Virologic Analysis of Non-B, Non-C Hepatocellular Carcinoma in Japan: Frequent Involvement of Hepatitis B Virus


Serum and liver tissues from hepatitis B surface antigen-negative/anti-hepatitis C virus (HCV)-negative (non-B, non-C) hepatocellular carcinoma (HCC) patients in Japan were examined for the presence of hepatitis B virus (HBV), HCV, and TT virus (TTV) by polymerase chain reaction. The studies evaluated the contribution of these viruses to pathogenesis of HCC. HBV DNA was detected in the sera of 20 (47.6%) of 42 non-B, non-C HCC patients, which was significantly higher than in age-matched controls without liver disease (P < .001). In 8 of 12 patients with liver tissues available, HBV DNA was detected in cancerous and adjacent noncancerous liver tissues. No HCV RNA was detected. The positivity for TTV DNA was not significantly different between HCC patients and controls. These results indicate that HBV is associated with a substantial proportion of non-B, non-C HCC cases in Japan. The role of HBV in hepatocarcinogenesis in such patients needs to be clarified.

The discovery of the cDNA for hepatitis C virus (HCV) disclosed that the majority of hepatocellular carcinoma (HCC) cases are related either to hepatitis B virus (HBV) or HCV infection [1–3]. This is also so in Japan, where deaths due to HCC exceeded 30,000 in 1996, ranking third for the cause of death due to neoplasia [4]. However, numerous HCC patients worldwide have neither hepatitis B surface antigen (HBsAg) nor antibody to HCV (anti-HCV). In Japan, about 5% of HCC patients are “non-B, non-C” [1]. The HBV genome can be detected in persons with chronic liver disease in the absence of circulating HBsAg [5–11]. In a subset of these patients, HBV DNA or its replicative intermediate RNA is detected, although the prevalence varies among studies. A substantial number of HCC patients with HCV infection, however, may have been included in previous studies [5–11].

The prevalence of HBV in non-B, non-C HCC patients and the role of HBV in the development of HCC in such patients are not clear. In Japan, the prevalence of HBV and HCV infection is about 1.0% [12], and deaths due to HCC are common. In addition, the contribution of newly discovered hepatitis-associated viruses to the development of non-B, non-C HCC should be elucidated. Although we and others have shown that GB virus-C/hepatitis G virus (GBV-C/HGV) has no association with non-B, non-C HCC [13, 14], the association of TT virus (TTV) [15, 16] remains to be determined. In the present study, we used the polymerase chain reaction (PCR) to examine the presence of HBV DNA, HCV RNA, and TTV DNA in liver tissues and sera from non-B, non-C HCC patients.

Methods

Patients. We studied 42 patients (35 men, 7 women) with HCC who were admitted to our department at the University of Tokyo Hospital from June 1992 to December 1998 and were negative for both HBsAg and anti-HCV (non-B, non-C). Diagnosis of HCC was based on histologic, radiologic, and biochemical findings. Patients with autoimmune liver disease, hemochromatosis, and Wilson’s disease were excluded on the basis of clinical and biochemical findings. General characteristics of the 42 patients and age-matched controls without liver diseases are shown in table 1. Fourteen of the 42 patients had a history of surgery, and 5 had a history of blood transfusion. Seven patients had a history of excessive alcohol intake (>1000 kg of ethanol intake in total before diagnosis of HCC). Liver specimens were obtained from 12 of the 42 HCC patients at hepatectomy, 9 of whom had cirrhosis; the other 3 had moderate-to-severe chronic hepatitis as the underlying liver disease [17]. Clinical profiles of these 12 patients, including histology of the liver tissue, are shown in table 2. We also studied 4 HBsAg-positive HCC patients, including 2 who were positive for hepatitis B e antigen (HBeAg) and 2 who were positive for antibody to HBeAg (anti-HBe), as positive controls.
Table 1. General characteristics and hepatitis B virus and hepatitis C virus (HCV) serology of 42 “non-B, non-C” hepatocellular carcinoma (HCC) patients and 42 controls without liver diseases.

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Non-B, non-C HCC</th>
<th>Controls without liver diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>Age (years) ± SD</td>
<td>66.1 ± 10.9</td>
<td>64.2 ± 11.2</td>
</tr>
<tr>
<td>Sex (male: female)</td>
<td>35:7</td>
<td>34:8</td>
</tr>
<tr>
<td>HBsAgg</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anti-HBs /anti-HBc</td>
<td>26</td>
<td>32</td>
</tr>
<tr>
<td>Anti-Hbs/anti-HBc</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Anti-Hbs/anti-HBc</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Anti-HCV</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

NOTE. HBsAg, hepatitis B surface antigen; †, positive; ‡, negative.

Virus serology. HBsAg, antibody to HBsAg (anti-HBs), antibody to hepatitis B core antigen (anti-HBc), HBeAg, anti-HBc, and anti-HCV in sera were determined by commercial EIA kits (Dainabot, Tokyo), according to the manufacturer’s instructions.

Nucleic acid extraction. DNA was prepared from 50 µL of serum or 10 mg of liver tissue. The serum was digested for 2 h at 55°C with 20 µg of proteinase K (Boehringer, Mannheim, Germany) in 50 µL of STE solution (100 mM Tris/HCl [pH 8.0], 100 mM NaCl, 2 mM EDTA [pH 8.0], and 0.2% SDS). The liver samples were digested with 200 µg of proteinase K in 400 µL of STE solution under the same conditions as the serum samples. Total cellular DNA was extracted twice with phenol/chloroform and once with chloroform and then precipitated with ethanol. DNA pellets from the serum and liver were then dissolved in 20 µL and 50 µL of 10 mM Tris and HCl and 1 mM EDTA (pH 8.0), respectively. RNA was extracted from the serum or liver as previously described [18]. The RNA pellet from 50 µL of serum or from 10 mg of liver tissue was dissolved in 50 µL of distilled water, and equivalent amounts of RNA were subjected to amplification using the reverse transcriptase (RT)-PCR method.

PCR and Southern blotting. We subjected 5 µL of a nucleic acid solution from serum or 0.1 µg of DNA from liver tissue to PCR amplification. By use of 2 independent pairs of primers, each of which was set within the X or C region of the HBV genome according to the sequence of subtype adr [19], HBV DNA was amplified and subsequently detected by Southern blotting with specific probes (figure 1). Amplification was done in a thermal cycler for 40 cycles: 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min in 100 µL of reaction mixture containing 200 µM dNTPs, 1.0 µM of each primer, 1× PCR buffer (50 mM KCl, 10 mM Tris-HCl [pH 8.3], 1.5 mM MgCl2, and 0.001% [wt/vol] gelatin), and 2 U of Ampli-Taq polymerase (Perkin-Elmer Cetus, Norwalk, CT). The PCR products were separated in a 2% agarose gel and transferred onto a nylon membrane (Schleicher & Schuell, Dassel, Germany). The membrane was then hybridized with digoxigenin-labeled (Boehringer) oligonucleotide probes that were located between each pair of PCR primers in the HBV genome (figure 1). This PCR-Southern hybridization was sufficiently sensitive to detect ~10 copies of HBV DNA per reaction tube [20]. HCV RNA was detected by seminested RT-PCR as described elsewhere [18].

TTV DNA was detected by PCR as reported elsewhere [15, 16]. After DNA extraction from the serum or liver, 2.5 µL of DNA was subjected to PCR with the following final concentrations of reagents: 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl2, 0.001% (wt/vol) gelatin, 200 µM dNTPs, 1.0 µM of each primer, and 0.625 U of Ampli-Taq Gold (Perkin-Elmer Cetus). Two pairs of PCR primers were designed as described elsewhere [15, 16]: sense T801, GCTACGCTACAAACACGTG, and antisense T935, CTCGGGTGTGAAACTCAG, in the 5’-noncoding region [16]; sense NG061, GGCAACATGYTRTGGATAGACTGG, and antisense NG063, CTGGCATTTTACATTTCCAAGT, in the sequence of the N22 clone [15]. The former primer set is supposed to detect TTV DNA regardless of genotypes, and the latter is supposed to detect TTV DNA of genotype 1a [21]. A serum sample shown to be positive for TTV DNA by a previously described method [15, 16] was used as a positive control (gift of M. Mayumi, Jichi Medical School, Tochigi-ken, Japan).

Water and a serum sample from a healthy subject were used as negative controls. To avoid cross-contamination, steps for nucleic acid extraction, PCR, and post-PCR procedures were performed in separate rooms. In each experiment, all reagents were UV irradiated when appropriate, and filter pipette tips (USA/Scientific Plastics, Ocala, FL) were used to avoid cross-contamination. 

Table 2. Hepatitis B virus (HBV) DNA in the liver of “non-B, non-C” hepatocellular carcinoma (HCC) patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, years</th>
<th>History of transfusion/ surgery</th>
<th>Excessive alcohol intakea</th>
<th>Histology of HCC</th>
<th>Histology of underlying liver disease</th>
<th>HBsAg</th>
<th>anti-HBs</th>
<th>anti-HBc</th>
<th>HBV DNA in liver (HCC/nHCC)</th>
<th>HBV DNA in serum</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>65</td>
<td>–/+</td>
<td>–</td>
<td>PD</td>
<td>Cirrhosis</td>
<td>–</td>
<td>–</td>
<td>--</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>71</td>
<td>+/</td>
<td>–</td>
<td>MD</td>
<td>Cirrhosis</td>
<td>–</td>
<td>–</td>
<td>–/++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>75</td>
<td>–/+</td>
<td>–</td>
<td>MD</td>
<td>Cirrhosis</td>
<td>–</td>
<td>–</td>
<td>++/</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>64</td>
<td>–/+</td>
<td>–</td>
<td>MD</td>
<td>Cirrhosis</td>
<td>–</td>
<td>–</td>
<td>–/++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>68</td>
<td>–/+</td>
<td>–</td>
<td>MD</td>
<td>Cirrhosis</td>
<td>–</td>
<td>–</td>
<td>++/</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>76</td>
<td>–/</td>
<td>–</td>
<td>MD</td>
<td>Cirrhosis</td>
<td>–</td>
<td>–</td>
<td>++/</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>76</td>
<td>++/</td>
<td>–</td>
<td>MD</td>
<td>Cirrhosis</td>
<td>–</td>
<td>–</td>
<td>++/</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>65</td>
<td>–/</td>
<td>–</td>
<td>MD</td>
<td>Cirrhosis</td>
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<td>–</td>
<td>++/</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>61</td>
<td>–/+</td>
<td>–</td>
<td>PD</td>
<td>Cirrhosis</td>
<td>–</td>
<td>–</td>
<td>++/</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>58</td>
<td>++/</td>
<td>–</td>
<td>MD</td>
<td>Cirrhosis</td>
<td>–</td>
<td>+</td>
<td>–/</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>11</td>
<td>54</td>
<td>–/</td>
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<td>PD</td>
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<td>–/</td>
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<td>–</td>
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<tr>
<td>12</td>
<td>69</td>
<td>–/</td>
<td>–</td>
<td>MD</td>
<td>Cirrhosis</td>
<td>–</td>
<td>–</td>
<td>++/</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

NOTE. All patients were men. HBsAg, hepatitis B surface antigen; anti-HBs, antibody to HBsAg; anti-HBc, antibody to hepatitis B core antigen; nHCC, noncancerous tissue; –, negative; +, positive; PD, poorly differentiated; MD, moderately differentiated; moderate, chronic hepatitis with moderate fibrosis; severe, chronic hepatitis with severe fibrosis.

a Alcohol consumption >1000 kg in total before diagnosis of HCC.
Table 3. Positivity of hepatitis B virus (HBV) DNA in serum according to HBV serology.

<table>
<thead>
<tr>
<th>Serology</th>
<th>Non-B, non-C HCC</th>
<th>Controls without liver diseases</th>
<th>P (non-B, non-C HCC vs. controls)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HBs/anti-HBc</td>
<td>14/26 (53.8)</td>
<td>0/32</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Anti-HBs/anti-HBc'</td>
<td>4/9 (44.4)</td>
<td>1/6 (16.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Anti-HBs'/anti-HBc'</td>
<td>2/7 (28.6)</td>
<td>0/4</td>
<td>NS</td>
</tr>
<tr>
<td>Total</td>
<td>20/42 (47.6)</td>
<td>1/42 (2.4)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

NOTE. Data are no. positive/no. tested (%). HCC, hepatocellular carcinoma; NS, not statistically significant; †, positive; ‡, negative.
reaction mixture containing 200 μM each of dNTP and 1× PCR buffer and 2 U of Ampli-Taq with outer primers. A 2-μL aliquot from the amplified sample was then made up to 100 μL with the reaction mixture consisting of 1.0 μM of an internal seminested primer (figure 1), 200 μM each of dNTP and 1× PCR buffer, and 2 U of Ampli-Taq. The second-round amplification was performed for 25 cycles under the same conditions as for the first one. The PCR products were sequenced in both directions by the fluorescent dye terminator cycle sequencing method (DNA Sequencer 373A; Applied Biosystems, Foster City, CA).

Statistical analysis. We used Fisher’s exact test for statistical evaluations.

Results

Detection of HBV DNA in serum and liver. We categorized the patients into 3 groups according to the presence or absence of serum HBV markers. As shown in table 1, 26 of the 42 patients were negative for both anti-HBs and anti-HBc, 9 were positive for anti-HBc but negative for anti-HBs, and the remaining 7 were positive for both anti-HBs and anti-HBc. We first determined HBV DNA positivity of the sera from non-B, non-C HCC patients and control subjects without liver diseases. PCR with the primer sets in the X and C regions gave identical results. Among subjects who were negative for antibody to both HBsAg and anti-HBc, HBV DNA was detected in sera in 14 (53.8%) of 26 non-B, non-C HCC patients but not in 32 controls (P < .001). There were no significant differences in the positivity of HBV DNA in the sera between non-B, non-C HCC patients and control subjects among those who were negative for antibody to HBsAb but positive for antibody to HBcAg or those who were positive for antibody to HBsAg and HBcAg. In total, 20 (47.6%) of 42 non-B, non-C HCC patients were positive for serum HBV DNA, while only 1 (2.4%) of 42 controls was positive (P < .001; table 3).

Of the 12 patients with available liver tissues, HBV DNA was detected in cancerous (HCC) and/or noncancerous (NC) liver tissues in 8 (66.7%) (table 2). HBV DNA was detected in 5 of 6 liver tissues from anti-HBs/anti-HBc+ HCC patients. There was no association between HBV DNA positivity and histologic findings of HCC or underlying liver disease.

Semiquantification of HBV DNA by serial dilution. To determine the levels of HBV DNA in the liver tissues from the 8 patients positive for HBV DNA, semiquantification of HBV DNA was performed using the end point-dilution PCR method. Figure 2 shows the results of representative patients. Patient 1 had 10^6 GE/μg of HBV DNA both in HCC and NC liver tissues, which was the same as the titer of HBV DNA in huH2-2 cells and corresponded to >1 copy of HBV DNA per diploid host genome. Patient 12 had 10^5 GE/μg of DNA in HCC tissue and 10^4 GE/μg of DNA in NC tissue, the latter of which corresponded to ~0.1 copy of HBV DNA per diploid host genome. In general, HCC tissues retained ~1 copy of HBV DNA, while NC tissues occasionally had <1 copy of HBV DNA per diploid genome. In contrast, the titers of HBV DNA in liver tissues of HBsAg+/anti-HBe+ HCC patients were >10^7 GE/μg of DNA and much higher than those in non-B, non-C HCC patients (data not shown). However, 2 HBsAg+/anti-HBe+ HCC patients had titers of HBV DNA similar to those of non-B, non-C HCC patients (figure 2).

Detection of HBV RNA. To determine whether the HBV genome in the liver is transcribed, we examined the liver tissues of the patients for the presence or absence of HBV RNA.

Figure 2. Semiquantitative determination of hepatitis B virus DNA in liver tissues. DNA prepared from cancerous (C) or noncancerous (NC) liver tissues was used for semiquantification of HBV DNA. Patients 1 and 12 in table 3 and 1 patient with HCC positive for hepatitis B surface antigen (HBsAg+) and anti–hepatitis B (HB) e are shown as representative patients. Titer was expressed as genome equivalents (GE)/μg of liver DNA.
Figure 3. Detection of hepatitis B virus RNA in liver tissues. Prepared RNA was treated with DNase and subjected to reverse transcriptase-polymerase chain reaction (RT-PCR) or PCR using primers in the X region. Results are shown for patients 1 and 12 in table 3. Lane 1, sample from liver tissue subjected to RT-PCR; lane 2, sample from liver tissue subjected to PCR without prior reverse transcription; lane 3, water sample (negative control) subjected to RT-PCR. C, cancerous liver tissue; NC, noncancerous liver tissue.

Partial mapping and sequence analysis of HBV DNA. To further characterize the HBV DNA detected in the liver tissues, we carried out 2-stage PCR on the DNA prepared from liver samples, using 5 sets of primers that were located in the S, X, or pre-C-C region of the HBV genome (figure 1). As shown in figure 4, some DNA fragments in the S and pre-C-C region could not be amplified in several specimens from HCC or NC tissues.

We next determined the nucleotide sequences of the amplified HBV DNA fragments by the direct sequencing method. Figure 5 shows the nucleotide sequences of the entire S region in the HBV DNA from 3 patients. Several nucleotides in the sequences of HBV DNA differed among patients, but those from HCC and NC liver tissues were identical. HBV DNA in the NC tissue from patient 4 exhibited a number of mutations in the S region (figure 5A), which were concentrated in the region encoding the “a” determinant (a-loop) and resulted in amino acid (aa) changes: Thr → Pro at aa 123, Gln → Leu at aa 129, Thr → Ala at aa 131, Ser → Phe at aa 132, Met → Lys at aa 133, Cys → Gly at aa 138, Cys → Gly at aa 139, and Gly → Val at aa 145 (figure 5B).

HBV DNA in the HCC tissue from patient 6 also had an aa change from Gln → His at aa 129. The NC tissue from patient 12 had several nucleotide mutations in the S region, which did not result in aa changes in the a-loop. There were also some mutations in the nucleotide sequences in the X and pre-C-C regions. For example, nucleotide sequences in the C region of HBV DNA in HCC tissue from patient 2 showed a 12-bp deletion, and those from patient 7 showed several aa changes in the latter half of the core antigen. However, there were no mutations in the determinant for the core antigen around aa 80 [23] or the core gene promoter (data not shown) [24]. Thus, the absence of HBV markers may be explained by the mutations and/or deletions of HBV genome in some cases, such as in patient 4 for HBsAg. However, in most other patients, there was no major gene aberration that might confer a change in the antigenicity of HBsAg or the core antigen.

Detection of HCV RNA and TTV DNA in serum and liver. HCV RNA was not detected in any of the 42 serum samples or 12 HCC/NC liver tissues from non-B, non-C HCC patients. We compared the positivity of TTV DNA in non-B, non-C HCC patients and in control persons without liver diseases. With primers in the 5'-noncoding region, which is supposed to be nonspecific for genotypes, TTV DNA was detected in the sera of 35 (83.8%) of 42 non-B, non-C HCC patients and in 40 (95.2%) of 42 control subjects (statistically not significant [NS]). With primers in the N22 region, which is supposed to be specific for genotype 1a [21], TTV DNA was detected in the sera of 16 (38.0%) of 42 non-B, non-C HCC patients and in 13 (31.0%) of 42 control subjects (NS). TTV DNA was detected in the liver tissues of 8 of 12 patients with 5'-NC primers and in 2 of 12 with N22 primers, which was similar to the TTV DNA positivity in the serum.

Discussion

Our analysis revealed that HBV DNA was detected frequently in serum or liver samples from non-B, non-C HCC patients in Japan. Control subjects without liver diseases had a very low HBV DNA positivity in serum despite a similar positivity of anti-HBs and anti-HBc. In contrast, HCV RNA
was not detected in non-B, non-C HCC patients, and the prevalence of TTV DNA among non-B, non-C HCC patients did not differ significantly from those among controls. These results, together with the absence of involvement of GBV-C/HGV in non-B, non-C HCC, as we [13] and others [14] previously showed, indicate that among the hepatitis viruses and candidate viruses for hepatitis, only HBV may be associated with the development of HCC.

HBV DNA has been reported in the serum or liver of HBsAg, non-B HCC patients [5±11], but the prevalence of HBV DNA is not yet clear in non-B, non-C HCC patients, who lack both HBsAg and anti-HCV in serum. In studies that used the PCR technique, HBV DNA was detected in 30%±50% of the serum or liver samples from non-B HCC patients, some of whom were also positive for HCV RNA [9±11]. From the data in 2 previous reports on non-B HCC, the HBV DNA positivity of serum from non-B, non-C HCC patients can be calculated as 40.0% in 1 study [9] and 58.3% in another [10]. In those studies, however, few patients were analyzed (10 and 12, respectively). In the current study, we examined sera from 42 non-B, non-C patients and found HBV DNA in 20 (47.6%), a prevalence similar to that of serum HBV DNA in the previous studies. HBV DNA positivity was higher in liver tissues (66.7%) than in serum (47.6%), which may result from a difference in the copy number of HBV DNA between the serum and liver or from the presence of an integrated form of HBV DNA in the liver tissues without circulating HBV particles.

The titers of HBV DNA in the liver tissues computed from the results of the end point-dilution methods were generally low: ~1 copy per cell in the HCC tissues and <1 copy per cell in NC liver tissues. Of note, in HBsAg/anti-HBe+ HCC patients, the titers of HBV DNA were similar to those in HBsAg- HCC patients. Moreover, HBV RNA was detected in HCC tissues, indicating that the HBV genome was transcribed in HCC tissues. However, on the basis of our data, we could not determine whether the HBV DNA was integrated into the host genome or existed in an episomal form in the virus particles.

Mapping of the HBV DNA from the liver tissues revealed the failure of amplification in some parts of the HBV genome. This may be due to several factors: first, a deletion in the corresponding fragment of HBV DNA in the liver tissue; second, mutations or variations in the nucleotide sequences of the HBV genome, which hampered the amplification by PCR with our primers; or third, the copy number of HBV DNA may be too low to be detected by PCR with a certain set of primers (a difference in sensitivity). Although we could not determine which factor applied, it is notable that the primers within the X (1400F, 1627R, and 1569R) or C region (2267F, 2241R, and 2402R) were set in the regions that were relatively conserved among different HBV clones [19, 25]. Variations in the nucleotide sequences may be responsible for the inability to amplify HBV DNA. This may explain why HBV DNA was amplified with the primers indicated above but not with primers that were set outside of those regions (patients 1 and 4). This is consistent with the results of sequence analysis, which revealed a number of variations in the nucleotide sequences in the HBV DNA of some HCC patients.

We also found HBV DNA in HCC tissues from patients who lacked any HBV markers. The detection of HBV DNA in the liver tissues of patients without any HBV markers has been described previously [5, 7, 22, 26±28]. Mutations in the nucleotide sequences in the region encoding HBsAg have been considered to be responsible for the absence of HBsAg in serum in some patients [27]. However, 1 study found no significant changes in the nucleotide sequences of regions encoding HBsAg or the core antigen that could explain the absence of detection of the antigen or antibody in such non-HCC patients [26]. In the present study, we found a number of nucleotide mutations that resulted in changes of aa sequences around the “a” determinant of HBsAg in the analysis of 1 patient (figure 5). How-
Figure 5. Sequence analysis of the entire S region of specimens from representative patients with hepatocellular carcinoma. Left column, nucleotide sequences. Right column, amino acid sequences. The HBV DNA obtained from noncancerous liver tissue of patient 4 had several mutations in the “a” determinant region. HBV-ADR, hepatitis B virus subtype adr [18]; NC, noncancerous; C, cancerous; AA, amino acid.

ever, few such mutations were found in other patients. We were also not able to find mutations that could confer changes in the antigenicity of HBcAg. The reason for the absence of anti-HBc in patient sera is not clear, but it may be due to a very low level of antigen production in these patients. Active cytotoxic T lymphocytes against HBV, which were demonstrated in HBsAg+ subjects long after recovery from acute hepatitis B [29, 30], may suppress viral replication, contain HBV within the liver, and prevent it from interacting with the host B cell system.

In the present study, HCV RNA was not detected in sera or liver tissues from non-B, non-C HCC patients. This finding is similar to results of our previous studies of chronic hepatitis patients, in which no HCV RNA was detected in liver tissues
from patients with chronic liver disease without anti-HCV [31]. TTV, which was found in the serum of a patient with posttransfusion non–A–E hepatitis [15], may be another candidate for the development of non-B, non-C HCC. In the present study, however, we found no difference in the prevalence of TTV DNA between non-B, non-C HCC patients and control subjects by PCR when we used either a genotype 1a–specific or genotype-non-specific primer set. This finding does not indicate a role for TTV in the carcinogenesis of non-B, non-C HCC. These results are compatible with a recent report that showed no association between TTV infection and the development of HCC [32].

A substantial proportion of non-B, non-C HCC patients in Japan, who were HBsAg-antibody positive, expressed HBV DNA in HCC or NC liver tissues and sera. Other hepatitis viruses and putative candidates for hepatitis viruses were not associated with HCC. In the pathogenesis of HCC associated with HBV infection, the integration of the HBV genome and the production of the HBx protein of HBV in the liver tissue have been suggested to play an important role [33–36]. Further studies are necessary to clarify the role of the “occult” HBV in the development of non-B, non-C HCC.

References


28. Zhang YY, Hansson BG, Kuo LS, Widdell A, Nordenfelt E. Hepatitis B virus DNA in serum and liver is commonly found in Chinese patients with chronic liver disease despite the absence of antibodies to HBsAg. Hepatology 1993;17:538–44.


