HTATIP2 Represents an Innovative Bio-marker in Hepatocellular Carcinoma Detection

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Abstract

Background: This study was designed to investigate the serum level of HIV-1 Tat interactive protein 2 (*HTATIP2*) mRNA in hepatocellular carcinoma (HCC) patients and its diagnostic significance in the disease.

Methods: The serum *HTATIP2* mRNA level was determined by quantitative real-time polymerase chain reaction (qRT-PCR). The relationship between *HTATIP2* expression and clinical parameters was analyzed using Chi-square test. Receiver operating characteristics (ROC) curve was adopted to estimate the diagnostic role of serum *HTATIP2* in HCC.

Results: HCC patients showed a significantly lower serum level of *HTATIP2* than the healthy control ($P<0.001$). The level of *HTATIP2* was closely associated with venous invasion ($P=0.011$), lymph node metastasis ($P=0.007$) and TNM stage ($P=0.016$). ROC curve demonstrated that *HTATIP2* could discriminate between HCC patients and healthy individuals at the optimal cutoff point of 2.39. Besides, the AUC was 0.892, with the corresponding sensitivity and specificity of 83.90% and 84.37%, respectively.

Conclusions: *HTATIP2* is negatively expressed in HCC and may be a diagnostic biomarker for this disease.

Background

Hepatocellular carcinoma (HCC) is one of the most frequently diagnosed liver cancers, with severe mortality [1, 2]. There are several well known risk factors for HCC, such as alcohol abuse, hepatitis C virus (HCV), hepatitis (HBV) and fatty liver [3]. Until now, a variety of therapeutic strategies are available for HCC patients, such as surgical resection, local ablation therapy, chemoembolization, liver transplantation and molecular target therapy [4, 5]. Despite slight improvements in clinical outcomes, the five-year survival of HCC patients is still dismal [6]. The low survival rate may be attributed to delay in diagnosis [7]. Unfortunately, the majority of HCC patients are diagnosed with advanced stages owing to the lack of efficient screening methods [8, 9]. Therefore, it is urgently needed to investigate novel and effective diagnostic biomarkers for HCC to improve the prognosis of the patients.

HIV-1 Tat interactive protein 2 (*HTATIP2*), also known as *TIP30* or *CC3*, is located on human chromosome 11p15.1, and codes a 30kD human cellular protein [10, 11]. The gene is initially identified as a metastasis suppressor in human variant small cell lung carcinoma (v-SCLC) [12, 13]. *HTATIP2* is ubiquitously expressed in normal tissues, whereas its expression patterns are aberrant in diseases tissues. An increasing number of studies have demonstrated that *HTATIP2* acts as a tumor suppressor in various cancers via regulating a series of biological processes, including cell growth, apoptosis, and angiogenesis [14-16]. For instances, in pancreatic cancer, down-regulated *HTATIP2* expression could facilitate cancer cell invasion and metastasis via controlling the expression of Snail family members [17]. In esophageal squamous cell carcinoma, inhibiting the expression of *HTATIP2* was able to active EMT, thus promoting migrative and invasive abilities of the cancer cells [18]. The anti-tumor action of *HTATIP2* in HCC was

also reported in the previous tumor investigations. The study carried out by Lu et al. reported that up-regulation of *HTATIP2* through Aspirin treatment was able to weaken invasiveness of HCC cells [19]. However, the diagnostic performance of *HTATIP2* in HCC remained poorly known.

In the present study, we examined the serum level of *HTATIP2* in HCC patients and explored its diagnostic roles in this disease.

Methods

Patients and serum specimens

A total of 118 patients who were pathologically diagnosed with HCC were recruited from Harrison international Peace Hospital. The clinical features of patients were listed in **Table 1**. All the patients were classified based on the World Health Organization (WHO). None of the patients had received any treatments, such as radio- or chemo- therapy. In addition, 64 healthy donors who were age and gender matched with the patients were enrolled in this study. 5 mL peripheral blood was collected from each participant on the morning after fasting for 8-10h. The serum samples were prepared from peripheral blood through centrifugation and stored in EDTA-containing tubes at -80°C. This study was approved by the Ethics Committee of Harrison international Peace Hospital. Each participant signed the informed consent before blood collection.

RNA isolation and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from serum samples using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacture's instructions. Then the total RNA was used to synthesis the first chain of cDNA by PrimerScript RT reagent kit (Takara, China). QRT-PCR was applied to detect the relative mRNA level of *HTATIP2* in serum specimens. The reaction was carried out by SYBR Premix Ex Taq (Takara, China) in the 7500 Real Time PCR system (Applied Biosystems) under optimal conditions. All assays were conducted in triple. β -actin was taken as an internal reference. The primer sequences were as follows: *HTATIP2*, forward 5'-TCACCTTCGACGAGGAAGCT-3', reverse 5'-GCTCTGCAGACTTCAGACCA-3'; β -actin, forward 5'-CGTGGACATCCGTAAAGACC-3', reverse 5'-ACATCTGCTGGAAGGTGGAC-3'. The expression of *HTATIP2* mRNA was calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

All data were statistically analyzed by SPSS 18.0 software and Graphpad Prism 5.0. Student's t-test was adopted to evaluate the expression difference of *HTATIP2* mRNA between HCC patients and healthy controls. The associations between *HTATIP2* expression and clinical parameters of patients were determined by Chi-square test. Receiver operating characteristics (ROC) curve was generated to estimate the diagnostic sensitivity and specificity of *HTATIP2* in HCC. $P < 0.05$ was considered significant.

Table 1. Relationship of *HTATIP2* expression and clinical features

Clinical characteristics	Case NO.	<i>HTATIP2</i> expression		χ^2	<i>P</i> value
		Low (n=79)	High (n=39)		
Age (year)				3.579	0.059
<60	55	32	23		
≥ 60	63	47	16		
Gender				1.534	0.216
Male	64	46	18		
Female	54	33	21		
Serum AFP (ng/ml)				2.260	0.133
≥ 400	57	42	15		
<400	61	37	24		
Etiology				3.158	0.076
HBV	53	40	13		
HCV	65	39	26		
Venous invasion				6.472	0.011
Present	62	48	14		
Absent	56	31	25		
Lymph node metastasis				7.235	0.007
Negative	64	36	28		
Positive	54	43	11		
TNM stage				5.833	0.016
I,II	60	34	26		
III,IV	58	45	13		

Results

Expression of *HTATIP2* mRNA was decreased in HCC serum

To determine the expression of *HTATIP2* mRNA in HCC patients, we conducted the qRT-PCR test. As shown in **Figure 1**, the expression of *HTATIP2* mRNA in HCC serum samples was significantly lower than that in healthy controls (1.83 ± 0.59 vs 3.12 ± 0.80 , $P < 0.0001$).

Association between *HTATIP2* expression and clinical characteristics

The patients were divided into high expression group (n=39) and low expression group (n=79), based on their median expression level of *HTATIP2*. Chi-square test was applied to estimate the association between *HTATIP2* mRNA level and clinical characteristics of the patients. As listed in **Table 1**, the decreased expression of *HTATIP2* was significantly correlated with venous invasion ($P=0.011$), positive lymph node metastasis ($P=0.007$) and advanced TNM stage ($P=0.016$). However, no significant relationship was found between *HTATIP2* expression and age ($P=0.059$), serum AFP ($P=0.133$), gender ($P=0.216$) or etiology ($P=0.076$).

Diagnostic performance of *HTATIP2* in HCC

ROC curve was built to assess the diagnostic value of serum *HTATIP2* in HCC. The curve demonstrated that the value of AUC was 0.892 with the corresponding specificity and sensitivity of 84.37% and 83.90%, respectively. Besides, the cut-off value of *HTATIP2* for HCC diagnosis was 2.39. All these results indicated that serum *HTATIP2* might be a potential diagnostic biomarker in HCC (**Figure 2**).

Discussion

HCC is a prevalent malignancy with insidious onset. Tumor stage at diagnosis and appropriate therapy are key for outcomes of HCC patients [7]. To date, there is no available tool for early detection of the disease, due to the unclear etiology and pathogenesis [20]. Consequently, most of the patients are diagnosed at an advanced stage, resulting in low survival rate. The detection of biomarkers in body tissues or fluids are considered as a promising way to improve the diagnostic accuracy of HCC [21]. Serum AFP (alpha fetoprotein) is one of the major biomarkers for HCC diagnosis currently. Despite of high specificity, the low sensitivity of AFP may lead to missed diagnosis [22]. Hence, more specific and sensitivity tumor markers are urgently needed for HCC research.

HTATIP2 is a tumor metastasis-related gene which is primitively discovery by Shtivelman et al. [23]. *HTATIP2* plays inhibitory roles in tumorigenesis via regulating various processes, such as inhibiting tumor growth, inducing cancer cell apoptosis, weakening athletic ability, and so on [14-16]. As a tumor suppressor gene, *HTATIP2* has been reported to be involved in the development and progression of various cancers. Chen et al. reported that promoter methylation caused down-regulation of *HTATIP2* that contributed to tumor progression of colorectal cancer [24]. Tong et al. suggested that *HTATIP2* was down-regulated in lung cancer and its decreased expression showed significant association with metastatic potential of the disease [25]. In this study, we determined the expression pattern of *HTATIP2* in HCC, as well as its functional roles in tumor progression.

In the current study, we found that *HTATIP2* mRNA level was down-regulated in HCC patients compared with the healthy controls. Besides, chi-square test demonstrated that the reduced expression of *HTATIP2* might contribute to positive venous invasion, lymph node metastasis and advanced TNM stage. The above findings hinted that *HTATIP2*, as a tumor suppressor, was involved in the development and

progression of HCC. The conclusion was consistent with the previous studies. A related study carried out by Zhu et al. demonstrated that *HTATIP2* was decreased in HCC patients and its expression level showed close association with E-cadherin expression. Cell experiments proved that down-regulated *HTATIP2* expression contributed to activated EMT, as well as enhanced invasion and motility of HCC cells, suggesting the inhibitory function of the gene in malignant development and progression of HCC [26].

As an important tumor suppressor, *HTATIP2* was proved to play a predictive role in tumor initiation and progression. In pancreatic ductal adenocarcinoma, decreased expression of *HTATIP2* predicted poor prognosis for the patients [17]. Among gastric cancer patients, reduced expression of *HTATIP2* represented dismal overall survival[27]. In the present study, we evaluated the diagnostic value of *HTATIP2* in HCC. ROC curve demonstrated that *HTATIP2* could discriminate between HCC patients and healthy controls with satisfactory sensitivity and specificity. Though we had confirmed the low expression of *HTATIP2* in HCC patients and identified its diagnostic role in this malignancy, its precise mechanism on this disease was still not well understood. Accumulating evidences proved that *HTATIP2* might be linked with the development and progression of angiogenesis in HCC, which was realized through modulating the transcription of angiogenesis regulator [28]. Besides, it was also proposed that *HTATIP2* served as a tumor suppressor via inducing cell apoptosis in HCC [29]. All the related researches could provide theoretical foundations for our further studies.

Conclusion

In conclusion, *HTATIP2* expression is significantly lower in HCC patients than that in the healthy controls. The expression level of *HTATIP2* may efficiently discriminate HCC patients from healthy controls.

Abbreviations

HIV-1 Tat interactive protein 2 (*HTATIP2*)

hepatocellular carcinoma (HCC)

quantitative real-time polymerase chain reaction (qRT-PCR)

Receiver operating characteristics (ROC)

hepatitis C virus (HCV)

variant small cell lung carcinoma (v-SCLC)

World Health Organization (WHO)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of Harrison international Peace Hospital and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materials The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests The authors declare that they have no competing interests.

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Authors' contributions X.Z. and X.L. conceived and designed the experiments; X.Z. and H.B. conceived and performed the experiments; G.L. and N.L. prepared figures. H.L., J.D. and J.Z. wrote the main manuscript text. All authors reviewed the manuscript.

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Figures

Figure 1

The mRNA level of serum HTATIP2 in HCC patients and healthy controls was determined using the qRT-PCR. It was found that HTATIP2 was up-regulated in HCC patients compared with healthy control. ***: suggested $P < 0.0001$.

Figure 2

The diagnostic accuracy of serum HTATIP2 in HCC was investigated using ROC analysis. The AUC was 0.892, suggesting that serum HTATIP2 could be employed as a diagnostic biomarker for HCC. The cut-off value of HTATIP2 for HCC diagnosis was 2.39, with the sensitivity of 84.4% and the specificity of 83.9%.