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

Guang-Neng Zhu, Li Zuo, Qing Zhou, Su-Mei Zhang, Hua-Qing Zhu, Shu-Yu Gui, Yuan Wang

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).

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.C (p53 gene exon 11) in 1 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(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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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 °C 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/chloroform 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 °C. After phenol-chloroform extraction, DNA was precipitated with cold ethanol overnight at -20 °C. After centrifugation, the pellet was dried and resuspended in 50 µL TE buffer (Tris-EDTA buffer). DNA was stored at -20 °C 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 25 g/L
xylene cyanol FF, 2.5 g/L bromophenol blue, and 300 g/L glycerin. The mixture was denatured at 95 °C 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. 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 and 22q11.2-22q12.1 by five microsatellite markers, and the rate of LOH was 20%(4/20), 15%(3/20), 50%(8/20), 10%(2/20), respectively (Table 2). Homozygous deletion was observed in 8 of 20 cases (40%) for the marker D10S579, 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 the 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...
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).

in human hepatocellular carcinomas (C) as compared to non-tumor liver tissues (N). The arrows show the location of the missing products of 22q11.2-22q12.1. Lane 1, 100 bp DNA marker; Lanes 2-8, PCR products of 22q11.2-22q12.1 (A) and CDK4 gene (B, as a control) amplified from HCC genomic DNA. Lines 6 and 7, homozygous deletion. D: PCR products of 10q22-10q23. Lane 1, 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 4, 6-8, homozygous deletion. E: PCR products of p53 exon 11. Line 1, 100 bp DNA marker; Lanes 2-8, PCR products of p53 exon 11 (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. Lane 1, 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 marker; 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 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. Lanes 5 and 7, homozygous deletion. D: PCR products of 10q22-10q23. Lane 1, 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. Lanes 6 and 7, homozygous deletion. E: PCR products of 22q11.2-22q12.1. Lane 1, 100 bp DNA marker; 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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