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World J Gastroenterol 2004;10(6):830-833
World Journal of Gastroenterology
 ISSN 1007-9327

• LIVER CANCER •

Expression of transforming growth factor-α and hepatitis B surface antigen in human hepatocellular carcinoma tissues and its significance

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Received: 2003-12-19 **Accepted:** 2004-01-08

Abstract

AIM: To evaluate the expression of transforming growth factor-alpha (TGF-α) and hepatitis B surface antigen (HBsAg) in human hepatocellular carcinoma (HCC) tissues and its significance.

METHODS: Seventy specimens of HCC tissues were detected by immunohistochemical method. Five specimens of normal human liver tissues were used as control.

RESULTS: The TGF-α positive expression rates in HCC and its surrounding tissues were 74.3%(52/70) and 88.1%(52/59), respectively. TGF-α positive granules were mainly in the cytoplasm and fewer existed on the karyotheca. The TGF-α positive expressing rate in well differentiated HCC was significantly higher than that in moderately and poorly differentiated HCC ($P<0.05$). The TGF-α positive expression also was observed in intrahepatic bile ducts (part of those were hyperplastic ducts). The HBsAg positive expression rates in HCC and its surrounding tissues were 21.4%(15/70) and 79.7%(47/59), respectively. HBsAg positive granules were in the cytoplasm, inclusion and on the karyotheca. There was a prominent positive correlation between TGF-α and HBsAg expression in HCC surrounding tissues ($P<0.05$, $\gamma=0.34$). TGF-α was usually existed with HBsAg in regenerated and/or dysplastic liver cells. In the five normal liver tissues, TGF-α and HBsAg were not detectable in hepatocytes and bile ducts.

CONCLUSION: Hepatitis B virus infection is closely related with hepatocarcinogenesis. The overexpression of TGF-α in the liver seems to be associated with the regeneration of hepatocytes injured by HBsAg. The continued expression of TGF-α might lead to dysplasia of liver cells and development of HCC. Furthermore, TGF-α might play a role in morphogenesis and regeneration of intrahepatic bile ducts.

Zhang J, Wang WL, Li Q, Qiao Q. Expression of transforming growth factor-α and hepatitis B surface antigen in human hepatocellular carcinoma tissues and its significance. *World J Gastroenterol* 2004; 10(6): 830-833

http://www.wjgnet.com/1007-9327/10/830.asp

INTRODUCTION

Hepatocellular carcinoma (HCC) is a common malignant tumor in China with poor prognosis[1-8]. Substantial evidences have supported the concept that hepatitis B virus (HBV) infection is a causative factor for HCC[9-12]. The idiographic mechanisms of HBV in hepatocarcinogenesis had not been clearly defined yet. Transforming growth factor-alpha (TGF-α) is a multi-peptide, which can promote cellular proliferation and transformation[13-15]. The normal hepatocytes almost had no expression of TGF-α mRNA except the epitheliums of bile ducts in human normal liver tissues[16]. It was also thought to be an autocrine regulator of normal growth and regeneration in the rat liver. There was also a close relationship between TGF-α and hepatocarcinogenesis[17-19]. In order to find the relationship between TGF-α and HBV infection in hepatocarcinogenesis, the expression of TGF-α and HBsAg in HCC tissues was studied by immunohistochemical method. Such studies have been rarely reported in China.

MATERIALS AND METHODS

Specimens

Tissue samples were obtained from seventy cases of HCC in Xijing Hospital from January1988 to January 1995. Among them, fifty-nine cases of HCC had surrounding tissues. Five cases of normal human liver tissues were obtained from autopsy (excluding liver disease). All specimens were fixed in 40 g/L methanal solution, embedded in wax, and cut into 5 μm thick serial sections.

Reagents and methods

Mouse anti-human TGF-α was purchased from Santa Cruze, UAS. Mouse anti-human HBsAg and SABC kit were purchased from Wuhan Boster Co. Ltd. The expression of TGF-α and HBsAg was detected by SABC immunohistochemical method. In the control group, the primary antibody was substituted by PBS or normal mouse serum. All paraffin embedded sections were deparaffinized and rehydrated, and pretreated for 20 min at 75 ℃ in a microwave oven. After being treated with 1 mL/L H_2O_2 for 30 min to block the endogenous peroxidase, the sections were incubated with 20 mL/L fetal calf serum for 30 min to reduce nonspecific binding. Then the primary HBsAg and TGF-α antibodies were applied to the sections and incubated at 4 ℃ overnight. The sections were subsequently incubated with goat anti mouse IgG at 37 ℃ for 30 min, followed by incubation with SABC at 37 ℃ for 30 min, and stained with DAB-H_2O_2 for 5-10 min and counterstained with hematoxylin. Results estimation: the specimens with no positive cells or positive cells <10% were negative (-); with light brownish yellow cells or positive cells between 10-50% were weakly positive (±); with deep brownish yellow cells or positive cells >50% were positive (+).

Statistical analysis

Chi-square test was used to analyse the results. The expression of TGF-α and HBsAg were compared by analysis of coherence.

RESULTS

The expression of TGF-α and HBsAg in HCC

The TGF-α positive expression rates in HCC and its surrounding tissues were 74.3%(52/70) and 88.1%(52/59), respectively. The positive cells mainly existed diffusely and fewer existed in foci. The positive stainings were like brown-yellow granules, and were mainly located in the cytoplasm and/or fewer located on the karyotheca (Figure 1). There was a prominent difference in TGF-α distribution between HCC and its surrounding tissues (χ^2=3.93, P<0.05). The TGF-α positive expression rate in well differentiated HCC was significantly higher than that in moderately and poorly differentiated HCC (χ^2=9.11, P<0.05, Table 1). TGF-α positive expression also was observed in intrahepatic bile ducts (part of those were hyperplastic ducts). In the five cases of normal liver tissues, TGF-α was undetectable in hepatocytes and bile ducts.

Table 1 TGF-α expression in different HCC differentiation

Differentiation	*n*	Positive cases	Positive rate (%)
Well	15	15	100.0
Moderately	48	34	70.8
Poorly	7	3	42.9

The HBsAg positive expression rates in HCC and its surrounding tissues were 21.4%(15/70) and 79.7%(47/59), respectively. The positive cells mainly existed diffusely and fewer existed in foci. The positive stainings were like brown-yellow granules or floccules. They were located in the cytoplasm, inclusion and on the karyotheca (Figure 2). There was a prominent difference in HBsAg distribution between HCC and its surrounding tissues (χ^2=43.5, P<0.05). HBsAg expression was negative in the five cases of normal liver tissues.

The PBS blank controls and normal mouse serum substituted controls were negative for immunohistochemical staining.

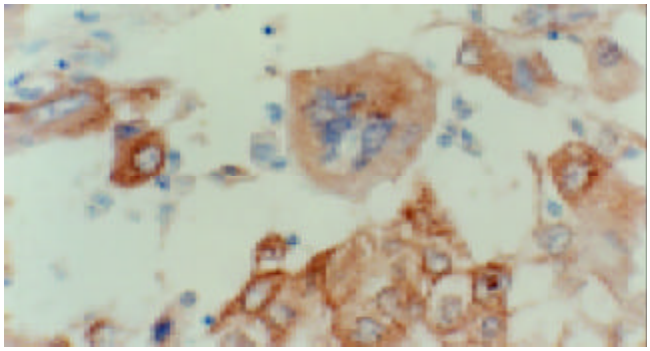

Figure 1 TGF-α positive stainings as brownish yellow granules in the cytoplasm of HCC tumor cells. SABC ×400.

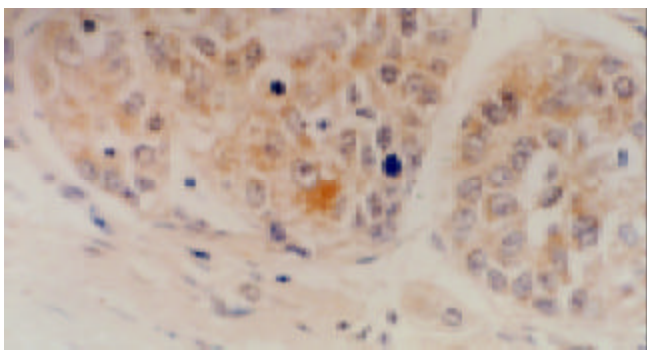

Figure 2 HBsAg positive stainings as brownish yellow granules in the cytoplasm of HCC tumor cells. SABC ×400.

Relationship between TGF-α and HBsAg expression

There was not a prominent correlation between TGF-α and HBsAg expression in HCC tissues, but there was a prominent positive correlation between TGF-α and HBsAg expression in HCC surrounding tissues (P<0.05, γ=0.34, Table 2). TGF-α was usually existed with HBsAg in regenerated and/or dysplastic liver cells. These cells had increscent or double nucleus, prominent or anomalous nucleolus, high ratio of nucleus/ cytoplasm, *etc*. (Figures 3 and 4).

Table 2 Relation between TGF-α and HBsAg expression in HCC surrounding tissues

TGF-α	*n*	HBsAg +	HBsAg -
+	52	44	8
-	7	3	4
Total	59	47	12

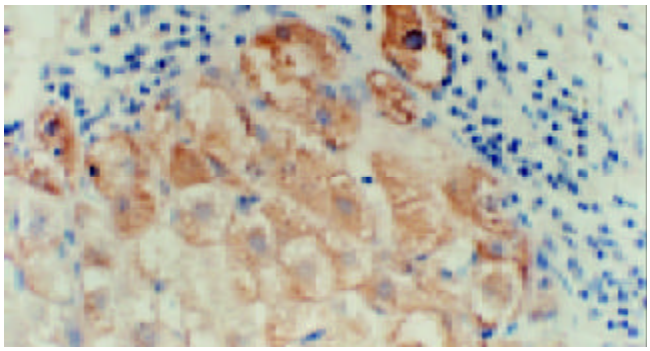

Figure 3 Location of immunohistochemical staining of TGF-α expression in regenerated and / or dysplastic liver cells. SABC ×400.

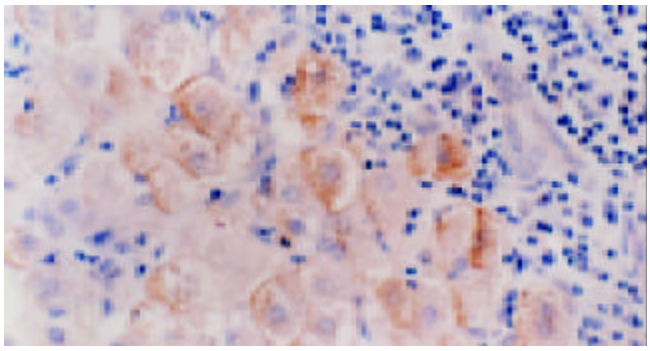

Figure 4 Location of immunohistochemical staining of HBsAg expression in regenerated and / or dysplastic liver cells. SABC ×400.

DISCUSSION

TGF-α was found in culture medium of fibroblasts transformed by some retroviruses in 1970s and nominated because of its ability to transform and induce the renal fibroblasts to proliferate. It is composed of 50 amino acid residues, with a 30% to 40% amino acid homology to epidermal growth factor (EGF), and binds the EGF receptor in the cellular membrane[20]. TGF-α was secreted by many transformed cells and involved in embryonic development. In human cancers, studies showed that TGF-α could serve as a tumor marker and as a marker for the malignant potential of a tumor[21]. Thus far, the types of carcinomas with which abnormal TGF-α expression has been associated include liver[22], gastrointestinal[23], breast[24], skin[25], lung[26], brain[27] and ovarian cancers[28]. TGF-α might play a role in the processes involved with tumor initiation and

growth. In cell lines, TGF-α has been found to be associated with autocrine and/or paracrine types of cellular growth initiation and with increased levels of oncogene expression[29,30]. It could promote the hepatocellular proliferation, differentiation, regeneration and tumor cell growth[31]. Transgenic mice that overexpressed TGF-α developed liver tumors between 12 and 15 months of age[22]. In our study, we found the TGF-α expression in the HCC and its surrounding tissues was significantly higher than that of normal liver tissues. The positive rate of TGF-α expression in well differentiated HCC was higher than that in moderately and poorly differentiated HCC. These may suggest the involvement of TGF-α in cellular transformation and provide a supporting evidence for the autocrine stimulation model. Increased expression of TGF-α might be the events of human primary hepatocarcinogenesis. In the later stages of HCC, there might be the other factors responsible for decreasing expression of TGF-α. Furthermore, we also found that intrahepatic bile ducts (part of these were hyperplasia ducts) were stained positive for TGF-α, suggesting that TGF-α might play a role in morphogenesis and regeneration of intrahepatic bile ducts.

Up to now, the close relationship among hepatitis B virus, liver cirrhosis and HCC has been approved[32], but the mechanisms have not been clearly defined yet. One hypothesis is that chronic HBV infection caused prolonged liver cellular injury, inflammation and cellular death. The hepatic regeneration after liver necrosis, along with DNA damage from genotoxic agents generated during the inflammatory response, was thought to increase the risk of HCC development[33]. Because of HBV replication in liver cells, HBV major envelope protein, HBsAg was often overexpressed. It accumulated to toxic levels in the endoplasmic reticulum of hepatocytes. This could lead to the chronic injury of hepatocytes, inflammation and cellular death by immune reaction[34]. In our study, we found the positive expression rate of HBsAg in the HCC surrounding tissues was higher than that of HCC tissues. There was a prominent difference in HBsAg distribution between the HCC and its surrounding tissues (χ^2=43.5, P<0.05). This suggested HBsAg might have more closely involved in hepatic cells injury than in hepatocarcinogenesis.

TGF-α is a potent mitogen for hepatocytes. It can induce hepatocytes to proliferate. Though we did not found the correlation between TGF-α and HBsAg in the HCC tissues, we found there was a prominent positive correlation between TGF-α and HBsAg in the HCC surrounding tissues. They were usually existed in regenerated and/or dysplastic liver cells. These cells had increscent or double nucleus, prominent or anomalous nucleolus and high ratio of nucleus/cytoplasm, *etc*. These data suggest that TGF-α overexpression in the liver seems to be associated with the hepatocellular regeneration injured by HBsAg. HBsAg may up-regulate the expression of TGF-α. Continued overexpression of TGF-α could lead to hepatocellular proliferation and dysplasia. These might have contributed to hepatocarcinogenesis and HCC growth.

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Edited by Gupta MK **Proofread by** Xu FM