Hepatocellular carcinoma mutation

SIR — In hepatocellular carcinoma (HCC), mutations within the p53 gene occur selectively at the third position of codon 249 (refs 1,2), unlike other human cancers where many different codons have been involved (ref. 3 and references therein). The patients in refs 1 and 2 came from areas of the world (South Africa and Qidong province of China) where there is a high exposure to hepatitis B virus (HBV) and potentially a high exposure to aflatoxin B₁. It has been suggested that aflatoxin B₁ is responsible for the observed mutations^{1,2}, but all patients who carried p53 mutations had been or were infected with HBV. HBV infection could have been the cause of these p53 mutations, or more likely, the interaction between HBV and aflatoxin B₁ could be the necessary prerequisite for the generation of these changes in codon 249. It is thus of great interest to determine whether codon 249 mutations occur in people with HCC who have not been exposed either to HBV or to high levels of aflatoxin B₁, or have been exposed to HBV in the absence of aflatoxin B₁.

We have screened 16 HCC DNA samples, from patients whose demographic details are listed in the table, for mutations at codon 249 of the p53 gene. Any changes to the second or third bases of codon 249 in exon 7 of the p53 gene result in the abolition of a restriction site for the enzyme *Hae*III (ref. 2). We have combined the polymerase chain reaction

(PCR) with restriction analysis to study possible mutations of this codon in our HCC samples. Oligonucleotide primers (upstream 5'-TCTCCTAGGTTGGCTCTGACT-3' and downstream 5'-TCCTGACCTGGAGTCTTCCAG-3') were used to amplify a 125-base-pair fragment containing all of exon 7 and part of the flanking introns⁴. We carried out PCR on 100–200 ng of HCC DNA using 50 pmol of each primer, 2 U *Taq* polymerase (Promega) in 1 × *Taq* polymerase buffer (Promega) in a total volume of 50 µl. We performed 30 cycles at 94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s. The products were run through 1% Seakem ME (FMC) + 2% NuSieve GTG (FMC) agarose gels and stained with ethidium-bromide.

The 125-base-pair fragments containing exon 7 were excised from the gel and eluted from the agarose by centrifugation⁵. We added MgCl₂ to the eluate to find concentration of 10mM and precipitated DNA by addition of two volumes of ethanol and leaving at –20 °C overnight.

The DNA was pelleted by centrifugation for 15 min, washed once with 70% ethanol, desiccated and resuspended in 7 µl 10 mM Tris, 1 mM EDTA, pH 8. These fragments were then restricted

for 3.5 h at 37 °C with 10 U *Hae*III (Toyobo) in a final volume of 10 µl. The resulting digests were then electrophoresed through 1.5% Seakem ME +2.5% NuSieve GTG agarose gels. Wild-type p53 alleles would result in cleavage by *Hae*III at codon 249, giving products of 83 and 42 base pairs using the primers indicated above. Any changes at codon 249 at position 2 or 3

Nine representative HCC samples digested with *Hae*III. Each shows bands at 42 (lower) and 83 base pairs (upper), indicating total cleavage of the original (unmutated) 125-base-pair PCR fragments. Right-hand lane, marker fragments, generated by *Hinf*I cleavage of phage ϕ X174 DNA.

would result in destruction of the *Hae*III site and an uncleaved product of 125 base pairs.

All our HCC samples (see figure) were cleaved by *Hae*III at codon 249 of p53. Thus none of the patients was found to have mutations at this position. Our results therefore support the postulated role^{1,2} of aflatoxin B₁ in the generation of specific mutations of the p53 gene and, in addition, provide evidence to suggest that HBV infection alone does not contribute to these base changes.

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Scientific Correspondence

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DNA FROM PATIENTS WITH HEPATOCELLULAR CARCINOMA

Patient	Sex/Age	Ethnic origin	Cirrhosis	Histology	Grade	Predisposing factor	Presentation
HBV–							
1	F44	Caucasian	–	pleo	2		early
2	F36	Caucasian	–	trab	1		early
3	M71	Caucasian	–	trab	2		late
4	M67	Caucasian	+	trab	2	haem	late
5	M68	Caucasian	+	pseu	1–3	alco	late
6	M76	Caucasian	+	trab	3		late
7	M79	Caucasian	+	trab	2	haem	late
8	M68	Caucasian	+	trab	2–3	haem	late
HBV+							
9	M51	Melanesian	–	trab	2		early
10	M52	Aborigine	+	trab	1		early
11	M52	Caucasian	+	trab	1		early
12	M29	Melanesian	+	pleo	3		early
13	M57	Vietnamese	+	trab	1		late
14	M35	Melanesian	–	pseu	2		late
15	F69	Caucasian	+	pseu	1		late
16	M36	Caucasian	+	trab	2		late

Histology is as follows: trabecular (trab), pleomorphic (pleo), pseudoglandular (pseu), fibrolamell (fibr).

Tumours were graded as follows: grade 1, well differentiated; grade 2, moderately well differentiated; grade 3, poorly differentiated.

Haem, haemochromatosis; alco, alcoholism.

Time of presentation: early, for example, found incidentally at transplantation or screening for alpha-fetoprotein and tumour less than 2 cm in diameter.

Eight patients had been exposed to HBV and eight had not. None of the patients had accumulated a high exposure to aflatoxin B₁.