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Interleukin 8 and vascular endothelial growth factor — prognostic factors in human gastric carcinomas?

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Abstract

Gastric carcinoma cells express potent angiogenic factors including vascular endothelial growth factor (VEGF). We previously reported that interleukin-8 (IL-8) acts as an angiogenic factor for human gastric carcinomas. More recently, we found that IL-8 upregulates matrix metalloproteinase-9 (MMP-9) expression and increases invasive activity of gastric carcinoma cells. The purpose of this study was to determine whether the expression of IL-8 and VEGF correlates with clinicopathological parameters in human gastric carcinomas. IL-8 and VEGF expression levels were measured by an enzyme-linked immunosorbent assay (ELISA) in 56 gastric carcinomas and the surrounding normal mucosa. Macroscopic and histopathological tumour findings, presence of metastasis and prognosis were obtained from the patient records and endoscopic, surgical and pathological reports. IL-8 protein levels were higher in most neoplasms than in the corresponding normal mucosal tissue. In contrast, VEGF expression in the tumours was similar to that in normal mucosa. The IL-8 level in the neoplasms correlated significantly with the depth of invasion, venous invasion and lymphatic invasion. VEGF expression in the tumours correlated well with the depth of invasion and lymph node metastasis. No correlation between IL-8 and VEGF expression in the tumours was observed. The survival rates of patients with tumours displaying high IL-8 and VEGF expression levels were significantly lower ($P<0.05$) than those of patients with tumours displaying low IL-8 and VEGF expression. The results suggest that IL-8 and VEGF may be independent and important prognostic factors in human gastric carcinomas.

Keywords: Angiogenesis; Gastric carcinoma; IL-8; VEGF

1. Introduction

Angiogenesis is required for tumour growth and progression and it is involved in metastasis [1–3]. The process results from an imbalance between positive and negative angiogenic regulators released by both tumour cells and host cells [4]. Tumour vascularisation correlates directly with the prognosis of patients with breast carcinoma [5], lung carcinoma [6], prostatic carcinoma [7], malignant melanoma [8], and gastric carcinoma [9].

Gastric carcinoma is one of the most common malignancies in the world and is the leading cause of death in Japan. Gastric carcinoma cells express several angiogenetic factors such as basic fibroblast growth factor (bFGF) [10] and vascular endothelial growth factor (VEGF) [11,12]. We previously reported that gastric carcinoma cells express interleukin-8 (IL-8) and that IL-8 expression levels correlate directly with tumour vascularity [13]. More recently, we found that IL-8 up-regulates matrix metalloproteinase-9 (MMP-9) expression and increases the invasive activity of gastric carcinoma cells [14]. VEGF expression has also been shown to correlate with tumour vascularity in well-differentiated type (intestinal type) gastric adenocarcinomas [12].

We investigated whether IL-8 and VEGF expression levels play a role in the progression of gastric carcinoma. We also investigated whether these angiogenic factors were associated with various clinicopathological features.

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2. Patients and methods

2.1. Patients and tumour specimens

This study included 56 randomly selected Japanese patients with primary gastric cancer who underwent surgical or endoscopic mucosal resection at Hiroshima University Hospital between 1996 and 1998. Patients ranged in age from 36 to 83 years (mean 62.1 years). Of these patients, 31 (55%) had stage I disease, 13 (23%) had stage II disease, and 12 (21%) had stage III disease. The distribution of pathological data for the entire population is listed in Table 1. Patients treated preoperatively with cytotoxic drugs were not included in this study. 22 patients (39%) were treated with tegaful at 600 mg/day after surgery. At the time of endoscopy, at least two biopsy specimens were taken from the gastric carcinoma and the surrounding normal mucosa. One specimen was snap-frozen in liquid nitrogen for enzyme-linked immunosorbent assay (ELISA) and the other was placed in formalin and processed for routine histopathology. Tumour stage and histological classification were determined according to the criteria of the Japanese Research Society for Gastric Cancer [15].

2.2. Immunohistochemical staining (IHC)

Consecutive 4-μm sections were cut from each specimen. Sections were immunostained for IL-8 and VEGF. Immunohistochemical staining was performed by the immunoperoxidase technique following trypsinisation. Primary antibodies included rabbit anti-IL-8 polyclonal IgG (Otsuka, Tokushima, Japan) diluted 200-fold in phosphate-buffered saline (PBS) [13] and rabbit anti-VEGF polyclonal IgG (Santa Cruz Biotechnology, Santa Cruz, CA) diluted 300-fold in PBS [11]. Non-specific staining was determined with IgG from normal rabbit serum.

Table 1
IL-8 and VEGF expression levels with respect to clinicopathological features of gastric carcinoma

Clinicopathological feature		IL-8 pg/mg-protein	VEGF pg/mg-protein
Histological type			
Intestinal	(31)	2406.7±742.4	123.1±18.1
Diffuse	(25)	938.2±272.2	158.9±27.8
Depth of invasion			
m	(10)	312.9±138.7*	102.1±16.0
sm	(14)	2266.4±1273.9*	80.9±22.3*
mp, ss	(32)	1949.3±514.6*	179.0±22.8*
Lymphatic invasion			
Positive	(40)	2415.4±718.4*	141.9±19.8
Negative	(16)	443.9±128.3*	83.0±12.8
Venous invasion			
Positive	(32)	2252.3±815.6*	156.1±25.1
Negative	(24)	1264.3±568.7*	87.7±9.8
Lymph node metastasis			
Positive	(23)	2599.6±1111.2	183.9±20.2*
Negative	(33)	1259.8±453.8	92.5±11.2*

IL-8, interleukin-8; VEGF, vascular endothelial growth factor; m, mucosa; sm, submucosa; mp, muscularis propria; ss, subserosa.
Numbers in parentheses are number of cases.
*$P<0.05$.

2.3. ELISA

Tissue specimens were pooled and immediately homogenised in 2.0 ml of PBS (pH 7.4). Levels of IL-8 and VEGF in homogenates of gastric biopsy specimens were assayed by ELISA, as previously described [16], with IL-8 and VEGF monoclonal antibodies. The assay detection limit was 20 pg/ml, and intra-assay variation was 4.1–4.5% for IL-8 and 7.9–9.3% for VEGF.

2.4. Statistical analysis

Statistical significance was determined by Student *t*-test, or Mann–Whitney *U*-test. Survival rates were calculated starting from the day of surgery. Overall survival curves were drawn according to the Kaplan–Meier method, and differences were analysed by the Log-rank test. A multivariate analysis was performed using logistic regression to investigate the independence of the risk factors identified as significant by univariate analyses. The significance level was set at 5% for all analyses.

3. Results

3.1. IL-8 and VEGF expression in gastric carcinoma

We initially examined IL-8 and VEGF protein levels in human gastric carcinomas using ELISA. Levels of IL-8 protein were significantly higher in most neoplasms (1736.3±436.0) than in normal gastric mucosa (142.6±22.3), but the levels of VEGF expression were

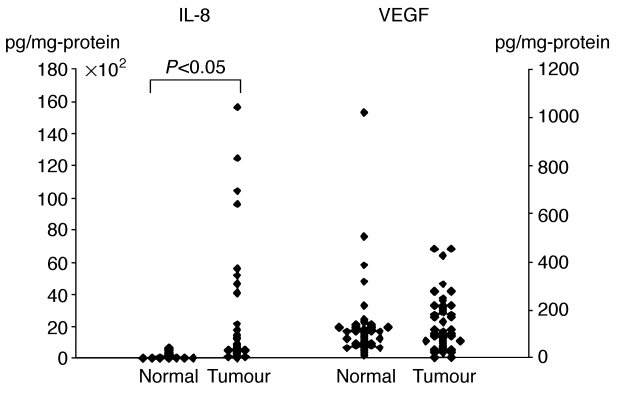

Fig. 1. The level of IL-8 expression (but not VEGF expression) is significantly higher in carcinoma tissues than in normal corresponding gastric mucosa. The left scale is for IL-8 content, the right scale is for VEGF content.

similar in both the tumours and normal mucosa (Fig. 1). No correlation (Spearman correlation $r=0.096$, $P<0.05$) between IL-8 and VEGF expression in the tumours was observed.

3.2. Localisation of IL-8 and VEGF in the gastric carcinoma tissues

IHC of cancer cells revealed that both IL-8 and VEGF were localised in the cytoplasm (Fig. 2). Faint VEGF immunoreactivity was also present in some fibroblasts, smooth muscle cells, inflammatory cells and vascular endothelial cells. In contrast, control sections showed minimal staining in normal gastric epithelium (data not shown). These results agree with our previous findings from *in situ* mRNA hybridisation analysis. [11,13].

3.3. IL-8, VEGF and clinicopathological characteristics

Expression of IL-8 in the gastric carcinoma tissues significantly correlated with the depth of invasion, venous invasion and lymphatic invasion (Table 1). VEGF expression in the tumours correlated with depth of invasion and lymph node metastasis. There was no correlation between IL-8 or VEGF expression and histological type.

3.4. Correlation between IL-8 or VEGF expression and survival rate

To determine whether IL-8 or VEGF expression predicts prognosis in patients with gastric carcinoma, we selected 56 patients that were followed-up in our hospital, and a Kaplan–Meier analysis was performed. The mean follow-up time for the 44 surviving patients was 21.8 months (range 1.5–39.4 months). The remaining 12 patients died between 0.8 and 21.3 months (mean 9.6 months). The survival rate of patients with high tumour VEGF expression was significantly lower than that of patients with low tumour VEGF expression (Fig. 3b). The group with high IL-8 expression showed a poorer prognosis, but statistical significance was not demonstrated (Fig. 3a). All patients with low levels of IL-8 and VEGF showed no recurrence of disease (Fig. 3c).

In the univariate analyses, three factors were found to be related to poor prognosis: venous invasion ($P=0.022$), lymph node metastasis ($P=0.001$), and the expression of VEGF ($P=0.032$) (Table 2). In the multivariate analyses, lymph node metastasis was again found to be significantly associated with poor prognosis. However, the expression of VEGF and venous invasion showed non-significant trends.

4. Discussion

Angiogenesis involves the formation of new blood vessels from the existing vasculature. This process is essential for tumour growth and metastasis and is controlled by chemical signals called angiogenic factors [4]. The complex process of angiogenesis is believed to involve the breaking down of the basement membrane of an existing vessel, migration of endothelial cells and the formation of a new basement membrane for the new vessel [17]. It is likely that multiple factors stimulate or inhibit angiogenesis. Some angiogenic factors act

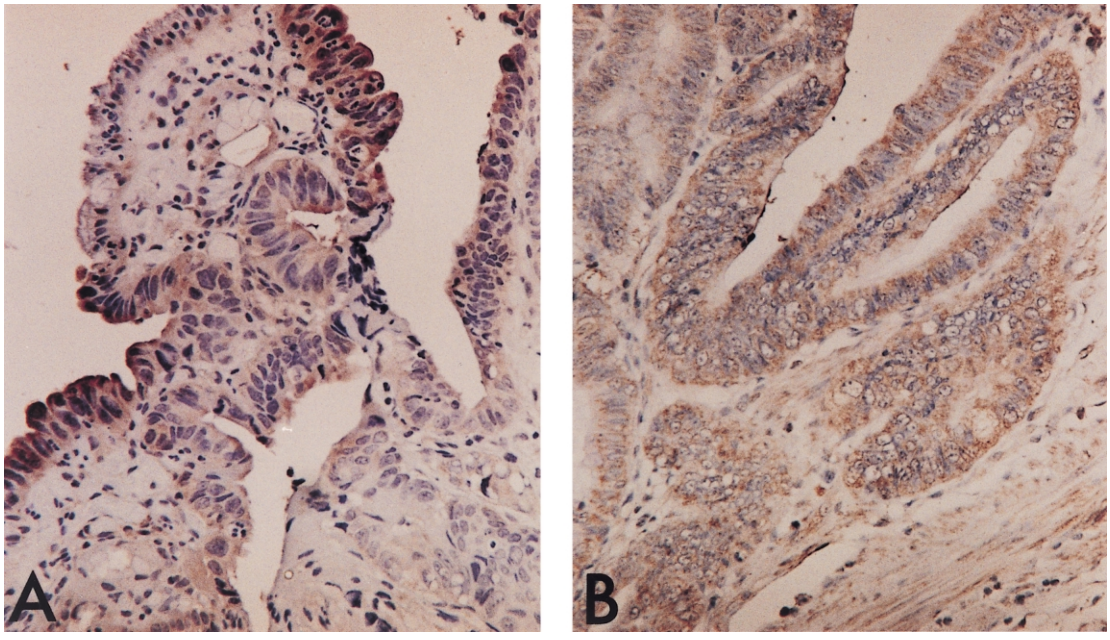

Fig. 2. Representative staining of IL-8 (a) and VEGF (b) in the cytoplasm of the tumour cells. Faint VEGF immunoreactivity was present in some of the fibroblasts, smooth muscle cells, inflammatory cells and vascular endothelial cells.

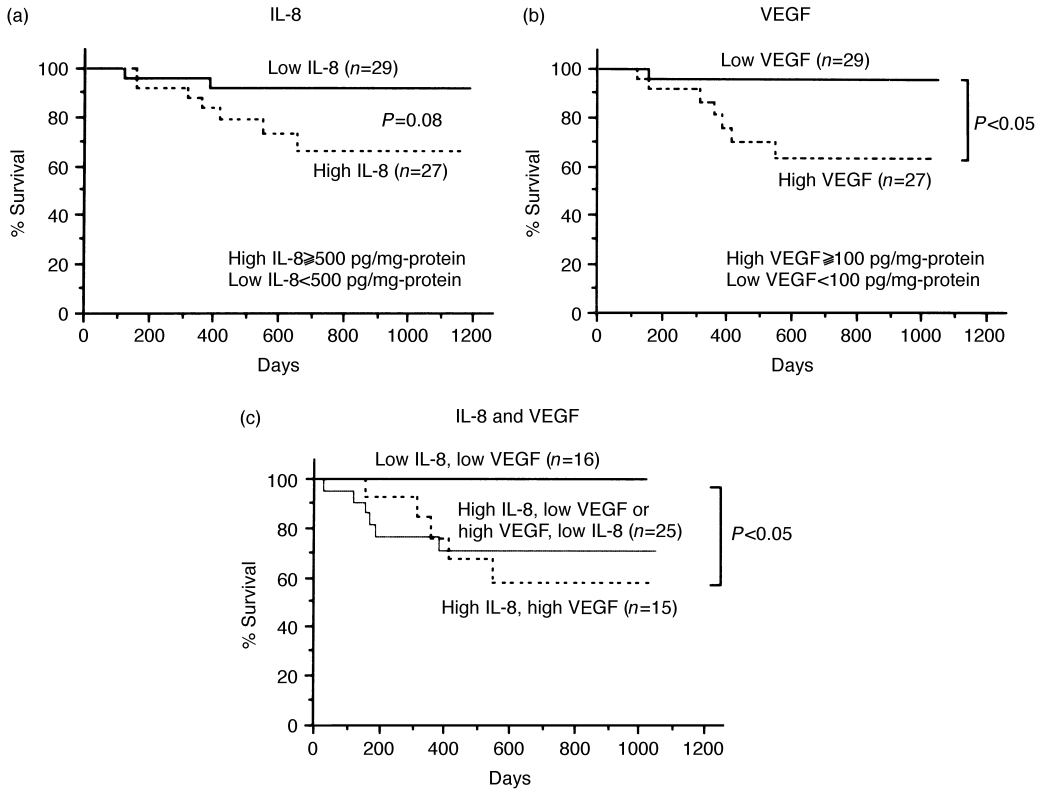

Fig. 3. Overall survival of patients after therapy. The survival rate of patients with high IL-8 and high VEGF expression in the tumours was significantly lower than that of patients with low IL-8 and low VEGF expression in the tumours.

Table 2
Univariate analysis of prognostic factors for patients with gastric carcinoma

Prognostic factors	*P* value
Histological type	0.201
Venous invasion	0.022
Lymphatic invasion	0.165
Lymph node metastasis	0.001
Expression of IL-8 (< 500 pg/mg-protein or more)	0.145
Expression of VEGF (< 100 pg/mg-protein or more)	0.032

IL-8, interleukin-8; VEGF, vascular endothelial growth factor.

directly on endothelial cells, other factors act indirectly via local inflammatory cells.

A variety of growth factors and cytokines are reported to regulate one or more key angiogenetic events. IL-8 is a multifunctional cytokine originally identified as a leucocyte chemoattractant [18,19]. Studies have revealed that IL-8 can also induce haptotactic migration of tumour cells [20], proliferation of keratinocytes and melanoma cells [21,22], and angiogenesis [23,24]. Human recombinant IL-8 can induce proliferation and migration of human umbilical vein endothelial cells and is potently angiogenic when implanted in rat cornea. IL-8 is a known angiogenic factor for human lung [25], prostate [26], and bladder carcinomas [27]. We recently found that surgical specimens of human gastric carcinomas overexpressed IL-8 in comparison to corresponding normal mucosa and that *IL-8* mRNA levels correlated directly with tumour vascularity [13]. Furthermore, transfection of gastric carcinoma cells with the *IL-8* gene enhanced the tumorigenic and angiogenic potential of these cells in the gastric wall of nude mice [28]. However, whether IL-8 expression is associated with clinicopathological parameters such as tumour stage and prognosis has not been reported.

VEGF, one of the most important angiogenic factors, is a dimeric glycoprotein with a relative mobility from 34 to 50 KDa and is synthesised by both tumour and normal cells [29]. VEGF functions in a paracrine fashion by interacting with the specific endothelial cell receptors flt-1 and KDR [30]. It is also a strong permeability factor [31]. We have previously reported that the signals of VEGF expression were detected in cytoplasm of tumour cells by both *in situ* mRNA hybridisation and IHC [11]. Vessel density correlated with VEGF expression and the presence of endothelial KDR in intestinal-type gastric carcinoma [12]. Although IL-8 and VEGF have been characterised individually, nothing is known about coexpression of these factors in human gastric carcinomas.

In this study, we examined IL-8 and VEGF expression levels in gastric carcinomas to elucidate their role in tumour progression. Consistent with our previously reported findings, IL-8, (but not VEGF), expression was significantly higher in tumours than in normal mucosa.

The level of IL-8 in the neoplasms correlated significantly with the depth of invasion, venous invasion and lymphatic invasion. VEGF expression was enhanced at more advanced tumour stages, and the expression level correlated with lymph node metastasis. We could not observe relationship between IL-8 and VEGF protein levels, indicating the expression of these angiogenic factors is likely to be independent. Furthermore, the survival rate of patients with higher levels of IL-8 and VEGF in the tumours was significantly lower than that of patients with lower IL-8 and VEGF expression levels in the tumours.

In the univariate analyses, VEGF but not IL-8 expression was found to be related to poor prognosis (Table 2). Although there was a tendency for shorter survival in the high IL-8 group, this correlation did not reach statistical significance (Fig. 3). Because IL-8 is a multifunctional cytokine which can act as a chemoattractant, immunological reaction between tumor cells and host cells may influence prognosis of the patients.

O'Brien and colleagues identified at least two distinct angiogenic pathways, the platelet derived-endothelial cell growth factor (PD-ECGF) pathway in invasive carcinoma and the VEGF pathway in early superficial carcinoma, in different stages of bladder cancer [32]. Eisma and coworkers showed that human head and neck squamous carcinoma concurrently express IL-8 and VEGF, and that the levels of these factors were associated with disease progression [33]. These findings suggest that concurrent increases in the levels of several angiogenic factors are important to disease progression.

In conclusion, we demonstrated that gastric carcinoma cells express both IL-8 and VEGF and that high expression levels are associated with advanced disease and poor prognosis. Therefore, measurement of IL-8 and VEGF in tumour tissues may be used to predict disease recurrence and the prognosis of gastric carcinoma patients.

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