J Korean Med Sci 2003; 18: 501-4
ISSN 1011-8934

A Single Nucleotide Polymorphism in the *E-cadherin* Gene Promoter −160 is Not Associated with Risk of Korean Gastric Cancer

Recently, the −160 C/A polymorphism, located within the regulatory region of *E-cadherin* promoter, has been shown to influence *E-cadherin* transcription by altering transcription factor binding. We examined the effect of this polymorphism on risk of gastric cancer and on histological classification of intestinal- and diffuse-type gastric cancer in 146 normal healthy individuals and 292 Korean gastric cancer patients. Genomic DNA samples were examined by polymerase chain reaction (PCR)-single strand conformational polymorphism (SSCP)-sequencing and confirmed by restriction fragment length polymorphism (RFLP). Unexpectedly, there was no significant difference in the genotype frequencies of the polymorphism between normal control and gastric cancer patients (χ^2 test, p=0.433). The estimated odd ratio of C/C to A/A genotype in gastric cancer cases was 1.07 (95% confidence interval, 0.396-2.870). We also found no evidence for differences in risk for the intestinal- and diffuse-type gastric cancer. These results suggest that the −160 C/A polymorphism of the *E-cadherin* has no direct effect on the risk of Korean gastric cancer development and on its histological classification.

Key Words : *Cadherins; Polymorphism, Single Nucleotide; Stomach Neoplasms; Disease Susceptibility*

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Received : 4 February 2003
Accepted : 1 April 2003

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*This work was supported by Korea Research Foundation Grant (2001-041-F00060).

INTRODUCTION

Gastric cancer occurs with a high incidence in Asia and is one of the leading causes of cancer deaths worldwide. Although it is well known that environmental factors such as dietary habit and *Helicobacter pylori* infection are associated with the risk of gastric cancer, host genetic factors may be one of the critical factors in gastric carcinogenesis.

Cell to cell adhesion plays a critical role in the development and maintenance of complex differentiated epithelial tissues and structures in multicellular organisms. Interference with cell attachment, independence of growth control, and increased migration have long been implicated during the neoplastic process (1, 2). The cadherins constitute a large family of cell membrane glycoproteins involved in the calcium-dependent cell-cell adhesion molecules (3). The human E-cadherin, so called CDH1, the major cadherin molecule expressed by epithelial cells, maps to chromosome 16q22 (4) and serves as the prime mediator of epithelial cell adhesion through homotypic interactions of its extracellular domain. There are overwhelming genetic data to support the role of E-cadherin as a tumor invasion suppressor in epithelial cells. Structural abnormalities and loss of expression of the E-cadherin were shown to disrupt E-cadherin-mediated intercellular adhesion and are frequently associated with high tumor grade, infiltrative growth, and lymph node metastasis in a variety of human malignancies, including diffuse-type gastric cancer, hepatocellular carcinomas and lobular carcinomas of the breast (5-8). It has also been shown that loss of *E-cadherin* expression in a transgenic mouse model is associated with the development of invasive carcinoma from well differentiated adenoma (9).

Interestingly, nucleotide variations in DNA sequence, especially in promoter and protein encoding region of a gene, are very important to the function and transcriptional efficiency of the gene (10, 11). The *E-cadherin* gene encoding E-cadherin is highly polymorphic, and several diallelic polymorphisms have been reported. Three of these are in the promoter region at positions −347, -163, and −160 numbering from the transcription start site, representing G→GA, T→△T, and C→A transversion, respectively (11, 12). Recently, it has been reported that the polymorphism, located -160 upstream from the *E-cadherin* transcription site, showed different transcriptional binding strength and the transcriptional activity in vitro (11). Thus, the A allele promoter variant was regarded as a potential genetic marker that can identify those individuals at higher risk of gastric cancer. Furthermore, E-cadherin pro-

moter polymorphism at position −160 has been associated with an increased susceptibility to sporadic diffuse-type gastric cancer (13). However, It has been reported that the A allele was suggested to be protective against gastric cancer in Taiwanese (14) and that the −160 promoter polymorphism is not associated with risk of gastric cancer (15). Thus, it is worthwhile to investigate the genotype specific risk of −160 promoter polymorphism in Korean gastric cancer patients. In the present study, we examined the effect of this polymorphism on risk of gastric cancer and on histological classification of intestinal- and diffuse-type gastric cancer in Korean.

MATERIAL AND METHODS

Samples

A total of 292 paraffin-embedded sporadic gastric carcinoma specimens were obtained from College of Medicine, the Catholic University of Korea. No patient had a family history of gastric cancer. Hematoxylin & eosin (H&E)- stained histological sections were reviewed in each case. Gastric carcinomas were classified according to the Lauren's criteria (16): 165 carcinomas were of the intestinal-type and 127 tumors of the diffuse-type. In addition, 146 healthy individuals were also included as normal controls in this study.

DNA extraction

Normal gastric mucosa or lymphocytes were selectively procured from H&E-stained slides using a 30 G1/2 hypodermic needle (Becton Dickinson, Franklin Lake, NJ). DNA extraction was performed by a modified single step DNA extraction method, as described previously (17).

Allelic analysis

The allele frequencies of −160 promoter polymorphism of *E-cadherin* gene were analyzed by polymerase chain reaction (PCR)-single strand conformational polymorphism (SSCP) analysis. Genomic DNAs were amplified with the primer covering the promoter region of the *E-cadherin*. The primer sequences were as follows; forward primer, 5′-ATCAGAACCGTGCAGGTCCCATAA-3′ and reverse, 5′-GTTCACCTGCCGGCCACAG-3′. Each PCR was performed under standard conditions in a 10 μL reaction mixture containing 1 μL of template DNA, 0.5 μM of each primer, 0.2 μM of each deoxynucleotide triphosphate, 1.5 μM $MgCl_2$, 0.4 unit of *Taq* polymerase, 0.5 μCi of [^{32}P] dCTP (Amersham, Buckinghamshire, U.K.), and 1 μL of 10× buffer. The reaction mixture was denatured for 1 min at 94°C and incubated for 30 cycles (denaturing for 40 sec at 94°C, annealing for 40 sec at 60°C, and extending for 40 sec at 72°C). Final extension was continued for 5 min at 72°C. After amplification, PCR products

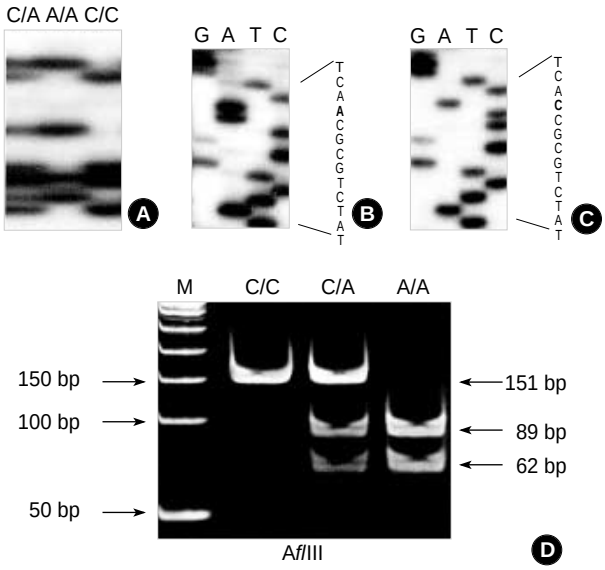

Fig. 1. Genotype analysis at −160 promoter polymorphic site of *E-cadherin* by SSCP-sequencing and RFLP. SSCP band patterns of heterozygote with C/A and homozygote with A/A and C/C (**A**); Sequencing showing homozygotes with A/A (**B**) and C/C (**C**); the PCR products were digested with *Afl*III restriction endonuclease. If the recognition site was present, amplified DNA (151 bp in size) produced two fragments, 89 bp and 62 bp (**D**).

were denatured for 5 min at 95°C at 1:1 dilution of sample buffer containing 98% formamide/5 mmol/L NaOH and were loaded onto a SSCP gel (FMC Mutation Detection Enhancement system; Intermountain Scientific, Kaysville, UT, U.S.A.) with 10% glycerol. Samples were electrophoresed at 8 W at room temperature overnight. After electrophoresis, the gels were transferred to 3 mm Whatman paper and dried, and autoradiography was performed with Kodak X-OMAT film (Eastman Kodak, Rochester, NY, U.S.A.) (Fig. 1A). For the detection of allele sequence, DNA bands were cut out from the dried gel, and reamplified for 30 cycles using the same primer set. Sequencing of the PCR products was carried out using the cyclic sequencing kit (Perkin-Elmer, Foster City, CA, U.S.A.) according to the manufacturer's recommendation (Fig. 1B, C). We also performed PCR-restriction fragment length polymorphism (RFLP) to confirm the PCR-SSCP results. After amplification with the same primer set, the PCR products were digested with 5 U of restriction enzyme *Hph*I or *Afl*III at 37°C for 16 hr. The C allele had an *Hph*I recognition site, whereas the A allele created an *Afl*III site. DNA fragments then were eletrophoresed on a 10% polyacrylamide gel (Fig. 1D).

Statistical analysis

The chi-square test for association was used to test difference of the genotype frequencies between normal controls and gastric cancer patients, and between two histologic types. The

Table 1. Genotype frequencies at −160 promoter polymorphic site of *E-cadherin* gene in healthy individuals and gastric cancer patients

Subject	Total	C/C	C/A	A/A	χ^2 (df=2)	*p*-value*
Control	146	85 (58.2%)	55 (37.7%)	6 (4.1%)		
Gastric cancer†	292	186 (63.7%)	92 (31.5%)	14 (4.8%)	1.674	0.433
Intestinal	165	111 (67.3%)	45 (27.3%)	9 (5.4%)	3.903	0.142
Diffuse	127	75 (59.1%)	47 (37.0%)	5 (3.9%)	0.021	0.989

*, *p*-value compared with normal control; †, intestinal- and diffuse-type.

Table 2. Genotype specific risks with odd ratio and 95% confidence interval*

Subject	Genotype at −160 promoter of *E-cadherin*		
	C/A	A/A	A allele
Gastric cancer†	0.764 (0.502-1.165)	1.066 (0.396-2.870)	0.869 (0.619-1.219)
Intestinal	0.627 (0.386-1.017)	1.149 (0.394-3.352)	0.792 (0.538-1.167)
Diffuse	0.968 (0.589-1.594)	0.944 (0.277-3.221)	0.972 (0.650-1.452)

*, compared with common homozygote (C/C); †, intestinal- and diffuse-type.

genotype specific risks were estimated as odds ratios with associated 95% confidence interval (CI).

RESULTS

The genotype frequencies at −160 promoter polymorphism of *E-cadherin* in Korean gastric cancer cases and controls are summarized in Table 1. The frequency of genotype C/C was 58.2%, C/A was 37.7%, and A/A was 4.1% in normal healthy individuals, showing that C allele is more common than A allele in Korean population. Interestingly, there was no significant difference in the frequency of genotypes between control and gastric cancer patients, indicating no associations between the *E-cadherin* specific genotype and gastric cancer in Korean (χ^2 test, p=0.433). In addition, we could not find any evidence for significant differences between intestinal- and diffuse-type gastric cancers (χ^2 test, p=0.196). Statistically, there was also no significant difference between intestinal-type gastric cancers and normal controls (χ^2 test, p=0.142) and between diffuse-type and controls (χ^2 test, p=0.989).

Genotype specific risks are shown in Table 2. The estimated odds ratio of A/A to C/C genotype in gastric cancer was 1.066 (95% CI, 0.396-2.870) and the odd ratio in diffuse histologic type was 0.944 (95% CI, 0.277-3.221). The odd ratio of A allele to C allele in diffuse-type gastric cancer was 0.972 (95% CI, 0.650-1.452).

In addition, we found the T deletion polymorphism at −163 promoter of *E-cadherin* in 3 of 146 healthy individuals and 2 of 292 gastric cancer cases, respectively.

DISCUSSION

Many common diseases in humans, especially cancer, are not caused by one genetic variation within a single gene, but are determined by complex interactions among multiple genes, environmental and lifestyle factors. Genetic factors confer susceptibility or resistance to a disease and influence the severity or progression of disease. Host factors, including polymorphism at the genes involved in tumorigenesis, may partly explain the difference in individual susceptibility of cancer occurrence (18). Single nucleotide polymorphisms (SNPs) are common DNA sequence variations among individuals. They promise to significantly advance our ability to understand and treat human disease, including cancer. SNP profiles that are characteristic of a variety of cancers will be established and provide fundamental understanding of many cancers, thus providing new therapeutic targets.

Development of malignant tumors is in part characterized by the ability of a tumor cell to overcome cell-cell adhesion and to invade surrounding tissue. E-cadherin, the main adhesion molecule of epithelia, has been implicated in carcinogenesis because it is frequently lost in human epithelial cancers. Recently, Li et al. (11) characterized a C/A polymorphism located 160 upstream from the *E-cadherin* transcription start site and found the A-allele to have reduced transcriptional activity of the C-allele in vitro. Thus, A-type promoter variant may be regarded as a candidate cancer susceptibility polymorphism. However, recent epidemiological studies failed to demonstrate a correlation between the *E-cadherin* promoter variant and breast (19) or colorectal cancer (20) or gastric cancer (15). In the present study, there is no significant difference in the *E-cadherin* −160 promoter genotype between normal healthy individuals and gastric cancer patients (Table 1). Therefore, it is likely that −160 promoter polymorphism of the *E-cadherin* gene may not be associated with risk for gastric cancer among Koreans and that this allele variation should not be a genetic marker to identify those individuals at higher risk for gastric cancer.

Histologically, gastric cancer can be divided into intestinal and diffuse subtypes according to the presence of glandular structure formation by tumor cells. Interestingly, the genotyping data in the Italian group showed an association between the promoter –160A allele and an increased risk of sporadic diffuse-type gastric cancer (13). When we tried to determine whether this polymorphism is important with respect to the different histologic types of gastric cancer, we did not observe any impact of *E-cadherin* genotype on histologic type, suggesting that the presence of A allele in heterozygote C/A and homozygote A/A genotype did not affect the expression of E-cadherin protein. Several reasons may account for this discrepancy. The influence of a susceptibility gene on disease risk may depend on environmental factors and lifestyles. The different frequency of *H. pylori* infection and different genetic background between Italian and Korean populations may to some extent explain the different risk estimates associated with the –160 variant. In addition, no direct correlation between mRNA expression and protein level by post-transcriptional regulation may help us understand this discrepancy (21).

A genetic marker has to be relevant to the pathogenesis of the disease in question and to occur at a sufficiently high frequency to make its screening worthwhile. Since our data indicate that –160 promoter polymorphism of the *E-cadherin* gene is not sufficient to form the basis of a screening program for Korean gastric cancer, addition of other relevant markers is essential in refining this genetic strategy. In addition, a rigorous case-control study on a large scale is mandatory to elucidate the relationship between *E-cadherin* genotype and gastric cancer risk.

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