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ORIGINAL ARTICLE

Hepatocellular carcinoma suppressor 1 promoter hypermethylation in serum. A diagnostic and prognostic study in hepatitis B

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Summary
Background: Liver cancer ranks as the second leading cause of cancer-related mortality in man worldwide, and hepatocellular carcinoma (HCC) is the most prevalent malignant neoplasm of the liver. The sensitivity of alpha-fetoprotein (AFP) as an HCC diagnostic marker for HCC diagnosis is 39—65%, and one-third patients with HCC are missed using AFP. New biomarkers are needed to diagnose HCC at an earlier stage and to individualize treatment strategies. Hepatocellular carcinoma suppressor 1 (HCCS1) is a newly identified liver tumor suppressor gene.
Objective: Our study evaluated the diagnostic value of serum HCCS1 promoter methylation in patients with HCC associated with hepatitis B.
Methods: We determined the methylation status of serum HCCS1 promoter in 120 patients with HCC, 146 patients with chronic hepatitis B (CHB) and 27 healthy controls (HCs) by methylation-specific polymerase chain reaction (MSP). Evaluation of a cohort with 63 patients with HCC and 44 patients with CHB was set as a validation dataset.

Abbreviations: HCCS1, Hepatocellular carcinoma suppressor 1; HCC, Hepatocellular carcinoma; AFP, Alpha-fetoprotein; CHB, chronic hepatitis B; HCs, healthy controls; MSP, methylation-specific polymerase chain reaction; TNM, tumor node metastasis; US, ultrasound; MRI, magnetic resonance imaging; CT, computed tomography; cfDNA, cell-free DNA; AASLD, American Association for the Study of Liver Diseases; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HIV, human immunodeficiency virus; NAFLD, non-alcoholic fatty liver diseases; AUC, area under the receiver operating characteristic curves; ROC, receiver operating characteristic curve.

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Results: The frequency of HCCS1 promoter methylation in patients with HCC was significantly higher than that in patients with CHB ($P < 0.001$) and HCs ($P < 0.001$), and was associated with tumor node-metastasis (TNM) stage ($P = 0.01$). The sensitivity of serum HCCS1 promoter methylation for discriminating patients with HCC from CHB was 62.5% and that of AFP alone was 55%. Notably, the sensitivity of serum HCCS1 promoter methylation plus AFP level was 81.7%.
Conclusion: HCCS1 has potential as a biomarker for diagnosis and prognosis of patients with HCC.

Introduction

Hepatocellular carcinoma (HCC) is the most prevalent malignant neoplasm of the liver and liver cancer ranks as the second leading cause of cancer-related mortality in humans worldwide, most notably in developing countries [1]. Although the management of HCC has improved in recent years, the mean survival of patients is usually between 6 and 20 months. In addition, the long-term prognosis is poor with reported 5-year survival rates as low as 17%, mainly because of late diagnosis [2,3]. Currently, diagnosis of HCC primarily depends on alpha-fetoprotein (AFP) levels and non-invasive standard imaging methods, including ultrasound (US) [4]. Advances in magnetic resonance imaging (MRI) and computed tomography (CT) have greatly improved imaging of focal hypervascular masses consistent with HCC. However, these techniques are also limited by their restricted penetration depth imposed by tissue turbidity, which limits the use of high-resolution microscope [5]. As a matter of fact, the accuracy of AFP levels is limited by a modest 39—65% sensitivity and 76—94% specificity, and only 10% to 20% of patients with early-stage HCC have abnormal AFP serum levels. Consequently, a majority of cases with early-stage HCC are missed on screening based on AFP levels alone [6]. In addition, serum AFP levels are probably false positive in patients with benign liver diseases, such as hepatitis and cirrhosis [7]. New biomarkers are needed to detect HCC at an earlier stage and to individualize treatment strategies.

Most circulating cell-free DNA (cfDNA) derives from apoptotic and necrotic cells but some are released by living eukaryotic cells [8]. Significant increases of serum cfDNA have been reported in patients with cancer, including those with HCC, compared with healthy controls (HCs) [9]. Aberrant cfDNA methylation, a common epigenetic modification, has been described in most types of cancer and clinical applications are under investigation [10,11].

DNA methylation involves the addition of methyl group to the fifth carbon of cytosine residues within a cytosine-guanine (GC) dinucleotide, frequently referred to CpG, is widely spread in human genome [12]. Previous studies have shown that the detection of methylated cfDNA may be a useful tool to assess prognosis in cancer patients, including those with HCC [13,14]. We previously showed significantly increased frequencies of methylated promoters of TFPI2, IGFBP7 and MT1M had significant higher frequency of methylation in the serum of patients with HCC and suggested that they might be used to predict HCC progression [15—17]. Recently, aberrant methylation of several genes, including *GSTP1*, *p16*, *RASSF1A*, and *APC*, has been reported in the sera/plasma of patients with HCC, thus, they are candidate biomarkers as well [18—21]. However, to the best of our knowledge, the methylation status of hepatocellular carcinoma suppressor 1 (HCCS1) promoter has not been evaluated as an HCC biomarker.

HCCS1 is a novel tumor suppressor gene located in the high-frequency loss of heterozygosity (LOH) region of chromosome 17p13.3 in HCC [22]. HCCS1 has a high-frequency of mutation and significantly lower expression in HCC than in non-cancerous liver tissue. HCCS1 has been shown to significantly decrease the efficiency of colony formation in vitro and to inhibit tumor growth in vivo [23]. If HCCS1, as an HCC tumor suppressor gene, is involved in the occurrence and development of HCC, HCCS1 downregulation might promote HCC progression.

DNA methylation might act to maintain stable expression of specific genes via mitosis [24], by contributing to establishment of a silent chromatin state via interactions with proteins that modify nucleosomes [25]. Consequently, we considered that HCCS1 downregulation might be related to methylation of HCCS1 promoter. However, we were unable to find any previous reports of the methylation of the promoter of HCCS1 in serum of patients with malignant disease, including HCC. This study was designed to determine whether HCCS1 methylation could be detected in the serum of patients with HCC and if it might be used as a non-invasive biomarker of HCC.

Materials and methods

Study populations

We collected a total of 293 serum samples from 120 patients with HCC associated with hepatitis B, 146 patients with chronic hepatitis B (CHB) and 27 healthy controls (HCs). The patients were identified by clinical and laboratory evaluation. Patients with HCC associated with hepatitis B and CHB were recruited from November 2013 through April 2015 in the Department of Hepatology, Qilu Hospital of Shandong University. Treated specimens were stored at −80 °C. HCC was diagnosed following the 2010 update of the American Association for the Study of Liver Diseases (AASLD) Practice Guidelines for Management of hepatocellular carcinoma [26]. Patients with CHB were diagnosed as positive