

Pathogenesis of cholangiocarcinoma in the porta hepatis and infection of hepatitis virus

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OBJECTIVE: To study the correlation between human cholangiocarcinoma in the porta hepatis and the infection of hepatitis virus.

METHODS: Immunohistochemistry was used to detect HBxAg and HCV-C protein in formalin-fixed and paraffin-embedded samples taken from 68 patients with cholangiocarcinoma in the porta hepatis. The findings were reviewed against the clinical records of the patients.

RESULTS: Six patients (8.8%) were positive for HBxAg and 24 (35%) for HCV-C protein, respectively. One patient was positive for both HBxAg and HCV-C protein. There were statistically differences in the extent of differentiation, invasion, lymph-node metastasis, and treatment between the patients with cholangiocarcinomas in the porta hepatis with HB(C)V infection and those without infection.

CONCLUSIONS: HB(C)V infection is correlated to the development of cholangiocarcinoma in the porta hepatis. The tumor with HB(C)V infection may have a higher malignancy biologically and poorer prognosis. HBxAg and HCV-C protein may play an important role in the pathogenesis of cholangiocarcinoma in the porta hepatis.

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Key words: cholangiocarcinoma; porta hepatis; hepatitis B virus; hepatitis C-like virus

Introduction

Infections of hepatitis C virus (HCV) and hepatitis B virus (HBV) are risk factors for the development of hepatocellular carcinoma. Recent studies have detected HBV DNA, HCV RNA, HBV or HCV antigens in cholangiocarcinoma patients,^[1-4] but the correlation between hepatitis virus and cholangiocarcinoma is not clear. To study the correlation between cholangiocarcinoma in the porta hepatis and hepatitis virus, we used immunohistochemistry to detect HBxAg and HCV-C antigen in formalin-fixed and paraffin-embedded samples taken

from 68 patients with cholangiocarcinoma in the porta hepatis, and reviewed the clinical records of these patients.

Methods

Patients

Formalin-fixed and paraffin-embedded samples were collected from 68 patients with cholangiocarcinoma in the porta hepatis treated at the First Hospital of China Medical University, Qingdao, Qingdao Municipal Hospital of Qingdao University, Qingdao, and Tongji Hospital of Tongji Medical University, Wuhan, from 1990 to 1999. These patients (42 men and 26 women) aged from 32 to 69 years (median 58.2 years) underwent radical resection (41 patients) or palliative operation (27) respectively. The types of this cholangiocarcinoma included tubular adenocarcinoma (28 patients), papillary adenocarcinoma (18), mucoid carcinoma

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(10), and non-differentiated carcinoma (12). The tumors were well, moderate or poor differentiated in 38, 10 and 20 patients, respectively. UICC classification revealed T₁ in 32 patients, T₂ in 16, and T₃ in 20. Lymph-node metastasis occurred in 42 patients and non lymph-node metastasis in 26.

Serological markers and risk factors for HBV and HCV

Serological markers for HBV and HCV were tested by ELISA assay. Positive serological markers for HBV and HCV were detected in 13 and 8 patients respectively, and 2 patients were both HBV and HCV positive. The risk factors for HBV or HCV infection in the porta hepatitis included HBV infection, HCV history, transfusion history, opera-

tion history, mother or mate with HBV infection or HCV history, and history of close contact with HBV or HCV patients. Thirty-eight patients had two or more risk factors and 30 less than two risk factors.

Detection methods

Five-micron sections were dewaxed in xylene and rehydrated. Endogenous peroxidase was blocked for 15 minutes with 3% hydrogen peroxide (H₂O₂) in phosphate-buffered saline (PBS) at room temperature. The sections were blocked with a combination of normal mouse serum and then incubated with anti-HBx or anti-HCV-C protein (dilution 1:50, mouse anti-HBx and anti-HCV core proteins by Chemicon Co., USA). This procedure was fol-

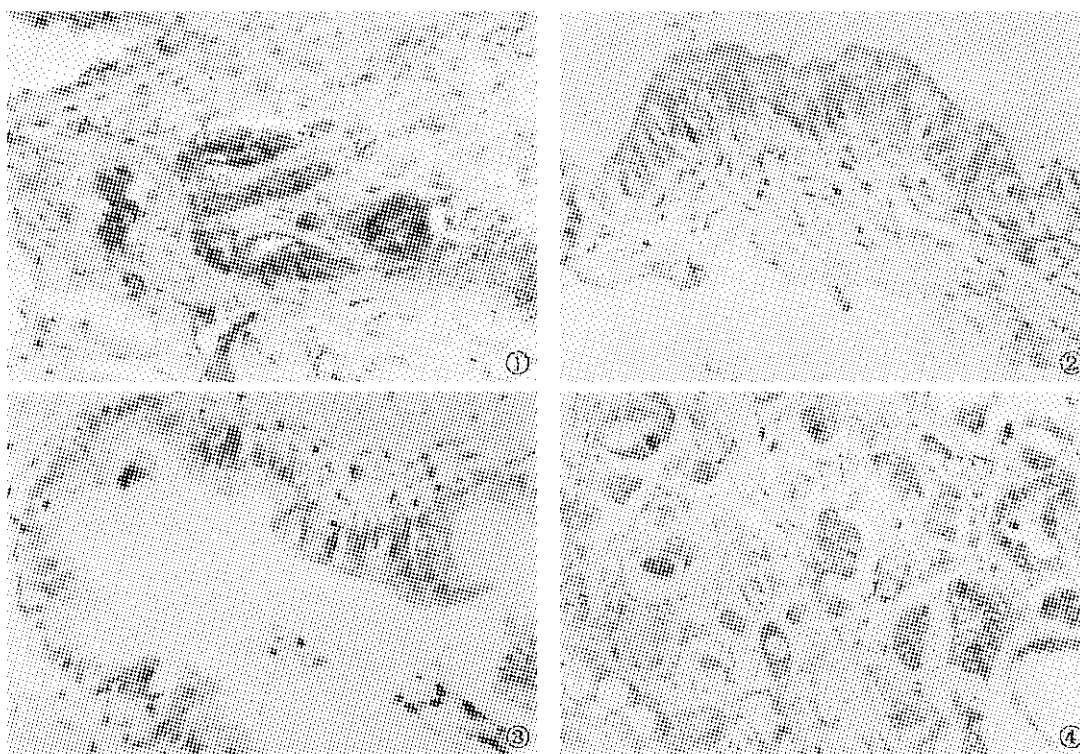


Fig. 1. HBxAg in cholangiocarcinoma detected using SABC method. The positive signals were located in the cytoplasm, nuclei and distributed in clusters (original magnification $\times 200$).

Fig. 2. HBxAg in cholangiocarcinoma detected using SABC method. The positive signals were located in the cytoplasm, nuclei and distributed scatteredly (original magnification $\times 200$).

Fig. 3. HCV-C protein in cholangiocarcinoma detected using ABC method. The positive signals were located in the cytoplasm and distributed in clusters (original magnification $\times 200$).

Fig. 4. HCV-C protein in cholangiocarcinoma detected using ABC method. The positive signals were located in the cytoplasm and distributed scatteredly (original magnification $\times 200$).

lowed in turn by biotinylated-conjugated sheep anti-mouse IgG (Boster Co., USA). The complex was visualized by a diaminobenzidine (Boster Co., USA). The specificity of the reaction was confirmed by 1) examination of uninfected liver tissue and liver tissue for HBV or HCV-infected patients; 2) replacement of the primary mouse antiserum with preimmune serum; 3) single substitution of each antibody layer with the diluent PBS.

Statistical analysis

The differences were analyzed using χ^2 test.

Results

Six patients (8.8%) were HBxAg positive and 24 (35%) HCV-C protein positive. One patient was both HBxAg and HCV-C protein positive. Both HBxAg and HCV-C protein localized to the cytoplasm of cholangic epithelial cells. Moreover, HBxAg was detected partly in the nuclei. It was crisp and finely granular (Figs. 1-4).

The positive expression of HBxAg or HCV-C protein were observed in 22 patients of 38 patients with two or more risk factors. However, 7 patients were positive among 30 patients with less than two risk factors ($P < 0.01$).

The positive expression of HBxAg or HCV-C protein was noted in 13 (31.7%) of 41 patients undergoing radical resection. Moreover, 16 (59.3%) were positive among 27 patients undergoing palliative operation ($P < 0.05$).

The positive expression of HBxAg or HCV-C protein was statistically different in the extent of differentiation, invasion, and lymph-node metastasis ($P < 0.05$, < 0.05 , < 0.01), but not related to the pathological type of cholangiocarcinoma in the porta hepatis ($P > 0.05$, Table 1).

The expression of HBxAg was observed in 6 (8.8%) of the 68 patients with cholangiocarcinoma in the porta hepatis. Five patients showed HBxAg positive expression in 13 patients with positive HBV-serological markers ($P < 0.01$). Moreover, the expression of HCV-C protein was seen in 24 (35%) of the 68 patients with this cholangiocarcinoma. Six patients (75%) showed positive expres-

Table 1. The expression of HBxAg or HCV-C proteins and pathological characteristics of cholangiocarcinoma in the porta hepatis

Pathological characteristics	n	HBxAg or HCV-C protein expression		P
		+	Positive rate (%)	
Pathological type				
Tubular carcinoma	28	10	32.1	
Papillary carcinoma	18	8	44.4	
Mucoid carcinoma	10	6	60.0	
Non-differentiated carcinoma	12	5	41.7	>0.05
Differentiation				
Well and moderate	48	15	31.3	
Poor	20	14	70.0	<0.01
Invasion				
T ₁	32	11	34.3	
T ₂	16	5	31.3	
T ₃	20	13	65.0*	<0.05
Lymph node metastasis				
N ₀	26	8	30.8	
N ₁	42	21	50.0	<0.05

* : The positive expression of HBxAg or HCV-C protein was statistically different between T₃ and T₁, T₂ ($P < 0.05$).

Table 2. The expression of HBxAg or HCV-C proteins and serological markers of cholangiocarcinoma in the porta hepatis

Serological markers	n	HBxAg or HCV-C protein expression	
		+	Positive rate (%)
HBsAg HBeAg HBcAb (+)	1	1	100
HBsAg HBeAg (+)	1	0	0
HBsAg HBcAb (+)	1	0	0
HBsAg (+)	8	3	37.5
HCVAb (+)	6	5	83.3
HCVAb HBsAg (+)	1	1*	100
HCVAb HBsAg HBcAb (+)	1	0	0

* both HBxAg and HCV-C proteins were positively expressed.

sion of HCV-C protein in 8 patients with positive HCV-serological markers ($P < 0.05$, Table 2).

Discussion

Cholangiocarcinoma is a cancer in incidence next to liver cancer in the hepatobiliary system. Two-

thirds of such tumors are located in the porta hepatitis, one-fourth in the mid and low segments of the common bile duct, and the others in the liver. The incidence and mortality of cholangiocarcinoma of the porta hepatitis are increasing worldwide, with an annual incidence of 3000 in the USA. Cholelithiasis, cystic dilation of the biliary system, ulcerative colitis, and primary sclerosing cholangitis are thought to be the risk factors for cholangiocarcinoma of the porta hepatitis. Recent studies^[1-4] found that the infection of HBV and HCV is related to the development of this cholangiocarcinoma, but the correlation between hepatitis virus and the tumor has not been elucidated.

In the past, HBV and HCV were considered as hepatotropic viruses and viral replication and cellular injury were confined to the liver. Recent findings suggested that HBV and HCV may replicate in tissues other than in hepatocytes. Many researchers found the presence of HBV DNA, HCV RNA and HBVAg or HCVAg in the lymph nodes, pancreas, ovary, kidney, heart, and bile duct epithelial cells^[5] and confirmed that the infection of HBV or HCV may lead to virus cholecystitis or cholangitis. The infection of HBV or HCV causes bile duct damage and loss, which is characterized by swelling of epithelial cells, steatosis, lymphocytic infiltration of the bile duct.^[6]

Changes after infection of hepatitis virus showed that several proteins encoded in the HBV and HCV genomes, and the function of HBxAg and HCV-C proteins play important roles in carcinogenesis. HBxAg could alter directly or indirectly the cellular genes by strong transactivation of oncogenes including C-myc, H-ras, K-ras, IGF-II and inactivation of the tumor suppressor gene-p53.^[7] HCV-C proteins could act as a transcriptional regulator of viral and cellular promoters to potentially disrupt normal cellular functions.^[7] Cooperating with ras oncogenes, these C proteins may transform primary rat embryo fibroblasts to a tumorigenic phenotype.^[7] Also they cause anti-apoptosis by inactivation of the tumor suppressor gene-p53 and activation of NF- κ B. Moreover, they implicate a mechanism by which HCV may invade the host's immune system, leading to viral persistence and possibly to carcinogenesis.^[8,9] Thus, HCV-C proteins

play a major role in the malignant transformation of cells.

In our study, the expression of viral proteins was noted in 6 patients (8.8%) with HBxAg positive in 24 patients (35%) with HCV-C protein positive. One patient was both HBxAg and HCV-C protein positive. The positive expression rate of the 68 patients with cholangiocarcinoma in the porta hepatitis was 42.6% (29/68). This suggests that HB(C)V infection is correlated with the development of this tumor. The expression of HBx-Ag or HCV-C protein was related to the risk factors for HBV or HCV infection in patients with cholangiocarcinoma in the porta hepatitis ($P < 0.01$). Moreover, there are significant differences in the extent of differentiation, invasion, lymph-node metastasis, and treatment between the patients with this cholangiocarcinoma with HB(C)V infection and those without infection. Patients with cholangiocarcinoma in the porta hepatitis with HB(C)V infection may have a higher malignancy biologically and poorer prognosis. In our study, the expression of HCV-C protein in this cholangiocarcinoma was higher than the expression of HCV-serological markers ($P < 0.05$). However, the expression of HBxAg was lower than that of the HBV-serological markers ($P < 0.01$). It is suggested that HBxAg and HCV-C protein may play an important role in the pathogenesis of cholangiocarcinoma in the porta hepatitis.

Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

References

- 1 Ltermann G. Immunohistochemical study of HBV antigen in 338 liver cell carcinomas. *J Gastroenterol* 1999;37:329-342.
- 2 Lu HY, Ye MQ, Thung SN, et al. Detection of hepatitis C virus RNA sequences in cholangiocarcinomas in Chinese and American patients. *Chin Med J* 2000; 113:1138-1141.
- 3 Wang WL, Wang CJ, Wang BF. Significance of HCV gene and its antigen expression in human primary intrahepatic cholangiocarcinoma. *Shijie Huaren Xiaohua Za*

- Zhi 2001;9:542-545.
- 4 Liu XF, Zou SQ, Qiu FZ. Construction of HCV-core gene vector and its expression in cholangiocarcinoma. *World J Gastroenterol* 2002;8:135-138.
 - 5 Yoffe B, Noonan CA. Hepatitis virus new and evolving issues. *Dis Sci* 1992;37:1-9.
 - 6 Goldin RD, Patel NK, Thomas HE. Hepatitis C and bile duct loss. *J Clin pathol* 1996;49:836-838.
 - 7 Ray RB, lagging LM, Meyer K, et al. Transcriptional regulation of cellular and viral promoters by the hepatitis C virus core protein. *Virus Res* 1995;37:209-220.
 - 8 Ray RB, Robert S, Keith M, et al. Communication-transcriptional repression of p53 promoter by hepatitis C virus core protein. *J Biol Chem* 1997;272:10983-10989.
 - 9 Marusawa H, Hijikata M, Chiba J, et al. Hepatitis C virus core protein inhibits fas-and tumor necrosis factor alpha-mediated apoptosis via NF- κ B activation. *J Virol* 1999;73:4713-4720.

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