

Immunohistochemical detection of HCV infection in patients with hepatocellular carcinoma and other liver diseases *

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Subject headings Hepatitis C; carcinoma, hepatocellular; immunohistochemistry; liver neoplasms; liver diseases

Abstract

AIM To detect HCV infection in patients with HCC and other liver diseases by the immunohistochemical method.

METHODS The expression of HCV antigen was identified by means of LSAB (labelled streptavidin-biotin) method using anti-NS3 monoclonal antibody.

RESULTS The positive rates of HCV antigen in the three groups of HCC, liver cirrhosis and hepatitis were 13.5% (7/52), 12.5% (2/16), and 10% (4/40) respectively, while in the samples from patients with constitutional jaundice and normal liver samples, no HCV antigen was found. HCV antigen could be seen in the nuclei and/or cytoplasm of carcinoma cells and/or pericarcinoma hepatocytes. In HCC, HCV antigen was more often seen in nuclei than in cytoplasm. The positive rate of HCV antigen in pericarcinoma tissues was higher than that in cancerous tissues.

CONCLUSION HCV is associated with HCC, and HCV infection enhances the development of liver diseases. HCV affects the initiative period of HCC and induces the malignant phenotypic alteration of hepatocytes.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the common malignant tumors which ranks the third in cancer mortality in our country, while its etiology and carcinogenesis are still far from clearly identified. The association of hepatitis C virus (HCV) infection with HCC has been indicated by serosurvey^[1,2], but studies at cellular level in detecting HCV antigen in liver tissues to demonstrate this association have so far been rare. The significance of results from a few immunohistochemical studies reported is quite limited because of the use of polyclonal antibodies and a small number of cases. In order to reveal the HCV infection status in HCC and other liver diseases and to explore the relationship between HCV and HCC at cellular level, we detected immunohistochemically the HCV antigen expression in cancerous tissues and liver tissues of 116 cases of different liver diseases (mainly HCC) using LSAB (labelled streptavidin-biotin) method and monoclonal antibody against NS3 antigen of HCV.

MATERIALS AND METHODS

Patients and samples

Liver tissue samples were obtained from inpatients during resection of their HCC in the Department of Abdominal Surgery of Affiliated Tumor Hospital of Sun Yat-Sen University of Medical Sciences in the period from April 1993 to February 1994 and the preserved paraffin embedded samples by liver biopsy in the Department of Pathology of First Affiliated Hospital of Xinjiang Medical College from 1986 to 1990 were also collected for study. The clinical data are shown in Table 1. The diagnosis was made according to pathological examination, clinical data and laboratory assay.

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Table 1 Clinical data of 116 cases of liver diseases

Groups	Cases	Male/female	Age (years) ($\bar{x} \pm s$)	ALT (IU/L) ($\bar{x} \pm s$)
HCC	52	41/11	42 \pm 2	126 \pm 93
Cir.	16	10/6	42 \pm 6	171 \pm 68
CH	40	28/12	35 \pm 9	269 \pm 51
CJ	8	5/3	23 \pm 1	76 \pm 45

HCC: hepatocellular carcinoma; Cir: cirrhosis; CH: chronic hepatitis; CJ: constitutional jaundice

Reagents

The monoclonal antibody against NS3 antigen of HCV, prepared by Bionikes Co. U. S. A. , was kindly provided by the Virology Institute of Chinese Academy of Preventive Medicine; LSAB (labelled streptavidin-biotin) kit and monoclonal antibody to HBsAg were obtained commercially (LSAB kit from DAKO, anti-HBs from Clinical Immunology Lab of Tongji Medical University).

Immunohistochemical methods

The tissue sections were dewaxed routinely and then treated as follows: 0.3% H₂O₂-methanol blocking for 20min, phosphate-buffered NaCl solution (PBS) washing, incubation with monoclonal antibody against NS3 of HCV overnight at 4°C, continuing the following procedure according to the instruction of LSAB kit, DAB staining.

The negative controls included: ①substitution of monoclonal antibody against NS3 of HCV with unrelated antibody (anti-HBs) or PBS, ②exclusion of incubation with antibody against NS3 of HCV, and ③normal liver samples as controls.

RESULTS

The positive rates of HCV antigen in the groups of different liver diseases

In the groups of HCC, cirrhosis and chronic hepatitis, the positive rates of HCV Ag were 13.5% (7/52), 12.5% (2/16) and 10% (4/40) respectively, while in the samples from constitutional jaundice and normal liver no HCV Ag was found. No positive staining was shown in the negative controls except with anti-HBs as the first antibody (the HBsAg staining was located differently, as compared with HCV antigen).

Location and distribution of HCV antigen in tissues

HCV Ag could be seen in the nuclei and/or cytoplasm of cancer cells and/or pericancerous hepatocytes of HCC. In the 7 positive cases of HCC, HCV Ag was more often seen in nucleus than in cytoplasm (5 cases in nuclei, only 2 cases in cytoplasm). This difference was not significant in the other groups. The HCV Ag positive cells were scattered singly or gathered in small groups in some parts. The detecting rate of HCV Ag in cancer tissues was 5.8% (3/52), whereas in the pericancerous tissues it was as high as 12.5% (6/48), significantly higher than that in the former.

DISCUSSION

Since the application of ELISA and PCR techniques in the detection of HCV antibody and HCV RNA,

the view that HCV infection was an important risk factor for the genesis of HCC was supported by many studies which were mainly carried out in HBV non-endemic areas such as Japan and Europe^[3,4]. Even in China, as an HBV hyperendemic country, the association of HCV infection with HCC was also preliminarily demonstrated, but the published investigations up to now were almost all serological studies. Detection of HCV antigen at cellular level in liver tissues to demonstrate this association has been reported rarely. The specificity of the results from a few immunohistochemical studies reported was quite limited because of the use of polyclonal antibody derived from patient's serum, and the consistency of the results from different studies was quite poor^[5]. We detected HCV antigen expression in cancerous tissues and pericancerous tissues of HCC using LSAB method and monoclonal antibody against NS3 antigen of HCV, and our results supported the association of HCV infection with HCC. The positive rate of HCV antigen in pericancerous hepatocytes was higher than that in cancer tissues, similar to the results of HCV RNA detection reported by Ohkoshi^[6], and this phenomenon gives us two hints: HCV probably affects mainly the initiative period of HCC development and enhances malignant phenotypic alteration of normal hepatocytes; and as soon as the malignant alteration has taken place, the compatibility of hepatocytes to HCV decreases. The positive rates of HCV antigen in cases of HCC and cirrhosis were higher than those in cases of chronic hepatitis, constitutional jaundice and normal liver tissues and this result suggests that HCV infection may enhance the development of liver diseases. It is common knowledge that HCV, as a non-reverse transcription RNA virus, completes its proliferation cycle in cytoplasm, and so the expression of HCV antigen in nucleus is not related with the proliferation cycle itself. Whether HCV NS3 antigen, as a "counter-modulating" factor, gets into the nucleus to influence expression and regulation of host cell genes and to induce malignant alteration of cells remains to be studied further in the future.

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