Hepatitis B Virus Integration Event in Human Chromosome 17p near the p53 Gene Identifies the Region of the Chromosome Commonly Deleted in Virus-positive Hepatocellular Carcinomas

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ABSTRACT

The development of hepatocellular carcinoma (HCC) presumably occurs in multiple steps and is influenced by numerous factors. Hepatitis B virus (HBV) is strongly associated with the development of HCC in people chronically infected with the virus, but the mechanism of viral involvement remains unclear. One possibility is that the gross chromosomal alterations frequently observed in HCC DNA at the site of HBV integration may alter the expression of important nearby cellular genes. We previously reported the cloning and characterization of a HBV insert from a Chinese HCC. The viral insert mapped to chromosome 17p11.2-12, and cellular sequences were duplicated at the site of viral integration. In the present study a DNA probe derived from cellular DNA sequences adjacent to the previously characterized HBV insert was used to analyze a set of 19 matched normal liver and HBV-positive hepatoma samples obtained from the same region of China, near Shanghai. Tumor-specific DNA changes were detected in two additional HCCs, suggesting that the small region of chromosome 17p defined by the flanking cell DNA probe is commonly altered in hepatomas. Restriction fragment length polymorphism studies demonstrated that the loss of one copy of portions of chromosome 17 occurred in 10 (53%) of the 19 patients. The loss of one allele of the p53 gene (located on chromosome 17p13) occurred in at least 6 (60%) of the 10 patients who were heterozygous at the p53 locus. As the p53 gene is known to possess tumor suppressor activity, the functional loss of this gene may be a significant step in the development of a subset of HCCs. High levels of allele loss also were detected for chromosomes 8q (4 of 9; 44%) and 16p (5 of 6; 83%) and may indicate the presence of additional cellular genes whose functional loss is important in the development of HCCs.

INTRODUCTION

HCC is one of the most common human cancers in Africa and Southeast Asia (1-5). Although a strong correlation exists between chronic infection with HBV and the development of HCC (4, 6, 7), the actual mechanism by which HBV might contribute to tumor formation remains unknown. HBV sequences are found integrated in the HMW DNA of most HCCs obtained from geographic areas with high endemic HBV chronic infection rates (3, 8). Analyses of integrated HBV inserts cloned from HCCs have revealed that gross chromosomal abnormalities (including deletions, duplications, and translocations) are common at the site of viral integration (9-17). The functional effects of such cellular DNA changes clearly will depend on the identity and extent of disruption of nearby genes. Although the activation of cellular oncogenes does not appear to be a common feature of HBV-associated HCC (3, 5), the possibility exists that recessive tumor suppressor genes may play important roles in the development of liver cancer.

Tumor-specific chromosome losses have been detected in many human tumors by cytogenetics and RFLP studies (18-24). The loss of a specific chromosomal fragment in a significant number of tumors has been thought to result from a selection process that favored cells that had lost a gene(s) whose expression was growth inhibitory. RFLP studies on human liver tumors have identified regions of specific allele loss on chromosomes 11p (25), 13q (25), 4 (26,27), and 16q (27); these observations are consistent with the idea that the loss of genes on one or more of these chromosomes may be an important step in the development of some HCCs.

We previously described the cloning and characterization of a HBV insert from a Chinese HCC. The viral insert mapped to chromosome 17p11.2-12, and cellular sequences were duplicated at the site of viral integration (14). In the present study we used a DNA probe derived from the cellular DNA adjacent to the previously characterized HBV insert to analyze a set of 19 matched normal liver and HBV-positive hepatoma tissue samples obtained from the same region of China. Tumor-specific DNA changes were detected in two additional HCCs, suggesting that the small region of chromosome 17p defined by the flanking cell DNA probe is frequently altered in hepatomas. RFLP studies demonstrated that the loss of one copy of portions of chromosome 17 occurred often in our sample population, including the loss of one allele of the p53 gene. As the p53 gene is known to possess tumor suppressor activity (28-30), the functional loss of this gene may be a significant step in the development of a subset of HBV-positive HCCs.

MATERIALS AND METHODS

Tissue Samples and Preparation of Cellular DNA. Hepatoma tissue and adjacent nontumorous liver tissue were obtained by surgical resection from patients in the Qidong Liver Cancer Institute located near Shanghai, People's Republic of China. Surgical specimens were placed immediately into liquid nitrogen and were stored at -80° until transported frozen to the United States. HMW DNA was purified from tissues as described (8). Characteristics of the 19 members of the sample population are summarized in Table 1. Patients ranged in age from 26 to 64 years, the majority were male, and all resected tumors contained integrated HBV sequences.

Probe DNA. Plasmids containing human DNA sequences able to detect RFLPs were kindly provided by David H. Ledbetter (Institute for Molecular Genetics, Baylor College of Medicine) or were obtained from the American Type Culture Collection. Specific information regarding probe designation, chromosome localization, and restriction enzyme used is listed in Table 2. The 3.2-kilobase HBV probe was generated from a cloned head-to-tail dimer of HBV (subtype adw)
Table 1 Characteristics of Chinese patients from whom normal liver and hepatocellular carcinoma tissue samples were obtained

<table>
<thead>
<tr>
<th>Sample*</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Serum HBsAg</th>
<th>No. of integrated HBV inserts in tumor^†</th>
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<tbody>
<tr>
<td>88-1</td>
<td>M</td>
<td>36</td>
<td>+</td>
<td>5</td>
</tr>
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<td>88-2</td>
<td>F</td>
<td>31</td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td>88-4</td>
<td>M</td>
<td>56</td>
<td>NA^*</td>
<td>6</td>
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<td>M</td>
<td>36</td>
<td>NA</td>
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<td>26</td>
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<td>64</td>
<td>NA</td>
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<td>88-14</td>
<td>M</td>
<td>48</td>
<td>NA</td>
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<td>88-15</td>
<td>M</td>
<td>45</td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td>88-16</td>
<td>M</td>
<td>33</td>
<td>+</td>
<td>2</td>
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<td>88-17</td>
<td>F</td>
<td>50</td>
<td>NA</td>
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<td>1</td>
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<td>2</td>
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<td>F</td>
<td>37</td>
<td>+</td>
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<tr>
<td>87-27</td>
<td>M</td>
<td>52</td>
<td>+</td>
<td>2</td>
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<td>87-28</td>
<td>F</td>
<td>40</td>
<td>+</td>
<td>2</td>
</tr>
</tbody>
</table>

* Each sample was assigned a two-part number. The first two digits indicate the year in which it was collected; this is followed by a patient number.

† Hybridization analyses performed on EcoRI-digested genomic DNA using the 3.2-kilobase genomic-length HBV probe.

Table 2 DNA probes for detection of specific allele loss in HBV-associated human HCC

Table 2 DNA probes for detection of specific allele loss in HBV-associated human HCC

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Gene or probe</th>
<th>Restriction enzyme</th>
<th>No. of alleles (size in kilobases)</th>
<th>Ref.</th>
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<tbody>
<tr>
<td>3</td>
<td>EFD64.1</td>
<td>TaqI</td>
<td>3 (1.7-1.9)</td>
<td>Nakamura et al. (65)</td>
</tr>
<tr>
<td>3</td>
<td>EFD64.2</td>
<td>MspI, EcoRI</td>
<td>&gt;2 (2.6-4.5)</td>
<td>Nakamura et al. (66)</td>
</tr>
<tr>
<td>3</td>
<td>YNM2</td>
<td>MspI, EcoRI</td>
<td>&gt;5 (3.2-3.6)</td>
<td>Nakamura et al. (66)</td>
</tr>
<tr>
<td>13q14</td>
<td>RB5</td>
<td>EcoRI</td>
<td>2 (2.2-10)</td>
<td>Fung et al. (67)</td>
</tr>
<tr>
<td>13q14</td>
<td>RB3</td>
<td>HindIII</td>
<td>2 (1.1-19)</td>
<td>Fung et al. (67)</td>
</tr>
<tr>
<td>17p13.3</td>
<td>YNZ22</td>
<td>RslI</td>
<td>&gt;10 (1.3-2.3)</td>
<td>Nakamura et al. (65)</td>
</tr>
<tr>
<td>17p13.3</td>
<td>144D6</td>
<td>RslI</td>
<td>&gt;14 (1.7-5.3)</td>
<td>Kondoleon et al. (69)</td>
</tr>
<tr>
<td>17p13.1</td>
<td>p53</td>
<td>BanII</td>
<td>2 (1.5, 2.9)</td>
<td>McBride et al. (33); Kidd et al. (70)</td>
</tr>
<tr>
<td>17p13</td>
<td>10.5</td>
<td>HindIII</td>
<td>2 (4.9, 5.3)</td>
<td>Schwartz et al. (71)</td>
</tr>
<tr>
<td>17p13</td>
<td>10.2</td>
<td>MspI</td>
<td>2 (2.1, 2.9)</td>
<td>Barker et al. (72)</td>
</tr>
<tr>
<td>17p11.2</td>
<td>YNM67</td>
<td>RslI, TaqI</td>
<td>2 (1.3, 3.8)</td>
<td>Nakamura et al. (66)</td>
</tr>
<tr>
<td>17p11.2-12</td>
<td>T7/BglII</td>
<td>EcoRI</td>
<td>4 (9.0, 9.9)</td>
<td>Zhou et al. (14)</td>
</tr>
<tr>
<td>17q12</td>
<td>HH1H2O2</td>
<td>RslI</td>
<td>2 (1.9, 2.5)</td>
<td>Nakamura et al. (66)</td>
</tr>
<tr>
<td>17q24.3-25.1</td>
<td>HH1H152</td>
<td>BamHI</td>
<td>2 (9.6, 10.5)</td>
<td>Nakamura et al. (66)</td>
</tr>
<tr>
<td>17q24.3-25.1</td>
<td>RMU3</td>
<td>TaqI</td>
<td>6 (3.2-3.8)</td>
<td>Nakamura et al. (66)</td>
</tr>
<tr>
<td>17q24.3-25.1</td>
<td>THH59</td>
<td>TaqI</td>
<td>&gt;8 (3.4-4.0)</td>
<td>Nakamura et al. (66)</td>
</tr>
<tr>
<td>19</td>
<td>MCT6</td>
<td>TaqI</td>
<td>5 (8.5-15)</td>
<td>Nierman and Magliot (73)</td>
</tr>
</tbody>
</table>

* Certain probes of single-copy DNA can detect multiple alleles of that DNA because of the presence of variable number tandem repeats associated with that locus (65).

^† In addition to noted allele sizes, two smaller bands in common to both alleles may be detected.

RESULTS

Tumor-specific Alterations of Chromosome 17p in HBV-positive HCCs from China. Our laboratory previously described a HBV insert cloned from a human hepatoma. The viral insert mapped to chromosome 17p11.2-12 and consisted of an inverted repeat structure in which adjacent cellular sequences (at least 3 kilobases) were duplicated. To determine if this region of chromosome 17p was altered in other liver tumor DNAs, a set of 19 matched nontumorous and HCC tissues obtained from China was analyzed using a DNA probe [T7/BglII-(1.0)] derived from the cellular DNA that flanked the previously characterized HBV insert. HMW cellular DNA was digested with EcoRI and analyzed by Southern hybridization. A 9-kilobase band of hybridization was observed in the normal and tumor DNAs of all patients (Fig. 1, patients 1, 7, 8, 16, 27, and 28). An additional 4-kilobase band was detected in the normal DNAs of seven patients (Fig. 1, patients 8, 27, and 28), and this 4-kilobase band was absent from the tumor DNAs of two patients (Fig. 1, patients 8 and 27). This result suggests that this small region of chromosome 17p DNA is altered in at least two other HBV-positive HCCs.

Loss of Chromosome 17 Alleles in HCC. To determine the extent and frequency of chromosome 17-specific DNA changes in our sample population of 19 Chinese patients, we next used a panel of characterized RFLP DNA probes (Table 2). Genomic DNAs purified from matched nontumorous and HCC samples were digested with restriction enzymes appropriate for the detection of fragment length polymorphisms (Table 2). All 19 patients analyzed were heterozygous at one or more loci on chromosome 17 (Fig. 24, patients 88–5N, 88–6N, and 88–7N; Fig. 2B, patient 88–5N; Fig. 2C, patient 88–18N). Twelve DNA probes to 32P-labeled probe DNA was performed as described previously (14).

Computer Analysis of Autoradiograms. The presence of small amounts of normal tissue in tumor samples sometimes may contribute a weak autoradiographic signal for one allele in the tumor sample. In such instances, densitometer scanning was performed using a Visage 60 computer and the Whole-Band analysis program (BioImage, Ann Arbor, MI) to compare the intensity of both alleles in normal and tumor tissue DNA. In this report, a decrease in intensity of the alleles of at least 50% in the tumor sample as compared to the normal DNA of the same patient was considered to indicate loss of heterozygosity.
and 27. Patients numbered 1, 7, 8, 16, 27, and 28 correspond to
probe) (14), derived from cellular sequences adjacent to a HBV insert, was used
digested with EcoRI, and filters were prepared as described in “Materials and
Methods.” A 32P-labeled DNA probe (designated 1.0-kilobase flanking DNA
probe derived from cellular sequences adjacent to a HBV insert. Paired DNAsamples from nontumorous liver (/V) and matched hepatoma (7”) tissues were
assessed. Association with the development of several human cancers (35).

1). Note the loss of the 4-kilobase band from the tumor (7") DNA of patients 8
chromosome 17p is a frequent event in these Chinese HCCs.
pressor gene. The loss of normal p53 gene structure has been
results demonstrate that the loss of one copy of portions
specific LOH for 17p markers (Fig. 2A, arrowheads). These
patients were heterozygous at one or more loci on the long arm
of chromosome 17 (17q), and 2 (17%) of the 12 patients
demonstrated a LOH for a 17q marker (Fig. 2C, patients 88-
T). In contrast, LOH for markers on 17p occurred much
more frequently. Ten (53%) of the 19 patients had a tumor-
specific allelle loss in our sample population. Ten of the 19 patients
were heterozygous for the two alleles of p53 (Fig. 3, patients
88-1N, 88-5N, 88-7N, 88-8N, and 88-18N), and a tumor-
specific loss of one allele was detected in 6 (60%) of the 10
patients (Fig. 3, patients 88-1T, 88-5T, 88-7T, 88-8T, and
88-18T). Quantitative densitometric scanning confirmed
the p53 allele loss (>50% reduction in the deleted allele;
data not shown), with the faint hybridization signal from the
missing allele presumably due to small amounts of contaminating
DNA from nontumorous tissue. Although the remaining
p53 allele appears normal by digestion with BanII, EcoRI, and
HindIII (data not shown), the possibility exists that a point
mutation in the remaining allele may have affected the func-
tional loss of p53 in those tumors.

Loss of Heterozygosity on Chromosomes 3, 8, 16, and 19 in
the HBV-positive HCCs. The finding of chromosome 17p allele
loss in 53% of the sample population is similar to the loss rate
reported for other tumors with known involvements of tumor
suppressor genes (18, 36). The absence of cytogenetic data on
noncultured HCC cells makes it difficult to predict the rate of
nonspecific chromosome loss. Similar RFLP studies of human
hepatomas (25, 26), as well as of non-HCC human tumors,
suggest that allele losses of 20–25% may be attributed to
genome instability during tumor progression. To assess the
specificity of the 17p LOH in this sample population, we
performed RFLP analyses using probes from four additional
chromosomes. DNA probes specific for chromosomes 3 and 19
revealed <10% LOH (Table 3). In contrast, much higher levels
of allele loss were noted for chromosomes 8q (4 of 9 patients;
44% LOH) and 16p (5 of 6 patients; 83% LOH). The LOH for
8q and 16p is higher than the 20–25% expected for random
chromosome loss (25, 26) and may indicate the presence of

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Incidence of LOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>3q</td>
<td>1/11 (9)</td>
</tr>
<tr>
<td>8q</td>
<td>4/9 (44)</td>
</tr>
<tr>
<td>16p</td>
<td>5/6 (83)</td>
</tr>
<tr>
<td>19</td>
<td>0/4 (0)</td>
</tr>
</tbody>
</table>

* Chromosome location of probes used in RFLP analyses.
\( ^{\text{a}} \) Incidence of loss is expressed as the number of patients with tumor-specific
LOH per total number of patients showing constitutional heterozygosity.
\( ^{\text{b}} \) Data for chromosome 3 were derived from two chromosome 3-specific probes,
EFD64.1 and EFD64.2.
\( ^{\text{c}} \) Numbers in parentheses, percentages.

Fig. 2. Tumor-specific loss of heterozygosity for chromosome 17 markers in
HBV-positive hepatoma DNA. Representative autoradiograms of RFLP analyses. Paired DNA samples from nontumorous liver (N) and matched hepatoma (T) tissues were digested with the restriction enzyme appropriate for the probe used (see Table 2). Patients were identified by number according to the assigned code (see Table 1). The numbers (1 or 2) at the left of each panel identify the two
alleles, with the number 1 arbitrarily assigned to the larger of the two alleles.
Tumor-specific loss is indicated by the arrowheads. Probes used were 144-D6 (A),
YNZ22 (B), and HHH1202 (C).

Fig. 3. Tumor-specific loss of p53 heterozygosity in HBV-positive hepatomas.
Representative autoradiograms of RFLP analyses. Paired DNA samples from
nontumorous liver (N) and matched hepatoma (T) tissues were digested with
BanII and were analyzed by Southern hybridization using a p53 complementary
DNA probe. Patients were identified by number (e.g., 88-5, 88-6) according to
the assigned code (Table 1). The numbers (1 or 2) at the left of each panel identify
the two p53 alleles, with the number 1 arbitrarily assigned to the larger of the
two alleles. Two additional smaller bands present in each lane are in common to
both alleles (70). Tumor-specific allelle loss is indicated by the stars. The loss of a
p53 allelle (>50% reduction in the deleted allelle) was confirmed by quantitative
densitometer scanning, as described in "Materials and Methods."

Table 3 Loss of heterozygosity on chromosomes 3, 8, 16, and 19 in HBV-positive
HCCs from China

\[ \text{Chromosome} \quad \text{Incidence of LOH} \]
\[ 3q \quad 1/11 (9) \]
\[ 8q \quad 4/9 (44) \]
\[ 16p \quad 5/6 (83) \]
\[ 19 \quad 0/4 (0) \]

\( ^{\text{a}} \) Chromosome location of probes used in RFLP analyses.
\( ^{\text{b}} \) Incidence of loss is expressed as the number of patients with tumor-specific
LOH per total number of patients showing constitutional heterozygosity.
\( ^{\text{c}} \) Data for chromosome 3 were derived from two chromosome 3-specific probes,
EFD64.1 and EFD64.2.
\( ^{\text{d}} \) Numbers in parentheses, percentages.
additional cellular genes whose functional loss is important in the development of some HCCs.

**Pattern of Allele Loss in Patients with Chromosome 17 Deletions.** Chromosome 17 allele loss was detected in a total of 10 Chinese hepatoma patients. Analysis of the pattern of allele loss among all 19 patients revealed that the proximal boundary of common deletion differed among the patients and was not clearly defined (Fig. 4). Patient 88-28 showed an apparently unaffected 17p12 region [detected by the T7/Bg-E(1.0) probe; Fig. 1] but lost alleles distal to that region. Another patient (88-1) retained both alleles detected by YNM67 (map position 17p11.2) but lost alleles from the more distal 17p13.1 (p53) and 17p13.3 (YNZZ2). Chromosome 17 markers lost in other patients included a YNM67 allele (Fig. 4, patients 5, 18, and 19). The chromosomal breakpoints differed from patient to patient, suggesting that the frequent loss of p53 in this sample population was not due to a fragile site in the chromosome.

Of the 10 patients heterozygous at the p53 locus, 6 (60%) demonstrated a tumor-specific loss of one allele. Two additional patients who were not heterozygous at the p53 locus (88-6 and 88-17) lost markers both distal and proximal to the p53 locus and therefore most probably lost a p53 allele. Other patients (88-19 and 88-26) lost 17p markers (detected by probes p10.5 and YNM67; both proximal to p53) but were homozygous at all remaining 17p loci. Future quantitative densitometric scanning analyses may be able to measure possible p53 allele losses in these four additional patients.

Several patients had LOH for markers on chromosomes 8q and 16p. All four patients with 8q LOH had concomitant LOH for markers on chromosome 17 (Fig. 4, patients 8, 17, 19, and 28). In contrast, two cases of chromosome 16p LOH were from patients with 17p LOH (Fig. 4, patients 6 and 18), and the remaining three were from patients with an apparently intact chromosome 17 (Fig. 4, patients 4, 15, and 27). None of the 19 patients displayed LOH for markers on all three chromosomes. Preliminary analysis of the sample population did not reveal abnormalities in the retinoblastoma gene (located on chromosome 13q14.1; data not shown).

**DISCUSSION**

The development of HCC probably occurs in multiple steps and is influenced by multiple factors. HBV is one important etiological factor in certain parts of the world, such as China and Africa, where chronic infection with HBV is strongly associated with the development of HCC. The specific role of HBV in the development of HCC remains unknown. The activation of known oncogenes is not a common feature of HBV-positive HCC (3). A HBV random integration may, in rare instances, prompt a change in the expression of a critical regulatory gene by insertional mutagenesis (37-39). However, it is possible that the functional loss of cellular genes may contribute change(s) important in the development of liver cancer. Consistent with the latter, chromosomal alterations are frequently observed at the site of viral integration in HCC.

RFLP studies on HCCs revealed tumor-specific LOH on several chromosomes, including 11p [43% LOH (25)], 13q [50% LOH (25)], 4 [58% LOH (26) and 50% LOH (27)], and 16q [57% LOH (27)]. In our study of 19 matched liver (nontumor) and HCC samples from China, a similar level of loss for chromosome 17p (53% LOH) was detected. The loss of markers from chromosome 17 was recently demonstrated for other human cancers, including colon cancer (36), astrocytoma (40-42), bladder cancer (43), osteosarcoma (44, 45), lung cancer (46-49), neurofibrosarcoma (50), and breast cancer (51). One interpretation of specific allele loss in a significant portion of tumors is that a tumor suppressor gene may be located on that chromosome. The p53 gene, a known tumor suppressor gene (28-30, 35), is believed to be the target of the 17p chromosome deletions in many human cancers (24, 36).

The 17p deletions found in our sample population include the loss of one allele of the p53 gene. Six (60%) of the 10 patients who were heterozygous at the p53 locus had a tumor-specific loss of one allele of the p53 gene, and the pattern of chromosomal changes in an additional four patients was consistent with p53 allele loss. Thus, it is possible that a total of 10 patients in this sample population of 19 may have lost one copy of the p53 gene as a result of gross chromosomal altera-

Fig. 4. Pattern of allele loss among 19 Chinese HBV-positive hepatoma patients. Various DNA probes (listed at the left, with chromosome location given in parentheses) were used for RFLP analyses of matched nontumorous and hepatoma tissue samples. Results of the Southern hybridization assays are listed below the respective patient identification number. —, homozygosity at that locus in the nontumorous tissue DNA of the patient; O, the presence of both alleles (heterozygosity) in tumor DNA; Ø, tumor-specific loss of one allele.
tions. The normal role of p53 in growth regulation is currently unclear, and its involvement in cell transformation appears to be complex (28, 29). In keeping with current models of carcinogenesis, which presume that the inactivation of a tumor suppressor gene is a two-step process (19, 52), the first event in a p53-related pathway of transformation is believed to be a missense mutation (53) that results in an “activated” p53 known to contribute to transformation (28–30, 35). The second event in p53 loss most probably occurs during the selection of a cell that has deleted the remaining wild-type p53 allele. Such a scenario for p53 involvement in the development of HCC would predict that tumors in which an allele of p53 has been deleted will contain a point mutation in the remaining p53 allele. Point mutations that have been documented in remaining p53 alleles in several human cancers support this hypothesis (36, 45, 49, 50, 53).

In addition to the frequent LOH for chromosome 17p markers in this population of HCC patients, LOH for markers on chromosomes 8q (4/9; 44% LOH) and 16p (5/6; 83% LOH) was higher than expected for changes due to general genomic instability during tumor progression. Of the known genes localized to chromosome 8q, at least three (c-myc, c-yes, and c-mos) have documented roles in other tumor systems (54–56). Interestingly, c-myc is a frequent target for insertional mutagenesis by the woodchuck hepatitis virus during hepatocarcinogenesis in that animal (38). The chromosome 16p13 is a chromosomal breakpoint in two rearrangements found in a subgroup of patients with acute myelomonocytic leukemia (57). Although the possible role of genes residing on 8q and 16p in hepatocarcinogenesis remains unclear, our preliminary findings define chromosomal regions that warrant further analysis with more comprehensive panels of RFLP probes.

The involvement of HBV in the observed chromosomal losses remains unknown. In the present study, flanking cellular sequences from a HBV insert (localized to chromosome 17p12) identified a region of the chromosome that was altered in other HCCs. Our collection of HCCs with 17p deletions contains multiple HBV inserts (1 to 4/tumor). Although chromosome 17 is one of the preferred targets for HBV integration (5), we have no evidence that the 17p deletions we describe are associated with HBV integrations. Similarly, the frequent HBV integration events noted for chromosome 11 (5) have not been linked directly to the significant chromosome 11p LOH (25). High levels of LOH also have been found for chromosome 4 (26, 27), recently shown to be the site of a HBV integration into the cyclin A gene (39). It is evident that the analysis of cloned HBV inserts has been useful in the identification of regions of chromosomes commonly altered during hepatocarcinogenesis.

Multiple genetic changes are required for the development of human liver cancer, and it is possible that HBV integration may facilitate one or more of those changes in certain tumors. Our working hypothesis is that the functional effects of HBV integration may differ from tumor to tumor, and, indeed, no single common integration site for HBV has been identified. A HBV random integration event may, in rare instances, prompt a change in the expression of a critical regulatory cell gene by insertional mutagenesis. The transactivating potentials of a truncated HBV surface antigen (58, 59) and of the X gene product (60–64) may, on occasion, transactivate cellular gene expression and thereby alter cell growth control. The functional loss of one or more cellular tumor suppressor genes is presumably a necessary step in the emergence of liver cancer, and this inactivation may be accomplished either by HBV integration events or by spontaneous mutations.

ACKNOWLEDGMENTS

The technical assistance of Ling Tian is gratefully acknowledged.

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*Cancer Res* 1991;51:49-54.

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