Predictive value of vascular endothelial growth factor polymorphisms on the risk of renal cell carcinomas

W. Xian, H. Zheng and W.J. Wu

The Third Affiliated Hospital of Xinxiang Medical University, Xinxiang, China

Corresponding author: X. Wei
E-mail: xianwei555@126.com

Received August 19, 2014
Accepted January 22, 2015
Published July 13, 2015
DOI http://dx.doi.org/10.4238/2015.July.13.8

ABSTRACT. We conducted a case-control study in a Chinese population to assess whether 5 common single-nucleotide polymorphisms in the vascular endothelial growth factor gene (VEGF) affect the risk of renal cell carcinoma (RCC). The study population included 266 RCC patients who were newly diagnosed and histologically confirmed to have RCC as well as 532 cancer-free controls. Genotyping of VEGF -2578C/A, -1156G/A, +1612G/A, +936C/T, and -634G/C was conducted by polymerase chain reaction-restriction fragment length polymorphism. RCC patients were more likely to have higher body mass index, and have a habit of tobacco smoking as well as suffer from diabetes. Conditional logistic regression analyses showed that individuals with the AA genotype and A allele of -2578C/A significantly increased the risk of RCC when compared with the CC genotype. Individuals carrying the CT and TT genotypes of +936C/T were correlated with an increased risk of RCC compared to the CC genotype. The T allele of +936C/T was associ-
ated with an increased risk of RCC. The -2578C/A and +936C/T polymorphisms in the VEGF gene may play a role in the etiology of RCC.

**Key words:** Polymorphism; Renal cell carcinomas; Vascular endothelial growth factor

**INTRODUCTION**

Renal cell carcinoma (RCC) is a serious malignant tumor and the 3rd leading cause of death in genitourinary malignant tumor cases, accounting for 2-3% of all cancers (Jemal et al., 2009). It is estimated that the annual incidence of RCC is 37.7 men and 16.6 women per 10^5 individuals in Chinese population (Yang et al., 2005).

It is well-known that development of RCC is a complex, multistep, and multifactorial process, and involves multiple environmental and genetic factors (McLaughlin et al., 1995; Bergström et al., 2001; Bjørg et al., 2004; Hunt et al., 2005; Bellocco et al., 2012). Previous studies showed that cigarette smoking, alcohol drinking, obesity, a history of hypertension, occupational exposures, and physical activity as well as family history of cancer play important roles in RCC development (McLaughlin et al., 1995; Bergström et al., 2001; Bjørg et al., 2004; Hunt et al., 2005; Bellocco et al., 2012).

Previous studies have shown that cancer stem cells can play a key role in causing tumors (McLaughlin et al., 1995; Mu et al., 2014). With developments in molecular biology, many studies have reported that numerous genetic factors are involved in the development of RCC, such as glutathione S-transferases, C-X-C chemokine receptor type 4, miR-34b/c, and leptin receptor genes (Cai et al., 2013; Mu et al., 2014; Zhang et al., 2014; Jia et al., 2014).

Vascular endothelial growth factor (VEGF) is a common pro-angiogenic growth factor and it is one of the most potent endothelial cell mitogens (Motzer et al., 2007; Patard et al., 2008). Stimulation of VEGF under hypoxic conditions is involved in prolonging the lifetime of malignant cells, which play a critical role in tumor growth and invasion and the development of metastases of malignant tumor. It is well-known that single-nucleotide polymorphisms (SNPs) in VEGF can affect the expression of this gene. Previous studies showed that -2578C/A, -1156G/A, +1612G/A, +936C/T, and -634G/C are 5 common SNPs in VEGF, and they are reported to play a role in VEGF protein synthesis (Maeda et al., 2013).

Several previous studies reported that polymorphisms in VEGF play an important role in the development of several kinds of cancers such as bladder cancer, breast cancer, lung cancer, and renal cell carcinoma (Ajaz et al., 2011; Maeda et al., 2013; Kapahi et al., 2014; Yang et al., 2014). However, few studies have examined the association between VEGF polymorphisms and RCC risk, and the results are inconsistent (Zhang et al., 2013; Zhong et al., 2014). A recent meta-analysis revealed no association between VEGF polymorphisms and RCC risk because of limitations of the studies (Zhang et al., 2013).

Therefore, we conducted a case-control study in a Chinese population and assessed whether the 5 common SNPs affect RCC risk.
MATERIAL AND METHODS

Study population

A hospital-based case-control design was used in this study. All patients were histologically confirmed to have RCC at the Third Affiliated Hospital of Xinxiang Medical University between January 2011 and January 2013. The study population consisted of 266 RCC patients who were newly diagnosed and histologically confirmed to have RCC. The 532 cancer-free controls were randomly recruited from a pool of individuals who came to receive a health check-up in the health check-up center of the same hospital, and the control subjects were free from any cancer, and 2 healthy control subjects were matched to 1 case by gender and age.

All cases and control subjects signed an informed consent form before participating in this study, and the protocol of this study was approved by the institutional Ethics Committee of the Third Affiliated Hospital of Xinxiang Medical University.

Data collection

We collected data regarding the demographic and clinical characteristics from a self-designed questionnaire or medical records, including gender, age, body mass index (BMI), smoking status, diabetes status, histology, metastasis, and tumor stage.

Blood samples and genotyping

Case and control subjects were asked to provide a 5-mL blood sample, and 0.5 mg/mL ethylenediaminetetraacetic acid was used as an anticoagulant and samples were stored at -70°C until use. Genomic DNA was isolated from peripheral blood using the TIANamp Blood DNA Kit (Tiangen, Beijing, China) according to the manufacturer instructions. Five SNPs of the \textit{VEGF} gene were detected by polymerase chain reaction-restriction fragment length polymorphism according to manufacturer instructions, including \textit{VEGF} -2578C/A, -1156G/A, +1612G/A, +936C/T, and -634G/C.

The primers and probes used for -2578C/A, -1156G/A, +1612G/A, +936C/T, and -634G/C in the \textit{VEGF} gene were designed using the Sequenom Assay Design 3.1 software (Sequenom, San Diego, CA, USA). The amplification program consisted of: preliminary denaturation at 95°C for 10 min, followed by 35 cycles of denaturation at 95°C for 30 s, 62°C for 30 s, and 72°C for 30 s, and a final extension at 72°C for 10 min. We also randomly selected 5% of the cases and control subjects to repeat genotyping of the 5 SNPs, and the results confirmed the previous results.

Statistical analysis

All statistical analyses were conducted using the STATA version 9.0 statistical
VEGF polymorphisms and RCC

software. Continuous and categorical variables are reported as means ± SD and N (%) of subjects and analyzed by the Student t-test. Categorical variables are reported as frequencies and percentage of study participants and analyzed by the χ²-test, respectively. The Hardy-Weinberg equilibrium of VEGF -2578C/A, -1156G/A, +1612G/A, +936C/T, and -634G/C genotype frequencies between groups in control subjects were compared by the χ²-test. Differences in demographic and clinical factors between groups were compared by the χ²-test. Unconditional logistic regression was conducted to assess the effects of VEGF -2578C/A, -1156G/A, +1612G/A, +936C/T, and -634G/C on the risk of RCC. The results are reported as odds ratio (OR) and their corresponding 95% confidence intervals (CIs). All P values were 2-sided, and a P value was regarded as statistically significant when it was less than 0.05.

RESULTS

There were 87 females and 179 males in the RCC case group, while there were 174 females and 358 males in the control group (Table 1). The mean age of cases and control subjects were 60.4 ± 11.4 and 59.2 ± 10.6 years, respectively. RCC patients were more likely to have a higher BMI and have a habit of tobacco smoking as well as suffer from diabetes.

Among the RCC patients, 217 patients (81.6%) had clear cell RCC and 12 (4.5%) had papillary RCC. Fifty-seven patients (21.4%) showed metastasis, 182 (68.4%) were at stage I-II, and 84 (31.6%) were at III-IV.

The genotype distributions of -2578C/A, -1156G/A, +1612G/A, +936C/T, and -634G/C in RCC cases and control subjects are shown in Table 2. We found that the genotype distributions of -2578C/A, -1156G/A, +1612G/A, +936C/T, and -634G/C were in Hardy-Weinberg equilibrium in the control subjects. Conditional logistic regression analyses showed that individuals with the AA genotype of -2578C/A significantly increased the risk of RCC when compared with the CC genotype, with OR (95%CI) of 1.84 (1.15-2.93). Moreover, the A allele of -2578C/A was associated with a significantly higher risk of RCC compared to the C allele (OR = 1.37, 95%CI = 1.09-1.70).

Individuals carrying the CT and TT genotype of +936C/T were correlated with an increased risk of RCC compared to the CC genotype, with ORs (95%CI) of 1.51 (1.05-2.17) and 1.93 (1.25-2.97), respectively. The T allele of +936C/T was associated with an increased risk of RCC (OR = 1.43, 95%CI = 1.15-1.77). However, we found no significant role of -1156G/A, +1612G/A, and -634G/C on the risk of RCC.

Stratification analysis on the association between SNPs of VEGF and demographic characteristics in RCC showed that the AA genotype of -2578C/A was associated with an increased risk of RCC in females, as well as those with higher age, higher BMI, and non-diabetes status (Table 3). Moreover, we found that the GG genotype of +936C/T was associated with a higher risk of RCC in females, as well as those with higher BMI, a smoking habit, and non-diabetes status. The GC genotype of +936C/T was correlated with an increased risk of RCC in those with higher BMI and a smoking habit. Based on interaction analysis, we only found a significant interaction between the -2578C/A and +936C/T polymorphisms and smoking status on the risk of RCC (P for interaction were 0.008).
Table 1. Demographic and clinical variables among RCC cases and controls.

<table>
<thead>
<tr>
<th>Variables</th>
<th>RCC cases</th>
<th>%</th>
<th>Controls</th>
<th>%</th>
<th>Student t-test or χ²-test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean age</td>
<td>60.4 ± 11.4</td>
<td>59.2 ± 10.6</td>
<td>1.47</td>
<td>0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;60</td>
<td>129</td>
<td>48.5</td>
<td>280</td>
<td>52.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥60</td>
<td>137</td>
<td>51.5</td>
<td>252</td>
<td>47.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>87</td>
<td>32.7</td>
<td>174</td>
<td>32.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>179</td>
<td>67.3</td>
<td>358</td>
<td>67.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;24</td>
<td>116</td>
<td>43.6</td>
<td>395</td>
<td>74.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥24</td>
<td>150</td>
<td>56.4</td>
<td>137</td>
<td>25.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Smoking status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever</td>
<td>113</td>
<td>42.5</td>
<td>167</td>
<td>31.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>153</td>
<td>57.5</td>
<td>365</td>
<td>68.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Diabetes status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>231</td>
<td>86.8</td>
<td>510</td>
<td>95.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>35</td>
<td>13.2</td>
<td>22</td>
<td>4.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Histology</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clear cell</td>
<td>217</td>
<td>81.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papillary</td>
<td>12</td>
<td>4.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>37</td>
<td>13.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Metastasis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>209</td>
<td>78.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>57</td>
<td>21.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tumor stage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-II</td>
<td>182</td>
<td>68.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III-IV</td>
<td>84</td>
<td>31.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Genotype distribution of 5 SNPs in the VEGF gene between cases and controls.

<table>
<thead>
<tr>
<th>VEGF</th>
<th>Genotype</th>
<th>Osteosarcoma group (N = 266)</th>
<th>%</th>
<th>Control group (N = 532)</th>
<th>%</th>
<th>HWE</th>
<th>OR (95%CI) ¹</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>-2578C/A</td>
<td>CC</td>
<td>99</td>
<td>37.2</td>
<td>243</td>
<td>45.7</td>
<td>1.0 (Ref.)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>119</td>
<td>44.7</td>
<td>225</td>
<td>42.3</td>
<td>1.30 (0.93-1.82)</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>48</td>
<td>18.0</td>
<td>64</td>
<td>12.0</td>
<td>0.29</td>
<td>1.84 (1.15-2.93)</td>
<td>0.006</td>
</tr>
<tr>
<td>Allele</td>
<td>C</td>
<td>317</td>
<td>59.6</td>
<td>711</td>
<td>66.8</td>
<td>1.0 (Ref.)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>215</td>
<td>40.4</td>
<td>353</td>
<td>33.2</td>
<td>1.37 (1.09-1.70)</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>-1156G/A</td>
<td>AA</td>
<td>115</td>
<td>43.2</td>
<td>232</td>
<td>43.6</td>
<td>1.0 (Ref.)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>112</td>
<td>42.1</td>
<td>220</td>
<td>41.4</td>
<td>1.03 (0.74-1.43)</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td>G</td>
<td>39</td>
<td>14.7</td>
<td>80</td>
<td>15.0</td>
<td>0.18</td>
<td>0.87 (0.55-1.38)</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>342</td>
<td>64.3</td>
<td>684</td>
<td>64.4</td>
<td>1.0 (Ref.)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>+1612G/A</td>
<td>CC</td>
<td>113</td>
<td>42.5</td>
<td>248</td>
<td>46.6</td>
<td>1.0 (Ref.)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td>C</td>
<td>123</td>
<td>46.2</td>
<td>243</td>
<td>45.7</td>
<td>1.11 (0.80-1.53)</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>183</td>
<td>34.4</td>
<td>325</td>
<td>30.5</td>
<td>1.19 (0.95-1.50)</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>-634G/C</td>
<td>CC</td>
<td>104</td>
<td>39.1</td>
<td>227</td>
<td>42.7</td>
<td>1.0 (Ref.)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td>G</td>
<td>342</td>
<td>65.6</td>
<td>739</td>
<td>69.5</td>
<td>1.0 (Ref.)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>139</td>
<td>34.4</td>
<td>325</td>
<td>30.5</td>
<td>1.19 (0.95-1.50)</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>+936C/T</td>
<td>CC</td>
<td>70</td>
<td>26.3</td>
<td>196</td>
<td>36.8</td>
<td>1.0 (Ref.)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td>C</td>
<td>276</td>
<td>50.2</td>
<td>628</td>
<td>59.0</td>
<td>1.0 (Ref.)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>265</td>
<td>49.8</td>
<td>436</td>
<td>41.0</td>
<td>1.43 (1.15-1.77)</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

HWE: Hardy-Weinberg equilibrium. ¹Adjusted for age, gender, BMI, smoking status, and diabetes.
Table 3. Stratification analysis for the association between 5 SNPs and demographic characteristics on the risk of RCC.

<table>
<thead>
<tr>
<th>Variable</th>
<th>-2578C/A OR (95%CI) P</th>
<th>+936C/T OR (95%CI) P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC CA AA CA vs CC AA vs CC</td>
<td>CC CT TT CT vs CC TT vs CC</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤20</td>
<td>47/122 56/118 26/40 1.23 (0.76-2.01) 0.38 1.69 (0.88-3.19) 0.08</td>
<td>33.99 61/125 35/57 1.46 (0.86-2.50) 0.13 1.84 (1.0-3.41) 0.04</td>
</tr>
<tr>
<td>&gt;20</td>
<td>52/121 63/107 22/24 1.37 (0.85-2.20) 0.17 2.13 (1.06-4.36) 0.02</td>
<td>37.97 66/111 34/43 1.56 (0.93-2.62) 0.07 2.07 (1.10-3.89) 0.01</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>33/80 39/72 15/22 1.31 (0.72-2.40) 0.34 1.65 (0.70-3.81) 0.2</td>
<td>23.82 41/75 23/37 1.47 (0.77-2.86) 0.21 1.68 (0.78-3.61) 0.15</td>
</tr>
<tr>
<td>Female</td>
<td>66/163 80/153 33/42 1.29 (0.85-1.95) 0.2 1.94 (1.06-3.57) 0.01</td>
<td>47.134 86/161 46/63 1.52 (0.96-2.40) 0.05 2.08 (1.22-3.60) 0.004</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;24</td>
<td>44/183 49/159 23/53 1.28 (0.79-2.08) 0.29 1.80 (0.95-3.37) 0.05</td>
<td>31/136 55/184 30/75 1.31 (0.78-2.32) 0.28 1.75 (0.95-3.25) 0.05</td>
</tr>
<tr>
<td>≥24</td>
<td>55/60 70/66 11/25 1.16 (0.68-1.96) 0.57 2.48 (1.06-6.10) 0.02</td>
<td>39/60 72/52 39/25 2.13 (1.20-7.58) 0.005 2.4 (1.20-4.82) 0.007</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever</td>
<td>41/76 49/69 23/22 1.32 (0.75-2.31) 0.31 1.94 (0.91-4.13) 0.06</td>
<td>33/59 53/75 27/33 1.26 (0.70-2.38) 0.41 1.46 (0.71-2.99) 0.26</td>
</tr>
<tr>
<td>Never</td>
<td>58/167 70/156 25/42 1.29 (0.84-1.99) 0.22 1.71 (0.91-3.26) 0.07</td>
<td>37/137 74/161 42/67 1.70 (1.06-2.77) 0.02 2.32 (1.32-4.08) 0.001</td>
</tr>
<tr>
<td>Diabetes status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>87/232 104/220 40/58 1.26 (0.88-1.80) 0.27 1.84 (1.13-3.02) 0.01</td>
<td>60/188 107/225 64/97 1.49 (1.01-2.20) 0.03 2.07 (1.31-3.25) &lt;0.001</td>
</tr>
<tr>
<td>Yes</td>
<td>12/11 5/15 8/6 2.75 (0.63-12.79) 0.12 1.22 (0.27-5.77) 0.77</td>
<td>10/8 11/20 5/3 1.45 (0.37-5.55) 0.53 1.33 (0.18-11.10) 0.74</td>
</tr>
</tbody>
</table>
DISCUSSION

Angiogenesis is a key factor in the development of many malignant tumors. VEGF expression can play a role in regulating angiogenesis by promoting endothelial cell proliferation and regulating the extracellular matrix in the blood vessels (Roy et al., 2006; Kushner and Bautch, 2013). Several functional SNPs in the VEGF gene can alter the expression levels of VEGF protein in cancer cells, thus influencing the tumor angiogenic activity and accelerating carcinogenesis such as bladder cancer, breast cancer, lung cancer, colorectal cancer, prostate cancer, ovarian cancer, and osteosarcoma (Li et al., 2010; Jaiswal et al., 2013; Kapahi et al., 2014; Deng et al., 2014; Lau et al., 2014; Chen et al., 2014; Wang et al., 2014). Jaiswal et al. (2013) reported that VEGF polymorphisms may play a significant role in mediating bladder cancer. Kapahi et al. (2014) examined an Indian population and found that the VEGF +936C>T and +405C>G polymorphisms were associated with an increased risk of breast cancer. Lau et al. (2014) conducted a study in a Malaysian population and found that the VEGF gene polymorphism has a role in the development of colorectal cancer. These studies showed that VEGF polymorphisms can influence the development of cancer, possibly by altering VEGF expression.

Several studies have examined the association between VEGF polymorphisms and RCC risk, but the results have been inconsistent (Kawai et al., 2007; Bruyère et al., 2010; Ajaz et al., 2011; Sáenz-López et al., 2013). Sáenz-López et al. (2013) conducted a case-control study to assess the role of 4 SNPs in VEGF in the risk of RCC, and they found no significant association between them. Ajaz et al. (2011) found that the VEGF -2578 A-allele and A-carrier genotypes were associated with an increased risk of RCC. Kawai et al. (2007) suggested that the -2578C/A and -1156G/A polymorphisms may affect RCC progression or prognosis. Bruyère et al. (2010) found no significant association between the +936C/T polymorphism and risk of RCC. Our study showed that the -2578C/A and +936C/T polymorphisms in the VEGF gene may play a role in the development of RCC, which did not agree with the results of previous studies. The discrepancy in these results may be caused by differences in ethnicities, study design, and sample size.

Our study found that the -2578C/A and +936C/T polymorphisms interact with smoking habits. Mechanisms for the interaction between smoking habit and VEGF polymorphism may be caused by altering expression or protein activity (Conklin et al., 2002; Kanda and Watanabe, 2007). The -2578C/A and +936C/T polymorphisms were correlated with variation in VEGF expression and protein production. Tobacco smoking can play a role in stimulating both angiogenesis and VEGF expression, and thus promote the procarcinogenic effect of angiogenesis (Wong et al., 2007). Therefore, tobacco smoking and VEGF activity can have multiple effects on the risk of RCC.

There were several limitations to our study. First, RCC cases and controls were selected from 1 hospital, and hospital-based cases may not be representative of other populations. However, the controls were a random sample from a pool of individuals who came to receive a health check-up, which may represent the general population. Third, the risk of RCC may be modified by many other genetic factors in the angiogenesis pathway, in addition to VEGF. Therefore, further studies including more subjects are needed to confirm the association between VEGF gene polymorphisms and the risk of RCC.

In this case-control study, we found that the -2578C/A and +936C/T polymorphisms in the VEGF gene may play a role in the etiology of RCC. Further large-sample studies are needed to confirm these associations.
REFERENCES


