Gastric Cancer (2005) 8:155–163
DOI 10.1007/s10120-005-0329-4

Original article

VEGF significance in peritoneal recurrence from gastric cancer

Keishiro Aoyagi, Kikuo Kouhuji, Shojiro Yano, Motoshi Miyagi, Takuya Imaizumi, Jinryo Takeda, and Kazuo Shirouzu

Department of Surgery, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan

Abstract

Background. **In gastric cancer, the management of peritoneal dissemination in the Peritoneal cavity is extremely important; however, peritoneal dissemination in the final stage of gastric cancer remains untreatable. Peritoneal dissemination involves several steps, including tumor-cell attachment, invasion, and growth in the peritoneum. Many cytokines, growth factors, matrix metalloproteinases (MMPs), and angiogenic factors play important roles in these steps. So far, few studies have investigated the correlation, if any, between peritoneal dissemination and the angiogenic factor, vascular endothelial growth factor (VEGF).**

Methods. **Immunohistochemical staining, using the avidin-biotin peroxidase complex method, was performed on slides of surgical specimens from 40 patients with stage II gastric cancer with serosal invasion, who underwent surgery at our hospital between 1990 and 2000. Anti-human VEGF rabbit polyclonal IgG was used as the primary antibody. VEGF expression was classified in one of four categories depending on the percentage of tumor-cell staining (P). VEGF expression was also classified in one of three categories depending on the staining intensity (I). The VEGF expression score was calculated as P × I.**

Results. **There were ten patients with peritoneal recurrence. Of these, seven had macroscopic type-4 scirrhous-type gastric carcinoma. In the immunohistochemical study, the VEGF score of patients with peritoneal recurrence was 9.40 ± 2.46; on the other hand, that of patients without peritoneal recurrence was 3.47 ± 2.36. The VEGF score of patients with peritoneal recurrence was significantly higher than that of patients without peritoneal recurrence. In patients with macroscopic type 4, the VEGF score of those with peritoneal recurrence was 9.14 ± 2.19, while on the other hand, that of the patients without peritoneal recurrence was 3.80 ± 3.03. The VEGF score of these patients with peritoneal recurrence was significantly higher than that of those without peritoneal recurrence. The survival rate in the VEGF low-expression group was significantly higher than that in the VEGF high-expression group. Multivariate analysis showed that the VEGF score was a significant parameter of peritoneal recurrence.**

Conclusion. **These results suggested that VEGF was correlated with peritoneal metastasis from gastric cancer, and that VEGF was a useful indicator of peritoneal recurrence.**

Key words VEGF · Peritoneal recurrence · Gastric cancer

Offprint requests to: K. Aoyagi
Received: September 15, 2004 / Accepted: February 28, 2005

Introduction

In gastric cancer, the management of dissemination in the peritoneal cavity is extremely important. However, peritoneal dissemination at the final stage of gastric cancer remains untreatable. Peritoneal dissemination involves several steps, including tumor-cell attachment, invasion, and growth in the peritoneum. Many cytokines, growth factors, matrix metalloproteinases (MMPs), and angiogenic factors play important roles in these steps. Angiogenesis plays an important role in embryogenesis and tumorigenesis. So far, few studies have investigated the correlation, if any, between peritoneal dissemination and the angiogenic factor, vascular endothelial growth factor (VEGF). VEGF has been reported to enhance the permeability of tumor vessels [1], to induce serine protease or metalloproteases [2,3], and to inhibit the apoptosis of endothelial cells [4,5] and the maturation of dendritic cells [6].

The aim of the present study was to clarify the significance of VEGF in peritoneal metastasis of gastric cancer. VEGF was analyzed, using immunohistochemical staining on slides of surgical specimens.

Patients materials, and methods

Patients

Between 1990 and 2000, 1570 patients with gastric cancer were treated at Kurume University Hospital, and

1256 patients underwent curative resection in this period. There were 65 patients with stage II gastric cancer with serosal invasion (T3N0H0P0M0), as defined in the *Japanese classification of gastric carcinoma* [7].

Immunohistochemical analyses for VEGF and CD34 were performed on slides of the surgical specimens from 40 patients with stage II gastric cancer with serosal invasion who had no other neoplasm and who had undergone D2 or D3 lymph node dissection, excluding resection of multiple gastric cancer, and excluding resection of remnant stomach cancer. Twenty-four patients were men, and 16 were women. The mean age was 59.7 years, with age range from 35 to 79 years. There were 2 patients with macroscopic type IIc, 13 patients with type 2, 13 patients with type 3, and 12 patients with type 4.

Immunohistochemical staining

CD34 and VEGF were analyzed using immunohistochemical staining and the avidin-biotin-peroxidase complex technique (Vectastain ABC Kit; Vector, Burlingame, CA, USA). Briefly, paraffin sections were deparaffinized in xylene and rehydrated through graded ethanol solutions. The sections were washed with phosphate-buffered saline (PBS), treated with 0.3% hydrogen peroxide in methanol for 30 min to block endogenous peroxidase, and then incubated with the primary antibody in a humidified chamber at 4°C overnight. As the primary antibody, the mouse monoclonal antibody QB-END/10 (BMA Biomedicals, Augst, Switzerland) for CD34, diluted at 1:10, and the rabbit polyclonal antibody A-20 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for VEGF, diluted at 1:200, were used. Sections were washed three times with PBS, then incubated with biotinylated horse anti-mouse/anti-rabbit immunoglobulin G antibody for 30 min, washed again three times with PBS, and then incubated with avidin-biotinylated peroxidase complex for 30 min. After three additional washings with PBS, staining was developed by incubating the sections in 3-amino-9-ethylcarbazole (Vector) for 5 min. The sections were then counterstained with hematoxylin and mounted.

Tumor vessels

Immunoreactivity of CD34 was recognized in the endothelium. Positive staining of small tubule-like formation for CD34 was defined as a microvessel. Vessels that were recognized along the invasive cancer nests or cancer cells were counted. The tumor vessel count was defined as the mean number of tumor vessels in a 400-times magnified field from ten arbitrary microscopic fields.

VEGF score

The cell types showing positive staining for VEGF were defined morphologically by H&E staining, using the serial sections. VEGF expression was classified as one of four categories, depending on the percentage of tumor cells stained (P): category 1, less than 25% of cells stained; category 2, from 25% to 49% stained; category 3, from 50% to 74% stained; and category 4, 75% or more cells stained. VEGF expression was also classified as one of three categories depending on the staining intensity (I): 1, weak; 2, moderate; 3, strong. A VEGF expression score was calculated, as $P \times I$. A pathologist confirmed the staining intensity of the tumor cells. A patient for whom the VEGF score was higher than the mean VEGF score was defined as being in the VEGF high-expression group, while all other patients for whom the VEGF score was lower than the mean VEGF score, were defined as being in the VEGF low-expression group.

Statistical analysis

Student's *t*-test and the χ^2 test were used to analyze the data for significant differences, and any difference was considered statistically significant when the *P* value was less than 0.05. The cumulative survival rate was calculated with the Kaplan-Meier method. The significance of any difference between the survival curves was determined using the Cox-Mantel test, and any difference was considered significant at the 5% level. Various factors, including age, sex, tumor size, type of lymph node dissection, macroscopic type, histological type, venous invasion, number of tumor vessels, and VEGF score were evaluated, by univariate analysis, for any independent contributions to peritoneal recurrence, using Fisher's exact test or the χ^2 test. Significant factors were extracted for further analysis, carried out using multivariate analysis with a logistic regression method. The statistical analyses were performed using a statistical analysis computer program (Stat View 5.0; SAS Institute, Cary, NC, USA). The *P* value level of significance was set at 0.05.

Results

Patients

There were ten patients with peritoneal recurrence; the peritoneal recurrence was recognized as being type 4 in seven patients, and type 3 in the other three (Table 1). No recurrence was seen in lymph nodes or in the liver, in any patient (Table 1).

Table 1. The incidence of recurrence

Macroscopic type	IIc ($n = 2$)	2 ($n = 13$)	3 ($n = 13$)	4 ($n = 12$)	Total ($n = 40$)
Peritoneum	0	0	3 (23.1%)	7 (58.3%)	10 (25.0%)
Lymph node	0	0	0	0	0
Liver	0	0	0	0	0
Local	0	0	1 (7.7%)	0	1 (2.5%)
Total	0	0	4 (30.8%)	7 (58.3%)	11 (27.5%)

Table 2. Immunoreactivity of VEGF, and the incidence of peritoneal recurrence

Category	Number of patients with peritoneal recurrence (%)
Percentage of positive tumor cells (P)	
1, <25%	0/8 (0)
2, ≧25% and <50%	0/17 (0)
3, ≧50% and <75%	4/8 (50.0)**
4, ≧75%	6/7 (85.7)*
Staining intensity (I)	
1, Weak	0/13 (0)
2, Moderate	4/17 (23.5)
3, Strong	6/10 (60.0)*

P: * P < 0.001 vs ≧25% and <50%, * P < 0.005 vs <25%, ** P < 0.01 vs ≧25% and <50%
I: * $P < 0.01$ vs weak; * $P < 0.05$ vs moderate

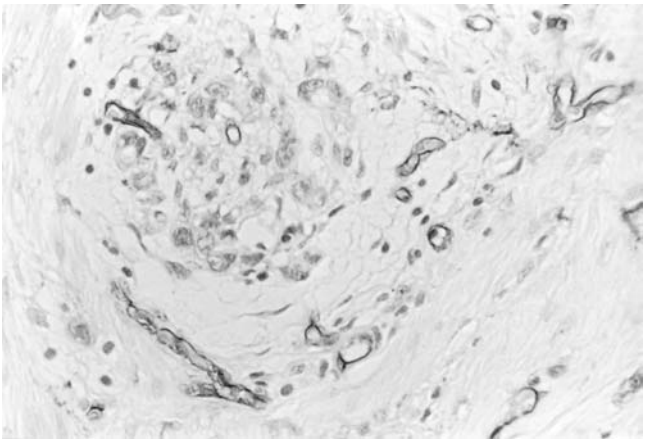

Fig. 1. Immunoreactivity for CD34 is recognized in the endothelium. CD34, ×400

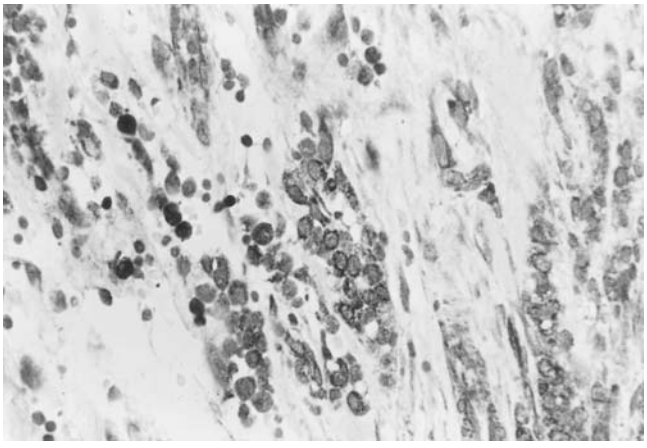

Fig. 2. Immunoreactivity for vascular endothelial growth factor (VEGF) is mainly identified as supranuclear staining or diffused staining in the cytoplasm of cancer cells. VEGF, ×400

Immunohistochemical staining

Immunoreactivity for CD34 was recognized in the endothelium (Fig. 1). Immunoreactivity for VEGF was mainly identified as supranuclear staining or diffused staining in the cytoplasm of cancer cells (Fig. 2), and VEGF was observed in some epithelial cells, lymphocytes, and macrophages. The incidence of patients with peritoneal recurrence according to the percentage of positive tumor staining (P) was 85.7% (6/7) in category 4, 50% (4/8) in category 3, and 0% in both category 2 (0/17) and category 1 (0/8; Table 2). The incidence of peritoneal recurrence in category 4, and in category 3, was significantly higher than that in category 2 ($P < 0.001$; $P < 0.01$, respectively). The incidence of peritoneal recurrence in category 4 was significantly higher than that in category 1 ($P < 0.005$; Table 2). The incidence of peritoneal recurrence according to staining intensity (I) was 60.0% (6/10) in those with strong intensity, 23.5% (4/17) in those with moderate intensity, and

0% (0/13) in those with weak staining intensity (Table 2). The incidence of peritoneal recurrence in those with strong intensity was significantly higher than that in those with moderate or weak intensity ($P < 0.05$; $P < 0.01$, respectively; Table 2). Based on the VEGF expression score according to the criteria P × I, VEGF high expression was found in 16 patients, and low expression in 24 (Table 3). In patients with type 3 or type 4, VEGF high expression was found in 11, and low expression in 14 (Table 3). In patients with type 4, VEGF high expression was found in 7, and low expression in 5 (Table 3).

Table 3. VEGF expression

Category	Number of patients (%)
VEGF expression score (P × I)	
Low	24 (60.0)
High	16 (40.0)
Type 3 and type 4	
Low	14 (56.0)
High	11 (44.0)
Type 4	
Low	5 (41.6)
High	7 (58.3)

Tumor vessels

The mean number of tumor vessels involved in macroscopic type IIc, type 2, type 3, and type 4 cancers was 24.0 ± 5.09, 18.0 ± 5.99, 24.9 ± 6.02, and 27.2 ± 11.9, respectively (Table 4). The mean number of tumor vessels in type 3 or type 4 was significantly higher than that in type 2 ($P < 0.01$, and $P < 0.05$, respectively). The mean number of tumor vessels in patients with peritoneal recurrence was 28.9 ± 12.7, and that in patients without peritoneal recurrence was 22.5 ± 5.65 (Table 5). In patients with type 3 or type 4, the mean number of tumor vessels in those with peritoneal recurrence was 28.9 ± 12.7, and that in those without peritoneal recurrence was 24.1 ± 5.61 (Table 6). In patients with type 4, the mean number of tumor vessels in those with peritoneal recurrence was 29.8 ± 14.8, and that in those without peritoneal recurrence was 23.6 ± 5.85 (Table 7). There was no significant correlation between the number of tumor vessels and peritoneal recurrence.

Venous invasion

The rate of venous invasion in patients with type IIc, type 2, type 3, and type 4, was 0% (0/2), 61.5% (8/13),

Table 4. The mean number of tumor vessels, VEGF score, and the incidence of venous invasion according to macroscopic type

Macroscopic type	IIc ($n = 2$)	2 ($n = 13$)	3 ($n = 13$)	4 ($n = 12$)
Tumor vessels	24.0 ± 5.09	18.0 ± 5.99	24.9 ± 6.02*	27.2 ± 11.9**
VEGF score	2.00 ± 0.00	2.92 ± 1.71	5.62 ± 3.80**	6.92 ± 3.68**
Venous invasion	0	8 (61.5%)	8 (61.5%)	4 (33.3%)

* $P < 0.01$ vs type 2; ** $P < 0.05$ vs type 2

Table 5. The mean number of tumor vessels, VEGF score, and the incidence of venous invasion according to peritoneal recurrence

	Peritoneal recurrence ($n = 10$)	No peritoneal recurrence ($n = 30$)
Tumor vessels	28.9 ± 12.7	22.5 ± 5.65
VEGF score	9.40 ± 2.46*	3.47 ± 2.36
Venous invasion	6 (60.0%)	14 (46.7%)

* $P < 0.001$ vs no peritoneal recurrence

Table 6. The mean number of tumor vessels, VEGF score, and the incidence of venous invasion according to peritoneal recurrence (type 3 and type 4)

	Peritoneal recurrence ($n = 10$)	No peritoneal recurrence ($n = 15$)
Tumor vessels	28.9 ± 12.7	24.1 ± 5.61
VEGF score	9.40 ± 2.46*	4.13 ± 2.83
Venous invasion	6 (60.0%)	6 (40.0%)

* $P < 0.001$ vs no peritoneal recurrence

Table 7. The mean number of tumor vessels, VEGF score, and the incidence of venous invasion according to peritoneal recurrence (type 4)

	Peritoneal recurrence ($n = 7$)	No peritoneal recurrence ($n = 5$)
Tumor vessels	29.8 ± 14.8	23.6 ± 5.85
VEGF score	9.14 ± 2.19*	3.80 ± 3.03
Venous invasion	4 (57.1%)	0

* $P < 0.05$ vs no peritoneal recurrence

Table 8. The mean number of tumor vessels and the incidence of venous invasion according to the expression of VEGF

	VEGF high-expression group ($n = 16$)	VEGF low-expression group ($n = 24$)
Tumor vessels	27.3 ± 11.4	21.9 ± 4.83
Venous invasion	9 (56.3%)	11 (45.8%)

Table 9. The mean number of tumor vessels and the incidence of venous invasion according to the expression of VEGF in patients with macroscopic type 3 or type 4 cancers

	VEGF high-expression group ($n = 11$)	VEGF low-expression group ($n = 14$)
Tumor vessels	28.0 ± 12.2	24.5 ± 6.01
Venous invasion	6 (54.5%)	4 (28.6%)

61.5% (8/13), and 33.3% (4/12), respectively (Table 4). There was no significant difference in this rate among the various macroscopic types. The rate of venous invasion in patients with peritoneal recurrence was 60.0% (6/10), and that in patients without peritoneal recurrence was 46.7% (14/30; Table 5). In patients with type 3 or type 4, the rate of venous invasion in those with peritoneal recurrence was 60.0% (6/10), and that in those without peritoneal recurrence was 40.0% (6/15; Table 6). In patients with type 4, the rate of venous invasion in those with peritoneal recurrence was 57.1% (4/7), and that in those without peritoneal recurrence was 0% (0/5; Table 7), with no significant correlation between the rate of venous invasion and peritoneal recurrence.

The VEGF score

The VEGF score in patients with type IIc, type 2, type 3, and type 4 was 2.00 ± 0.00, 2.92 ± 1.71, 5.62 ± 3.80, and 6.92 ± 3.68, respectively (Table 4). The VEGF score in patients with type 3 or type 4 was significantly higher than that in patients with type 2 ($P < 0.05$, both). The VEGF score in patients with peritoneal recurrence was 9.40 ± 2.46, and that in those without peritoneal recurrence was 3.47 ± 2.36 (Table 5). In patients with type 3 or type 4, the VEGF score in those with peritoneal recurrence was 9.40 ± 2.46, and that in those without peritoneal recurrence was 4.13 ± 2.83 (Table 6). In patients with type 4, the VEGF score in those with peritoneal recurrence was 9.14 ± 2.19, and that in those without peritoneal recurrence was 3.80 ± 3.03 (Table 7); the VEGF score in patients with peritoneal recurrence was significantly higher than that in patients without peritoneal recurrence ($P < 0.001$). In patients with type 3 or type 4, and separately in those with type 4, the VEGF score in patients with peritoneal recurrence was significantly higher than that in those without peritoneal recurrence ($P < 0.001$; $P < 0.05$, respectively).

The number of tumor vessels and VEGF expression

The mean number of tumor vessels in the VEGF high-expression group was 27.3 ± 11.4, and that in the VEGF low-expression group was 21.9 ± 4.83 (Table 8). The mean number of tumor vessels in the VEGF high-expression group tended to be higher than that in the VEGF low-expression group. In patients with type 3 or type 4, the mean number of tumor vessels in the VEGF high-expression group was 28.0 ± 12.2, and that in the VEGF low-expression group was 24.5 ± 6.01 (Table 9), with no significant difference between the VEGF high-

Table 10. The mean number of tumor vessels and the incidence of venous invasion according to the expression of VEGF in patients with macroscopic type 4 cancer

	VEGF high-expression group ($n = 7$)	VEGF low-expression group ($n = 5$)
Tumor vessels	32.1 ± 13.6	20.4 ± 3.14
Venous invasion	3 (42.9%)	1 (20.0%)

Table 11. Univariate and multivariate analyses

	Peritoneal recurrence		Univariate analysis		Multivariate analysis	
	+	−	χ^2	*P* value	χ^2	*P* value
Sex						
Male	3	21	3.932	0.0474	0.062	0.8035
Female	7	9				
Age (years)						
≧60	5	17	0.003	>0.9999	—	—
<60	5	13				
Tumor size (mm)						
≧100	6	8	2.488	0.1294	—	—
<100	4	22				
Type of lymph node dissection						
D2	4	17	0.416	0.7062	—	—
D3	6	13				
Macroscopic type						
0-IIc	0	2	10.936	0.0139	5.751	0.0165
2	0	13				
3	3	10				
4	7	5				
Histology						
Differentiated	0	7	2.559	0.1693	—	—
Undifferentiated	10	23				
Venous invasion						
v (−)	4	16	0.219	0.7164	—	—
v (+)	6	14				
VEGF score						
Low	0	24	18.720	<0.0001	13.524	0.0002
High	10	6				
Tumor vessels (number)						
<17	5	15	0.578	0.6722	—	—
≧17	5	15				

expression group and the VEGF low-expression group. In patients with type 4, the mean number of tumor vessels in the VEGF high-expression group was 32.1 ± 13.6, and that in the VEGF low-expression group was 20.4 ± 3.14 (Table 10), with the mean number of tumor vessels in the VEGF high-expression group tending to be higher than that in the VEGF low-expression group.

Venous invasion and VEGF expression

The rate of venous invasion in the VEGF high-expression group was 56.3% (9/16), and that in the VEGF low-expression group was 45.8% (11/24; Table 8). In patients with type 3 or type 4, the rate of venous invasion in the VEGF high-expression group was 54.5% (6/11), and that in the VEGF low-expression group was 28.6% (4/14; Table 9). In patients with type 4, the rate of venous invasion in the VEGF high-expression group was 42.9% (3/7), and that in the VEGF low-expression group was 20.0% (1/5; Table 10), with no significant correlation between the rate of venous invasion and VEGF expression.

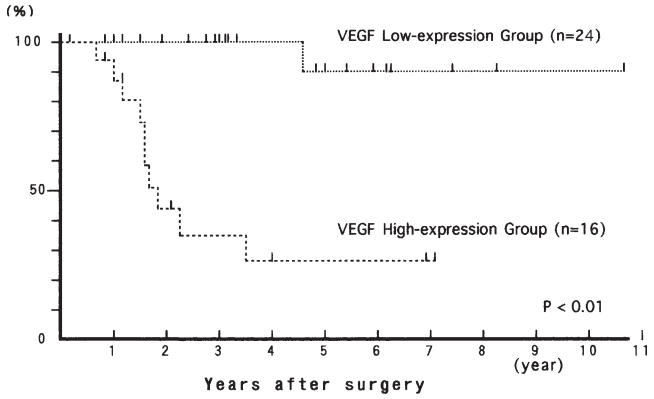

Fig. 3. The survival curve of the VEGF low-expression group was significantly higher than that of the VEGF high-expression group ($P < 0.01$)

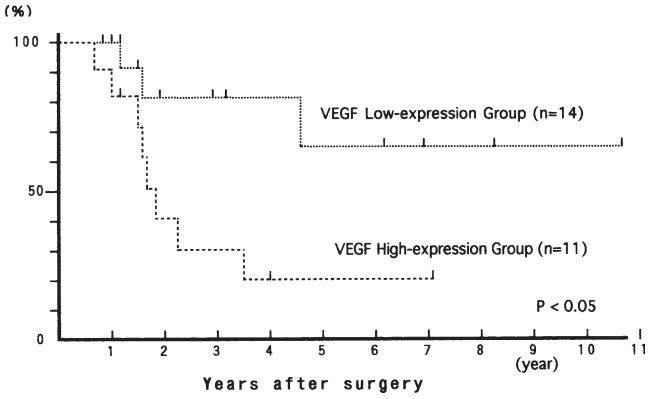

Fig. 4. Survival curves for patients with macroscopic type 3 and type 4 cancers. The survival curve of the VEGF low-expression group was significantly higher than that of the VEGF high-expression group ($P < 0.05$)

Multivariate analysis

Multivariate analysis was conducted for the three parameters (sex, macroscopic type, and VEGF score) that had been found to be significant by univariate analysis (Table 11). The multivariate analysis showed that the VEGF score, and the macroscopic type, but not sex, were significant parameters of peritoneal recurrence (Table 11).

Survival curves

The 5-year-survival rate was 90.0% in the VEGF low-expression group, and 26.3% in the VEGF high-expression group. The survival curve in the VEGF low-expression group was significantly higher than that in the VEGF high-expression group (Fig. 3; $P < 0.01$). In patients with type 3 or type 4, the 5-year-survival rate was 65.2% in the VEGF low-expression group, and 20.5% in the VEGF high-expression group. The survival curve in the VEGF low-expression group was significantly higher than that in the VEGF high-expression group (Fig. 4; $P < 0.05$). In patients with type 4, the 5-year survival rate was 75.0% in the VEGF low-expression group, and 14.3% in the VEGF high-expression group; the survival curve in the VEGF low-expression group tended to be higher than that in the VEGF high-expression group, but there was no significant difference (Fig. 5).

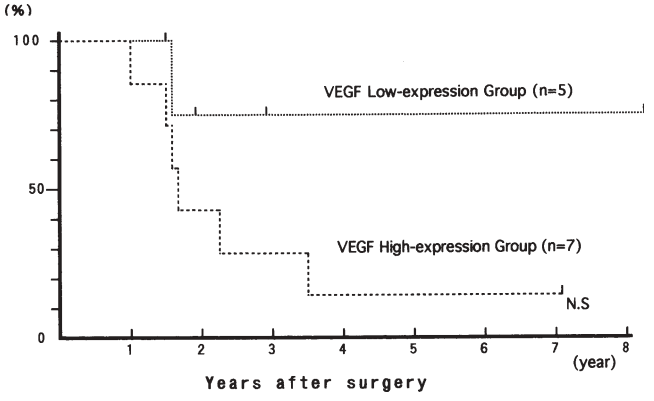

Fig. 5. Survival curves for patients with macroscopic type 4 cancer. The survival curve of the VEGF low-expression group tended to be higher than that of the VEGF high-expression group, but with no significant difference. *N.S*, not significant

Discussion

Scirrhous carcinoma of the stomach, known as diffusely infiltrative carcinoma or Borrman's type-IV carcinoma, or linitis plastica-type carcinoma, is characterized clinically as having the worst prognosis among the various types of gastric cancer, because it is frequently associated with metastases to lymph nodes and with peritoneal dissemination. The mechanism of peritoneal dissemination has not yet been clearly defined. Many reports have suggested that it occurs via the attachment of free cancer cells to the peritoneum [8–10]. So far, few studies have investigated the correlation, if any, between cancer cells and mesothelial cells during proliferation following attachment. Angiogenesis is a key component in various steps of human cancer development and spread [11]. Previous reports have indicated that the presence of angiogenic factors is an essential event in the development of peritoneal metastasis [12–14]. VEGF, as well as functioning as a growth factor, is also able to function as a vascular permeability factor. Increased permeability of blood vessels facilitates the extravasation of proteins and the formation of ascites [15–17]. In previous reports, the expression level of VEGF has been found to be directly associated with the

production of ascites and carcinomatosis [17,18]. Indeed, Kraft et al. [19] reported that VEGF may play an important role in tumor progression and the formation of malignant effusions. Mesiano et al. [20] demonstrated that the neutralization of VEGF activity may have clinical applications in inhibiting malignant ascites formation in ovarian cancer. Cancer cells transfected with VEGF have shown an increased potential for the development of tumorigenesis in a xenograft model [13]. Moreover, the functional inhibition of VEGF by neutralizing monoclonal antibody to VEGF [20] or by soluble VEGF receptor (R)-1 [14] effectively suppressed the development of peritoneal dissemination. In addition, VEGF levels were markedly elevated in malignant ascites [21]. We have found that the VEGF level in the medium of a gastric cancer cell line with a high potential for peritoneal metastasis was significantly higher than that in a gastric cancer cell line with a low potential for peritoneal metastasis (data not shown). These studies have provided clear evidence that VEGF is an essential element in the development of peritoneal metastasis. Tokuhara et al. [22] multiplied the proportion of VEGF-stained cells by the intensity of VEGF staining to evaluate VEGF expression in colorectal cancer, and they discovered a significant positive correlation between the vascular expression of E26 transformation-specific-1 (Ets-1), which influences angiogenesis, and VEGF, or pyrimidine nucleoside phosphorylase (dThdPase) activity in the tumor. In the present study, not only the percentage of tumor cells stained for VEGF but also the staining intensity was correlated with peritoneal recurrence, Therefore, we included the degree of staining intensity of VEGF, to calculate the VEGF score, in the 40 patients with stage II gastric cancer with serosal invasion that we investigated. No recurrence was recognized in any lymph node or in the liver in any of the patients. Peritoneal recurrences were recognized in patients with macroscopic type 4 or type 3, but were not recognized in those with type 2 or type IIc. The VEGF score of patients with type 3 or type 4 was significantly higher than that in patients with type 2. The VEGF score in patients with peritoneal recurrence was significantly higher than that in those without peritoneal recurrence. Even in patients with type 4, the VEGF score in those with peritoneal recurrence was significantly higher than that in patients without peritoneal recurrence. These results suggested that the VEGF score was a useful marker. Moreover, multivariate analysis showed that the VEGF score was a significant indicator of peritoneal recurrence. Therefore, we speculate that treatment with VEGF antibody might prevent peritoneal recurrence of gastric cancer. Konno et al. [23] reported that, in gastric cancer patients, VEGF levels in the peripheral veins were higher in patients with venous invasion. In our study, the mean number of tumor vessels patients with in type 3 or type 4 was significantly higher than that in patients with type 2, and the mean number of tumor vessels in those with high expression of VEGF tended to be greater than that in those with low VEGF expression. These results suggested that the expression of VEGF in tumor cells was correlated with the number of tumor vessels. The incidence of venous invasion in patients with high expression of VEGF was higher than that in patients with low expression of VEGF, but without significance. If a larger number of patients were to be studied, VEGF might be found to be significantly correlated with the incidence of venous invasion.

References

1. Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am J Pathol 1995;50:1209–39.
2. Pepper MS, Ferrara N, Orci L, Montesano R. Vascular endothelial growth factor (VEGF) induces plasminogen activators and plasminogen activator inhibitor-1 in microvascular endothelial cells. Biochem Biophys Res Commun 1991;181:902–6.
3. Unemori EN, Ferrara N, Bauer EA, Amento EP. Vascular endothelial growth factor induces interstitial collagenase expression in human endothelial cells. J Cell Physiol 1992;153:557–62.
4. Shaheen RM, Davis DW, Liu W, Zebrowski BK, Wilson MR, Bucanan CD, et al. Antiangiogenic therapy targeting the tyrosine kinase receptor for vascular endothelial growth factor receptor inhibits the growth of colon cancer liver metastasis and induces tumor and endothelial cell apoptosis. Cancer Res 1999;59:5412–6.
5. Gerber HP, McMurtrey A, Kowalski J, Yan M, Keyt BA, Dixit V, et al. Vascular endothelial growth factor regulates endothelial cell survival through the phosphatidylinositol 3'-kinase/Akt signal transduction pathway: requirement for Klk-1/FDR activation. J Biol Chem 1998;273:30336–43.
6. Lissoni P, Malugani F, Bonfanti A, Bucovec R, Secondino S, Brivio F, et al. Abnormally enhanced blood concentrations of vascular endothelial growth factor (VEGF) in metastatic cancer patients and their relation to circulating dendritic cells, IL-12 and endothelin-1. J Biol Regul Homeost Agents 2001;15:140–4.
7. Japanese Gastric Cancer Association. Japanese classification of gastric carcinoma. 13th ed. Tokyo: Kanehara; 1999.
8. Jonjic N, Peri G, Bernasconi S, Sciacca FL, Colotta F, Pelicci P, et al. Expression of adhesion molecules and chemotactic cytokines in cultured human mesothelial cells. J Exp Med 1992;176:1165–74.
9. Nishimura S, Chung YS, Yashiro M, Inoue T, Sowa M. CD44H plays an important role in peritioneal dissemination of scirrhous gastric cancer cells. Jpn J Cancer Res 1996;87:1235–44.
10. Nakashio T, Narita T, Akiyama S, Kasai Y, Kondo K, Ito K, et al. Adhesion molecules and TGF-β 1 are involved in the peritoneal dissemination of NUGC-4 human gastric cancer cells. Int J Cancer 1997;70:612–8.
11. Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med 1971;285:1182–6.
12. Yoneda J, Kuniyasu H, Crispens MA, Price JE, Bucana CD, Fidler IJ. Expression of angiogenesis-related genes and progression of human ovarian carcinomas in nude mice. J Natl Cancer Inst 1998;90:447–54.
13. Kondo Y, Arii S, Mori A, Furutani M, Chiba T, Imamura M. Enhancement of angiogenesis, tumor growth, and metastasis by transfection of vascular endothelial growth factor into Lo Vo human colon cancer cell line. Clin Cancer Res 2000;6:622–30.

14. Mori A, Arii S, Furutani M, Mizumoto M, Uchida S, Furuyama H, et al. Soluble Flt-1 gene therapy for peritoneal metastases using HVJ-cationic liposomes. Gene Ther 2000;7:1027–33.
15. Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science 1983;219: 983–5.
16. Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am J Pathol 1995;146: 1029–39.
17. Nagy JA, Masse EM, Herzberg KT, Meyers MS, Yeo KT, Yeo TK, et al. Pathogenesis of ascites tumor growth: vascular permeability factor, vascular hyperpermeability, and ascites fluid accumulation. Cancer Res 1995;55:360–8.
18. Boocock CA, Charnock-Jones DS, Sharkey AM, McLaren J, Barker PJ, Wright KA, et al. Expression of vascular endothelial growth factor and its receptors flt and KDR in ovarian carcinoma. J Natl Cancer Inst 1995;87:506–16.
19. Kraft A, Weindel K, Ochs A, Marth C, Zmija J, Schumacher P, et al. Vascular endothelial growth factor in the sera and effusion of patients with malignant and nonmalignant disease. Cancer 1999;85:178–87.
20. Mesiano S, Ferrara N, Jaffe RB. Role of vascular growth factor in ovarian cancer: inhibition of ascites formation by immunoneutralization. Am J Pathol 1998;153:1249–56.
21. Zebrowski BK, Liu W, Ramirez K, Akagi Y, Mills GB, Ellis LM. Markedly elevated levels of vascular endothelial growth factor in malignant ascites. Ann Surg Oncol 1999;6:373–8.
22. Tokuhara K, Ogata Y, Nakagawa M, Shirouzu K. Est-1 expression in vascular endothelial cells as an angiogenic and prognostic factor in colorectal carcinoma. Int Surg 2003;88:25–33.
23. Konno H, Ohta M, Baba M, Suzuki S, Nakamura S. The role of circulating IL-8 and VEGF protein in the progression of gastric cancer. Cancer Sci 2003;94:735–40.