High Frequency of K-ras Mutations in Biliary Duct Carcinomas of Cases with a Long Common Channel in the Papilla of Vater

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Abstract

The frequency of K-ras mutation in biliary duct carcinomas in different locations and the relationship to the form of the junction of the pancreaticobiliary duct (JPBD) are not understood clearly. These points were investigated in the present study. Thirty-seven biliary duct carcinomas in patients without anomalous JPBD were investigated for K-ras mutations. Regarding location, 12 were hilar, 4 in the upper, 11 in the middle, and 10 in the lower portion of the duct. Furthermore, with 14 cases for which the form of the JPBD could be confirmed by endoscopic retrograde cholangiopancreatography or postoperative cholangiography, division was made into two types: those with a long common channel (>5 mm) in the papilla of Vater (type 1, n = 4), and the other with a shorter or nonapparent common channel (type 2, n = 10). The overall frequency of K-ras mutation was 30%, the incidence gradually increasing from upper to lower regions. K-ras mutations were significantly more frequent in biliary duct carcinomas associated with long common channels (P < 0.05). These results suggest that a long common channel may bear a relation to K-ras mutations in biliary duct carcinogenesis, presumably through its influence on pancreatic juice regurgitation.

Introduction

Biliary tract carcinomas are relatively rare, but their prognosis is poor. With application of molecular biology techniques, K-ras mutations have been found to be frequent in various tumors, especially in pancreatic carcinomas (1–5), even at the step of mucinous cell hyperplasia (6). With biliary tract carcinomas, in contrast, there is no definite consensus regarding K-ras mutations (3, 7–15), although recent studies demonstrated more frequent detection in gallbladder carcinomas in patients with than without an AJPBD3 (16, 17). Therefore, it seems that K-ras mutations might bear a relation with pancreatic juice. In the cases without AJPBD, however, little information is available concerning the relationship between K-ras mutation and the form of the JPBD or the tumor location.

In this study, we, therefore, concentrated attention on the JPBD and point of origin in the bile duct of a series of bile duct carcinomas.

Materials and Methods

Tissue Samples. Resected specimens of 37 biliary duct carcinomas (12 hilar, 4 upper, 11 middle, 10 lower) in patients without AJPBD were obtained from the Cancer Institute Hospital. The tissues had all been fixed in formalin and embedded in paraffin wax.

Types of Pancreatobiliary Duct Junction. In this study, the two types of JPBD were: one with a common channel (>5 mm in length) (type 1), and the other with shorter (<5 mm) or a nonapparent common channel (type 2) on endoscopic retrograde cholangiopancreatography or postoperative cholangiopancreatography (Fig. 1). Fourteen cases for which the form of the JPBD could be confirmed were analyzed, 4 of type 1 and 10 of type 2.

DNA Extraction. Carcinoma tissues were microdissected from 20-μm formalin-fixed paraffin-embedded sections as described previously (6), deparaffinized with xylene three times, cleared with ethanol twice, completely dried, and digested with proteinase K. The resultant lysates were used directly for the PCR.

PCR Amplification and Detection of K-ras Mutations. The protocol used to analyze the tissue for point mutations in codon 12 of K-ras has been described in detail elsewhere (6). DNA isolated from surgical materials was used for PCR using primers A (5′-GCTCTGCTGAAAATGACTGA-3′) and D (5′-TAGCTGTATCGTCAAGGCAC-3′). The resulting PCR products were dot-blotted onto seven different nylon membranes, and each of these separate membranes was hybridized with allele-specific oligonucleotide probe for the wild-type K-ras sequence or for one of six possible activating point mutations in codon 12. Positive controls included cloned wild-type and mutant sequences.

Statistical Analysis. Categorical variables were analyzed using the Fisher’s exact probability test. Ps of <0.05 were considered significant.

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3 The abbreviation used is: AJPBD, anomalous junction of the pancreaticobiliary duct.

Fig. 1 Examples of two types in JPBD. a, type 1. A common channel is apparent (arrowhead). b, type 2. No common channel is detectable.
**Results**

K-ras mutations were detected in 11 of 37 samples (30%; Fig. 2), at frequencies of 17% (2 of 12) in hilar, 25% (1 of 4) in upper, 27% (3 of 11) in middle, and 50% (5 of 10) in lower biliary duct lesions, respectively (Fig. 3). The incidence, thus, gradually increased from upper to the lower biliary duct.

Four point mutation types were found in this study: GGT to GAT in six cases, to GTT in three cases, and to GCT and TGT each in one case (Fig. 2). No relationship between the type of mutation and the location was evident.

The frequencies of the K-ras mutation in type 1 and type 2 cases were 75% (3 of 4) and 10% (1 of 10), respectively (Table 1; \( P < 0.05 \), Fisher’s exact test). In type 1, GGT to GAT mutations were detected in two cases, and to TGT in one case. In type 2, the single mutation was a GGT to GAT.

**Discussion**

The results of our present investigation clearly showed that K-ras mutations are more frequently detected in cases with a long common channel in the region of the papilla of Vater. Furthermore, the frequency of K-ras mutations gradually increased from the upper to lower bile duct, suggesting that the JPBD form and the location might have a relation with K-ras mutation in biliary duct carcinoma.

According to previous studies, K-ras mutations are very frequent in pancreatic carcinomas and gallbladder carcinomas associated with AJPBD (1–5, 16). Moreover, mutations were detected even in mucous cell hyperplasia of pancreas and in noncancerous epithelium of the gallbladder and common bile duct with AJPBD (6, 17, 18). In light of those findings, it might be presumed that K-ras mutation has a relation to pancreatic juice exposure. In the biliary tract system, reflux of pancreatic juice into the bile duct is influenced by the function of Oddi’s sphincter and the form of the JPBD (19). It has been reported that a longer common channel in the papilla of Vater might predispose to regurgitation of pancreatic juice into the bile duct (20). The high frequency of K-ras mutations found in biliary duct carcinomas is associated with a long common channel; the present study is, therefore, very indicative with regard to the role of pancreatic juice.

![Fig. 2. A dot blot hybridization analysis of K-ras mutations in biliary duct carcinoma. Top, left, letters (a-g) represent positive controls. Sample numbers 1–12 were hilar, 13–16 upper, 17–27 middle, and 28–37 lower portion tumors. GGT is the wild type, and the others are mutant types.](image)

![Fig. 3. Frequencies of K-ras mutation in tumors at different locations. The data for gallbladder carcinomas are from our previous report. A gradual increase in frequency is evident from the upper to lower regions. CBD, common bile duct; GB, gallbladder; Panc, pancreas; Du, duodenum.](image)

**Table 1** Relationship between K-ras mutations and JPBD type

<table>
<thead>
<tr>
<th>K-ras mutation</th>
<th>(+)</th>
<th>(-)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1</td>
<td>3 (75%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1 (25%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4</td>
</tr>
<tr>
<td>Type 2</td>
<td>1 (10%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9 (90%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10</td>
</tr>
</tbody>
</table>

<sup>a</sup> \( P < 0.05 \).
As for the site of origin in the bile duct, our data are in line with the report of Motojima et al. (3) that K-ras mutations were more frequent in lower than middle or upper biliary duct carcinomas. These results again support a role for pancreatic juice.

In the future, to confirm this hypothesis, analysis of the relationship between the concentration of amylase in bile juice and K-ras again support a role for pancreatic juice.

In conclusion, the form of the long common channel in the papilla of Vater seems linked to K-ras mutations in biliary duct carcinomas.

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