ABSTRACT

Hepatocellular carcinoma, sometimes shows multiple tumor nodules, therefore poses a problem of differential diagnosis between cancers of multifocal origin and those of metastatic origin. Conventionally, pathological criteria have been used for this purpose, but these are largely subjective. In order to facilitate more objective differential diagnosis of multiple hepatocellular carcinoma, we used the pattern of mutation of the p53 gene as a marker for each tumor nodule. We studied 58 nodules from 26 cases of multiple hepatocellular carcinoma using polymerase chain reaction-single strand conformation polymorphism analysis, a simple method for detecting mutations. p53 gene mutations were detected in 65% (17 of 26) of cases. The internodule mutation patterns were heterogeneous in 11 cases and homogeneous in 6, enabling a multifocal origin to be diagnosed in the former and a metastatic origin in the latter at the genetic level. Moreover, the origin of recurrent tumors was determined from the mutation pattern. It is concluded that analysis of p53 mutations seems to be useful for differentiating the origin of multiple cancers, since the information it yields is essentially objective.

INTRODUCTION

The frequent presence of multiple nodules in patients with HCC often provides a problem of differentiation between cancer of multifocal origin and different cancer foci originating from a single tumor through metastasis. This issue is important from a clinical viewpoint, since these two categories correspond to different stages of the disease, and therefore subsequent treatment and prognosis would also differ. However, clinical studies of this aspect have not been adequate up to now because of the difficulty involved in differential diagnosis of multiple cancers.

Pathological criteria, based mainly on macroscopic and histological observations, have been proposed for differential diagnosis of multiple HCC (1). Differentiation is relatively easy when nodules show different histology, but in cases with similar histology and/or when tumors are closely adjacent it becomes very complicated. In any event, the final diagnosis is left to the subjective judgment of a pathologist where objective evidence is lacking. This uncertainty has prevented the advance of clinical and basic studies of multiple HCC. Therefore, objective methods for the differential diagnosis of multiple cancers and experimental demonstration of the validity of existing pathological criteria are clearly desirable.

As an objective approach, some genetic methods for analysis of multifocal HCC have already been applied, but they have been far from satisfactory. One example is Southern blot analysis of the integration pattern of hepatitis B virus in multiple HCC (2, 3) but this is applicable to only a small percentage of HCCs which harbor the virus (4).

In order to find a quicker and more reliable method for the differential diagnosis of multiple HCC, we focused on the mutation pattern of the p53 gene. Since an accumulation of various genetic alterations has been demonstrated in carcinoma cells (5), we reasoned that genetic abnormalities in different nodules would probably differ in cases of multifocal origin. Mutations of the p53 gene have been observed in 29–36% of single-nodule HCCs (6), and a higher frequency would be expected in cases with multiple nodules.

In the present study we analyzed the p53 mutation pattern in 58 nodules from 26 cases of HCC by the PCR-SSCP (7), a simple and sensitive method for detection of DNA mutations. We report here that analysis of the p53 mutation pattern could be a useful marker for the differential diagnosis of multiple cancers.

MATERIALS AND METHODS

Patients and DNA Extraction. Fifty-eight samples of tumor tissue obtained from 26 patients who had undergone surgical treatment for multiple HCC at the National Cancer Center Hospital, Tokyo, were studied. Four cases each involved 3 nodules, and the remaining 22 cases had 2 nodules each. The main tumor nodule in each case, i.e., the larger and/or less differentiated one, was named T1. In both case 2 and case 5, another tumor was obtained at a second operation, and this was an intrahepatic recurrent nodule and an abdominal lymph node metastasis, respectively (see Table 1). We classified the cases into 3 groups according to the pathological criteria (1, 2, 8, 9) shown in Fig. 1: multifocal (19 cases); metastatic (4 cases); undetermined (3 cases). DNA was extracted by digestion with proteinase K, extraction with phenol/ chloroform, and precipitation with ethanol (10).

PCR-SSCP Analysis. Because 98% of p53 gene mutations in different cancers have been found in exons 5–8 (11), we focused our study on these exons. The PCR-SSCP method described by Orita et al. (7) was used to detect the presence and pattern of p53 gene mutation.

The oligonucleotide primers for amplification of exons 5–8 were designed based on the published sequence (12):

Exon 5:
5' GGAATTCCTTCTTCTGAGTAC3',
5' GGAATTGCCCAGCTCACATCG3'

Exon 6:
5' GGAATTCGATGTCACGATGGG3',
5' GGAATTCAGTTGCAAACCAGACCTCAGG3'

Exon 7:
5' GGAATTCCTCTTCTTGACGATGGG3',
5' GGAATTCGATGTCACGATGGG3'

Exon 8:
5' GGAATTCCTTATCTGAGTGGGTGAA3',
5' GGAATTCCTCTTATCTGAGTGGGTGAA3'

One hundred ng of genomic DNA were subjected to 35 cycles of PCR (94°C, 55°C, 72°C for 0.5, 0.5, 1 min, respectively) in 10 µl of solution

1 To whom requests for reprints should be addressed.
2 Supported by a Foreign Research Fellowship from the Foundation for Promotion of Cancer Research, Tokyo, Japan. Present address: Institute of Pathology, Verona University, Strada Le Grazie, 37134 Verona, Italy.
3 Awardee of a Research Resident Fellowship.
4 Supported by a Grant-in-Aid for the Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health and Welfare of Japan.
Table 1  p53 mutation pattern in multiple hepatocellular carcinomas

<table>
<thead>
<tr>
<th>Case</th>
<th>Nodule</th>
<th>Diagnosis</th>
<th>Size (cm)</th>
<th>Differentiation</th>
<th>Abnormal band</th>
<th>Exon</th>
<th>Codon</th>
<th>Nucleotide</th>
<th>Amino acid</th>
<th>Mutation pattern*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T1</td>
<td>Multifocal</td>
<td>1.9</td>
<td>Well</td>
<td>+</td>
<td>8</td>
<td>282</td>
<td>CGG→TGG</td>
<td>Arg→Trp</td>
<td>A-0</td>
</tr>
<tr>
<td>T2</td>
<td>1.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>T1</td>
<td>Multifocal</td>
<td>6.5</td>
<td>Poor</td>
<td>+</td>
<td>7</td>
<td>241</td>
<td>TCC→GCC</td>
<td>Ser→Ala</td>
<td>A-B</td>
</tr>
<tr>
<td>T2</td>
<td>5.5</td>
<td>Poor</td>
<td></td>
<td></td>
<td>+</td>
<td>7</td>
<td>249</td>
<td>AGG→TGG</td>
<td>Arg→Trp</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>2.2</td>
<td>Moderate</td>
<td></td>
<td></td>
<td>+</td>
<td>7</td>
<td>249</td>
<td>AGG→TGG</td>
<td>Arg→Trp</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>T1</td>
<td>Multifocal</td>
<td>7.6</td>
<td>Moderate</td>
<td>+</td>
<td>5</td>
<td>176</td>
<td>TGC→AGC</td>
<td>Cys→Ser</td>
<td>A-0</td>
</tr>
<tr>
<td>T2</td>
<td>2.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>T1</td>
<td>Multifocal</td>
<td>4.3</td>
<td>Poor</td>
<td>+</td>
<td>7</td>
<td>239</td>
<td>AAC→AGC</td>
<td>Asn→Ser</td>
<td>A-0</td>
</tr>
<tr>
<td>T2</td>
<td>1.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>T1</td>
<td>Multifocal</td>
<td>2.0</td>
<td>Poor</td>
<td>+</td>
<td>7</td>
<td>236</td>
<td>TAC→TGC</td>
<td>Tyr→Cys</td>
<td>A-0</td>
</tr>
<tr>
<td>T2</td>
<td>2.6</td>
<td>Well</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LN</td>
<td>7.0</td>
<td>Poor</td>
<td></td>
<td></td>
<td>+</td>
<td>7</td>
<td>236</td>
<td>TAC→TGC</td>
<td>Tyr→Cys</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>T1</td>
<td>Multifocal</td>
<td>2.7</td>
<td>Moderate</td>
<td>+</td>
<td>5</td>
<td>130</td>
<td>CTC→CGC</td>
<td>Leu→Arg</td>
<td>A-0</td>
</tr>
<tr>
<td>T2</td>
<td>1.8</td>
<td>Moderate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>1.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>T1</td>
<td>Multifocal</td>
<td>3.8</td>
<td>Poor</td>
<td>+</td>
<td>7</td>
<td>248</td>
<td>CGG→CAG</td>
<td>Arg→Gln</td>
<td>A-0</td>
</tr>
<tr>
<td>T2</td>
<td>3.1</td>
<td>Moderate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>1.8</td>
<td>Poor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>T1</td>
<td>Multifocal</td>
<td>4.5</td>
<td>Poor</td>
<td>+</td>
<td>5</td>
<td>157-159</td>
<td>7-base pair del</td>
<td>Frame shift</td>
<td>A-0</td>
</tr>
<tr>
<td>T2</td>
<td>1.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>T1</td>
<td>Multifocal</td>
<td>3.0</td>
<td>Poor</td>
<td>+</td>
<td>7</td>
<td>237-243</td>
<td>16-base pair del</td>
<td>Frame shift</td>
<td>A-0</td>
</tr>
<tr>
<td>T2</td>
<td>1.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>T1</td>
<td>Multifocal</td>
<td>5.2</td>
<td>Moderate</td>
<td>+</td>
<td>5</td>
<td>141</td>
<td>1-base pair del</td>
<td>Frame shift</td>
<td>A-B</td>
</tr>
<tr>
<td>T2</td>
<td>3.6</td>
<td>Combined</td>
<td></td>
<td></td>
<td>+</td>
<td>7</td>
<td>249</td>
<td>AGG→AGT</td>
<td>Arg→Ser</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>T1</td>
<td>Multifocal</td>
<td>2.8</td>
<td>Moderate</td>
<td>+</td>
<td>5</td>
<td>163</td>
<td>TAC→AAC</td>
<td>Tyr→Asn</td>
<td>A-0</td>
</tr>
<tr>
<td>T2</td>
<td>2.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>T1</td>
<td>Metastatic</td>
<td>10.5</td>
<td>Moderate</td>
<td>+</td>
<td>7</td>
<td>245</td>
<td>GCC→TGC</td>
<td>Gly→Cys</td>
<td>A-A</td>
</tr>
<tr>
<td>T2</td>
<td>0.8</td>
<td>Moderate</td>
<td></td>
<td></td>
<td>+</td>
<td>7</td>
<td>245</td>
<td>GCC→TGC</td>
<td>Gly→Cys</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>T1</td>
<td>Metastatic</td>
<td>9.0</td>
<td>Moderate</td>
<td>+</td>
<td>7</td>
<td>242</td>
<td>TGC→TTC</td>
<td>Cys→Phe</td>
<td>A-A</td>
</tr>
<tr>
<td>T2</td>
<td>4.5</td>
<td>Moderate</td>
<td></td>
<td></td>
<td>+</td>
<td>7</td>
<td>242</td>
<td>TGC→TTC</td>
<td>Cys→Phe</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>T1</td>
<td>Metastatic</td>
<td>6.5</td>
<td>Poor</td>
<td>+</td>
<td>6</td>
<td>219</td>
<td>1-base pair del</td>
<td>Frame shift</td>
<td>A-A</td>
</tr>
<tr>
<td>T2</td>
<td>1.2</td>
<td>Poor</td>
<td></td>
<td></td>
<td>+</td>
<td>6</td>
<td>219</td>
<td>1-base pair del</td>
<td>Frame shift</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>T1</td>
<td>Undetermined</td>
<td>4.5</td>
<td>Poor</td>
<td>+</td>
<td>8</td>
<td>273</td>
<td>CGT→TGT</td>
<td>Arg→Cys</td>
<td>A-A</td>
</tr>
<tr>
<td>T2</td>
<td>3.9</td>
<td>Poor</td>
<td></td>
<td></td>
<td>+</td>
<td>8</td>
<td>273</td>
<td>CGT→TGT</td>
<td>Arg→Cys</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>T1</td>
<td>Undetermined</td>
<td>4.0</td>
<td>Poor</td>
<td>+</td>
<td>8</td>
<td>249</td>
<td>AGG→TGG</td>
<td>Arg→Trp</td>
<td>A-A</td>
</tr>
<tr>
<td>T2</td>
<td>0.9</td>
<td>Moderate</td>
<td></td>
<td></td>
<td>+</td>
<td>8</td>
<td>249</td>
<td>AGG→TGG</td>
<td>Arg→Trp</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>T1</td>
<td>Undetermined</td>
<td>4.5</td>
<td>Poor</td>
<td>+</td>
<td>8</td>
<td>278</td>
<td>CCT→CCT</td>
<td>Pro→Arg</td>
<td>A-A</td>
</tr>
<tr>
<td>T2</td>
<td>1.8</td>
<td>Moderate</td>
<td></td>
<td></td>
<td>+</td>
<td>8</td>
<td>278</td>
<td>CCT→CCT</td>
<td>Pro→Arg</td>
<td></td>
</tr>
</tbody>
</table>

* A-A, same mutation in different nodules; A-B, different mutations in different nodules; A-0, mutation in one of the nodules; R, recurrent liver tumor resected 8 months after the previous operation; LN, abdominal lymph node metastasis found 2 years after the previous operation; Combined, combined histology of cholangiocellular and hepatocellular carcinoma.

Direct DNA Sequencing. Abnormal bands detected by SSCP analysis were eluted from the gel and amplified by 55 cycles of asymmetrical (20:1 primer ratio) PCR (14). The single strand products were purified in a Centricon 30 microconcentrator (Amicon, Beverly, MA) and subjected to sequencing using a 7-deaza-GTP Sequenase version 2 kit (United States Biochemicals, Cleveland, OH) with 5'-end-labeled primers. The sequencing primers for sense (s) and antisense (a) of each exon were as follows:

5-s  5'-TCTTCGCTGACTCTCCCT3',
5-a  5'-AGCAGCTCCTACATCGAT3',
6-s  5'-CCTGATGCTCTCAGTT3',
6-a  5'-TGCAAACCAGACTCGAG3',

containing 0.25 pmol each of 5'-end-labeled primers (13), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.01% gelatin, 20 μM deoxynucleoside triphosphates, and 0.5 unit of Taq polymerase (Perkin Elmer/Cetus, Norwalk, CT).

The PCR products were diluted 1:100 in loading solution (96% formamide, 20 mM EDTA, 0.05% xylene cyanol, and bromophenol blue), denatured at 85°C for 3 min, and applied (1 μl/lane) to 6% polyacrylamide/Tris-borate EDTA gel with and without 5% (v/v) glycerol. Electrophoresis was performed at 30 W for 2.5–5 h at room temperature with vigorous air cooling. The gel was dried on filter paper and exposed to X-ray film (Kodak XR-P-1) at room temperature for 2–12 h.

Direct DNA Sequencing. Abnormal bands detected by SSCP analysis were eluted from the gel and amplified by 55 cycles of asymmetrical (20:1 primer ratio) PCR (14). The single strand products were purified in a Centricon 30 microconcentrator (Amicon, Beverly, MA) and subjected to sequencing using a 7-deaza-GTP Sequenase version 2 kit (United States Biochemicals, Cleveland, OH) with 5'-end-labeled primers. The sequencing primers for sense (s) and antisense (a) of each exon were as follows:

5-s  5'-TCTTCGCTGACTCTCCCT3',
5-a  5'-AGCAGCTCCTACATCGAT3',
6-s  5'-CCTGATGCTCTCAGTT3',
6-a  5'-TGCAAACCAGACTCGAG3',

3675
p53 mutations obtained differed from case to case. Only cases
of well differentiated peripheral lesions (2, 9).

The range over which mutations occurred was spread over 456 base pairs of codons 130-282. It is worth noting that the
recurrent hepatic nodule in case 2 showed the same mutation
as that of nodule T1 and the lymph node metastasis in case 5
displayed the same mutation as that of nodule T2 and the lymph node metastasis in case 5.

The products were analyzed in 8% polyacrylamide/Tris-borate EDTA
gel containing 5 M urea.

RESULTS

Seventeen of the 26 (65%) multiple HCC cases showed
abnormally shifted bands in at least one of their nodules upon
SCCP analysis of the p53 gene. The remaining 9 cases were
negative. All of the abnormal bands were analyzed further by
direct DNA sequencing, and the results are summarized in
Table 1. The positive cases were classified into three groups
according to the pattern of mutation between nodules as follows
(see Table 1 and representative cases are presented in Fig. 1):

- Group 1, A-A pattern: same mutation in different nodules (6 cases);
- Group 2, A-B pattern: different mutations in different nodules (2 cases);
- Group 3, A-0 pattern: mutation in one of the nodules (9 cases).

The range over which mutations occurred was spread over 456 base pairs of codons 130–282. It is worth noting that the
p53 mutations obtained differed from case to case. Only cases
16 and 2 (nodule T2) displayed the same mutation, although
case 2 also possessed a different mutation in nodule Tl.

Of the two samples obtained at a second operation, the
recurrent hepatic nodule in case 2 showed the same mutation
as that of nodule T2 and the lymph node metastasis in case 5
displayed the same mutation as that of nodule T1.

A comparison between the pathological diagnosis and the
p53 mutation pattern is as follows. In 19 cases of pathologically
multifocal HCC, p53 mutation was observed in 11 (58%). The
A-B pattern was detected in 2 cases, and the other 9 cases showed the A-0 pattern, but none of multifocal cases showed
the A-A pattern. Three (75%) of 4 pathologically metastatic
cases were positive for the p53 mutation, and their patterns
were all A-A. All 3 of the pathologically unclassified cases
were also informative in the present analysis, showing the A-A
pattern.

DISCUSSION

The main concept that we propose here is the application
of gene mutation to provide a marker of each tumor nodule. p53
gene mutation analysis of cases of multiple HCC was demonstr-
ated to be a good method for objective differentiation be-
tween multifocal and metastatic multiple tumor nodules. In
fact, as many as 65% (17 of 26) of multiple HCCs showed the
p53 gene mutation in at least one nodule. The mutations
differed from case to case, showing a large diversity that served
as a useful marker for each tumor nodule.

Our analysis showed the existence of three different patterns of p53 mutation in multiple HCC. Patterns A-B and A-0
indicating heterogeneity of the p53 mutation between nodules,
with regard to type and presence, respectively, suggest different
origins from different cellular clones. Therefore, the cases showing
these two genetic patterns could be diagnosed as multifocal
in origin. On the other hand, nodules showing the same genetic
abnormality in cases with an A-A pattern are likely to be derived
from the same clone, and thus metastatic in origin.

However, a few further possibilities should be considered.
One is that a case with metastatic nodules from a p53 mutation-
negative main tumor could show an A-0 pattern when one of
the nodules acquires the mutation through later progression.
Although p53 abnormalities are regarded as a late event in
cancer progression, they usually occur earlier than the forma-
tion of metastasis in a genetic model for colorectal tumorigen-
esis (15). Moreover, the mutations in cases with an A-0 pattern
were always observed in T1 (primary, larger and/or less differ-
entiated tumors) and never in T2 or T3 (secondary, smaller
and/or less advanced tumors), and the possibility that T2 or T3
may have arisen as metastatic tumors from T1 should be very
small. None of 11 T2 or T3 tumors with A-0 patterns presented
portal vein tumor thrombi while HCC metastasizes mainly through
portal veins. In addition, 6 of these 11 tumors were
differentiated including 2 early HCCs which rarely show
metastasis (1, 8). From these findings, the possibility that T2
giving rise to the T1 should be very small again. Therefore,
we considered that the genetic heterogeneity found in cases
with an A-0 pattern is mainly due to the multifocal origin of
the different nodules. Another possibility is that cases of multifocal
HCC could show an A-A pattern by possessing the same
mutation by chance. However, this possibility will be extremely
low at least in Japanese cases of HCC, since in this investigation
and that of Murakami et al. (6) all but one of the mutations
differed among the examined cases. It was recently reported
that p53 mutations of HCC in certain areas of China and South
Africa, where there is heavy exposure to aflatoxin B1, were
almost all localized to codon 249, but this could be an excep-
tional phenomenon related to the special etiology (16, 17).

The usefulness of our approach is supported also by the fact
that it was possible to determine a metastatic or multifocal
origin in three of four cases where morphological analysis was
not helpful. Because these cases showed A-A patterns, we were
able to recognize their metastatic origin. Another notable result
of our study was that the origin of recurrent tumors and
metastasis was clarified through analysis of the p53 mutation
pattern. That is, the same mutation of R as that of T2 in case
2 and of LN with that of T1 in case 5 suggested their metastatic
origin from one of the nodules of the respective primary HCCs.

Furthermore, by comparing the differential diagnosis of mul-
tiple HCCs based on morphological information and the results
of genetic analysis, no discrepancy was observed among the
comparable cases, i.e., those positive for the p53 mutation. This
lends objective support to the validity of the pathological cri-
eria used at the National Cancer Center Hospital, Tokyo
(shown in Fig. 1) for the differential diagnosis of multiple
HCCs. However, the clinical need for a preoperative (prether-
apeutic) objective diagnosis of a large number of cases can be
efficiently met by genetic analysis of p53 mutations in biopsy

Fig. 1. Morphological criteria for differential diagnosis of multiple hepatocel-
ular carcinoma. These criteria are based on the following findings: 1, HCC
metastasizes mainly through the portal vein (1); 2, HCC at an early stage does
not show metastasis (1, 8); 3, primary HCC often shows morphological evidence
of well differentiated peripheral lesions (2, 9).

Table 1. The positive cases were classified into three groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-A</td>
<td>Tumor apparently growing from portal vein tumor thrombi or</td>
</tr>
<tr>
<td></td>
<td>Multiple early HCCs or concurrent early and advanced HCCs</td>
</tr>
<tr>
<td></td>
<td>Presence of peripheral area of very well differentiated HCC in both lesions or in the smaller one</td>
</tr>
<tr>
<td></td>
<td>Multiple HCCs of obviously different histology</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Metastatic origin</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Multifocal origin</td>
</tr>
<tr>
<td></td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Undetermined</td>
</tr>
</tbody>
</table>

3676
Fig. 2. Cases representative of the three mutation patterns of the p53 gene in multiple hepatocellular carcinoma. For SSCP analysis DNAs from different tumors of the same patient (T1 and T2) and a normal control (N) were analyzed for abnormalities of p53 gene exons. Cases were considered positive for mutation when bands different from that of normal controls were observed (arrowhead). For DNA sequencing analysis (lower half of each pattern panel) the sequence obtained from the abnormal bands evident on SSCP analysis are shown. Each sequence is shown 5' (bottom) to 3' (top) for the noncoding strand for exon 7 and the coding strand for exon 6. The codons at which the mutation occurs are indicated. For exon 7, the indicated letters are the translated coding sequence obtained from the results. A-A pattern, different nodules with the same mutation; A-B pattern, different nodules with different mutations; A-0 pattern, only one of the nodules has a mutation.

material obtained by fine-needle aspiration.

The PCR-SSCP method has up to now been the easiest and quickest method for detecting point mutations and allows immediate comparisons to be made among different tumors without sequencing. In the present study, although we sequenced all the samples that were positive upon SSCP analysis, a simpler approach involving only PCR-SSCP would be more than adequate for determining whether the gene mutations are different or the same in different samples.

The present approach should prove applicable to differential diagnosis of other multiple cancers such as those of the colon, lung, and bladder, in which p53 abnormalities are also observed frequently (11). However, use of p53 may not be decisive, since p53 abnormalities are not present in every case and are considered to be a relatively late event in cancer progression (6, 15). When the genetic alterations involved in the early stage of carcinogenesis are elucidated, analysis of these genes may yield much accurate information. Finally, we expect that clinical studies of multiple cancer will advance, aided by the objective diagnosis provided by the present method, in conjunction with existing pathological criteria.

REFERENCES

2. Tsuda, H., Hirohashi, S., Shimosato, Y., Terada, M., and Hasegawa, H. Clonal origin of atypical adenomatous hyperplasia of the liver and clonal...
GENETIC DIFFERENTIATION OF MULTIPLE CANCERS


Mutation Pattern of the \textit{p53} Gene as a Diagnostic Marker for Multiple Hepatocellular Carcinoma


\textbf{Updated version} Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/52/13/3674

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

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

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