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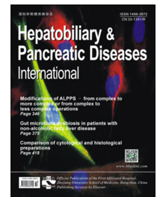

Original Article/ Liver

17-beta-hydroxysteroid dehydrogenase 13 inhibits the progression and recurrence of hepatocellular carcinoma

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ABSTRACT

Background: Our previous study showed that 17-beta-hydroxysteroid dehydrogenase 13 (HSD17B13) is down-regulated in hepatocellular carcinoma (HCC). But its function in HCC remains unknown. This study aimed to reveal the function of HSD17B13 and its clinical significance in HCC.
Methods: mRNA levels of HSD17B13 were analyzed in cohort 1 (30 normal, 30 HBV cirrhosis, 60 HBV-related HCC and 60 peritumoral tissue) by real-time PCR. HSD17B13 protein was evaluated in cohort 2 (15 normal, 33 HBV-cirrhosis, 12 dysplastic nodules, 34 HBV-related HCC, and 9 metastatic HCC) using immunohistochemistry. The association between HSD17B13 and the survival of HCC patients was analyzed in cohort 3 ($n = 88$). The inhibitory mechanism of HSD17B13 on HCC was explored .
Results: The mRNA of HSD17B13 and its protein expression were significantly down-regulated in HCC compared to non-tumor specimens ($P < 0.001$). The sensitivity, specificity and area under curve (AUC) values of HSD17B13 expression levels for HCC detection were 81.7%, 83.7% and 0.856, respectively ($P < 0.001$). Lower HSD17B13 in peritumoral tissue was an independent risk factor of worse recurrence free survival of HCC patients (HR: 0.41; 95% CI: 0.20–0.83; $P = 0.014$). The study in Huh 7 and SK-HEP-1 cells showed that HSD17B13 induced an accumulation of cells in G1 phase and reduction of cells in S and G2 phases via up-regulating the expression of P21, P27 and MMP2.
Conclusions: Lower HSD17B13 in peritumoral tissues was associated with worse recurrence free survival and overall survival of HCC patients. HSD17B13 delayed G1/S progression of HCC cells. HSD17B13 may be a therapeutic target for the treatment of HCC.
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Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide [1,2]. Majority of the HCC patients have chronic hepatitis B virus infection in China [3]. It has been reported that 15%–20% of chronic hepatitis B patients progress to cirrhosis within 5 years and 2.8% of the cirrhotic patients developed HCC annually [4]. Hepatocarcinogenesis is a typical multistage process characterized by chronic viral infection, liver cirrhosis and HCC [5].

The isobaric tags for relative and absolute quantitation (iTRAQ) and two-dimensional liquid chromatography-tandem mass spectrometry (2D LC-MS/MS) is one of the most important methods to identify protein markers of tumors [6]. In our previous study, the iTRAQ-2D LC-MS/MS was used to quantitatively analyze the protein alternations in multistep HBV-related hepatocarcinogenesis: healthy subjects, patients with HBV hepatitis, patients with HBV cirrhosis, patients with HCC and their peritumoral tissues [7]. And we found that 17-beta-hydroxysteroid dehydrogenase 13 (HSD17B13) was down-regulated in HCC tissues. HSD17B13 is known as short-chain dehydrogenase/reductase 9 which is a lipid droplet associated protein [8], and its up-expression has been proven in human liver [9]. Xing et al. also reported that HSD17B13 was down-regulated in HCC [10]. Therefore, the protein HSD17B13 might be a potential biomarker for HCC and play an important role in HCC. However, the role of HSD17B13 in HCC remains unclear. Thus, we aimed to investigate HSD17B13 expression and its role in HCC.

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Methods

Clinical samples

The samples used in this study included three independent cohorts. Cohort 1 including 180 samples (30 normal, 30 HBV cirrhosis, 60 HBV-related HCC and 60 peritumoral tissue) was used to determine the mRNA expression of HSD17B13. Cohort 2 consisted of a series of liver samples (15 normal livers, 33 HBV cirrhotic livers, 12 dysplastic nodules, 34 HBV-related HCC, and 9 metastatic HCC), which was used to investigate clinical diagnostic value of HSD17B13. Cohort 3 contained 88 HCC and matched peritumoral livers. Tissue samples in cohort 1 were immediately snap-frozen in liquid nitrogen post-operatively and stored at −80 °C until use. The liver tissues of the cohort 2 and 3 were formalin-fixed and paraffin-embedded for immunohistochemistry. The diagnosis of HCC was made histologically. Samples and data were obtained from patients with HCC who underwent a partial hepatectomy at the First Affiliated Hospital, Zhejiang University School of Medicine. This study was approved by the Ethical Committee of First Affiliated Hospital, Zhejiang University School of Medicine. Each patient provided written informed consent.

Real-time PCR

Total RNA was isolated from tissue samples preserved at −80 °C using Trizol (Qiagen, Duesseldorf, Germany). Good quality RNA was reverse transcribed using a cDNA kit (Vazyme, Piscataway, USA) and real-time PCR was performed using the ABI 7500 fast system (Applied Biosystems, Waltham, USA) according to the manufacturer's protocol. Gene specific primers are forward sequence: 5'-AGC CGA TCT TCT CAG CAC CAA G-3'; reverse sequence: 5'-CTG AAG CCA CTG TGA CGA TGT G-3'. Gene expression was measured in triplicate under optimized PCR conditions: one denaturing cycle at 95 °C for 3 min, followed by 40 amplification cycles at 95 °C for 15 s and 60 °C for 30 s, and lastly a melting curve cycle at 95 °C for 15 s, 60 °C for 15 s and 95 °C for 15 s. Relative gene expression was normalized to GAPDH and reported as $2-\triangle CT$, with $\triangle CT = Ct(\text{target gene}) - Ct(\text{GAPDH})$.

Immunohistochemical staining

The HSD17B13 protein expression was stained in paraffin-embedded samples from cohort 2 and 3. Briefly, 4 µm thick sections of tissues was placed in ethylenediamine tetra-acetic acid (EDTA) buffer for antigen retrieve using a microwave oven after deparaffinization. Then sections were incubated in methanol containing 0.5% hydrogen peroxide for 20 min to block endogenous peroxidase and further blocked in normal protein block serum solution. The anti-HSD17B13 antibody (Abcam, Cambridge, USA) was added to the sections at 4 °C overnight, and then washed by PBS buffer for 3 times (5 minutes of each time) at room temperature. It further followed by incubating with HRP-conjugated secondary antibodies (Biotech Inc., Wuhan, China) at room temperature for 1 h. Finally, the sections were subjected to DAB staining and hematoxylin background staining. A negative control was obtained by replacing the primary antibody with a normal murine or rabbit IgG. Immunoreactivity for proteins was scored using a semi-quantitative method by evaluating the number of positive cells over the total number of liver cells. Under high magnification, 10 fields were random selected and the positive staining cells were identified as having higher intensity of brown–yellow staining of cytoplasm than that of background non-specific staining. Scores were assigned by using 5% increments (0%, 5%, 10%...100%) by two experienced pathologists scored the results independently, as reported [7]. According to the proportion of positive staining cells, the staining intensities of samples were identified as negative (<5%), positive+ (5%-29%), positive ++ (30%-74%), positive +++ (≥75%).

Cell cycle and apoptosis analysis, and western blotting

Human HCC cell lines SK-HEP-1 and Huh7 (Chinese Academy of Sciences, Shanghai, China) were used in the present study. They were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Gibco, CA, USA) in a 37 °C incubator with 5% CO_2. The cells were harvested and seeded into 6-well plates and transfected with lentivirus-HSD17B13 and the control (Shanghai Genechem). After 48 h, cells were collected for flow cytometry. Experiment was performed three times. The detailed methods referred to the previous publication [11]. The cultured cells were collected in RIPA lysis buffer with 10 mL/mL protease inhibitor cocktail (Sigma, St. Louis, USA). The extracted proteins were separated by SDS-PAGE and transferred to PVDF membranes. After being blocked with TBS-T buffer containing 5% non-fat powder milk for 2 h, the membranes were then immunoblotted using primary antibodies against actin (Servicebio, Wuhan, China), Bax (Abcam), Bcl-2 (Cell Signaling Technology, Danvers, MA, USA), Caspase9 (Servicebio), P21 (Servicebio), P27 (Boster, Wuhan, China), MMP2 (Servicebio), MMP9 (Servicebio), E-Cadherin (Cell Signaling Technology), N-Cadherin (Servicebio), Ki67 (Abcam), and CD31 (Servicebio). Immunoreactive bands on the blots were visualized with enhanced chemiluminescence reagent ECL kit (Biological Industries, Kibbutz Beit-Haemek, Israel). The experiment was performed in at least three independent experiments.

Statistical analysis

Qualitative variables were analyzed by the Fisher's exact test and Pearson's Chi-square test. Quantitative variables were analyzed by Student's t test. Receiver operating characteristic curves (ROC) were used to assess the diagnostic value. Survival curves were computed using the Kaplan–Meier method, and compared by the log-rank test. Cox proportional hazards regression was carried out to identify the independent factors which significantly impact patient survival. Statistical analysis was performed by SPSS version 18.0 for windows (SPSS, Chicago, IL, USA) and all statistical tests were two-sided, and a P value <0.05 was considered statistically significant.

Results

HSD17B13 mRNA expression in series nodules during hepatocarcinogenesis

The HSD17B13 mRNA expression was determined in normal livers (n = 30), cirrhosis (n = 30), HCC (n = 60) and peritumoral tissues (n = 60) using real-time PCR. The mRNA levels of the HSD17B13 are showed in Fig. 1. We observed that HSD17B13 are obviously lower in HCC than other groups (P < 0.001). There are no difference among the normal livers, cirrhosis, and peritumoral tissues (P > 0.05).

Protein expression pattern of HSD17B13

To validate whether HSD17B13 protein is exclusively down-expressed in HCC tissues, we examined its expression pattern in cohort 2 containing a series of liver tissues (15 normal livers, 33 HBV cirrhotic livers, 12 dysplastic nodules, 34 HBV-related HCC, and 9 metastatic HCC) via immunohistochemistry. The immunoreactivity of HSD17B13 was observed primarily in the hepatocellular cell cytoplasm and down-regulated in HCC and metastasis

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