Null Results in Brief

No Association Between *EGF* Gene Polymorphism and Gastric Cancer

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Abstract

The etiology of gastric cancer is not well-understood. Epidermal growth factor (EGF) transduces growth signals to mitogen-activated protein kinase via RAS and BRAF, and EGF/EGF receptor interaction is important for tumor growth and progression. Previous studies have reported that the EGF +61 (A/G) single nucleotide polymorphism in the 5′-untranslated region of the *EGF* gene is functional, and is associated with gastric cancer and various malignancy. Individuals with the EGF A/A genotype produce less EGF than individuals with G/G or G/A. We investigated a single nucleotide polymorphism at exon 1 of EGF, named rs4444903 in NCI dbSNP, in 454 Japanese subjects undergoing a health checkup and 202 patients with gastric cancer. Genotype was determined by PCR with confronting two-pair primers. Results showed that EGF polymorphism was not associated with gastric cancer but that the EGF A/A genotype showed a protective effect (odds ratios, 0.58; 95% confidence interval, 0.29-1.17 relative to G/G). Furthermore, when we divided cases into two groups, a differentiated type and an undifferentiated type, the A/A and G/A combined was found to be lower frequency in the latter type than in the former type without significance (OR, 0.81; 95% confidence interval, 0.44-1.49 relative to G/G). As is the case with any malignancy, other factors are involved, including environmental and host factors. The present results show that although EGF is necessary for cancer, it is not sufficient. We also found ethnic heterogeneity in the functional EGF polymorphism. Because the relationship between EGF polymorphism and malignancy remains inconsistent, confirmation of the role of EGF polymorphism in gastric cancer requires a much larger study. (Cancer Epidemiol Biomarkers Prev 2005;14(10):2454–6)

Introduction

Gastric cancer remains the world's second most common malignancy, accounting for a large proportion of cancer cases in Asia, Latin America, and some countries in Europe (1). Although the etiology of gastric cancer is not well-understood, nutritional, microbial, and genetic factors acting in a multistep, multifactorial process have been suggested (2).

Epidermal growth factor (EGF) encodes a ligand for the EGF receptor (EGFR), a receptor tyrosine kinase that transduces growth signals to mitogen-activated protein kinase via RAS and BRAF. This EGF/EGFR interaction is important for tumor growth and progression.

Previous studies have reported that the *EGF* +61 (*A/G*) single nucleotide polymorphism in the 5′-untranslated region of the *EGF* gene is functional, and is associated with malignant melanoma and glioblastoma multiforme (3, 4). As the EGFR is highly expressed in a variety of solid tumors and is associated with a poor response to treatment, disease progression, and poor survival, inhibition of EGFR is logical as an anticancer strategy (5), and the multifunctional cytokine EGF thus represents a molecular therapeutic target for various cancers (5-9).

Although little is known about the association between gastric cancer and *EGF* polymorphism, the previous study reported that the A-G polymorphism of *EGF* is involved not only in the occurrence but also in the malignant progression of gastric cancer (10). In the present study, we have investigated the association between *EGF* polymorphism and gastric cancer in a case-control study.

Materials and Methods

Study Population. Detailed information of the characteristics of the healthy control and gastric cancer patients in this study has been published. Briefly, the control group was composed of 454 health checkup examinees (126 males and 328 females) ages 35 to 85 years, with no history of cancer, who attended a health checkup program supported by the Nagoya Municipal Government in August and September 2000 (11). The case group consisted of 202 patients (134 males and 68 females) ages 33 to 94 years, with a pathologically confirmed diagnosis of gastric adenocarcinoma, who underwent tumor resection in hospitals affiliated with Nagoya University (12). Informed consent was obtained from all subjects. Approval for the study was given by the relevant ethical committees.

EGF Genotyping. DNA was extracted from the buffy coat fraction with the Qiagen QIAamp DNA Blood Mini Kit (Qiagen Inc., Valencia, CA). A single nucleotide polymorphism at exon 1 of *EGF*, named rs4444903 in NCI dbSNP, was genotyped by PCR with confronting two-pair primers. The primers were F1: 5′ CTG TGT GGA GGA ATT GCC C; R1, 5′ AAC TGA TGG AAA GTT CCA GCC; F2, 5′ CCC CAA TCC AAG GGT TGT A; and R2, 5′ TGA CAA TTC ACA GAG TTT AAC AGC C. Genomic DNA was applied in a volume of 25 μL with 0.12 mmol/L deoxynucleotide triphosphates, 25 pmol

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of each primer, 0.5 units of AmpliTaq Gold (Perkin-Elmer Corp., Foster City, CA), and 2.5 μL 10× PCR buffer including 15 mmol/L $MgCl_2$. The PCR was done with initial denaturation at 95°C for 10 minutes, followed by 30 cycles of denaturation at 95°C for 1 minute, annealing at 60°C for 1 minute and extension at 72°C for 5 minutes. Final extension was at 72°C for 5 minutes.

Statistical Analysis. To estimate the gastric cancer risk associated with *EGF* genotypes, we used a χ^2 test and determined odds ratios (ORs) adjusted for sex and age with 95% confidence intervals (CIs) using unconditional logistic regression analysis. *EGF* polymorphism was tested using the Hardy-Weinberg equilibrium. Calculations were done using the computer program STATA v. 7 (STATA Corp, College Station, TX).

Results

Seven of 454 healthy controls could not be genotyped. Well-differentiated adenocarcinoma (well), moderately differentiated adenocarcinoma (mod), poorly differentiated adenocarcinoma (por), and signet ring cell carcinoma (sig) represented 48, 65, 50, and 23 of the 202 cases, respectively. Information on gastric cancer phenotype were not available for 16 cases.

The genotype distribution of the control group fits the Hardy-Weinberg equilibrium ($\chi^2 = 0.38$; $P = 0.54$). Table 1 shows the distribution of the *EGF* genotype in the gastric cancer patients and healthy controls. The odds ratio of the *A/A* genotype for gastric cancer relative to *G/G* was not significant but showed a protective effect (odds ratio, 0.58; 95% confidence interval, 0.29-1.17). *A* allele frequency in the patients was lower than in the controls, at 28.7% and 31.3%, respectively, although this difference was not significant ($P = 0.34$).

When *EGF* genotype frequency was stratified among patients according to histologic phenotype, *A* allele frequency showed a tendency to be low in all phenotypes (Table 2). Furthermore, when we divided four tumor phenotypes into two groups according to Nakanura et al. (13), namely a differentiated type including well and mod, and an undifferentiated type including por and sig, the *A/A* and *G/A* combined was found to be lower frequency in the latter type than in the former type without significance (odds ratio, 0.81; 95% confidence interval, 0.44-1.49 relative to *G/G*).

Discussion

In the present study, although individuals with the *EGF A/A* genotype produced less EGF than those with *G/G* or *G/A* (3), *EGF* polymorphism was associated with neither susceptibility to gastric cancer nor with severity, as indicated by cancer cell phenotype. Our study had 80% power to detect an absolute difference in the frequency of *G/A* and *A/A*, given 43% in the case and 53% in the control.

With regard to genotype frequencies, we saw no significant difference in *EGF* frequencies between our study controls and those of Hamai et al. (10), confirming the distribution of genotypes in Japan. Our control genotype frequencies were somewhat different from those of Shahbazi et al. (3) and McCarron et al. (14), which represented genotype distributions in U.K. Caucasians. Our low frequency of the *A/A* genotype in the case was in keeping with the results presented by Shahbazi et al. and Hamai et al. (3, 10), although not significant. It has been biologically shown that EGFR is highly expressed in a variety of solid tumors and that the *EGF* +61 gene is a functional polymorphism. As is the case for any malignancy, other factors are involved, including environmental and host factors. Although EGF is necessary for cancer, it might not be sufficient itself.

In summary, our study shows that a polymorphism in the *EGF* gene is not associated with gastric cancer. We have also found ethnic heterogeneity in the functional *EGF* polymorphism, namely that the distribution of the *EGF* gene differs between Caucasians and Japanese. Because the relationship of *EGF* polymorphism with malignancy remains inconsistent, confirmation of the role of *EGF* polymorphism in gastric cancer requires much larger studies.

Table 1. *EGF* genotype and allele frequency in patients with gastric cancer and healthy controls

EGF polymorphism	Cases (*n*)	Controls* (*n*)	Odds ratio† (95% confidence interval)
Genotype			
G/G	100 (49.5%)	215 (47.8%)	1.00 (Reference)
G/A	88 (43.6%)	188 (41.8%)	1.05 (0.72-1.54)
A/A	14 (6.9%)	47 (10.4%)	0.58 (0.29-1.17)
G/A and *A/A*	102 (50.5%)	235 (52.2%)	0.95 (0.66-1.37)
Allele			
G	288 (71.3%)	618 (68.7%)	1.00 (Reference)
A	116 (28.7%)	282 (31.3%)	0.87 (0.66-1.16)

*Seven of the 454 participants could not be genotyped.
†Adjusted for age and sex by unconditional logistic regression.

Table 2. Genotype frequency according to gastric cancer cell phenotype

EGF polymorphism	Phenotype			
	well (*n* = 48)	mod (*n* = 65)	por (*n* = 50)	sig (*n* = 23)
Genotype				
G/G	26 (54.2%)	25 (38.5%)	25 (50.0%)	13 (56.5%)
G/A	22 (45.8%)	35 (53.9%)	19 (38.0%)	9 (39.1%)
A/A	0 (0%)	5 (7.7%)	6 (12.0%)	1 (4.4%)
Allele				
G	74 (77.1%)	85 (65.4%)	69 (69.0%)	35 (76.1%)
A	22 (22.9%)	45 (34.6%)	31 (31.0%)	11 (23.9%)

Study Limitations

The study has three limitations: (*a*) the function of this polymorphism in the Japanese have not been investigated. (*b*) Because we did not have information on environmental factors such as smoking habit and alcohol consumption et al., we could not assess the interactions between those environmental factors and *EGF* polymorphism. (*c*) *EGFR* gene polymorphisms affecting EGFR activity were not examined.

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