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Advances in Brief

Mutations in the *BRCA2* Gene in Hepatocellular Carcinomas[1]

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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[3] (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.

Materials and Methods

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 by dideoxy chain termination with T7 DNA polymerase, using gene-specific primers to identify the nature of the mutation. All results were confirmed by two independent experiments.

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′-ACGAACCAAACCTATTT AAAACT-3′) in exon 3 and 8RTR (5′-AGCAGTAGTATCATGAGGAAAT-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. 1*a*). 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. 1*b*); 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, and this patient was without infections of hepatitis B and C virus. In patient 194, a 6-bp deletion was detected in the intronic sequence 39 bp downstream of the last nucleotide of exon 6 (Fig. 1*c*). Because this mutation was not present in the constitutional DNA of the patient, the alteration was considered to have occurred as a somatic event. Family background for patient 194 was also unclear because of limited medical information. No mRNA from this tumor was available, so we were unable to learn whether the deletion affected splicing of the transcript. However, many examples of

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

Table 1 *Mutations in the BRCA2 gene in HCCs*

Patient	Exon	Nucleotide change	Effect of mutation	Frequency in the screened samples			
				HCC	PC	BC[a]	NC
Germ line							
40	18	AAG→AAT	Lys 2729 Asn	1/60	0/36	0/100	0/44
244	10	AAA→CAA	Lys 322 Gln	1/60	0/36	0/100	0/44
Somatic							
194	Intron 6	TTGAGAATTT→ATTT (6-bp deletion)	NE				

[a] BC, breast cancer; NC, normal control population; NE, not examined.

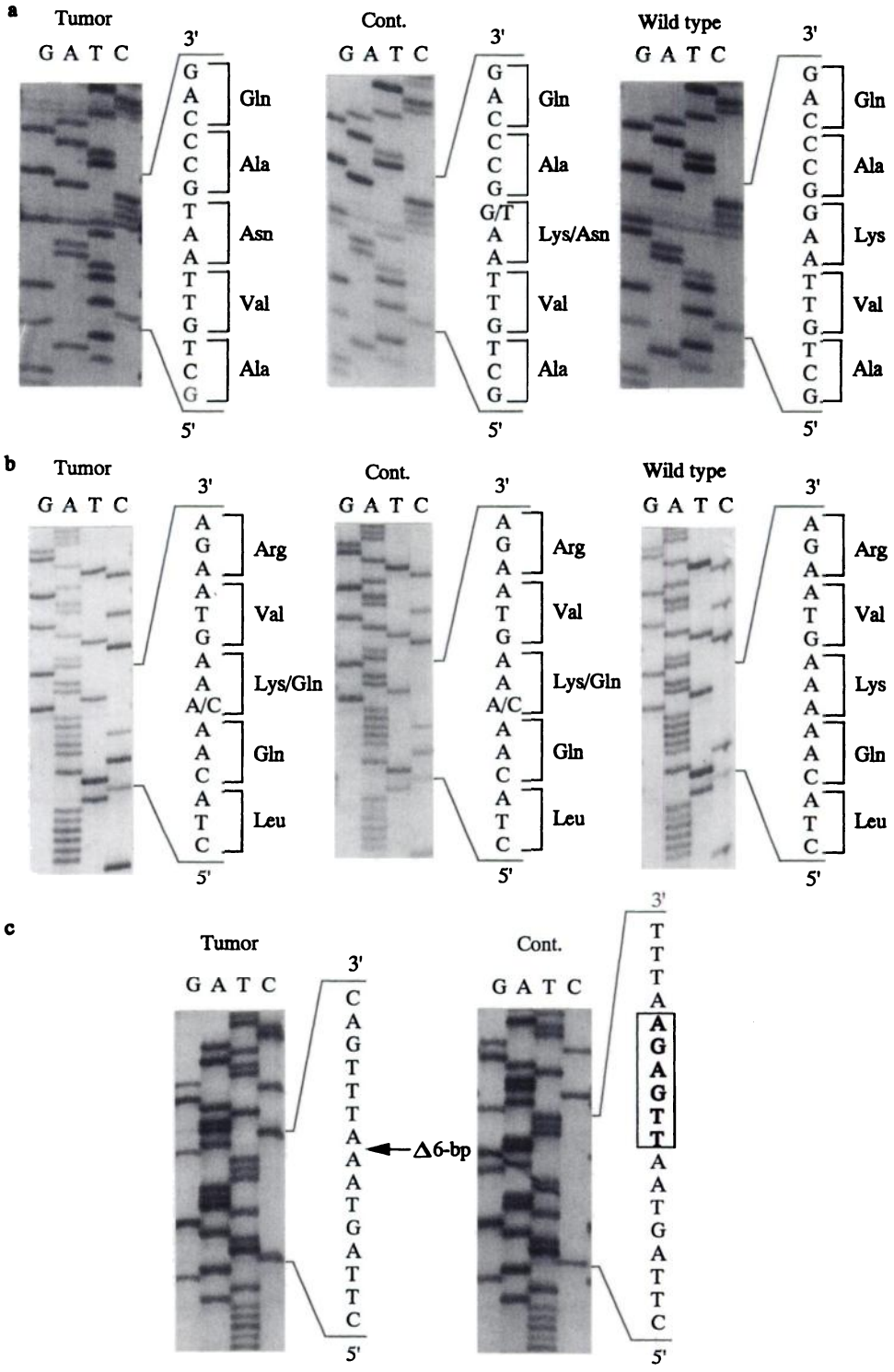

Fig. 1. Sequence analysis of PCR products of tumor DNAs and corresponding normal DNAs that showed variants by SSCP. *a*, in patient 40, missense mutation of AAG→AAT at codon 2729 in tumor (*Tumor*) DNA and corresponding constitutional (*Cont.*) DNA. *b*, in patient 244, a missense mutation of AAA→CAA 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.

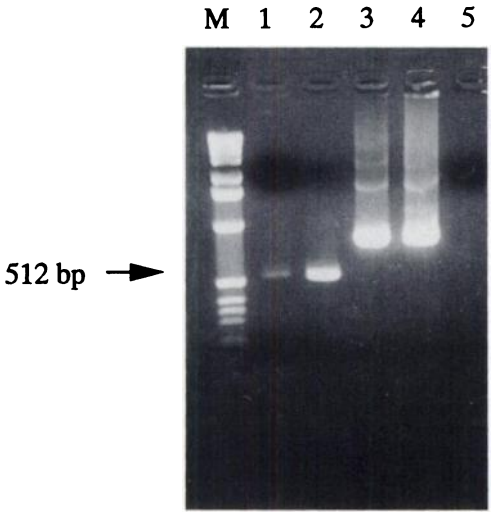

Fig. 2. Expression of *BRCA2* in human adult liver by RT-PCR analysis. A *BRCA2* cDNA fragment spanning exons 3–8 was amplified. As a control, β-actin PCR primers (forward primer, 5′-CTGGCTGGCCGGGACTG-3′; reverse primer, 5′-CTATTA-AAAAAACAACAATGTGCAAT-3′) were used to detect β-actin mRNA on the same 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.

intronic 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 in the *BRCA2* gene thus far found in 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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