Mutations in the BRCA2 Gene in Hepatocellular Carcinomas

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Abstract

To investigate whether the BRCA2 gene plays a role in carcinogenesis of hepatocellular carcinomas or pancreatic cancers in view of frequent losses of heterozygosity on chromosome 13q12-13 in those tumors, we screened the entire coding region of this gene for mutations in 60 hepatocellular carcinomas and 36 pancreatic cancers. No alteration was found in any of the pancreatic cancers examined, but three mutations were identified in hepatocellular carcinomas; one was a 6-bp somatic deletion within intron 6. The other two mutations we identified in hepatocellular carcinomas were missense mutations in the germ line, although all BRCA2 mutations thus far detected in patients with familial breast cancers likewise have been deletions. None of 194 other patients with cancers or 44 normal controls exhibited either mutation. Combined with our demonstration of BRCA2 expression in adult liver tissue, the evidence implies that inactivation of BRCA2 may play some role in development or progression of hepatocellular carcinoma and might predispose carriers of mutant alleles to liver malignancies.

Introduction

Tumor suppressor genes play important roles in regulation of cell growth and differentiation. Characterization of various human cancers on the basis of molecular genetics has disclosed involvement of altered tumor suppressor genes in the genesis and progression of carcinoma (1). We and others previously reported loss of heterozygosity analyses of HCCs (2) and PCs (3) that disclosed frequent allelic losses involving chromosome 13q. By means of deletion mapping, we identified two regions of this chromosomal arm that were commonly deleted in HCCs, one that encompassed the RB1 locus at q14 and the other proximal to it (2). A report by Zhang et al. (4) that somatic mutation of the RB gene in HCCs was infrequent (although nearly half of the cells in a given tumor cells lacked RB protein) implied the involvement of a tumor suppressor gene other than RB in these tumors. Recently, the BRCA2 gene, the mutant alleles of which, in a germ line, can predispose carriers to breast cancer, was isolated from a region proximal to the RB locus on chromosome 13, i.e., q12-13 (5). Furthermore, homozygous deletion of the BRCA2 region in a PC cell line has been reported (6). Hence, we assumed that the BRCA2 gene would be a candidate for involvement in the carcinogenesis of HCC and PC.

Genomic DNAs of HCCs. Genomic DNAs from HCCs and corresponding noncancerous liver tissues were prepared from 60 patients by the methods described previously (7).

Mutation Screening. The entire coding sequence of BRCA2, and associated exon-intron boundary sequences, were examined by PCR-SSCP analysis and PCR-multiplex SSCP analysis. The primers used for SSCP analyses and PCR conditions has been described (8). When variant bands were detected in SSCP analyses, PCR products of the tumor DNAs and the corresponding normal DNAs were subcloned into pT7-Blue T (Novagen). Their nucleotide sequences were determined for any variant bands.

RT-PCR Analysis. Polyadenylated RNAs derived from human adult liver and mammary gland (Clontech) were reverse transcribed using random primers and Superscript II reverse transcriptase (Life Technologies, Inc.) to prepare first-strand cDNA. Aliquots of each cDNA were amplified using primers 3RTF (5'-AACCGAAACACTTATTT AAAACT-3') in exon 3 and 8RTF (5'-AGCAGTAGATTCATAGAAAT-3') in exon 8, respectively. The expected size of each PCR product was 512 bp. The PCR was performed under the same conditions as the PCR-SSCP analysis. Part of the β-actin cDNA was amplified as a control for the RT-PCR experiments.

Results and Discussion

We first screened for mutations in the entire coding region of BRCA2, as well as exon-intron boundaries, in DNAs isolated from 60 HCCs and 36 PCs using a PCR-SSCP and a PCR-multiplex SSCP analysis technique. DNA sequences were determined for any PCR products that showed variant patterns by SSCP. Mutations were found in three of the HCCs (Table 1). Two of these alterations were also present in corresponding normal tissues of the respective patients: patient 40 carried a germ-line missense mutation, a G-to-T transition at the third nucleotide of codon 2729, which had substituted asparagine for lysine (Fig. 1a). DNA from patient 244 contained a missense mutation, an A-to-C transversion at the first nucleotide of codon 322, which altered lysine to glutamine (Fig. 1b); as in patient 40, a charged amino acid had been replaced with a noncharged amino acid. Because neither of these alterations was present in the other 58 HCCs or in the 36 PCs, 100 breast cancers (8), or 44 normal control subjects examined (Table 1), they may represent new disease-associated mutations. However, the possibility that they may be rare polymorphisms still remained. We found no family history in patient 244, with infection of hepatitis C virus, who had HCC at an age of 71; family background for patient 40 was unclear because of limited medical information. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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The abbreviations used are: HCC, hepatocellular carcinoma; PC, pancreatic cancer; SSCP, single-strand conformation polymorphism; RT, reverse transcription; ER, estrogen receptor; RB, retinoblastoma.

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Table 1 Mutations in the BRCA2 gene in HCCs

<table>
<thead>
<tr>
<th>Patient</th>
<th>Exon</th>
<th>Nucleotide change</th>
<th>Effect of mutation</th>
<th>HCC</th>
<th>PC</th>
<th>BC&lt;sup&gt;a&lt;/sup&gt;</th>
<th>NC</th>
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<tbody>
<tr>
<td>Germ line</td>
<td></td>
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</tr>
<tr>
<td>40</td>
<td>18</td>
<td>AAG→AAT</td>
<td>Lys 2729 Asn</td>
<td>1/60</td>
<td>0/36</td>
<td>0/100</td>
<td>0/44</td>
</tr>
<tr>
<td>244</td>
<td>10</td>
<td>AAA→CAA</td>
<td>Lys 322 Gln</td>
<td>1/60</td>
<td>0/36</td>
<td>0/100</td>
<td>0/44</td>
</tr>
<tr>
<td>Somatic</td>
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<tr>
<td>194</td>
<td>Intron 6</td>
<td>TIGAGAATT→ATT</td>
<td>(6-bp deletion)</td>
<td>NE</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

<sup>a</sup> BC, breast cancer; NC, normal control population; NE, not examined.

Fig. 1. Sequence analysis of PCR products of tumor DNAs and corresponding normal DNAs that showed variants by SSCP. a, in patient 40, missense mutation of AAG→AAT at codon 2729 in tumor (Tumor) DNA and corresponding constitutional (Cont.) DNA. b, in patient 244, a missense mutation of AAA→CAA at codon 322 in tumor (Tumor) DNA and corresponding constitutional (Cont.) DNA. Wild type, normal control sequences. c, in patient 194, a 6-bp deletion in the intronic sequence 39 bp downstream of the last nucleotide of exon 6 in tumor (Tumor) DNA.
cDNA fragment spanning exons 3—8 was amplified. As a control, -actin PCR primers (forward primer, 5'-CTGCTGGCCGCCCACGTG-3'; reverse primer, 5'-CTATTT AAAACATCAACACGTTGCAA-3') were used to detect -actin mRNA on the same tissue. M, 1-kb ladder; Lane 1, normal liver (BRCA2); Lane 2, mammary gland (BRCA2); Lane 3, normal liver (actin); Lane 4, mammary gland (actin); Lane 5, negative control.

Fig. 2. Expression of BRCA2 in human adult liver by RT-PCR analysis. A BRCA2 cDNA fragment spanning exons 3—8 was amplified. As a control, -actin PCR primers (forward primer, 5'-CTGCTGGCCGCCCACGTG-3'; reverse primer, 5'-CTATTT AAAACATCAACACGTTGCAA-3') were used to detect -actin mRNA on the same tissue. M, 1-kb ladder; Lane 1, normal liver (BRCA2); Lane 2, mammary gland (BRCA2); Lane 3, normal liver (actin); Lane 4, mammary gland (actin); Lane 5, negative control.

intrinsic mutations causing aberrant splicing have been reported, and it is entirely possible that the 6-bp deletion in tumor 194 may interfere with normal splicing of BRCA2. Moreover, in patients 40 and 194, significant reduction of signal from the normal nucleotide in the sequence analysis indicated that the DNA of these tumors was likely to have undergone two-hit mutation of the BRCA2 gene.

To investigate expression of BRCA2 in human liver, we performed a RT-PCR experiment using a fragment of BRCA2 spanning exons 3—8. Because a product of the expected size (512 bp) was observed, we assume that liver expresses the BRCA2 gene, although the level of expression in liver is much lower than in the mammary gland (Fig. 2).

This report demonstrates the first evidence that BRCA2 may play some role in malignancies other than breast cancer. Whether the novel germ-line mutations found in two patients with liver tumors had caused predisposition to HCC is still unclear, however. All the germ-line mutations found in primary breast cancer families have been deletions and nonsense mutations (5, 9—13), leading to truncation and inactivation of the gene product, but both of the germ-line alterations of BRCA2 found in our HCC patients were missense mutations. This type of change might affect the activity of the gene product; alternatively, BRCA2 proteins derived from the missense alleles might function in a dominant-negative fashion in liver cells.

Our data showing that BRCA2 is expressed in normal adult liver (Fig. 2) indicate that this gene is likely to play some role in liver cells. Villa et al. (14) reported that part of the hormone-binding domain of the ER gene was mutated in HCCs that had developed in male patients; it also has been reported that the concentration of ER in serum is elevated during progression of HCC in males (15, 16). In view of these facts, we suggest that mutational events in estrogen, ER, and BRCA2 genes may be involved in hepatocarcinogenesis, and that BRCA2 may function as a tumor suppressor in a hormone-related pathway in the liver as well as in mammary tissue.

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References
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