Integrity of p53 in Hepatitis B x Antigen-positive and -negative Hepatocellular Carcinomas

M. S. Greenblatt, M. A. Feitelson, M. Zhu, W. P. Bennett, J. A. Welsh, R. Jones, A. Borkowski, and C. C. Harris

ABSTRACT

Inactivation of the tumor suppressor p53 seems to be important to the pathogenesis of hepatocellular carcinoma (HCC) associated with chronic hepatitis B virus infection. Although this inactivation may be due to mutations in the p53 gene, recent evidence suggests that the hepatitis B virus-encoded X antigen (HBxAg) binds to and inactivates wild-type p53. Hence, experiments were designed to test the hypothesis that there is a low frequency of p53 mutations in HBxAg-positive HCC. HBxAg and p53 were assayed by immunohistochemistry (IHC) in HCC and nontumor liver from 16 Chinese patients, half of whom were hepatitis B surface antigen carriers. HBxAg was detectable in tumor and/or nontumor cells from all patients by IHC; six of these samples also had detectable p53. To determine whether p53 detection by IHC, and hence stabilization, is associated with mutation, sequencing of p53 exons 5—8 was performed with each patient sample. Wild-type sequences were found in 13 of 16 HBxAg-positive cases (81%). Hence, HBxAg is a common marker of HCC that correlates with the persistence of wild-type p53 among both carriers and noncarriers. The low frequency of p53 mutations in HCC in these patients implies that p53 Inactivation may occur predominantly by complex formation with HBxAg.

INTRODUCTION

Risk factors for HCC identified by epidemiological studies include chronic infections with HBV, HCV, and prolonged dietary consumption of aflatoxins. In case-control studies, the relative risk for HCC in chronic HBV carriers was 5—30 (1, 2), whereas in a large prospective study in Taiwan, the relative risk for chronic HBV carriers was over 200 (3). More recently, it has been shown that the relative risk for development of HCC increases synergistically among individuals exposed to both aflatoxins and HBV (4, 5). Hepatocellular regeneration accompanying antiviral inflammatory responses among patients with chronic active hepatitis is also a critical factor in the pathogenesis of HCC, although other characteristics of the host-virus relationship, such as genetic variation in the virus (6), are also likely to contribute to pathogenesis.

There are many possible mechanisms whereby HBV may cause HCC (7—9). Mechanisms that depend upon cis-activation of cellular oncogenes, growth factors, growth factor receptors, and so forth have been reported (10—15) but only seem to be operative in a small percentage of the cases studied. Alternatively, it has been proposed that the virus-encoded trans-activator, HBxAg, may stimulate the expression of cellular genes involved in transformation (16—20), but it is not clear what the targets of HBxAg trans-activation are or whether these presumed targets are consistently activated in the majority of HBV-associated HCCs. A putative role of HBxAg in neoplastic transformation is especially appealing in light of the fact that HBxAg stimulates progression through the cell cycle (21, 22), transforms a mouse hepatocyte cell line (23), and causes tumors in at least one strain of transgenic mice (24, 25). The finding that HBxAg functions by binding to other cellular proteins but not to nucleic acids (26—29) suggests that HBxAg binding partners in the cell may be important for transformation. This idea is further supported by the finding that HBxAg complexes with the tumor suppressor protein p53 both in vivo and in vitro, resulting in p53 inactivation (30—32), and that such inactivation correlates with the blocking of p53-dependent apoptosis (33). The binding of HBxAg to p53 occurs at the COOH terminus of the latter in the region responsible for p53 oligomerization, which, in turn, is important for p53 function (31).

There are several additional lines of evidence implicating that the integrity of p53 is compromised in HCC. For example, loss of heterozygosity at chromosome 17p (which includes the p53 locus; Refs. 34 and 35), HBV DNA integration near the p53 locus (36), and p53 mutations (reviewed in Ref. 37) documented in some HCCs disrupt normal p53 function. In addition, point mutations in the p53 gene have been found in half of the HCCs associated with long-term AFB1 exposure (reviewed in Ref. 38) and in 20—25% of non-AFB1-associated HCCs. Whereas in non-AFB1-associated HCCs, a variety of mutations are seen at different sites within the gene, almost all of the mutations in AFB1-associated tumors are G:C—T:A transversions at codon 249 (AGG—GTG changing Arg—Ser; Refs. 37—39). Such mutations are consistent with patterns of AFB1-induced mutagenesis in vitro and occur in both HBV-positive and -negative tumors with equal frequency (37, 40). This specific transversion has also been documented at higher than expected frequencies in nontumor liver from patients living in areas of high AFB1 prevalence independent of HBV infection (40—42). The finding of p53 mutations in a number of human hepatoma cell lines (42) and the finding that transfection of wild-type p53 back into some of these cell lines suppresses the transformed phenotype (43) further suggest that hepatocellular transformation is linked to the loss of both the structural and functional integrity of p53. The in vitro findings that HBxAg seems to inactivate p53/ERCC3 complexes associated with transcription coupled repair (31) and inactivates an UV light-induced DNA repair enzyme by direct binding (29) are compatible with the hypothesis that HBxAg may promote genetic instability and the accumulation of p53 mutations.

To determine whether HBxAg inactivation of p53 occurs by direct binding to wild-type p53 and/or by promoting p53 mutations, p53 was characterized by IHC and exon sequencing from the tumor tissues of 16 HCC patients. These patients were selected from Xi'an province, a region of China in which aflatoxin contamination of foodstuffs is low and the mortality from HCC is only one-half to one-third that of regions with high levels of AFB1 contamination (e.g., Qidong, Jiangsu province). The correlation between HBxAg and p53 in tumors by IHC, combined with the low incidence of p53 mutations in HBxAg-positive liver and tumor sections, is compatible with the hypothesis that wild-type p53 is functionally inactivated by binding to HBxAg and not by mutation in HBxAg-positive livers and tumors.
MATERIALS AND METHODS

Tumors. Formalin-fixed paraffin-embedded tissues were obtained from 16 HCCs from Xi'an, People's Republic of China. Serial sections from the blocks were cut and evaluated by H&E staining, IHC, and microdissection of tumor tissue for molecular analysis.

Markers of HBV Infection. HBsAg in serum was detected by a commercially available assay (Abbott Laboratories, Chicago, IL). HBsAg in tissue was detected by staining using a commercially available kit according to the instructions of the manufacturer (histogram-peroxidase-antiperoxidase kits; Biogenex, Dublin, CA). Integrated HBV DNA was determined by analysis of cellular DNA by Southern blot hybridization. DNA was extracted from approximately 3 g of liver tissue using the proteinase K-phenol-chloroform method and then analyzed by electrophoresis on 1% agarose gels, either untreated or after digestion with EcoRI or HindIII. DNA was transferred to Zetabind membranes (Cuno Corp., Meriden, CT) and hybridized with [32P]dCTP-labeled full-length HBV DNA probe.

IHC for HBsAg and p53. Five-μm sections were examined for HBsAg and p53 proteins by peroxidase staining of an avidin-biotin complex conjugated to horseradish peroxidase (Vectastain ABC kit; Vector Laboratories, Burlingame, CA), as described previously (30, 44). Staining was carried out according to the manufacturer's recommendations. IHC for HBsAg was performed using a polyclonal rabbit antibody to a HBx-specific synthetic peptide (DuPont, Boston, MA). Each exon was sequenced in both directions, and two separate PCR products were examined for each sample in which a mutation was found. Complete coding sequences including splice sites were examined.

Statistical Analysis. Comparisons between two characteristics were carried out using Fisher's exact test. Relationships were considered statistically significant when P < 0.05 in a two-tailed analysis.

RESULTS

The Presence of Markers of HBV Infection. Serum HBsAg was determined for all cases. Among the 16 HCC patients, 8 (50%) were positive for HBsAg in the serum, whereas the others lacked serological evidence of HBV infection (Table 1). Due to the limited availability of frozen liver tissue, only 10 cases were studied for HBV DNA by Southern blot hybridization. Seven of these 10 cases (70%) demonstrated HBV DNA integration by Southern blotting in the liver and/or tumor (Table 1). Among carriers, most of the livers positive by Southern blot hybridization also had detectable HBsAg by IHC. However, only three of seven tumor samples from carriers who were tested had detectable HBsAg by IHC (Table 1). Hence, HBsAg was a common marker in the liver but not in the tumor tissue of these HBsAg carriers. Among patients serologically negative for HBV, two of four tested were positive by Southern blot hybridization; one of these also had detectable HBsAg in the liver, but none of those stained had detectable HBsAg in tumor tissue. These results indicate the presence of underlying HBV infection in some seronegative patients, which is in accordance with earlier observations (48, 49).

Both tumor and nontumor tissue, sometimes in the same section, were available for immunohistochemical evaluation of HBsAg. Among the 16 cases stained, nontumor tissue from 13 patients (81%) was clearly positive in more than 25% of the hepatocytes, whereas an

| Table 1 Summary of HBV and p53 markers in individual HCC patients

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>serum</th>
<th>T</th>
<th>NT</th>
<th>HBV DNA a (liver)</th>
<th>HBsAg IHC c</th>
<th>Int</th>
<th>Dist</th>
<th>Pat</th>
<th>p53 DNA sequence</th>
<th>p53 amino acid change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td>WT</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>WT</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>WT</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>WT</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td>WT</td>
<td>None</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>GTG→TAG at 236 d</td>
<td>Tyr→stop</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>WT</td>
<td>None</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>WT</td>
<td>None</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>WT</td>
<td>None</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td>WT</td>
<td>None</td>
</tr>
<tr>
<td>11</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td>WT</td>
<td>None</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>AGG→AGT at 249 e</td>
<td>Arg→Ser</td>
</tr>
<tr>
<td>13</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>GAG→TAG at 298 f</td>
<td>Glu→stop</td>
</tr>
<tr>
<td>14</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>WT</td>
<td>None</td>
</tr>
<tr>
<td>15</td>
<td>-</td>
<td>WT</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>WT</td>
<td>None</td>
</tr>
<tr>
<td>16</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>WT</td>
<td>None</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>11</td>
<td>11</td>
<td>10</td>
<td>6</td>
<td>3 patients with mutations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| a | T, tumor; NT, nontumor; Int, p53 staining intensity; Dist, staining distribution; Pat, pattern of staining; ND, not done; WT, wild-type. |
| b | The presence of HBV DNA in the liver was determined by Southern blot hybridization using full-length HBV DNA as probe. |
| c | Immunohistochemical staining for HBsAg was evaluated in the following way: −, negative; ±, scattered positive cells (<10% of cells); +, positive (>10% of cells). |
| d | p53 staining intensity in tumor cells was evaluated in the following way: −, negative; +, positive; ++, strongly positive. The staining distribution was evaluated as follows: ±, staining in <10% of cells; +, staining in 10–70% of cells; ++, staining in >70% of cells. The pattern of staining was evaluated as follows: ±, sporadic positive cells; +, focal or clustered staining; ++, diffuse or widespread staining. |
| e | Liver samples demonstrate HBsAg in the nuclei of tumor cells and in hepatocytes adjacent to the tumor. |
| f | Codon base change and codon number where mutations occur. |
additional 2 cases (12%) had detectable staining in less than 10% of the hepatocytes (Fig. 1). In all cases, staining was cytoplasmic. In some cases, the staining was diffuse over many cells in the section, whereas in other cases, the staining was lobular within clusters of cells. The 2 cases that had HBxAg detectable in less than 10% of the cells were characterized by staining in single cells scattered throughout the tissue section (Fig. 1). In patients 2, 3, 9, and 12 (Table 1), HBxAg was also observed in the nuclei of hepatocytes, especially in cells adjacent to the tumor (Fig. 1). HBxAg was detected in liver and/or tumor cells in all patients, regardless of their serum HBsAg status (Table 1). In cases in which the tumor and adjacent nontumor tissues were HBxAg positive, the staining in the nontumor was almost always more intense than in the adjacent tumor tissue (Fig. 1), suggesting differences in the levels of HBxAg expression in transformed compared to nontransformed cells. Furthermore, the finding of HBxAg-negative HCC in five patients who stained positive for this antigen in surrounding liver (Table 1) is consistent with the idea that persistent HBxAg expression, as detectable by

![Fig. 1. Patterns of HBxAg staining in tumor and surrounding nontumor liver tissue. A, cytoplasmic staining in many hepatocytes from a HBsAg carrier with chronic active hepatitis (patient 2). B, cytoplasmic staining in scattered hepatocytes from a HBsAg-negative patient with HCC (patient 10). C, tumor (top) and adjacent nontumor (bottom) tissues from a carrier (patient 3). Note that some of the intensely stained hepatocytes adjacent to the tumor are also positive for nuclear HBxAg (arrows). D, tumor (left) and adjacent nontumor (right) tissues from a carrier (patient 5). Note that the staining intensity is much greater in the nontumor compartment. E, normal human liver stained with anti-HBx. F, tumor and surrounding tissue from the patient in D stained with preimmune serum. Bar in A, 100 nm for all panels.](cancerres.aacrjournals.org)
IHC, is not required for tumor maintenance. The specificity of staining for these experiments was verified by controls (45) in which: (a) preimmune serum was used in place of primary antibody; (b) the immunizing X antigen synthetic peptide was used to block the binding of anti-HBx to the tissue; (c) normal liver powder failed to reduce the binding of primary antibody to the sample; (d) irrelevant peptide antibodies did not stain the tissues; and (e) normal (uninfected) liver did not stain with primary antibodies against HBxAg. Examples of some of these controls are presented in Fig. 1. Hence, HBxAg is a common marker in the liver and tumors of HCC patients, whether or not they had detectable HBsAg in the serum (Table 1). These observations are also in accordance with earlier results (50).

**p53 Expression and Mutation.** p53 staining was detected exclusively in the nuclei of tumor cells from six patients (Fig. 2). In patients 2, 3, 9, and 16, the staining was detected in single cells and cell clusters, whereas in patient 12, the staining was detected in a larger percentage of cells. In patient 13, more than 70% of the tumor cells had detectable p53 (Table 1). All p53-positive tumors also had detectable HBxAg (Table 1). Further analysis showed that there was a statistically significant relationship between p53 and HBxAg staining in tumor cells ($P = 0.039$; Table 2). Four of six patients who had detectable p53 by IHC also had evidence of nuclear HBxAg in both tumor cells and hepatocytes directly surrounding tumor nodules (Figs. 1 and 2). Although this relationship between nuclear HBxAg and p53 in tumor cells was statistically significant ($P = 0.0036$), the staining patterns for these two antigens in consecutive tissue sections only partially overlapped. Unlike tumor tissue, the detection of cytoplasmic HBxAg in non-tumor tissue had no predictive value on the possibility of finding p53 in the tumor by IHC, although tumor cells developed from nontransformed counterparts (Table 2). The presence of p53 in tumor tissue also failed to correlate with HBsAg status in the blood ($P = 0.31$). Hence, p53 staining occurs in a subset of HCC cases and correlates with HBxAg in tumor but not with HBxAg in nontumor or HBsAg in serum.

The status of p53 was further examined by PCR amplification and direct sequencing of DNA spanning exons 4–8 extracted from paraffin blocks. p53 mutations were identified in 3 of 16 patients (Table 1). Patient 13, who was most strongly positive for p53 by IHC, had a mutation in the 3rd bp of codon 249, which resulted in an Arg$\rightarrow$Ser mutation consistent with chronic aflatoxin exposure (Table 1; Fig. 3). The point mutations in patients 6 and 14 resulted in the creation of a premature translation stop codon in each case and are consistent with the idea that p53 stabilization may be due to mechanisms other than p53 mutation. These results are consistent with independent findings describing a lack of correlation between p53 IHC and mutant p53 genotype in neoplastic liver (51).

The finding of a statistically significant relationship between HBxAg and p53 staining in tumor tissue (Table 2) is consistent with the hypothesis that the binding of HBxAg to wild-type p53, and not mutant p53, is relevant to hepatocarcinogenesis. These findings are further supported by the statistically significant correlation between HBxAg staining in nontumor and wild-type p53 sequences (Table 2), suggesting that the persistence of HBxAg in the liver correlates with the corresponding persistence of wild-type p53 sequences during the pathogenesis of this tumor type.

**DISCUSSION**

This is the first study that compares the immunohistochemical staining of HBxAg and p53 in HCCs and further extends the analysis to the sequencing of p53 exons 4–8 in these same patients. The results demonstrate that HBV infection seems to be a common feature of HBsAg-negative HCC, as detected by Southern blot hybridization and/or immunohistochemical detection of HBxAg. These observations confirm and extend previous findings (48, 50) and suggest that underlying HBV is associated with HCC in HBsAg-negative cases, many of which are now being ascribed as being largely due to HCV (52, 53). The recent finding of HBV enhancer II region deletion variants in sera from patients serologically negative for HBV markers
who also have non-A, non-B hepatitis and cirrhosis (54, 55) is also consistent with the observation that HBV may be present in a considerable proportion of cases with HBV serologically negative chronic liver diseases. Although these findings do not diminish the important association between HCV and HCC, the high prevalence of HBxAg in the livers of HBsAg seronegative patients with HCC may be of etiological and prognostic significance.

The finding that HBxAg binds to both wild-type p53 and several mutants of p53 (31), in and of itself, does not clarify whether such binding is biologically relevant to the pathogenesis of HCC. However, the statistically significant correlation between HBxAg and p53 staining as well as between HBxAg staining in nontumor cells and wild-type p53 sequences within exons 4—8 suggests that HBxAg binding to wild-type p53 plays an important role in the pathogenesis of this tumor type. Indeed, previous work with fresh liver and tumor samples from these same patients demonstrated that HBxAg and p53 could be coimmunoprecipitated with both anti-x and anti-p53 (30), strongly suggesting that they form physical complexes. The results with human HCC samples reported herein also help to validate observations in other systems in which HBxAg has been shown to inactivate wild-type but not mutant p53 function (31, 56). This is especially pertinent to the binding of HBxAg to p53 in X transgenic mice that develop HCC (24, 32) because these mice have essentially only wild-type p53. The correlation between HBxAg and p53 staining in these mice, however, differs from that in this study, in part because the mice represent a “synchronized system,” whereas human HCC does not. For example, the X transgene encompassing the entire X open reading frame is integrated into the same position within resting, fully differentiated hepatocytes in the transgenic mice, but none of these characteristics apply to different cells in a chronically infected human liver. These differences may contribute to the apparent lack of extensive HBxAg and p53 colocalization in consecutive slides from tumors. Other work, demonstrating that HBxAg potentially binds a variety of other host proteins (26—29), may also contribute to the apparent discordance of HBxAg and p53 staining by IHC. Although the X transgenic mice seem to inactivate wild-type p53 by cytoplasmic translocation, and little evidence exists to support a predominantly cytoplasmic localization of p53 in human HCC (51), the fact that HBxAg binds to and inactivates wild-type p53 serves to link the pathogenesis of HCC in both the human and murine contexts. In both cases, persistent high expression of HBxAg was also associated with the development of HCC (32, 45, 57). Similar observations have also

![DNA sequence analysis of wild-type and point mutations in p53 found among some of the patients in this study.](image-url)

Fig. 3. DNA sequence analysis of wild-type and point mutations in p53 found among some of the patients in this study. A, wild-type p53 (patient 2). B, mutation at codon 249 (from patient 13). C, wild-type p53 (patient 3). D, Mutation at codon 236 (patient 6).
been reported in human HCC (58, 59). Whether sustained HBxAg overexpression results in the stabilization of p53 in wild-type or mutant conformation is an open question but is consistent with previous observations demonstrating a lack of correlation between p53 staining and p53 mutations in HCC (51). The statistically significant relationship between HBxAg and p53 expression in HCC (Table 2), especially in the nuclei of tumor cells, is also consistent with the hypothesis that HBxAg complexes with and stabilizes wild-type p53.

In light of these results, there is considerable support for the hypothesis that p53 inactivation is important to the pathogenesis of HCC (37, 60). For example, there is evidence for the appearance of p53 mutations both early and late in the pathogenesis of HCC. Although p53 point mutations have been found in nontumor liver, suggesting that they occur early in the process of tumor formation (41), other studies have suggested that they are associated with tumor progression (39, 60). However, non-AFB1-exposed populations with HBV-associated HCC have reportedly low frequencies of p53 mutations (37, 61—63), as observed in this study (Table 1). The latter result is supported by the lack of correlation between HBxAg staining in nontumor cells and missense p53 mutations, as determined by p53 exon sequencing (Table 2), suggesting that persistent HBxAg expression does not result in the accumulation of p53 mutations. Given the importance of p53 structural and corresponding functional integrity to the pathogenesis of this tumor type, it is proposed that there are two sequential steps of p53 inactivation that characterize the development and progression of HCC. The first step would involve inactivation of wild-type p53 by HBxAg. This would most likely occur in HBsAg carriers with progressive chronic liver disease, in which increased integration of HBV DNA fragments (including the X gene) would occur over a period of many years. It is proposed that when HBxAg becomes overexpressed over many years of infection (56—58), it would finally saturate the intracellular p53 stores, resulting in uncontrolled proliferation characteristic of HCC instead of controlled replication and regeneration. The finding that more that 70% of HBsAg carriers with cirrhosis and dysplasia have pronounced nuclear localization of HBxAg (57) suggests that in preneoplastic tissues, HBxAg may complex with and inactivate p53. This saturation of p53 by HBxAg is short-circuited in the X transgenic mice (32) without the need to have X gene sequences integrate during the course of chronic liver disease. If the binding of HBxAg to wild-type p53 alters p53 conformation and/or increases the half-life of p53 in tissue, it may partially account for the detection of p53 in tumors by IHC both here (Table 1) and elsewhere (51) and/or permit the detection of HBxAg–altered p53 by antibodies against mutant forms. The latter result may explain the apparent correlation between mutant p53 and HBxAg by IHC in independent work (64). HBxAg and p53 are only two proteins of a multicomponent system involved in the control of hepatocellular growth. Other proteins that bind to HBxAg and to p53 are likely to affect the subcellular localization and functional properties of each. In this context, it is likely that these other binding proteins contribute to the imperfect correlation between HBxAg and p53 by IHC. For example, the binding of HBxAg to a putative UV-induced DNA repair protein (29) and its putative inactivation of the p53/ERCC3 coupled transcription-repair mechanism (31) may allow the accumulation of mutations in p53 and elsewhere in the genome among rapidly dividing cells. These mutations accumulate during tumor progression. At this point in the pathogenic pathway, it is proposed that p53 inactivation by HBxAg would no longer provide a selection advantage (i.e., it would not be rate-limiting in the stimulation of hepatocellular proliferation) and that the correlation between HBxAg and p53 staining would break down. Some p53 mutants that exhibit oncogenic activity would further enhance the growth and expansion of their clones, accounting for the presence of p53 mutations in some cases (three in this study) that stain positive for HBxAg. In addition, the observation that HBxAg expression under selected conditions is incompatible with hepatocellular viability implies that HBxAg-negative HCC nodules would arise and predominate. The finding of HBxAg-negative HCC nodules in this and previous studies (57), combined with the lack of HBxAg-positive HCCs and HBxAg/p53 complex formation among tumors with documented p53 mutations (65), is consistent with this model. Hence, it is proposed that HBxAg inactivation of wild-type p53 and p53 inactivation by mutation are important to distinct stages in the pathogenesis of HCC.

Although the observations from this and previous studies (30—33, 56) suggest that the binding and inactivation of wild-type p53 by HBxAg is an important step in the mechanism of hepatocarcinogenesis, they do not exclude additional steps or completely alternative mechanisms as being operative in the pathogenesis of this tumor type. For example, the variable integration of HBV DNA during the course of chronic infection among different patients who develop HCC may result in the production of little or no HBxAg in some cases. If sustained high levels of HBxAg made from an integrated template are required for inactivation of p53, then the relatively low levels of HBxAg staining in the livers and tumors of patients 15 and 16 from this study, for example, suggest that other properties of HBxAg or other mechanisms of HCC may be more important in these cases. The uneven geographical exposure of human populations to aflatoxin, toxins that are metabolized by the liver, alcohol, HCV, and other chemical hepatocarcinogens further underscores that hepatocarcinogenesis may be mediated by different mechanisms under different circumstances. The recent finding that HBxAg may block p53-mediated apoptosis (33) suggests that if such a mechanism is operative in HCC, it may facilitate the action of other viruses and/or chemicals in the pathogenesis of this tumor type. On the other hand, the finding that the woodchuck hepatitis virus is a complete carcinogen (66) suggests that the chronic carrier state and the associated liver disease are necessary and sufficient for the development of HCC (3, 7—9), but that in nature, one or more of the other factors mentioned above may also contribute. Hence, the HBxAg-p53 relationship is likely to be one of several steps important to the development of HCC.

REFERENCES

11. Hatada, I., Tokino, T., Ochiya, T., and Matsubara, K. Co-amplification of integrated

4 M. A. Feitelson, unpublished observations.


Integrity of p53 in Hepatitis B x Antigen-positive and -negative Hepatocellular Carcinomas

M. S. Greenblatt, M. A. Feitelson, M. Zhu, et al.

Cancer Res 1997;57:426-432.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/57/3/426

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.