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WJG, 1999 February; 5(1):45-46
World Journal of Gastroenterology
 ISSN 1007-9327

Effect of HCV NS_3 protein on *p*53 protein expression in hep atocarcinogenesis *

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Subject headings liver neoplasms; oncogenes; hepatitis virus; *p*53 protein

Abstract

AIM To investigate hepatocarcinogenesis by detecting the effect of HCV NS_3 protein on *p*53 protein expression in hepatocellular carcinoma (HCC) and pericarcinomatous liver tissue (PCLT).

METHODS The expression of HCV NS_3 and *p*53 protein was det ected with immunohistochemical technique (SP method) in specimens of HCC and PCLT from 47 patients with negative HBV.

RESULTS The positive rate of HCV NS_3 protein was lower in HCC (62%) than in PCLT (83%) ($P < 0.025$). The better differentiaton of cancer cells, the stronger expression of HCV NS_3 protein ($P < 0.025$). The positive rate of *p*53 protein in HCC (81%) was higher than in PCLT (47%) ($P < 0.025$). The worse differentiaton of cancer cells, the stronger expression of *p*53 protein ($P < 0.05$). The *p*53 protein expression was not correlated with the HCV NS_3 protein expression in HCC ($P > 0.5$), whereas their expression was closely related to PCLT ($P<0.01$), and the expression rate of p53 protein in the cases of positive HCV NS_3 protein was higher than that in the cases of negative HCV NS-3 protein.

CONCLUSION HCV NS_3 protein may exert its hepatocarcinogenic effect in early stage on host cells by endogenous pathway which may bring about mutation of *p*53 gene and transformation of hepatocytes.

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***Project supported by the Health Ministry Science Foundation of China, No.94-120**

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Received 1998-09-10

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common human cancers in the world. Recently, the HCV infection was found to be an etiological factor to HCC. HCV is a RNA virus bearing no reverse transcriptase activity. Therefore, in stead of "promoter insertion", or "insertion mutagenesis", the HCV NS_3 expression of HCV gene may play an essential role in transfomation of hepatocytes[1]. *p*53 gene, an oncogene, has been intensively investigated recently. Mutation of this gene was found to be related to HCC in a variety of studies. Inorder to understand the relationship among *p*53 protein, HCV NS_3 protein and HCC, we studied the effect of HCV NS_3 protein on expression of *p*53 protein in HCC and pericarcinomatous liver tissue (PCLT).

MATERIALS AND METHODS

Tissue samples

HCC and PCLT were obtained by surgical resection from 47 patients in Xiangya Hospital and the Second Affiliated Hospital of Hunan Medical University, China. Forty patients were males and 7 females. Their age ranged 33 to 67 years (mean, 52 years), and all patients were negative for HBsAg serological marks. The tissues were fixed in 10% formalin and embedded in paraffin.

Reagents

Anti-HCV NS_3 protein MAb was purchased from GIB Comp. (Beijing, China), anti-*p*53 protein MAb and SP detection kit from Maixing Comp. (Fuzhou, China).

Immunohistochemistry

Five μm tissue sections were deparaffinized and washed in 0. 05mol/L PBS, handled with 20g/L H_2O_2 and treated with microwave. According to SP method, the tissues were detected with immunohistochemical technique. HCV RNA(+) biopsy liver tissues and breast cancer tissues were used as positive control of HCV NS_3 protein and *p*53 protein respectively. PBS was used as substitutes of Mabs for negative control groups.

Histological assessment

Semi-quantity analysis was performed as Formonitz[2] described.

Statistical analysis

The difference between each group was analyzed by Chi-square test.

RESULTS

Expression and distribution of HCV NS3 protein in HCC and PCLT

Among 47 cases, the positive rate of HCV NS_3 protein in HCC was 62% (29/47), and the positive cells were clustered or diffused in HCC. The positive signal was localized in cytoplasm. The expression strength of HCV NS_3 protein in HCC was related to the degree of carcinoma cell differentiation ($P < 0.05$). The better differentiaton of cancer cells, the stronger expression of HCV NS_3 protein ($P < 0.05$). The positive rate (83%) and the expression strength of HCV NS_3 protein in HCC were higher than those in PCLT. The distribution of positive cells in PCLT appeared large patchy or diffused.

Expression and distribution of p53 protein in HCC and PCLT

Of the 47 cases, the positive rate of *p*53 protein in HCC was 81% (38/47), and the positive cells appeared focal or patchy in HCC. The positive signal was in the nuclei. The expression strength of *p*53 protein in HCC was correlated with the degree of carcinomatous cell differentiation ($P < 0.05$). The worse differe ntiaton of cancer cells, the stronger expression of *p*53 protein ($P < 0.05$). The positive rate (47%) and the expression strength of *p*53 protein in HCC were lower than those in PCLT. The positive cells in PCLT was scattering in distribution.

Table 1 The expression strength of HCV NS protein and *p*53 protei n in HCC and PCLT

	n	HCV NS_3 protein					*p*53 protein				
		-	+	++	+++	Positive rate(%)	-	+	++	+++	Positive rate(%)
HCC											
I	8	2	0	3	3	6/8	4	2	2	0	4/8
II	10	2	1	2	5	8/10	2	3	4	1	8/10
III	18	8	3	6	1	56	2	0	6	10	89
IV	11	6	3	2	0	46	1	1	3	6	91
PCLT											
Normal	3	2	1	0	0	1/3	3	0	0	0	0
Hepatitis	23	4	2	5	12	83	13	8	2	0	44
Cirrhosis	21	2	2	6	11	91	9	7	5	0	57

Relationship between p53 protein expression and HCV NS3 protein

In HCC, the expression rate of *p*53 protein in 29 cases of positive HCV NS_3 protein was 83% (24/29), and in 18 cases of negative HCV NS_3 prote in was 78% (14/18), the difference between the former and the latter being not significant ($P>0.1$). In PCLT, the expression rate of *p*53 protein in 39 cases of positive HCV NS_3 protein was 54% (21/39), and in 8 cases of negative HCV NS_3 protein was only 13% (1/8), the difference between the former and the latter being significant ($P<0.05$).

DISCUSSION

Chronic infection with HCV is strongly associated with the development of HCC. HCV causes HCC by expressing protein, especially HCV NS_3 protein[1,3]. Nevertheless, the exact molecular mechanism remains quite unknown. Our results showed that the positive rate and expression strength of HCV NS_3 protein in PCLT were higher than those in HCC, and the expression strength of HCV NS_3 protein in HCC was related to the degree of carcinoma cell differentiation. The better differentiaton of cancer cells, the stronger expression of HCV NS_3 protein. It is indicated that the cellular internal environment for HCV replication is disturbed with cancer growth. HCV may be eliminated at the final stage of hepato cytes transformation because the virus without reverse transcriptase is unable to integrate into the host hepatocytes genome. On the other hand, the positive rate and expression strength of *p*53 protein in PCLT were lower than those in HCC. The expression strength of *p*53 protein in HCC was related to the degree of carcinoma cell differentiation. The worse differentiaton of cancer cells, the stronger expression of *p*53 protein. It is suggested that *p*53 protein may play a role in the morphological change and the differentiated degree of cancer cells in HCC, thus detecting *p*53 protein expression is of benefit to HCC prognosis.

Effect of HCV NS_3 protein on mutation of *p*53 gene was not confirmed, although HCV infection may result in mutation of *p*53 gene[4]. This study revealed that there was no relationship between *p*53 protein and HCV NS_3 prote in in HCC. In PCLT, *p*53 protein expression was positive in 21 of 39 cases of pos itive HCV NS_3 protein while only one case of *p*53 protein expression in 8 cases of negative HCV NS_3 protein was positive. HCV NS_3 protein may exert its hepatocarcinogenic effect in early stage on host cells by endogenous pathway which may bring about mutation of *p*53 gene and transformation of hepatocytes.

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Edited by MA Jing-Yun