Vascular endothelial growth factor gene polymorphisms associated with prognosis for patients with gastric cancer


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Background: The present study analyzed vascular endothelial growth factor (VEGF) gene polymorphisms and their impact on the prognosis for patients with gastric cancer.

Patients and methods: Five hundred and three consecutive patients with surgically resected gastric adenocarcinoma were enrolled in the present study. The genomic DNA was extracted from paraffin-embedded tissue and four VEGF (−406T > C, −116G > A, +405G > C, and +936C > T) gene polymorphisms were determined using a polymerase chain reaction-restriction fragment length polymorphism assay.

Results: The survival analysis showed no association of three VEGF gene polymorphisms with the prognosis. For the +936C > T polymorphism, the T/T genotype, however, had a worse overall survival (OS) compared with the C/C genotype (P = 0.037). The −406T/C or C/C genotype was a poor prognostic factor in patients with stage 0 or I gastric cancer (OS: hazard ratio (HR) = 3.96, disease-free survival (DFS): HR = 4.87). In the haplotype analysis, the CACC haplotype was associated with a significantly worse survival when compared with the TGGC haplotype (OS: HR = 1.72, DFS: HR = 1.73).

Conclusions: VEGF gene polymorphisms were found to be an independent prognostic marker for patients with gastric cancer. Consequently, the analysis of VEGF gene polymorphisms can help identify patient subgroups at high risk of a poor disease outcome.

Key words: gastric cancer, gene, polymorphism, prognosis, VEGF

introduction

Although gastric cancer is increasingly being diagnosed at an early stage, resulting in 10-year survival rates of between 80% and 95% in the Far East, gastric adenocarcinoma is still the second leading cause of cancer-related death worldwide [1, 2].

Angiogenesis, the formation of new blood vessels from endothelial precursors, is a prerequisite for the growth and progression of solid malignancies, and the vascular endothelial growth factor (VEGF) superfamily of endothelial growth factors has been identified to critically influence tumor-related angiogenesis [3, 4]. Clinical studies have demonstrated that an increased expression of VEGF or its family is associated with the grade of angiogenesis and the prognosis for various human cancers [5–8]. In particular, with gastric cancer, the expression of VEGF or VEGF-C, which are intimately involved in the regulation of the lymphangiogenic process, has been reported to be correlated with a poor prognosis [9–11]. Furthermore, Juttner et al. [12] found that the presence of VEGF-D and its receptor vascular endothelial growth factor receptor 3 was associated with lymphatic metastasis, reduced patient survival, and poor prognosis after the curative resection of gastric adenocarcinomas. Given these results, VEGF or its family would seem to play an important role in lymphangiogenesis and lymphatic tumor spread, thereby affecting the prognosis of gastric cancer.

The VEGF gene is assigned to chromosome 6p12-p21 and consists of eight exons separated by seven introns that exhibit alternative splicing to form a family of proteins [13, 14]. Several polymorphisms have been described in the VEGF gene, and some of these variants (−406T > C, −116G > A, and +405G > C, transcription start site counted as +1) in the promoter or 5′-untranslated region and +936C > T in the 3′-untranslated region) found to be associated with variations in VEGF protein production [15–17]. In clinical studies, these polymorphisms have been reported to be involved in the development of solid
tumors, such as melanoma [18], lung [19], prostate [20], and breast cancer [21], where angiogenesis is critical in the pathogenesis of the disease. Moreover, recent studies have demonstrated that genetic polymorphisms can be used to predict the clinical outcomes of gastric [22], breast [23, 24], colon [25], and pancreatic cancer [26].

No study, however, has yet been published that has investigated the single nucleotide polymorphisms (SNPs) of the VEGF gene and their relationship to the clinical outcomes of gastric cancer. Therefore, the present study analyzed four VEGF gene polymorphisms and their impact on the prognosis for patients with gastric adenocarcinoma.

**materials and methods**

**study population**

All the tissues investigated in this study were obtained from consecutive Korean patients (n = 503) who had undergone a surgical gastrectomy between January 2000 and December 2001 at Kyungpook National University Hospital (Daegu, Korea). Written informed consent for gene expression analyses including SNPs was received from all the patients before surgery, and the study was approved by the Institutional Research Board at Kyungpook National University Hospital. The diagnosis and staging of gastric carcinoma were assessed according to the World Health Organization classifications [27] and tumor–node–metastasis (TNM) classifications set out by the American Joint Committee on Cancer [28].

**genotyping of VEGF gene polymorphisms**

The genomic DNA was extracted from paraffin-embedded tumor bearing tissue using a Wizard genomic DNA purification kit (Promega, Madison, WI). The VEGF and VEGF-C were classified by estimating the percentage of epithelial expression analyses including SNPs was received from all the patients before surgery, and the study was approved by the Institutional Research Board at Kyungpook National University Hospital. The diagnosis and staging of gastric carcinoma were assessed according to the World Health Organization classifications [27] and tumor–node–metastasis (TNM) classifications set out by the American Joint Committee on Cancer [28].

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**statistical analysis**

The genotypes for each SNP were analyzed as a three-group categoric variable (referent model), and those were also grouped according to the dominant and recessive model. The haplotypes and their frequencies were estimated using the Bayesian algorithm in the phase program [29], which is available at http://www.stat.washington.edu/stephens/phase.html. The correlation between the level of VEGF or VEGF-C protein expression and four VEGF gene polymorphisms was analyzed using chi-square test and logistic regression analysis. Overall survival (OS) was measured from the day of surgery until the date of death or last follow-up. Disease-free survival (DFS) was calculated from the day of surgery until recurrence or death from any cause. The survival estimates were calculated using the Kaplan–Meier method. The differences in OS or DFS according to the four VEGF gene polymorphisms were analyzed using log-rank tests. Cox proportional hazards regression model was used for the multivariate survival analyses, and the analyses were always adjusted for age (<60 versus ≥60 years), sex (male versus female), stage (0–IV), and adjuvant chemotherapy (i.e., versus oral versus not received). The hazard ratio (HR) and 95% confidence interval (CI) were also estimated. The multivariate analyses were carried out on 492 out of 503 patients, as haplotype data were unavailable for 11 patients.

A cut-off P value of 0.05 was adopted for all the statistical analyses. The statistical data were obtained using an SPSS software package (SPSS 11.5 Inc., Chicago, IL) or SAS Genetic software (SAS Institute, Cary, NC).

**results**

**patient characteristics and survival analysis**

The median age of the patients was 60 (range, 25–83) years, and 337 (67.0%) patients were male. Curative resections (gastrectomy with more than D1 lymph node dissection) were carried out in 479 (97.2%) patients, while the others received a palliative gastrectomy. The pathologic stages after the surgical
Phenotype and effects on survival

The four VEGF gene polymorphisms were successfully amplified in 98%-99% of the cases. The frequencies of each genotype are shown in Table 1. The −460T > C and +405G > C or +936C > T polymorphisms exhibited a strong linkage disequilibrium (correlation coefficient, R = 0.26; Lewontin’s D’, D’ = 0.79 or correlation coefficient, R = 0.39; Lewontin’s D’, D’ = 0.91) in the study population, whereas the linkage with the −116G > A polymorphism was weaker (correlation coefficient, R = 0.09; Lewontin’s D’, D’ = 0.65). The survival analysis showed no association between the three VEGF gene polymorphisms (−460T > C, −116G > A, and +405G > C) and the prognosis. For the +936C > T polymorphism, the T/T genotype, however, exhibited a worse OS compared with the C/C genotype, however, exhibited a worse OS compared with the C/C genotype (HR = 3.23; 95% CI, 1.13–9.25; P = 0.037), although no significant difference was observed in the dominant or recessive model (Table 1). When confined to the patients with stage II–IV diseases, 167 (75.9%) patients received adjuvant chemotherapy with 5-FU for 1 year or just oral 5-FU for 1 year (n = 86). At the time of last analysis (January 2006), 111 patients had experienced a disease relapse and 103 patients had died as a result of gastric cancer. The death of 6 patients, however, was not related to gastric cancer. The estimated 5-year OS and DFS for all the patients was 78.4 ± 1.85% and 77.4 ± 1.87%, respectively.

Table 1. Overall and disease-free survival according to four VEGF gene polymorphisms in all patients

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. (%)</th>
<th>Overall survival</th>
<th>Disease-free survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HR</td>
<td>95% CI</td>
</tr>
<tr>
<td>−460T &gt; C polymorphism</td>
<td>500</td>
<td>1.017</td>
<td>0.852–1.876</td>
</tr>
<tr>
<td>T/T</td>
<td>259 (51.8)</td>
<td>1.264</td>
<td>1.003–6.719</td>
</tr>
<tr>
<td>C/C</td>
<td>224 (44.8)</td>
<td>2.596</td>
<td>0.936–6.342</td>
</tr>
<tr>
<td>C/C</td>
<td>17 (3.4)</td>
<td>1.300</td>
<td>0.878–1.926</td>
</tr>
<tr>
<td>−116G &gt; A polymorphism</td>
<td>496</td>
<td>0.191</td>
<td>0.878–1.926</td>
</tr>
<tr>
<td>G/G</td>
<td>339 (68.3)</td>
<td>1</td>
<td>0.878–1.926</td>
</tr>
<tr>
<td>G/A</td>
<td>157 (31.7)</td>
<td>1</td>
<td>0.878–1.926</td>
</tr>
<tr>
<td>A/A</td>
<td>0</td>
<td>1</td>
<td>0.878–1.926</td>
</tr>
<tr>
<td>+405G &gt; C polymorphism</td>
<td>499</td>
<td>0.389</td>
<td>0.495–1.134</td>
</tr>
<tr>
<td>G/G</td>
<td>150 (30.1)</td>
<td>0.749</td>
<td>0.373–1.675</td>
</tr>
<tr>
<td>C/C</td>
<td>312 (62.5)</td>
<td>0.790</td>
<td>0.373–1.675</td>
</tr>
<tr>
<td>+936C &gt; T polymorphism</td>
<td>498</td>
<td>0.037</td>
<td>0.500–1.209</td>
</tr>
<tr>
<td>C/T</td>
<td>337 (67.7)</td>
<td>1</td>
<td>0.500–1.209</td>
</tr>
<tr>
<td>T/T</td>
<td>152 (30.5)</td>
<td>0.777</td>
<td>0.500–1.209</td>
</tr>
<tr>
<td></td>
<td>9 (1.8)</td>
<td>3.231</td>
<td>1.128–9.252</td>
</tr>
</tbody>
</table>

P values correspond to multivariate Cox model adjusted for age, sex, tumor–node–metastasis stage, and adjuvant chemotherapy. VEGF, vascular endothelial growth factor; HR, hazard ratio; CI, confidential interval.
shown). Also, no correlation was observed with any other clinicopathologic variables. Finally, no association between the grade of immunostaining and the OS or DFS was observed (Table 4).

**discussIon**

We have investigated the prognostic impact of four VEGF gene polymorphisms in quite a large population of patients with surgically resected gastric adenocarcinoma. The current study demonstrated that CAGG haplotype or $-460 \text{T/C} \rightarrow \text{C/C}$ genotype was a poor prognostic factor in these patients.

The role of genetic polymorphisms, which is an important determinant of endogenous causes of cancer, in relation to the risk for gastric cancer has attracted increasing interest, probably because of advances in DNA analysis technologies and human genome knowledge. Most previous studies, however, have only addressed the effect of genetic variants of metabolic enzymes and inflammation mediators [30]. Since VEGF or its family plays a critical role in tumor-related angiogenesis, the association of VEGF gene polymorphisms with the risk or prognosis of several solid tumors, such as melanoma [18], lung [19], prostate [20], and breast cancer [21, 23, 24], has already been demonstrated.

The genotype frequencies of $-460 \text{T} > \text{C}$, $+405 \text{G} > \text{C}$ or $+936 \text{C} > \text{T}$ in the present study were similar to those previously reported for the Korean [19], Chinese [23], and European populations [16, 21, 24], whereas the frequency of the $-116 \text{G/G}$ genotype was higher than that for European patients (68.3% versus 42.1%) [24]. The $-460 \text{T} > \text{C}$ and $+405 \text{G} > \text{C}$ polymorphisms exhibited a strong linkage disequilibrium in the present study population, while the linkage between the $-460 \text{T} > \text{C}$ and $-116 \text{G} > \text{A}$ polymorphisms was weak, which is consistent with findings from an earlier study [15, 19, 23].

In the present study, the CACC haplotype was associated with a significantly worse survival when compared with the TGCC haplotype in patients with surgically resected gastric cancer (OS: $HR = 1.72$, DFS: $HR = 1.73$), and the $-460 \text{T/C}$ or C/C genotype was a poor prognostic factor in patients with stage 0 or I gastric cancer (OS: $HR = 3.957$, DFS: $HR = 4.870$). In a recent study by Lu et al. [23] that also evaluated the effects of three VEGF gene polymorphisms (without the $-116 \text{G/G}$ polymorphism) in a Chinese population, the CACC haplotype was associated with a significantly worse survival when compared with the TGGC haplotype (OS: $HR = 1.72$, DFS: $HR = 1.73$). In a recent study by Lu et al. [23] that also evaluated the effects of three VEGF gene polymorphisms

<table>
<thead>
<tr>
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<th>No. (%)</th>
<th>Overall survival</th>
<th>Disease-free survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HR 95% CI</td>
<td>P value</td>
</tr>
<tr>
<td>$-460 \text{T} &gt; \text{C}$ polymorphism</td>
<td>282</td>
<td>T/T 147 (52.1) 1</td>
<td>0.104 1</td>
</tr>
<tr>
<td>Referent model</td>
<td></td>
<td>T/C 127 (45.0) 4.102 1,118–15.046</td>
<td>5.029 1.406–17.993</td>
</tr>
<tr>
<td>C/C 8 (2.8)</td>
<td></td>
<td>T/C–C/C 135 (47.9) 3.957 1,078–14.521</td>
<td>4.870 1.360–17.432</td>
</tr>
<tr>
<td>Dominant model for C alleles</td>
<td></td>
<td>T/T 147 (52.1) 1</td>
<td>0.038 1</td>
</tr>
<tr>
<td>T/C–C/C 135 (47.9)</td>
<td></td>
<td></td>
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</tbody>
</table>

$P$ values correspond to multivariate Cox model adjusted for age, sex, and tumor–node–metastasis stage.

HR, hazard ratio; CI, confidential interval.

Figure 1. Overall survival (A) and disease-free survival (B) curves according to $-460 \text{T} > \text{C}$ polymorphism in patients with stage 0 or I gastric cancer. $P$ values correspond to dominant model in multivariate Cox model adjusted for age, sex, and tumor–node–metastasis stage.
and +405 G/G genotypes and higher among the patients with the −460T/+405C/+936C haplotype. One possible explanation for these results is that the DNA sequence variations in the VEGF gene may alter VEGF production and/or activity, thereby causing interindividual differences in the lymphangiogenesis and lymphatic tumor spread. Given the homogenous ethnic background of Korean patients, any potential confounding effect due to ethnicity is likely to be small in the present study.

The correlation between an elevated preoperative serum VEGF concentration and the poor survival in patients with gastric cancer was already demonstrated in previous study [31]. A few studies, however, have reported that VEGF gene polymorphisms are associated with VEGF production. Nonetheless, the results are inconsistent. Awata et al. [32] reported that individuals with the +405 C/C genotype had a higher fasting serum VEGF level than those with other genotypes, and that they carried an increased risk of diabetic retinopathy. Meanwhile, Watson et al. [15] documented that the +405G allele is associated with higher lipopolysaccharide-stimulated VEGF production by peripheral blood mononuclear cells than the +405C allele. In a recent in vitro study [17], carrying a haplotype containing the −460C/+405G polymorphisms was found to significantly increase basal VEGF promoter activity and phorbol ester-induced responsiveness compared with the presence of a haplotype containing the −460T/+405C polymorphisms.

In the present study, any of the four VEGF gene polymorphisms was not correlated with the level of VEGF or VEGF-C protein expression. Koukourakis et al. [33] reported that −2578 C/C, −634 G/G, and −1154 A/A and G/A genotype (from translation start site) in the VEGF gene were linked with low VEGF protein expression in non-small cell lung cancer (NSCLC). They indicate that inherited variations in VEGF sequence, that also characterize the tumor genome, are determinants of the molecular VEGF phenotype in NSCLC and, consequently, of the intratumoral vascular density. Sample size, however, was small (36 cases) and VEGF −460T > C, +405G > C, and +936C>T polymorphisms were not evaluated in their study. Accordingly, further studies are needed to elucidate the relationship between the VEGF gene polymorphisms and serum VEGF level or VEGF protein expression.

Recently, several VEGF inhibitors, including bevacizumab, a monoclonal antibody against VEGF, have actively progressed into clinical trials for different tumor types, and the results are very encouraging [34]. Thus, if the prediction of individual angiogenic potential on the basis of VEGF genotypes is possible, the efficacy of antiangiogenic treatment could be further enhanced.

### Table 3. Overall and disease-free survival according to common haplotypes of four VEGF gene polymorphisms in all patients

<table>
<thead>
<tr>
<th>Haplotype (−460T &gt; C/−116G &gt; A/+405G &gt; C/+936C &gt; T)</th>
<th>Haplotype frequency (%)</th>
<th>Overall survival HR 95% CI P value</th>
<th>Disease-free survival HR 95% CI P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGGC 46.2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>TGGT 7.9</td>
<td>1.160 0.667–2.017 0.600</td>
<td>1.240 0.723–2.126 0.434</td>
<td></td>
</tr>
<tr>
<td>TGCC 17.0</td>
<td>0.995 0.659–1.502 0.979</td>
<td>1.047 0.701–1.563 0.824</td>
<td></td>
</tr>
<tr>
<td>CGCC 5.6</td>
<td>1.251 0.681–2.299 0.471</td>
<td>1.129 0.600–2.124 0.707</td>
<td></td>
</tr>
<tr>
<td>CACC 6.5</td>
<td>1.719 1.022–2.893 0.041</td>
<td>1.733 1.031–2.915 0.038</td>
<td></td>
</tr>
</tbody>
</table>

*P* values correspond to multivariate Cox model adjusted for age, sex, tumor–node–metastasis stage, and adjuvant chemotherapy.

**Table 3.** Overall and disease-free survival according to common haplotypes of four VEGF gene polymorphisms in all patients

**Figure 2.** Overall survival (A) and disease-free survival (B) curves according to haplotypes in all patients with gastric cancer. *P* values correspond to multivariate Cox model adjusted for age, sex, tumor–node–metastasis stage, and adjuvant chemotherapy.
Table 4. Survival analysis according to the level of VEGF and VEGF-C protein expression

<table>
<thead>
<tr>
<th>No.</th>
<th>%</th>
<th>Overall survival HR (95% CI)</th>
<th>P value</th>
<th>Disease-free survival HR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor cells expressing VEGF</td>
<td>374</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 0 (≤10%)</td>
<td>37</td>
<td>9.9</td>
<td>1</td>
<td>0.234</td>
<td>1</td>
</tr>
<tr>
<td>Grade I/II (&gt;10%)</td>
<td>337</td>
<td>91.1</td>
<td>1.747 (0.696–4.384)</td>
<td>1.844 (0.737–4.617)</td>
<td></td>
</tr>
<tr>
<td>Tumor cells expressing VEGF-C</td>
<td>371</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 0 (≤10%)</td>
<td>93</td>
<td>25.1</td>
<td>1</td>
<td>0.076</td>
<td>1</td>
</tr>
<tr>
<td>Grade I/II (&gt;10%)</td>
<td>278</td>
<td>74.9</td>
<td>1.562 (0.954–2.558)</td>
<td>1.429 (0.897–2.277)</td>
<td></td>
</tr>
</tbody>
</table>

P values correspond to multivariate Cox model adjusted for age, sex, tumor–node–metastasis stage, and adjuvant chemotherapy.

VEGF, vascular endothelial growth factor; VEGF-C, vascular endothelial growth factor C; HR, hazard ratio; CI, confidential interval.

In conclusion, VEGF gene polymorphisms were found to be an independent prognostic marker for Korean patients with surgically resected gastric adenocarcinoma. Consequently, in addition to the pathologic stage, the analysis of VEGF gene polymorphisms may help identify patient subgroups at high risk for a poor disease outcome, thereby helping to refine therapeutic decisions in gastric cancer.

acknowledgements

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references