PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

WJG, 2000 February; 6(1):134-135
World Journal of Gastroenterology
 ISSN 1007-9327

Overexpression of *P*-glycoprotein in hepatocellular carcinoma and its clinical implication

Kong XB, Yang ZK, Liang LJ, Huang JF and **Lin HL**

Subject headings liver neoplasms; carcinoma, hepatocellular; P-glycoprotein; multidrug resistance-1 gene;immunohistochemistry

INTRODUCTION

Most advanced hepatocellular garcinoma (HCC) is insensitive to most anticancer drugs which might be related to the high frequency of expression of the multidrug resistance-1 (MDR1) gene[1] and its product, P-glycoprotein (P-gp)[2]. P-gp expression may also be concerned with tumor progression and differentiation[3]. In the present study, we investgated P-gp expression and assessed the relationship between expression level of P-gp and the clinico-pathological parameters of HCC by immunohistochemistry in combination with computer-imaging analysis.

MATERIALS AND METHODS

Patients and specimens

The data were from 47 HCC resected specimens, from June 1996 to July 1997, including 47 tumor tissues and 33 adjacent tissues, 39 men and 8 women, age 25-74 years (mean 49 years). No one received preoperative chemotherapy. Of the 47 tumors, 72% were >5 cm, and 28% were ⩽5 cm, 40% and 26% were accompanied by involvement of portal veins with presence of satellite nodules and intrahepatic metastasis, respectively. The HCC consisted of well and poorly differentiated types and 11 normal liver tissues were obtained at surgery from patients without chronic liver disease as control.

Xian Bing Kong[1], Zu Kui Yang[1], Li Jian Liang[2], Jie-Fu Huang[2] and Han Liang Lin[3]
[1]Department of Surgery, The First Affiliated Hospital, Chongqing University of Medical Sciences, Chongqing 400016, China
[2]Division of Hepatobiliary Surgery, The First Affiliated Hospital, Sun Yat-Sen University of Medical Sciences, Guangzhou 510080, Guangdong Province, China
[3]Department of Pathology, Sun Yat-Sen University of Medical Sciences, Guangzhou 510089, Guangdong Province, China
Dr. Xian Bing Kong, male, born in 1961-10-17 in Pengxi County, Sichuan Province, graduated from Chongqing University of Medical Sciences as postgraduate in 1989, having 8 papers published.
Supported in part by the China Medical Board of New York, Inc., USA, Grant No.90-534
Correspondence to: Dr. Xian Bing Kong, Department of Surgery, The First Affiliated Hospital, Chongqing University of Medical Sciences, Chongqing 400016, China
Tel. +86-23-68899866
Received 1999-06-23 **Accepted** 1999-09-18

Immunohistochemical stainings for P-gp

Serial sections of 4ìm in thickness were prepared and immunohistochemical examination was performed by the streptavidin- biotin-complex (SABC) method using monoclonal antibody JSB1 (Boehringer Mannheim). The negative control was processed by substituting PBS for the primary antibody. The positive control was a breast cancer sample shown to express P-gp by SABC method.

Quantitative analysis

Immunoprecipitates were analyzed using an Axiotron microscope (Zeiss, Germany) and a 3CCD camera (JVC, Japan), and then processed by an image analysis system (IBAS, Kontron, Germany). According to Shen's method[4], only the gray level and the area of defined area A and the phase á of positive products in A need to be tested. Positive unit (PU) was tested and calculated with the formula in A: $PU = 100\times|G_\alpha\text{-}G_A/(1\text{-}A_{A\alpha})\times G_{max}$. G_α and G_A denote respectively the mean gray level of the studied structure α and the test area A. $A_{A\alpha}$ means the area density of phase α in A. G_{max} is the maximum gray level of the instrument. PU that reflected intensity and area of the phase α of positive products in A represented *P*-gp ex pression level. Five times were performed at random in a section to obtain the mean PU.

Statistical analysis

PU of *P*-gp was expressed as mean±SD. The data were analyzed statistically by means of Student's *t* test. Statistical significance was defined as a two-sided *P* value of $P<0.05$.

RESULTS

Positive expression of P-gp in HCC, adjacent to tumor and normal liver tissues

P-gppositive cells were not uniformly distributed, the intensity of immunoreactivity was also variable, staining was mainly on the cellular membrane. In normal liver, *P*-gp was present on the bile canalicular surface of hepatocytes and the luminal surface of bile duct epithelial cells. In *P*-gp-positive HCC, the staining was observed on the contact surface between tumor cells resembling the canalicular staining pattern (Figure 1). *P*-gp expression was found in 70% (33/ 47) of HCC tissues and 66% (22/33) of adjacent tissues. The staining was mild in three normal liver tissues

specimens. A significant increase in *P*-gp expression level was observed in HCC tissues ($P<0.01$) and those adjacent to the tumor ($P<0.01$) but without significant difference ($P>0.05$).

P-gp expression level in relation to clinico-pathological parameters in HCC

The expression level of *P*-gp in HCC with local invasion was significantly higher than that in HCC without ($P<0.05$). Moreover, it was significantly higher in HCC of the well differentiated type than that of poorly differentiated ($P<0.01$). Recurrence of HCC also had a significantly higher *P*-gp expression than that without ($P<0.01$). On the other hand, there were no relationships between *P*-gp expression level and the tumor size ($P>0.05$).

DISCUSSION

Hepatocytes exclusively express *P*-gp on the bile canalicular surface, it is a M_r170 000 membrane protein responsible for pumping lipophilic anticancer drugs such as doxorubicine and vincristine out of tumor cells[5], resulting in chemotherapeutic effect poor. Recent reports showed increased expression of *P*-gp in HCC was significantly associated with non-responders[6]. Our results indicated more than two thirds of the samples were positive for *P*-gp in HCC and adjacent tissues, this suggested that *P*-gp overexpression be probably responsible for the so-called innate drug resistance of HCC or non responder.

The fact that *P*-gp expression level was significantly higher in well differentiated than poorly differentiated HCC ($P < 0.01$), suggested the well differentiated HCC have a stronger potential of *P*-gp expression and a tendency to produce resistance to anticancer drugs more easily. It is worth trial to treat with anticancer drugs in combination with a reverse agents of MDR1 gene or *P*-gp modulator as verapamil, preferably supported by data on *P*-gp expression.

The intrahepatic spreading, metastasis and postoperative recurrence were closely related to the biologic behavior of tumor cells, etc, about 43.5% of patients with small HCC recurred within 5 years after surgical resection[7]. Recently, *P*-gp overexpression had been demonstrated in cancer with recurrence[8]. So far there have been no reports on *P*-gp expression in HCC recurrence. In this study, hepatic recurrence had a significantly higher *P*-gp expression level than that without ($P<0.01$). These results revealed that recurrence of hepatoma probably more easily to acquire multidrug resistance, thereby, the response to chemotherapy was likely unsatisfactory.

Our study further showed *P*-gp expression level of invasive HCC was significantly higher than that of non-invasive HCC($P<0.05$), suggesting that a high *P*-gp expression level was well associated with the invasiveness of HCC, and not related to the size of the tumor whether large or small.

REFERENCES

1 Gottesman M. How cancer cells evade chemotherapy. *Cancer Res*, 1993;53:747-763
2 Park J, Lee S, Hong I. MDR1 gene expression: its effect on drug resistance to doxorubicin in hepatocellular carcinoma cell lines. *J Natl Cancer Inst*, 1994;86:700-708
3 Chin KV, Ueda K, Pastan I. Modulation of activity of the prooter on the human MDR-1 gene by Ras and P53. *Science*,1992;255:459-462
4 Shen H. Study on the quantitative method of immunohistochemistry (3). *Chin J Histocytochem*,1995;4:89-91
5 Goldstein LJ.MDR gene expression in solid tumors.*Eur J Cancer*, 96;32A:1039-1047
6 Chou YY, Cheng AL, Hsu HC. Expression of P-glycoprotein and P53 in advanced hepatocellular carcinoma treated by single agent chemotherapy: clinical correlation.*J Gastroenterol Hepatol*,1997; 12:569-575
7 Tang ZY. Studies on small hepatocellular carcinoma clinical aspect and molecular biology. *Hepatol Rapid Literat Rev*,1996;26:11-19
8 Gregorcyk S, Kang Y, Brandt D.P-gp expression as a predictor of breast cancer recurrence.*Ann Surg Oncol*,1996;3:8-14

Edited by Wu XN
Proofread by Miao QH