Influence of HBcAg in liver cell plasma on expression of transforming growth factor-beta 1 in liver tissue of low-grade chronic hepatitis B patients

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INTRODUCTION
The incidence rate of chronic hepatitis B (CHB) is high in China. The status of virus replication and the process of hepatic fibrosis are regarded as important. At present, TGF-β1 is known not only as a cytokine which adjusts proliferation, development, conversion, and differentiation of cells, but also as an important transmitter of hepatic fibrosis. It plays an important role in the formation of cirrhosis[1-3]. The synthesis and degradation of extracellular matrix (ECM) are adjusted by it[4,5]. This study was to observe the influence of HBcAg on the expression of TGF-β1 in liver tissue of low-grade CHB patients.

MATERIALS AND METHODS

Reagents
Mouse anti-human TGF-β1 antibody was purchased from Fuzhou Maxim Biotechnology Co., Ltd (Lot No.: 30212238L). The PV-9000 kit was provided by Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd. Rabbit anti-human HBcAg was purchased from Fuzhou Maxim Biotechnology Co., Ltd.

Clinical data
A total of 93 low-grade CHB patients (68 males and 25 females, mean age 33.3 years, ranging from 17 to 56 years) were analyzed. HBcAg was expressed as plasma type in the liver tissue of 50 cases and no HBcAg was expressed in 43 cases. The diagnosis of all cases was coincident with the program of prevention and cure for viral hepatitis[6].

Liver biopsy
The liver biopsy was taken from 93 cases under B ultrasound guidance. The liver tissue longer than 1.0 cm and without break was fixed by formaldehyde solution and embedded in paraffin. Six serial sections (4 μm thick) were prepared for HE, Masson, Gordon Sweet, HBsAg, HbcAg, and TGF-β1.
Table 1 Influence of HBcAg in liver cell plasma on the expression of TGF-β1 in liver tissue of low-grade CHB patients

| Groups                          | Expression of TGF-β1 protein by semi-quantitative scoring (Score) | Total |
|--------------------------------|---------------------------------------------------------------|-------|
| HBCAg expression in cell plasma | 2  2  19  23  4                                              | 50    |
| No HBCAg expression in liver tissue | 4  2  13  20  4                                              | 43    |

Immunohistochemistry

TGF-β1 antigen was repaired by microwave in pH 6.0 citrate solutions. The next procedure was performed according to the instructions of PV-9000 kit. TGF-β1 was observed randomly at least in five portal areas under 200 light microscope and the expression of TGF-β1 was evaluated with semi-quantitative scoring method: score 0: no stain or no cell was hyperchromatic or the positive cells were less than 1% of total liver tissues; score 1-4: the areas of positive cells in hepatic lobules, hepatic sinusoid, portal areas, and fibrous plate were 1%-9%, 10%-15%, 16%-20% and more than 20% of total liver tissues, respectively.

Statistical analysis

Statistical analyses were carried out with the rank test.

RESULTS

TGF-β1 expression

The positive cells of TGF-β1 were mainly distributed over the focal necrosis and the active fibrosis areas. They were mainly expressed in the interstitial cells of hepatic sinusoid and the inflammatory cells of portal areas. Some bile duct cells and plasma hepatocytes were also expressed.

HBcAg expression

In the 93 low-grade CHB patients, HBcAg was expressed in cell plasma but not in the liver tissue. There was no significant difference between the two groups (H = 0.004, P>0.05, Table 1).

DISCUSSION

TGF-β1 is a cluster of active polypeptides with closely correlative structures and similar functions. Five isomers (TGF-β1-5) have been found though TGF-β1 is the main content of TGF-β in human liver and has important functions. It mainly comes from the Kupffer cells (KCs) though hepatic stellate cells (HSCs) can autocrine TGF-β1. TGF-β1 can transfer anti-signals of cell cycle with Smad molecules and inhibit gene transcription of cell cycle correlative proteins. It can inhibit the expression of cyclin related to P70s6k through P70s6k (serine/threonine kinase). The upregulation of connective tissue growth factor (CTGF) expression is related to TGF-β1 and it may be the core of activation in HSCs. TGF-β1 can inhibit the proliferation of quiescent HSCs but cannot inhibit the activated HSCs. In CHB, its serum level is increased and its expression in the liver is reinforced. TGF-β1 is one of the network cytokines related to hepatic fibrosis and can accelerate the synthesis of ECM and inhibit the degradation of ECM. Following the expression of TGF-β1, the proliferating cell nuclear antigen (PCNA) decreases in the liver. TGF-β1 can inhibit regeneration of hepatocytes and accelerate apoptosis of hepatocytes, but not HCC cells. Powell et al showed that the risk of developing cirrhosis is higher in hyper-expression than in hypo-expression of TGF-β1.

Some scholars have found that the expression of TGF-β1 in the liver tissues is not related with HBcAg and HBV DNA in the serum of CHB patients. In our study, TGF-β1 in the liver tissue of low-grade CHB patients did not influence the status of inflammation and fibrosis with the comparability improved. We found that the expression of TGF-β1 evaluated by semi-quantitative scoring was not related with HBcAg expression in liver cell plasma of low-grade chronic hepatitis B. We suppose that the expression of some cytokines is not related with hepatitis B virus possibly due to the role of virus replication and body immune response.

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