Urotensin II receptor as a potential biomarker for the prognosis of hepatocellular carcinoma patients

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Abstract. Urotensin II and the associated urotensin II receptor (UTR) are important in the carcinogenesis of hepatocellular carcinoma (HCC). However, the clinical significance of UTR remains to be elucidated. The aim of the present study was to investigate if UTR exhibits the potential to act as a biomarker to predict the prognosis of HCC patients. The effects of UTR on motility and invasion of HCC cells were additionally investigated. UTR expression levels were determined by immunohistochemistry, in 83 HCC patients that previously underwent curative liver resection. The association between UTR levels and clinicopathological data were analyzed. *In vitro*, the expressions of UTR in QSG-7701, BEL-7402 and MHCC-97H cell lines were determined via western blotting. Small interfering (si)RNA was used to downregulate UTR in BEL-7402 and MHCC-97H cell lines, and the effects of UTR on tumor cell motility were tested by Transwell assay. UTR expression was associated with tumor number, size, histology and tumor node metastasis/Barcelona Clinic Liver Cancer HCC stage. UTR expression levels were additionally associated with recurrence-free and overall survival in HCC patients by Kaplan-Meier curve analysis ($P<0.0001$). *In vitro*, UTR expression levels were increased in BEL-7402 and MHCC-97H cell lines, compared with QSG-7701 ($P<0.05$). siRNA-mediated silencing of the UTR gene significantly inhibited cell motility in BEL-7402 and MHCC-97H cells. The results indicated that UTR may be regarded as a novel biomarker to predict outcomes following radical liver resection and as a potential therapeutic target to inhibit invasion and metastasis of HCC.

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common malignancy and the third cause of tumor associated deaths worldwide (1). The curative therapies currently available for HCC are liver resection, local ablative treatments and liver transplantation (2). Due to limited donors, rigorous indication for local ablative treatments, liver tumor resection is the main curative treatment for HCC in clinical practices (3). However, the recurrence rate after hepatic resection was over 70% in 5 years (4). The high recurrence and metastasis are closely related to poor prognosis of HCC patients after radical treatment. Currently, there is no effective way to radically prevent recurrence and metastasis of HCC. Therefore, it is of great clinical significance to accurately determine the prognosis and take the corresponding individualized treatment after surgery, which will be helpful to improve the long-term survival of HCC patients.

Human urotensin II (UII), isolated from the urophysis of teleost fish, is an undecapeptide (H-Glu-Thr-Pro-Asp-c [Cys-Phe-Trp-Lys-Tyr-Cys]-Val-OH). The orphan G-protein coupled receptor 14 was identified as the urotensin II receptor (UTR) (5). As a powerful vasoactive peptide in mammals, initially UII/UTR is reported to play an important role in portal hypertension, renal disease and heart failure (5). Recent studies demonstrated that UII/UTR may involve in tumorigenesis and tumor progression in many malignant tumors (6), such as adrenal gland neoplasms (7), breast carcinoma (8), pulmonary adenocarcinoma (9), renal cell carcinoma and colon carcinoma (10). Our previous study demonstrated that UII

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Abbreviations: AFP, α-fetoprotein; HCC, hepatocellular carcinoma; OS, overall survival; RFS, recurrence-free survival; UII, urotensin II; UTR, urotensin II receptor; IHC, Immunohistochemistry; siRNA, small interfering RNA

Key words: urotensin II receptor, hepatocellular carcinoma, motility, prognosis

and UTR are up-regulated in rat HCC model and human HCC tissue, exogenous UII can increase hepatic oval cell and HCC cell proliferation *in vitro* (11-13). All these results indicated that UII played an important role in initiation and progression of HCC. And in our preliminary experiment, we also found that the intensity of UTR expression on HCC tissues varies from patient to patient. So, we wonder whether UTR has a clinical significance in HCC patients and whether UTR plays a role on HCC development.

The aim, in the present study, was to determine whether UTR could be as a biomarker to predict the outcomes of HCC patients underwent radical treatment. The effects of UTR on HCC cell motility and invasion was also explored.

Materials and methods

Patients and tissue samples. HCC patients underwent curative resection at Beijing Youan Hospital (Beijing, China) from January 2010 to March 2013 were enrolled. Patients had a history of malignancy, or previous anticancer therapy, or detectable distant metastases, or carrying tumor residual after surgery, or received special treatment (such as gene therapy, molecular targeted drug therapy) during follow-up, were excluded from the present study. HCC were staged according to the TNM staging system of the Union for International Cancer Control/American Joint Committee on Cancer (AJCC, 7th edition), and Barcelona Clinic Liver Cancer (BCLC) staging system. The clinicopathological characteristics of patients were retrieved from the medical records and summarized in Table I. The study was approved by the Ethics Committee of Beijing Youan Hospital, Capital Medical University. Written informed consent was obtained from each patient.

Follow-up and endpoints. All enrolled patients were follow-up in real-life clinical practice by outpatient clinic or telephone. The primary endpoints were death or 3 years (36 months) follow-up, and the secondary endpoint was HCC recurrence.

In total, 14 (16.87%) patients were lost to follow-up. Forty patients (48.19%) recurrence and 18 patients (21.69%) death were observed during 3 years follow-up.

Immunohistochemistry (IHC). Formalin-fixed, paraffin-embedded HCC tissues samples were collected from the 83 patients above-mentioned. The immunohistostaining (IHS) was routinely done. In briefly, the sections were incubated with a specific antibody against UTR 1:200 [GPR14 (M-250): sc-28998; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA] at 4˚C for overnight and then incubated with a second antibody with broad spectrum (Invitrogen, Carlsbad, CA, USA) at 37˚C for 20 min. After washed by PBS for three times, the visualization signal was used by a 3,3'-diaminobenzidine and counterstained with hematoxylin. The result of IHS was separately determined by two experienced pathologists. UTR scores were determined by assessing both staining intensity and the proportion of positively stained tumor cells. IHS intensity was divided into 0, no positive staining; 1, positive staining was weak yellow; 2, positive staining was yellow and 3, strong positive staining was brown. The mean percentage of positive tumor cells was determined in five fields under x400 magnification. UTR positive expression was estimated as staining intensity x mean percentage of positive tumor cells. The scores ≤4 was regarded as UTR low expressions and 5-12 as UTR high expressions.

Western blot analysis. The UTR protein concentration was determined using a BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA) as described in the manufacturer's manual. Lysate protein (80 μg) was separated on 10% SDS-PAGE gels and transferred to a polyvinylidene fluoride membrane. After blotting, the membrane was blocked in 5% skim milk in TBST for 1 h at room temperature and then incubated with the specific primary antibody against UTR (1:500) and GAPDH (1:5,000) at 4˚C overnight. After well washed by TBST, membranes were then incubated with horseradish perxoidase-conjugated secondary antibody (1:5,000). The membrane was developed by enhanced chemiluminescence detecting reagents. And densities of specific proteins were normalized according to the amount of total protein and GAPDH.

Cell lines. Human hepatic cell line QSG-7701, human HCC cell line BEL-7402 (obtained from the Cell Bank of the Chinese Academy of Sciences, Shanghai, China), and MHCC-97H cells (human HCC cell lines with high metastatic potential, established at the Liver Cancer Institute, Zhongshan Hospital, Fudan University, Shanghai, China) were maintained in Dulbecco's modified Eagle's medium containing 10% of fetal calf serum and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin). The cells were harvested in the logarithmic phase of growth and serum-starved for 8 h before used in experiments outlined below.

Small interfering RNA-mediated UTR gene silencing. The expression of human UTR was knocked down using small interfering RNA (siRNA) duplexes (two sequences and one control siRNA). The two pre-designed siRNA (1-sense, 5'CCA UGUACGUCUACGUGGUTRT-3' and antisense, 5'-ACCACG UAGACGUACAUGGAG-3'; 2-sense, 5'ACGCAACCCUCA ACAGCUCtt-3' and antisense, 5'-GAGCUGUUGAGGGU UGCGUtg-3') were bought from Life Technologies Corp., (Carlsbad, CA, USA). Fluorescein Conjugate (A: sc-36869, Santa Cruz Biotechnology, Inc.) was used as control. Cells (10^5) in the exponential growth phase were inoculated in 6-well plates and cultured for 24 h. According to the manufacturer's recommended protocol, cells were serum-starved for 8 h before transfected with 10 μM siRNA in serum free medium (Opti-MEM; Gibco, Grand Island, NY, USA). The result of transfection was analyzed by western blot analysis.

In vitro invasion and motility assays. Transfected cells ($2x10^4$) in 200 μl serum free medium were added to the upper compartment of MilliCell (12 mm diameter with 8 μm pores) chambers which were pre-coated with Matrigel (Corning Inc., Corning, NY, USA), and the chambers were placed into 24-well plates with 0.5 ml complete medium. After 24 h cultivation at 37˚C, chambers were taken out and washed with PBS (5 times, each for 5 min) after the medium was discarded. Immediately, the chambers were fixed in 24-well plates with 1 ml absolute ethyl alcohol for 15 min. washed with PBS for 3 times, the chambers were then stained with 1 ml crystal violet (0.1%) for another

Table I. Correlation between UTR expressions and clinicopathologic variables of patients with HCC.

Variables	Total (n=83)	Expression of UTR		P-value[a]
		Low (n=44)	High (n=39)	
Sex (%)				0.948
Male	70 (84.34)	37 (84.09)	33 (84.62)	
Female	13 (15.66)	7 (15.91)	6 (15.38)	
Age (years) (%)				0.089
≤50	45 (54.22)	20 (45.45)	25 (64.10)	
>50	38 (45.78)	24 (54.55)	14 (35.90)	
Underlying liver disease (%)				0.432
HBV	76 (91.57)	39 (88.64)	37 (94.87)	
HCV	5 (6.02)	4 (9.09)	1 (2.56)	
Others	2 (2.41)	1 (2.27)	1 (2.56)	
Tumor location (%)				0.271
Right lobe	61 (73.49)	32 (72.73)	29 (74.36)	
Left lobe	16 (19.28)	9 (20.45)	7 (17.95)	
Bilateral	4 (4.82)	1 (2.27)	3 (7.69)	
Caudate	2 (2.41)	2 (4.55)	0 (0)	
Tumor number (%)				0.002[b]
Single	60 (72.29)	38 (86.36)	22 (56.41)	
Multiple	23 (29.71)	6 (13.64)	17 (43.59)	
Tumor size (cm)				0.017[b]
<3	30 (36.14)	22 (50.00)	8 (20.51)	
3-5	31 (37.35)	14 (31.82)	17 (43.59)	
>5	22 (26.51)	8 (18.18)	14 (35.90)	
AFP (ng/ml) (%)				0.296
<20	36 (43.37)	21 (47.73)	15 (38.46)	
20-400	22 (26.51)	13 (29.55)	9 (23.08)	
>400	25 (30.12)	10 (22.73)	15 (38.46)	
TNM stage (%)				0.000[b]
I+II	57 (68.67)	41 (93.18)	16 (41.03)	
III+IV	26 (31.33)	3 (6.82)	23 (58.97)	
BCLC HCC stage (%)				0.000[b]
A	42 (50.60)	30 (68.18)	12 (30.77)	
B	22 (26.51)	12 (27.27)	10 (25.64)	
C	19 (22.89)	2 (4.55)	17 (43.59)	
Child-Pugh class (%)				0.139
A	75 (90.36)	42 (95.45)	33 (84.62)	
B	8 (9.64)	2 (4.55)	6 (15.38)	
Histologic grade (%)				0.021[b]
Poorly	18 (21.69)	5 (11.36)	13 (33.33)	
Moderate	52 (62.65)	29 (65.91)	23 (58.97)	
Well	13 (15.66)	10 (22.73)	3 (7.69)	
Tumor recurrence (%)				0.000[b]
No	43 (51.81)	31 (74.45)	12 (30.77)	
Yes	40 (48.19)	13 (29.55)	27 (69.23)	
Mortality (%)				0.000[b]
No	65 (78.31)	41 (93.18)	24 (61.54)	
Yes	18 (21.69)	3 (6.82)	15 (38.46)	

[a]P-value comparison between lower UTR expression and higher UTR expression; [b]P<0.05. UTR, urotensin II receptor; HCC, hepatocellular carcinoma; HBV, hepatitis B; HCV, hepatitis C; AFP, α-fetoprotein; BCLC, Barcelona Clinic Liver Cancer.

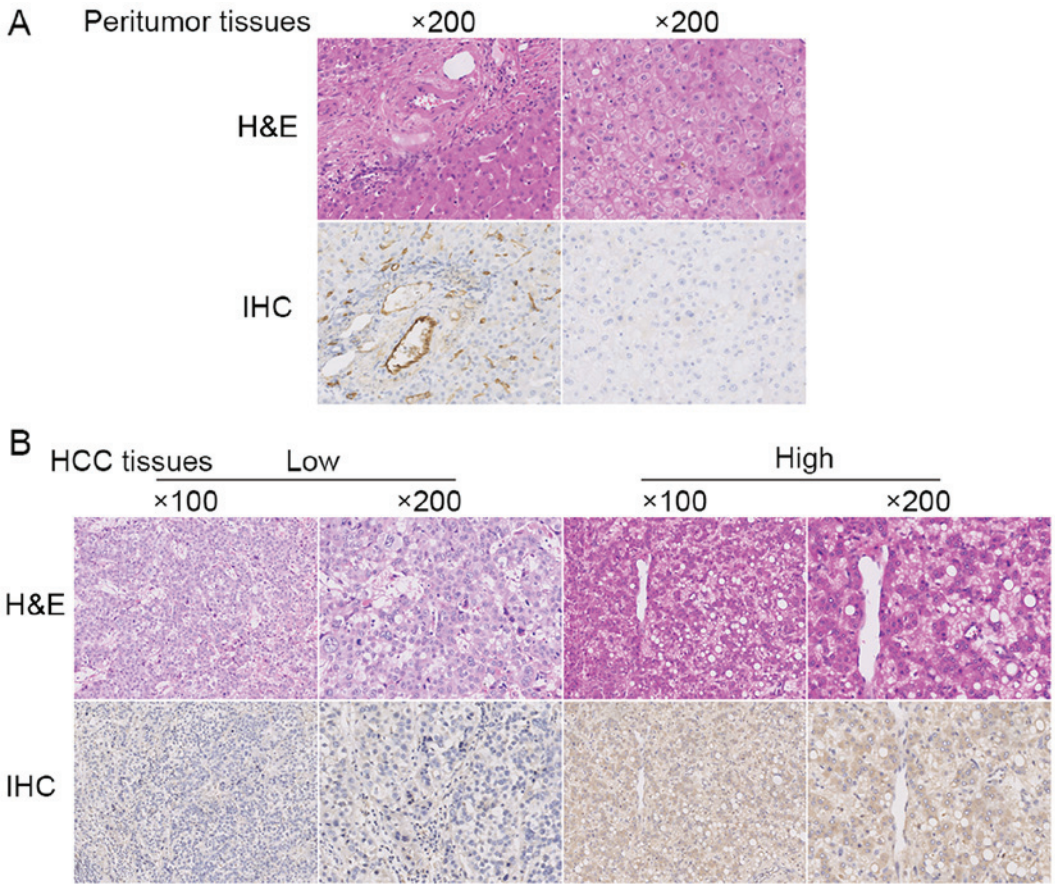

Figure 1. UTR expressions in (A) peritumor and (B) HCC tissues. UTR, urotensin II receptor; HCC, hepatocellular carcinoma; H&E, hematoxylin and eosin staining; IHC, immunohistochemistry; low, low UTR expression; high, high UTR expression.

15 min. Cells were well washed with PBS buffer. Then cells in the upper compartment of chambers were wiped away, and which in the lower compartment of chambers were observed by light microscope. The amount of five high power fields per chamber were counted and mean value was calculated. The invasive activity was quantified from at least three individual chambers. The migration assay is similarly performed using invasion assay excepting that no Matrigel was used.

Statistical analysis. Data were analyzed with SPSS Statistics software, version 22.0 (IBM Corporation, Armonk, NY, USA). Differences among the categorical variables and quantitative variables were analyzed using Chi-square and the paired Wilcoxon signed rank test/unpaired t-test, respectively. Univariate and multivariate Cox proportional hazards analyses were used to assess the effects of various factors on prognosis. Kaplan-Meier analysis was used to assess recurrence-free survival (RFS)/overall survival (OS), and log-rank tests were used to compare them between the subgroups. All P-values were two-sided, and $P<0.05$ was considered to be statistically significant.

Results

The clinical characteristics of enrolling patients. In a total of 83 enrolled HCC patients, 77 (92.77%) patients underwent lobectomy and 8 (7.23%) patients underwent hemihepatectomy. There were 70 men and 13 women (84.34% vs. 15.66%). The age of 45 (54.22%) patients were under 50 years. A total of 76 (91.57%) patients had chronic hepatitis B, and 5 (6.02%) had chronic hepatitis C. Majority (73.49%) of the tumor was located at right lobe. A total of 23 (29.71%) patients had more than one tumor in their liver. The tumor sizes in 30 (36.14%) patients were no more than 3 cm, while in 22 (26.51%) patients were more than 5 cm. Serum α-fetoprotein (AFP) levels in 36 (43.37%) patients were less than 20 ng/ml, that in 22 (26.51%) patients range between 20 to 400 ng/ml, and in 25 (30.12%) patients were above 400 ng/ml. According to TNM stage classification, 57 (68.67%) patients were I or II stage. While according to BCLC staging, 42 (50.60%) patients were stage A, 22 (26.51%) stage B, 19 (22.89%) stage C. Poor differentiation was found in 18 (21.69%) patients, and moderate differentiation was found in 52 (62.65%) patients, and well differentiation was found in 13 (15.66%) patients.

UTR expression characteristic on tissues. In the peritumor tissues, UTR staining was positive in the vessels and portal area, and the little staining or negative in the normal liver cells (Fig. 1A). Whereas in HCC tissues, UTR staining was detected positive in the cytomembrane and cytoplasm of HCC cells (Fig. 1B).

Relationship between expressions of UTR and clinicopathological characteristics. Based on UTR expressions determined by IHC, the HCC patients were divided into two subgroups: low UTR expressions group (N=44) and high UTR expressions

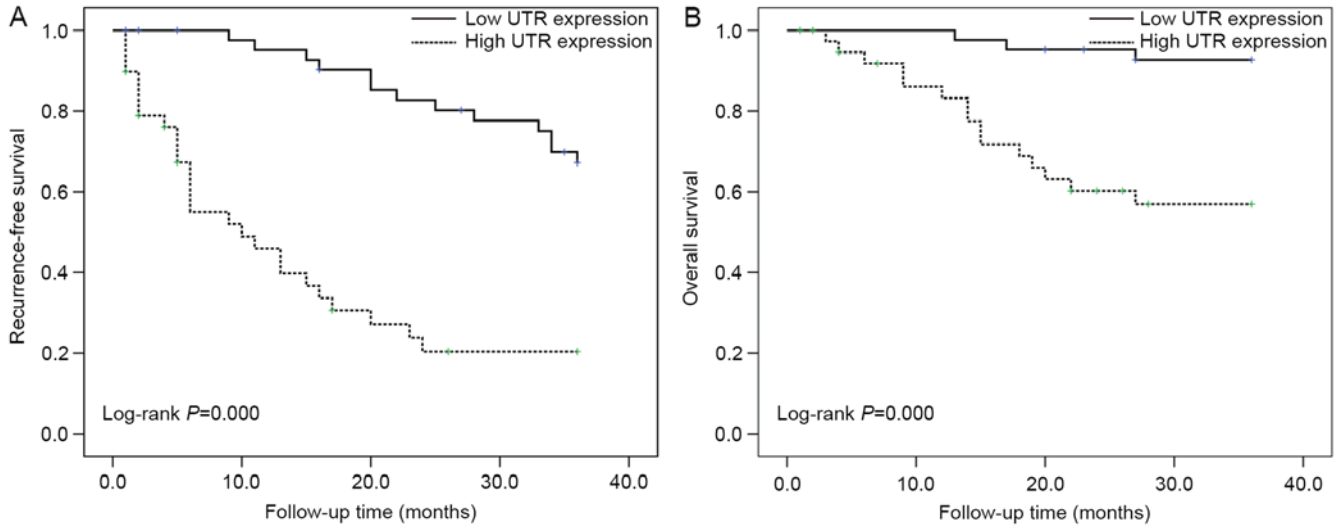

Figure 2. The relationship between UTR expression and survival. Kaplan-Meier curve for (A) recurrence-free survival and (B) overall survival for the low and high expression of UTR patients with hepatocellular carcinoma. UTR, urotensin II receptor.

group (N=39). The clinicopathological characteristics between the two subgroups were shown in Table I. Those with high expression levels of UTR had higher stage of HCC progression, such as higher TNM stage and BCLC stage (P=0.000, P=0.000), more multiple tumor, bigger tumor size, poorer histologic grade, high tumor recurrence and mortality (P=0.004, 0.026, 0.021, 0.000 and 0.000, respectively). There were no correlation between UTR expressions and sex, age, chronic liver disease, tumor location, serum AFP, and Child-Pugh class (P>0.05).

RFS and OS. The relationship between RFS/OS and UTR expressions were summarized in Table II. Three parameters including tumor number (P=0.009), TNM stage (P=0.000), and high expression of UTR (P=0.000) were significantly related to RFS. The TNM stage, Child-pugh class and UTR high expressions (P=0.002, P=0.013 and P=0.001) were also significantly related to OS. It found that UTR high expressions were an independent prognostic factor for RFS and OS (P=0.004 and 0.038, respectively). The Kaplan-Meier curve and log-rank test also indicated that UTR expressions was associated with RFS and OS in HCC patients (P=0.000 and P=0.000; Fig. 2A and B).

siRNA-mediated UTR gene silencing. First, we determined the expression of UTR in two HCC cell lines and one normal cell line using western blot analysis. We found higher UTR expressions in the HCC cell lines (BEL-7402 and MHCC-97H) than in normal cell lines (QSG-7701) (Fig. 3A). Then, we knocked down UTR gene using two defined siRNAs (methods above-mentioned) in BEL-7402 and MHCC-97H cells, respectively. The level of UTR protein, normalized by GAPDH was obviously reduced compared with that in the negative control cells (Fig. 3B). Furthermore, Transwell invasion assays revealed that silencing UTR expression decreased the invasion of the BEL-7402 and MHCC-97H cells compared to the control cells (P<0.05; Fig. 3C). Silencing UTR expression was also decreased the migration of HCC cells (P<0.05; Fig. 3D). These results indicated the positive role of UTR in migration and invasion of human HCC cell lines *in vitro*.

Discussion

Our study shows that UTR is the potential biomarker to predict the prognosis in HCC patients after radical liver resection, and patient with a higher expression of UTR always has a worse prognosis than that with a lower expression of UTR. The evidence are as follows: Firstly, we demonstrate that UTR expression was associated with HCC malignant features, such as HCC stage, tumor number and tumor size; secondly, patients with a higher UTR expression level tend towards a high recurrence and mortality rate after resection, and survival curves (RFS and OS) showed significant difference between the two subgroups; finally, univariate and multivariate analysis found that UTR was an independent risk factor for predicting RFS and OS. In the light of these results, we suggest those patients with high UTR expression should be closely monitored or taken prophylactic treatments, such as molecular targeted drug therapy.

In several other malignancies, upregulation of UTR has been observed to have a relationship with poor prognosis (6). Federico *et al* (10) reported that UTR may play a role in colon carcinogenesis, when they found that UTR is expressed at a higher positive rate in colon adenocarcinomas than in adenomatous polyps and normal epithelial cells (65-90, 30-48 and 5-30%, respectively). De Cobelli *et al* (14) and Grieco *et al* (15) suggested that UTR could be considered as prognostic marker in human prostate carcinoma patients. Franco *et al* (16) reported that UTR expression determines prognosis of bladder cancer, through discriminating non-muscle-invasive bladder transitional cancer (NMIBC) from muscle-invasive bladder transitional cancer and predicting the risk of relapses in NMIBCs. Consistent with these findings, we found that high UTR expression level was associated with poor prognosis in HCC patients after radical liver resection.

Migration and invasion are the embodiment of tumor metastasis ability. Evidence shows that tumor metastasis occurs at an early stage (17-19), and suggests that early metastasis is one of the major causes of recurrence and poor prognosis after

Table II. Univariate and multivariate Cox proportional hazards analyses of recurrence-free survival and overall survival.

		Recurrence-free survival						Overall survival					
		Univariate analysis			Multivariate analysis			Univariate analysis			Multivariate analysis		
Variables	Categories	Risk ratio	95% CI	P-value	Risk ratio	95% CI	P-value	Risk ratio	95% Cl	P-value	Risk ratio	95% CI	P-value
Sex	Male	Reference group						Reference group					
	Female	0.653	0.256-1.668	0.373				0.574	0.132-2.496	0.459			
Age (years)	≤50	Reference group						Reference group					
	>50	1.030	0.554-1.917	0.925				0.711	0.276-1.836	0.481			
Liver disease	HBV	Reference group						Reference group					
	HCV/others	0.611	0.147-2.534	0.497				1.319	0.303-5.737	0.712			
Tumor location	Right lobe	Reference group						Reference group					
	Other lobes	0.865	0.423-1.770	0.691				0.514	0.149-1.778	0.293			
Tumor nubmer	Single	Reference group						Reference group					
	multiple	2.356	1.235-4.495	0.009[a]	1.403	0.706-2.787	0.333	1.860	0.720-4.802	0.200			
Tumor size	<3	Reference group						Reference group					
	3-5	1.409	0.621-3.196	0.412				4.135	0.859-19.913	0.077			
	>5	4.128	1.911-8.918	0.000[a]				8.025	1.731-37.216	0.008[a]			
AFP	<20	Reference group						Reference group					
	20-400	1.367	0.612-3.051	0.446				0.781	0.195-3.123	0.727			
	>400	2.419	1.158-5.055	0.019[a]				2.617	0.930-7.360	0.068			
TNM stage	I-II	Reference group						Reference group					
	III	5.604	2.870-10.942	0.000[a]	2.756	1.275-5.957	0.010[a]	4.663	1.802-12.066	0.002[a]	1.912	0.647-5.650	0.241
BCLC HCC stage	A	Reference group						Reference group					
	B	1.785	0.857-3.719	0.122				1.181	0.282-4.943	0.820			
	C	3.312	1.518-7.226	0.003[a]				6.360	2.163-18.701	0.001[a]			
Child-Pugh class	A	Reference group						Reference group					
	B	0.930	0.286-3.025	0.904				3.705	1.316-10.432	0.013[a]	1.822	0.627-5.293	0.270
Histologic grade	Poorly	Reference group						Reference group					
	Moderate/well	0.841	0.386-1.832	0.663				0.518	0.194-1.382	0.189			
UTR expression	Low	Reference group						Reference group					
	High	5.480	2.771-10.839	0.000[a]	3.275	1.454-7.376	0.004[a]	7.603	2.195-26.334	0.001[a]	4.578	1.087-19.277	0.038[a]

[a]P<0.05. Tumor number, TNM stage and UTR were the adjustment factors for multivariate Cox proportional hazards analyses of recurrence-free survival. TNM stage, Child-Pugh class and UTR were the adjustment factors for multivariate Cox proportional hazards analyses of overall survival. CI, confidence intervals; AFP, α-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; HCC, hepatocellular carcinoma; UTR, urotensin II receptor; HBV, hepatitis B; HCV, hepatitis C.

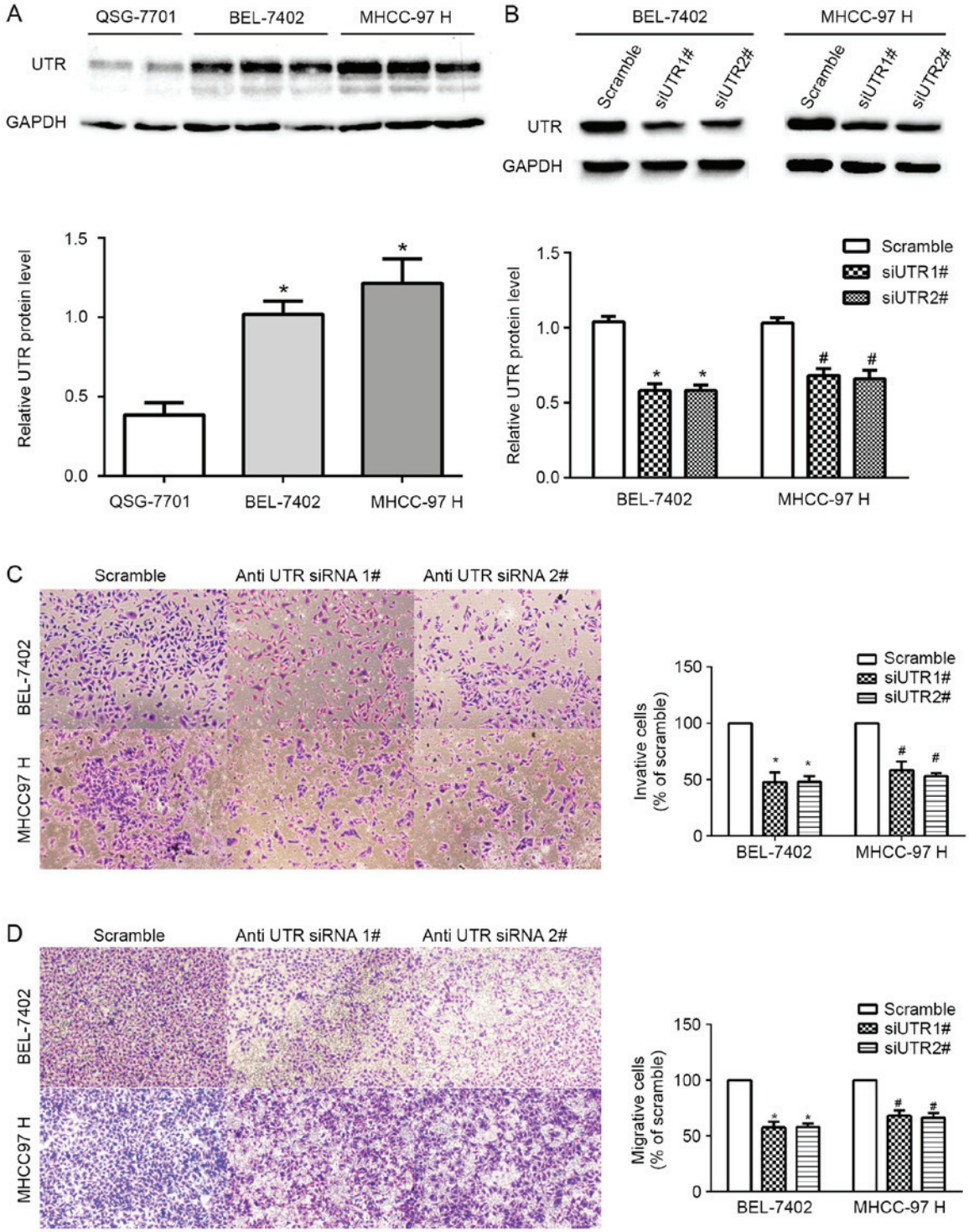

Figure 3. UTR-gene-silencing inhibits HCC cell migration and invasion. (A) UTR high expressions in BEL-7402, MHCC-97H compared with QSG-7701. (B) UTR expression decreased in BEL-7402, MHCC-97H transfected with siRNA compared with control vector. UTR protein was measured using western blot analysis. The data was expressed as mean ± SD. *P<0.05; #P<0.05 vs. QSG-7701 (in A) and scramble (in B-D). (C) Invasion and (D) migration measured by Transwell assays was weakened in both HCC cell lines treated by siRNA. UTR, urotensin II receptor; HCC, hepatocellular carcinoma.

surgical resection (20). Moreover, several studies have reported that UTR can stimulate the migration and invasion of many malignant cell lines. For example, it is reported that UTR is involved in the regulation of motility and invasion of colon cancer (10), bladder cancer cells (16) and prostate adenocarcinoma cells (15). Furthermore, we found a positive correlation between UTR expression and HCC cells metastatic potential (UTR levels, MHCC-97H > BEL-7402 >> QSG-7701) *in vitro* study. Therefore, we speculate UTR may mediate cell invasion and migration in HCC cells. In order to investigate our speculation, we used two siRNAs to downregulate UTR level and then monitored cell motility behavior *in vitro*. Consistent with our speculation, the results showed that UTR knockdown in HCC cells reduced migration and invasion. Our results are complementary to our previous studies, in which UII/UTR system is found expressing differences between tumor and peri-tumor,

and promoting tumorigenesis in hepatic progenitor cell (9). These studies together suggest UTR as a target for future HCC therapies because it plays an important role in tumorigenesis and tumor progression.

In our study, information of the caval/portal thrombosis during the follow-up were not included. It would be better to correlate them to UTR expression at the moment of the HCC recurrence. In clinical practice, it is difficult to evaluate wheather or not caval/portal thrombosis exist, especially in patients followed-up by telephone, without obtaining all-around medical information. And in future study, it is possible to carry out the correlation between UTR and caval/portal thrombosis in selected patients with good compliance.

The drawbacks of this study cannot be ignored. Due to small sample size and retrospective study, a more comprehensive analysis was not done for the risk factors determining poor OS and RFS, such as platelet count, microscopic vascular invasion, anatomic resection, grade of inflammation and antiviral therapy.

In conclusion, our novelty findings indicate that UTR may be regarded as a novel biomarker to predict outcomes after radical liver resection. It is also suggested that UTR as a potential therapeutic target inhibited invasion and metastasis of HCC.

Poor prognosis of hepatocellular carcinoma (HCC) patients is closely related to high recurrence, invasion and metastasis after radical treatment. In this study, we reported some novelty findings that urotensin II receptor (UTR) overexpression was associated with tumor number and size, histology, TNM/BCLC HCC stage, recurrence and mortality, and also correlated with recurrence-free survival and overall survival in HCC patients underwent curative liver resection. Furthermore, siRNA-mediated silencing UTR expression inhibited HCC cell motility and Invasion. Our novelty findings indicate that UTR may be regarded as a novel biomarker to predict outcomes after radical liver resection and as a potential therapeutic target for HCC.

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