Relationship between LAPTM4B gene polymorphism and susceptibility of gastric cancer

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Background: A novel gene called LAPTM4B (lysosome-associated protein transmembrane 4β) was mapped to 8q22, and contains seven exons. The 2.25-kb messenger RNA of the gene encodes a putative lysosome-associated protein with four transmembrane regions. There are two alleles of the gene, named as LAPTM4B*1 and LAPTM4B*2. Allele *1 differs from allele *2 in that it contains only one copy of a 19-bp sequence in the 5' untranslated region (UTR), whereas this sequence is duplicated and tandemly arranged in allele *2. Studies showed that the allelic variation of LAPTM4B was associated with the genetic susceptibility of hepatocellular carcinoma but not with that of esophageal squamous cell carcinoma. This study was designed to investigate the possible association between the allelic variation of LAPTM4B and the genetic susceptibility of gastric cancer.

Materials and methods: The genotype of LAPTM4B was analyzed in 350 unrelated healthy adult individuals and 214 patients with gastric cancer by utilizing polymerase chain reaction based on specific primers. The genotypic distribution of LAPTM4B was analyzed by χ² test.

Results: The allelic frequencies of the *2 were 33.88% and 24.14% in the gastric cancer group and the healthy control group, respectively, which was significantly different between the two groups (P < 0.001). There was a significant difference in the overall genotypic distribution between the patients and the controls (P = 0.023). The risk of suffering from gastric cancer was increased 1.819 times in the individuals of the *1/2 genotype [95% confidence interval (CI) 1.273–2.601] and 2.387 times in the individuals of the *2/2 genotype [95% CI 1.195–4.767] compared with the *1/1 genotype. No association between the genotypic distribution of LAPTM4B and the clinical information on patients of gastric cancer such as age, pathological type, differentiation classification of TNM, and infection of hepatitis B virus was shown.

Conclusion: This study indicated that allele *2 of LAPTM4B might be the risk factor of gastric cancer, which could be associated with genetic susceptibility of gastric cancer.

Key words: gastric cancer, gene polymorphism, LAPTM4B, susceptibility

introduction

Lysosome-associated protein transmembrane 4β (LAPTM4B), a novel gene overexpressed in most hepatocellular carcinomas (HCCs) and their cell lines, showed that up-regulation of the gene correlated significantly with differentiation of HCC. Deregulation of the gene may contribute to the development of HCC. The LAPTM4B fragment was first screened out by fluorescent differential-display polymerase chain reaction (PCR) [1], and confirmed by slot blot [2–4]. It was overexpressed in HCCs compared with paired noncancerous liver or normal liver tissues [5, 6]. Significantly high expression was also noticed in poorly differentiated HCCs when compared with moderately or well-differentiated HCCs. LAPTM4B was mapped to 8q22, and contains seven exons. The 2.25-kb messenger RNA (mRNA) of the gene encodes a lysosome-associated protein with four transmembrane regions.

There are two alleles of the gene, named as LAPTM4B*1 and LAPTM4B*2 (GenBank accession No.: AY219176 and AY219177, respectively). Allele *1 differs from allele *2 in that it contains only one copy of a 19-bp sequence in the 5' UTR, whereas this sequence is duplicated and tandemly arranged in allele *2. The mRNA of allele *1 can only start translation at nt 157, because there are in-frame stop codons at nt 40 and nt 103. The mRNA of allele *2 would start translation at nt 17 which would produce an extra stretch of amino acids at N-terminus of LAPTM4B.

Previous studies showed that the frequencies of LAPTM4B*2/2-type allele of HCC were significantly higher than the normal population which indicated that LAPTM4B*2/2 was associated with the genetic susceptibility of HCC [7] and its gene polymorphism was associated with the certain tumor
particularity, but not all the genetic susceptibility of tumors was related with LAPTM4B gene.

Several studies have shown that LAPTM4B was highly expressed in gastric cancer tissues and less expressed in normal gastric tissues. The relationship between LAPTM4B gene polymorphism and susceptibility of gastric cancer, however, is still not clear. Previous studies suggested that the allelic variation of LAPTM4B was associated with the genetic susceptibility of HCC and lung cancer [8], but not with that of esophageal squamous cell carcinoma (ESCC). The available information makes it plausible to hypothesize that there was possible association between the allelic variation of LAPTM4B and the genetic susceptibility of gastric cancer. Here we are showing the results of a case–control study to examine the hypothesis.

**materials and methods**

**cases**

Eligible cases were newly diagnosed gastric cancer patients seen at Beijing Cancer Hospital from January 2004 to 30 September 2005. The diagnosis of gastric cancer was based on the criteria of tumor, node, metastasis (TNM) classification system formulated by American Joint Committee on Cancer and International Union Against Cancer in 2003. Patients consented in writing to participate in the study. Pathology reports were obtained after gastric resection or upon discharge to confirm diagnoses of gastric cancer. We approached 280 consecutive patients with gastric cancer. For this LAPTM4B polymorphism study, high-quality genomic DNA isolated from blood from 214 patients was obtained.

**controls**

Eligible controls were healthy individuals recruited from the employees at Beijing Cancer Hospital and Medical Health Center in Peking University. They met the same eligibility criteria, except for never having a diagnosis or history of cancer. The nurse interviewer approached blood donors, explained the study, and asked them to read the study description and sign the informed consent form if they agreed to participate. For controls who were patients at Beijing Cancer Hospital, medical charts were examined to ensure that the individuals did not have a prior history of cancer. For other controls, their cancer history was asked in the face-to-face interview. A total of 350 potential participants were approached.

**blood sample collection and storage**

Two milliliters of blood sample was collected from cases and controls who were willing to donate samples for genotype assays. All blood specimens were stored in a –20°C freezer at the Department of Clinical Laboratory and Department of Immunology, the School of Oncology, Peking University.

**DNA extraction**

Genomic DNA from human peripheral white blood cells was isolated for LAPTM4B assays. Two milliliters of each blood sample was collected in EDTA tubes kept at –20°C. Then the blood was thawed in a water bath at room temperature and 1 ml was transferred to a centrifuge tube. To this, 0.5 ml of phosphate-buffered saline was added at room temperature. The blood was centrifuged at 3500 g for 15 minutes at room temperature. This step was repeated twice. Then the supernatant was removed, which contained lysed red cells, by aspiration. The pellet was resuspended in 0.5 ml of lysis buffer. The solution was incubated for 1 h at 37°C, and proteinase K (20 mg/ml) was added to a final concentration of 100 μg/ml. A glass rod was used to mix the enzyme solution gently into the viscous lysates of cells. The lysates were incubated in a water bath for 3 hours at 50°C. DNA isolation followed a standard phenol–chloroform extraction and ethanol precipitation procedure.

**PCR analysis of LAPTM4B polymorphisms**

Genotyping for the LAPTM4B was determined by PCR and agarose gel electrophoresis analysis using the specific primers P1 (forward) 5’-GGCGACTAGGGACTGGGGA 3’ (nt 72–92) and P2 (reverse) 5’-CGAGAGCTCCAGCTCTGCCC 3’ (nt 255–275) [7]. PCR cycles were carried out in a Perkin–Elmer thermocycler with the following cycle conditions: initial denaturation at 95°C for 5 minutes following 35 cycles of denaturation at 94°C for 30 seconds, annealing at 62°C for 30 seconds, and extension at 72°C for 30 seconds. The final extension step was for 5 minutes at 72°C. Each PCR was done in a final reaction volume of 25 μl containing 100 ng of genomic DNA, 10 pmol of each of the forward and reverse primers, 0.2 mmol/l of each deoxynucleoside triphosphate, 1.5 mmol/l MgCl₂, 50 mmol/l KCl, 10 mmol/l Tris–HCl (pH 8.3), and 2.5 U Taq DNA polymerase (Promega; Beijing, China). Human β-actin was used as positive inner control. The forward primer was 5’-AACGAGGAGCCATGAGGAAGG-3’ and the reverse primer 5’-GGTACTCAGTCAGGGTC-3’. The PCR products were analyzed by separation in a 2% agarose gel and visualized with ethidium bromide. The homozygous *1/*1 genotype was identified by a 204-bp band; the homozygous *2/*2 genotype was identified by the presence of a 223-bp band. The heterozygous *1/*2 type exhibited the two bands, 204 and 223 bp. The β-actin PCR product will be 318 bp long.

**statistical analysis**

Statistical Analysis Software Version 13.0 was used for all statistical analyses. The distribution of selected demographic variables was analyzed by χ² test or Fisher’s exact test was used when the number of variables expected per cell was less than five, and Fisher’s exact test was used to determine the significance of the deviation. The corrected P values were obtained by multiplying the uncorrected P values with the number of comparisons. P < 0.05 was taken as the level of significance. The relationships between gastric cancer and putative risk factors were measured using odds ratios (ORs) and the 95% confidence intervals (CIs) that were derived from logistical regression analysis. Dummy variables were used to estimate the OR for each category of exposure in logistical regression analysis. On the basis of the distributions of variables and prior knowledge of the risk factors for gastric cancer, age, pathological type, differentiation classification of TNM, and infection of hepatitis B virus (HBV) were adjusted for in the logistical regression model. Stratified analysis was used to evaluate the potential interaction effects between LAPTM4B variant and smoking status. Departures from multiplicative interaction effects between LAPTM4B and other potential risk or protective factors were evaluated. The null hypotheses for multiplicativity were tested. Testing of the multiplicative interaction was done by the likelihood ratio test. Departure from multiplicative effects was assessed by inclusion of main effect variables and their product terms in the logistical regression model.

**results**

We tested 214 gastric cancer subjects and 350 normal control subjects for the 19-bp polymorphism of the LAPTM4B gene. Three kinds of genotypes of the LAPTM4B polymorphisms were found (Figure 1). From Figure 1B, 204-bp band for homozygous genotype *1/*1 and 223-bp band for homozygous genotype *2/*2 could be seen. The heterozygous genotype *1/*2 exhibited the two bands, that is 223- and 204-bp bands. The up band in each lane, which was β-actin-positive control, showed 318-bp PCR product. The mean (± standard deviation) age of the case and
control groups was 50.91 (±9.71) and 49.75 (±9.39) years, respectively. Characteristics of the case and control subjects are presented in Table 1. There were no significant statistical differences in age and gender between the case and control groups.

A total of 214 gastric cancer subjects and 350 normal control subjects were investigated. The distribution of genotype frequencies of LAPTM4B gene promoter region polymorphism in gastric cancer and normal control subjects is illustrated in Table 2. There were no significant deviations from Hardy–Weinberg equilibrium in the two samples (gastric cancer patients and controls).

To examine the effect of having at least one *2 allele on the risk of gastric cancer, we found that the adjusted OR of *2/2 and *1/2 versus genotype *1/1 was 1.887 (95% CI 1.337–2.664).

The allelic frequencies of the *2 were 33.88% and 24.14% in the gastric cancer group and the healthy control group, respectively, which was significantly different between the two groups (P < 0.001). There was a significant difference in the overall genotypic distribution between the patients and the controls (P = 0.023). (These data are not shown in the table since it is difficult to put in. It was done by t-test.) The risk of suffering from gastric cancer was increased 1.819 times in the individuals of the *1/2 genotype (95% CI 1.273–2.601) and 2.387 times in the individuals of the *2/2 genotype of LAPTM4B (95% CI 1.195–4.767) compared with the *1/1 genotype.

We detected a significant difference in the distribution of the *2/2 genotype in the LAPTM4B gene between gastric cancer patients and normal controls (P = 0.014). The frequency of the homozygote for the *2 allele, which was with the two copies of the 19-bp sequences in the LAPTM4B, increased significantly in gastric cancer patients, compared with normal controls (P < 0.001).

In addition, the association between allele frequencies and genotypes of the patients was investigated on one hand and the genotypic distribution of LAPTM4B in relation to clinicopathological and other variables in case group, such as age, pathological type, differentiation classification of TNM, and infection of HBV, was done on the other hand (Table 3). After adjusting for age, smoking, and drinking, no significant association was observed. No association was found between the genotypic distribution of LAPTM4B and the clinical information on patients of gastric cancer such as age, pathological type, differentiation classification of TNM, and infection of HBV.

**discussion**

Studies showed that LAPTM4B was an important novel gene associated with proliferation and differentiation of cells. In normal cells, it may also play important roles such as regulation of cell proliferation and survival. LAPTM4B was widely expressed in human tissues. Its expression was high in heart, skeletal muscle, kidney, and testis; moderate in ovary, kidney, and pancreas; low in liver, spleen, and thymus; but lowest in lung and peripheral leukocytes. Furthermore, the expression levels of LAPTM4B were significantly related to the differentiation status of HCCs. Its expression was highest in poorly differentiated HCCs, high in moderately differentiated

**Table 2.** Allelic frequencies and distribution of genotype of LAPTM4B gene polymorphisms in case and control groups

<table>
<thead>
<tr>
<th>Allele frequencies</th>
<th>Cases (%)</th>
<th>Controls (%)</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>*1</td>
<td>283 (66.12)</td>
<td>531 (75.86)</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>*2</td>
<td>145 (33.88)</td>
<td>169 (24.14)</td>
<td>1.819 (1.273–2.601)</td>
<td>0.001</td>
</tr>
<tr>
<td>Total</td>
<td>428 (100.00)</td>
<td>700 (100.00)</td>
<td>0.016</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1.** General characterization of case and control groups

<table>
<thead>
<tr>
<th>Case (n = 214)</th>
<th>Control (n = 350)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (year ± SD)</td>
<td>50.91 ± 9.71</td>
<td>49.75 ± 9.39</td>
</tr>
<tr>
<td>Gender n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>153 (71.50)</td>
<td>225 (64.30)</td>
</tr>
<tr>
<td>Female</td>
<td>61 (28.50)</td>
<td>125 (35.70)</td>
</tr>
</tbody>
</table>

*X2* test.

SD, standard deviation.
increased risk of gastric cancer compared with normal group.

The putative protein of LAPTM4B is highly conserved, with *2/2 genotype (OR = 2.387; 95% CI = 1.195–4.767; \( P = 0.014 \)). These results showed that allele *2 was associated with genetic susceptibility of gastric cancer. No difference, however, was observed in the frequencies of LAPTM4B genotypes in patients with ESC comparing with the corresponding controls, indicating that allele *2 was associated specifically with the susceptibility of the certain tumors.

The distribution of genotypes of LAPTM4B in relation to clinicopathological and other variables in case group is shown in Table 3.
It is also 46% homologous at the amino acid level to a human lysosome-associated transmembrane-4 protein, LAPTM4A. LAPTM4B shares a number of characteristics with other lysosome-associated proteins such as LAPTM5 [18]. These clues inspired us to investigate the functions of LAPTM4B and its roles in hepatocarcinogenesis.

LAPTM4B-overexpressing NIH3T3 cells displayed an increased cell growth and proliferation, reduced serum dependence, as well as changes in cell morphology [25]. Therefore, the LAPTM4B gene possibly promotes the cell cycle from G1 to S by cyclin E [26], accelerates cell proliferation, and causes cell malignancy. Therefore, further elucidation of the role of LAPTM4B and its correlation with the differentiation status of hepatocellular carcinoma. World J Gastroenterol 2005; 11 (18): 2704–2708.

**Figure 2.** Comparison of the putative proteins encoded by LAPTM4B*1 and LAPTM4B*2. Only partial sequences of the first exon are shown. The sequence numbers of the first nucleotide (left) and the final amino acid (right) in each row are indicated. The nucleotide sequences are numbered with the putative transcription start site as +1. In-frame stop codons are underlined and marked by symbols #, the 19-bp sequences in both of the alleles are underlined.

Messenger RNA (mRNA) of allele *1 can only start translation at nt 157, because there are in-frame stop codons at nt 40 and nt 103. The mRNA of allele *2 would start translation at nt 17 which would produce an extra stretch of amino acids (33 aa, gray-shaded letters) at N-terminus of LAPTM4B.

<table>
<thead>
<tr>
<th>Allele</th>
<th>Sequence</th>
<th>Description</th>
</tr>
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**acknowledgements**

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**references**


**original article**

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