Loss of constitutional heterozygosity on chromosomes 5 and 17 in cholangiocarcinoma

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Summary  It has been established that loss of tumour suppressor genes is crucial in carcinogenesis. There has been no reported study on searching for tumour suppressor genes in cholangiocarcinomas as yet. In order to investigate the loss of heterozygosity (LOH), which may represent such gene loss, in cholangiocarcinoma, we studied 14 patients with this tumour using restriction fragment length polymorphism analysis. Twenty-two probes assigned to chromosomes 1, 5, 7, 9, 11, 12, 13, 14, 16, 17 and 18 were used. Allelic losses were found in chromosomal regions 5q35-qter and 17p13. Loss of genetic material in these regions in cholangiocarcinoma was shared with hepatocellular carcinoma. Probes for other chromosomes have as yet shown no consistent LOH. In conclusion, this study for the first time showed LOH on chromosomes 5 and 17 in cholangiocarcinoma.

Cholangiocarcinoma, the intrahepatic bile duct carcinoma, is thought to arise from the same stem cell as hepatocellular carcinoma (HCC) (Sell & Dunsford, 1989). Cholangiocarcinoma is reported as occurring less frequently than HCC in most parts of the world. The prognosis of cholangiocarcinoma is poor, with the majority of patients dying 6–12 months after diagnosis. The overall survival rate in treated cases at 5 years is below 9% (Czerniak & Blumgart, 1989).

There is a growing realisation that cancer is a set of fundamentally genetic diseases (Lasko et al., 1991). Multiple genetic alterations including the activation of oncogenes and the inactivation of tumour suppressor genes are important in carcinogenesis. Tumour suppressor genes are normal cellular genes whose products are thought to be inhibitors of the uncontrolled cellular proliferation characteristic of cancer. Several tumour suppressor genes have been cloned, including the RB1 (Friend et al., 1986), p53 (Oren et al., 1981), WT1 (Call et al., 1990), NF1 (Wallace et al., 1990; Viskochil et al., 1990; Cawthon et al., 1990) and APC (Kinzler et al., 1991a; Groden et al., 1991) genes. DCC was cloned and could prove to be a candidate suppressor gene (Fearon et al., 1990). Introduction of a normal tumour suppressor gene, for example the RB1 gene, into tumour cells can inhibit tumorigenesis (Bookstein et al., 1990).

Inactivation of tumour suppressor genes can occur via a variety of mechanisms including allele loss and mutation. One of the most widely used techniques for detection of tumour suppressor gene loss is the demonstration of consistent allele loss or loss of heterozygosity (LOH), in tumour cells. This is achieved by using a battery of restriction fragment length polymorphism (RFLP) probes to analyse DNAs from paired samples of non-tumour and tumour tissues (Lasko et al., 1991). A variety of tumours, including both childhood and common adult malignancies, exhibit LOH (Lasko et al., 1991).

Expression of oncogenes, including ras, myc and erbB-2, and point mutations at K-ras codons 12 and 61 have been reported in a high proportion of cholangiocarcinomas (Voravud et al., 1989; Tada et al., 1990). Cyto genetic studies on two cholangiocarcinoma cell lines revealed several chromosomal abnormalities (Storto et al., 1990). To our knowledge, however, there has been no reported study of loss of heterozygosity in cholangiocarcinomas as yet. Here we report the first study of LOH in cholangiocarcinoma with 22 RFLP probes assigned to 11 chromosomes.

Materials and methods

Patients and biopsies

Fourteen patients with cholangiocarcinoma were studied. All underwent resection of their tumours. None of the patients received chemotherapy or radiotherapy before surgery. Surgical biopsies from tumoral and non-tumoral liver tissues were snap frozen in liquid nitrogen at the time of operation. Lymphocytes from peripheral blood obtained pre-operatively were also used as a source of normal DNA. Tissue was stored at −70°C until DNA extraction. A portion of each tumour sample was examined histologically to confirm the type of tumour present.

DNA extraction and analysis

DNA was prepared from blood and tissue samples by standard phenol/chloroform methods (Sambrook et al., 1989). Southern analyses were done as previously described (Ding et al., 1991). The 22 RFLP probes for chromosomes 1, 5, 7, 9, 11, 12, 13, 14, 16, 17 and 18 and the appropriate restriction enzymes are listed in Table I. If two alleles appeared as two separate bands in the resultant autoradiograph of the constitutional DNA, the patient was considered 'informative', or heterozygous, for the particular marker. Complete deletion or loss of intensity of one band in the tumour DNA indicated an allele loss, or an LOH. The loss of band intensity was confirmed by examination of the autoradiographs with densitometry. A cutoff level of 50% or more of allele intensity was considered as evidence of LOH.

Results

Table I shows the overall pattern of allele loss in cholangiocarcinoma. Overall, 164/229 Southern blots were informative (heterozygosity: 71.6%) and the overall LOH was 17 out of 164 informative cases (10.4%). Figure 1 shows representative examples of allele loss.

As shown in Table I, the 14 cholangiocarcinomas had a higher rate of LOH on chromosomes 5 and 17 than on other chromosomes. Allelic losses were shown in two out of 14 informative cases (14.3%) for the region of the short arm of chromosome 1 (1p33-35) detected by the probe AM51, three

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Table I  Loss of constitutional heterozygosity in human cholangiocarcinoma

| Probe     | Chromosomal region | Enzyme used | LOH* |
|-----------|--------------------|-------------|------|
| cMS621    | 5p                 | HinfI       | 0/4  |
| cMS626    | 5q21               | MspI        | 0/7  |
| L5-71     | 7pter-q22          | HinfI       | 0/12 |
| 54-D      | 7q21               | MspI        | 0/6  |
| YN5.48    | 7q21-22            | MspI        | 0/4  |
| AM58      | 9q35-qter          | HinfI       | 3/10 |
| AM531     | 7pter-q22          | HinfI       | 1/13 |
| pAg3      | 7q31.3-qter        | HinfI       | 0/12 |
| ECB27     | 11p15              | BamHI       | 0/3  |
| pMS51     | 11q13              | HaeIII      | 0/7  |
| ECM43     | 12q24.3-qter       | HinfI       | 1/11 |
| p3.8R     | 13q14.2             | HindIII     | 0/7  |
| cMS626    | 13q                 | AluI        | 0/5  |
| cMS627    | 14q                 | AluI        | 0/5  |
| 3’HVR     | 16p13.3             | PvuII       | 0/8  |
| pU81147   | 16q22.1             | TaqI        | 0/3  |
| p144-D6   | 17p13               | Rsal        | 4/9  |
| pYNZ.22   | 17p13               | Rsal        | 2/5  |
| cMS440    | 18q                 | HaeIII      | 0/2  |

*No. with LOH/No. of informative cases. *References for probes: cMS621, cMS626, cMS8, pAg3 and cMS43: Wong et al., 1987; cMS621, cMS627 and cMS440: Armour et al., 1990; EBC27; Varesco et al., 1989; L5-71; Kizilbash et al., 1991b; 54-D: Kizilbash et al., 1991a; YN5.48: Nakamura et al., 1988a; EFD126.3: Nakamura et al., 1987; H-ras: Krontiris et al., 1985; pMS51: Armour et al., 1989; P3.8R: Friend et al., 1986; 3’HVR; Higgs et al., 1986; pU81148: van der Straten et al., 1983; p144-D6; Kondoleon et al., 1987; pYNZ.22: Nakamura et al., 1988b.

Figure 1  Representative autoradiographs of Southern hybridisations with λMS8 (5q35-qter) and pYNZ.22 (17p13). B = blood lymphocyte DNA; N = non-tumour tissue DNA; T = tumour tissue DNA. The autoradiographs show allele losses in tumour DNA (indicated by arrows).

Table II  Allele loss on chromosome 5 in cholangiocarcinomas and colonic metastases in liver

| Patients | cMS621 (5p) | ECB27 (5q21) | L5-71 (MCC) | 54-D (APC) | YN5.48 | λMS8 (5q35-qter) |
|----------|-------------|--------------|-------------|------------|--------|-----------------|
| Cholangiocarcinoma | 1.2          | 1.2          | 1.2         | 1.2        | 1.2    | (1,2)           |
| 2        | 1.2         | 1.2         | 1.2         | 1.2        |        |                 |
| 3        | -           | 1.2         | 1.2         | -          | 1.2    |                 |
| 4        | 1.2         | 1.2         | -           | 1.2        |        |                 |
| 5        | -           | -           | 1.2         | 1.2        |        |                 |
| 6        | 1.2         | 1.2         | 1.2         | -          |        |                 |
| 7        | nd          | 1.2         | 1.2         | nd         | 1.2    |                 |
| 8        | nd          | 1.2         | 1.2         | nd         | 1.2    |                 |
| 9        | nd          | nd          | 1.2         | nd         | 1.2    |                 |
| 10       | nd          | nd          | 1.2         | nd         | 1.2    |                 |
| 11       | nd          | nd          | nd          | nd         | 1.2    |                 |
| 12       | nd          | nd          | nd          | nd         | 1.2    |                 |
| 13       | nd          | nd          | nd          | nd         | 1.2    |                 |
| 14       | nd          | nd          | nd          | nd         | 1.2    |                 |
| Total no | 6            | 7           | 10          | 12         | 7      | 14              |
| Heterozygosity | 4            | 5           | 7           | 6          | 4      | 10              |
| Allele loss | 0            | 0           | 0           | 0          | 0      | 3               |

Colonic metastasis

| 15       | 1.2         | 1.2         | 1.2         | 1.2        | 1.2    | (1,2)           |
| 16       | 1.2         | 1.2         | (1,2)       | (1,2)      | (1,2)  | (1,2)           |
| 17       | 1.2         | (1,2)       | -           | 1.2        |        |                 |
| 18       | 1.2         | (1,2)       | (1,2)       | 1.2        |        |                 |
| 19       | -           | -           | -           | 1.2        |        |                 |
| 20       | 1.2         | (1,2)       | 1.2         | (1,2)      | (1,2)  | (1,2)           |
| 21       | -           | -           | -           | (1,2)      |        |                 |
| Total no | 7            | 7           | 7           | 7          | 7      | 7               |
| Heterozygosity | 5            | 1           | 3           | 4          | 5      | 5               |
| Allele loss | 0            | 1           | 2           | 3          | 4      | 2               |

Heterozygosity in the constitutional DNA (non-informative pattern is indicated as a dash; where the normal tissue was informative the tumour genotype is shown in the table. Heterozygosity is indicated by 1.2. The continued presence of the larger allelic restriction fragment is indicated by ‘1’ and ‘2’ indicates continued presence of the smaller allelic fragment. Allele loss (deletion or reduction of intensity of a band) is indicated by (0). ‘nd’ indicates no data.
Discussion

This is the first study on loss of heterozygosity in cholangiocarcinomas. Three out of 22 probes revealed a relatively high rate of LOH in two chromosomal regions, namely, 5q35-qter (30%) and 17p13 (44.4% and 40%). There were also allelic losses at 1p33-35 (2/14, 14.3%), 1q42-43 (23.1%), 7pter-q22 (1/13, 7.7%), 9q34 (1/11, 9.1%) and 12q43-3.4 (1/11, 9.1%), but these lower values might represent random losses since rapid division of malignant cells can produce loss of heterozygosity at a certain region by chance (Lasko et al., 1991).

We have previously reported allelic losses at 1q42-43 and 17p13 in hepatocellular carcinoma with liver cirrhosis and at 5q35-qter and 17p13 in HCC without liver cirrhosis (Ding et al., 1991). Hence it is of interest to find LOH at 5q35-qter and 17p13 in cholangiocarcinoma in this study. It has been proposed that HCC and cholangiocarcinoma arise from the same pluripotent liver stem cell (Sell & Dunsford, 1989). These two types of primary liver malignancies, therefore, may share similar genetic changes. Allele loss on chromosome 17p is shared with other tumours and may be involved in tumour progression (Sager, 1989; Lasko et al., 1991). Loss of heterozygosity at 5q35-qter in both HCC and cholangiocarcinoma thus might represent a common genetic change in the development of the two tumours. Further study is needed to confirm this finding. This investigation reports the results of 14 patients collected simultaneously from two active liver centres over 3 years. The scarcity of this material highlights the difficulty of surgical resection of intrahepatic cholangiocarcinoma. Most patients present usually at such an advanced stage that precludes surgical resection. The familial adenomatous polyposis coli (APC) gene is located at 5q21 and the gene has been cloned (Kinzler et al., 1991; Groden et al., 1991). We previously compared the pattern of allele loss in non-cirrhotic HCC with that of hepatic metastases from colorectal cancers using various probe for chromosome 5q (Ding et al., 1991). The majority of LOH in hepatic metastases from colorectal cancers was found at the region 5q21-22 while the LOH in non cirrhotic HCC was at 5q35-qter. In the present study on cholangiocarcinomas allele loss also occurred at 5q35-qter. However, probes from 5q21-22, including a cDNA probe from APC gene, did not show any allele loss in cholangiocarcinoma (Tables I and II). The possible 5q35-qter region involved in both HCC and cholangiocarcinoma appears to be distinct from that encompassing APC. This is supported by the finding that the three patients exhibiting allele loss at 5q35-qter with the probe AM58 have shown no allele loss with the probe 54-D from the APC gene.

There has been no reported direct cytogenetic study as yet on cholangiocarcinoma tissue. Chromosome study on two cholangiocarcinoma cell lines showed a number of abnormalities (Sarto et al., 1990). It is of particular interest that chromosome 5 was among the most commonly involved chromosomes in structural abnormalities in both cell lines. This finding and the results of RFLP analysis in this study suggest that mutation or deletion of a possible tumour suppressor gene located on chromosome 5, distal to 5q21-22, may play a role in the development of cholangiocarcinoma.

Recently, loss or mutation of the p53 tumour suppressor gene at chromosome 17p has been seen at a very high frequency in a variety of human malignancies (Weinberg, 1991). Loss of heterozygosity occurred in four out of nine cholangiocarcinoma shown by p144-D6, and in two of five shown by pYN22, in this study. Both probes are assigned to the region of 17p13, near the locus of the p53 tumour suppressor gene. This finding makes it likely that loss of the p53 gene is also involved in the development of cholangiocarcinoma. It will be of interest to know if there is any overexpression of mutant p53 or point mutation of the p53 gene in cholangiocarcinoma.

In conclusion, further study should show allelic losses on chromosomes 5q35-qter and 17p13 in cholangiocarcinoma. These losses are shared with HCC.

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