Correlation between the EGF gene intronic polymorphism, rs2298979, and colorectal cancer

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Abstract. Colorectal cancer (CRC) is an important disorder that results from genetic and epigenetic alterations in one colonic epithelial cell. Epidermal growth factor (EGF) is critical in the development of tumors in epithelial tissues. Variations in the DNA sequence of the EGF gene may be particularly significant with regard to susceptibility to CRC. The present study aimed to investigate the effect of the EGF gene single nucleotide polymorphism (SNP), rs2298979, on CRC. In this prospective study, 220 samples were collected from patients with CRC and compared with 220 matched healthy controls. Genotyping was performed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, and the result was validated by direct sequencing. A significant correlation was observed between the rs2298979 variant in the EGF gene and CRC. The frequency of the A/G genotype in the control group was higher than in the patients with sporadic CRC [odds ratio (OR), 0.488; 95% confidence interval (CI), 0.307-0.774; P=0.002]. In this study there were no individuals with a G/G genotype. Although the frequency of the G and A alleles was similar in the healthy control and CRC patient groups, individuals with the A/G genotype were less susceptible to CRC compared with those with the A/A genotype.

Introduction

Colorectal cancer (CRC) originates from one colonic epithelial cell due to an accumulation of genetic and epigenetic changes that lead to malignancy (1). The incidence of CRC has a varied distribution in the different geographical regions and a significant difference has been observed between developed and developing countries (2,3). A number of studies have shown show that CRC is becoming increasingly common in Asian countries (4,5,6). According to the statistics issued by the Iranian Ministry of Health, ~1,130 patients suffering from CRC succumbed in 2006, and there was no significant difference between the mortality rates of male and female patients (7). The causes of CRC may include genetic and environmental factors. Based on the observed molecular genetic alterations, it has been indicated that CRC is a heterogeneous disease (8). For the development of more appropriate diagnostic methods, there is a requirement for studies to identify new molecular markers in the affected populations. Epidermal growth factor (EGF) is one of the most important factors leading to the development of tumors and plays a significant role in the uncontrolled reproduction of cancerous cells (9). EGF is an important regulator of cell survival, and in numerous types of cancer, including breast, prostate, pancreas, colorectal, lung and head and neck, an increase in EGF and ErbB, a member of the EGF receptor family, has been demonstrated (10-12). The EGF gene is located on chromosome 4q25-27 and produces various transcripts, the largest of which has 24 exons and 23 introns (12,13). Genetic factors play a key role in the susceptibility to diseases, resistance against medications and interference in the interaction with peripheral factors. Single nucleotide polymorphisms (SNPs) are the most common genetic changes (14). As the polymorphisms of the EGF gene affect the susceptibility to numerous types of cancer, the majority of the case-control studies on EGF genes have been conducted on exonic polymorphisms and untranslated regions (UTRs) (12,15-17). In the present study, the hypothesis that the noncoding polymorphism, rs2298979, may be used to predict the susceptibility to CRC was tested in the Iranian population.

Materials and methods

Study population. Genetic analysis was conducted on a population of 220 patients suffering from sporadic CRC
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and 220 normal individuals who had been referred to the Research Institute for Gastroenterology and Liver Diseases (RIGLD), Taleghani Hospital, Shahid Beheshti University of Medical Sciences (Tehran, Iran). Patients with a family history of hereditary non-polyposis CRC (HNPCC) and familial adenomatous polyposis (FAP) were excluded from this study. The patients and healthy individuals were all of Iranian nationality. A colonoscopy was performed on all participants in the patient and control groups, the medical normality of the control group was confirmed and the histological diagnosis of the pathologist was approved by the gastroenterologist. The parameters of age, gender and cigarette smoking status were controlled in the patient and control groups and the stage of CRC was determined in the patient group. The tumor stage was classified according to the tumor-node-metastasis (TNM) classification of the Union for International Cancer Control (UICC; Table I). This study was conducted under the approval of the ethics committee of the Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Sciences (Tehran, Iran).

**DNA extraction.** Subsequent to obtaining a letter of consent from each individual, 5 ml peripheral blood was collected and stored at 4°C in a bottle containing EDTA. Genomic DNA was extracted as soon as possible following sampling using the standard salting out method (18). The quality of the extracted DNA was then assessed using a NanoDrop spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA).

**EGF rs2298979 gene polymorphism genotyping.** Two specific primers were designed. The polymorphism was determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The characteristics and sequence of the primers are shown in Table II. The 768-bp
DNA fragment that was located in the intron region of the EGF gene was amplified using the specific primers (Table III). The PCR products were digested by *Pci*I endonuclease enzyme (recognition sequence A/CATGT) for 6 h at 37˚C. In order to observe the digested fragments, the RFLP solution was separated on a 3% agarose gel and stained with ethidium bromide.

Table III. Technical data for the rs2298979 polymorphism detection method.

| Factor                                      | Value |
|---------------------------------------------|-------|
| PCR reaction condition, no. of cycles      |       |
| 94˚C for 5 min                              | 1     |
| 94˚C for 45 sec                             | 30    |
| 65˚C for 40 sec                             | -     |
| 72˚C for 45 sec                             | -     |
| 72˚C for 5 min                              | 1     |
| Master mix, µl                              |       |
| 10X PCR buffer                              | 2.50  |
| MgCl₂ (50 mM)                               | 0.75  |
| Primer-forward (10 mM)                      | 1.00  |
| Primer-reverse (10 mM)                      | 1.00  |
| dNTP (10 mM)                                | 0.50  |
| Taq polymerase (5 U/µl)                     | 0.50  |
| Water                                       | 17.75 |
| Restriction enzyme                          | *Pci*I |
| Restriction enzyme time, h                  | 6     |
| Restriction pattern length, bp              |       |
| A                                           | 768   |
| G                                           | 508+260 |
| Agarose gel concentration, %                | 3     |

PCR, polymerase chain reaction; dNTP, deoxyribonucleotide triphosphate.

Table IV. Colorectal cancer risks correlated with rs2298979 SNP that were examined in the present study.

| Genetics | Patients, n (%) | Controls, n (%) | P-value | OR (95% CI) | Unadjusted | Adjusted | P-value |
|----------|----------------|----------------|---------|-------------|------------|----------|---------|
|          |                 |                |         |             | Unadjusted | Adjusted |         |
| Genotype |                 |                |         |             |            |          |         |
| A/A      | 63 (28.6)       | 36 (16.4)      | -       | 1.00 (Reference) | 1.00 (Reference) | -    |
| A/G      | 157 (71.4)      | 184 (83.6)     | 0.002   | 0.488 (0.307-0.774) | 0.449 (0.263-0.767) | 0.003 |
| Alleles  |                 |                |         |             |            |          |         |
| A        | 283 (64.3)      | 256 (58.2)     | -       | 1.00 (Reference) | -          | -    |
| G        | 157 (35.7)      | 184 (41.8)     | 0.062   | 0.772 (0.588-1.013) | -          | -    |

Patients, n=220; controls, n=220. SNP, single nucleotide polymorphism; CI, confidence interval; OR, odds ratio.

Table V. Tumor-stage specific distribution of EGF rs2298979 genotypes among patients with CRC.

| Genotype | Stage I, n (%) | Stage II, n (%) | Stage III, n (%) | Stage IV, n (%) | P-value |
|----------|----------------|----------------|------------------|----------------|---------|
| A/A      | 29 (64.4)      | 38 (67.9)      | 49 (75.4)        | 41 (75.9)      | 0.483   |
| A/G      | 16 (35.6)      | 18 (32.1)      | 16 (24.6)        | 13 (24.1)      | -       |

EGF, epithelial growth factor; CRC, colorectal cancer.
The anatomical distribution investigated risk of CRC-gene in the patient group were less susceptible to CRC-polymorphism is located in intron 1 of the studied for the first time in the present study. The rs2298979 polymorphism of the of our knowledge the correlation between the rs2298979 polymorphism of the results revealed that the polymorphism +61A/G polymorphism in the 5'UTR of the gene. The mechanism of this correlation is unknown. The region where the SNP is located may be part of the regulatory sequence (21). The majority of studies on human genome sequences focus on protein coding regions (exons) (22). The protein coding regions in combination with the UTRs account for only 2% of the human genome. It has been revealed that the non-coding regions of the genome are effective in development, natural physiology and pathogenic processes (22). In addition, it has been demonstrated that there are SNPs present in intron regions that have a significant correlation with cancer and different types of diseases. In a study by Millar et al (23), it was revealed that the +1169A/T polymorphism located within intron 4 of the growth hormone I (GH1) gene was significantly correlated with the decrease in the circulation of growth hormone and the risk of cancer of the large intestine (23). Furthermore, Li et al (24) investigated the affect of two polymorphisms, +45G15G (T/G) and +276 (G/T), located in exon 1 and intron 4 of the adiponectin gene, respectively, in patients suffering from polycystic ovary syndrome (PCOS). No significant correlation was revealed between the disease and the +45G15G (T/G) polymorphism. By contrast, there was a statistically significant correlation between the +276 (G/T) polymorphism, located in intron 4, and PCOS disease (P=0.0126) (24). In conclusion, the present study demonstrates that the carriers of the A/G genotype among patients with CRC are less susceptible to the risk of cancer in comparison with the carriers of A/A genotype. Further studies on the intron regions of the EGF gene, particularly the rs2298979 polymorphism, are required in other populations.

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