HMLH1 gene mutation in gastric cancer patients and their kindred

Jian-Hua Li, Xian-Zhe Shi, Shen Lü, Min Liu, Wan-Ming Cui, Li-Na Liu, Jing Jiang, Guo-Wang Xu

AIM: To study the status of hMLH1 gene point mutations of gastric kindreds and gastric cancer patients from northern China, and to find out gene mutation status in the population susceptible to gastric cancer.

METHODS: Blood samples of 120 members from five gastric cancer families, 56 sporadic gastric cancer patients and control individuals were collected. After DNA extraction, the mutations of exon 8 and exon 12 of hMLH1 gene were investigated by PCR-SSCP-CE, followed by DNA sequencing.

RESULTS: In the five kindreds, the mutation frequency was 25% (5/16) for the probands and 18% (19/104) for the non-cancerous members, which were significantly higher than the controls ($P<0.01$ $\chi^2 = 7.71$, $P<0.01$ $\chi^2 = 8.65$, respectively). In the sporadic gastric cancer, the mutation frequency was 7% (4/56), which was similar to that (5/100) in the healthy controls. The mutation point of exon 8 was at 219 codon of hMLH1 gene (A-G), resulting in a substitution of Ile-Val (ATC-GTC), whereas the mutation of exon 12 was at 384 codon of hMLH1 gene (T-A) resulting in a substitution of Asp-Val (GTT-GAT), which were the same as previously found in hereditary nonpolyposis colorectal carcinoma.

CONCLUSION: The members of gastric cancer families from northern China may have similar genetic background of hMLH1 gene mutation as those of hereditary nonpolyposis colorectal carcinoma.
there was no first-degree relative affected by gastric cancer in those sporadic patients (all were adenocarcinomas) aged 42-64 years, 36 male and 20 female and healthy controls aged 18-60 years, 60 male and 40 female.

**DNA extraction**

Genomic DNA was extracted from blood using the DNA extraction kit (Huashun Co., Ltd, Shanghai).

**PCR of hMLH1 gene in exon 8 and exon 12**

Primer sequences used were as follows: for exon 8, 5'-AM-ACAGACTTTGCTACCAGAATGG-3' (Forward) and 5'-FAM-TGTCTATTCTCTGTGACAAATGG-3' (Reverse) and for exon 12, 5'-FAM-CTCAGCCATGAGACAAATAAATCC-3' (Forward) and 5'-FAM-GGTCCCCAAATATGTGATGG-3' (Reverse). Fluorescence-labeled primers and PCR amplification kits were obtained from TaKaRa Biotechnology (Dalian, China). The reaction mixture contained 10 mmol/L Tris-HCl (pH 9.0), 50 mmol/L KCl, 1.5 mmol/L MgCl₂, 200 µmol/L dNTPs, 1 mmol/L each primer, 100-200 ng of the extracted genomic DNA and 1.25 U Taq DNA polymerase in a total volume of 25 µL. PCR was performed with a Perkin-Elmer Model 2700 PCR system (Foster City, CA, USA) with the following polymerase chain reaction (PCR) program: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 40 s, annealing at 55 °C for 40 s, extension at 72 °C for 1 min, and a final elongation step at 72 °C for 5 min.

**SSCP analysis by capillary electrophoresis**

Single-strand conformation polymorphism (SSCP) of DNA was analyzed by the method of capillary electrophoresis (CE). A P/ACE MDQ capillary electrophoresis instrument (Beckman, Palo Alto, CA, USA) with argon ion LIF detector (CE). A P/ACE MDQ capillary electrophoresis instrument was analyzed by the method of capillary electrophoresis Single-strand conformation polymorphism (SSCP) of DNA SSCP analysis by capillary electrophoresis nonfluorescent forward primer and the Big Dye terminator sequenced. The PCR for sequencing was performed using the PCR for sequencing was performed using the cycle sequencing ready reaction kit (Perkin-Elmer, ABI Prism) under the following conditions: initial denaturation at 96 °C for 2 min, 25 cycles at 96 °C for 10 s, annealing at 50 °C for 5 s, extension at 60 °C for 2 min, and a final elongation step at 60 °C for 7 min, in a 2 700 thermal cycler (Perkin-Elmer). The products were purified by an ethanol/NaAc method, and then CE-sequencing was conducted using ABI Prism 310 (Perkin-Elmer, ABI Prism).

**RESULTS**

Of the 16 probands in the five kindreds, two cases were found mutant at exon 8, two cases were found mutant at exon 12 and the total mutation frequency was 25% (5/16). Meanwhile 12 cases were found mutant at exon 8, seven cases were found mutant at exon 12 and the total mutation frequency was 18% (19/104) in the familial noncancerous members. In the 56 cases of sporadic gastric cancer, two cases were found mutant at exon 8, two cases were found mutant at exon 12 and the total mutation frequency was 7% (4/56), which was similar to the healthy control individuals but being significantly lower than both the probands and familial members in the kindreds (Table 1). The mutation point of exon 8 was at 219 codon of hMLH1 gene (A-G), resulting in a substitution of Ile-Val (ATC-GTC) (Figures 1A and 2A), whereas the mutation of exon 12 was at 384 codon of hMLH1 gene (T-A), resulting in a substitution of Asp-Val (GTT-GAT) (Figures 1B and 2B), which was the same as previously found in HNPC.

**CE-sequencing**

The samples suspected to be mutant by CE-SSCP were sequenced. The PCR for sequencing was performed using nonfluorescent forward primer and the Big Dye terminator cycle sequencing ready reaction kit (Perkin-Elmer, ABI Prism) under the following conditions: initial denaturation at 96 °C for 2 min, 25 cycles at 96 °C for 10 s, annealing at 50 °C for 5 s, extension at 60 °C for 2 min, and a final elongation step at 60 °C for 7 min, in a 2 700 thermal cycler (Perkin-Elmer). The products were purified by an ethanol/NaAc method, and then CE-sequencing was conducted using ABI Prism 310 (Perkin-Elmer, ABI Prism).

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**Table 1** Comparison of hMLH1 mutation among the gastric cancer kindreds, sporadic gastric cancer patients, and control individuals

| Groups                        | Cases | Exon 8 | Exon 12 | HMLH1 mutation | Mutation rate (%) |
|-------------------------------|-------|--------|---------|----------------|------------------|
| Proband of kindreds           | 16    | 2      | 2       |                | 25 b             |
| Non-cancerous members of kindreds | 104   | 12     | 7       |                | 18 a             |
| Sporadic gastric cancer patients | 56    | 2      | 2       |                | 7                |
| Control individuals           | 100   | 2      | 3       |                | 5                |

bP<0.01  χ² = 7.71, aP<0.01  χ² = 8.65 vs control individuals.
DISCUSSION

Mismatch repair (MMR) plays a central role in maintaining genomic stability by repairing DNA replication errors and inhibiting recombination between non-identical sequences[7,8]. Loss of mismatch repair causes destabilization of the genome and results in high mutation frequency[8]. hMLH1 gene is a prominent component in the human mismatch repair system and its dysfunction is involved in a number of patients with HNPCC[9,10]. It has been reported that germline mutations of MMR are identified in nearly 80% of the patients with HNPCC and almost 60% of the mutations are in hMLH1[10,11]. According to statistics, about 8-10% of gastric cancer cases are related to an inherited familial genetic factor[12]. Up to 3-fold increases in risk for gastric cancer among relatives of gastric cancer patients are consistent[12]. The gene background of gastric cancer susceptibility is not sufficiently known to us, especially in China, although the hereditary tendency is even higher.

We primarily detected exon 8 and exon 12 of the hMLH1 gene in 16 probands’ peripheral blood sample of five gastric cancer kindreds and found the same mutations that had previously been discovered in HNPCC. The mutation frequency was significantly higher than that of the control individuals and it was true of those familial members. It was proposed that the gene background is characterized by hMLH1 mutation in gastric cancer kindreds and that the presence of hMLH1 mutation is associated with an increased risk of developing gastric cancer and those carrying mutant hMLH1 gene are prone to develop gastric cancer even at an early age. What about sporadic gastric cancer? To answer this question, we extended the samples by analyzing 56 sporadic gastric cancer patients with their blood samples. To our surprise, the mutation frequency in the blood samples were similar to control individuals, which was significantly lower than both the probands and noncancerous members in the five kindreds’ blood samples. It seems that hMLH1 gene mutation is not a characteristic of sporadic gastric cancer.

From the above-mentioned, it can be concluded that there may be similar hMLH1 gene mutation as HNPCC in somatic cells in gastric cancer kindreds, but not in those in sporadic gastric cancer patients. The mutations of hMLH1 gene may be involved in tumorigenesis of hereditary gastric cancer in northern China.

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