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• BRIEF REPORTS •

Loss of heterozygosity on chromosome 10q22-10q23 and 22q11.2-22q12.1 and *p53* gene in primary hepatocellular carcinoma

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Abstract

AIM: To analyze loss of heterozygosity (LOH) and homozygous deletion on *p53* gene (exon2-3, 4 and 11), chromosome 10q22-10q23 and 22q11.2 -22q12.1 in human hepatocellular carcinoma (HCC).

METHODS: PCR and PCR-based microsatellite polymorphism analysis techniques were used.

RESULTS: LOH was observed at D10S579 (10q22-10q23) in 4 of 20 tumors (20%), at D22S421 (22q11.2-22q12.1) in 3 of 20(15%), at TP53.A (*p53* gene exon 2-3) in 4 of 20 (20%), at TP53.B (*p53* gene exon 4) in 6 of 20(30%), and at TP53.G *(p53* gene exon 11) in 0 of 20(0%). Homozygous deletion was detected at 10q22-10q23(8/20; 40%), 22q11.2-22q12.1(8/20; 40%), p53 gene exon 2-3(0/20;0%), *p53* gene exon 4(6/20; 30%), and *p53* gene exon 11(2/20; 10%).

CONCLUSION: There might be unidentified tumor suppressor genes on chromosome 10q22-10q23 and 22q11. 2-22q12.1 that contribute to the pathogenesis and development of HCC.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a primary liver malignancy with high mortality. It is among the most common malignancies worldwide, especially in Asia, Africa and Southern Europe[1]. It has been generally accepted that HCC is highly associated with chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection or alcohol intake which induces cirrhosis[2]. High intake of aflatoxin B found in many kinds of food is also reported to be a risk factor for HCC[3,4]. Like other solid tumors, It has been proposed that hepatocarcinogenesis and metastasis of HCC is a multi-step process requiring the accumulation of genetic alterations, but the precise molecular pathogenesis is far from clear.

Loss of heterozygosity (LOH) analysis has become an effective way to identify informative loci and candidate tumor suppressor genes (TSGs). Molecular chromosomal studies of tumors by using polymerase chain reaction (PCR) -based polymorphic markers can detect small loci of anomalies that may harbor TSGs. Search for novel TSGs is based largely on the identification of common regions of deletion on chromosomes. LOH has been found in many types of tumors, including HCC. LOH in HCC has been detected on chromosomal arms 1p, 2q, 4p, 4q, 5q, 6q, 8p, 8q, 9p, 9q, 11p, 13q, 16p, 16q and 17p[5-11]. However, deletion of 10q22-10q23, and 22q11.2-22q12.1 and p53 gene exon 2-3 and 11 in HCC has not been investigated.

In the present study, we detected LOH and homozygous deletion on chromosome 10q, and chromosome 22q near the NF2 gene locus, and *p53* gene locus in 20 cases of HCC.

MATERIALS AND METHODS

Specimens

Surgical specimens of HCC were collected from the First Affiliated Hospital of Anhui Medical University and the Affiliated Hospital of Bengbu Medical College. The patients were born and grew in different places of Anhui Province, China. Both tumor and corresponding non-tumor liver tissues were immediately put into liquid nitrogen after separation and then stored at -80 ℃ until DNA extraction. Diagnosis of HCC was confirmed by pathological examination.

DNA extraction

Genomic DNA was extracted from tissues with the standard proteinase K-phenol/choloroform method. To each of the samples, 500 μL of DNA extraction buffer containing 200 mmol/L NaCl, 10 g/L sodium dodecyl sulfate, 2 mmol/L EDTA, 0.1 mol/L Tris-HCl was added during the process of homogenization. After 0.2 mg/mL proteinase K was added, the sample was shaken for 12 h at 37 ℃. After phenol-chloroform extraction, DNA was precipitated with cold ethanol overnight at -20 ℃. After centrifugation, the pellet was dried and resuspended in 50 μL TE buffer (Tris-EDTA buffer). DNA was stored at -20 ℃ until polymerase chain reaction (PCR) amplification was performed.

Pcr amplification

PCR amplification primer pairs for p53 gene, 10q22-10q23 and 22q11.2-22q12.1 are as follows (Table 1).

Polyacrylamide gel electrophoresis

PCR product (12 μL) was mixed with 3 μL 950 g/L deionized formamide and 3 μL DNA loading buffer containing 2.5 g/L

Table 1 PCR amplification primer pairs for p53 gene, 10q22-10q23 and 22q11.2-22q12.1

Markers name	Forward	Reverse	Annealing (T℃)	Size (bp)
TP53.A1/TP53.A2 (p53 gene exon 2-3)	TGGATCCTCTTGCAGCAGCC	AACCCTTGTCCTTACCAGAA	54	270
TP53.B1/TP53.B2 (p53 gene exon 4)	ATCTACAGTCCCCCTTGCCGGC	AACTGACCGTGCAAGTCA	57	296
TP53.G1/TP53.G2 (p53 gene exon 11)	TCTCCTACAGCCACCTGAAG	CTGACGCACACCTATTGCAA	58	122
D10S579	CCGATCAATGAGGAGTGCC	ATACACCCAGCCAATGCTGC	60	260
D22S421	CTGCTGCCCCTAACATATCAC	GGCCAGGAGTGTCTGAATTTTA	65	163
CDK4[1]	GGAGGTCGGTACCAGAGTG	CATGTAGACCAGGACAGG	60	364

[1]The aim of PCR amplification of CDK4 gene was to confirm that genomic DNA had been truly extracted from all samples. These primer sequences were retrieved from the Genome Database (http://gdbwww.gdb.org). PCR amplification was performed in a 50 μL reaction volume containing 400 ng template DNA, 0.2 mmol/L of each deoxynucleotide triphosphate, 20 mmol/L of each primer, 1.5 mmol/L $MgCl_2$, 1× reaction buffer and 2 U Taq DNA polymerase. The reaction mixture was denatured for 5 min at 94℃. DNA was subsequently amplified for 35 cycles with 94℃ for 30 s, 54-65℃ for 30 s, 72℃ for 40 s, and a final extension at 72℃ for 8 min. PCR product (8 μL) was electrophoresed in a 20 g/L agarose gel, visualized by staining with ethidium bromide and ultraviolet illumination, and documented by a computer-linked camera. The target DNA fragments were confirmed by comparing to a 100 bp DNA ladder.

Table 2 Clinical and genetic features of 20 patients with HCC (*, LOH; △, homozygous deletion; O, no deletion; +, HBsAg positive; -, HBsAg negative)

Case No.	Age (yr)	Sex	HBsAg	TP53.A	TP53.B	TP53.G	D10S579	D22S421
1	49	M	+	*	*	O	*	O
2	55	M	+	O	△	O	O	△
3	39	M	+	O	△	O	△	△
4	55	F	+	*	O	O	O	*
5	72	F	+	O	*	△	△	O
6	40	M	+	O	O	O	O	O
7	34	F	+	O	*	O	△	△
8	56	M	+	O	O	O	O	O
9	27	F	_	O	*	O	△	O
10	50	M	+	O	O	O	O	△
11	48	M	+	O	△	O	△	△
12	52	M	+	O	O	O	△	O
13	63	F	+	O	△	△	△	△
14	60	M	+	O	△	O	*	△
15	65	M	+	O	O	O	*	O
16	34	M	+	O	*	O	O	O
17	64	M	+	*	*	O	*	*
18	52	F	+	O	O	O	△	O
19	32	F	+	O	△	O	O	△
20	38	M	+	*	O	O	O	*
LOH rate (%)				20	30	0	20	15
Homozygous deletion rate (%)				0	30	10	40	40

xylene cyanol FF, 2.5 g/L bromophenol blue, and 300 g/L glycerin. The mixture was denatured at 95 ℃ for 5 min, put onto ice for 10 min, loaded onto 80 g/L denaturing polyacrylamide gel containing 3.3 mol/L urea and then electrophoresed at 100 V for 2 h. The gel was silver-stained[13]. LOH was determined by visual evaluation, which compared the allele bands from tumors and the corresponding non-tumor tissues. The complete loss of one polymorphic allele from those seen in the paired control DNA was scored as allelic loss by three independent observers. PCR reactions were performed twice to confirm LOH.

RESULTS

HCC tumor and corresponding non-tumor liver tissues of 20 patients were studied for LOH on 10q22-10q23 (D10S579), 22q11.2-22q12.1 (D22S421), and 17p13.1 by five microsatellite markers, and the rate of LOH was 20%(4/20), 15%(3/20), 50%(10/20), respectively (Table 2). Homozygous deletion was observed in 8 of 20 cases (40%) for the marker D10S579, 8 of 20 cases (40%) for D22S421, 6 of 20 cases (30%) for TP53.B, 2 of 20 cases (10%) for TP53.G, and in 0 of 20 cases (0%) for the marker TP53.A.

Results of 20 g/L agarose gel electrophoresis are shown in Figure 1. LOH in tumor and corresponding non-tumor liver tissues are shown in Figure 2.

DISCUSSION

HCC is one of most malignant tumors. The mechanism of hepato-carcinogenesis is a multi-factor and multi-step process requiring the accumulation of genetic alterations, including chromosomal aberration, oncogene activation, inactivation of TSGs and abnormality of growth factors and growth factor

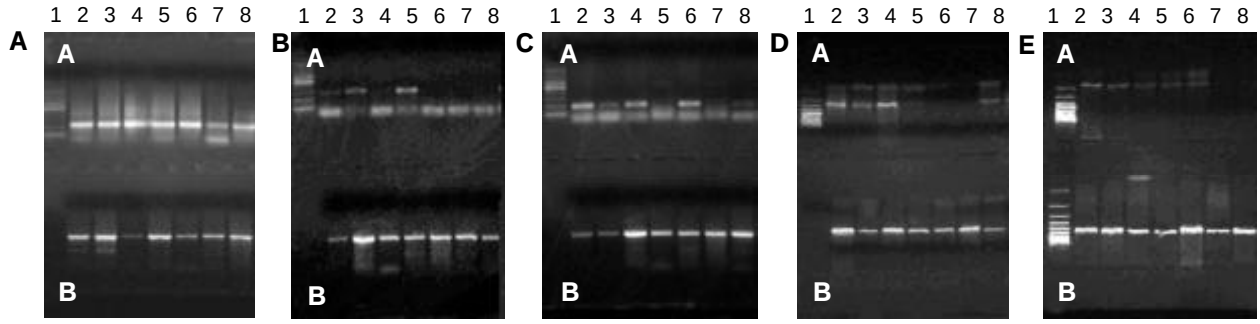

Figure 1 Agarose gel electrophoresis of PCR products of p53 gene exons 2-3, 4, 11, and chromosome 10q22-10q23 and 22q11.2-22q12.1. A: PCR products of p53 exon 2-3. Lane 1, 100 bp DNA marker; Lanes 2-8, PCR products of p53 exon2-3 (A) and CDK4 gene (B, as a control) amplified from HCC genomic DNA. No homozygous deletion of p53 exon 2-3 was found in all HCC specimens. B: PCR products of p53 exon 4. Lane 1, 100 bp DNA marker; Lanes 2-8, PCR products of p53 exon4 (A) and CDK4 gene (B, as a control) amplified from HCC genomic DNA. Lines 4, 6-8, homozygous deletion. C: PCR products of p53 exon11. Line 1, 100 bp DNA ladder; Lanes 2-8, PCR products of p53 exon11 (A) and CDK4 gene (B, as a control) amplified from HCC genomic DNA. Lanes 5 and 7, homozygous deletion. D: PCR products of 10q22-10q23. Lane1, 100 bp DNA marker; Lanes 2-8, PCR products of 10q22-10q23 (A) and CDK4 gene (B, as a control) amplified from HCC genomic DNA. Lines 6 and 7, homozygous deletion. E: PCR products of 22q11.2-22q12.1. Lane 1, 100 bp DNA ladder; Lanes 2-8, PCR products of 22q11.2-22q12.1 (A) and CDK4 gene (B, as a control) amplified from HCC genomic DNA. Lanes 7 and 8, homozygous deletion.

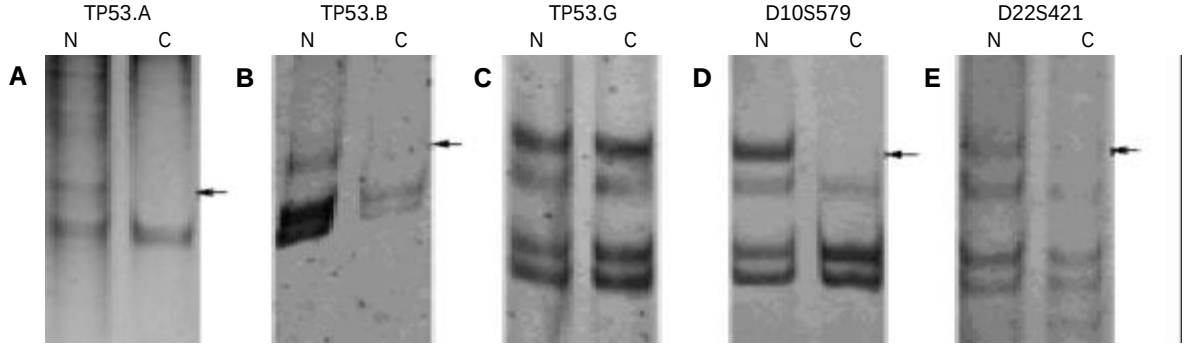

Figure 2 Representative illustrations of LOH detected with the microsatellite markers TP53.A, TP53.B, TP53.G, D10S579, D22S421 in human hepatocellular carcinomas (C) as compared to non-tumor liver tissues (N). The arrows show the location of the missing alleles. A: TP53.A (case 4); B: TP53.B (case 17); C: TP53.G (case 19,no LOH); D: D10S579 (case 17); E: D22S421 (case 20).

receptors. Of these factors, inactivation of TSGs is a very important factor.

Allelic loss on chromosome 17p is among the most common genetic abnormalities in many human cancers. *p53* gene is thought to be the gene associated with the genesis of these cancer types, including HCC[12]. *p53* is activated in response to DNA damage, inducing either cell cycle arrest to permit DNA repair or apoptosis. Loss of p53 function occurs mainly through allelic deletions at chromosome 17p13, where *p53* gene is located. In human HCC, LOH at chromosome 17p13 has been reported in 25-60% of tumors, and the worldwide prevalence of p53 mutation is around 28% with, however, important geographic variations. In this study, LOH was observed at exon 2 and 3 (TP53.A) and exon 4 (TP53.B), of the gene in 20% and 30% of HCC cases, respectively, but not detected at exon 11 (TP53.G). In addition, all but one (19/20) patients were positive with HBsAg. These data also support the idea that LOH at *p53* gene and HBV infection are highly associated with the pathogenesis and development of HCC.

LOH on D10S579 has been reported in renal cell carcinoma (RCC)[13]. We investigated 20 HCCs in the present study, and found four cases had LOH and eight cases had homozygous deletion on 10q22-10q23(D10S579). Our finding suggests that on 10q22-10q23, there might be unidentified TSG(s) that plays an important role in the pathogenesis of hepatocellular carcinoma.

22q11.2-22q12.1(D22S421) is near the locus of *NF2* gene. *NF2* (neurofibromatosis 2) gene, which is located on chromosome 22q12.2-22q12.2, is postulated to be a tumor suppressor gene. It encodes for a protein with 595 amino acids, designated as merlin or schwannomin which belongs to a family of cytoskeletal proteins. The majority of *NF2* gene mutations are deletions, insertions, and point mutations, all of which lead to a nonfunctional, truncated protein[14].

LOH at the *NF2* locus has been observed in many tumors, including schwannoma[15], meningioma[16], malignant mesothelioma[17], gastrointestinal stromal tumor[18], colorectal carcinoma[19]. However, Handel-Fernandez *et al*.[20] found that there was no LOH at *NF2* gene in pancreatic adenocarcinoma, but 37% of the cases had deletions which were clustered into two separate areas of chromosome 22 - one proximal and one distal to *NF2* gene. In the present study, we detected LOH on 22q11.2-22q12.1 in three of 20 HCCs and homozygous deletion on 22q11.2-22q12.1 in eight of 20 HCCs. Our finding suggests that 22q11.2-22q12.1 likely contains an unidentified tumor suppressor gene that contributes to the pathogenesis and the development of HCC, that the region plays an important role of cis-acting element similar to *NF2* gene, or that it acts the part of trans-acting factor similar to other TSGs, such as *p53* gene.

In conclusion, we have obtained important new information on LOH and homozygous deletion in chromosome 10q, 22q and 17p, in a subset of HCC. Inactivation of p53 gene and unidentified tumor suppressor gene(s), present in regions of 10q22-10q23 and 22q11.2-22q12.1, may play an important role in the pathogenesis of HCC.

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