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Original Article

Ube2s expression is elevated in hepatocellular carcinoma and predicts poor prognosis of the patients

Qi Li[1], Yang Zhao[2], Xiuying Shi[1], Chuifeng Fan[1]

[1]*Department of Pathology, First Affiliated Hospital and College of Basic Medical Sciences of China Medical University, Shenyang, China;* [2]*Department of Hepatobiliary and Spleenary Surgery, The Affiliated Shengjing Hospital, China Medical University, Shenyang, China*

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Abstract: Ube2s belongs to the ubiquitin-conjugating (E2) enzyme family of the ubiquitin system. Expression and function of Ube2s in human malignancies was largely unknown. Here we report our investigation of Ube2s expression in human non-tumor liver and hepatocellular carcinoma tissues using immunohistochemistry. Ube2s expression was detected in nuclear and cytoplasm. The positive rate of Ube2s in normal liver tissues was 19.2% (5/26). The positive rate of Ube2s expression in liver tissues with hepatocirrhosis (33.3% (6/18)) was higher than that in normal liver tissues (P<0.05). Ube2s expression in hepatocellular carcinoma (59.1% (39/66)) was higher than that in normal liver tissues and liver tissues with hepatocirrhosis (P<0.05). There were 35 cases (53.0%) showing nuclear expression, 21 cases (31.8%) showing cytoplasm expression, 17 cases (25.8%) showing both nuclear and cytoplasm expression, 18 cases (27.3%) showing only nuclear expression and 4 cases (6.1%) showing only cytoplasm expression in hepatocellular carcinoma tissues. Ube2s expression was significantly associated with higher AFP levels (>100 ng/ml), advanced TNM stages (II+III), higher ABCL stages (B+C) in hepatocellular carcinoma (P<0.05). Kaplan Meier analysis showed that Ube2s expression was significantly associated with shorter survival time of patients (*P*<0.05). These results indicate that Ube2s may be involved in oncogenesis and development of hepatocellular carcinoma and may be a potential cancer marker and therapy target.

Keywords: Hepatocellular carcinoma, IHC, prognosis, Ube2s, liver

Introduction

Hepatocellular carcinoma is one of the leading causes of death by malignant tumors all around the world. To understand the molecular mechanism and molecules involved in cancer development is important for developing clinical target therapy. Ube2s is an ubiquitin-conjugating (E2) enzyme of the ubiquitin system [1-4]. Recently, more and more E2s were found to have notable roles in carcinogenesis [5-7]. Ubiquitin-Conjugating Enzyme E2T was found to promote gastric cancer development [5]. Agboola's study indicates that Ube2s is marker in breast carcinoma predicting poorer clinical outcome [6]. Perrotta's study showed that Ube2s was overexpressed in lung carcinoma and associated with decreased cancer differentiation [7]. However Ube2s expression in human malignancies including hepatocellular carcinoma and its function was largely known. Ube2s was proved to be a novel co-worker in anaphase-promoting complex (APC) ubiquitination pathway [2]. Ube2s was found to play important roles during the meiotic division of mouse oocytes through regulating (APC/C) activity [8]. Ayesha's study showed that Ube2s was highly expressed in breast carcinoma and associated with clinical development [9]. In this study we investigate Ube2s expression in human hepatocellular carcinoma and its clinicalpathological and prognostic significance.

Materials and methods

Tissue samples

Hepatocellular carcinoma tissues and non-tumor liver tissues were obtained from patients between 2010 and 2012 from the Shengjing Hospital of China Medical University. The tumors were diagnosed and classified accord-

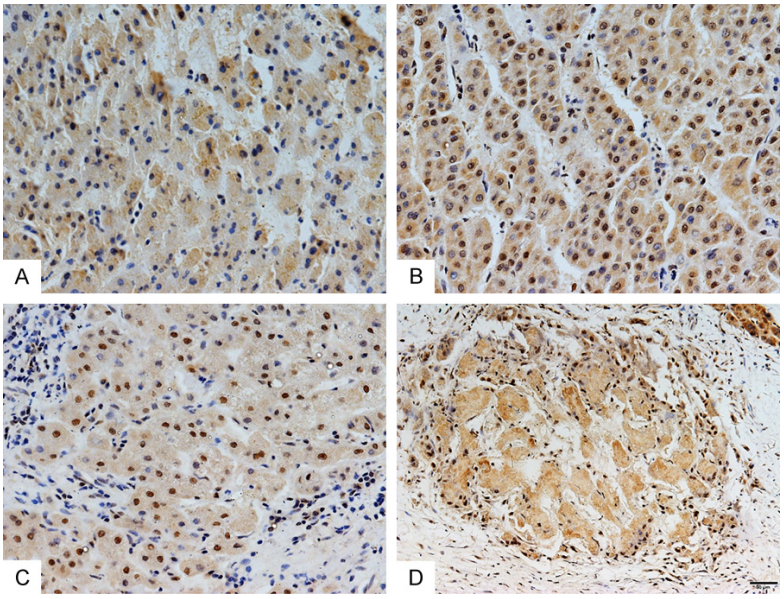

Figure 1. Ube2s expression in non-tumor liver tissues. Cytoplasm and nuclear expression of Ube2s was detected in both normal liver tissues (A, B) and liver tissues with cirrhosis (C, D). (A) Shows only cytoplasmic immunostaining in normal liver tissues. (B) Shows both cytoplasm and nuclear immunostaining in normal liver tissues. (C) Shows only nuclear immunostaining of Ube2s in liver tissues with cirrhosis. (D) Shows both cytoplasm and nuclear immunostaining in liver tissues with cirrhosis.

ing to WHO classification of tumors of the digestive system, 4th edition [10]. This study was performed under the regulations of the Institutional Review Board of China Medical University. Informed consent was obtained from all enrolled patients.

Immunohistochemistry (IHC)

IHC was performed as described previously [11] using the SP IHC kit (Maixin Biotechnology, Fuzhou, Fujian, China). The sections were incubated with primary mouse anti-human Ube2s monoclonal antibody (sc-390917, Santa Cruz, USA; dilution 1:50) overnight at 4°C. Scoring of IHC was based on two parameters as described previously [11]: the proportion of immunopositive cells and their intensity of immunoreactivity. A final immunoreactivity score of each section was obtained by multiplying the two individual scores and final scores of 2 or more were considered as positive.

Statistical analysis

All data was analyzed using the SPSS statistical software package version 22.0 (SPSS Inc., Chicago, IL, USA). Pearson's Chi-Square test was used to analyze the association between Ube2s expression and clinicopathological factors. McNemar's test was used to compare Ube2s expression in non-tumor liver tissues and hepatocellular carcinoma tissues. Kaplan-Meier test was used to analyze association between Ube2s expression and patients' survival time. *P*-values <0.05 were considered significant.

Results

Ube2s expression in Non-tumor liver tissues

Immunostaining of Ube2s in normal liver tissues was detected in cytoplasm and nuclear (**Figure 1**). The positive rate of Ube2s expression in normal liver tissues was 19.2% (5/26). There were 4 cases with both cytoplasm and nuclear immunostaining and 1 case with only cytoplasm immunostaining. Immunostaining of Ube2s was also detected in cytoplasm or nuclear in liver tissues with hepatocirrhosis (**Figure 1**). The positive rate of Ube2s expression in liver tissues with hepatocirrhosis was 33.3% (6/18) and was significantly higher than that in normal liver tissues ($P<0.05$). There were 4 cases showing both nuclear and cytoplasm expression of Ube2s and 2 cases showing only nuclear expression.

Ube2s expression in hepatocellular carcinoma tissues

Ube2s expression was detected in cytoplasm and nuclear in hepatocellular carcinoma (**Figure 2**). The total positive rate of Ube2s expression in hepatocellular carcinoma was 59.1% (39/66) (**Table 2**). There were 35 cases (53.0%) showing nuclear immunostaining and 21 cases (31.8%) showing cytoplasm immunostaining. There were 17 cases (25.8%) showing both nuclear and cytoplasm immunostaining. There were 18 cases (27.3%) showing only nuclear immunostaining and 4 cases (6.1%) showing

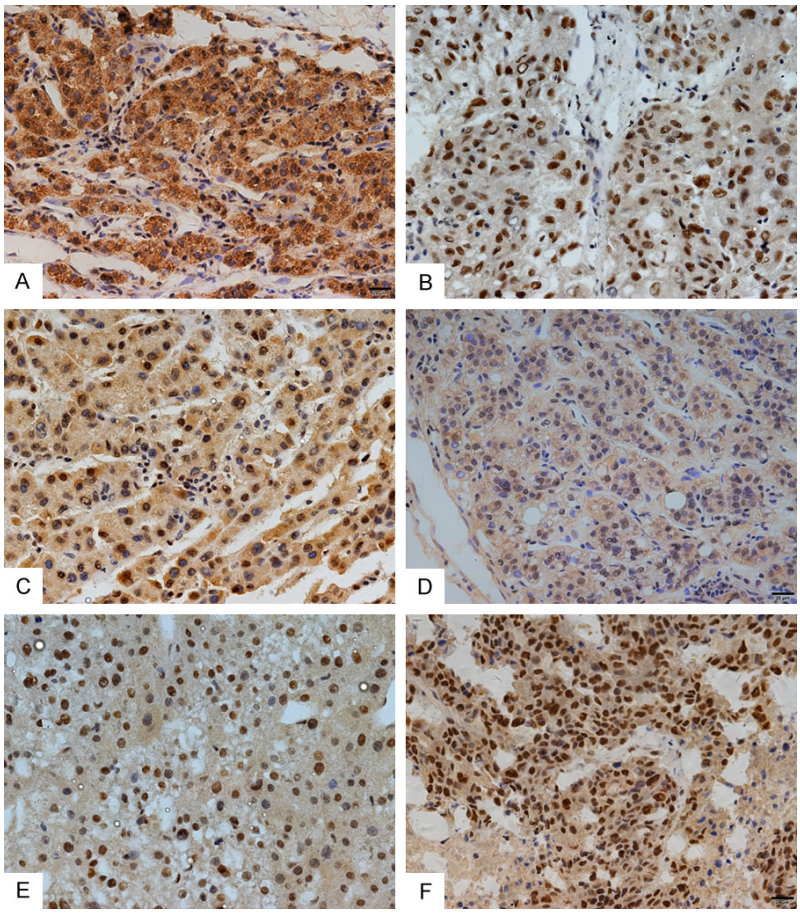

Figure 2. Expression of Ube2s in hepatocellular carcinoma. Immunostaining was detected in cytoplasm and nuclear. Cancer tissues showing only cytoplasmic immunostaining (A). Cancer tissues showing only nuclear immunostaining (B). Cancer tissues showing both cytoplasm and nuclear immunostaining (C). Cancer tissues with high differentiation showing weak cytoplasm and nuclear immunostaining (D). Cancer tissues with media differentiation showing strong nuclear immunostaining (E). Cancer tissues with poor differentiation showing strong nuclear immunostaining (F).

Table 1. The positive status of UBE2s in nuclear and cytoplasm in hepatocellular carcinoma

		Cytoplasm expression	
		+	-
Nuclear expression	+	17 (25.8%)	18 (27.3%)
	-	4 (6.1%)	27 (40.9%)

Summary					
Total positive	Nuclear positive	Cytoplasm positive	Nuclear only	Cytoplasm only	Both Nuclear and cytoplasm
39 (59.1%)	35 (53.0%)	21 (31.8%)	18 (27.3%)	4 (6.1%)	17 (25.8%)

only cytoplasm immunostaining. The positive status of Ube2s expression in nuclear and cytoplasm in hepatocellular carcinoma was summarized in **Table 1**. Expression of Ube2s in hepatocellular carcinoma was significantly higher than that in normal liver tissues and liver tissues with cirrhosis ($P<0.05$).

Clinicalpathological significance of Ube2s expression in hepatocellular carcinoma

The clinicopathological analysis of Ube2s expression in hepatocellular carcinoma was shown in **Table 3**. Ube2s expression was significantly associated with higher AFP level (>100 ng/ml). Ube2s expression was also significantly associated with lower differentiation of cancer ($P<0.05$). Hepatocellular carcinoma with media or poor differentiation was significantly higher than those with well differentiation (**Figure 2** and **Table 3**). Elevated Ube2s expression was also associated with advanced TNM stages (II+III), BCLC stages (B+C) and large tumor size (>5 cm) ($P<0.05$). Ube2s expression was more frequently seen in patients with multiple tumors ($P=0.050$ and was very close) and portal vein invasion ($P=0.144$) though not significantly.

Correlation between Ube2s expression in hepatocellular carcinoma and patients' clinical outcome

The survival time of the patients with hepatocellular carcinoma ranged from 6 to 60 months. The overall mean survival time was 32.8±15.8 months. The survival time of patients with hepatocellular carcinoma and the association with Ube2s expression was shown in **Table 3** and **Figure 3**.

Table 2. Clinicopathological significance of Ube2s expression in hepatocellular carcinoma

variables	All patients	Ube2s expression		p^*
		Negative	Positive	
Total	66	27	39	
Age (y)				
≤50	18	9	9	0.358
>50	48	18	30	
Gender				
Male	53	23	30	0.407
Female	13	4	9	
HbsAg				
Positive	48	22	26	0.184
Negative	18	5	13	
AFP				
>100 ng/mL	28	7	21	0.024
<100 ng/mL	38	20	18	
Hepatocirrhosis				
Yes	27	10	17	0.594
No	39	17	22	
Grade				
Well	18	13	5	0.002
Moderate and poor	48	14	34	
TNM stage				
I	42	22	20	0.012
II/III	24	5	19	
BCLC stage				
A	29	18	11	0.002
B/C	37	9	28	
Tumor number				
Multiple	9	1	8	0.050
Single	57	26	31	
Tumor size				
≤5 cm	33	18	15	0.024
>5 cm	33	9	24	
Portal vein invasion				
Yes	13	3	10	0.144
No	53	24	29	

*p values were obtained with the X^2 test.

The survival time of patients with hepatocellular carcinoma was significantly associated with Ube2s expression. The survival time of patients with Ube2s expression (29.4±2.6 months) was significantly shorter than that without Ube2s expression (59.2±2.7 months) (**Figure 3A**) (P< 0.05). When we analyzed the significance of Ube2s expression in different subcellular location, we found that Ube2s expression in nuclear, cytoplasm, nuclear only and both nuclear and cytoplasm all showed significant association with shorter survival time of the patients (P<0.05).

Discussion

Ube2s belongs to the E2 enzymes which provide binding sites for E1 and E3 enzymes, and activated ubiquitin's. So far, Ube2s expression in human malignancies was largely unknown. Here we found that Ube2s expression was significantly elevated in liver tissues with hepatocirrhosis than in normal liver tissues. As hepatocirrhosis was well known as a precancerous lesion [12], it is possible that Ube2s may be involved in oncogenesis of hepatocellular carcinoma, though the related mechanism needs to be further studied. Hepatitis B virus infection is the most frequent factor related to hepatocellular carcinoma. Our study shows that there isn't significant association between HbsAg and Ube2s expression. It indicates that Hepatitis B virus infection is not likable to be the cause for Ube2s expression. But whether Ube2s is involved in oncogenesis through Hepatitis B virus is not clear. We found that Ube2s expression in hepatocellular carcinoma was significantly associated with high AFP levels. It may be because Ube2s expression was significantly related to lower differentiation of hepatocellular carcinoma. Wang's study indicates that Ube2s may inhibit differentiation of the mouse embryonic stem cells and reinforce their pluripotency possibly through promoting SOX-2 proteosomal degradation [13]. However, the association between Ube2s expression and poor differentiation need to be further investigated. It is unknown if it is because cancer cells with poor differentiation more frequently generate this protein, or the protein helps to maintain the poor differentiation, or the both.

We found in this study that Ube2s was elevated in hepatocellular carcinoma compared to non-tumor liver tissues and significantly associated advanced TNM and ABCL stages which indi-

Table 3. Survival time of patients with hepatocellular carcinoma and the association with Ube2s expression

	Positive		Nuclear (+)		Cytoplasm (+)		Only nuclear (+)		Nuclear and cytoplasm (+)		Only cytoplasm (+)	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Time (m)	29.4	59.2	28.5	53.4	30.3	45.0	28.6	45.3	28.4	44.8	35.8	41.1
X^2	15.981		16.044		4.161		6.342		4.965		0.000	
p	0.000		0.000		0.041		0.012		0.026		0.994	

The *p* and X^2 values were obtained by Kaplan-Meier analysis.

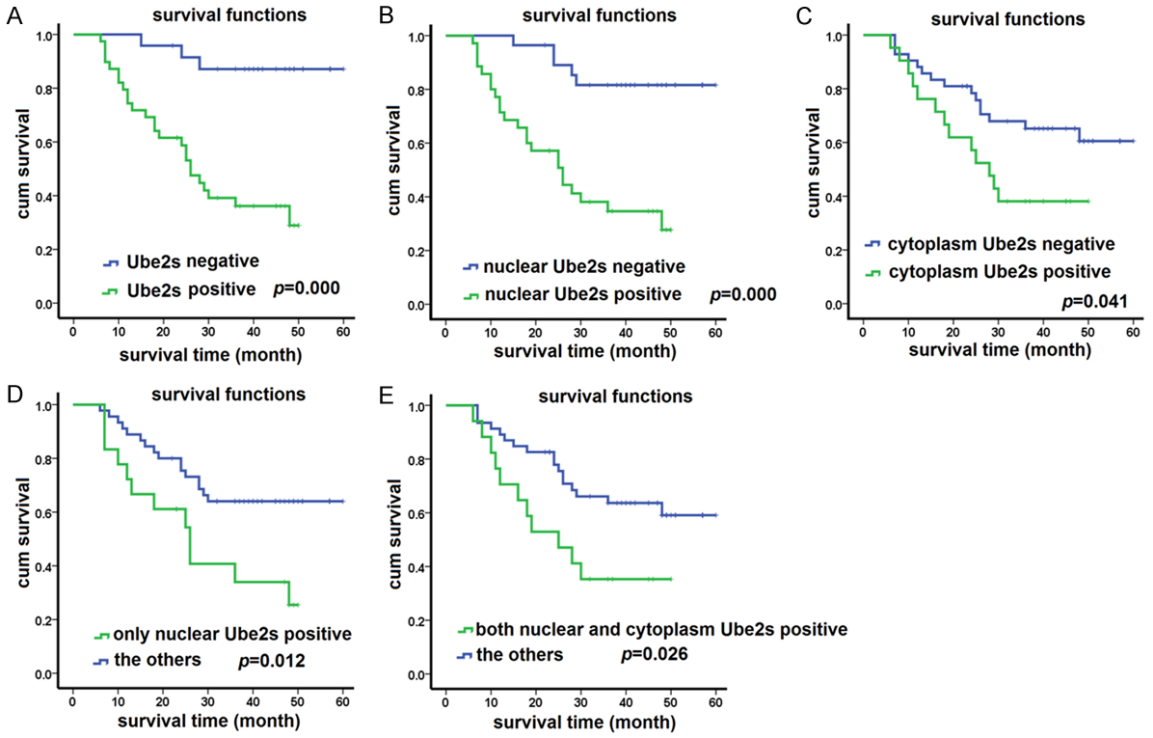

Figure 3. Correlation between Ube2s expression in hepatocellular carcinoma and patients' clinical outcome. The survival time of patients with Ube2s expression in hepatocellular carcinoma was significantly shorter than that without Ube2s expression (A) (P<0.05). The survival time of patients with nuclear Ube2s expression was significantly shorter than that without nuclear Ube2s expression (B) (P<0.05). The survival time of patients with cytoplasm Ube2s expression was significantly shorter than that without cytoplasm Ube2s expression (C) (P<0.05). Ube2s expression in only nuclear was also associated with shorter survival times of patients (D) (P<0.05). The survival time of patients with both nuclear and cytoplasm Ube2s expression was significantly shorter than the others (E) (P<0.05).

cates that it may be involved in development of this cancer. However the function of Ube2s and the related mechanism in human malignancies remain not fully understood. Hu's study indicates that Ube2s play important roles in regulating chemotherapy resistance in glioblastoma [14]. Ube2s was also found to be critical for maintenance of self-renewal of the mouse embryonic stem cells [13]. The study indicated that Ube2s may play this role by regulating Sox2 stability through ubiquitating it and promoting its degradation [13]. Whether Ube2s could similarly play a role in self-renewal of cancer stem cells needs to be clarified. Ube2s was identified as a co-worker of the APC/C ubiquitin ligase and play important roles in regulating drug-induced spindle-assembly checkpoint activation [1]. Aneuploidy is frequently seen in cancer cells. It is also important to investigate whether Ube2s could impact spindle-assembly checkpoint activation without drug. Our study shows that Ube2s expression in hepatocellular

carcinoma was significantly associated with large tumor size. It indicates that Ube2s may have a role in promoting cancer cell proliferation. But whether spindle-assembly checkpoint activation was involved in the mechanism is not clear.

In the current study we found that Ube2s expression in hepatocellular carcinoma was significantly associated with shorter survival time of the patients. Ube2s expression in nuclear and cytoplasm in cancer cells both predict poorer clinical outcome. However, as most of the cases with cytoplasm Ube2s expression (80.95%, 17/21) were also positive for nuclear Ube2s and the number of the patients with cytoplasm expression of Ube2s only was too small, it was not clear whether cytoplasm expression of Ube2s only is a factor to predict patients' poor clinical outcome though the survival time of patients with cytoplasm Ube2s expression only (35.8±4.71 months) was actually shorter than the others (41.1±2.84 months). However as the group of patients with both nuclear and cytoplasm Ube2s expression also had shorter survival time, it indicates that at least cytoplasm Ube2s is not likable to impair the function of nuclear Ube2s related to cancer development.

Conclusions

Ube2s expression was elevated in hepatocellular carcinoma compared to normal liver tissues. Higher Ube2s expression was correlated with cancer development and patients' poor clinical outcome. Ube2s may be a potential cancer marker in hepatocellular carcinoma.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Chuifeng Fan, Department of Pathology, First Affiliated Hospital and College of Basic Medical Sciences of China Medical University, Shenyang 110001, China. Tel: +86 24 23261638; Fax: +86 24 23261638; E-mail: fanchuifeng2013@163.com

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