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Expression of Interleukin-8 Correlates with Vascularity in Human Gastric Carcinomas

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Interleukin (IL)-8 is a multifunctional cytokine that can stimulate the division of endothelial cells. We examined the expression of IL-8 mRNA using Northern blot analysis and *in situ* mRNA hybridization (ISH) and protein production using enzyme-linked immunosorbent assay and immunohistochemistry in 8 human gastric carcinoma cell lines and 39 gastric carcinomas and corresponding normal mucosa (34 surgical specimens and 5 biopsy specimens). Of the 8 human gastric carcinoma cell lines, 6 expressed 1.8-kb IL-8 mRNA and secreted various levels of IL-8 protein. The expression of IL-8 by TMK-1 cells was induced by exposure to IL-1 α, epidermal growth factor, and transforming growth factor-α, shown previously to be autocrine growth stimulators for human gastric carcinoma cells. In tumor tissues, most of the tumors (28 of 34 surgical specimens and 4 of 5 biopsy specimens) expressed IL-8 at higher levels than the corresponding normal mucosa. ISH and immunohistochemical analyses revealed that IL-8 mRNA and protein were localized in the cytoplasm of tumor cells. The number of blood vessels in the gastric carcinomas was determined by using antibodies against CD34. The level of IL-8 mRNA in the neoplasms strongly correlated with vascularization (Spearman correlation, $r = 0.812$; $P = 0.001$). The data suggest that IL-8 produced by tumor cells may regulate neovascularization and, hence, the growth and spread of human gastric carcinoma. *(Am J Pathol 1998, 152:93–100)*

Angiogenesis, which is a prerequisite for tumor growth and metastasis, depends on the production of angiogenic factors by host and tumor cells.[1,2] Increased vascularity enhances the growth of primary neoplasms and provides an avenue for hematogenous metastasis. Weidner et al[3] reported a direct correlation between the incidence of metastasis and the number and density of blood vessels in invasive breast carcinomas. Similar studies have confirmed this finding in other carcinomas, including lung,[4] prostate,[5] cervical,[6] colon,[7] and gastric[8,9] carcinomas, and melanoma.[10]

Our research has focused on the relation between angiogenesis and gastric carcinomas in particular and on the effects of various cytokines on the growth and metastasis of this cancer.[11,12] We have previously reported that interleukin (IL)-1α[13] and members of the epidermal growth factor (EGF) family, including EGF, transforming growth factor (TGF)-α,[14] *cripto*,[15] and amphiregulin,[16] are overexpressed by gastric carcinoma cells and act as autocrine growth stimulators. Gastric carcinoma cells also produce angiogenic factors such as basic fibroblast growth factor (bFGF)[17] and vascular endothelial growth factor (VEGF),[9] but their exact role in the induction of neovascularization remains unclear.

Further efforts to isolate inducers of angiogenesis in gastric carcinoma have led us to IL-8. IL-8 is a multifunctional cytokine shown to induce angiogenesis,[18,19] haptotactic migration,[20] and proliferation of keratinocytes and melanoma cells.[21,22] As angiogenesis, migration, and cell proliferation are all important components of the metastatic process, the data suggest that IL-8 expression by tumor cells could influence their metastatic capacities.[23] Singh et al[24] reported that the expression level of IL-8 by human melanoma cells correlates with their metastatic potential, but the exact role of IL-8 in this process remained unclear.

To determine whether IL-8 plays a role in the progression of gastric carcinomas, we examined its expression and regulation in human gastric carcinoma cell lines and fresh tumor specimens. We also examined its expression and regulation in cells treated with cytokines, and we attempted to correlate vessel density with IL-8 production by tumors.

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Materials and Methods

Cell Cultures and Tumor Tissues

Eight cell lines established from human gastric carcinomas were maintained in RPMI-1640 (Nissui Co., Tokyo, Japan) with 10% fetal bovine serum (FBS; M. A. Bioproducts, Walkersville, MD). The TMK-1 cell line (poorly differentiated adenocarcinoma) was established in our laboratory.[25] HSC-39 and KATO-III cell lines established from signet ring cell carcinomas were kindly provided by Dr. K. Yanagihara (Hiroshima University, Hiroshima, Japan)[26] and Dr. M. Sekiguchi (University of Tokyo, Tokyo, Japan), respectively. The other five gastric carcinoma cell lines (MKN-1, adenosquamous carcinoma; MKN-7, MKN-28, and MKN-74, well differentiated adenocarcinomas; and MKN-45, poorly differentiated adenocarcinoma) were provided by Dr. T. Suzuki (Fukushima Medical College, Fukushima, Japan). Human umbilical vein endothelial cells (HUVECs) were also used.

A total of 39 cases of gastric carcinoma were examined. Surgical specimens of 34 cases and biopsy specimens of 5 cases were examined by Northern blot analysis and enzyme-linked immunosorbent assay (ELISA), respectively. There was corresponding normal tissue for each tumor sample. We confirmed microscopically using cryostat sections that the tumor tissue specimens consisted mainly of carcinoma tissue and that non-neoplastic mucosa did not exhibit any tumor cell invasion or show significant inflammatory involvement. The definition of stage grouping and the histological classification were made according to the criteria of the Japanese Research Society for Gastric Cancer.[27]

IL-1α, EGF, and TGF-α Treatment of Gastric Carcinoma Cells

Human recombinant EGF and TGF-α were kindly provided by Wakunaga Pharmaceutical Co. (Hiroshima, Japan). After 24 hours of serum starvation, 10 U/ml IL-1α (Otsuka, Tokushima, Japan) or 1 nmol/L EGF or TGF-α was added to the cultures. The TMK-1 cells were treated for 0 (control), 1, 3, and 12 hours. Five micrograms of polyadenylated RNA was subjected to Northern blot analysis. After 48 hours of treatment with EGF or TGF-α (1 nmol/L or 10 nmol/L), cultured supernatants of TMK-1 were assayed for the presence of IL-8 protein by ELISA.

Northern Blot Analysis

Polyadenylated mRNA was extracted from gastric carcinoma cell lines and surgical specimens using the FastTrack mRNA isolation kit (Invitrogen, San Diego, CA). mRNA was electrophoresed on 1% denaturing formaldehyde/agarose gel, electrotransferred at 0.6 A to a GeneScreen nylon membrane (DuPont, Boston, MA), and ultraviolet cross-linked with 120,000 mJ/cm^2 using a UV Stratalinker 1800 (Stratagene, La Jolla, CA). Hybridizations were performed as described previously.[28] Nylon filters were washed at 65°C with 30 mmol/L NaCl, 3 mmol/L sodium citrate (pH 7.2), and 0.1% sodium dodecyl sulfate (w/v).

The cDNA probes used in these analyses were a 1.3-kb *Pst*I cDNA fragment corresponding to rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH; Clontech, Palo Alto, CA) and a 0.5-kb *Eco*RI cDNA fragment corresponding to human IL-8 (kindly provided by Dr. N. Mukaida, Kanazawa, Japan). Each cDNA fragment was purified by agarose gel electrophoresis, recovered using GeneClean (BIO 101, La Jolla, CA), and radiolabeled using the random primer technique with ^{32}P-labeled deoxyribonucleotide triphosphates.[29]

ELISA for IL-8 Protein

Tissue specimens were pooled and immediately homogenized in 2.0 ml of phosphate-buffered saline (pH 7.4). Supernatants, obtained by centrifugation (1800 rpm for 10 minutes), were frozen until assayed. IL-8 levels in cell-free culture supernatants from different gastric carcinoma cells and homogenates of gastric biopsy specimens were assayed by ELISA as described previously,[30] using an IL-8 monoclonal antibody. The detection limit of the assay was 20 pg/ml, and inter- and intra-assay variations were 4.1 to 4.5% and 7.9 to 9.3%, respectively.

Immunohistochemical Staining (IHC)

Archival paraffin blocks of 20 cases were available. Consecutive 4-μm sections were cut from each study block. Sections were immunostained for IL-8 and CD34 (specific for endothelial cells). IHC was performed by the immunoperoxidase technique with minor modification.[8,31] Antibodies used were a rabbit polyclonal antibody (Otsuka, Tokushima, Japan) at a 1:200 dilution for IL-8 and a mouse monoclonal antibody (Nichirei, Tokyo, Japan) for CD34. Negative controls were done using nonspecific IgG as the primary antibody.

In Situ *mRNA Hybridization (ISH) Analysis*

ISH was performed as described previously[32,33] with minor modification. Briefly, an IL-8-specific oligonucleotide probe was designed complementary to the 5′ end of human IL-8 mRNA transcript.[24] The DNA oligonucleotide sequence 5′-CTC-CAC-AAC-CCT-CTG-CAC-CC-3′ was of the antisense orientation and hence complementary to IL-8 mRNA.[24] To verify the integrity and lack of degradation of mRNA in each sample, we used a d(T)$_{20}$ oligonucleotide. All DNA probes were synthesized with six biotin molecules (hyperbiotinylated) at the 3′ end via direct coupling using standard phosphoramidite chemistry (Research Genetics, Huntsville, AL). ISH was carried out using the Microprobe manual staining system (Fisher Scientific, Pittsburgh, PA).[34,35] A positive reaction in this assay stained red. Control for endogenous alkaline phosphatase included treatment of sample in the absence of the biotinylated probe and use of chromogen alone. To check the specificity of the hybridization signal, the following controls were used: RNAse pretreatment of tissue sections, substitution of the antisense probe with a biotin-

labeled sense probe, and competition assay with unlabeled antisense probes. No or markedly decreased signals were obtained after either of these treatments.[24]

In Vitro *Growth Assay*

Cells (5×10^3) were plated into multiple 38-mm^2 wells of 96-well plates (Falcon Laboratories, McLean, VA) in RPMI medium without serum in the absence or presence of recombinant human IL-8 (Otsuka, Tokushima, Japan). The cells were cultured for 2 days, and their proliferation was determined by an MTT assay.[36] Fifty microliters of MTT (40 μg/ml) was added to each well, incubated for 1 hour, aspirated, and dissolved in dimethylsulfoxide. The intensity of color adduct formation was measured using an ELISA plate reader. The percentage of increase in cell growth was calculated as follows: % of growth stimulation = $(B - A)/A \times 100$, where A is the A_{540} of the control cultures and B is the A_{540} of test cultures.

Evaluation of Vessel Counting

The vessel count was assessed by light microscopy in areas of the tumor containing the highest numbers of capillaries and small venules at the invasive edge. The highly vascular areas were identified by scanning tumor sections at low power (×40 and ×100). After six areas of highest neovascularization were identified, vessel count was performed on a ×400 field (40× objective and 10× ocular), and the average counts of the six fields were determined. As Weidner et al[3] described, vessel lumens were not necessary for a structure to be defined as a vessel. The microvessels were counted by two investigators (Y. Kitadai and S. Yamamoto) who had no knowledge of the IL-8 expression of the tumors.

Statistical Analysis

Differences in IL-8 protein levels were analyzed by Student's *t*-test. Correlation between the microvessel counts and the IL-8 expression was examined by the Spearman rank correlation coefficient.

Results

Expression and Secretion of IL-8 by Human Gastric Carcinoma Cell Lines

The expression of IL-8 mRNA and secretion of IL-8 protein into cultured medium by gastric carcinoma cell lines were examined by Northern blot analysis and ELISA, respectively. Of eight gastric carcinoma cell lines, six constitutively expressed a 1.8-kb IL-8 mRNA at various levels (Figure 1A). In most cultured human gastric carcinoma cells, IL-8 protein production directly correlated with mRNA expression levels (Figure 1B). KATO-III, MKN-1, and MKN-7 cells expressed IL-8 mRNA and secreted IL-8 protein into cultured medium at high levels, whereas IL-8 production by MKN-45 and TMK-1 was very low. IL-8 mRNA and protein were below detectable levels in HSC-39 cells. Southern blot analysis was performed to detect potential alterations in the IL-8 gene. Neither gene amplification nor rearrangement was observed in the gastric carcinoma cell lines (data not shown).

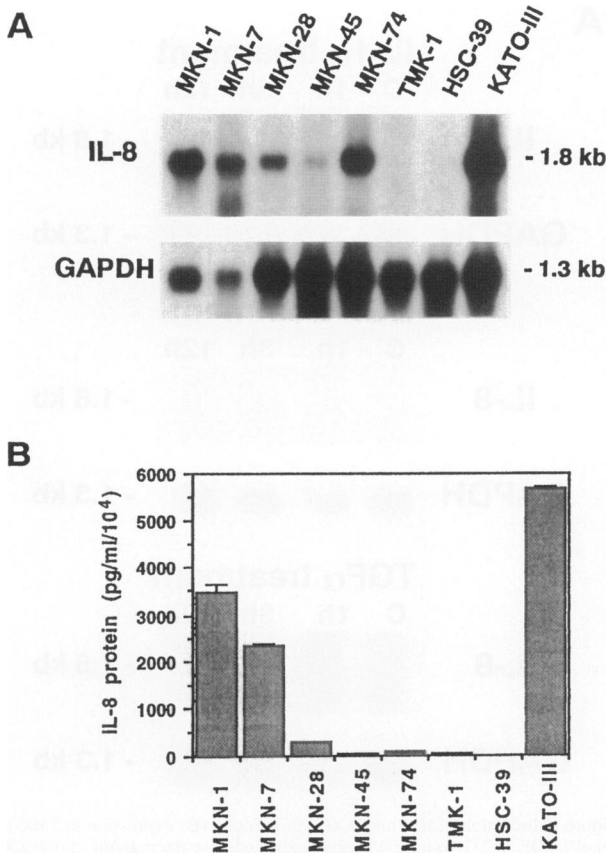

Figure 1. Expression of IL-8 mRNA (**A**) and protein (**B**) by gastric carcinoma cell lines. **A:** Five micrograms of polyadenylated selected RNA was subjected to Northern blot analysis by using ^{32}P-labeled IL-8 cDNA as described in Materials and Methods. A GAPDH probe was used as an internal control. **B:** Gastric carcinoma cells were incubated in supplemented medium containing 10% FBS. Culture supernatants were collected after 48 hours and assayed for the presence of IL-8 by ELISA as described in Materials and Methods. Values are the mean ± SE of duplicate samples.

Induction of IL-8 by IL-1α, EGF, and TGF-α

It is known that various types of inflammatory stimuli, including IL-1 and tumor necrosis factor (TNF), can induce IL-8 production by a wide variety of cells.[37,38] Gastric carcinoma cell lines express IL-1α, EGF, and TGF-α, which act as autocrine growth stimulators.[13,14] These factors induce the expression of various growth factor/receptor genes by gastric carcinoma cells.[13,14,16] Therefore, we examined whether IL-1α, EGF, and TGF-α induced the expression of IL-8 by TMK-1 cells (low endogenous IL-8). The time course of IL-8 mRNA induction was determined by Northern blot analysis. The expression of IL-8 mRNA in TMK-1 cells was apparently induced by IL-1α incubation for 1 hour (Figure 2A). EGF and TGF-α also induced the expression of IL-8 mRNA by TMK-1 cells; detectable levels appeared 1 hour after

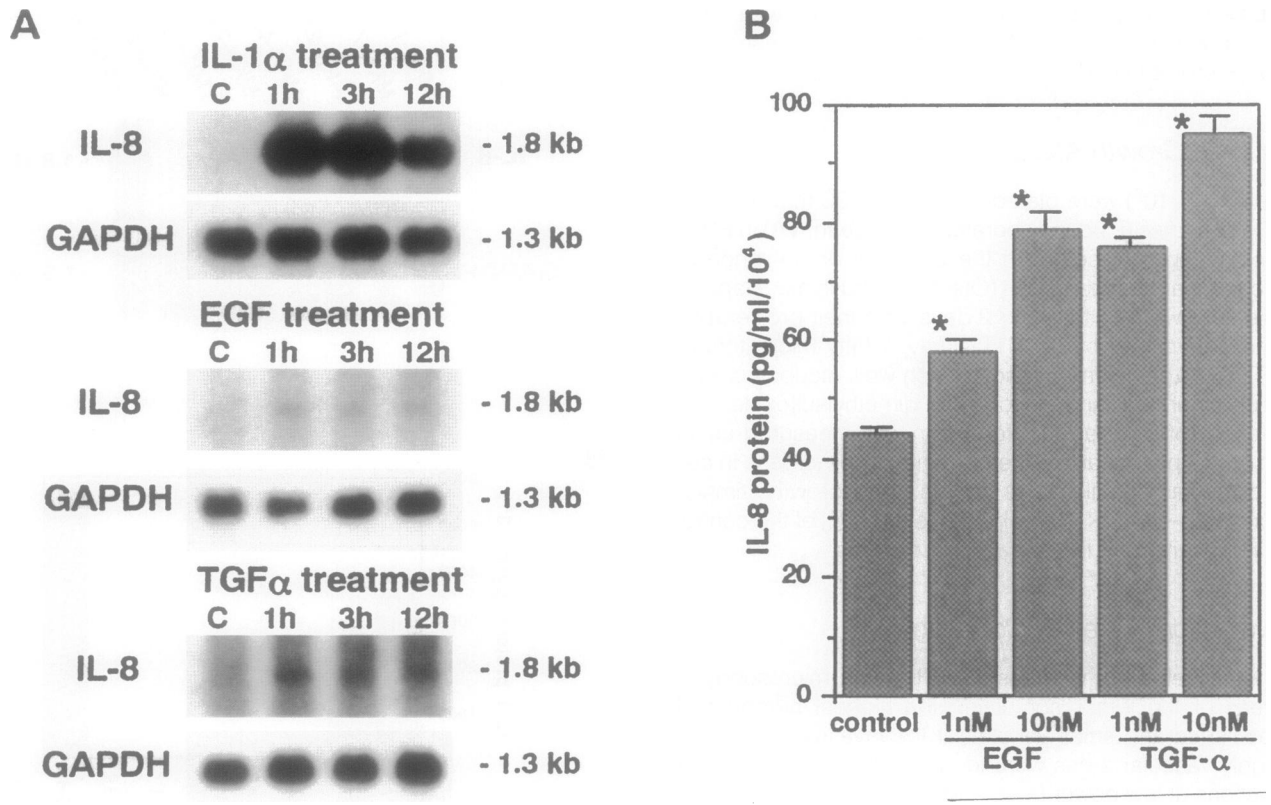

Figure 2. Induction of IL-8 mRNA (**A**) and protein (**B**) expression in TMK-1 cells by IL-1α, EGF, and TGF-α. **A:** TMK-1 cells were treated with 10 U/ml IL-1α, 1 nmol/L EGF, or TGF-α for the periods indicated. Five micrograms of polyadenylated selected RNA was subjected to Northern blot analysis. A GAPDH probe was applied as an internal control. **B:** TMK-1 cells were cultured for 48 hours with 10% FBS in the presence of indicated concentrations of EGF and TGF-α. Culture supernatants were assayed for the presence of IL-8 by ELISA as described in Materials and Methods. Values are the mean ± SE of triplicate samples. *Significantly different from control by Student's *t*-test ($P < 0.01$).

treatment and continued until 12 hours after treatment (Figure 2A). To confirm the IL-8 induction by EGF and TGF-α, IL-8 protein levels in culture supernatants were measured by ELISA 48 hours after the treatment. The IL-8 protein level was significantly increased by EGF and TGF-α treatment ($P < 0.01$; Figure 2B).

Effect of IL-8 on Proliferation of Gastric Carcinoma Cell Lines

It has been reported that IL-8 is an autocrine growth factor for melanoma cells.[22,24] We next analyzed whether IL-8 could stimulate the growth of gastric carcinoma cells and endothelial cells. TMK-1 (low endogenous IL-8), MKN-1 (high endogenous IL-8), and endothelial-derived HUVECs were cultured in the presence or absence of recombinant IL-8. Cell proliferation was determined 48 hours later by MTT assay. No significant difference was observed in the proliferation of either TMK-1 or MKN-1 cells in the presence or absence of exogenous IL-8 (Table 1). In contrast, recombinant IL-8 remarkably stimulated the *in vitro* growth of HUVECs.

Expression of IL-8 in Tumor Tissues

We next examined the level of IL-8 mRNA in 34 surgical specimens by Northern blot analysis. The clinicopathological findings and the IL-8 mRNA ratio between gastric carcinoma tissues and corresponding normal mucosal are summarized in Table 2. Representative autoradiographs of Northern blot analysis for IL-8 are shown in Figure 3. The specific IL-8 transcript was detected not only in the tumor tissues but also in the corresponding normal mucosa. IL-8 mRNA was expressed in the gastric carcinoma tissues at various levels. In 28 of the 34 cases (82.4%), the level of IL-8 mRNA in the tumor tissue was higher than that in the corresponding normal mucosa (T/N > 1.0). To confirm these results at protein levels, we measured IL-8 protein levels of gastric biopsy specimens

Table 1. Effect of IL-8 on *in Vitro* Growth Analysis of Gastric Carcinoma Cells

IL-8 (ng/ml)	Growth stimulation (% of control)		
	TMK-1 cells	MKN-1 cells	HUVEC cells
0 (control)	100 ± 3.1	100 ± 2.5	100 ± 33.4
0.1	101.3 ± 4.4	102.3 ± 3.5	267.2 ± 23.7
1.0	101.8 ± 2.0	103.6 ± 4.2	312.8 ± 27.5

TMK-1, MKN-1, and HUVEC cells (5×10^3 cells/well) were incubated with medium alone or medium containing different doses of recombinant human IL-8. After 48 hours, growth stimulation was determined by the MTT assay as described Materials and Methods. Values are mean ± SD of triplicate cultures. This is one representative experiment of three. The percentage of growth stimulation was calculated as compared with the data of cells without treatment.

Table 2. Clinical Features and IL-8 mRNA Levels in 34 Primary Human Gastric Carcinomas

Case	Age (years)	Sex	Histology*	Stage†	mRNA expression‡ (T/N ratio)	Vessel counts in the tumor
226	59	M	Well	1	2.9	
243	79	M	Well	1	5.2	50
244	72	M	Well	2	4.8	60
245	51	M	Well	2	1.0	54
246	44	M	Well	2	5.1	59
248	73	M	Well	2	10.3	65
304	36	M	Well	4	2.7	
504	53	M	Well	3	3.7	27
526	67	F	Well	3	1.0	40
528	79	F	Well	3	10.8	
4502	52	M	Well	3	15.3	91
4803	58	F	Well	2	1.0	31
216	71	F	Poorly	3	7.2	
219	27	F	Poorly	4	1.0	26
220	63	F	Poorly	3	4.2	
249	45	F	Poorly	4	8.6	63
289	79	F	Poorly	4	4.2	36
291	62	M	Poorly	3	1.4	
292	72	F	Poorly	4	1.0	
294	37	F	Poorly	4	3.4	
295	51	F	Poorly	4	4.2	
303	61	M	Poorly	4	1.0	
309	59	M	Poorly	4	2.9	50
507	62	F	Poorly	4	3.4	
508	58	M	Poorly	4	2.9	
509	63	F	Poorly	2	4.3	
514	50	M	Poorly	4	1.2	44
523	71	M	Poorly	3	1.4	36
524	57	F	Poorly	4	1.3	20
525	55	M	Poorly	3	1.3	25
527	41	F	Poorly	2	3.4	41
529	41	M	Poorly	2	1.5	
1957	42	M	Poorly	4	1.9	58
5390	70	F	Poorly	4	8.6	78

M, male; F, female.
*According to Japanese Classification of Gastric Cancer.[25] Well, well differentiated adenocarcinoma including papillary and tubular adenocarcinoma; poorly, poorly differentiated adenocarcinoma including signet ring cell carcinoma and mucinous adenocarcinoma.
†According to Japanese Classification of Gastric Cancer.[25]
‡The ratio of densitometric measurements of autoradiographic signals from Northern blot analysis in colon carcinoma tissues (T) and corresponding normal tissues (N).

by ELISA. In four of the five cases, mucosal IL-8 protein levels were higher in the tumor tissues than the corresponding normal mucosa (Table 3).

Localization of IL-8 in Gastric Carcinoma Tissues

To confirm the expression of IL-8 mRNA and protein in cancer cells, ISH and IHC were performed on the gastric carcinoma cell lines and tissues. MKN-1 cells (high endogenous IL-8) demonstrated an intense histochemical reaction with the IL-8-specific antisense probe (Figure 4A) and IL-8 antibody (Figure 4B), but TMK-1 cells (low endogenous IL-8) did not (data not shown). In surgical

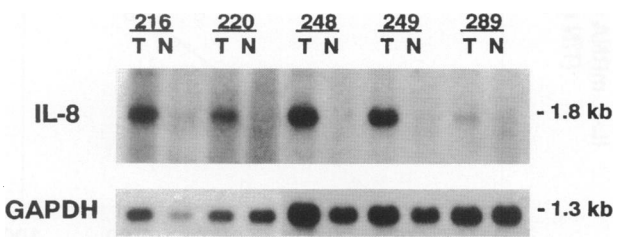

Figure 3. Expression of IL-8 mRNA in human gastric carcinomas. Five micrograms of polyadenylated selected RNA was analyzed as in Figure 1. The signal intensity was measured by densitometric scanning. Numbers above the lanes are case numbers. T, tumor tissue; N, corresponding normal mucosa. A GAPDH probe was applied as an internal control.

Table 3. Clinical Features and IL-8 Protein Levels in Five Primary Human Gastric Carcinomas

Case	Age (years)	Sex	Histology*	IL-8 protein levels (pg/μg protein)† Tumor tissue	IL-8 protein levels (pg/μg protein)† Normal mucosa
B1	58	M	Poorly	0.60	0.22
B2	42	F	Poorly	0.14	0.13
B3	69	M	Poorly	1.60	UD
B4	72	M	Poorly	12.7	0.26
B5	52	M	Well	10.6	0.29

M, male; F, female; UD, undetectable level.
*According to Japanese Classification of Gastric Cancer.[25] Well, well differentiated adenocarcinoma including papillary and tubular adenocarcinoma; poorly, poorly differentiated adenocarcinoma including signet ring cell carcinoma and mucinous adenocarcinoma.
†IL-8 protein levels of the biopsy specimens were assayed by ELISA as described in Materials and Methods.

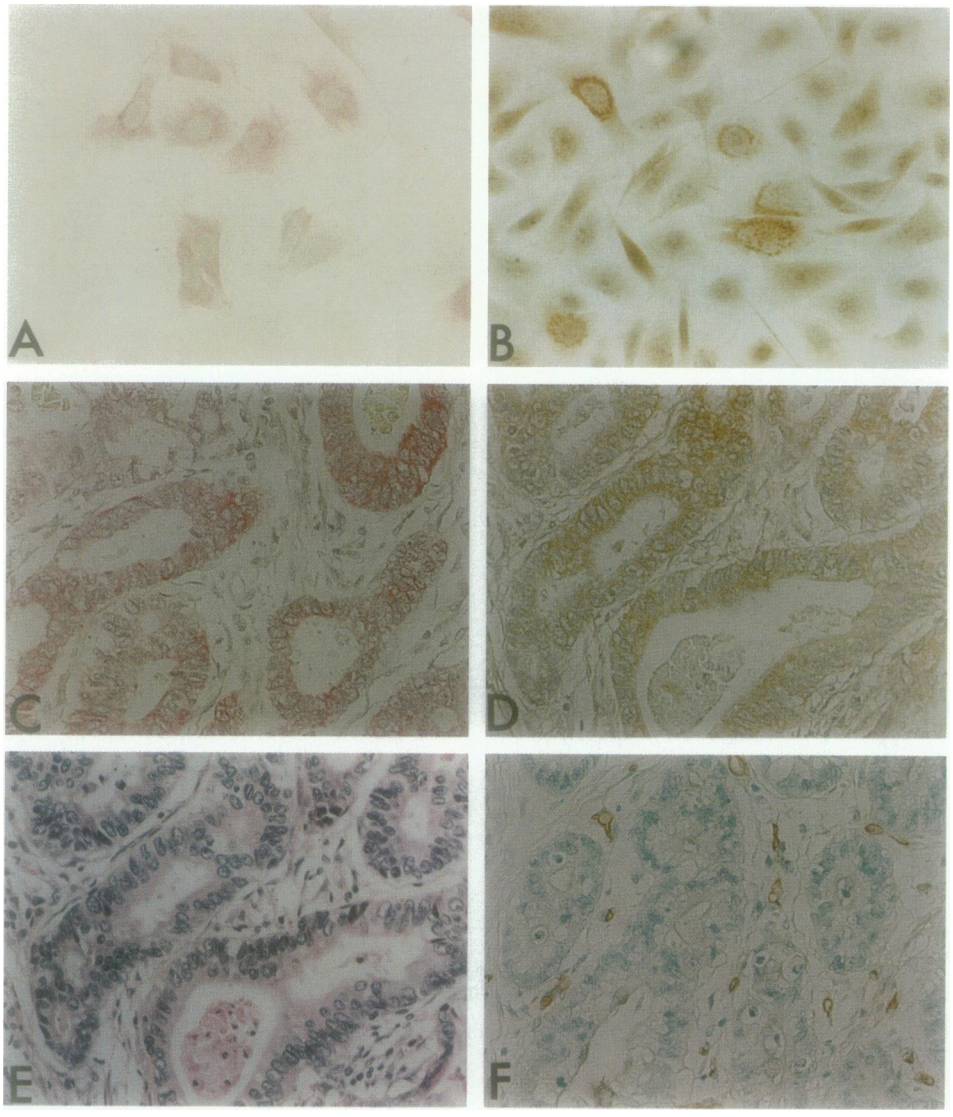

Figure 4. ISH (**A** and **C**) and IHC (**B** and **D**) for IL-8 of MKN-1 cells (**A** and **B**) and surgical tissue (case 243; **C** and **D**). IL-8 mRNA and protein are detected within cytoplasm of tumor cells. **E** and **F**: Hematoxylin and eosin (H&E) staining (**E**) and IHC of endothelial cells with antibodies against CD34 (**F**) for case 243. Magnification, ×250.

tissues, IL-8 mRNA and protein were detected in variable numbers of cancer cells (Figure 4, C and D). Normal epithelial cells and normal stromal cells showed minimal IL-8 staining with ISH and IHC (data not shown).

Correlation between Vessel Count and IL-8 mRNA Expression

To prove that IL-8 is an important angiogenic factor for human gastric carcinoma, we next performed immunohistochemistry against CD34 (endothelial cell specific), counted microvessel number, and correlated it with the levels of IL-8 mRNA expression. Archival paraffin sections of 20 cases were available for this experiment. A representative picture of immunohistochemistry for CD34 is shown in Figure 4F. As shown in Figure 5, a strong correlation between tumor vessel count and IL-8 mRNA

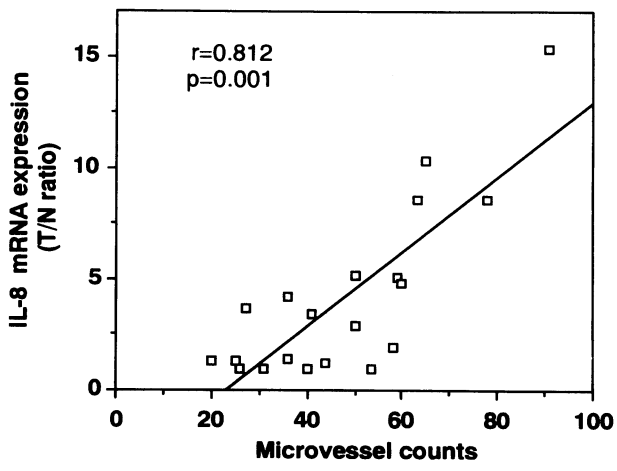

Figure 5. Relationship between IL-8 mRNA expression and microvessel counts obtained by staining for CD34. Spearman rank correlation test demonstrated that $r = 0.812$ ($P = 0.001$).

expression was observed (Spearman correlation r = 0.812; P = 0.001).

Discussion

Recent studies have demonstrated that tumor vascularization directly correlates with the prognosis of patients with breast carcinoma,[3] lung carcinoma,[4] prostatic carcinoma,[5] malignant melanoma,[10] and gastric carcinoma.[8] Numerous angiogenic factors have been described, but the ones responsible for angiogenesis in gastric carcinoma remain unknown. One of the possibilities, IL-8, was originally identified as a leukocyte chemoattractant.[37,38] As is the case with most cytokines, subsequent work has uncovered its multifunctionality. IL-8 can induce migration in some tumor cells[20] and has been implicated in the induction of angiogenesis in such diverse diseases as psoriasis,[39] rheumatoid arthritis,[40] and some malignant disease.[1,2] IL-8 is a known angiogenic factor for human lung carcinoma[41,42] and is also produced by melanoma,[24] bladder,[43] and prostate[44] carcinomas. Arenberg et al[45] has recently reported that IL-8 acts as a promoter of tumor growth for human non-small-cell lung carcinoma through its angiogenic properties.

In the present study, expression of IL-8 was examined in gastric carcinoma. IL-8 mRNA was constitutively expressed by most gastric carcinoma cell lines at various levels. In the surgical specimens of gastric carcinoma, 82.4% (28 of 34) exhibited higher levels of IL-8 mRNA than the corresponding normal mucosa. These results were confirmed at the protein level. IHC and ISH analyses showed that IL-8 was mainly produced by gastric carcinoma cells in the tumor tissues. More interestingly, we found that the levels of IL-8 mRNA strongly correlated with the number of microvessels in the tumor area (Spearman correlation, r = 0.812; P = 0.001). Therefore, IL-8 may be responsible for angiogenesis in human gastric carcinoma.

It has been reported that IL-8 induces proliferation of keratinocytes and melanoma cells.[21,22] IL-8 acts as an autocrine growth factor for melanoma cells, and its expression directly correlates with metastatic potential in nude mice.[24] We examined the effect of IL-8 on proliferation, but a stimulatory effect was observed only in HUVECs (endothelial cells), not in TMK-1 and MKN-1 cells (gastric carcinoma cell lines). This finding suggests that IL-8 produced by cancer cells may act as a paracrine factor for endothelial cells but not as an autocrine growth factor.

Most of the gastric carcinoma tissues overexpressed IL-8 mRNA as compared with their corresponding normal mucosa; however, no gross alteration of the IL-8 gene was detected by Southern blot analysis (data not shown). Previous studies have shown that IL-8 expression is regulated by the specific organ microenvironment[31] and that IL-1 and TNF-α induce IL-8 expression.[37,38] The present study demonstrates that exogenous IL-1α, EGF, and TGF-α induce IL-8 expression by TMK-1 cells. Gastric carcinoma cells express endogenous EGF, TGF-α,[14] and IL-1[13] as well, which might result in the constitutive overexpression of IL-8 in tumor cells via an autocrine mechanism. In addition, the levels of EGF, TGF-α, and EGF receptor are well correlated with biological malignancy of gastric carcinoma.[11,14] Therefore, EGF and TGF-α may act not only as autocrine growth factors but also as inducers of angiogenic factor expression; these mechanisms may explain how they promote tumor growth *in vivo*.

Previous studies have shown that *Helicobacter pylori* is etiologically associated with gastric carcinoma.[46–48] IL-8 production in gastric mucosa is significantly increased by *H. pylori* infection, and eradication of *H. pylori* results in a decrease in IL-8 level.[49] Incubation with *H. pylori* results in the significant induction of IL-8 mRNA and protein by gastric carcinoma cell lines.[50] Uemura et al[51] recently reported that *H. pylori* eradication inhibits the growth of the intestinal type of gastric carcinoma; however, the mechanism is not known. It would be of great interest to elucidate whether *H. pylori* eradication decreases IL-8 expression levels and the number of vessels in a tumor.

In conclusion, this study has demonstrated that IL-8 expression levels correlate with vessel density in gastric carcinoma. Our study was performed with a relatively small number of fresh specimens. A large study of archival materials should determine whether the levels of IL-8 directly correlate with disease recurrence and patient survival. The identification of factors that correlate with angiogenesis in gastric carcinoma may provide a basis for experiments targeting these angiogenic factors to inhibit vascularization of tumors. It is possible that IL-8 may be used as a target for antiangiogenic therapy in the future.

Acknowledgments

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