
Short Communication

DETECTION OF HEPATITIS C VIRUS cDNA SEQUENCE BY THE POLYMERASE CHAIN REACTION IN HEPATOCELLULAR CARCINOMA TISSUES

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SUMMARY: We found the presence of hepatitis C virus (HCV) infection in liver tissues of hepatocellular carcinoma (HCC) patients who had antibodies to HCV but no serological markers for hepatitis B virus infection by the sensitive reverse transcription/polymerase chain reaction (R/PCR) method. The primers used were derived from the non-structural (NS) 3 and/or the structural (C/E) region. Amplified cDNA sequences of HCV were detected in either cancerous or non-cancerous portion of liver tissues from four out of eight HCC patients with primers of NS3 region. Similar but less efficient results were obtained with primers of C/E region. These results indicate that HCV persists in the liver tissue of HCC. A possible role of persistent infection of HCV for the development of HCC is discussed.
The cDNA of the HCV genome was recently cloned and identified (1). An assay system for circulating antibodies to HCV was developed by use of an HCV antigen synthesized in recombinant yeast (2). The results obtained from this assay have shown that HCV is the major causative agent of transfusion-associated non-A, non-B hepatitis (2-4), and also a high prevalence of HCV antibodies among HCC patients who had no serological markers for hepatitis B virus infection (non-B) (5,6). A close association between HCV infection and development of HCC has been suggested (5,6). Furthermore, HCC developed in a chimpanzee after inoculation of human plasma containing non-A, non-B hepatitis agent (7). To study the mechanism of development of HCC, it is necessary to examine the liver tissues of HCC patients for HCV. Recently, cDNA fragments of the HCV genome were cloned from Japanese HCV carriers (8,9). A comparison in nucleotide sequences of Japanese and USA isolates has revealed that there are some heterogeneities of the viral genome between the two isolates. With the nucleotide sequence available, it is now possible to detect the genome of HCV in blood or liver tissues of infected patients (10).

Liver tissues were obtained from autopsy samples of eight non-B HCC patients who had antibodies to HCV. Cancerous and non-cancerous portions were taken from each patient and confirmed histologically. RNA was extracted by the guanidine thiocyanate/cesium fluorotriacetate method (11) from each tissue sample. About 4 µg of RNA was used for cDNA synthesis with 10 units of reverse transcriptase (Bio-Rad, Richmond, CA) and with an antisense primer, J513A (5'-CGTATGAGACACTTCCACAT-3'). The cDNA was amplified by PCR (12) after addition of sense primer, J469S (5'-GTCACTCAGACGGTGATTT-3'), encompassing the 440 base pairs (bp) of the non-structural protein region 3 (NS) as previously described (8,9). Another set of primers, J135A (5'-ACAGCTTTGTGGGATCCGGAG-3') and 71S (5'-GCCGACCTCATGGGTTACAT-3'), which bound the 440 bp of the structural protein region [C/E: putative core and envelope regions (13), K. Takeuchi et al.; manuscript submitted] was also used. Thirty cycles of PCR were carried out as follows: denaturation for 1.5 min at 95 C, annealing of primers for 1.5 min at 55 C, and extension for 2 min at 70 C. The amplified cDNA fragment was electrophoresed on a 2% agarose gel and then transferred to a nylon membrane by a vacuum blotting system (LKB, Bromma, Sweden). A specific signal was identified by Southern blot hybridization under the stringent condition with a 32P-labeled probe encompassing target regions of NS3 or C/E. The probe in the
Fig. 1. Detection of cDNA sequences of the HCV genome from liver tissues of HCCs. RNAs were prepared from cancerous (T) and non-cancerous (N) portions of autopsy samples of HCC patients who had antibodies to HCV. Products of R/PCR using either NS3 or C/E primers were electrophoresed on a 2% agarose gel, and analyzed by Southern blot hybridization with a probe encompassing either NS3 (A) or C/E region (B). HCC patients were indicated by numbers 1-8. The arrow indicates the position of the amplified 440 bp cDNA fragment of NS3 or C/E region.
NS3 region was about 620 bp cDNA fragment from pU1-4652 clone digested with EcoRI and Pst I (9). The probe in the C/E region was about 1,300 bp cDNA fragment from pSR316 digested with Kpn I and Pst I (S. Harada et al., manuscript submitted). When NS3 region was examined as a target of R/PCR (Fig. 1A), HCV sequences were detected in four samples of the non-cancerous portion (patients Nos. 1, 2, 4, and 8) from eight HCC patients. Amplified cDNA sequences of HCV were also detected in two samples of cancerous portion (patient No. 2 and 8). When C/E region was examined (Fig. 1B), three samples of either cancerous or non-cancerous portion from eight HCCs were also positive (patients Nos. 1, 2, and 4), which is in good agreement with the results with NS3 region. The efficiency of detection with primers of NS3 region was better for the sample from patient No. 8 than with those of C/E region. This may have been due to the different degrees of conservation of nucleotide sequence between the NS3 and structural protein region (9), because mismatching of the primer and template may result in inefficient detection by R/PCR. Unexpected signals were also visible in HCV-positive lanes. It remained unclear what caused them. The partial nucleotide sequence analysis (about 200 nucleotides) of the NS3 cDNA fragments detected here revealed that 2 to 4% of sequence differences of the HCV genome were observed among four positive liver tissues, whereas the nucleotide sequences were identical between the cancerous and non-cancerous portions derived from the same patient (W.-H. Chou et al., manuscript in preparation). No specific signals of the HCV genome were detected by Northern blot hybridization of these RNA preparations (data not shown); the amount of the virus in HCCs must have been extremely low. No integrated HCV genome in cellular DNA of cancerous portion of HCCs was detected by ordinary Southern blot hybridization or after PCR amplification with primers of NS3 region (data not shown).

Over 50% of HCV infections develop eventually into chronic hepatitis (14). Persistent infection of long duration of the liver may cause repeated tissue injury and regeneration of hepatocytes. It may finally lead to the development of HCC through cirrhosis (15). Our present data indicate that HCV persists in cancerous and non-cancerous liver tissues of HCC. It is noteworthy that patients Nos. 1, 4 and 8 had previous histories of blood transfusion approximately 30 years ago. Most likely, they were infected with HCV on these occasions (16). These results as well as such a high prevalence of HCV antibody in non-B HCC patients (6) suggest that HCV is an important factor in pathogenesis of HCC.
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REFERENCES


