The role of circulating IL-8 and VEGF protein in the progression of gastric cancer

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Both vascular endothelial growth factor (VEGF) and interleukin 8 (IL-8) play an important role in the progression of gastric cancer (GC). In this study, we investigated whether circulating levels of VEGF or IL-8 in drainage veins of GC patients were correlated with any clinicopathological factors. Thirty-seven patients with primary GC who underwent gastrectomy at our department between 1999 and 2002 were analyzed. Blood samples were drawn from a peripheral vein just before surgery and from a drainage vein immediately after laparotomy. Plasma VEGF levels were significantly higher than those in 10 healthy controls. There was no correlation between VEGF levels in drainage veins and any clinicopathological variable, whereas there was a significant relationship in the case of VEGF levels in peripheral veins; the levels were higher in patients with venous invasion. We found a significant relationship between IL-8 levels in drainage veins and both tumor size and lymph node metastasis, whereas no significant relationship between IL-8 levels in peripheral veins and any variable was found. There was no correlation between VEGF and IL-8 levels in drainage veins. Large tumors, deeply invasive tumors, lymph node involvement, venous invasion and high IL-8 levels in drainage veins were all significantly associated with shorter disease-free survival, although multivariate analysis revealed that lymph node involvement was the only independent prognostic factor. In conclusion, the measurement of IL-8 levels in drainage veins of GC patients may reflect production mainly by the primary lesion and is valuable as an indicator of risk for recurrent disease. (Cancer Sci 2003; 94: 735–740)

Vascular endothelial growth factor (VEGF) and interleukin 8 (IL-8) contribute to the progression of gastrointestinal tumors, as well as to the induction of tumor angiogenesis.[1–3] VEGF has been reported to enhance the permeability of tumor vessels,[4] to induce serine protease or metalloproteases,[5, 6] and to inhibit apoptosis of endothelial cells[7, 8] and maturation of dendritic cells.[9] IL-8, a member of the C-X-C cytokine family, is produced by various cells, including neutrophils, macrophages, endothelial cells, and cancer cells. IL-8 has mitogenic activity, enhances cell adhesion, and upregulates metalloprotease-9.[10] Many clinical studies have shown that the protein levels of VEGF or IL-8 are correlated with clinicopathological factors and that they have a significant impact on survival. The relationship of VEGF levels to metastasis and/or poor outcome has been demonstrated in patients with various malignancies, including breast cancer,[11] small cell lung cancer,[12] gastrointestinal tumors (colorectal cancer, CRC),[13, 14] clear cell renal cancer,[15] ovarian cancer,[16, 17] hepatocellular carcinoma,[18] esophageal cancer,[19, 20] nasopharyngeal carcinoma[21] and gastric cancer (GC).[22] Recently the plasma concentration of VEGF was demonstrated to be more reliable for assessing metastasis, survival or tumor angiogenesis than the serum concentration in several cancers,[23–26] since the serum level was influenced by VEGF released from platelets and white blood cells during clotting.[27] A series of studies concerning the relationship of plasma IL-8 levels with clinicopathological factors in patients with solid tumors found that high levels of IL-8 were correlated with some clinicopathological factors, and that the patients tended to have a poor prognosis, although the effect was not statistically significant.[28]

On the other hand, there have been only a few studies of VEGF or IL-8 protein levels in the drainage veins of patients with solid tumors. In CRC, high IL-8 levels in the drainage veins were correlated with liver metastasis.[29] In this study, we investigated whether circulating VEGF or IL-8 levels in drainage veins of GC patients were correlated with any clinicopathological factors, and whether they had any impact on survival, since the role of IL-8 in the progression of GC, among various gastrointestinal malignancies, has been identified most clearly.[28–30]

Materials and Methods

Patients. Thirty-seven patients with primary GC who underwent gastrectomy at the Second Department of Surgery, Hamamatsu University School of Medicine, between 1999 and 2001 were analyzed. The patients ranged in age from 25 to 83 years (average: 59.2 years), and there were 27 men and 10 women. None of the patients had received chemotherapy or radiotherapy before surgery. Ten healthy volunteers ranging in age from 27 to 48 years (average: 34.2 years) were also studied as controls. The Japanese Classification of Gastric Carcinoma was used for pathological diagnosis and for the classification of variables. Ten patients had stage Ia cancer, 6 patients stage Ib, 6 patients stage II, 6 patients stage IIIa, 3 patients stage IIIb, and 6 patients stage IV. Informed consent was obtained from each patient for the procedure.

Blood samples. Blood samples from a peripheral vein for measurement of circulating VEGF were drawn on the morning of the operation day, just before surgery. Immediately after laparotomy, blood samples from a drainage vein were drawn from one of the marginal veins that branched directly to the stomach in which the primary lesion was located.

Both sets of blood samples were collected in tubes with and without sodium ethylenediaminetetraacetic acid (EDTA-Na), then centrifuged immediately at 3000 rpm for 10 min, and the plasma samples were stored at −80°C until used for the measurement of VEGF and IL-8. All samples were used for assay within a few months.

Measurement of plasma VEGF and serum IL-8 levels. The samples were analyzed for VEGF and IL-8 by a quantitative sandwich enzyme immunoassay technique, using an ELISA kit for VEGF (Quantikine human VEGF, R&D Systems, Minneapolis, MN), and an ELISA kit for IL-8 ("PeliKine Compact," TFB Inc., Amsterdam, The Netherlands). The limits of sensitivity were 9.0 pg/ml for VEGF and 1.0 pg/ml for IL-8.

Statistical analysis. Results are expressed as mean±SE. The relationships between circulating levels of VEGF and IL-8 and various clinicopathological factors were analyzed using the Mann-Whitney *U* test. The correlation between the levels in pe-

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Table 1. Relationship between circulating VEGF levels and clinicopathological variables in 37 patients with GC

Variables	No. of patients	VEGF levels (pg/ml)			
		Drainage veins	*P* value	Peripheral veins	*P* value
Total	37	38.8±42.8		45.0±46.6	
Gender					
men	27	32.5±28.4		34.7±29.5	
women	10	55.9±67.5	0.141	72.7±70.8	0.0252
Histology					
differentiated	12	31.2±22.8		35.6±30.0	
others	25	42.5±49.7	0.462	49.5±52.7	0.4055
Tumor size					
≤40 mm	18	43.2±53.6		37.2±37.1	
>40 mm	19	34.7±30.2	0.5576	52.3±54.1	0.3319
Depth of tumor					
≤mp	16	43.1±56.1		39.4±39.9	
>mp	21	35.6±30.2	0.6040	49.2±51.7	0.5319
Lymph node involvement					
negative	18	41.6±52.2		40.6±39.1	
positive	19	36.2±32.8	0.7064	49.1±53.5	0.5853
Lymphatic invasion					
negative	16	36.4±30.8		32.9±24.1	
positive	21	40.7±50.8	0.7652	54.2±57.2	0.1726
Venous invasion					
negative	20	29.5±29.1		29.5±28.8	
positive	17	49.9±53.6	0.1510	63.1±57.0	0.0267
MVD					
≤5/field	23	31.8±29.7		47.9±50.8	
>5/field	13	52.0±59.9	0.1819	36.2±38.8	0.4797

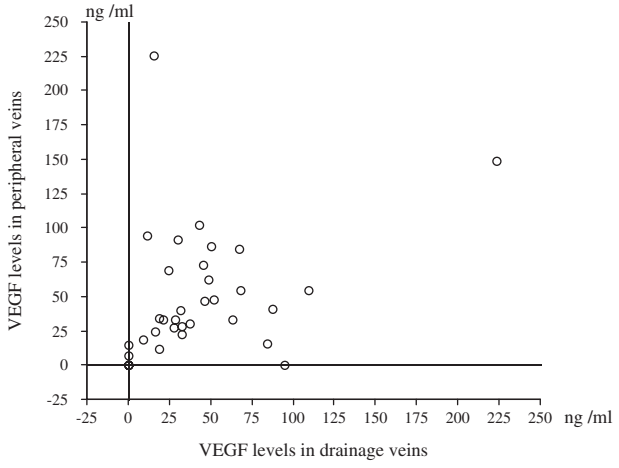

Fig. 1. A significant correlation was found between VEGF levels in the drainage veins and VEGF levels in the peripheral veins (r=0.388, P=0.0168).

ripheral veins and in drainage veins, and between VEGF and IL-8 levels was analyzed using the Kruskal-Wallis test. Disease-free survival was analyzed with the log rank test, and multivariate analysis was done using the Cox proportional hazards regression model. The criterion of statistical significance was $P<0.05$. Statview version 4.5 software application (Abacus Concepts) was used for all analyses.

Results

Relationship between VEGF levels in drainage veins or peripheral veins and clinicopathological variables in patients with GC. There was no correlation of VEGF levels in drainage veins with any clinicopathological variable (Table 1). VEGF levels in drainage veins were not higher than those in peripheral veins (38.83±42.80 ng/ml in drainage versus 44.97±46.60 ng/ml in peripheral veins), although there was a significant relationship between the two (r=0.388, P=0.0168, Fig. 1). The VEGF levels in peripheral veins were higher in the patients with venous invasion (Table 1). Furthermore, peripheral levels of VEGF were correlated moderately with the number (r=0.44, P=0.0064) and ratio (r=0.445, P=0.0058) of metastatic lymph nodes.

Relationship between IL-8 levels in drainage veins or peripheral veins and clinicopathological variables in patients with GC. A significant relationship of IL-8 levels in drainage veins with tumor size and lymph node metastasis was observed, whereas there was no significant relationship between IL-8 levels in peripheral veins and any of the examined variables (Table 2). Interestingly, IL-8 levels in drainage veins were higher than those in peripheral veins (3.65±4.07 and 2.32±5.24), but the difference was not statistically significant (P=0.1904). There was no significant correlation of IL-8 levels in drainage veins with the levels in peripheral veins (r=0.167, P=0.3262, Fig. 2).

Correlation between VEGF levels and IL-8 levels. As shown in Fig. 3, there was no correlation between VEGF and IL-8 levels in drainage veins (r=−0.056, P=0.7433).

Disease-free survival (DFS) of GC patients. The clinical relevance of different clinicopathological variables, together with VEGF and IL-8 levels, for DFS was evaluated by univariate analysis in the patients for whom survival information was available. Tumors larger than 40 mm, deeply invasive tumors (deeper than the muscle layer), lymph node involvement, venous invasion and high IL-8 level in drainage veins (>3.65 ng/ml) were all associated significantly with shorter DFS (P=0.0365, Fig. 4, Table 3). VEGF levels or IL-8 levels in peripheral veins were not significant prognostic factors. Multivariate analysis re-

Table 2. Relationship between circulating IL-8 levels and clinicopathological variables in 37 patients with GC

Variables	No. of patients	IL-8 levels (pg/ml)			
		Drainage veins	*P* value	Peripheral veins	*P* value
Gender					
men	27	3.33±3.34		2.73±5.60	
women	10	4.50±5.76	0.4475	1.21±2.00	0.4405
Histology					
differentiated	12	2.93±2.50		1.55±2.19	
others	25	3.99±4.65	0.4676	2.79±6.31	0.5144
Tumor size					
≤40 mm	18	1.62±2.24		1.04±2.00	
>40 mm	19	5.57±4.53	0.0020	3.52±6.92	0.1528
Depth of tumor					
≤mp	16	2.27±2.18		1.27±2.07	
>mp	21	4.70±1.06	0.072	3.11±6.68	0.2974
Lymph node involvement					
negative	18	2.26±2.14		1.33±2.05	
positive	19	4.96±5.02	0.0423	3.25±7.00	0.2715
Lymphatic invasion					
negative	16	2.93±4.24		1.07±1.52	
positive	21	4.20±3.96	0.3564	3.27±6.74	0.2111
Venous invasion					
negative	20	3.51±4.64		1.80±2.17	
positive	17	3.81±3.69	0.8278	2.92±7.44	0.5245
MVD					
≤5/field	23	3.31±3.48		2.84±6.49	
>5/field	13	4.09±5.19	0.5953	1.32±1.85	0.4173

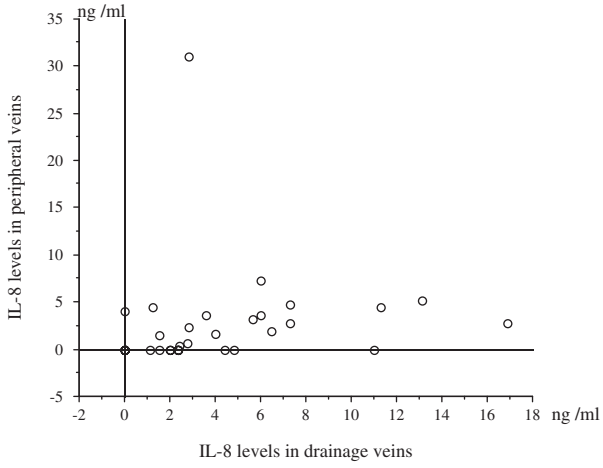

Fig. 2. No significant correlation was found between IL-8 levels in the drainage veins and IL-8 levels in the peripheral veins (r=0.167, P=0.3262).

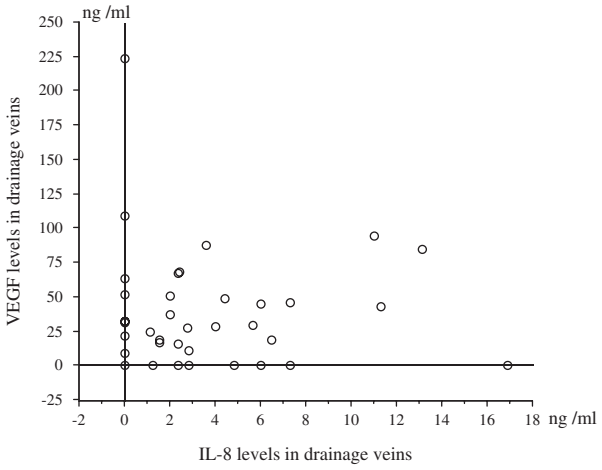

Fig. 3. No significant correlation was found between VEGF and IL-8 levels in the drainage veins (r=0.056, P=0.7433).

vealed that lymph node involvement was the only independent prognostic factor (Table 4), and that a high IL-8 level was not an independent factor.

Discussion

Our results highlight the significance of IL-8 levels in drainage veins. IL-8 levels in the drainage veins in patients with GC were shown to have significant relationships with tumor size, lymph node metastasis and DFS, whereas IL-8 levels in peripheral veins had no such relationship. Although some previous studies have shown a significant relationship between IL-8 levels in peripheral veins and clinicopathological variables in GC, the present study demonstrated that IL-8 levels in drainage veins reflect the clinicopathological status of GC more sensitively than those in peripheral veins. Few studies of the relationship between IL-8 in the drainage veins of solid tumors and clinicopathological factors have been reported. Haraguchi *et al*.[31] reported that in 20 patients with Dukes' C CRC, IL-8 levels in drainage veins were significantly higher in the patients with liver metastases than in those without. We did not assess the relationship between IL-8 levels and hematogenic metastasis, since no GC patient had synchronous hematogenic metastasis, which is common in recurrent CRC, but not in GC. On the other hand, our results showed a positive relationship between high IL-8 levels and lymph node metastasis, which is common in patients with recurrent GC as well as peritoneal dissemination. These findings suggest that the IL-8 level in drainage veins is more valuable as a prognostic indicator or risk factor

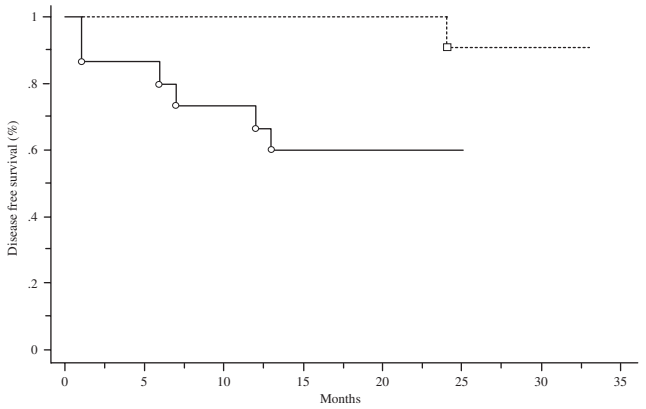

Fig. 4. Relationship of DFS to IL-8 levels in the drainage veins. High IL-8 level in the drainage veins was significantly associated with a shorter DFS (P=0.0365). Solid line: high (>3.65 ng/ml) IL-8 group, dotted line: low (≤3.65 ng/ml) IL-8 group.

for recurrent disease than that in peripheral veins in both GC and CRC.

Kitadai *et al*. observed that IL-8 expression was higher in GC tissue than in normal adjacent tissues, and showed that IL-8 mRNA and protein were localized in the cytoplasm of GC cells.[30] They also demonstrated the production of IL-8 from GC cells *in vitro* and the existence of IL-8 receptors in both cancer cells and endothelial cells. These findings suggest that IL-8 acts through both autocrine and paracrine pathways.

A significant relationship between IL-8 levels in drainage veins and microvessel density (MVD) was observed in CRC. IL-8 in GC patients is also thought to play a role in angiogenesis. Transfection of IL-8 into a human GC cell line with a low level of endogenous IL-8 increased angiogenesis and tumorigenesis in nude mice, whereas it did not affect the proliferative ability of the cells.[29] We were unable to demonstrate a positive relationship between IL-8 levels and MVD. The study of other angiogenic factors and/or the assessment of tumor-specific vasculature may be useful for clarifying the role of IL-8 in tumor angiogenesis in GC.

Besides the induction of angiogenesis, various biological actions of IL-8 in the progression or metastasis of solid tumors have been reported. The overexpression of IL-8 mRNA and protein in breast cancer cell line was reported to enhance bone metastasis, presumably through increased adhesion and invasion.[32] Human neutralizing antibodies against IL-8 inhibited metalloprotease-2 activity and reduced invasive activity as well as angiogenesis, resulting in the suppression of metastasis in a melanoma cell line.[33] It has also been reported that tumor necrosis factor (TNF)-α induced IL-8 secretion in a colon cancer cell line, and that this was responsible for regulating the expression of CD44.[34] These results indicate that IL-8 has various biological effects on the progression or metastasis of tumors, and our present findings suggest that IL-8 in GC patients is responsible for local growth or lymph node metastasis, presumably by enhancement of local invasion or adhesion, not by enhancement of angiogenesis.

Our results demonstrated that VEGF levels in drainage veins were not always higher than those in peripheral veins, though there was a positive relationship between the two VEGF levels. There is a large body of clinical and experimental evidence for the role of VEGF in the progression of solid tumors, and the clinical significance of VEGF in solid tumors has been demonstrated not only immunohistochemically, but also quantitatively. The present results also demonstrated the significant relationship of peripheral VEGF levels with clinicopathological factors, including the number and ratio of metastatic lymph nodes, and vascular invasion. However, the VEGF levels in the drainage veins were not related significantly to any clinicopathological factor, indicating that the supply of VEGF protein from the primary tumor to the circulating blood did not increase the VEGF levels in the drainage veins much above those in the peripheral veins. This suggests that peripheral VEGF levels are more stable and more reliable as a risk factor for recurrence than those in the drainage veins. On the other hand, IL-8 levels in the drainage veins are correlated well to the tumor status compared with those in the peripheral levels, indicating that IL-8 may be produced mainly by the primary lesion. However, IL-8 levels in peripheral veins was not correlated well to the tumor

Table 3. Impact of variables on DFS by univariate analysis in GC patients

Variables	No. of patients	P value
Gender		
men	25	
women	10	0.9682
Histology		
differentiated	12	
others	23	0.6903
Tumor size		
≤40 mm	24	
>40 mm	11	0.0189
Depth of tumor		
≤mp	16	
>mp	19	0.0224
Lymph node involvement		
negative	17	
positive	18	0.0013
Lymphatic invasion		
negative	16	
positive	19	0.1042
Venous invasion		
negative	19	
positive	16	0.0448
MVD		
≤5/field	22	
>5/field	11	0.3072
VEGF in drainage veins		
≤45.0 ng/ml	21	
>45.0 ng/ml	14	0.3464
VEGF in peripheral veins		
≤38.8 ng/ml	22	
>38.8 ng/ml	13	0.7126
IL-8 in drainage veins		
≤3.65 ng/ml	22	
>3.65 ng/ml	13	0.0213
IL-8 in peripheral veins		
≤2.32 ng/ml	24	
>2.32 ng/ml	11	0.3337

Table 4. Cox proportional hazards model of DFS

Variables	P value	Odds ratio
Lymph node involvement (positive or negative)	0.0285	3.73
Tumor size (≤40 mm or >40 mm)	0.1419	2.10
IL-8 levels in drainage veins (≤3.65 ng/ml or>3.65 ng/ml)	0.3185	1.61
Depth of tumor (≤mp or>mp)	0.3366	1.58
Venous invasion (positive or negative)	0.5181	1.36

status, presumably because systemic IL-8 production may be influenced by various factors, including inflammatory stimuli in GC patients.

In addition, a large amount of VEGF proteins is stored in the platelets[35] and a positive relationship of circulating VEGF levels and platelet count was reported in CRC.[36] The significance of serum or plasma VEGF levels may be different among different malignancies and clinicopathological variables.

Although a positive correlation between IL-8 levels and VEGF levels in drainage veins was not observed in the present study, administration of exogenous IL-8 to MKN-1 GC cells has been shown to enhance their expression of epidermal growth factor, metalloproteinase-9 and VEGF mRNAs.[11] It is still unknown whether IL-8 and VEGF enhance tumor progression synergistically. Additional studies are needed to clarify the role of IL-8 in GC progression, since IL-8 has various biological functions, including the promotion of invasion and loss of cell adhesion.

Recent studies have shown that *Helicobacter pylori* infection upregulates IL-8 transcription. The adhesion of *H. pylori* to gastric epithelial cells was reported to activate nuclear factor kappa B (NFκB) and stimulate IL-8 production.[37] Yamaoka *et al*. reported a remarkable increase of IL-6 and IL-8 levels in GC tissues, and concluded that IL-8 levels in GC tissues are largely independent of *H. pylori* infection.[38]

In conclusion, the IL-8 level measured in drainage veins may reflect IL-8 production mainly by the primary lesion, and is valuable as an prognostic indicator or a risk factor for recurrence in GC patients, whereas the peripheral level of IL-8 is not.

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