Regional mapping to 4q32.1 by in situ hybridization of a DNA domain rearranged in human liver cancer

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Summary. Recently, a unique cellular DNA segment, representing the normal allele counterpart of hepatitis B virus integration site, has been isolated. It has allowed the identification of a cellular domain in which rearrangements occur in approximately 10% of primary liver tumours. We here report on the assignment of this probe, D4S112, by in situ hybridization to band 4q32.1.

Introduction

The carcinogenic factors involved in the development of human primary liver cancer (mostly hepatocellular carcinoma (HCC)) include chronic hepatitis B virus (HBV) infection, chemical carcinogens (mycotoxins), liver cirrhosis and hormonal status (Beasley and Hwang 1984).

Integration of the HBV DNA has been demonstrated in the tumorous tissues and might be involved in liver cell transformation (Bréchot et al. 1981). The analysis of cellular DNA sequences at the HBV DNA integration sites showed that different mechanisms are likely to be involved (Nagaya et al. 1987; De Thé et al. 1987). Deletions have been frequently identified on various chromosomes (Nagaya et al. 1987; Rogler et al. 1985; Tokino et al. 1987), while a 17:18 translocation was demonstrated in a tumour (Hino et al. 1986). In addition, the viral DNA was recently shown, in one tumour, to integrate in the DNA binding domain of a gene sharing homology with the retinoic acid and steroid hormone receptor genes (Dejean et al. 1986; De Thé et al. 1987). Finally, in woodchucks with liver cancer related to a chronic hepadna viral infection, integration of the woodchuck hepatitis virus (WHV) was recently identified, in two different tumours, in the 5' and 3' regions of the myc oncogene (Hsu et al. 1987). We have recently identified a cellular DNA domain in which rearrangements occur in approximately 10% of primary liver tumours (Pasquinelli et al. 1988). This probe, D4S112, is a unique cellular DNA segment of 1.9 kb, which represents the normal allele counterpart of the HBV integration site (Pasquinelli et al. 1988). This EcoRI–HindIII fragment was subcloned from a bacteriophage lambda L47.1 vector into a Bluescribe plasmid vector. Southern blot analysis using this probe after EcoRI and HindIII digestion, showed 7-kb and 6-kb DNA bands. The insert was tritium labelled to a specific activity of 3.4 x 10⁸ cpmp/µg using a modification of the random primer technique with the Klenow fragment of DNA polymerase I.

In situ hybridization

Hybridization of the probe to the chromosome preparations was carried out essentially as described by Mattei et al. (1985). Slides were first treated with ribonuclease A at 100 µg/ml in 2 x SSC (1 x SSC is 0.15 M NaCl, 15 mM sodium citrate) at 37°C for 1 h and ethanol dehydrated. Chromosomal DNA was denatured by incubating at 70°C in 70% formamide, 2 x SSC pH 7.0 for 2 min and dehydrated in cold ethanol. The probe was precipitated by ammonium acetate and ethanol, and resuspended in TE (10 mM Tris, 1 mM EDTA). Hybridization fluid was as follows: 50% formamide, 2 x SSC, 40 mM sodium phosphate pH 6.0, 0.1% sodium dodecyl sulfate, 1 x Denhart's solution, 1 mg/ml denatured and sonicated herring DNA and 10 M dextran sulfate. Various concentrations (15 and 30 µg/ml) of probe were used and the hybridization mixture was denatured by heating at 70°C for 10 min, followed by rapid cooling on ice. A 50-µl aliquot of probe was placed on each of ten slides and incubated overnight at 37°C under a 24 x 55 mm coverslip in 2 x SSC saturated environment.
After hybridization the slides were washed in a 50% formamide, 2 × SSC then in 2 × SSC at 40°C, then in 2 × SSC at 4°C. A final wash in 0.1 × SSC at 4°C was performed before ethanol dehydration.

Slides were dipped in Kodak NTB 2 nuclear track (Eastman) diluted 1 : 1 with water and exposed at 4°C for 5–10 days. They were developed for 1.30 min in Dektol D19 and fixed in Ilford Hypam.

Staining and R-banding

After hybridization and autoradiography the slides were initially stained with a Giemsa solution (2%, pH 6.5 for 15 min) and photographed to localize silver grains. Chromosome banding was subsequently obtained by staining with Hoechst 33258 (150 µg/ml), exposure to long-wave UV light in 2 × SSC, and staining in Giemsa (2%, pH 6.8 for 20 min). Metaphases were then photographed again for chromosome identification. This two-step procedure was needed because slippling of silver grains could arise during this banding procedure.

Results

The distribution of silver grains located on chromosomes or touching them is shown in Fig. 1. A total of 115 metaphases were scored and 253 grains were counted, i.e. an average of 2.2 grains per mitosis. Among these 253 grains, 70 were located on chromosome 4 with a clustering on the proximal part of band 4q32, probably 4q32.1 (Figs. 2, 3). Background grains were randomly distributed.

Discussion

The normal allele counterpart of this HBV integration site has previously been assigned to chromosome 4 with a panel of human–mouse somatic cell hybrids (Pasquinelli et al. 1988). Using this probe (D4S122) and the technique of in situ hybridization, we have confirmed its assignment and localized this