*Liver 1999: 19: 305–311*
*Printed in Denmark . All rights reserved*



Liver
*ISSN 0106-9543*

# Loss of heterozygosity and microsatellite instability in human non-neoplastic hepatic lesions

Tsopanomichalou M, Kouroumalis E, Ergazaki M, Spandidos DA. Loss of heterozygosity and microsatellite instability in human non-neoplastic hepatic lesions.
Liver 1999: 19: 305–311. © Munksgaard, 1999

**Abstract:** *Aims/background:* Carcinogenesis is thought to be a multistage process that occurs as a result of mutations in oncogenes and tumor suppressor genes. One way to monitor a vast range of these changes is by microsatellite PCR amplification that detects loss of heterozygosity and microsatellite instability between normal and tumor specimens of the same subject. Viral cirrhosis is considered a strong predisposing factor for the development of liver cancer. The aim of the study therefore was to examine precancerous hepatic lesions and compare them with others not considered as high risk for hepatocellular carcinoma. *Methods:* We examined 43 subjects for 19 microsatellite markers spanning chromosomes 1, 9 and 17. Normal specimens were blood samples that were compared to liver needle biopsies. Samples were classified according to histological features as non-cancerous (10 cases) and pre-cancerous (33 cases, chronic hepatitis and cirrhosis). *Results:* Our results indicate that there is a tendency of increased chromosomal alteration as lesions become chronic. Samples from patients with antibodies to antibodies for hepatitis C virus show more alterations than hepatitis B positive samples. Steatohepatitis, a disease of unknown etiology, appears to have a high number of microsatellite abnormalities. *Conclusions:* Microsatellite APOA2 located on chromosome 1, shows a statistically significant increase in the rate of loss of heterozygosity as liver lesions become more severe, indicating the presence of tumor suppressor genes which may be involved in the development of these lesions.

**Maria Tsopanomichalou[1], Elias Kouroumalis[2], Maria Ergazaki[1] and Demetrios A. Spandidos[1]**

[1]Laboratory of Clinical Virology, [2]Dept of Gastroenterology, Medical School, University of Crete, Heraklion, PO Box 1393, Greece

Key words: loss of heterozygosity – microsatellite instability – hepatic lesions

Prof. D. A. Spandidos, Laboratory of Clinical Virology, Medical School, University of Crete, Heraklion, PO Box 1393, Greece

Received 10 July 1998, accepted 31 December 1998

Genetic alterations are commonly associated with malignancies of most human tissues (1). The rate of spontaneous mutations in human normal cells is insufficient to account for the high frequencies observed in cancer cells (2, 3). In order to explain these findings two hypotheses have been formed: the mutator phenotype and mutation-driven clonal repopulation. These are not mutually exclusive since both may be required to account for the frequency of mutations in cancers. A third emerging concept is that genomes of cancer cells are unstable and the instability results in a cascade of mutations some of which enable cancer cells to bypass host regulatory processes. This bypass could involve the overexpression of oncogenes or the loss of function of tumor suppressor genes. Mismatch repair genes have also been implicated due to an early deficit in the correction of errors in DNA replication that occurs more frequently in repetitive sequences (4).

These repetitive nucleotide sequences of two to four nucleotides, termed microsatellites, are located between genes and have been classified as "junk" DNA. Microsatellites are estimated to number between 35000 and 100000 copies in the human genome, even by the most conservative estimates (5). The mutations of microsatellites do not exhibit a mutator phenotype to the carrier and they are likely hot spots for mutagenesis and these mutations might be a marker of increased replicative errors throughout the genome of cancer cells (6).

Hepatocellular carcinoma (HCC) is one of the most common causes of cancer mortality world-

wide. The mechanisms of liver oncogenesis have not been elucidated although the role of viral infection, that is, hepatitis B and C viruses, has been extensively studied. Microsatellite technology and RFLP analysis, have contributed to the identification of chromosomal alterations to some chromosomal regions (7–12) and the implication of specific genes (13–17).

There is evidence that chronic hepatitis and liver cirrhosis are steps leading to HCC and they are often referred to as pre-cancerous stages. Our study monitored the chromosomal alterations occurring in each of these groups in respect to chromosomes 1, 9, and 17. The selection of these chromosomes was based on bibliographic data of their involvement in HCC in earlier (chromosome 1) or later stages (chromosome 17). Nineteen microsatellite markers were used in 43 samples. Our results show that alterations accumulate as the lesion becomes more persistent. This finding agrees with the current concept of carcinogenesis (1).

## Materials and Methods

### Specimens

Samples (needle biopsy and blood specimens) from 43 patients were collected at the University Hospital of Heraklion, Crete. Tissue samples were stored at −70°C and blood samples at 4°C until use. Sera were tested for HCV antibodies with both Ortho and Abbott ELISA kits. Confirmation was carried out with RIBA III Western blot (Ortho). HBsAg specific antibodies were detected with IMx automated system (Abbott). All serological tests were performed at the Laboratory of Clinical Virology, University Hospital of Crete.

### Patient classification

The samples were classified using standard histological criteria as non-cancerous lesions [steatohepatitis, non cirrhotic primary biliary cirrhosis, biliary obstruction etc.] and pre-cancerous lesions [chronic mild, chronic severe hepatitis and cirrhosis] (Table 1). Steatohepatitis was not due to alcohol abuse and included only patients without diabetes or obesity. Cirrhosis was of viral etiology in seven cases, due to alcohol abuse in two, and two cases were primary biliary cirrhosis (PBC).

### RNA extraction

HCV RNA was extracted by Trizol LS reagent (Gibco) according to the instructions of the manufacturer. Briefly, 250 μl of serum was mixed with 750 μl of Trizol and 120 μl of chloroform. Samples were centrifuged and the aqueous phase removed. RNA precipitation was accomplished by equal volume of isopropanol. RNA was washed with 70% ethanol and resuspended in 20 μl DEPC treated $H_2O$.

For tissue RNA extraction, Trizol (Gibco) for solid samples was used, followed by the procedure described above.

### Reverse transcription, PCR and detection of HCV PCR product

Reverse transcription was performed with Superscript reverse transcriptase (Gibco) as described previously (18).

### DNA extraction

Blood samples (approximately 10cc), were collected in tubes containing EDTA, and needle biopsies were extracted according to a previously described protocol (19).

### HBV PCR and detection

DNA was amplified using 0.5 μl of DNA extract and specific primers as described previously (20).

### PCR and LOH analysis

Microsatellite repeat primers were obtained from Institute of Molecular Biology and Biotechnology (Forthe, Heraklion, Crete, Greece) and Research Genetics (Huntsville, AL, USA).

The microsatellite primers used for chromosome 17 were: TP53, D17S379, D17S29, THRA1, D17S250, D17S579, D17S113, TK1. The microsatellite primers used for chromosome 9 were D9S144, D9S200, D9S166, D9S51, D9S103. The microsatellite primers used for chromosome 1 were D1S165, CTRM, D1S116, D1S305, APOA2 and ACTN2. Their characteristicts can be retrieved from the Human Genome Database (http://www.gdb.org)

10μl of each PCR product was electrophoresed on a non-denaturating 8% polyacrylamide gel at 250V and visualised by silver staining.

Microsatellite instability was scored in all samples analyzed by a demonstration of a shift of one or both alleles in the tissue DNA specimen as compared with the blood DNA specimen. Likewise, LOH was identified as the absence of one allele in the tissue DNA compared to the blood counterpart.

### Statistical evaluation

Statistical analysis was performed using SPSS for Windows Version 6. Chi-square test and Fisher test were used when appropriate.

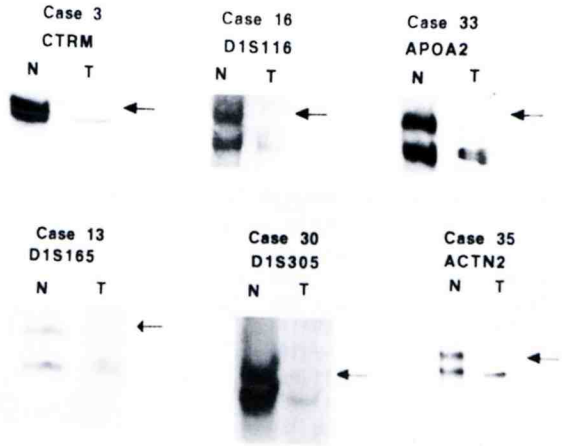


*Fig. 1.* Characteristic examples of LOH on chromosome 1. Patient number is shown next to each example. N, normal blood cells; T, tissue – liver.

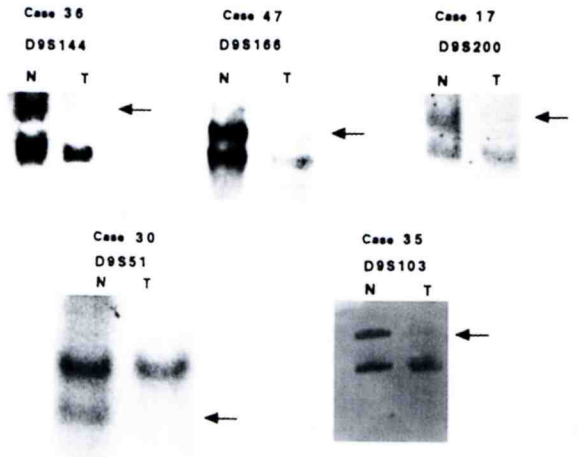


*Fig. 2.* Characteristic examples of LOH on chromosome 9. Patient number is shown next to each example. N, normal blood cells; T, tissue – liver.

## Results

Forty-three needle liver biopsies and blood samples were collected and classified as follows: the non-cancerous group [non-alcoholic steatohepatitis ($n$=4); non-cirrhotic PBC ($n$=2); acute hepatitis ($n$=1); biliary obstruction ($n$=1); drug hepatitis ($n$=1); normal ($n$=1)] and the pre-cancerous group [HCV positive chronic mild hepatitis ($n$=4); HBV positive chronic mild hepatitis ($n$=2); HCV positive chronic severe hepatitis ($n$=9); HBV positive chronic severe hepatitis ($n$=5), nonA-E chronic severe hepatitis ($n$=2) and cirrhosis ($n$=11)]. Nineteen microsatellite markers spanning most of the three chromosomes 1, 9 and 17 were chosen. Figures 1, 2 and 3 present characteristic

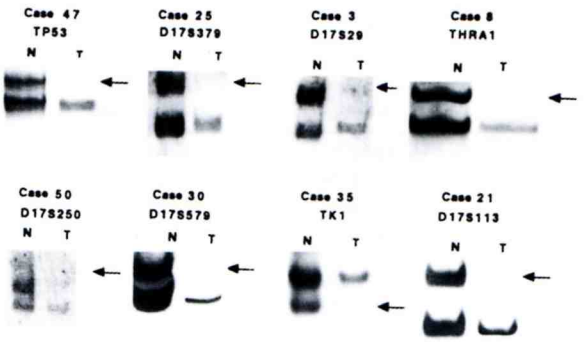


*Fig. 3.* Characteristic examples of LOH on chromosome 17. Patient number is shown next to each example. N, normal blood cells; T, tissue – liver.

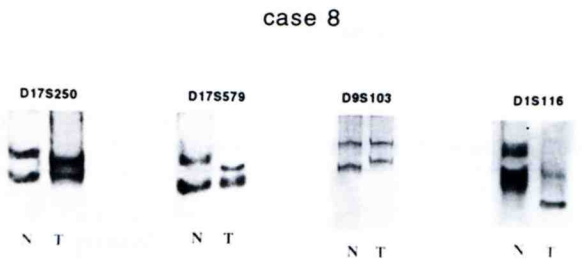


*Fig. 4.* Microsatellite instability in case No 8 for markers D17S250, D17S579, D9S103 and D1S116. N, normal blood cells; T, tissue – liver.

examples of LOH for chromosomes 1, 9 and 17, respectively, while Fig. 4 presents MI in one patient for different microsatellite markers. The group of precancerous lesions was further divided into anti-HCV positive and HBsAg positive subjects. The subdivision was based on antigen-antibody status before PCR was performed.

HBV DNA detection by PCR was in accordance to antigen-antibody data whereas HCV RNA was detected in 10 of the 18 tissue samples and only in 5 of the respective blood samples (data not shown). Due to the small sample number, no correlation can be made between viral genome detection and chromosomal lesions. In this study, loss of heterozygosity (LOH) was evaluated in each case, whereas microsatellite (MI) phenomenon was accepted only if at least two markers exhibited MI.

Table 1 presents the results of loss of heterozygosity per histological group. Markers D9S51, CTRM and APOA2 present a gradual increase of the observed LOH as histology becomes more severe. Markers TP53, THRA1, D17S250, D17S113,

Table 1. LOH results per marker for each histological group

| Marker | Non-cancerous LOH/No. of cases (%) | Pre-cancerous chronic LOH/No. of cases (%) | Pre-cancerous cirrhosis LOH/No. of cases (%) |
|---|---|---|---|
| TP53 | 1/8 (13) | 4/19 (21) | 1/6 (17) |
| D17S379 | 0/7 (0) | 4/12 (33) | 2/7 (29) |
| D17S29 | 1/6 (17) | 3/19 (16) | 0/8 (0) |
| THRA1 | 1/10 (10) | 2/20 (10) | 1/10 (10) |
| D17S250 | 1/10 (10) | 2/22 (9) | 2/11 (18) |
| D17S579 | 1/10 (10) | 1/22 (5) | 3/11 (27) |
| D17S113 | 1/9 (11) | 2/20 (10) | 1/9 (11) |
| TK1 | 1/7 (14) | 2/19 (11) | 1/9 (11) |
| D9S144 | 3/8 (38) | 1/19 (5) | 0/11 (0) |
| D9S200 | 2/9 (22) | 2/21 (10) | 2/11 (18) |
| D9S166 | 2/9 (22) | 1/19 (5) | 1/8 (13) |
| D9S51 | 0/9 (0) | 0/21 (0) | 1/9 (11) |
| D9S103 | 3/10 (30) | 1/20 (5) | 4/9 (44) |
| D1S165 | 1/9 (11) | 1/19 (5) | 2/8 (25) |
| CTRM | 0/6 (0) | 2/19 (11) | 1/8 (13) |
| D1S116 | 0/8 (0) | 0/21 (0) | 0/7 (0) |
| D1S305 | 1/9 (11) | 2/19 (11) | 1/7 (15) |
| APOA2 | 0/9 (0) | 0/16 (0) | 3/8 (38) |
| ACTN2 | 1/7 (14) | 1/16 (6) | 1/7 (14) |

Table 2. LOH results per marker in the pre-cancerous subgroup

| Marker | Type of lesion: Chronic mild LOH/No. of cases (%) | Chronic severe LOH/No. of cases (%) | cirrhosis LOH/No. of cases (%) |
|---|---|---|---|
| TP53 | 1/5 (20) | 3/14 (21) | 1/6 (17) |
| D17S379 | 1/2 (50) | 1/10 (10) | 2/7 (29) |
| D17S29 | 1/5 (20) | 2/14 (14) | 0/8 (0) |
| THRA1 | 1/5 (20) | 1/16 (6) | 1/10 (10) |
| D17S250 | 1/6 (16) | 2/16 (13) | 2/11 (18) |
| D17S579 | 0/6 (0) | 1/16 (6) | 3/11 (27) |
| D17S113 | 1/5 (20) | 2/15 (13) | 1/9 (11) |
| D9S144 | 0/5 (0) | 1/14 (7) | 0/9 (0) |
| D9S200 | 1/5 (20) | 1/16 (6) | 2/11 (1) |
| D9S166 | 0/6 (0) | 1/14 (7) | 1/9 (11) |
| D9S51 | 0/5 (0) | 0/16 (0) | 1/10 (10) |
| D9S103 | 0/5 (0) | 1/16 (6) | 4/9 (44) |
| D1S165 | 0/5 (0) | 1/14 (7) | 2/9 (22) |
| CTRM | 0/5 (0) | 2/14 (14) | 1/7 (14) |
| D1S116 | 0/5 (0) | 0/16 (0) | 0/9 (0) |
| D1S305 | 1/5 (20) | 1/14 (7) | 1/7 (14) |
| APOA2 | 0/4 (0) | 0/12 (0) | 3/8 (38) |
| ACTN2 | 0/4 (0) | 0/8 (0) | 1/6 (17) |

TK1, D9S116, D1S305, ACTN2 were unaffected by histology. Markers D17S29, D17S579, D9S200, D9S103 and D1S165 present fluctuations without a specific pattern. Marker D9S144 shows higher LOH percentages in the non-cancerous group.

LOH was statistically significant for D9S144 ($p=0.015$) and APOA2 ($p=0.05$). For D9S51 and CTRM there was a strong tendency but it was not significant.

Further analysis of LOH in chronic hepatitis groups is presented in Table 2. The pattern described above becomes more obvious, since a gradual increase in prevalence is observed for markers D17S579, D9S51, D9S103, D1S165, APOA2 and ACTN2. However, markers D17S579, D9S103, D1S165 and ACTN2 showed an increased percentage of LOH in non-cancerous lesions as well (Table 2) and therefore could not account for progression to HCC.

Additional classification of the precancerous

Table 3. LOH results per marker in pre-cancerous lesions

| Marker | anti-HCV(+) LOH/No. of cases (%) | HBsAg(+) LOH/No. of cases (%) | Other[1] LOH/No. of cases (%) |
|---|---|---|---|
| TP53 | 2/13 (15) | 2/8 (25) | 1/5 (20) |
| D17S379 | 2/8 (25) | 1/8 (13) | 1/5 (20) |
| D17S29 | 2/14 (14) | 1/9 (11) | 0/4 (0) |
| THRA1 | 1/16 (6) | 1/9 (11) | 1/5 (20) |
| D17S250 | 3/18 (17) | 0/9 (0) | 1/6 (17) |
| D17S579 | 4/18 (22) | 0/9 (0) | 0/5 (0) |
| D17S113 | 2/15 (13) | 1/9 (11) | 0/5 (0) |
| TK1 | 2/15 (13) | 0/8 (0) | 0/5 (0) |
| D9S144 | 1/14 (7) | 0/9 (0) | 0/5 (0) |
| D9S200 | 2/16 (13) | 0/9 (0) | 1/6 (17) |
| D9S166 | 2/14 (14) | 0/9 (0) | 0/4 (0) |
| D9S51 | 0/16 (0) | 0/9 (0) | 1/5 (20) |
| D9S103 | 3/14 (21) | 0/9 (0) | 2/6 (33) |
| D1S165 | 2/15 (13) | 0/7 (0) | 1/5 (20) |
| CRTM | 2/15 (13) | 1/9 (11) | 0/5 (0) |
| D1S116 | 0/15 (0) | 0/9 (0) | 0/6 (0) |
| D1S305 | 1/12 (8) | 2/9 (22) | 0/5 (0) |
| APOA2 | 1/11 (9) | 1/9 (11) | 1/5 (20) |
| ACTN2 | 1/10 (10) | 0/9 (0) | 1/4 (25) |

[1]Includes all other lesions which have not been positive to anti-HCV or HBsAg.

lesions (chronic and cirrhotic), based on antibody status, was carried out (Table 3) in order to assess the impact of an individual viral infection. Markers D17S579, TK1, D9S144 and D9S166 had LOH only in anti-HCV positive samples. Markers D17S29, D17S579, D17S113, TK1, D9S144, D9S166, CTRM, D1S116 and D1S305 appear to have LOH only when markers for viral infection are present. The overall percentage of LOH in anti-HCV positive samples is higher than in HBsAg positive samples.

The results of our analysis indicate that LOH is often observed in hepatic lesions. The results of markers D9S51, CTRM and APOA2 agree with the concept of accumulating mutations during the progression of disease.

In summary, statistically significant rise of LOH is observed in the case of APOA2 marker, while D9S51 and CTRM show a positive but not significant trend. The subjects that show LOH, are positive to HCV RNA, in both serum and biopsy, and to HBV DNA respectively.

MI is a common phenomenon in hepatic lesions reaching 73% in cirrhotic patients (Table 4). It is worth noting that a high percentage of MI is also observed in the group of non-cancerous samples (50%), particularly steatohepatitis, a non-cancerous lesion of unknown etiology.

## Discussion

Liver tumors, like other human tumors, arise from a cascade of genetic events involving oncogenes and tumor suppressor genes that results in decreasing stability of the genome and ultimately in a malignant phenotype. If these steps could be monitored, intervention in the carcinogenic process might be possible.

Many studies (7–17) have focused on hepatocellular carcinoma and have already described abnormalities on chromosomes 1, 4, 8, 9, 13, 16, and 17. Our aim was to monitor the accumulation of mutations through the study of different stages of precancerous hepatic lesions (compared to non cancerous) that might lead to HCC and assess the impact of viral infection to HCC development.

We selected three chromosomes that are known to present high rates of LOH (chr1, chr9, chr17) and contain important regulatory genes such as p53 gene, located on the 17p arm and p73 on the 1p arm, postulated as significant in HCC progression (16, 17, 21, 22).

Microsatellite markers APOA2 and, to a lesser extent, D9S51 and CTRM represent regions that may be important in hepatocellular carcinoma development and progression. HCV chronic carriers tend to exhibit higher mutational rates than HBV

Table 4. Microsatellite instability (MI) according to each histological group

| Histological group | MI/No. of cases (%) |
|---|---|
| Non-cancerous lesions | 5/10 (50) |
| Mild chronic | 2/6 (33) |
| Chronic severe | 6/16 (38) |
| Cirrhosis | 8/11 (73) |

chronic carriers, though HBV is known to integrate in the human genome (17, 21, 23, 24), while HCV does not.

Our results indicate that the region 1q21–1q23 (marker APOA2) might play a critical role in the development and progression to hepatocellular carcinoma. The changes in biochemical parameters measured in the blood samples can be considered to reflect each hepatic lesion. Altered parameters comprise albumin, prothrombin, aminotransferases (ALT, AST), γ-GT, alkaline phosphatase, bilirubin, etc. It is believed that these biochemical abnormalities are the outcome of the hepatic malfunction (25). However, growing evidence suggests that biochemical abnormalities may be caused directly by hepatotropic viruses themselves (26). HCV core protein was found to be colocalised with APOA2 and both were located on lipid droplets. In this context, HCV may play an important role in liver steatosis. Recently, the newly discovered hepatitis virus HGV-C was found to be associated with lipoproteins and this finding was associated with immune escape mechanism. HGV-C hepatitis virus is related to HCV genome and we speculate that lipoprotein association is a common finding in viral liver disease (27). APOA2 marker is an intragenic marker (it amplifies nucleotides 2531–2921) and LOH at the marker represents impairment of the APOA2 gene, while, the histopathological features of HCV reveal extensive steatosis of the liver. The fact that we observed high rates of LOH at this locus in association with advanced hepatic lesions enhances the notion that APOA2 is crucial for the progression of hepatic lesions. HCV core protein could trigger alternative metabolic pathways that cause hepatic malfunction. Moreover, we should note that none of the steatohepatitis subjects (pre-cancerous group) presented LOH at APOA2, possibly because this locus is associated with viral steatosis or extensive liver damage.

The 9p22 region includes the IFN cluster, known to influence the progression of viral hepatitis. Impairment of this region could be one of the reasons for the persistence of HBV and HCV infections (28).

The statistically significant high rate of LOH of D9S144 in non-cancerous lesions has to be further analysed. We speculate that it represents a region implicated in other hepatic lesions such as steatohepatitis but not in HCC development.

MI was high in chronic severe hepatitis suggesting that DNA repair genes have been impaired. However, MI was also high in non-cancerous lesions, mostly steatohepatitis, which is an unusual observation. The nature of the non-cancerous lesions could be responsible for these results.

The mechanism of HCV viral persistence has not yet been elucidated but it is generally believed that HCV related cancer is caused by cytotoxic viral protein activity as DNA interaction does not occur, as with HBV or other viruses (3). The APOA2 impairment and the increase of lipid concentration in hepatocytes could account for such a response.

On the other hand, the tendency of elevated LOH in the region of IFN cluster (D9S51 marker) permits us to speculate that IFN gene impairment could account for persistent virulence. A correlation between IFN genes and tumor-suppressor genes has alredy been postulated (28). Double-stranded-RNA-activated protein kinase (PKR) is known to be induced by interferon. Adenovirus and Epstein-Barr virus also impair the action of interferon. Thus a closer look at interferon genes and their malfunction could provide useful insights into virally-induced tumorigenesis.

In summary, LOH and MI appear to increase with increasing histological severity of hepatic lesions. Findings in steatohepatitis suggest that chromosomal abnormalities might play a role in the pathogenesis of this disease.

### Acknowledgements

We would like to thank Dr G Alexandrakis and Dr N. Charoulakis for collection of the samples, and G. M. Mavromanolakis for the statistical support.

### Abbreviations

HCC, hepatocellular carcinoma; LOH, loss of heterozygosity; MI, microsatellite instability; PBC, primary biliary cirrhosis.

## References

1. Solomon E, Borrow J, Goddard A D. Chromosome aberrations and cancer. Science 1991; 254: 1153–60.
2. Loeb L A. A mutator phenotype may be required for multistage carcinogenesis. Cancer Res 1991; 51: 3075–9.
3. Loeb L A. Microsatellite instability: marker of a mutator phenotype in cancer. Cancer Res 1994; 54: 5059–63.
4. Mao L, Lee D J, Tockman M S, Erozan Y S, Askin F, Sidransky D. Microsatellite alterations as clonal markers for the detection of human cancer. Proc Natl Acad Sci USA 1994; 91: 9871–75.
5. Koreth J, O'Leary J J, McGee J O' D. Microsatellites and PCR genomic analysis. J Pathol 1996; 178: 239–48.
6. zur Hausen H. Viruses in human cancers. Science 1991; 254: 1167–73.
7. Fujimori M, Tokino T, Hino O, et al. Allelotype study of primary hepatocellular carcinoma. Cancer Res 1991; 51: 89–93.
8. Buetow K H, Murray J C, Israel J L, et al. Loss of heterozygosity suggests tumor suppressor gene responsible

for primary hepatocellular carcinoma. Proc Natl Acad Sci USA 1989; 86: 8852–6.
9. Tsuda H, Zhang W, Shimosato Y, et al. Allelle loss on chromosome 16 associated with progression of human hepatocellular carcinoma. Proc Natl Acad Sci USA 1990; 87: 6791–4.
10. Nagai H, Pineau P, Tiollais P, Buendia M A, Dejean A. Comprehensive allelotyping of human hepatocellular carcinoma. Oncogene 1997; 14: 2927–33.
11. Zhang X, Xu H J, Murakami Y, et al. Deletions of chromosome 13q, mutations in *Retinoblastoma 1* and Retinoblastoma protein state in human hepatocellular carcinoma. Cancer Res 1994; 54: 4177–82.
12. Yeh S H, Chen P J, Chen H L, Lai M Y, Wang C C, Chen D S. Frequent genetic alterations at the distal region of chromosome 1p in human hepatocellular carcinomas. Cancer Res 1994; 54: 4188–92.
13. Yeh S H, Chen P J, Lai M Y, Chen D S. Allelic loss on chromosome 4q and 16q in hepatocellular carcinoma: Association with elevated α-fetoprotein production. Gastroenterology 1996; 110: 184–92.
14. Kuroki T, Fujiwara Y, Nakamori S, Imaoka S, Kanematsu T, Nakamura Y. Evidence for the presence of two tumour suppressor genes for hepatocellular carcinoma on chromosome 13q. Br J Cancer 1995; 72: 383–5.
15. Zhang X, Xu H J, Murakami Y, et al. Deletions of chromosome 13q, mutations in *Retinoblastoma 1* and retinoblastoma protein state in human hepatocellular carcinoma. Cancer Res 1994; 54: 4177–82.
16. Murakami Y, Hayashi K, Hirohashi S, Sekiya T. Aberrations of the tumor suppressor p53 and Retinoblastoma genes in human hepatocellular carcinomas. Cancer Res 1991; 51: 5520–1.
17. Walker G J, Hayward N K, Falvey S, Cooksley W G E. Loss of somatic heterozygosity in hepatocellular carcinoma. Cancer Res. 1991; 51: 4367–70.
18. Tsopanomichalou M, Ergazaki M, Linardopoulos S, Kouroumalis E, Spandidos D A. Detection of hepatitis C virus in sera and genotyping according to the 5′non-coding region. Oncol Rep 1997; 4: 1171–4.
19. Kiaris H, Jones A S, Spandidos D A, Vaughan E D, Field J K. Loss of heterozygosity on chromosome 8 in squamous cell carcinomas of the head and neck. Int J Oncol 1994; 5: 1243–8.
20. Nicolaidou A, Liloglou T, Malliri A, et al. Detection of hepatitis B virus DNA and mutations in K-ras and p53 in human hepatocellular carcinomas. Int J Oncol 1993; 3: 593–6.
21. Slagle B L, Zhou Y Z, Butel J S. Hepatitis B virus integration event in human chromosome 17p near the p53 gene identifies the region of the chromosome commonly deleted in virus positive hepatocellular carcinomas. Cancer Res 1991; 51: 49–54.
22. Kaghad M, Bonnet H, Yang A, et al. Monoallelically expressed gene related to p53 at 1p36, a region frequently deleted in Neuroblastoma and other human cancers. Cell 1997; 90: 809–19.
23. Rogler C E, Sherman M, Su C Y, et al. Deletion in chromosome 11p associated with a hepatitis B integration site in hepatocellular carcinoma. Science 1985; 230: 319–22.
24. Greenblatt M S, Feitelson M A, Zhu M, et al. Integrity of p53 in hepatitis B × antigen-positive and negative hepatocellular carcinomas. Cancer Res 1997; 57: 426–32.
25. Fiore G, Fera G, Napoli N, Vella F, Schiraldi O. Liver steatosis and chronic hepatitis C: a spurious association? Eur J Gastroenterol Hepatol 1996; 8: 125–9.
26. Barba G, Harper F, Harada T, et al. Hepatitis C virus core protein shows a cytoplasmic location and associates to cellular lipid storage droplets. Proc Natl Acad Sci USA 1997; 94: 1200–5.
27. Sato K, Tanaka T, Okamoto H, Miyakawa Y, Mayumi M. Association of circulating hepatitis G virus with lipoproteins for a lack of binding with antibodies. Biochem Biophys Res Commun 1996; 229: 719–25.
28. Lengyel P. Tumor suppressor genes: News about the interferon connection. Proc Natl Acad Sci USA 1993; 90: 5893–5.