The Detection of Hepatitis B Virus (HBV) in HBsAg Negative Individuals with Primary Liver Cancer

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The importance of chronic hepatitis B virus (HBV) infection in the development of primary liver cancer has been established by epidemiological studies. However, the evidence for a direct role of the virus in liver carcinogenesis is still tentative. In addition, the findings of HBV DNA sequences in HBsAg-negative subjects with liver cancer has been reported, although it is controversial. Here we report the use of the polymerase chain reaction to detect HBV DNA in the serum and liver of HBsAg-negative patients. This technique allows both for the detection and cloning of HBV variants. In addition, the demonstration of HBV DNA and RNA molecules in HCC of HBsAg-negative individuals as determined by standard techniques reinforces the role of HBV in the pathogenesis of this tumor.

KEY WORDS: HBV; PCR; liver cancer; hybridization; cloning and sequencing.

Extensive epidemiological studies (1–4) have shown a clear association between chronic hepatitis B virus (HBV) infection and primary liver cancer (PLC). The evidence consists of: 1) the superposition of geographical areas with a high incidence of primary liver cancer and those with a high prevalence of HBV infection, 2) an increase of HBV serological markers in subjects with liver cancer as compared to the general population of the same areas, and 3) an increased risk of PLC in HBsAg-positive subjects in prospective studies conducted in Taiwan (2) and Japan (3). HBV is thought to induce liver cancer by the combination of at least two mechanisms. First, HBV induces liver cirrhosis, which is found in 80–90% of PLC and thought to be a cofactor in the pathogenesis of liver cancer. Second, molecular studies have indicated a direct role of HBV in the liver carcinogenesis as integrated HBV DNA sequences are present in PLC cells and presumably might modify cellular gene expression both by producing large chromosomal rearrangements as well as insertional mutagenesis (5–16). In addition, the viral protein X may act as a transactivating factor (17, 18).

The role of HBV in the pathogenesis of HBsAg-negative subjects with liver cancer is debatable. Indeed hepatitis B virus DNA sequences have been detected in the liver and serum of patients with chronic hepatitis and hepatocellular carcinoma in whom HBsAg was not identified using conventional assays (19–27). Recently, patients with primary liver cancer occurring in a histologically normal liver have been studied for the presence of HBV DNA; only a minority of such patients have been found to be HBV DNA positive (5/22) (21). It appears clear that there is a small group of PLC that...
Fig 1. Comparison of results obtained with classical Southern blot hybridization assay between HBsAg-negative and -positive subjects with primary liver cancer. A: HBsAg-negative tumors: French alcoholic patient with no HBV marker. Injection of the serum to chimpanzee showed transmission of the HBV variant (23). Lanes A and B: HindIII (A) and EcoRI (B) digest of liver tumor cellular DNA. Hybridization with HBV probe (exposure time: 10 days). Lane C: Same filter as in lane B but rehybridized with a cellular probe corresponding to one copy per cell (exposure time: 3 days). Note that the observed band is much more intense that in lane B, thus reflecting the low copy number of HBV per cell (same results were obtained in low stringency conditions). HBsAg-positive tumors: Lanes D and E: EcoRI (D) and HindIII (E) digested cellular DNA hybridized with HBV probe (exposure time: 5 days). Lane F: TaqI digest, hybridized with the same cellular probe as in lane C (exposure time: 5 days). Again, although in an HBsAg positive tumor, there is evidence for a low HBV copy number. Lanes G–I: HindIII digest of nontumorous cirrhotic area (G), tumor (H), and a further nontumor area (I) of the same liver with HBsAg-positive HCC—hybridization with HBV probe (exposure time: 1 night). Lanes J and K: EcoRI (J) and HindIII (K) digest of the same tumor (exposure time: 5 days). This case is representative of that most frequently observed in HBsAg-positive tumors: there is a clear, easy to show, clonal proliferation of infected cells on lane H, as opposed to that observed on lane B.