

## Original Article

# Elevated expression of *UHRF1* predicts unfavorable prognosis for patients with hepatocellular carcinoma

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**Abstract:** Aims: The present study was designed to evaluate the different expression of ubiquitin-like with PHD and ring finger domains 1 (*UHRF1*) in hepatocellular carcinoma (HCC) tissues and the adjacent normal tissues, further explore the correlation between *UHRF1* expression and the prognosis of HCC patients. Methods: The *UHRF1* expression at protein level in HCC tissues and the adjacent normal tissues were measured by high performance liquid chromatography (HPLC). Chi-square test was used to estimate the relationship between *UHRF1* expression and clinicopathologic characteristics of HCC patients. The overall survival of HCC patients with diverse expression of *UHRF1* was measured by Kaplan-Meier analysis. Cox regression analysis was conducted to judge the prognostic value of *UHRF1* in HCC patients. Results: The *UHRF1* was over-expressed in HCC tissues compared with the adjacent normal tissues according to the outcome of HPLC ( $P < 0.001$ ). Besides, the *UHRF1* expression was tightly related to distant metastasis, cancer area, and HBV ( $P < 0.05$ ), but shared no correlation with gender, cirrhosis, and bilirubin ( $P > 0.05$ ). Patients with high *UHRF1* expression had a shorter overall survival time than those with low *UHRF1* expression ( $P < 0.001$ ). Cox regression analysis showed that *UHRF1* was significantly linked with the prognosis of HCC patients ( $P = 0.002$ , HR=5.807, 95% CI=1.901-17.742). Conclusion: *UHRF1* was over-expressed in HCC tissues compared to the adjacent normal tissues and *UHRF1* expression shared significant relevance with distant metastasis, cancer area and HBV. It could be an important and independent prognostic biomarker for HCC patients.

**Keywords:** Hepatocellular carcinoma, HPLC, *UHRF1*, prognosis

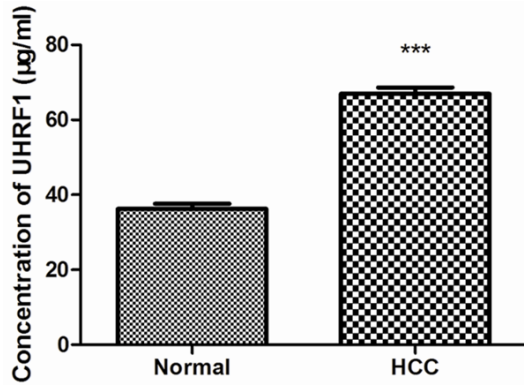
## Introduction

Hepatocellular carcinoma (HCC) is a malignant cancer that accounts for the majority of primary liver cancers [1]. HCC is one of the most common cancers and one of the leading causes of cancer-related deaths all over the world [2]. Every year, more than 600,000 patients are diagnosed as HCC and the 5-year survival rate of HCC patients is poor [3]. Virus infection including HBV and HCV has been regarded as the crucial factor for HCC in china [4, 5]. However, the pathogenesis of HCC is still unclear and concealed, the process of HCC is aggressive, invasive and rapid, and the prognosis of HCC is poor with a high mortality because of the diagnosis at an advanced stage [6, 7]. A diagnostic or prognostic marker is useful for the diagnosis or improving the overall survival of patients with HCC. However, the accurate indicators for the diagnosis and prognosis were

scarce. Consequently, it is urgent to develop a novel biomarker for therapy and prognosis of HCC.

Ubiquitinlike with PHD and ring finger domain 1 (*UHRF1*), which is also known as *ICBP90/Np95*, encodes multi-functional domain containing proteins with a length of 793 amino acids [8, 9]. *UHRF1* could not only regulate the cell proliferation but also maintain DNA methylation and play an important role on the high order of chromatin via the SRA domain and TTD domain [10-15]. It is also considered as an apoptotic-gene and abnormal expressed in various cancers including breast cancer, colorectal cancer, bladder cancer and pancreatic cancer [16-19]. All these studies have suggested that *UHRF1* may play an important role in the progression of human cancers. Besides, Zhuo et al. have demonstrated that *MEG3* regulates HCC cell proliferation and apoptosis and its over-expression

## Correlation between *UHRF1* expression and the prognosis of HCC patients



**Figure 1.** The expression of *UHRF1* in HCC tissues and the adjacent normal tissues were detected by HPLC. The result presented that *UHRF1* expression was strongly increased in HCC tissues compared with the adjacent normal tissues ( $P < 0.001$ ).

was thought to be a mechanism underlying DNA hypomethylation in HCC. Meanwhile, *UHRF1* had been proven to be a controlling gene for *MEG3* which might be a prognostic marker for predicting the survival of HCC patients. [20, 21]. However, according to the information we have, the prognostic role of *UHRF1* in HCC has never been reported.

In this study, we aimed at detecting the expression of *UHRF1* in HCC and evaluating whether it could be an innovated prognostic marker for HCC patients.

### Materials and methods

#### *Patients and specimens*

A total of sixty eight HCC patients, including 29 cases infected with HBV, were recruited from Hepatobiliary Department of Internal Medicine in Henan Provincial People's Hospital from 2008 to 2009 in this study. All patients had not received any radiotherapy or chemotherapy before surgery. The study was performed upon the approval of Ethics Committee of Henan Provincial People's Hospital and all patients were asked to sign the informed consents in advance.

The HCC tissues and adjacent tissues were collected from HCC patients and frozen by liquid nitrogen immediately. Then the tissues were stored at  $-80^{\circ}\text{C}$  for use. In addition, a 5-years follow-up was conducted with a telephone or questionnaire. The overall survival time of patients was defined from the day of surgery to

the day of death. The clinicopathologic characteristics including gender, liver cirrhosis, bilirubin, distant metastasis, cancer area and HBV were recorded. Patients died from unexpected events or other disease were eliminated from our study.

#### *The detection of UHRF1 protein via high performance liquid chromatography*

10 g HCC tissues and the adjacent normal tissues were selected from each specimen, respectively. The homogenate was prepared with the addition of 20 mL methyl cyanides to the tissues and then centrifugated at 4000 r/min for 10 min. The supernatant was collected and the precipitation was dropped. The extracting solution was affiliated and dried with rotary evaporator, followed by dissolution with 1 mL mobile phase for use. 100 RP  $\text{C}_{18}$  column (12×4 mm, 5  $\mu\text{m}$ ) was applied and the column temperature was maintained at  $35^{\circ}\text{C}$ . The mobile phase included 20% methyl cyanides, 30% methanol and 50% water with a flow speed of 1 mL/min, and the detection wavelength was at 365 nm. 0.5 g *UHRF1* protein was dissolved with mobile phase in a 10 mL volumetric flask and used as standard solution for the HPLC.

#### *Statistical analysis*

All data were analyzed by SPSS 18.0 software. The difference of *UHRF1* protein expression in HCC tissues and adjacent tissues was estimated by students *t* test. While the relationship between *UHRF1* protein expression and clinicopathologic characteristics was estimated by Chi-square test. Kaplan-Meier analysis was adopted to delineate the overall survival time of HCC patients with different *UHRF1* protein expression. The prognostic value of *UHRF1* was evaluated by Cox regression analysis. It was considered to be significant different when *P* value was less than 0.05.

### Results

#### *Up-regulation of UHRF1 protein in HCC tissues*

The *UHRF1* protein expression in HCC tissues and adjacent normal tissues was determined by HPLC method. As shown in **Figure 1**, the concentration of *UHRF1* protein in HCC tissues was  $66.98 \pm 13.46$  (mean  $\pm$  SD), which was significantly higher than that in the adjacent normal tissues ( $36.26 \pm 9.72$ ), indicating that *UHRF1*

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**Table 1.** Correlation between *UHRF1* expression and clinicopathologic characteristics

Characteristics	Case (n)	<i>UHRF1</i> protein Expression		$\chi^2$	P value
		High (n)	Low (n)		
Gender				0.180	0.671
Male	35	25	10		
Female	33	22	11		
Liver cirrhosis				1.240	0.265
Yes	36	27	9		
No	32	20	12		
Bilirubin ( $\mu\text{mol/L}$ )				1.722	0.189
$\leq 17.1$	34	21	13		
$> 17.1$	34	26	8		
Distant metastasis				4.607	0.032
Yes	39	31	8		
No	29	16	13		
Cancer area ( $\text{cm}^2$ )				4.168	0.041
$\leq 10$	36	21	15		
$> 10$	32	26	6		
HBV				4.408	0.036
Yes	29	24	5		
No	39	23	16		

low-up, 33 (70.2%) patients in high *UHRF1* protein expression group died, while only 4 (19.0%) died among those with low *UHRF1* protein expression. Kaplan-Meier analysis showed that the overall survival time of patients with high *UHRF1* protein expression was significantly shorter than those with low *UHRF1* protein expression (**Figure 2**, log rank test,  $P=0.000$ ). Cox regression analysis manifested that there was significant correlation between *UHRF1* protein expression and the prognosis of HCC patients (**Table 2**,  $P=0.002$ , HR=5.807, 95% CI=1.901-17.742). The results indicated that *UHRF1* could act as an independent prognostic biomarker for HCC patients.

was increased in HCC tissues ( $P<0.001$ ). This might intimate that *UHRF1* was an oncogene in HCC.

### Association between *UHRF1* protein expression and clinicopathologic characteristics of HCC patients

Further investigations were performed to evaluate the association between *UHRF1* expression and the clinicopathologic characteristics of HCC patients. All the enrolled patients were divided into two groups: the high group with *UHRF1* protein expression of more than 42.3, and the low group with *UHRF1* protein expression of less than or equal to 42.3. It was confirmed that *UHRF1* expression was significantly related to distant metastasis ( $P=0.032$ ), cancer area ( $P=0.041$ ), and HBV ( $P=0.036$ ) (**Table 1**). However, there was no statistical difference between *UHRF1* protein expression and gender, cirrhosis as well as the concentration of bilirubin ( $P>0.05$ ).

### Elevated protein expression of *UHRF1* was related to poor prognosis of HCC patients

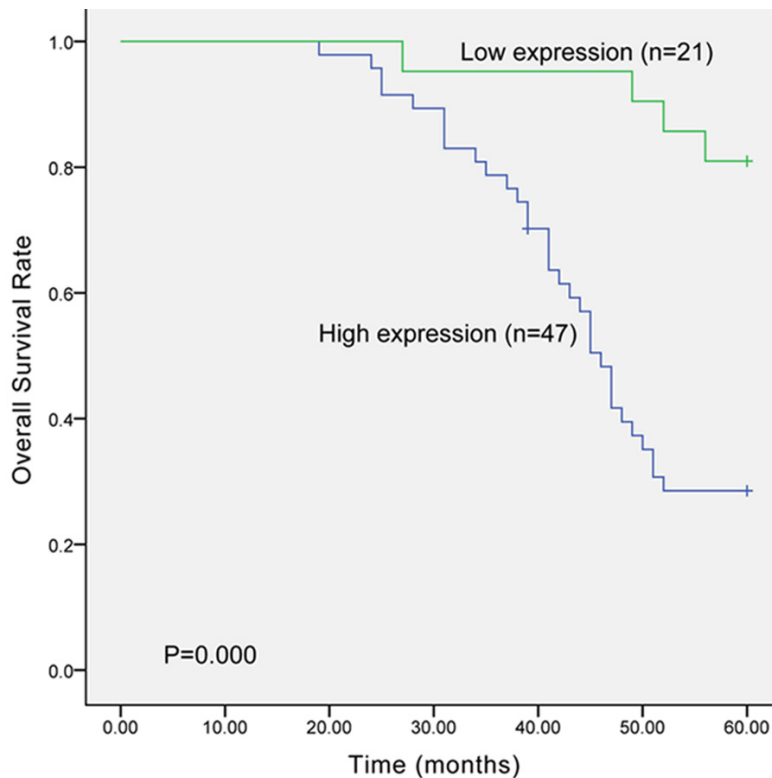
A follow-up ranged from 0 to 60 months was adapted to deeply research the prognostic value of *UHRF1* in HCC patients. During the fol-

## Discussion

HCC is of high malignancy and rapid development with a short average survival time [22]. Early HCC has no obvious symptoms thus patients are often diagnosed with advanced stage. The 5-year survival rate of HCC is only 14% [23]. Therapies for HCC in clinical are predominantly surgery, radiotherapy and chemotherapy. However, these therapies have weak effects on advanced stage patients and the prognosis of HCC patients was poor [24]. Therefore, early discovery, early diagnosis and early treatment are of urgent needs for HCC patients. Many researchers have engaged in finding some molecules that can be candidate biomarkers for therapy and prognosis of HCC and various biomarkers had already been found such as AFP, *miRNA* and *lncRNA* [25-28].

*UHRF1* encodes a series of a subfamily of ring-finger type E3 ubiquitin ligases. The protein can bind to specific DNA sequences to regulate chromatin structure and gene expression. Recently, much attention from investigators has been focused on the relationship between *UHRF1* expression and various cancers. A quantity of reports manifested that overexpression of *UHRF1* was observed in many kinds of cancer. For example, Cui et al. found

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**Figure 2.** The overall survival of HCC patients was evaluated by Kaplan-Meier analysis. The result indicated that the survival time of patients with high *UHRF1* protein expression was shorter than those with low *UHRF1* protein expression ( $P=0.000$ ).

**Table 2.** Multivariate analysis of *UHRF1* protein expression and clinicopathologic characteristics in the prognostic of HCC

Clinical characteristics	P value	HR	95% CI
Bilirubin	0.346	0.713	0.353-1.441
Cancer area	0.250	1.598	0.719-3.551
HBV	0.157	1.725	0.811-3.668
High <i>UHRF1</i> expression	0.002	5.807	1.901-17.742
Low <i>UHRF1</i> expression	-	-	-

that the up-regulated *UHRF1* in pancreatic cancer cells not only be an oncogene, but also could promote the cell growth, migration and metastasis [29]. In bladder cancer although *UHRF1* expression was also increased, but it played a role in inducing cell invasion [30]. The over-expression of *UHRF1* was also discovered in lung cancer, and it played an important role in the diagnosis of this cancer [31]. In addition, *UHRF1* over-expression was observed in HCC in previous study, but the relationship between *UHRF1* expression and clinicopathological characteristics as well as the prognostic value

of *UHRF1* in HCC patients was still unclear.

In the present study, we validated the expression level of *UHRF1* in HCC by HPLC. The over-expression of *UHRF1* was observed in HCC cases, especially in HBV infected cases, which was accordant with the previous description [21]. So we inferred that *UHRF1* might be an oncogene of HCC. Moreover, previous studies have found that *UHRF1* expression level was linked to the presence of lymph node metastasis, distal metastasis, and T stage [17, 31]. Yang et al. [18] have shown that in bladder cancer, *UHRF1* expression was positively related with tumor grade. Thus in our study, we analyzed the relationship between *UHRF1* expression and clinicopathologic characteristics in HCC. Distant metastasis, cancer area and HBV infection were proved to be influential factors for the protein expression of *UHRF1*. Based on the result, *UHRF1* was suggested to be a participator in the development of HCC.

The prognostic value of *UHRF1* was studied in several cancers including bladder cancer [18], lung cancer [31], and prostate cancer

[32]. Furthermore, in bladder cancer [18] and lung cancer [31] patients, *UHRF1* could be used as an independent prognostic biomarker. However, the potential role of *UHRF1* in the prognosis of HCC is still unclear, and it is necessary to explore an effective prognostic marker for HCC patients. Thus we estimated the prognostic value of *UHRF1* in HCC. The overall survival time of patients with high *UHRF1* expression was shorter than those with low expression according to Kaplan-Meier analysis which manifested that *UHRF1* was related to the prognosis of HCC. Hence, the multivariate anal-

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ysis was performed and the result exhibited that high *UHRF1* expression increased the risk of HCC. The *UHRF1* was a vital factor for the prognosis of HCC and would be an independent prognostic biomarker for HCC patients.

In conclusion, combing with the previous findings, we have verified that the expression of *UHRF1* was increased in HCC and it could act as a potential prognostic indicator for HCC patients. However, the mechanism of *UHRF1* on HCC remains unclear which needs to be further investigated.

### Disclosure of conflict of interest

None.

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