Association of Genetic Polymorphisms in the VEGF Gene with Breast Cancer Survival

Hua Lu,1 Xiao-Ou Shu,1 Yong Cui,1 Nobuhiko Kataoka,1 Wanqing Wen,1 Qiuyin Cai,1 Zhi-Xian Ruan,2 Yu-Tang Gao,2 and Wei Zheng1

1Department of Medicine, Center for Health Services Research, Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, Tennessee and 2Department of Epidemiology, Shanghai Cancer Institute, Shanghai, China

Abstract

The vascular endothelial growth factor (VEGF) is an important regulator of angiogenesis and vascular permeability. VEGF overexpression has been associated with advanced stage and poor survival of several cancers. We evaluated the association of functional polymorphisms in the VEGF gene with breast cancer survival in a cohort of 1,193 breast cancer patients who were recruited as part of a population-based case-control study in Shanghai, China from 1996 to 1998 and followed for cancer recurrence and mortality between March 2000 and December 2002. Included in the study were three functional polymorphisms (C−460T, G+405C, and C+936T) in the VEGF gene. Carrying the −460C or +405G allele was associated with decreased overall survival. The age-adjusted hazard ratios (HR) were 1.5 (95% confidence interval [CI], 0.9-2.5) for −460CC genotype carriers and 1.6 (95% CI, 1.0-2.5) for −460TG genotype carriers compared with noncarriers. Further analyses showed that the −460T/+450C/+936C haplotype was related to increased survival (HR, 0.57; 95% CI, 0.4-0.9), whereas the −460C/+405G/+936T haplotype was associated with nonsignificantly decreased survival (HR, 2.1; 95% CI, −0.9 to 4.7). The C+936T polymorphism alone was not related to overall or disease-free survival. This study suggests that VEGF polymorphisms may be a significant genetic marker for breast cancer prognosis. (Cancer Res 2005; 65(12): 5015-9)

Introduction

The growth of solid tumors, including breast cancer tumors, depends on angiogenesis, the process by which new blood vessels develop from the endothelium of a preexisting vasculature. Tumors promote angiogenesis by secreting or activating angiogenic factors that stimulate endothelial migration, proliferation, and capillary morphogenesis. Newly formed blood vessels supply the tumor with nutrients and oxygen, dispose of the metabolic waste products of tumor cells, generate paracrine stimuli, and provide potential routes for tumor dissemination. Thus, tumor-induced angiogenesis plays a pivotal role in cancer progression and metastasis (1, 2).

The vascular endothelial growth factor (VEGF) is one of the most potent endothelial cell mitogens and plays a critical role in angiogenesis. VEGF specifically binds to two transmembrane VEGF receptor tyrosine kinases on endothelial cells to initiate intracellular signal transduction pathways that mediate angiogenesis and vascular permeability (3). Several lines of compelling evidence from in vitro and in vivo experiments have shown that increased VEGF expression is associated with tumor growth and metastasis, whereas the inhibition of VEGF signaling results in suppression of both tumor-induced angiogenesis and tumor growth (4). Clinically, high levels of blood, urinary, or intratumoral VEGF and increased microvessel density in tumors have been observed to be associated with advanced stage disease and worse prognosis for several types of tumors, including breast cancer (2, 5–8).

The VEGF gene is located on chromosome 6p21.3. At least 30 single-nucleotide polymorphisms (SNPs) in this gene have been described in the literature. Of particular note are three SNPs (G+405C in the 5′-untranslated region, C−460T in the promoter region, and C+936T in the 3′-untranslated region) that are common and are related to VEGF protein production (9–12). The +936T allele has been related to lower levels of plasma VEGF in healthy men (9) and women (10), whereas the +405G allele has been shown to significantly increase lipopolysaccharide-stimulated VEGF protein production in peripheral blood mononuclear cells (11). Furthermore, carrying a haplotype containing the +405G and −460C polymorphisms was found to significantly increase basal VEGF promoter activity and phorbol ester–induced responsiveness (12). These SNPs have been implicated in the risk for several types of tumors and other diseases with a putative angiogenic basis (10, 13–15). However, the effect of these polymorphisms on breast cancer prognosis has not been evaluated. Based on the biological and pathologic significance of VEGF, it is possible that functional genetic variations in the VEGF gene may contribute to the progression of breast cancer. To test this hypothesis, we evaluated the effects of these three functional polymorphisms (G+405C, C−460T, and C+936T) in the VEGF gene on breast cancer survival in a cohort of breast cancer patients who had participated in a population-based case-control study, the Shanghai Breast Cancer Study (16).

Materials and Methods

Participants. Of the 1,459 breast cancer patients who were recruited into the Shanghai Breast Cancer Study from August 1996 to March 1998 and ages 25 to 64 years, 1,455 were included in the survival study. Four subjects were excluded due to lack of adequate information for follow-up. The initial institutional diagnoses of breast cancer were confirmed by independent review of pathologic slides by two senior pathologists. A peripheral blood sample (10 mL from each woman) was obtained from 1,193 (82%) study participants at recruitment. The blood samples were typically processed within 6 hours of collection and stored at −70°C until the relevant bioassays were conducted. Information on cancer diagnosis, disease stage (tumor-node-metastasis stage, TNM stage), cancer treatments, and estrogen and progesterone receptor (ER/PR) status was abstracted from medical charts using a standard protocol.

Patients were followed for cancer recurrence and mortality between March 2000 and December 2002 with a combination of active follow-up and record linkage to the death certificates kept by the Vital Statistics Unit of the Shanghai Center for Disease Control and Prevention (17). Of the 1,455...
eligible patients, 1,290 (88.4%) were followed-up via in-person contact or by phone from March 2000 to December 2002. Among them, 200 patients were deceased. Through interview of patients, or next of kin for deceased patients, we obtained information on disease progress, recurrence, quality of life, and cause of death (if deceased). Survival status for the remaining 165 patients, respectively.

DNA isolation and genotyping assays. Genomic DNA was extracted from buffy coat fractions using a Puregene DNA Purification kit (Gentra Systems, Minneapolis, MN) following the manufacturer’s protocol. DNA concentration was measured using a PicoGreen dsDNA Quantitation Kit (Molecular Probes, Eugene, OR). The allelic discrimination of the VEGF gene polymorphisms was assessed with the ABI PRISM 7900 Sequence Detection Systems (Applied Biosystems, Foster City, CA), using the fluorogenic 5′-VIC-labeled Taqman Minor Groove Binder (MGB) probes. The wild-type Taqman MGB probes were FAM labeled; and the mutant probes were VIC labeled. The final volume for each reaction was 5 μL, consisting of 2.5 μL Taqman Universal PCR Master Mix (Applied Biosystems), 0.6 μL of each primer, 0.2 μL of each Taqman probe, and 2.5 ng genomic DNA. The PCR profile was an initial denaturation step at 95°C for 10 minutes and 40 to 55 cycles with 92°C for 15 seconds and 60°C for 1 minute. Fluorescent signals were measured at 60°C. The primers and probes for the G−405C (rs2010963) and the C−460T (rs833061) polymorphism assays (assay on demand), as well as for the C+936T (rs3025039) polymorphism assay (Assay-by-Design), were ordered from Applied Biosystems.

The laboratory staff was blind to the identity of the subjects. Quality control samples were included in the genotyping assays. Each 384-well plate contained four water, eight CEPH 1347-02 DNA, eight blinded quality control samples, and eight unblinded quality control samples. The blinded and unblinded quality control samples were taken from the second tube of study samples included in the study. The concordance for the blinded samples was >97%. Genotypes for polymorphisms of G−405C, C−460T, and C+936T in the VEGF gene were successfully determined for 1,091, 1,119, and 1,105 patients, respectively.

Statistical analysis. The primary outcomes used for this study were overall survival and disease-free survival. The end point for the overall survival analysis was any death and for the disease-free survival analysis was cancer recurrence/metastasis or death related to breast cancer. Survival time was calculated as the time from cancer diagnosis to the end points of cancer recurrence/metastasis or death related to breast cancer. Survival analysis was any death and for the disease-free survival analysis was any cancer event. The Kaplan-Meier method was used to compute 5-year survival rates and the log-rank test was applied to test the differences in survival across different genotypes. The Cox regression model was applied to evaluate the effect of the VEGF genotype on overall survival and disease-free survival with adjustments for age. Analyses were also conducted by stratifying the data by breast cancer characteristics of breast cancer to examine the potential interactive effects.
association of haplotypes and breast cancer survival was evaluated using a Cox model by treating each haplotype as a continuous variable with probabilistically assigned values, as recommended by Zaykin (19). All tests were based on two-sided probability.

Results

Table 1 shows selected demographic factors and known prognostic factors of breast cancer and the corresponding 5-year survival rates for the whole cohort of 1,455 breast cancer patients and the 1,119 subjects who were included in the genotype association study. As expected, older age and TNM stage were the major prognostic factors for survival. Virtually, all (99%) patients received surgery and 94% of patients received chemotherapy. Radiotherapy was related with poorer prognosis, an effect reflecting late stage at diagnosis. ER/PR status was not predictive of overall survival, although the point estimates were 30.0%, 32.5%, and 17.8%. The association of overall survival after diagnosis of breast cancer with the genotypes defined by these SNPs. No clear association was observed between C+936T polymorphism and overall survival or disease-free survival.

Haplotype analyses were conducted to evaluate the combined effect of the three polymorphisms on breast cancer survival (Table 3). The three most common haplotypes in this cohort of breast cancer patients were −460TT/+450G/+936C, −460TT/+450C/+936C, and −460C/+450G/+936C, with the respective frequencies being 30.0%, 32.5%, and 17.8%. The association of overall survival after diagnosis of breast cancer with decreased overall survival was observed, although the point estimates were not statistically significant. No significant associations between haplotypes and disease-free survival were observed.

The associations of the VEGF G+405C and C−460T genotypes with tumor characteristics at diagnosis were examined. We found that these polymorphisms were unrelated to the TNM, ER, and PR status of the cancer at diagnosis. Additional adjustment for those

---

Table 2. G+405C, C−460T, and C+936T polymorphisms of VEGF gene in association with breast cancer survival, the Shanghai Breast Cancer Study

<table>
<thead>
<tr>
<th>VEGF</th>
<th>Genotype</th>
<th>Cases</th>
<th>Overall survival</th>
<th>Disease-free survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Events</td>
<td>5-y survival %*</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C−460T</td>
<td>TT</td>
<td>614</td>
<td>92</td>
<td>85.7</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>417</td>
<td>70</td>
<td>83.4</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>88</td>
<td>19</td>
<td>78.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.19</td>
<td></td>
<td>0.11</td>
</tr>
<tr>
<td>G+405C</td>
<td>CC</td>
<td>191</td>
<td>26</td>
<td>87.8</td>
</tr>
<tr>
<td></td>
<td>GC</td>
<td>506</td>
<td>77</td>
<td>85.3</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>394</td>
<td>78</td>
<td>80.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.03</td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C+936T</td>
<td>CC</td>
<td>741</td>
<td>114</td>
<td>85.0</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>333</td>
<td>59</td>
<td>83.1</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>31</td>
<td>6</td>
<td>79.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.46</td>
<td></td>
<td>0.28</td>
</tr>
</tbody>
</table>

*Proportion of survival derived from Kaplan-Meier analysis.
†Adjusted for age.
‡Subjects with missing information on disease relapse (n = 165) were excluded from this analysis.

---

Downloaded from cancerres.aacrjournals.org on March 23, 2021. © 2005 American Association for Cancer Research.
tumor characteristics in the Cox regression model did not materially change the genotype-survival associations (data not shown). We found no major modifying effect of these tumor characteristics on the gene-survival associations (data not shown).

Discussion

Angiogenesis is not only essential for tumor growth but also plays a critical role in the invasion and metastasis of tumor cells. Angiogenesis is regulated by many growth factors, among which VEGF plays a central role and serves as an important prognostic factor in a variety of tumors, including breast cancers. A number of functional polymorphisms in the VEGF gene have been reported and have been associated with increased risk for several tumors (10, 14). To our knowledge, no previous study has reported an association of VEGF gene polymorphism with breast cancer survival.

The genotype frequencies of the G+405C and C+936T polymorphisms in the current study population are similar to those reported in Japanese and European populations (9, 10, 13). The frequency of the −460TT genotype (54.4%) in our study population, however, is higher than those reported in two previous, smaller studies (30% and 36.1%, based on <200 subjects; refs. 14, 15). We found that G+405C and C−460T polymorphisms are in strong linkage disequilibrium in our study population, consistent with findings from an earlier study (11). Survival after breast cancer diagnosis was lower among patients with the −460CC and +405GG genotypes and higher among subjects with the −460TT/+405CG/+936T haplotype. These findings are biologically plausible, given the pivotal role the VEGF gene plays in tumor growth and progression and the functional significance of these SNPs. It is well documented that overexpression of the VEGF gene and high blood or tumor levels of VEGF is associated with poor breast cancer progression (2, 8) and high levels of intratumoral VEGF predicts poor response to systemic therapy in advanced breast cancer (6, 7). The G+405C GG genotype has been previously linked to significantly increased VEGF production (11). The +936T allele of VEGF has also been reported to be associated with lower VEGF plasma levels in healthy subjects (9, 10) and with decreased risk for breast cancer (10). In a recent in vitro study, carrying a haplotype containing the +405G and −460C polymorphisms was found to significantly increase basal VEGF promoter activity and phorbol ester–induced responsiveness (12). Several VEGF inhibitors have currently progressed into cancer clinical trials in different tumor types, including breast cancer, and preliminary data are very encouraging (20). If the individual angiogenic potential could be predicted on the basis of VEGF genotypes, the efficacy of

Table 3. Association of VEGF haplotype and breast cancer survival, the Shanghai Breast Cancer Study

<table>
<thead>
<tr>
<th>Haplotypes (C−460T/G+405C/C+936T)</th>
<th>Haplotype frequency*</th>
<th>Overall (n = 1,129)</th>
<th>Disease-free (n = 1,006)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>P</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>TGC</td>
<td>30.0</td>
<td>1.2 (0.7-1.9)</td>
<td>0.54</td>
</tr>
<tr>
<td>TGT</td>
<td>5.1</td>
<td>1.4 (0.4-4.6)</td>
<td>0.59</td>
</tr>
<tr>
<td>TCC</td>
<td>32.5</td>
<td>0.6 (0.4-0.9)</td>
<td>0.02</td>
</tr>
<tr>
<td>TCT</td>
<td>6.0</td>
<td>0.9 (0.3-2.5)</td>
<td>0.76</td>
</tr>
<tr>
<td>CGC</td>
<td>17.8</td>
<td>1.4 (0.8-2.4)</td>
<td>0.23</td>
</tr>
<tr>
<td>CGT</td>
<td>6.4</td>
<td>2.1 (0.9-4.7)</td>
<td>0.08</td>
</tr>
<tr>
<td>CCC</td>
<td>1.8</td>
<td>0.3 (0.0-7.5)</td>
<td>0.48</td>
</tr>
<tr>
<td>CCT</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Haplotype frequency was derived using the program PHASE.

†HRs were derived from the Cox regression model by treating the probability of each haplotype as a continuous independent variable.
angiogenic treatment in solid tumors, including breast cancer, could be further enhanced.

It is worth mentioning that the associations in this study between the single SNPs or haplotypes of the VEGF gene and disease-free survival were not as clear as those observed for overall survival. However, the information on disease relapse and cause of death in this study was based on self-reports or reports from next of kin (for the deceased subjects). The misclassification in ascertaining the outcomes for disease-free survival is most likely to be undifferentiable, and thus could have biased results toward null. In addition, information on relapse was missing for 165 subjects whose survival status was established via linkage with the death registry. The reduced sample size in the disease-free survival analysis compromised the statistical power of this analysis.

In summary, we found that the +450G and −460C alleles in the VEGF gene were significantly associated with poorer survival after diagnosis of breast cancer, and that the −460T/+405C/+936C haplotype was associated with more favorable survival outcomes.

These findings were based on a large cohort of breast cancer patients who were systematically identified and recruited with a high response rate, which minimized potential selection bias. Given the homogeneous ethnic background of Chinese women living in Shanghai (>98% belong to a single ethnic group), any potential confounding effect due to ethnicity is likely to be small. The association found in this study is biologically plausible and may have significant implications for breast cancer treatment.

Acknowledgments


Grant support: National Cancer Institute grants USPHS RO1CA64277 and RO1CA80899.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Drs. QJ Dai and Fan Jin for their contributions in coordinating data and specimen collection in Shanghai, Bethanie Hull for her assistance in the preparation of this article, and all the study participants and research staff of the Shanghai Breast Cancer Study for support.

References

Association of Genetic Polymorphisms in the VEGF Gene with Breast Cancer Survival

Hua Lu, Xiao-Ou Shu, Yong Cui, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/65/12/5015

Cited articles
This article cites 20 articles, 8 of which you can access for free at:
http://cancerres.aacrjournals.org/content/65/12/5015.full#ref-list-1

Citing articles
This article has been cited by 17 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/65/12/5015.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/65/12/5015.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.