Mutation of p53 gene in Hepatocellular Carcinoma in Taiwan

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Abstract

To elucidate the role of p53 mutation in hepatocarcinogenesis in Taiwan, a hepatitis B viral infection hyperendemic area, exons 5 to 8 of the p53 gene in the tumor tissue of 61 hepatocellular carcinomas were amplified and sequenced. A total of 20 cases (32.8%) were found to have mutations; 36.6% (15 of 41) for the hepatitis B surface antigen positive group and 25.0% (5 of 20) for the hepatitis B surface antigen negative group. The corresponding normal liver showed no mutation. The mutation is widely distributed throughout exons 5 to 8. Only 4 cases (6.6%), all positive for hepatitis B surface antigen, had a specific hot spot mutation at codons 249 with G to T transversion. Our results show that scattered point mutations in p53 are not uncommon in hepatocellular carcinoma samples from Taiwan and may be important in the development of this cancer. However, the aflatoxin related specific mutation seems much less related to the genesis of hepatocellular carcinoma in Taiwan.

Introduction

HCC3 is one of the most common malignancies in the world (1). Chronic HBV infection, and dietary AFB1 contamination are considered as important etiological factors (2, 3). In recent years, the HCV infection was also implicated related to the genesis of HCC (4). Nevertheless, the precise molecular mechanism remained largely unknown. The p53 gene, which functions as an oncogene as well as a cancer suppressor gene, has been intensively investigated recently. Mutation of this gene has been identified in a variety of human cancers (5–8). Most of the mutations occurred randomly in this gene and tended to cluster in exons 5 to 8 (5–7), which is the most conserved region of the p53 gene. In HCC, a specific hot spot mutation at codon 249 of the p53 gene with G to T transversion was found in 9 HCC samples from Taiwan (18). In order to clarify this discrepancy and to elucidate the cause of hepatocarcinogenesis, we investigate the status of p53 mutation by studying the whole sequence of exons 5 to 8 of p53 gene in 61 HCC samples from Taiwan.

Materials and Methods

Materials. A total of 61 cases of pathology proved HCC were included in this study. In 60 of them, the tissues were obtained by surgical resection. Only one case was obtained by needle biopsy under ultrasound guidance (19, 20). Forty patients were males and 21 were females. The age ranged from 35 to 72 years (mean, 54 years). 41 were positive for HBsAg, and 20 were negative for HBsAg. Most of the HBsAg negative HCC patients were positive for antibody to HBsAg (anti-HBsAg) and/or antibody to hepatitis B core antigen (anti-HBcAg), and only 2 were negative for all HBV serological markers. Of the total 61 cases 21 were positive for antibody to HCV (anti-HCV). Among them, 17 were negative for HBsAg. The tumor size was less than 3 cm in 10 cases, between 3 and 5 cm in 20, between 5 and 10 cm in 23, and larger than 10 cm in the remaining 8. Most of the HCCs were detected by a prospective study for early detection of HCC in high risk subjects (19, 20). The nontumorous part was cirrhotic in 42, chronic persistent hepatitis in 14, congenital hepatic fibrosis in 1, and normal in 4.

Methods. The nucleotide sequences of exons 5 to 8 of the p53 gene from the tumor samples were studied. When the tumor was identified to have a mutation, the p53 sequences of the nontumorous liver tissue from the same individual were also evaluated.

DNA was extracted from all 61 tumor samples as described before (21). The RNA was reverse transcribed to cDNA using antisense primer P1035R: 5' ATTACGCTT CCTTACGATCT CAGAAGGCCT 3'. The cDNA was then subjected to a two step nested PCR (22). The primers used for the first PCR were P1035R (as above) and P273: 5' GCCCCTGTCA TCTTCTGTCC C1TCCCAGAA 3'. The primers used for the second PCR were P281 5' CATCTTCTGT CTTTCCAGAA 3' and P1004R 5' GCCCGGCGGA TCTGAAAGGG GATAATTCTT 3'.

Direct sequencing was performed with a commercial microtiter sequencing kit (Amersham, Buckinghamshire, United Kingdom) with slight modification of the original protocol. After PCR amplification, the PCR product was purified in a Centricon microconcentrator (Amicon, Danvers, MA). Four different primers were used as sequencing primer: P319: 5' TACGGTTCC GTCTGGGCTT 3'; P565: 5' GCCCCTGATC AGCATAT 3'; P654R20: 5' CACCACCA CTAATGCGAA 3'; P964R20: 5' GTCTTCTCT TGGGCTGGGA 3'. The primer-template mix was boiled for 5 min and then subjected to snap cooling in liquid nitrogen for 15 s. A labeling reaction was performed with [35S]-dATP at room temperature for 30 s and then the termination reactions were run at 37°C for 2 min. The reaction products were electrophoresed on a 8% polyacrylamide gel. After an overnight exposure, the film was read. When a mutation was identified, the reverse transcription, PCR, and sequence analysis were repeated to confirm the original results.

The hepatitis B markers were performed by radioimmunoassay (Ausria-II; Abbott Laboratories, North Chicago, IL). The anti-HCV was tested by a synthetic peptide based second generation anti-HCV immunoassay which included both the structural region and nonstructural regions as antigen (UBI HCV; United Biomedical Inc., Lake Success, NY) (21).

Results

Of the 61 cases with HCC, 20 (32.8%) were found to have mutations in exons 5 to 8 of the p53 gene of the tumor samples.

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: HCC, hepatocellular carcinoma; HBV, hepatitis B virus; AFB1, aflatoxin B1; HCV, hepatitis C virus; HBsAg, hepatitis B surface antigen; PCR, polymerase chain reaction.
Table 1: Clinical data and p53 mutation in 20 cases that had mutation

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>HBsAg</th>
<th>Anti-HCV</th>
<th>Tumor size (cm)</th>
<th>Codon change</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Liver state</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>56</td>
<td>P</td>
<td>P*</td>
<td>11.9</td>
<td>280</td>
<td>AGA → AGT</td>
<td>Arg → Ser</td>
<td>CPH</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>65</td>
<td>P</td>
<td>N</td>
<td>7.9</td>
<td>249</td>
<td>AGG → AGT</td>
<td>Arg → Ser</td>
<td>CPH</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>35</td>
<td>P</td>
<td>N</td>
<td>&gt;10</td>
<td>280</td>
<td>AGA → GGA</td>
<td>Arg → Gly</td>
<td>CIR</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>54</td>
<td>P</td>
<td>N</td>
<td>4.8</td>
<td>175</td>
<td>CGC → CAC/CGC</td>
<td>Arg → His</td>
<td>CIR</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>61</td>
<td>P</td>
<td>?</td>
<td>5.0</td>
<td>174</td>
<td>AGG → TGG</td>
<td>Arg → Trp</td>
<td>CPH</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>46</td>
<td>P</td>
<td>N</td>
<td>2.3</td>
<td>249</td>
<td>AGG → AGT</td>
<td>Arg → Ser</td>
<td>CIR</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>60</td>
<td>P</td>
<td>?</td>
<td>10</td>
<td>273</td>
<td>CGT → CTT</td>
<td>Arg → Leu</td>
<td>CIR</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>52</td>
<td>N</td>
<td>N</td>
<td>6.0</td>
<td>249</td>
<td>AGG → AGT/AGG</td>
<td>Arg → Ser</td>
<td>Normal</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>36</td>
<td>P</td>
<td>N</td>
<td>8</td>
<td>259</td>
<td>GAC → GTC</td>
<td>Arg → Val</td>
<td>CIR</td>
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<td>10</td>
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<td>P</td>
<td>P</td>
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<td>152</td>
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<tr>
<td>11</td>
<td>M</td>
<td>45</td>
<td>P</td>
<td>N</td>
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<td>TAC → CAC</td>
<td>Tyr → His</td>
<td>CIR</td>
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<tr>
<td>12</td>
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<td>N</td>
<td>N</td>
<td>8.0</td>
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<td>Gly → Arg</td>
<td>CPH</td>
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<tr>
<td>13</td>
<td>M</td>
<td>35</td>
<td>P</td>
<td>N</td>
<td>5.4</td>
<td>220</td>
<td>TAT → TGG/TAT</td>
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<td>CIR</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>54</td>
<td>P</td>
<td>P</td>
<td>7.8</td>
<td>249</td>
<td>AGG → AGT</td>
<td>Arg → Ser</td>
<td>CPH</td>
</tr>
<tr>
<td>15</td>
<td>M</td>
<td>59</td>
<td>P</td>
<td>N</td>
<td>7.4</td>
<td>179</td>
<td>CAT → CTT</td>
<td>His → Leu</td>
<td>CIR</td>
</tr>
<tr>
<td>16</td>
<td>F</td>
<td>53</td>
<td>N</td>
<td>N</td>
<td>3.5</td>
<td>174</td>
<td>AGG → TGG</td>
<td>Arg → Trp</td>
<td>CIR</td>
</tr>
<tr>
<td>17</td>
<td>F</td>
<td>53</td>
<td>N</td>
<td>P</td>
<td>2.0</td>
<td>159</td>
<td>GCC → CCC</td>
<td>Ala → Pro</td>
<td>CIR</td>
</tr>
<tr>
<td>18</td>
<td>F</td>
<td>57</td>
<td>N</td>
<td>P</td>
<td>4.8</td>
<td>248</td>
<td>CGA → CGT</td>
<td>Arg → Arg</td>
<td>CIR</td>
</tr>
<tr>
<td>19</td>
<td>M</td>
<td>58</td>
<td>N</td>
<td>P</td>
<td>5.4</td>
<td>162</td>
<td>ATC → TTC</td>
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<tr>
<td>20</td>
<td>M</td>
<td>63</td>
<td>N</td>
<td>P</td>
<td>2.3</td>
<td>280</td>
<td>AGA → AGT</td>
<td>Arg → Ser</td>
<td>CIR</td>
</tr>
</tbody>
</table>

* ? not done; CIR, cirrhosis; CPH, chronic persistent hepatitis.

In the HBsAg positive group, 15 of 41 (36.6%) had mutations in the tumor samples. Among them, 4 had mutation at codon 249, with G to T transversion of the third base (arginine to serine). The other mutations occurred randomly from codons 152 to 280; 2 cases had same mutations at codon 280 while the remaining mutations were noted in only one case each. The nucleotide changes most often occurred in G (7 cases) and A (6 cases) and most frequently involved amino acid arginine (9 cases).
In the HBsAg negative group, 5 of 20 (25.0%) had mutation in the tumors samples (Table 1). Four of the 5 cases were positive for anti-HCV. The mutations occurred from codons 159 to 280. There was no mutation at codon 249 in this HBsAg negative group. The amino acids changes involved arginine (2 cases with arginine to tryptophan and serine, respectively), alanine (to proline in one case), and isoleucine (to phenylalanine in one case). In the remaining one case, the mutation did not result in the substitution of the encoding amino acid (arginine).

Discussion

Taiwan is a HBV hyperendemic area and is highly prevalent in HCC (1-3). Contamination of food with AFB1 was also rather frequent in earlier investigations (14-16). In this study, we showed that p53 mutation in Taiwan was not uncommon. However, the mutation is distributed widely throughout exons 5 to 8; only 4, all positive for HBsAg, had a specific G to T transversion at the third base of codon 249. The frequency of codon 249 p53 mutations in this series is lower than that found in Qidong, China, and South Africa (9, 10); Nevertheless, our frequency is still higher than that found in the low AFB1 exposure area (11, 12). Thus although AFB1 might play an important role in the development of liver cancer in areas where both HBV infection and AFB1 contamination are frequent, its role in Taiwan seems much less important. The reason for the relatively lower rate of AFB1 related specific codon 249 mutation in our area might be due to the smaller severity of AFB1 exposure in Taiwan as compared to Qidong, China, and South Africa (9, 10, 17).

Except for the 4 cases in the HBsAg positive group with specific mutations at codon 249, the distribution pattern of the remaining mutations of the p53 gene seemed to have no specific difference from those of HBsAg negative group. Furthermore, in the 5 HBsAg negative cases with mutation, 4 had serological evidence of HCV infection. The cause of nonspecific p53 mutations in our HCC is unknown. Other environmental carcinogens besides AFB1 may be related to the mutation of p53 gene which in turn might interact with the HBV or HCV and lead to the genesis of HCC (23). On the contrary, the not uncommon frequencies of mutation of p53 might represent only one of the multiple steps involved in carcinogenesis such as in other human cancers (5-7, 24).

In this series, no mutations were noted in the corresponding nontumor part, suggesting that the p53 mutation in our HCC was somatic. In the 20 HCC samples with mutation, 17 had a single mutant band. This result most probably represents the achievement of a homozygous state resulting from the pairing of a mutant allele with a deletion in the remaining allele as in other cancers (5-7). In the remaining 3 cases, the sequencing gels revealed both the mutant as well as the wild type. The wild type may be derived from the contamination of tumor DNA by the normal stroma or liver DNA or that these tumors had a p53 mutation but no deletion of the remaining wild type p53 allele. Finally, 4 cases with the tumor size smaller than 3 cm had mutation, and 5 of the 20 cases with p53 mutations had no coexisting liver cirrhosis suggesting that p53 mutation might present as early as the very early stage of HCC and can occur irrespective of the underlying liver state. In conclusion, our results using a large series of HCC samples shows that, like other kinds of human solid tumor, scattered point mutations of p53 are rather common in HCC in Taiwan and may be important in the development of HCC. However, the AFB1 related specific mutation seems much less important in the genesis of HCC in Taiwan.

References

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