RESEARCH ARTICLE

Aberrant DNA Methylation of P16, MGMT, hMLH1 and hMSH2 Genes in Combination with the MTHFR C677T Genetic Polymorphism in Gastric Cancer

Hai-Lin Xiong, Xun-Qi Liu, Ai-Hua Sun, Ying He, Jun Li, Yuan Xia*

Abstract

Associations of P16, MGMT, hMLH1 and hMLH2 with gastric cancer and their relation with MTHFR status in gastric patients who were confirmed with pathological diagnosis were assessed. Aberrant DNA methylation of P16, MGMT, hMLH1 and hMLH2 and polymorphisms of MTHFR C677T were assayed. The proportional DNA hypermethylation in P16, MGMT, hMLH1 and hMLH2 in cancer tissues was significantly higher than in remote normal-appearing tissues. DNA hypermethylation of P16 and MGMT was correlated with the T and N stages. Individuals with homozygotes (TT) of MTHFR C677T had significant risk of hypermethylation of MGMT in cancer tissues [OR (95% CI)= 3.47(1.41-7.93)]. However, we did not find association between polymorphism in MTHFR C677T and risk of hypermethylation in P16, MGMT, hMLH1 and hMLH2 genes either in cancer or remote normal-appearing tissues. Aberrant hypermethylation of P16, MGMT, hMLH1 and hMLH2 could be predictive of gastric cancer.

Keywords: Aberrant DNA methylation - P16 - MGMT - hMLH1 - hMLH2 - gastric cancer

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Introduction

Gastric cancer is the second leading cause of cancer worldwide, and almost 800 thousands deaths worldwide every year (IARC, 2008). Almost half of the gastric cancer cases and deaths occur in China, and this cancer ranks the third most common cancer (IARC, 2008). The prognosis of gastric cancer was poor, with 20-30% of 5-year survival rate being attributable to the fact that most cases are diagnosed in an advanced stage. However, early detection of gastric cancer is warranted to improve the survival of this cancer. Infection with H.pylori is a well-established cause of gastric cancer, but variants in various genetic factors also influence the susceptibility of gastric cancer (IARC, 1994).

Folate is a water-soluble vitamin and naturally found in green leafy vegetables, cereals and fruits (Aune et al., 2011). It is reported that folate metabolism is associated with risk of gastric cancer, and thus variants of folate metabolizing genes may affect the susceptibility of gastric cancer (Miao et al., 2002; Götzé et al., 2007). Methylenetetrahydrofolate reductase (MTHFR) is an important enzyme in folate metabolism, and the MTHFR C677T polymorphisms are associated with a reduced activity of this protein and risk of gastric cancers (Froost et al., 1995; Bagley and Selhub, 1998; Neves et al., 2010; Saberi et al., 2012).

DNA methylation plays an important role in gene regulation, and is involved in gene expression, eukaryotes, chromatin configuration and structural stability of DNA, binding of transcriptional factors and other proteins, X chromosome inactivation, aging and carcinogenesis (Jones and Selhub, 1998). Alteration of DNA methylation in genome can be found in the cancer tissues, and induce the over-expression of oncogenes and silencing of tumor suppressor genes in the process of carcinogenesis. Till data, few studies have been conducted to understand the role of aberrant hypermethylation of cancer-related genes, such as P16, MGMT, hMLH1 and hMLH2, in the risk of gastric cancer. Therefore, we aimed to explore the association of P16, MGMT, hMLH1 and hMLH2 with gastric cancer and their relation with MTHFR polymorphism.

Materials and Methods

This study recruited gastric patients who were confirmed with pathological diagnosis between March 2009 and December 2011 were involved in our study. A total of 413 patients were included. Patients who were cardiac adenocarcinoma, secondary or recurrent tumors and a history of other malignant tumor as well as and a history of eradication therapy for H.pylori were excluded in our study. All patients were asked to provide their peripheral blood, and they had read and signed an informed consent form.
Table 1. Primers in the PCR Process

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Sequence (5′ -3′)</th>
<th>Size (bp)</th>
<th>Temperature</th>
</tr>
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<tbody>
<tr>
<td>P16</td>
<td>M F</td>
<td>TTATAGGGCGGGGCGATGCC</td>
<td>150</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>GACCCGAACCCAGCTGAAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>U F</td>
<td>TTATAGGGCGGGGCGATGGT</td>
<td>151</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>CAACCACAACACCAACTACATA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MGMT</td>
<td>M F</td>
<td>CTTGAGTCTCGTGGATGGTTT</td>
<td>143</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>GACCCGAACCCAGCTGAAA</td>
<td>81</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>U F</td>
<td>CTTGAGTCTCGTGGATGGTTT</td>
<td>93</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>CAACCACAACACCAACTACATA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hMLH2</td>
<td>M F</td>
<td>CTTGAGTCTCGTGGATGGTTT</td>
<td>134</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>GACCCGAACCCAGCTGAAA</td>
<td>61</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>U F</td>
<td>CTTGAGTCTCGTGGATGGTTT</td>
<td>124</td>
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<td></td>
<td>CAACCACAACACCAACTACATA</td>
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<td>CTTGAGTCTCGTGGATGGTTT</td>
<td>124</td>
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</tr>
<tr>
<td></td>
<td>CAACCACAACACCAACTACATA</td>
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</tbody>
</table>

All patients received surgery, cancer lesion and remote normal-appearing tissues of the patients were excised and stored at -70°C. Twenty normal gastric tissue samples were obtained under surgery and also stored.

Data extraction and quantification

All the patients were required to provide 5ml peripheral bloods, and were collected in 9 mL EDTA vacutainers and stored at -20°C until DNA extraction. DNA was isolated from the peripheral blood using a TIANamp blood DNA kit (Tiangen Biotech, Beijing, China). The methylation of P16, MGMT, hMLH1 and hMLH2 was determined by the method of methylation-specific PCR after sodium bisulfate modification of DNA (Herman et al., 1996; Wang et al., 2008). The pairs of primers were designed using Assay Design 3.1 software (Sequenom, San Diego, CA, USA; Table 1). The 1.5 to 2.0 ug of genomic DNA was dissolved in 50 uL H₂O and incubated into 5.5 uL 3 mol/L NaOH for 10 minutes at 37°C, and then treated by hydroquinone and 520 uL 3 mol/L NaHSO₄. After these procedures, the unmethylated cytosine was converted to uracil and determined as thymine by Taq polymerase during the PCR process according to the instruction.

Genotyping of MTHFR C677T genetic polymorphism was determined using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). The forward primer and backward primer were TGA AGG AGA AAG GTTTCT CGG GA and AGG AGC GTG CGG TGA GAG TG, respectively. Briefly, a total PCR reaction volume of 10 uL contained 200 ng of genomic DNA and 20 pmol of each primer. The PCR conditions were as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 65 s, 60°C for 65 s, and 72°C for 90 s; a final extension was performed at 72°C for 5 min. The PCR products included a 173-bp fragment of 677C/C wild-type homozygotes; 173-, 125- and 48-bp fragments of 677T/T heterozygotes; and 125- and 48-bp fragments of 677T/T homozygotes.

Statistical analysis

All analyses were performed by using the SPSS version 16.0 statistical software (SPSS, Chicago, IL, USA). Continuous variables are expressed as mean ± standard deviation (SD), whereas categorical variables are shown as frequencies and percentages. Demographic characteristics were compared between cases and controls by χ² and Student’s t tests. Odds ratios (OR) and their corresponding 95% confidence intervals (CI) were used to assess the association between DNA hypermethylation in P16, MGMT, hMLH1 and hMLH2 and gastric cancer risk. We analyzed the data using two-sided P values.

Results

A total of 457 gastric cancer patients were included in our study, and 413 patients were involved in the final analysis (participation rate: 90.3%; 222 males and 191 females). The average age of 413 patients were 52.8±10.3 years old. The DNA hypermethylation of P16, MGMT, hMLH1 and hMLH2 in cancer tissue and paracancerous normal tissue was shown in Table 1. The proportions of DNA hypermethylation in P16, MGMT, hMLH1 and hMLH2 in gastric cancer tissues or remote normal-appearing tissues was shown in Table 2. We found a significantly higher proportion of hypermethylation in P16 in patients with T4 and N1 TNM stage either cancer tissues or remote normal-appearing tissues. The hypermethylation of P16 and MGMT showed significant correlation with the different clinical characteristics either in cancer tissue or remote normal-appearing tissues (Table 2). We found a significantly higher proportion of hypermethylation of P16 in patients with T4 and N1 TNM stage either cancer tissues or remote normal-appearing tissues (P<0.05). Similarly, we found hypermethylation of MGMT had significantly higher proportion in N1 and M1 TNM stage both in cancer tissues and remote normal-appearing tissues (P<0.05).

The association of hypermethylation of P16, MGMT, hMLH1 and hMLH2 was determined using two-sided P values.
The present study indicated that individuals with methylation of P16 and MGMT was significantly higher in the cancer tissues, which proved DNA methylation may play a role in the development of gastric cancer.

In the present study, we found polymorphisms in MTHFR C677T may influence the DNA methylation status. The main reason might be the activity of folate metabolic enzyme which participates into the methylation process of DNA. Previous studies reported that individuals carrying variant genotypes CT or TT had a higher risk of methylation of MGMT in cancer tissues (Wang et al., 2008; Chen et al., 2012). Our results are in line with previous study.

In conclusion, we found the aberrant hypermethylation of P16, MGMT, hMLH1 and hMLH2 could be predictive biomarkers for detecting of gastric cancer. The aberrant hypermethylation of P16 and MGMT gene was associated with TNM stages, and the polymorphism of MTHFR C677T could influence the methylation of MGMT. Further large-scale studies are required to elucidate their association.

### References


changes in the global DNA methylation profile of leukocytes are linked to nutrition but are not associated with the MTHFR C677T genotype or to functional capacities. *PLoS One*, 7, e52570.


