br. J. Cancer, *71:* 271–277, 1995.

29. Morikawa, K., Walker, S. M., Jessup, J. M., and Fidler, I. J. *In vivo* selection of highly metastatic cells from surgical specimens of different human colon carcinomas implanted into nude mice. Cancer Res., *48:* 1943–1948, 1988.

30. Morikawa, K., Walker, S. M., Nakajima, M., Pathak, S., Jessup, J. M., and Fidler, I. J. Influence of organ environment on the growth, selection, and metastasis of human colon carcinoma cells in nude mice. Cancer Res., *48:* 6863–6871, 1988.

31. Singh, R. K., Ino, K., Varney, M., Heimann, D., and Talmadge, J. E. Immunoregulatory cytokines in bone marrow and peripheral blood stem cell products. Bone Marrow Transplant., *23:* 441–444, 1995.

32. Alley, M. C., Scudiero, D. A., Monks, A., Hersey, M. L., Czerwinski, M. J., Fine, D. L., Abbot, B. J., Mayo, J. G., Shoemaker, R. H., and Boyd, M. R. Feasibility of drug screening with panels of human tumor cell line using a microculture tetrazolium assay. Cancer Res., *48:* 589–602, 1988.

33. Hendrix, M. J., Seftor, E. A., Seftor, R. E., and Fidler, I. J. A simple quantitative assay for studying the invasive potential of high and low human metastatic variants. Cancer Lett., *38:* 137–147, 1987.

34. Chu, Y. W., Runyan, R. B., Oshima, R. G., and Hendrix, M. J. Expression of complete keratin filaments in mouse L cells augments cell migration and invasion. Proc. Natl. Acad. Sci. USA, *90:* 4261–4265, 1993.

35. Yokota, J. Tumor progression and metastasis. Carcinogenesis (Lond.), *21:* 497–503, 2000.

36. Kulke, R., Bornscheue, E., Schluter, C., Bartels, J., Rowert, J., Sticherling, M., and Christophers, E. The CXC receptor 2 is over-expressed in psoriatic epidermis. J. Investig. Dermatol., *110:* 90–94, 1998.

37. Nicolson, G. L. Metastatic tumor cell attachment and invasion assay utilizing vascular endothelial cell monolayers. J. Histochem. Cytochem., *30:* 214–220, 1982.

38. Cohen, M. C., Bereta, M., and Bereta, J. Effect of cytokines on tumour cell-endothelial interactions. Indian J. Biochem. Biophys., *34:* 199–204, 1997.

39. Voura, E. B., Sandig, M., and Siu, C. H. Cell-cell interactions during transendothelial migration of tumor cells. Microsc. Res. Tech. 1, *43:* 265–275, 1998.

40. Nicolson, G. L., Irimura, T., Nakajima, M., and Estrada, J. Metastatic cell attachment to and invasion of vascular endothelium and its underlying basal lamina using endothelial cell monolayers. Symp. Fundam. Cancer Res., *36:* 145–167, 1983.

41. Koch, A. E., Polverini, P. J., Kunkel, S. L., Harlow, L. A., Dipietro, L. A., Elner, V. M., Elner, S. G., and Strieter, R. M. Interleukin-8 as a macrophage-derived mediator of angiogenesis. Science (Wash. DC), *258:* 1798–1801, 1992.

42. Strieter, R. M., Kunkel, S. L., Elner, V. M., Martonyi, C. L., Koch, A. E., Polverini, P. J., and Elner, S. G. Interleukin-8. A corneal factor that induces neovascularization. Am. J. Pathol., *141:* 1279–1284, 1992.

43. Ueda, T., Shimada, E., and Urakawa, T. serum levels of cytokines in patients with colorectal cancer: possible involvement of interleukin-6 and interleukin-8 in hematogenous metastasis. J. Gastroenterol., *29:* 423–429, 1994.

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