Effect of Polymorphisms in the 3’-Untranslated Region (3’-UTR) of VEGF Gene on Gastric Pre-malignant Condition

TOMOMITSU TAHARA1, TOMIYASU ARISAWA3, TOMOYUKI SHIBATA1, MASAKATSU NAKAMURA1, HIROMI YAMASHITA1, DAISUKE YOSHIOKA1, MASAAKI OKUBO1, NAOKO MARUYAMA1, TOSHIKI KAMANO1, YOSHIKO KAMIYA1, HIROSHI FUJITA1, MITSUO NAGASAKA1, MASAMI IWATA1, KAZUYA TAKAHAMA1, MAKOTO WATANABE2, HIROSHI NAKANO2 and ICHIRO HIRATA1

1Department of Gastroenterology, 2Fujita Health University School of Medicine, Toyoake; 3Department of Gastroenterology, Kanazawa Medical University, Ishikawa, Japan

Abstract. Background: A complex interaction of host genetic and environmental factors may be relevant in Helicobacter pylori-related gastric carcinogenesis. We investigated the effect of VEGF gene polymorphisms on the risk of gastric pre-malignant condition. Patients and Methods: The G1612A and C936T polymorphisms in the 3’-untranslated region (3’-UTR) of the VEGF gene were genotyped in 337 cancer-free individuals. The presence of intestinal metaplasia in the gastric antrum was assessed in all. Gastritis scores were also assessed according to the updated Sydney system. Results: The 1612 GA genotype held a significantly higher incidence of intestinal metaplasia in H. pylori-positive individuals more than 65 years of age (OR=4.05, 95% CI=1.08-15.15, p=0.038). The degree of intestinal metaplasia was also higher among individuals with 1612 GA in the same generation (GG vs. GA; 0.98±0.87 vs. 1.55±0.96, p=0.026). Conclusion: The G1612A but not the C936T polymorphism of the VEGF gene is associated with gastric pre-malignant condition in older individuals.

Helicobacter pylori infection is now established as a critical event in the development of gastro-duodenal diseases such as peptic ulcer disease, chronic atrophic gastritis and gastric cancer (1-5). However, there is considerable variation in the extent of gastric inflammation and atrophy among H. pylori-infected patients, and only a small percentage of them actually develop peptic ulcer diseases and gastric cancer, suggesting that H. pylori infection is not always associated with the risk of gastro-duodenal diseases. Some genetic factors may also play crucial roles in the long-term outcome of H. pylori infection.

Neoangiogenesis, the development of new blood vessels from existing endothelial precursors, is a general pathophysiologically mechanism critically involved in the pathogenesis of inflammatory and ulcerative epithelial lesions, as well as malignant tumor growth and metastasis (6, 7). Among the pro-angiogenic factors known so far, vascular endothelial growth factor (VEGF) represents one of the most potent stimuli of neoangiogenesis (6-8).

In the stomach, enhanced VEGF gene expression has been identified to contribute to the healing of peptic lesions (9-11). In addition, gastric adenocarcinomas frequently display high levels of VEGF expression accompanied by an increased intratumoral microvessel density (12, 13). Moreover, high levels of VEGF expression have also been observed in gastric pre-malignant lesions such as chronic atrophic gastritis and intestinal metaplasia (14), suggesting that alterations of VEGF expression may also contribute early in the process of gastric carcinogenesis.

A recent study revealed the CC genotype of C936T polymorphism in the 3’-untranslated region (3’-UTR) of the VEGF gene was associated with an increased serum VEGF level as compared with the CT and TT genotypes (15). In addition, another polymorphism, G1612A, has been reported to be common in the Japanese (16, 17).

This study was designed to clarify the possible association between common polymorphisms in the 3’-UTR of the VEGF gene and the incidence of pre-cancerous lesion i.e. intestinal metaplasia, in a Japanese population. We also investigated the effect of VEGF polymorphisms on the histological severity of gastritis according to the updated Sydney system (18).
Patients and Methods

Study population. Three hundred and thirty-seven individuals attending the Endoscopy Center of Fujita Health University Hospital from January 2005 to May 2007 were enrolled. All underwent upper gastroscopy for their health check, secondary complete check up of stomach cancer following barium X-ray examination, or for the complaint of abdominal discomfort. Patients with severe systemic diseases, with malignancies in the stomach or other organs, who had received non-steroidal anti-inflammatory drugs, antibiotics, or H. pylori eradication treatment were excluded from this study. The Ethics Committee of Fujita Health University School of Medicine approved the protocol and written informed consent was obtained from all of the participants.

Detection of H. pylori infection. The H. pylori infection status was determined on the basis of histology, culture, the urea breath test (UBT) and serum antibody to H. pylori. Infection was diagnosed when at least one of these 4 tests was positive.

Genotyping. Genomic DNA was extracted from non-pathological gastric biopsies or peripheral blood using the standard phenol/choloroform method. The G1612A and C936T polymorphisms in the VEGF gene were determined by PCR-based restricted fragment length polymorphism assays as described previously (16, 19).

Histological examination. Biopsy specimens were obtained from the uninvolved mucosa of the gastric antrum for assessment of gastritis. The presence of intestinal metaplasia was assessed in all the samples. In addition, the extent of neutrophil infiltration, mononuclear cell infiltration, atrophy, and metaplasia was assessed according to the updated Sydney system (18), with each factor being scored from 0 (normal) to 3 (marked).

Statistical analysis. Genotype frequencies were calculated by direct counting. Differences of VEGF genotype frequencies between two groups were determined by the 2 statistics. The odds ratios (OR) and 95% confidence intervals (CI) were calculated by logistic regression analysis. Differences of gastritis scores between two groups were examined by the Mann-Whitney test. A probability value of less than 0.05 was considered statistically significant.

Results

Study population. The characteristics of the 337 participants are summarized in Table I. After gastroscopy, 82 patients were diagnosed as having active ulcer. Of all participants, intestinal metaplasia was detected in 126.

Table I. Characteristics of study participants.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>Total participants (n)</td>
<td>337</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>193/144</td>
</tr>
<tr>
<td>Mean age±SD (years)</td>
<td>59.9±13.1</td>
</tr>
<tr>
<td>H. pylori infection positive ratio (%)</td>
<td>65.3</td>
</tr>
<tr>
<td>Active ulcer (n)</td>
<td>82</td>
</tr>
<tr>
<td>Intestinal metaplasia (n)</td>
<td>126</td>
</tr>
</tbody>
</table>

The effect of VEGF genotype on the occurrence of intestinal metaplasia and on the degree of histological severity of gastritis in different generations. To further evaluate the effect of VEGF genotype on the occurrence of intestinal metaplasia and on the degree of histological severity of gastritis, we investigated the association of VEGF genotypes and occurrence of intestinal metaplasia as well as the degree of gastritis in different generations. Among H. pylori-infected individuals, the 1612 GA genotype was associated with a significantly higher incidence of intestinal metaplasia (IM) in those aged more than 65 years by logistic regression analysis with adjustment for age and sex. We also investigated the VEGF genotypes and histological severity of gastritis according to the updated Sydney system. But none of the scores were significantly different among different genotypes, neither in H. pylori-positive nor -negative individuals by Mann-Whitney U-test (Table III). Neither was any difference observed between VEGF genotypes and ulcer diseases (data not shown).

Discussion

In the present study, we have shown that the 1612 GA genotype was associated with the incidence of intestinal metaplasia in older H. pylori-positive individuals. In addition, we have also shown that the GA genotype was associated with the severity of intestinal metaplasia in the same individuals.

VEGF plays a major role in gastric mucosa repair and is overexpressed in gastric cancer. High levels of VEGF
expression have also been documented in *H. pylori*-infected gastric epithelial cells (20, 21), and gastric pre-malignant ones (14), suggesting that alterations of VEGF expression may be induced by *H. pylori*-related gastric mucosal inflammation and may contribute to early in the process of gastric carcinogenesis via angiogenesis. Our data of an association between the 1612 GA genotype and the incidence, as well as severity, of intestinal metaplasia in older *H. pylori*-positive individuals indicates that the same genotype may influence the gastric pre-malignant condition in the long-term outcome of the *H. pylori* infection.

The 3′-UTR of the *VEGF* gene has been proven to increase the stability of mRNA and be associated with the hypoxic induction of *VEGF* (17, 22-24). Recently, genes designed as Hu family have been identified and their products have been shown to bind the AU-rich element of 3′-UTR of several genes, including *VEGF* mRNA (17, 25-27). It is suggested that the proteins of the Hu family change the *VEGF* mRNA conformation so that the mRNA is not attacked by RNAase. Alterations in a few nucleotides in the 3′-UTR, such as single nucleotide polymorphism and triplet nucleotide repeats have been shown to be associated with the deregulation of affected genes (28). Therefore, it is suggested that the nucleotide polymorphism in the 3′-UTR may alter the mRNA conformational integrity.
resulting in genetic variation of the VEGF gene expression. From the functional perspective, Renner et al. reported that the 936 CC genotype of the VEGF gene is associated with a higher plasma VEGF level as compared with the CT and TT genotypes, while no specific genotype of G1612A influences the plasma VEGF level (15). On the other hand, Awata et al. reported that VEGF serum levels were significantly higher in healthy individuals with the CC genotype of the C634G polymorphism than in those with the other genotypes, while neither C936T nor G1612A influenced the VEGF level (17). Since there are no data of differences of VEGF proteins in different genotypes in H. pylori-infected human gastric mucosa, this should be clarified using well-designed future studies.

We have also investigated the association between VEGF polymorphisms and acute or chronic inflammation, and atrophy. But we did not find any association between VEGF polymorphism and the severity of these parameters. VEGF polymorphism may affect the risk of a pre-malignant condition in H. pylori-infected individuals but not the severity of acute or chronic inflammation, or atrophy.

Concerning the possible association of VEGF gene polymorphism and gastric carcinogenesis, several polymorphisms of the VEGF gene have been reported to be associated with the susceptibility and progression of gastric cancer. Tzanakis et al. showed a marginally significant association of the VEGF 634CC genotype with increased risk for gastric cancer development in Greek patients (29). On the other hand, Chae et al. showed that the 936T allele and TCT haplotype of VEGF -T460C, G405C, and C936T was associated with a decreased susceptibility to gastric cancer (30). More recently, Kim et al. found a possible association between the 936 TT genotype and worse overall survival, the -460 TC or CC genotype and a poor prognosis in early stage gastric cancer, and the CACC haplotype of VEGF -T460 C, -G116A, G405C, and C936T and worse survival in Korean patients (31). All these data suggest a potential association between VEGF polymorphisms and susceptibility and progression of gastric cancer. In addition, our data suggest that the VEGF genotype may also influence the gastric carcinogenesis in the early process of the pre-malignant stage.

The VEGF G1612A polymorphism shows variations in different ethnic groups (15). Moreover, other polymorphisms have been described in the VEGF gene that remained to be studied by us (29-31). Further studies will be needed in a larger and more ethnically diverse population to resolve the impact of VEGF polymorphisms on the gastric pre-malignant condition.

### References


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