Alterations of p16 and Prognosis in Biliary Tract Cancers from a Population-Based Study in China

Takashi Ueki,1 Ann W. Hsing,3 Yu-Tang Gao,4 Bing-Sheng Wang,5 Ming-Chang Shen,6 Jiaron Cheng,4 Jie Deng,4 Joseph F. Fraumeni, Jr.,3 and Asif Rashid1,2,7

1Department of Pathology and 2Oncology Center, The Johns Hopkins University School of Medicine, Baltimore, Maryland; 3Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland; 4Shanghai Cancer Institute, Shanghai, China; 5Department of General Surgery, Zhongshan Hospital, Shanghai, China; 6Department of Pathology, Shanghai Cancer Hospital, Shanghai, China; and 7Department of Pathology, University of Texas M. D. Anderson Cancer Center, Houston, Texas

ABSTRACT

Purpose: Biliary tract cancer is an uncommon malignancy with a poor survival rate. We evaluated p16 gene alteration as a prognostic marker for this disease.

Experimental Design: We studied p16 gene alterations by sequencing, methylation, and loss of heterozygosity of chromosome 9p in 118 biliary tract carcinomas, including 68 gallbladder cancers, 33 extrahepatic bile duct cancers, and 17 ampullary cancers. Survival was evaluated in 57 patients with gallbladder carcinomas, 27 with bile duct carcinomas, and 16 with ampullary carcinomas with and without somatic p16 alterations detected by two different methods.

Results: p16 gene alterations including silent mutations were present in 61.8% gallbladder cancers, 54.5% bile duct cancers, and 70.6% ampullary cancers, p16 gene nonsilent mutations, p16 methylation, and loss of chromosome 9p21-22 that targets p14, p15, and p16 genes were present in 13 of 53 (24.5%), 8 of 54 (14.8%), and 32 of 44 (72.7%) gallbladder tumors; 5 of 25 (20.0%), 5 of 31 (16.1%), and 12 of 21 (57.1%) bile duct tumors; and 3 of 13 (23.1%), 6 of 15 (40.0%), and 8 of 16 (50.0%) ampullary tumors, respectively. The mean survival of patients with gallbladder cancers without p16 alterations was 21.5 ± 14.8 months compared with 12.1 ± 11.4 months for patients with p16 alterations (P = 0.02).

Conclusions: Alteration of p16 gene alone or in combination with alterations of other tumor suppressor genes on chromosome 9p is a prognostic indicator in gallbladder carcinoma, with more favorable survival rates associated with carcinomas lacking p16 gene alterations.

INTRODUCTION

Biliary tract carcinomas are relatively uncommon in most parts of the world, although incidence is elevated in certain geographic locations and populations groups (1). From 1972 to 1994, biliary tract cancer was the most rapidly rising malignancy in Shanghai, the People’s Republic of China, with a 119% increase in men and 124% increase in women (2). Reasons for the rising incidence of biliary tract cancers in Shanghai are unclear, but nutritional changes and improvements in diagnosis and classification may contribute to this trend.

The p16 (INK4, MTS1, and CDKN2) tumor suppressor gene, which maps to chromosome 9p21-p22, is frequently inactivated in a wide variety of tumors by deletion, mutation, or CpG island methylation (3–7). p14 (ARF) and p15 genes are present in the same region of chromosome 9p as the p16 gene (8). p16 and p14 transcripts are produced from two separate promoters and use alternative first exons (1α and 1β, respectively) joined through the same splice acceptor site to exon 2 coding sequences but in a different reading frames (9–10). p16 is a cyclin-dependent kinase (CDK) inhibitor involved in regulation of the G1 checkpoint in the cell cycle (11). The p16 protein blocks the G1-S transition in the cells by binding to and preventing association of CDK4 and CDK6 with cyclin D1, leading to dephosphorylation of the retinoblastoma gene product and repression of transcription factor E2F (11, 12). The CDK4/cyclin D complex phosphorylates the product of the retinoblastoma gene, thereby promoting entry into the cell cycle. The p16 gene is inactivated by homozygous or heterozygous deletion, mutations of coding and adjoining noncoding regions, or methylation of CpG island present at the 5'-end of the gene in a wide variety of primary cancers and tumor cell lines (5–7, 13–17). Loss of expression of p16 because of methylation or deletion or loss of functional p16 protein because of mutations result in unregulated CDK4 activity, leading to persistent retinoblastoma phosphorylation and therefore uncontrolled cellular proliferation.

p16 gene alterations (18, 19), loss of heterozygosity of chromosome 9p (19–22) and loss of p16 protein expression (23) are reported in primary biliary tract cancers and cell lines derived from these cancers. To clarify the role of p16 inactivation in biliary tract carcinomas, we studied p16 gene alterations in a population-based study of these tumors by sequencing, methylation of CpG island present at the 5'-end of the p16 gene, and loss of heterozygosity (LOH) of chromosome 9p. We also evaluated p16 gene alteration status as a prognostic marker for biliary tract cancers.

MATERIALS AND METHODS

Patient Population. Patients with primary biliary tract cancer (ICD9 156) newly diagnosed between 1997 and 1999...
were identified through a rapid reporting system established between the Shanghai Cancer Institute and 30 collaborating hospitals in urban Shanghai. This reporting system recruited over 95% of patients with biliary tract cancers in Shanghai. A total of 118 patients with biliary tract carcinomas, including 68 gallbladder cancers, 33 bile duct cancers, and 17 ampullary cancers, were identified for analysis as part of a population-based case-control study. Eligibility criteria for recruitment of patients included the following: residents of urban Shanghai between 18 and 74 years of age diagnosed after April 1997 with gallbladder, extrahepatic bile duct, or ampullary carcinomas.

**Tissue Specimens.** Surgical pathology specimens were collected from patients with biliary tract cancers undergoing curative resection by pancreaticoduodenectomy or bile duct resection or biopsy of an advanced tumor. As part of the case-control study, 6 H&E-stained slides and 6 unstained slides of each patient were collected for each patient from the surgical pathology and surgery departments of the participating hospitals. In addition, a structured questionnaire was used to elicit information on demographic, clinical, and epidemiological variables. The anatomical location where the tumor was taken was recorded in a diagram completed by the local pathologist at the participating hospital. The histopathological slides were reviewed by two pathologists from Shanghai and were independently reviewed by one of us (A. R.). Classification was based on the WHO classification of tumors of the biliary tract (24). Duodenal and pancreatic carcinomas were excluded from the study. Medical records were abstracted for all cancer cases. Patients’ follow-up status was obtained by the Shanghai Cancer Institute by contacting the patients or their relatives from the date of diagnosis to March 2001.

**DNA Preparation.** Genomic DNA was extracted from tumor tissue by microdissection from three H&E-stained slides without a coverslip from formalin-fixed, paraffin-embedded blocks and prepared as described in previous studies (25). DNA from tumor tissue represented at least 50% tumor DNA. DNA from peripheral blood was used if available. From tumor tissue represented at least 50% tumor DNA. DNA from peripheral blood was used if available.

**RNase Cleavage Assay.** A RNase cleavage assay was used to screen for mutations of p16 gene according to the manufacturer’s instructions (Nonisotopic RNase cleavage assay; Ambion, Austin, TX). Exons 1 and 2 of the p16 gene were amplified. Exon 1 of the p16 gene was amplified using the forward and reverse amplification primers 5′-GAAGAAA-GAGGAGGGCTG-3′ and 5′-GAAGAAA-GAGGAGGGCTG-3′, respectively. The cycling conditions were as follows: 95°C for 10 min; 37 cycles of 95°C for 30 s; and 55°C for 45 s. Exon 2 of the p16 gene was amplified using the forward and reverse amplification primers 5′-ACACAAGCTTCTCTGACTCT-3′ and 5′-ACACAAGCTTCTCTGACTCT-3′, respectively. The cycling conditions were as follows: 95°C for 10 min; 38 cycles of 95°C for 30 s; and 60°C for 45 s. The T7 promoter sequence was introduced at both ends of the PCR product by nested PCR amplification. The PCR product was sequenced, and no mutations were found. PCR amplification of exon 1, exon 2, or both exons failed for 26 carcinomas.

**Sequencing of p16.** DNA sequencing was performed using the PCR product and amplification primers using a commercial DNA sequencing kit according to the manufacturer’s instructions (Sequitherm Excel; Epicenter Technologies, Madison, WI). The forward and reverse primers for sequencing were 5′-GCTGCGGTGGCTACGAG-3′ and 5′-ACTTTGCTCTTCAGAGTC-3′ for exon 1, and 5′-CTTCTTCTCTCGTACTGGG-3′ and 5′-AGCTTTGGAAGCTCCTAGGG-3′, respectively. Mutations were confirmed by reamplification of tumor DNA and sequencing in both directions. Germ-line mutations were excluded by sequencing DNA extracted from blood cells if available and by comparison with the previous reports of germ-line mutations (16–17).

**Bisulfite Treatment of DNA and Methylation-Specific PCR.** The methylation status of p16 was determined by bisulfite treatment of DNA followed by methylation-specific PCR as described, with modifications (26). In brief, 1 μg of microdissected genomic DNA was denatured with 2 M NaOH at 37°C for 10 min, followed by incubation with 3 M sodium bisulfite (pH 5.0) at 50°C for 16 h in the dark. After treatment, DNA was purified using the DNA Cleanup Kit (Promega) as recommended by the manufacturer, incubated with 3 M NaOH at room temperature for 5 min, precipitated with 10 M ammonium acetate and 100% ethanol, washed with 70% ethanol, and finally resuspended in 20 μl of distilled water. The primers and PCR conditions for p16 were the same as those reported by Herman et al. (26). In brief, 2 μl of bisulfite-treated DNA was used as template for PCR reactions using primers specific for methylated and unmethylated alleles. PCR products from methylated and unmethylated reactions were electrophoresed on 10% acrylamide gels and visualized by ethidium bromide staining (examples in Fig. 1A). Representative samples were reanalyzed to confirm the methylation status. DNA for bisulfite treatment was unavailable for 17 carcinomas.

**LOH of Chromosome 9p.** Fluorescent-labeled PCR amplification was performed using the markers on the short arm of chromosome 9 (D9S161, D9S259, D9S171, and D9S285, centromeric to telomeric). D9S161, D9S259, and D9S171 at 9p21 are centromeric to p16 gene locus, and D9S285 at 9p22-9p23 is
RESULTS

The p16 Gene Alterations. p16 gene alterations including p16 mutations (including silent mutations and/or p14 gene mutations due to a shared exon), methylation of the CpG island in the 5’-region of the p16 gene, and loss of chromosome 9p21-22 that targets p14, p15, and p16 genes were present in 72 (61.0%) of 118 biliary tract carcinomas, including 42 of 68 (61.8%) gallbladder cancers, 18 of 33 (54.5%) bile duct cancers, and 12 of 17 (70.6%) ampullary cancers (Fig. 2). The frequency of these alterations in gallbladder, bile duct, and ampullary carcinomas were 13 of 53 (24.5%), 5 of 25 (20.0%), and 3 of 13 (23.1%) for nonsilent p16 gene mutations, 8 of 54 (14.8%), 5 of 31 (16.1%), and 6 of 15 (40.0%) for p16 methylation, and 32 of 44 (72.7%), 12 of 21 (57.1%), and 8 of 16 (50.0%) for LOH of chromosome 9p, respectively. LOH of chromosome 9p was more prevalent in gallbladder carcinomas and bile duct carcinomas than p16 mutations or methylation of CpG islands in the 5’-region of the p16 gene (P = 0.000001 and P = 0.02, respectively). The frequency of LOH at D9S161, D9S259, D9S171, and D9S285 was 31 of 45 (68.9%), 23 of 31 (74.2%), 20 of 26 (76.9%), and 17 of 40 (42.5%) of informative cases, respectively.

p16 gene mutations are tabulated in Fig. 2. There were 36 mutations present in 18 gallbladder carcinomas, 6 bile duct carcinomas, and 3 ampullary carcinomas. The mutations included a noncoding mutation in the promoter region, 8 mutations in exon 1 and 27 mutations in exon 2 of p16 gene. Twelve carcinomas had silent mutations in p16 gene, but 9 were associated with other p16 alterations. As exon 2 of p16 gene also codes for exon 2 of p14 gene in a different reading frame, all missense and silent mutations in exon 2 of p16 gene were missense or silent mutations in exon 2 of p14 gene. There were 21 missense and 5 silent mutations of the p14 gene.

Among 32 coding single-base substitutions, all were present at G or C nucleotides; 30 (93.7%) were transitions (19 G to A and 11 C to T) and only 2 (6.3%) transversions (1 C to A and 1 G to T). Furthermore, 20 (62.5%) of the 32 mutations were present at non-CpG dinucleotides and 12 (37.5%) at CpG dinucleotides. Eleven of these 12 single-base substitutions at CpG dinucleotides were G-A or C-T transitions.

Three patients with gallbladder carcinomas, 2 with bile duct carcinomas, and 1 with ampullary carcinoma had two or more p16 and/or p14 mutations. In addition, of 98 carcinomas that were studied by two or more methods, 12 gallbladder carcinomas, 3 bile duct carcinomas, and 5 ampullary carcinomas had two or more p16 alterations (Fig. 2). The prevalence of p16 alterations was more frequent if all three methods were used: 24
Fig. 2  
*p16* alterations in 68 gallbladder, 33 bile duct, and 17 ampullary carcinomas. Carcinomas with any *p16* alterations had nonsilent *p16* mutation, *p16* methylation, or chromosome 9p loss; and carcinomas without any *p16* alterations had no alterations detected by at least two different methods.
of 31 (77.4%) gallbladder carcinomas, 12 of 16 (75.0%) bile duct carcinomas, and 9 of 12 (75.0%) ampullary carcinomas had \textit{p16} alterations in patients whose carcinomas were examined by all three methods—DNA sequencing, methylation, and LOH.

**Clinicopathological Characteristics and Survival Associated with \textit{p16} Alteration Status.** The clinicopathological characteristics of biliary tract carcinomas, \textit{p16} alteration status, and survival times were evaluated for 101 patients, including 57 patients with gallbladder carcinomas, 28 with bile duct carcinomas, and 16 with ampullary carcinomas with and without \textit{p16} alterations according to at least two different methods (Fig. 2). Two gallbladder carcinomas and one bile duct carcinoma with silent \textit{p16} mutations (with \textit{p14} mutations) without other \textit{p16} alterations were excluded. None of 4 (0%) adenosquamous gallbladder carcinomas had \textit{p16} alterations compared with 40 of 53 (75.5%) gallbladder carcinomas of other histological types ($P = 0.001$). The vital status, age or gender of patient, presence of cholelithiasis, and histological type of bile duct and ampullary carcinomas were not associated with \textit{p16} status.

The \textit{p16} Alteration Status and Survival. Patients whose carcinomas lacked \textit{p16} alterations had a better mean survival compared with those with alterations. The mean survival of patients whose carcinomas lacked \textit{p16} alterations was $20.5 \pm 13.4$ months, compared with $14.2 \pm 11.9$ months for those with alterations ($P = 0.04$; Fig. 3A).

As expected, clinical stage and histology of the carcinomas were significant prognostic factors. The overall mean survival of patients with stage I, II, III, and IV disease was $26.5 \pm 11.5, 17.9 \pm 12.1, 13.5 \pm 12.0, and 12.0 \pm 11.4$ months, respectively ($P = 0.009$). The mean survival of patients with \textit{p16} alterations versus those without was $26.5 \pm 11.5$ versus $29.2 \pm 12.3$ months for stage I carcinomas, $17.9 \pm 12.1$ versus $22.1 \pm 12.5$ months for stage II carcinomas, $13.8 \pm 12.3$ versus $18.3 \pm 15.4$ months for stage III carcinomas, and $12.0 \pm 11.4$ versus $17.5 \pm 13.5$ months for stage IV carcinomas, respectively ($P = 0.002$, Fig. 3B). Patients without \textit{p16} alterations had a better prognosis at each stage, particularly those with stage II–IV tumors. Thus, the mean survival of stage III and IV patients with biliary tract cancers lacking \textit{p16} alterations resembled that for stage II patients with \textit{p16} alterations.

The histology of carcinomas was also associated with prognosis and with \textit{p16} alteration status. The overall mean survival of patients with adenocarcinomas not otherwise speci-
Table 1  Prevalence of p16 alterations compared with patient demographics and carcinoma characteristics in 57 gallbladder, 27 bile duct, and 16 ampullary carcinomas

<table>
<thead>
<tr>
<th>p16 Alteration</th>
<th>Gallbladder carcinomas</th>
<th>Bile duct carcinomas</th>
<th>Ampullary carcinomas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absent (n = 17)</td>
<td>Present (n = 40)</td>
<td>Absent (n = 11)</td>
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<tr>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Age (mean ± SD yrs)</td>
<td>63.5 ± 11.5</td>
<td>65.7 ± 11.0</td>
<td>65.1 ± 8.7</td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
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<tr>
<td>Present</td>
<td>12 (70.6)</td>
<td>5 (29.4)</td>
<td>30 (75.0)</td>
</tr>
<tr>
<td>Absent</td>
<td>0</td>
<td>0</td>
<td>1 (9.1)</td>
</tr>
<tr>
<td>ChOLELITHIASIS</td>
<td>Present</td>
<td>Absent</td>
<td>Unknown</td>
</tr>
<tr>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Present</td>
<td>12 (70.6)</td>
<td>28 (70.0)</td>
<td>0</td>
</tr>
<tr>
<td>Absent</td>
<td>5 (29.4)</td>
<td>11 (27.5)</td>
<td>1 (9.1)</td>
</tr>
<tr>
<td>Histological type</td>
<td>Adenocarcinoma</td>
<td>Papillary</td>
<td>Mucinous</td>
</tr>
<tr>
<td>Present</td>
<td>9 (52.9)</td>
<td>6 (15.0)</td>
<td>1 (5.9)</td>
</tr>
<tr>
<td>Absent</td>
<td>29 (72.5)</td>
<td>0</td>
<td>3 (7.5)</td>
</tr>
<tr>
<td>Not otherwise specified</td>
<td>9 (81.8)</td>
<td>1 (9.1)</td>
<td>1 (9.1)</td>
</tr>
<tr>
<td>Papillary</td>
<td>14 (82.4)</td>
<td>0</td>
<td>1 (5.9)</td>
</tr>
<tr>
<td>Mucinous</td>
<td>1 (25.0)</td>
<td>0</td>
<td>1 (5.9)</td>
</tr>
<tr>
<td>Adenosquamous</td>
<td>3 (7.5)</td>
<td>0</td>
<td>1 (5.9)</td>
</tr>
<tr>
<td>Small cell carcinoma</td>
<td>1 (5.9)</td>
<td>0</td>
<td>1 (5.9)</td>
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<tr>
<td>Carcinosarcoma</td>
<td>0</td>
<td>1 (2.5)</td>
<td>0</td>
</tr>
<tr>
<td>Tumor-Node-Metastasis stage</td>
<td>Stage I</td>
<td>Stage II</td>
<td>Stage III</td>
</tr>
<tr>
<td>Present</td>
<td>4 (23.5)</td>
<td>3 (17.6)</td>
<td>3 (17.6)</td>
</tr>
<tr>
<td>Absent</td>
<td>22 (75.0)</td>
<td>17 (69.2)</td>
<td>17 (69.2)</td>
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<tr>
<td>Tumor stage</td>
<td>Present</td>
<td>Absent</td>
<td>Unknown</td>
</tr>
<tr>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Alive</td>
<td>7 (41.2)</td>
<td>7 (17.5)</td>
<td>0</td>
</tr>
<tr>
<td>Dead</td>
<td>10 (58.8)</td>
<td>32 (80.0)</td>
<td>0</td>
</tr>
<tr>
<td>Mean follow-up ± SD (mos)</td>
<td>21.5 ± 14.8</td>
<td>12.1 ± 11.4</td>
<td>18.0 ± 13.2</td>
</tr>
</tbody>
</table>

* Nine gallbladder, four bile duct, and one ampullary carcinomas without p16 alteration by only one method were excluded.

* Two gallbladder and one bile duct carcinomas with silent mutations without other p16 alterations were excluded.

* p = 0.001; adenosquamous carcinomas of the gallbladder versus gallbladder carcinomas of other histological type.

* p = 0.02, log-rank statistic.

Clinical studies in well-characterized population samples. We therefore evaluated alterations of the p16 gene in 118 gallbladder, extrahepatic bile duct and ampullary cancers from a population-based study. Patients with gallbladder carcinomas associated with p16 gene alterations and/or alterations of other tumor suppressor genes on chromosome 9p had a poorer survival, stage for stage, compared with those without p16 alterations.

It is possible that other genes such as p14 or p15 may contribute to the survival disadvantage associated with the pres-
ence of p16 mutations or LOH at chromosome 9p in our study. As mutations in exon 2 of p16 gene are also p14 gene mutations in a different reading frames (9–10), missense or silent mutations in exon 2 of the p16 gene were nonsense or missense mutations of the p14 gene in our study. Similarly, p14 and p15 genes are present in the same region of chromosome 9p as the p16 gene (8), and may be involved by allelic loss of chromosome 9p21–22 in our study. However, no p15 mutations were present in a previous study of biliary tract carcinomas (18), but involvement of p14 has not been evaluated. Overexpression of p14 induces G1- and G2-cell cycle arrest by a mechanism that is independent of CDKs (10). Chromosome 9p has additional tumor suppressor genes, which are deleted in a variety of other tumors (29), but their role in biliary tract cancer remains to be determined.

p16 gene mutations, methylation of CpG islands, and/or heterozygous deletions were present in 61.0% of the biliary tract carcinomas in our study. Homozygous deletion is another mechanism of p16 inactivation, but detection requires pure tumor DNA from cell lines or tumor xenografts (14). In our study, nonamplification of p16 gene was present in ten carcinomas by multiples PCR but also had LOH of chromosome 9p (data not shown). Other techniques such as real-time PCR or fluorescence in situ hybridization can be used to detect homozygous deletions in tumor tissue but require frozen tissue (30–33). Previous studies have reported p16 mutations in biliary tract carcinomas (18, 19) and LOH of chromosome 9p in gallbladder carcinomas (19–22). Most of the p16 mutations in the previous study were missense point mutations or silent mutations (18). Although these alterations predominated as well in our study, we also noted nonsense and silent mutations, along with a deletion resulting in frameshift mutation. Two or more mutations and/or alterations were present in 27 biliary tract carcinomas in our study, which probably represent inactivation of both alleles of the p16 gene.

In our study, the p16 alterations status of gallbladder carcinomas was associated with prognosis. Patients whose carcinomas lacked p16 alterations had a better mean overall survival compared with those whose carcinomas had p16 alterations. This difference in survival was associated with the stage of gallbladder cancer. In our study, the frequency of p16 alterations was similar in patients with gallbladder, bile duct, and ampullary carcinomas, but p16 alteration status was a significant prognostic factor only for patients with gallbladder carcinomas. In contrast, a recent study has shown loss or heterogeneous p16 protein expression was associated with poor survival among patients with bile duct and ampullary carcinomas (23). This difference in prognosis may be due to the geographical or racial differences in the study population and/or differences in the methodology of the two studies.

Comparison of p16 gene alterations in biliary tract cancers versus other malignancies of the hepatopancreaticobiliary system suggests site-specific differences in the mechanisms of p16 inactivation (34). Inactivation of p16 is frequent in pancreatic malignancies and occurs usually by homozygous or intragenic deletions and occasionally by promoter region hypermethylation (35–37). However, in hepatocellular carcinomas and intrahepatic cholangiocarcinomas, p16 is inactivated frequently by methylation of the promoter region rather than by homozygous deletions or gene mutations (38–41).

The majority of mutations present in our study have been described in previous compilations of p16 gene mutations (16–17). However, there are interesting differences in the p16 mutations in our study as compared with other studies. All single-base substitutions were present at C or G nucleotides, whereas 93.8% were transitions at G or C. In contrast, transitions at G or C appear to be responsible for 45% of the base substitutions in a number of malignancies, and for 57% of the base substitutions in melanomas (17). The type of mutation depends on the gene, site of tumor, and type of mutagenic exposure; for example, G to T transversions are common in the p53 gene but not in p16 in smoking-related lung carcinomas (16, 42). Distinctive mutation signatures from exposure to a specific carcinogen have been reported in the tandem base substitutions at dipyrimeridine sequences in UV-related skin cancers (42), G to T transversions at codon 249 of the p53 gene in hepatocellular carcinomas due to aflatoxins (43), and G to T transversions in p53 gene due to benzo(a)pyrene adducts in smoking-related lung carcinomas (44). C to T transitions due to deamination of 5-methylcytosine residues in methylated CpG dinucleotides can arise spontaneously or due to reactive oxygen species or nitric oxide (45–47).

In our study, the majority of point mutations were present at non-CpG dinucleotides. G/C to A/T transitions are seen after exposure to alkylating agents, primarily because of O6-methylguanine-DNA adducts (48–50). O6-Methylguanine-DNA methyltransferase protects against the mutagenic effects of alkylating agents by removing O6-methylguanine adducts from the DNA, and this activity is modulated by in vitro and in vivo levels of O6-methylguanine-DNA methyltransferase (50–53). It is possible that exposure to an alkylating agent may account for the increased G/C to A/T transitions in our study.

In conclusion, our study revealed that alterations of p16 gene and/or other tumor suppressor genes on chromosome 9p are frequent in biliary tract cancers and may cause p16 inactivation through gene mutations, methylation of the promoter region, and/or LOH of chromosome 9p. Patients with p16 alterations in their gallbladder carcinomas have a worse survival when compared with those without p16 alterations.

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REFERENCES

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