Clinical Significance of Plasma Level of Vascular Endothelial Growth Factor-C in Patients with Colorectal Cancer

Tatsuya Miyazaki¹, Norimichi Okada¹, Keiichiro Ishibashi¹, Kyouichi Ogata¹, Tomonori Ohsawa¹, Toru Ishiguro¹, Hiroshi Nakada¹, Masaru Yokoyama¹, Moriyuki Matsuki¹, Hiroyuki Kato², Hiroyuki Kuwano² and Hideyuki Ishida¹

¹Department of Digestive Tract and General Surgery, Saitama Medical Center, Saitama Medical University, Saitama and ²Department of General Surgical Science, Gunma University Graduate School, Graduate School of Medicine, Gunma, Japan

Received May 9, 2008; accepted September 5, 2008; published online October 15, 2008

Objective: Vascular endothelial growth factor (VEGF)-C is known to be associated with angiogenesis and lymphangiogenesis in various cancers. However, little is known about the clinical significance of determining the blood level of VEGF-C in patients with colorectal cancer.

Methods: Plasma levels of VEGF-C in patients with colorectal cancer (n = 127) and normal healthy volunteers (n = 23) were determined by the sandwich enzyme-linked immunosorbent assay.

Results: The plasma VEGF-C concentration did not significantly differ between patients with colorectal cancer and healthy controls (P = 0.53). However, subgroup analysis showed that deeper tumor invasion (P = 0.04), more severe lymphatic invasion (P = 0.03) and venous invasion (P < 0.01) were correlated with an elevated level of plasma VEGF-C. Among the patients (n = 109) who underwent potentially curative surgery, the plasma level of VEGF-C was higher in patients who developed recurrence (n = 35) than in those who did not (n = 74) (P = 0.04). In addition, disease-free (P = 0.02) and overall survival times (P = 0.02) were shorter in patients with a high level (>1840 pg/ml) of plasma VEGF-C than in those with a low level (≤1840 pg/ml) when the cut-off value was determined on the basis of the median value in colorectal cancer patients. Multivariate analysis with the Cox proportional hazard model demonstrated that the plasma VEGF-C level along with Dukes’ stage was an independent factor affecting overall survival (P = 0.03).

Conclusion: These results suggest that determining the plasma level of VEGF-C would be useful for predicting lymphatic invasion, venous invasion and poor outcome of patients with colorectal cancer.

Key words: vascular endothelial growth factor-C (VEGF-C) – colorectal cancer – prognosis

INTRODUCTION

Vascular endothelial growth factor (VEGF)-C is a member of the VEGF family of growth factors with angiogenic and lymphangiogenic function (1). VEGF-C and -D are secreted glycoproteins that are structurally similar, sharing areas of sequence similarity with one another and with the angiogenic growth factor VEGF-A (1,2). The role of VEGF-C in neoplasms has not been fully investigated, but some reports (3–5) have demonstrated increased aggressiveness of transfected cancer cell lines, intratumoral lymphangiogenesis, dilated and increased numbers of peritumoral lymphatics, enhanced lymph node metastasis and increased tumor angiogenesis in cancers expressing VEGF-C. In colorectal cancer, VEGF-C expression has been found to correlate with lymphatic invasion and lymph node metastasis (6,7). However, only a few reports (8–10) have described circulating levels of VEGF-C in patients with colorectal cancer. Also, little is known about the clinical significance of determining the levels of circulating VEGF-C in patients with colorectal cancer. In this study, we measured the levels...
of VEGF-C from preoperative plasma samples in patients with colorectal cancer and examined the clinical utility of the VEGF-C assay.

PATIENTS AND METHODS

PATIENTS

A total of 127 patients (73 males and 54 females) who underwent resection of primary colorectal cancer between January 2000 and December 2004 were included in this study. The median age of the patients was 61 years, with a range of 33–91 years. No patients had received blood transfusion, radiotherapy or chemotherapy before surgery. Control patients were recruited from healthy volunteers (15 males and 8 females) without known neoplasm, recent trauma, surgery, pregnancy or menstruation. The median age of the controls was 59 years with a range of 32–77 years. Clinicopathological factors were recorded in all cancer patients. Pathological status (primary tumor, regional lymph nodes and distant metastasis) was classified according to the sixth edition of the TNM classification of the International Union against Cancer (UICC) (11). Macroscopic appearance was classified into four types according to the Japanese Classification of Colorectal Carcinoma (12): Type 0, superficial, flat tumors with or without minimal elevation or depression; Type 1, Protruberant type; Type 2, Ulcerated type with a clear margin and Type 3; Ulcerated type with infiltration. Lymphatic and venous invasions were also classified into four grades, respectively, according to the Japanese classification of colorectal carcinoma: no invasion (ly0, v0), minimal invasion (ly1, v1), moderate invasion (ly2, v2) and severe invasion (ly3, v3). Histological subclassification (well-differentiated, moderately differentiated, poorly differentiated and mucinous adenocarcinoma) was also identified according to the Japanese classification of colorectal carcinoma (12). Written informed consent to participate in the study was obtained from all patients and controls. This study was approved by the Ethics Committee of Saitama Medical Center, Saitama Medical University (No. 192).

MEASUREMENT OF PLASMA LEVELS OF VEGF-C

Peripheral blood was collected from the colorectal cancer patients and from the control patients, within 7 days before surgery, and in tubes containing EDTA (ethylenediamine tetra-acetic acid). Blood samples were centrifuged at 1000 × g for 5 min within 2 h of collection. Plasma aliquots were separated and frozen at −40°C for later analysis. The levels of VEGF-C were determined using commercially available sandwich enzyme-linked immunosorbent assay kits (human VEGF-C ELISA kit; IBL Fujioka, Fujioka, Japan).

FOLLOW-UP

All of the patients with Dukes’ stages A (n = 17), B (n = 36) and C (n = 45) and 11 patients with Dukes’ stage D underwent potentially curative surgery.

Five Dukes’ A patients (29%), 31 Dukes’ B patients (86%) and 40 Dukes’ C patients (89%) received postoperative adjuvant chemotherapy comprising fluoropyrimidines such as UFT (Taiho Pharmaceutical, Tokyo, Japan), doxifluoridine (Japan Roche, Tokyo, Japan) or 5-fluorouracil (Kyowa Hakko Kogyo, Tokyo, Japan) plus leucovorin (Wyeth K.K., Tokyo, Japan). All the 11 Dukes’ D patients who underwent potentially curative surgery, also received postoperative chemotherapy comprising fluoropyrimidines. Eighteen Dukes’ D patients (62%), who underwent non-curative surgery, received postoperative chemotherapy comprising fluoropyrimidines and/or irinotecan hydrochloride (Yakult Honsha Co., Ltd, Tokyo, Japan). Patients were followed-up periodically, at intervals of 3–6 months, with clinical and laboratory examinations, including ultrasonography, computed tomography and chest radiography. The median follow-up period was 44 months (range: 1–94 months). Among 109 patients, who underwent potentially curative surgery (no residual tumors, as evaluated both macroscopically and microscopically), recurrence was confirmed in 55. The types of initial recurrence were hematogenous (liver, lung and bone) in 15, peritoneal in two, lymph nodal in two and locoregional in three, hematogenous plus lymph nodal in five, hematogenous plus peritoneal in four, hematogenous plus locoregional in three and peritoneal plus lymph nodal in one.

STATISTICAL ANALYSIS

Statistical analyses were performed using the Statview version 5.0, a software package (Statview, version 5.0., SAS, Institute Inc., NC, USA). Because of their skewed distributions, median values with ranges are provided to describe VEGF-C levels. Comparisons of continuous variables were performed by the Mann–Whitney U or Kruskal–Wallis test whichever was appropriate. Survival curves were generated by the Kaplan–Meier method and the differences in the survival curves were assessed by the log-rank test. Multivariate regression analysis by the Cox proportional hazard model was applied to determine independent factors affecting survival. The level of statistical significance was set at P < 0.05.

RESULTS

The median plasma VEGF-C concentration was 1839 pg/ml (157–9955 pg/ml) in the patients with colorectal cancer (n = 127) and 2094 pg/ml (40–4041.5 pg/ml) in the healthy controls (n = 23). The difference in the levels of VEGF-C between the two groups was not significant (P = 0.53) (Fig. 1). The correlations between the clinicopathological factors and the plasma levels of VEGF-C are summarized in...
Table 1. An elevated level of plasma VEGF-C was correlated with deeper invasion ($P = 0.04$), severe lymphatic ($P = 0.03$) and venous invasions ($P < 0.01$) by the primary tumor. The other factors, including gender, age, location, macroscopic type, histological differentiation, grade of lymph node metastasis, distant metastasis and Duke’s stage, did not correlate with the plasma level of VEGF-C. When the analysis was restricted to patients ($n = 109$) with Dukes’ stages A, B, C and D, who underwent potentially curative surgery, the plasma level of VEGF-C was higher in patients who subsequently developed recurrence ($n = 35$) than in those who did not ($n = 74, P = 0.04$) (Fig. 2). When the cut-off level of plasma VEGF-C was set at 1840 pg/ml based on the median level in patients with colorectal cancer, the cumulative 5-year disease-free survival rate was 75.3% for patients with a low level of VEGF-C and 51.6% for those with a high level. Disease-free survival time was shorter in patients with a high VEGF-C level ($P = 0.02$) (Fig. 3). When overall survival was assessed for all patients, the cumulative 5-year survival rate was 67.9% for patients with a low VEGF-C level ($n = 64$) and 48.3% for those with a high level ($n = 63$). Overall survival time was shorter in patients with a high VEGF-C level ($P = 0.02$) (Fig. 4). Multivariate analysis by the Cox proportional hazard model with forward stepwise selection revealed that, along with Duke’s stage ($P < 0.01$), the plasma level of VEGF-C tended to be an independent factor affecting disease-free survival ($P = 0.051$) and was a significantly independent factor affecting overall survival ($P = 0.03$) (Table 2).

**DISCUSSION**

We have shown that an elevated level of plasma VEGF-C correlated with deeper invasion, and more severe venous and lymphatic invasions of the primary tumor, although there was no significant difference in the plasma level between patients with colorectal cancer and the healthy controls. In addition, among patients with Duke’s stages A, B, C and D, who underwent potentially curative surgery, the level of

![Figure 1.](https://example.com/figure1.png)

Figure 1. Plasma levels of vascular endothelial growth factor (VEGF)-C in patients with colorectal cancer ($n = 127$) and healthy controls ($n = 23$).
plasma VEGF-C tended to be higher in patients who subsequently developed recurrence than in those who did not. Most interestingly, a high level of plasma VEGF-C, based on a cut-off value representing the median level in patients with cancer, was predictive of unfavorable disease-free and overall survival in the patients.

To our knowledge, there have been only two previous reports (9, 10) describing the relationship between clinicopathological factors and circulating levels of VEGF-C in patients with colorectal cancer, and these produced conflicting results. It was noteworthy that Duff et al. (9) reported the plasma VEGF-C level to be higher in the control group than in the group with colorectal cancer, and that this parameter did not correlate with any clinicopathological factors. In contrast, Xu et al. (10) reported that the serum level of VEGF-C was higher in patients with colorectal cancer than in healthy controls, and that patients with lymph node metastasis had higher levels of serum VEGF-C than those without lymph node metastasis.

We did not find any significant difference in the plasma level of VEGF-C between patients with colorectal cancer and the healthy controls. The reason for this was unclear, but the results concur with the report by Duff et al. (9). Although the choice of our control group may have influenced the results, they were selected for their similarity to the cancer patients to avoid bias with regard to age and sex distribution.

Given the association of the preoperative plasma VEGF-C level with the depth of tumor, lymphatic and venous invasions, we suggest that the preoperative plasma VEGF-C level might reflect the formation of metastatic foci through angiogenesis and lymphangiogenesis in the tissue surrounding the primary tumor, leading to poor patient survival. This is the first documentation of poor prognosis in colorectal cancer patients with a preoperatively high level of plasma VEGF-C. Our results concur with those obtained from patients with carcinoma of the esophagus (13) and uterine cervix (14). Kimura et al. (13) reported that the preoperative serum level of VEGF-C was useful for predicting lymph node metastasis and recurrence in patients with potentially curable squamous cell carcinoma of the esophagus.

<table>
<thead>
<tr>
<th>Table 2. Independent significant factors affecting disease-free survival by the Cox’ multivariate proportional hazard model</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Factors</strong></td>
</tr>
<tr>
<td>Disease-free survival</td>
</tr>
<tr>
<td>Dukes’ stage</td>
</tr>
<tr>
<td>A, B versus D</td>
</tr>
<tr>
<td>C versus D</td>
</tr>
<tr>
<td>Plasma VEGF-C level</td>
</tr>
<tr>
<td>Low versus High</td>
</tr>
<tr>
<td>Overall survival</td>
</tr>
<tr>
<td>Dukes’ stage</td>
</tr>
<tr>
<td>A, B versus D</td>
</tr>
<tr>
<td>C versus D</td>
</tr>
<tr>
<td>Plasma VEGF-C level</td>
</tr>
<tr>
<td>Low versus High</td>
</tr>
</tbody>
</table>

95% CI, 95% confidence interval; low, ≤1840 pg/ml; high, >1840 pg/ml.
Mitsuhashi et al. (14) reported that the pre-therapeutic serum levels of VEGF-C correlated with FIGO stage, tumor size and disease recurrence or persistence after treatment in patients with squamous cell carcinoma of the uterine cervix. As VEGF-C was the first specific lymphangiogenesis factor to be identified, acting predominantly through the VEGF receptor (VEGFR)-3, several reports have investigated the clinical significance of VEGFR-3 pathway inhibition (15,16). Chen et al. (17) demonstrated that the inhibition of tumor cell VEGF-C expression by stably transfected small interfering RNA reduced lymphangiogenesis, lymph node and lung metastasis of murine mammary cancers. As an alternative approach, soluble VEGFR-3 fusion protein has been shown to inhibit VEGF-C-induced tumor lymphangiogenesis and metastatic spread in a breast cancer xenotransplant model (18). Overexpression of soluble VEGFR-3 by lung cancer cells also reduced the number of intratumoral lymphatic vessels and the incidence of metastasis to draining lymph nodes (19), and ectopic overexpression of soluble VEGFR-3 in an immunocompetent rat mammary tumor model suppressed metastasis formation in lymph nodes and lungs (20). These findings indicate that the blockade of the VEGFR-3 pathway efficiently inhibits lymph node metastasis and likely also reduces the incidence of distant organ metastases. Colorectal cancer patients with an elevated blood level of VEGF-C may be good candidates for therapy involving blockade of the VEGFR-3 pathway.

In conclusion, our results suggest that the preoperative plasma level of VEGF-C would be a useful biomarker for prognostication in patients with colorectal cancer.

**Conflict of interest statement**

None declared.

**References**


2. Achen MG, Jeltsch M, Kukk E, Ma¨kinen T, Vitali A, Wilks AF, et al. Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor 2 (Flk1) and VEGF receptor 3 (Flt4). *Proc Natl Acad Sci USA* 1998;95:548–53.


