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The expression of *HtrA2* and its diagnostic value in patients with hepatocellular carcinoma

Honggang Wang[a], Fuguo Jiang[a], Furong Hao[b], Ruixue Ju[a,*]

Abstract

The purpose of this study was to detect the expression of high-temperature requirement A2 (*HtrA2*) and its diagnostic value in the patients with hepatocellular carcinoma (HCC).

The relative serum *HtrA2* expression at mRNA and protein level was severally detected by quantitative real-time polymerase chain reaction and western blot analysis in 198 HCC patients and 48 healthy controls. And its association with clinicopathological features was analyzed by chi-square test. The diagnostic value of *HtrA2* expression was estimated by establishing a receiver operating characteristic (ROC) curve.

Serum *HtrA2* was significantly higher in patients with HCC than that in healthy controls both at mRNA and protein levels ($P < .05$ for both). In addition, the high *HtrA2* expression was associated with large tumor size and advanced clinical stage. Furthermore, the value of the area under the ROC curve was 0.808 corresponding with a sensitivity of 65.2% and a specificity of 89.6%, revealed that *HtrA2* might be a diagnostic biomarker in HCC.

HtrA2 is upregulated and considered to be a potential biomarker for the diagnosis of patients with HCC.

Abbreviations: AUC = area under the ROC curve, HCC = hepatocellular carcinoma, HtrA2 = high-temperature requirement A2, qRT-PCR = quantitative real-time polymerase chain reaction, ROC = receiver operating characteristic.

Keywords: diagnosis, hepatocellular carcinoma, high-temperature requirement A2

1. Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide, representing the third leading cause of cancer-related deaths.[1,2] It has a high morbidity and mortality in the world.[3,4] This high mortality rate is mainly due to limited therapeutic options and difficulty in the early detection.[5] Although there are great advances in the treatments of HCC, the prognosis of patients is still unsatisfactory. There are no specific symptoms at early stages; thus, most of HCC patients develop to advanced stages when initial diagnosis. Moreover, the cancer shows high resistance to chemo and radiation therapies, and thus leading to high recurrence and metastasis which are unfavorable factors for prognosis in HCC.[6–8] Therefore, it is urgent to explore a novel diagnostic biomarker for HCC.

High-temperature requirement A2 (*HtrA2*) (also known as Omi) is a serine protease and chaperone protein that has a mitochondrial targeting sequence and transmembrane domain in the N-terminal region.[9] It was initially identified as a mammalian homologue of the *Escherichia coli* protein HtrA.[10,11] *HtrA2* is localized to the mitochondrial intermembrane space by a mitochondrion-targeting sequence. Several studies have described that *HtrA2* can influence the physiology of mitochondrial APP.[12–14] And its aberrant expression was also found in several diseases, such as endometrial cancer, ovarian cancers, and non–small cell lung cancer.[15–17] However, although its abnormal expression was also observed in HCC,[18,19] the diagnostic value of *HtrA2* in HCC remained unclear.

In this study, we detected the expression of *HtrA2* and explored its relationship with clinical factors of patients with HCC. Moreover, we investigated the clinical significance of *HtrA2* in the early detection of HCC.

2. Materials and methods

2.1. Patients and samples

One hundred ninety-eight patients who were diagnosed with HCC in Weifang People's Hospital were included in the study. None of them had received any radiotherapy or chemotherapy before sampling. Patients with any other synergetic syndromes, such as Parkinson disease, would be excluded from our study. In addition, 48 healthy subjects were taken as the healthy controls. Written informed consent was signed by each participant and the experiments were approved by the Ethics Committee of the hospital in advance. The investigations were conducted according to the Declaration of Helsinki Principles.

Five milliliter blood was collected from patients with HCC and healthy controls, respectively. Then the blood samples were stood and centrifuged at 3000 rpm for 10 minutes. The supernate (namely serum) was severally put into blood collection tubes of EDTA and stored at −80°C for use. The clinicopathologic characteristics of patients with HCC including age, sex, tumor size, historical grade, portal vein embolus, cirrhosis, HBsAg, AFP, and clinical stage were recorded in a database.

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[a] Clinical Laboratory, [b] Department of Radiotherapy, Weifang People's Hospital, Weifang 261041, Shandong, China.

[*] Correspondence: Ruixue Ju, Clinical Laboratory, Weifang People's Hospital, Weifang 261041, Shandong, China (e-mail: nncdg34@163.com).

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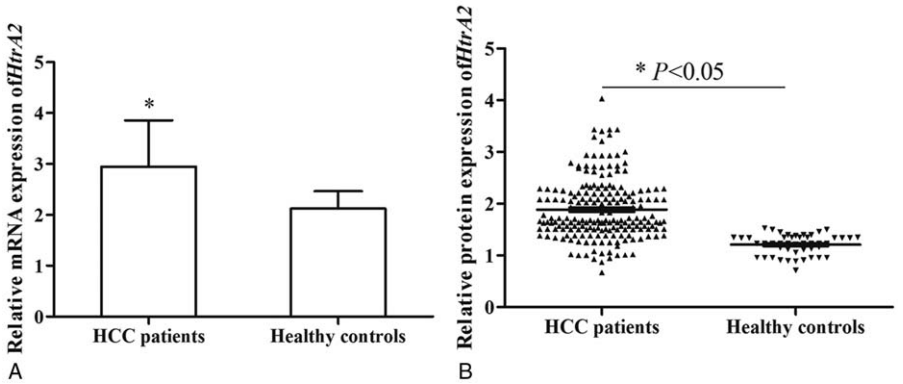

Figure 1. The relative expression of serum *HtrA2* in patients with HCC and healthy controls. Serum *HtrA2* expression was upregulated in patients with HCC compared to that in healthy controls both at mRNA (A) and protein (B) level. HCC =hepatocellular carcinoma, *HtrA2* = high-temperature requirement A2.

2.2. RNA extraction and quantitative real-time polymerase chain reaction analysis

Total RNA was extracted from the serum of patients with HCC and healthy controls using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions, respectively. Reverse transcription was conducted to synthesize the first chain of cDNA with Tagman MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Real-time polymerase chain reaction (RT-PCR) reaction was performed using FastStart Universal SYBR Green Master kit (Roche Diagnostics) and analyzed with an Applied Biosystems 7900 RT-PCR System. *GAPDH* was taken as internal controls. The relative mRNA expression of *HtrA2* was calculated using the method of $2^{-\Delta\Delta CT}$ as described previously.[20,21]

2.3. ELISA analysis

Total protein was severally isolated from the serum of patients with HCC and healthy controls. *HtrA2* protein expression was measured using an ELISA kit (Miltenyi Biotec, Bergisch Gladbach, Germany) in accordance with the manufacturer's instructions. The optical density at 450 nm was determined. Results are presented as the concentration of *HtrA2* (ng/mL) in samples.

2.4. Statistical analysis

Data were presented as mean ± SD. Statistical analyses were conducted with SPSS 18.0 software and the figures were designed by GraphPad Prism 5. The *HtrA2* expression differences between 2 groups were analyzed by Student *t* test. Chi-square test was used to estimate the relationship between *HtrA2* expression and clinical factors of patients with HCC. Receiver operating characteristic (ROC) curve was established to evaluate the diagnostic value of *HtrA2* in HCC. $P < .05$ was considered to be statistically significant.

3. Results

3.1. The expression of high-temperature requirement A2 was upregulated in patients with hepatocellular carcinoma

HtrA2 expression in 198 HCC patients and 48 healthy controls at mRNA and protein level were detected by qRT-PCR and western blot analysis, respectively. As shown in Figures 1A and B, compared with that in healthy controls, the relative *HtrA2* expression was significantly higher in the serum of patients with HCC both at the mRNA and protein levels ($P < .05$ for both, Fig. 1).

3.2. Association between high-temperature requirement A2 expression and clinical factors of patients with hepatocellular carcinoma

To explore whether *HtrA2* was related to the development of HCC, we analyzed the relationship between its expression and clinicopathological factors of patients with HCC. The result showed that the high *HtrA2* expression was distinctively associated with tumor size ($P < .001$) and clinical stage ($P < .001$). However, there was no association between *HtrA2* expression and historical grade, portal vein embolus, cirrhosis, HBsAg, or AFP (all $P > .05$, Table 1).

3.3. The diagnostic values of high-temperature requirement A2 in patients with hepatocellular carcinoma

To explore the clinical significance of *HtrA2* in HCC, a ROC curve was plotted. The outcome demonstrated that *HtrA2* had a high diagnostic value with an area under the ROC curve (AUC) of 0.808 combing with a sensitivity of 65.2% and a high specificity of 89.6% (Fig. 2). Moreover, the ideal cut-off value of *HtrA2* mRNA for HCC diagnosis was 2.455.

4. Discussion

HCC is one of the most prevalent causes of cancer-related death in the world. The majority of HCC cases result from cirrhotic livers and livers that have underwent repeated HBV and HCV infections.[22,23] It is well documented that hepatitis B or C infections are major risk factors for HCC, HCC and cirrhosis frequently coexist within the same liver.[24,25] The burden of HCC has been increasing with a doubling in its incidence rate in the past 10 years.[26] Most patients with HCC are diagnosed at a late stage, leading to poor prognosis with 5-year survival rate only 50% to 70% even after curative treatment.[27] Thus effective screening strategies are very critical which include a combination of ultrasound and molecular marker (s).

Table 1

Relationship between high-temperature requirement A2 expression and clinicopathological features of patients with hepatocellular carcinoma.

Characteristics	Cases (n = 198)	*HtrA2* expression		χ^2	*P*
		Low (n = 94)	High (n = 104)		
Tumor size				18.013	.000
≤5 cm	103	34	69		
>5 cm	95	60	35		
Historical grade				0.003	.954
Well differentiation	91	43	48		
Moderate and poor differentiation	107	51	56		
Portal vein embolus				0.164	.685
Positive	102	47	55		
Negative	96	47	49		
Cirrhosis				0.098	.754
Positive	95	44	51		
Negative	104	50	53		
HBsAg				0.176	.675
Positive	98	48	50		
Negative	100	46	54		
AFP				0.978	.323
Increasing	100	44	56		
Normal	98	50	48		
Clinical stage				30.402	.000
I–II	92	63	29		
III–IV	106	31	75		

HtrA2 = high-temperature requirement A2.

HtrA2, located on chromosome 2p12, is a apoptosis factor existing in the mitochondrial intermembrane space and apoptosis effect can be shown in a variety of tumor cells.[28,29] N-terminal 4 residue IAP of mature *HtrA2* bind with apoptosis protein inhibiting factor, relieving inhibitory effect of IAPs to cysteine aspartic acid protease.[10,30–32] Its abnormal expression has been reported to be involved in some cancers. Zeng et al[33] found that *HtrA2* played an important role in the development of bladder transitional cell carcinoma and its expression was upregulated. It was also revealed that *HtrA2* was upregulated in colon cancer in the study of Pruefer et al.[34]

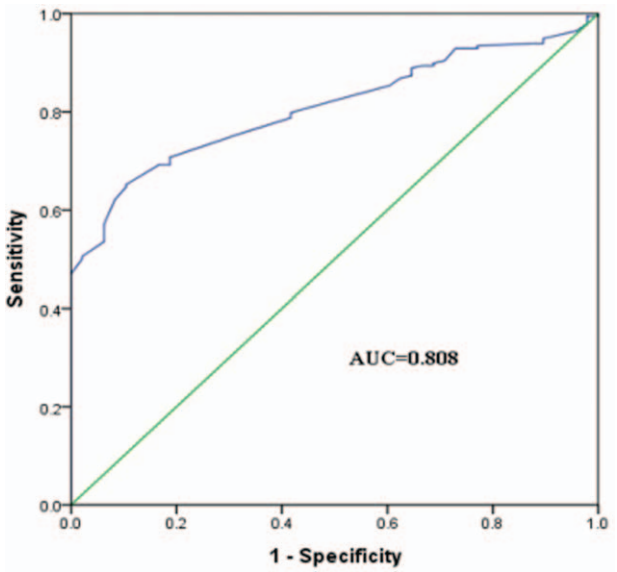

Figure 2. Receiver operating characteristic (ROC) curve was plotted to evaluate the accuracy of high-temperature requirement A2 (*HtrA2*) in discriminating patients with hepatocellular carcinoma (HCC) from healthy controls. AUC = area under the ROC curve.

In the present study, we detected the expression of *HtrA2* in patients with HCC and healthy controls. And we discovered that its level was significantly higher in the serum of patients with HCC than the healthy control which claimed that *HtrA2* was an oncogene in HCC. To further research the relationship of *HtrA2* expression with the development of HCC, we analyzed its correlation with clinical factors of patients with HCC. The high expression of *HtrA2* was considered to be significantly associated with large tumor size and advanced clinical stage. All the data revealed that *HtrA2* as an oncogene could promote the aggressive progression and development of HCC. Our results were consistent with the previous studies.[19] For example, Xu et al[19] have shown that Omi/HtrA2 was overexpressed in HCC tissues and the overexpression was associated with HCC differentiation, tumor size, clinical stage, and lymph node metastasis. Omi/HtrA2 expression may be a significant factor for HCC prognosis. However, it had also been proved that Omi/HtrA2 was a pro-apoptotic factor in HCC cell lines.[18] Omi/HtrA2 might play a dual role in progression of HCC. Further investigations will be required to explore the mechanisms underlying the function of Omi/HtrA2 in HCC, and thus describe the clinical roles of Omi/HtrA2 in the cancer all-roundly.

Then we investigated the diagnostic value of *HtrA2*. The ROC curve was designed and indicated that *HtrA2* could significantly distinguish the patients with HCC from the healthy control with an AUC of 0.808 as well as a high sensitivity and specificity. This evidence showed that *HtrA2* could be a potential marker for the diagnosis of HCC for the first time.

However, there are some limitations in our study. First, the sample size is relatively small and we need more samples to prove our experiment findings. Secondly, the molecular

mechanism of *HtrA2* acting on HCC needs to be clarified in the following research. In addition, despite of the high diagnostic accuracy and specificity, the sensitivity of *HtrA2* for HCC diagnosis was relatively low. The auxiliary diagnosis methods were needed to improve the diagnostic sensitivity of *HtrA2* in HCC. For instance, the study carried out by Kempkensteffen et al[35] reported that the expression patterns of *Smac/DIABLO* and *HtrA2* showed close association with development and progression of testicular germ cell tumors; moreover, their expression levels were strongly intercorrelated. Therefore, it was reasonable to estimate the 2 factors as biomarkers for patients with TCGT. Based on their study, further studies might be carried out to explore the combined diagnostic value of *HtrA2* with other reference genes.

In conclusion, the expression of *HtrA2* is increased and positively related to the tumor size and clinical stage of HCC. *HtrA2* may be a potential diagnostic biomarker in HCC.

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