

## Different DNA changes in primary and recurrent hepatocellular carcinoma

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### Abstract

DNA restriction fragment length polymorphism analysis was carried out on a primary and recurrent hepatocellular carcinoma in a hepatitis B virus negative patient. For the primary tumour, allele losses were found on the short arm of chromosome 17 (probe: p144-D6, 17p13) and the long arm of chromosome 5 with the probe Lambda MS8 (5q35-qter); other probes showed either no allele loss or a non-informative pattern. The recurrent cancer also showed allele loss with p144-D6, but not with Lambda MS8. In addition, the recurrent tumour had allele losses with Lambda MS43 (12q24.3-qter), pYNZ22 (17p13), and DNA rearrangement revealed by the probe Lambda MS32 (1q42-43), a pattern not seen in the primary lesion. These results indicate that the second hepatocellular carcinoma was of independent clonality and probably represents a de novo neoplasm rather than a recurrence.

(Gut 1992; 33: 1433-1435)

Hepatocellular carcinoma is one of the most lethal malignancies in the world. At present only surgical resection offers a chance for cure.<sup>1</sup> Intrahepatic tumour recurrence rates, after liver resection, however, can be as high as 50-82%.<sup>2,3</sup>

Information on the clonal origin of tumours is important for prevention of recurrence, as management strategies are formed based on the

exact cause of this. It has been shown that, genetically, recurrence in hepatocellular carcinoma can either be monoclonal, with recurrent tumours originating from the primary lesion, or polyclonal, where recurrent tumours represent de novo neoplasms.<sup>4</sup> Detecting the integrated hepatitis B virus DNA in hepatocellular carcinoma genome is an effective means of determining the clonal origin of hepatitis B virus related hepatocellular carcinoma.<sup>4,5</sup> This approach is not possible for hepatitis B virus negative hepatocellular carcinoma, however, which is more common than hepatitis B virus related hepatocellular carcinoma in the West. Using DNA restriction fragment length polymorphism analysis, we report a case where a recurrent hepatocellular carcinoma showed a different clonality from the primary neoplasm.

### Case report

A 74 year old man was noted to have a 10 cm mass in the left lobe of the liver on follow up ultrasonography for colorectal carcinoma. This was confirmed on computed tomography and biopsy showed it to be a well differentiated hepatocellular carcinoma. Laparotomy revealed a large lesion involving segments II, III, and IV of the liver, and a left hepatectomy was performed. Histology showed complete resection of tumour. A follow up computed tomography scan, performed 15 months later, revealed a 6 cm nodule in segment VI of the liver, with no extra hepatic

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Figure 1: Computed tomography scan showing recurrent tumour in segment VI of liver.

*Pattern of DNA changes in the primary and recurrent hepatocellular carcinoma*

Probe name	Chromosome region	First HCC	Second HCC	Probe References
Lambda MS1	1p33-35	1,2	1,2	8
Lambda MS32	1q42-43	-	R	8
Lambda MS8	5q35-qter	1,(2)	1,2	8
Lambda MS31	7pter-q22	1,2	1,2	8
p Lambda g3	7q31.3-qter	1,2	1,2	8
EFD126.3	9q34	1,2	1,2	9
pMS51	11q13	1,2	1,2	10
Lambda MS43	12q24.3-qter	1,2	(1),2	8
3'HVR	16p13.3	1,2	1,2	11
p144-D6	17p13	(1),2	(1),2	12
pYNZ22	17p13	1,2	(1),2	13

Homozygous pattern of normal DNA is indicated as a dash; where the normal DNA was heterozygous the tumour genotype is shown in the Table as '1,2'. The continued presence of the larger allele is indicated by '1,' and '2' indicates the continued presence of smaller allele. Allele loss is indicated by (. 'R' indicates DNA rearrangement. HCC=hepatocellular carcinoma.

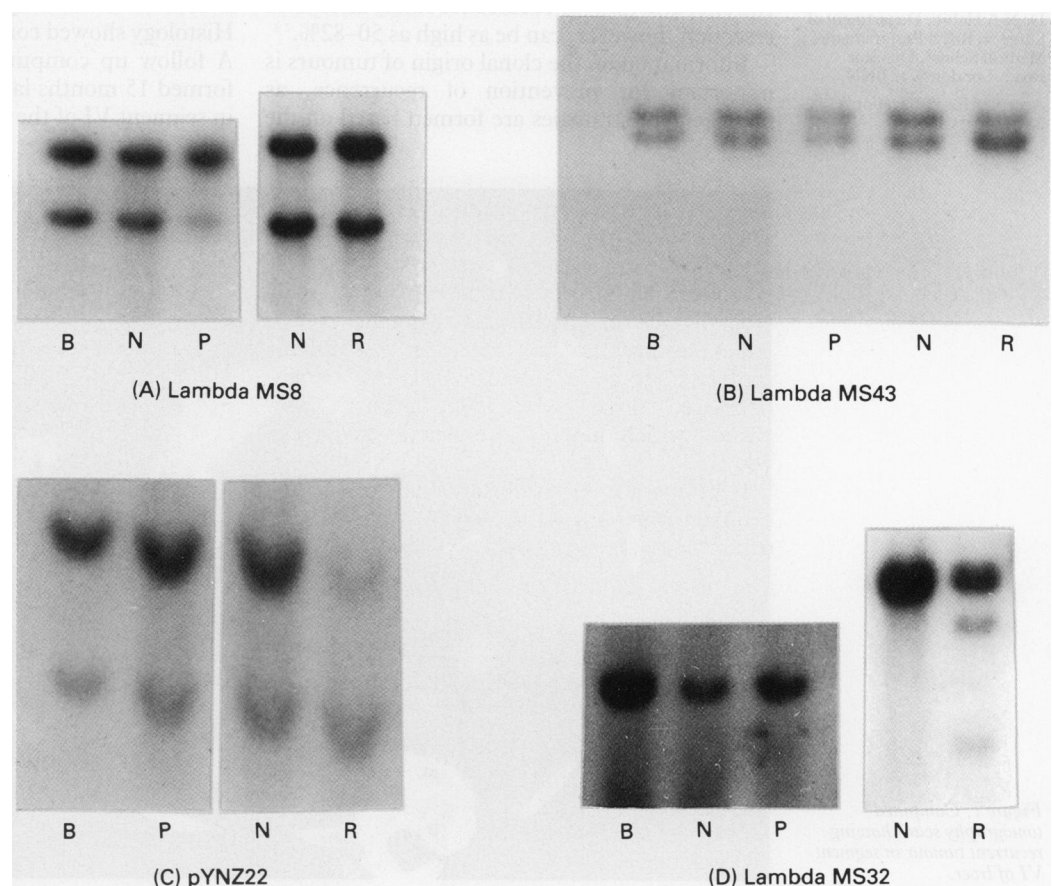
metastasis (Fig 1). These findings were confirmed at laparotomy and local resection of segment VI was undertaken. Histology showed this to be a recurrent hepatocellular carcinoma. Serum neurotensin was normal throughout. Hepatitis B virus status, determined by blood assay and Southern analysis of hepatic tissue DNA, using the hepatitis B virus genome probe pEco63, was negative. At both operations, biopsies from the tumour and non-tumour liver tissue were collected and snap frozen in liquid nitrogen. Lymphocytes from peripheral blood, obtained before any blood transfusion, were also used as a source of constitutional (normal) DNA. Tissue and blood were stored at  $-70^{\circ}\text{C}$  until DNA extraction.

DNA was prepared from blood and tissue samples by standard phenol/chloroform

methods.<sup>6</sup> Samples were digested with the appropriate restriction endonuclease and were size fractionated by electrophoresis through 0.9% agarose gels. The DNA was transferred to Hybond-N hybridisation filters (Amersham) according to the manufacturer's specifications. DNA probes recognising restriction fragment length polymorphisms were radiolabelled with alpha-<sup>32</sup>P- dCTP (3000 Ci/mol) by the random hexanucleotide primer method to a high specific activity.<sup>7</sup> Hybridisations were performed at  $65^{\circ}\text{C}$  in 1% sodium dodecyl sulphate, 1 M NaCl and 5% dextran sulphate (W:V) for 16–24 h. Filters were washed to stringency of  $2\times$  standard saline citrate 1% sodium dodecyl sulphate (W:V) at  $65^{\circ}\text{C}$  and were autoradiographed at  $-70^{\circ}\text{C}$  using Fuji: RX-L x-ray film.

DNA extracted from the tumours was compared with that from blood lymphocytes and normal liver. The detection of chromosomal DNA loss is dependent on the constitutional DNA being heterozygous for restriction fragment length polymorphisms at the various loci for which probes were available. Two bands are visible on the autoradiograph, representing different sized fragments of DNA. A deletion or loss of intensity of one of these bands in tumour DNA is indicative of loss of heterozygosity, or allele loss, in tumourigenesis. In some cases, rearrangement in tumour DNA can be shown with gain of one or more bands, compared with normal DNA. In this study, 11 probes assigned to chromosomes 1, 5, 7, 9, 11, 12, 16, and 17 were used (Table I).<sup>8-13</sup>

Of the 11 probes studied, three showed different patterns of loss of heterozygosity between the primary and recurrent hepatocellular carcinoma.



**Figure 2:** Autoradiographs of Southern hybridisation with: (a) Lambda MS8 (5q35-qter); (b) Lambda MS43 (12q24.3-qter); (c) pYNZ22 (17p13); (d) Lambda MS32 (1q42-43); B=Blood lymphocyte DNA; N=Non-tumour tissue DNA; P=Primary hepatocellular carcinoma DNA; R=Recurrent hepatocellular carcinoma DNA.

mas (Table I, Fig 2). The primary tumour had allele loss on the long arm of chromosome 5 (probe: Lambda MS8, 5q35-qter) and the short arm of chromosome 17 shown by the probe p144-D6 (17p13) but not by the probe pYNZ22 (17p13), while the recurrent hepatocellular carcinoma had allele losses on chromosome 12 shown by Lambda 43 (12q24.3-qter) and chromosome 17 shown by both p144/D6 and pYNZ22, but no allele loss on chromosome 5 (Table I, Fig 2a-c). This patient was shown as homozygous with the probe Lambda MS32, assigned on the long arm of chromosome 1 (1q42-43), for his normal and primary tumour DNA. DNA from the recurrent tumour showed three bands, however, a gain of two, indicating a rearrangement (Fig 2d). Thus it was clear that the clonality was different between primary and recurrent tumours.

### Discussion

DNA restriction fragment length polymorphism analysis in this patient showed differences in DNA changes between primary and recurrent hepatocellular carcinomas. The primary tumour had allele losses on chromosome 5 shown by Lambda MS8 and chromosome 17 shown by p144-D6, while the second tumour did not have allele loss on chromosome 5, but had additional allele losses on chromosomes 12 and 17 and rearrangement on chromosome 1. One of the possible explanations for the differences could be that a small subclone was not present initially in the sample of the first tumour and eventually grew up to form the recurrence, but as there were so many genetic changes present in the recurrence which were not initially there and the Lambda MS8 did not show allele loss, a balance of probabilities seemed that the latter tumour was not a progression from the first. More likely, both neoplasms were of different clonality.

Tumour recurrence can be the result of incomplete resection of the primary lesion, presence of satellite nodules at the time of primary resection, tumour seeding along needle biopsy track or genuine de novo tumour recurrence. DNA analysis in this patient allowed us to exclude the possibility of tumour seeding along the needle biopsy track of the original tumour. It also indicates that the first resection was indeed complete. It is of interest that this patient developed three independent primary neoplasms, which may indicate genetic predisposition.

It is imperative to study the clonal origin of the recurrent tumours, as strategies for preventing recurrence are dependent on causation. By

detecting the integrated hepatitis B virus DNA in hepatocellular carcinoma genome, Chen *et al* reported five pairs of primary and recurrent hepatocellular carcinoma; two showing the same clonal origin, while the other three were of different clonalities.<sup>4</sup> For the first group, more careful diagnostic and treatment procedures, especially extent of surgical resection, are essential in preventing recurrence, while for the second group, one has to identify means of blocking the function of or deleting the effects of persisting carcinogenetic factors, such as hepatitis B virus infection. Although the techniques for these are not available at present, recent continuing advances in the field of molecular biology and tumour suppressor gene research might lead to some benefit with gene therapy in the future.<sup>14</sup>

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