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Effect of HCV infection on expression of several cancer-associated gene products in HCC *

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Abstract

AIM To study hepatocarcinogenesis of hepatitis C virus (HCV).

METHODS Expression of HCV antigens (CP10, NS3 and NS5) and several cancer-associated gene products (ras p21, c-myc,c-erbB-2, mutated p53 and p16 protein) in the tissues of hepatocellular carcinoma (HCC, *n* = 46) and its surrounding liver tissue were studied by the ABC (avidin-biotin complex) immu nohistochemical method. The effect of HCV infection on expression of those gene products in HCC was analyzed by comparing HCV antigen-positive group with HCV antigen negative group.

RESULTS Positive immunostaining with one, two or three HCV antigens was found in 20 (43.5%) cases, with either of two or three HCV antigens in 16 (34.8%) cases, and with three HCV antigens in 9 (19.6%) cases. Deletion rate of p16 protein expression in HCC with positive HCV antigen (80%, 16/20) was significantly higher than that in HCC with negative HCV antigen. Where as no significant difference of the other gene product expression was observed between the two groups.

CONCLUSION HCV appears related to about one-third of cases of HCC in Chongqing, the southwest of China, and it may be involved in hepatocarcinogen esis by inhibiting the function of p16 gene, which acts as a negative regulator of cell cycle.

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INTRODUCTION

Our previous studies by seroepidemiological, molecular epidemiological and immunopathological methods have revealed that hepatitis C virus (HCV) infection is closely linked to development of hepatocellular carcinoma (HCC) and HCV may be the second important factor in association with HCC- etiology in Chongqing, the southwest of China[1-5]. But the molecular mechanisms involved in hepatocarcinogenesis of HCV remain poorly understood. Up to now, many authors believe that HCV can not directly change the structure of the host genes like hepatitis B virus by integration because HCV is a RNA virus. Therefore, the effect of HCV on factors of controlling cell growth and development is an important field in the hepatocarcinogenesis studies. In this study, expression of several oncogene and tumor suppressor gene products in HCV-associated and non-HCV-associa ted HCC was investigated, so as to identify if HCV infection can affect expression of these gene products.

MATERIALS AND METHODS

Specimens

HCC specimens of 46 cases were randomly selected from partial hepatectomy in 1994 in this hospital. Of them, 38 cases contained pericancerous liver tissues. All specimens were fixed in 100mL/L-formalin, embedded in paraffin and sequentially sectioned with a thickness of 5 μm.

Reagents

Mouse monoclonal antibodies (mAb) to HCV NS3 and NS5 were kindly provided by Professor TAO Qi-Min (The Institute of Hepatology, Beijing Medical University). Mouse mAb against HCV CP10 was kindly presented by Professor LI Meng-Dong (Department of Infectious Diseases, the Southwest Hospital). Mouse mAbs to human ras p21, C-myc, C-erbB-2 and mutated p53 protein were purchased from Fuzhou Maxim Biotechnical Company. Mouse McAb to human p16 protein was purchased from Beijing Zhongshan Biotechinical Company. Avidin-biotin complex (ABC) kits were purchased from Fuzhou Maxim Biotechnical Company and Vector company.

Immunostaining

Immunostaining of HCV antigens CP10, NS3, NS5 and cancer-associated gene produc ts ras p21, c-myc,

c-erbB-2, mutated p53 and p16 proteins was performed by the ABC method in each case. The procedures of ABC staining were taken according to the manufacturer's recommendations as previously described[5]. The color was developed with diaminobenzidine and hematoxylin. Positive and negative controls were simultaneously used to ensure specifity and reliability of the staining.

RESULTS

Expression of HCV antigens

In the 46 cases of HCC, positive HCV antigen was found in 20 (43.5%) cases, of which 4 cases with one positive HCV antigen, 7 cases with two positive HCV antig ens, and 9 cases with three positive HCV antigens. The positive staining of HCV antigen CP10, NS3 and NS5 in the cancer tissues was observed in 10 (21.7%), 10 (21.7%) and 7 (15.2%) cases, respectively, while in its pericancerous liver tissues in 14 (36.8%), 13 (34.2%) and 12 (31.6%) cases. Although the expression rates were higher in the pericancerous tissues than in the cancer tissues, no statistical significance was obtained (P>0.05) (Table 1). The immunostaining of each HCV antigen was mainly seen in HCC and hepatocyte cytoplasms, seldom in the cell membranes, none in the nuclei. The positive-staining cells were distributed mostly in scattered or focalized patterns, seldom in diffused pattern.

Table 1 Expression of HCV antigens in HCC tissue and its surrounding liver tissue (%, positive rate)

HCV antigens	Cancer	Non-cancer
CP10	21.7(10/46)	36.8(14/38)
NS3	21.7(10/46)	34.2(13/38)
NS5	15.2(7/46)	31.6(12/38)

The effect of HCV infection on expression of the gene products

On the one hand, positive rates of ras p21 and mutated p53 in HCC (58.7%, 27/46; 28.3%, 13/46) were significantly higher than in the pericancerous tissues (34.2%, 13/38; 7.9%, 3/38, $P < 0.05$), whereas the positive rate of p16 in HCC (41.3%, 19/46) was significantly lower than in the pericancerous tissues (63.2%, 24/38, $P < 0.05$). But the expression rates of c-myc and c-erbB-2 did not show significant difference between the cancer and pericancerous groups ($P > 0.05$). On the other hand, it attracted our attention that the positive rate of P16 protein in HCV antigen-positive HCC (20%, 4/20) significantly lower than in HCV antigen-negative HCC (57.7%, 15/ 26, $P < 0.025$), even though the expression rates of ras p21, C-myc, C-erbB-2 and mutated p53 showed no significant difference between HCV-associated and non HCV-associated HCC (Table 2).

Table 2 Relationship of HCV antigens with expression of cancer-associated gene products(CAGP) (*n*, positive cases)

HCV antigens	*n*	CAGP expression				
		p21	C-myc	C-erbB-2	p53	p16
Positive	20	11	11	9	5	4
Negative	26	15	20	13	8	15

DISCUSSION

In the previous studies, we found that HCV RNA could be detected in 36.6% (34/93) serum samples of patients with primary hepatic carcinoma and 37.5% (21/56) cases of HCC tissues[1,3]. In this study, using three McAbs to different HCV antigens and immunohistochemical ABC method, we found that the positive immunostaining with either one, two or three HCV antigens was found in 20 (43.5%) cases, with either two or three HCV antigens in 16 (34.8%) cases and with three HCV antigens in 9 (19.6%) cases among the 46 cases of HCC. The present data are consistent with our previous studies and further indicate that about one-third of HCC seems to be related to HCV infection in Chongqing, the southwest of China. Up to now, a lot of affirmative evidences in seroepidemiology, molecular epidemiology and immunopathology have been obtained concerning the association of HCV infection with HCC development in this area.

Recent studies have shown that the molecular mechanisms of hepatocarcinogenesis are involved in oncogene activation and anti-oncogene inactivation like many other tumors. The role of ras, c-myc, c-erbB-2, p53 and p16 gene in the development and progression of HCC have been noted by many workers. To understand the potential hepatocarcinogenesis of HCV, we studied the expression of these gene products in HCV-associated and non-HCV associated HCC tissues. The results showed that the expression of ras p21, c-myc, c-erbB-2 and mutat ed p53 was not significantly different between HCV antigen-positive and HCV antigen-negative groups, but the deletion rate of p16 protein expression in HCV antigen-positive HCC (80%, 16/20) was significantly higher than in HCV antigen-negative HCC (42.3%, 11/ 26, $P < 0.025$). It implicates that the molecular mechanisms involved in HCV hepatocarcinogenesis seems to be connected with the repression of p16 gene function.

The p16 gene is a new negative regulator of cell

cycle and tumor supressor gene found recently, which is located in chromosome 9p21 with 8.5kb long and encoding for a nucleus phosphoprotein with 16kD-P16 protein. P16 protein can bind to cycle-dependent kinase 4 (CDK4), preventing their interaction with cyclin D and thereby preventing cell cycle progression from G1 to S phase. Many authors proposed that when p16 gene function is repressed, the activity of cyclin D/ CDK4 complex will increase because of the CDK4 being free from the inhibition of P16 protein, thereafter cell proliferation will be out of control and tumor may develop at last[6,7]. Recently, Ray *et al* reported that HCV core protein can act as an effector in the promotion of cell growth by repression trancription of the another negative regulator of cell cycle and inhibitor of cyclin D/CDK4 complex p21 (WAF1/ Cip1/ Sid1) gene through unknown cellular factors[8]. Therefore, the role of p16 gene in molecular mechanisms of HCV hepatocarcinogenesis deserves further studies.

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REFERENCES

1 Yang JM, Liu WW, Jin HY. Relationship of hepatitis C virus infection with primary hepatic carcinoma: an investigation of serum HCV RNA in different population groups.*J Med Coll PLA*,1993;8:109-113
2 Yang JM, Liu WW, Jin HY. Study on the replicative intermediate of hepatitis C virus in the tissues of hepatocellular carcinoma. *Chin J Dig*,1994;14:210-212
3 Yang JM, Liu WW, Jin HY. Detection of hepatitis C virus RNA in formalinfixed, paraffin embedded liver tissues from patients with hepatocullar carcinoma and liver cirrhosis. *Chin J Cancer*,1994;13:299-301
4 Yang JM, Liu WW, Luo YH. Genotypic investigation of hepatitis C virus in patients with primary hepatic carcinoma, liver cirrhosis and hepatitis. *Chin J Infect Dis*, 1995;13:1-3
5 Wang RQ, Zhou ZC, Yang JM, Fang DC.Immunohistochemical study of hepatitis C virus NS5 antigen in the tissues of hepatocellular carcinoma and its surroundings. *Natl J Med China*, 1996;76:623
6 Foulkes WD, Flanders TY, Pollock PM, Hayward NK. The CDKN2A (p16) gene and human cancer. *Mol Med*, 1997;3:5-20
7 Biden K, Young J, Buttenshaw R, Searle J, Cooksley G, Xu DB, Leggett B. Frequency of mutation and deletion of the tumor suppressor gene CDKN2A (MTS1/p16) in hepatocellular carcinoma from an Australian population. *Hepatology*,1997;25:593-597
8 Ray RB, Steele R, Meyer K, Ray R. Hepatitis C virus core protein represses p21WAF1/Gip1/Sid1 promoter activity. *Gene*, 1998;208:331-336

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