

Original Article

High expression of long non-coding RNA GHET1 is associated with unfavorable prognosis in hepatocellular carcinoma

Jun Li, Jian Xie, Zuofu Liao, Yan Yu, Zhonghao Chen

Department of Surgery, Tongren Hospital, Shanghai Jiao Tong University School of Medicine, 1111 Xianxia Road, Shanghai 200336, China

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Abstract: Background: Recently, emerging evidence has suggested the role of long non-coding RNAs (lncRNAs) in human carcinogenesis. Herein, we aimed to detect the expression profiles and functional implications of lncRNA GHET1 in human HCC. Methods: GHET1 levels in a cohort of 179 pairs of HCC tissues and pair-matched noncancerous hepatic tissues were tested using quantitative real-time PCR (qRT-PCR). The correlations of GHET1 expression with clinicopathologic features and prognostic outcomes were also analyzed in these HCC patients. The effects of GHET1 on HCC cell proliferation, cell-cycle profiles, Cyclin D1 expression and cell migration/invasion *in vitro* were investigated through CCK-8, flow cytometer, Western blot and transwell assays, respectively. Results: We verified that GHET1 was significantly up-regulated in HCC compared with corresponding non-tumor tissues, and a high level of GHET1 was correlated with larger tumor size, advanced TNM stage, and positive vascular invasion. HCC patients with high GHET1 expression level had shorter OS and DFS than the low GHET1 expression group, and multivariate logistic regression analysis considered high GHET1 expression as an independent factor of unsatisfactory prognosis. Functionally, knock-down of GHET1 attenuated HCC cell proliferation *in vitro* through inducing G0/G1 arrest and inhibiting Cyclin D1 expression. In addition, knock-down of GHET1 also inhibited migration and invasion ability of HCC cells *in vitro*. Conclusions: Collectively, these data demonstrated that GHET1 is obviously increased in HCC tissues, and uncontrolled GHET1 overexpression might drive HCC progression. Therefore, GHET1 might serve as a novel indicator of unsatisfactory prognosis and a promising therapeutic target for HCC patients.

Keywords: Hepatocellular carcinoma, long non-coding RNAs, GHET1, cell growth, prognosis

Introduction

As one of the most prevailing malignant diseases, hepatocellular carcinoma (HCC) is the third frequent cause of cancer-associated deaths in the world [1, 2]. In recent years, liver resection, liver transplantation, chemotherapy and radiotherapy are common therapeutic approaches for HCC. However, because of the post-treatment recurrence and metastasis, the prognostic outcomes of HCC patients remain dismal, with 5-year cumulative tumor recurrence rate being 77-100% [3, 4]. Accordingly, it is urgent for us to elucidate the molecular mechanisms that involved in the proliferation, invasion and metastasis of HCC, and further improve the diagnostic and therapeutic approaches for HCC patients.

Over the past decade, high-throughput sequencing and bioinformatics have shown that in the human genome, more than 90% of transcripts can be transcribed into non-coding RNAs (ncRNAs) [5]. Long non-coding RNAs (lncRNAs), a cluster of evolutionarily conserved ncRNAs that are longer than 200 nucleotides in length with limited or no protein-coding capacity, were featured as exerting functions in multiple cellular processes, such as cell growth, proliferation and differentiation [6]. Importantly, dysregulated expression of lncRNAs is frequently implicated in multiple human diseases, including cancer [7], neurodegenerative diseases [8], and cardiovascular diseases [9]. Numerous lncRNAs have been found to be involved in the pathogenesis of HCC as oncogenes or onco-suppressive genes, such as

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Table 1. The sequences of primers

Gene name	Primer sequences
GHET1	
Forward	5'-CCCCACAAATGAAGACACT-3'
Reverse	5'-TTCCCAACACCCTATAAGAT-3'
GAPDH	
Forward	5'-GTCAACGGATTTGGTCTGTATT-3'
Reverse	5'-AGTCTTCTGGGTGGCAGTGAT-3'

AOC4P [10], TUSC7 [11], FTX [12], HULC [13] and lincRNA-p21 [14]. Therefore, identification of lncRNAs involved in HCC progression might help yield novel biomarkers for early detection and treatment in the future.

A currently identified lncRNA, gastric carcinoma high expressed transcript 1 (GHET1), a 1913-bp lncRNA that maps to chromosome 7q36.1, was found to be highly expressed in some types of human cancers, including gastric cancer [15], bladder cancer [16], and colorectal cancer [17]. However, the role of GHET1 in HCC has not been fully understood. In this article, for the first time we examined the expression level of GHET1 in HCC tissues and adjacent non-tumor tissues. The relationships between its expression and clinicopathological features were then analyzed in order to evaluate whether GHET1 expression could be a useful biomarker for HCC patients' prognosis. Then we performed loss-of-function studies to determine the effect of GHET1 on tumor growth, cell proliferation, and migration, and revealed that GHET1 promotes tumor growth, cell proliferation and migration in HCC.

Materials and methods

Human tissue specimens

179 sets of HCC tissues and adjacent normal hepatic tissues (≥ 3 cm away from tumor margin) were obtained from patients with HCC who underwent HCC resection at the Department of General Surgery of Shanghai Tongren Hospital (Shanghai, China) between March 2009 and November 2010. All candidates recruited to this trial did not undergo any pre-operative local or systemic treatment before the surgical excision. The preoperative clinical diagnosis of HCC met the diagnostic criteria of the American Association for the Study

of Liver Diseases. Histopathology was assessed independently by two trained clinical specialist. The relevant clinical variables of these patients, including age, gender, smoking status, drinking status, HBsAg status, AFP, ALT, liver cirrhosis, tumor size, tumor number, TNM stage, tumor differentiation and vascular invasion were documented in **Table 2**. The HCC and non-neoplastic tissue samples were obtained immediately and were snap-frozen in liquid nitrogen and stored at -80°C . Data on clinical follow-up were collected. All patients or their relatives gave their written consent, and the trial was approved by the Institutional Research Ethics Committee of Shanghai Tongren Hospital.

Cell culture

Herein, one normal liver cell line (L02), and four human HCC cell lines (HCCLM3, Huh7, SK-Hep1, and HepG2) were used. All the cell lines were purchased from the American Type Culture Collection (Manassas, VA). All cell lines were cultured in Dulbecco's modified Eagle's medium (Gibco, Grand Island, NY) containing heat-inactivated 10% fetal bovine serum (FBS; Hyclone, Thermo Fisher Scientific, Waltham, MA), 100 U/ml penicillin, and 100 $\mu\text{g}/\text{ml}$ streptomycin (Invitrogen, Carlsbad, CA), and maintained in a humidified chamber with 5% CO_2 at 37°C . These cells were free of mycoplasma contamination. Cells were used when they were in the logarithmic growth phase.

Quantitative real-time PCR (qRT-PCR)

Total RNA from cell lines or tissues was isolated using Trizol reagent (Invitrogen). The integrity of isolated RNA was determined by electrophoresis, and the amount of RNA was quantitatively determined using a 2000c Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). RNA was reverse transcribed to complementary DNA (cDNA) using the PrimeScript RT reagent Kit (TaKaRa, Dalian, China). qRT-PCR analyses were conducted using SYBR Green Realtime PCR Master Mix (Takara) with specific set of primers on Applied Biosystems 7300 Real Time PCR system (Applied Biosystems, Foster City, CA). Used oligonucleotide primer sequences were listed in **Table 1**. As a stable endogenous control, glyc-

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Table 2. Association of GHET1 expression with clinico-pathological features of HCC patients

Characteristics	Total number (n=179)	GHET1 expression		P value
		Low (n=101)	High (n=78)	
Age (years)				0.554
≤55	94	55	39	
>55	85	46	39	
Gender				0.435
Male	123	67	56	
Female	56	34	22	
Smoking status				0.684
Yes	41	22	19	
No	138	79	59	
Drinking status				0.292
Yes	58	36	22	
No	121	65	56	
HBsAg status				0.595
Positive	141	81	60	
Negative	38	20	18	
AFP (ng/ml)				0.512
≥20	83	49	34	
<20	96	52	44	
ALT (U/ml)				0.413
≤75	98	58	40	
>75	81	43	38	
Liver cirrhosis				0.297
Yes	114	61	53	
No	65	40	25	
Tumor size (cm)				0.010
<5	93	61	32	
≥5	86	40	46	
Tumor number				0.582
Single	127	70	57	
Multiple	52	31	21	
TNM stage				<0.001
I-II	109	81	28	
III-IV	70	20	50	
Tumor differentiation				0.248
Well	82	50	32	
Moderate	65	37	28	
Poor	32	14	18	
Vascular invasion				0.003
Yes	72	31	41	
No	107	70	37	

eraldehyde-3-phosphate dehydrogenase (GAPDH) was used for template normalization. The 2^{-ΔΔCt} method was employed to investigate the relative expression levels of genes [18].

Small interfering RNA (siRNA)

Knock-down of GHET1 was performed by transfecting cells with siRNA specifically targeting GHET1 (GenePharma, Shanghai, China). The GHET1 specific siRNA sequence was as follows: GHET1 siRNA (si-GHET1): CGGCAGGCATTAGAG-ATGAACAGCA. si-GHET1 and scrambled negative control siRNA (si-NC) were purchased from Invitrogen. Cells were grown to 70% confluency on six-well plates and transfected using the Lipofectamine 2000 transfection reagent (Invitrogen). After being transfected for 48 hours, cells were harvested for further analyses.

Cell proliferation assay

The *in vitro* cell proliferation of HCC cells was measured using the Cell counting kit 8 (CCK8; Beyotime Biotechnology, Haimen, China) assay. In brief, 48 hours after transfection, a total of 2×10³ cells were seeded in a 96-well plate and cultured at 37°C. At indicated time points, 10 μl CCK-8 (5 mg/ml) was added into the culture medium in each well. After 1 hour incubation at 37°C, the absorbance of each well was detected at 450 nm using a microplate reader (Bio-Tek Company, Winooski, VT).

Cell cycle analysis

After being transfected for 48 hours, cells were treated with propidium iodide (PI) staining using the CycleTEST PLUS DNA Reagent Kit (BD Biosciences). The cell-cycle profiles were measured at 488 nm on an EPICS 752 flow cytometer (Coulter, Hialeah, FL) equipped with CellQuest software (Becton Dickinson, San Jose, CA, USA). Data were expressed as percentage distribution of cells in G0/G1, S and G2/M phases of the cell cycle.

Western blot assay

Cells were lysed using RIPA buffer (Thermo scientific, Rockford, IL) containing a protease inhibitor cocktail (Roche, Shanghai, China). Protein concentration was measured by the Bradford method using Bradford reagent (Bio-Rad, Hercules, CA).

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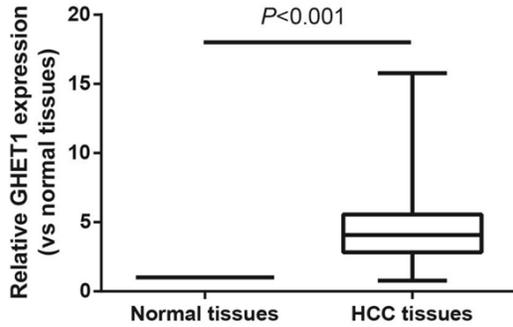


Figure 1. qRT-PCR analysis of GHET1 expression in HCC. GHET1 was significantly up-regulated in HCC tissues (n=179) compared to the adjacent non-tumor tissues (n=179). The $2^{-\Delta\Delta Ct}$ method was used to measure the relative GHET1 expression, which was normalized by the GAPDH expression level.

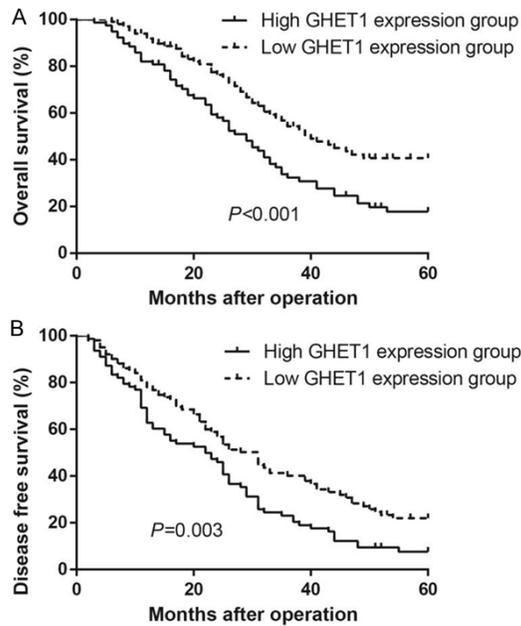


Figure 2. GHET1 expression affected overall survival and disease-free survival in HCC patients. All patients were divided into two groups according to the expression level of GHET1, then the prognostic significance of GHET1 was evaluated by Kaplan-Meier survival analysis and log-rank test. A: OS of patients with high expression of GHET1 was shorter than that with low expression of GHET1. B: DFS of patients with high expression of GHET1 was shorter than that with low expression of GHET1.

Lysates with equal protein concentrations were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred onto nitrocellulose membrane (Millipore, Billerica, MA), and then incubated with the spe-

cific primary antibody. After the blots were incubated with HRP-labeled second antibody (Jackson ImmunoResearch Laboratories, West Grove, PA), the signals were visualized by the enhanced chemiluminescence reagents (GE Healthcare Bio-Sciences Corp., Piscataway, NJ). ImageQuant 5.2 software (GE Healthcare, Piscataway, NJ) were used to quantify band intensities. Antibody against β -actin was from Sigma-Aldrich (Sigma Co., St. Louis, MO). Antibody against Cyclin D1 was from Cell Signaling Technology, Inc. (Danvers, MA).

Cell migration and invasion assay

1×10^5 cells in 100 μ l serum-free DMEM were placed on the non-coated membrane in the upper chamber (24-well insert; 8-mm pore size, Corning Costar Corp) with (for invasion assay) or without (for migration assay) Matrigel (BD Biosciences). 600 μ l DMEM containing 10% FBS was used as chemo-attractant in the bottom chamber. After incubation for 24 h, cells that did not migrate or invade through the pores were scraped out using cotton swabs, whereas the cells on the lower membrane surface were fixed with 95% ethanol and stained with 0.1% crystal violet. Finally, cells were counted from five random fields under a microscope.

Statistical analysis

All statistical analyses were conducted using SPSS version 16.0 software (SPSS Inc., Chicago, IL) and GraphPad Prism version 5 software (GraphPad Software, Inc., La Jolla, CA). The statistical significance between groups was determined using a two-tailed Student's *t* test. Associations between GHET1 levels and clinicopathologic characteristics were evaluated using Chi-square test. Survival curves for the HCC patients were assessed using the Kaplan-Meier method and the differences between groups were estimated using the log-rank test. Overall survival (OS) was calculated from the date of surgery to the date of death or last follow-up. Disease-free survival (DFS) was calculated from the date of surgery to the date of recurrence, metastasis, death or last follow-up. Univariate and multivariate logistic regression analyses were performed to analyze the independent prognostic indicators for DFS and OS. The data are expressed as the mean \pm

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Table 3. Univariate and multivariate logistic regression analysis of factors associated with overall survival of HCC patients

Characteristics	Univariate analysis			Multivariate analysis		
	Risk ratio	95% CI	P value	Risk ratio	95% CI	P value
Age (years)						
≤55 vs. >55	1.494	0.815-2.738	0.194			
Gender						
Male vs. Female	1.233	0.641-2.372	0.531			
Smoking status						
Yes vs. No	0.871	0.423-1.791	0.707			
Drinking status						
Yes vs. No	1.279	0.677-2.420	0.448			
HBsAg status						
Positive vs. Negative	1.305	0.617-2.762	0.487			
AFP (ng/ml)						
≥20 vs. <20	0.442	0.238-0.823	0.010	0.467	0.235-0.926	0.029
ALT (U/ml)						
≤75 vs. >75	1.420	0.773-2.608	0.259			
Liver cirrhosis						
Yes vs. No	0.771	0.414-1.435	0.411			
Tumor size (cm)						
<5 vs. ≥5	1.879	1.020-3.463	0.043	1.258	0.618-2.562	0.527
Tumor number						
Single vs. Multiple	1.475	0.748-2.906	0.262			
TNM stage						
I-II vs. III-IV	4.806	2.363-9.776	<0.001	2.185	0.899-5.315	0.085
Tumor differentiation						
Well vs. Moderate/Poor	1.931	1.051-3.548	0.034	1.427	0.712-2.858	0.316
Vascular invasion						
Yes vs. No	0.353	0.183-0.678	0.002	0.580	0.270-1.245	0.162
GHET1 expression						
High vs. Low	0.316	0.165-0.603	<0.001	0.457	0.215-0.970	0.042

standard deviation (SD) from at least three independent validations. All tests were two-sided, and a $P < 0.05$ was regarded statistically significant.

Results

Up-regulated expression of GHET1 in human HCC tissues

GHET1 has been found up-regulated in multiple human cancers. In this article, we firstly investigated GHET1 expression levels in 179 pairs of resected HCC samples and adjacent normal hepatic tissues as control using quantitative reverse transcription-PCR (qRT-PCR), and normalized to GAPDH expression. As dem-

onstrated in **Figure 1**, the relative levels of GHET1 in HCC specimens was remarkably higher than that in adjacent noncancerous hepatic tissues ($P < 0.001$). These findings revealed that GHET1 was strongly overexpressed in HCC tissues, and up-regulation of GHET1 may contribute to HCC tumorigenesis.

To explore the correlation of GHET1 expression with clinicopathological variables, the expression levels of GHET1 in HCC tissues were categorized into two groups, including the high expression group and the low expression group in accordance to the mean value. Clinicopathologic factors were analyzed in the high and low GHET1 expression groups, as recorded in **Table 2**. Tumor size ($P = 0.01$), TNM stage

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Table 4. Univariate and multivariate logistic regression analysis of factors associated with disease-free survival of HCC patients.

Characteristics	Univariate analysis			Multivariate analysis		
	Risk ratio	95% CI	P value	Risk ratio	95% CI	P value
Age (years)						
≤55 vs. >55	0.625	0.301-1.297	0.207			
Gender						
Male vs. Female	1.097	0.498-2.413	0.819			
Smoking status						
Yes vs. No	0.911	0.380-2.184	0.835			
Drinking status						
Yes vs. No	1.356	0.638-2.880	0.429			
HBsAg status						
Positive vs. Negative	1.197	0.480-2.985	0.700			
AFP (ng/ml)						
≥20 vs. <20	0.350	0.158-0.777	0.010	0.344	0.147-0.805	0.014
ALT (U/ml)						
≤75 vs. >75	1.969	0.918-4.223	0.082			
Liver cirrhosis						
Yes vs. No	0.601	0.288-1.252	0.174			
Tumor size (cm)						
<5 vs. ≥5	2.646	1.215-5.761	0.014	2.078	0.892-4.843	0.090
Tumor number						
Single vs. Multiple	1.988	0.812-4.868	0.133			
TNM stage						
I-II vs. III-IV	5.403	1.990-14.666	0.001	2.326	0.707-7.649	0.165
Tumor differentiation						
Well vs. Moderate/Poor	1.518	0.734-3.139	0.260			
Vascular invasion						
Yes vs. No	0.403	0.177-0.916	0.030	0.722	0.279-1.867	0.501
GHET1 expression						
High vs. Low	0.284	0.121-0.663	0.004	0.405	0.156-1.049	0.063

($P < 0.001$) and vascular invasion ($P = 0.003$) were significantly correlated with the GHET1 expression. However, we did not observe any remarkable correlation between GHET1 levels and other clinicopathological parameters, including age, gender, smoking status, drinking status, HBsAg status, AFP, ALT, liver cirrhosis, tumor number and tumor differentiation (all $P > 0.05$).

Correlations between GHET1 expression and prognosis of HCC patients

We further assessed whether GHET1 expression level associated with prognostic outcomes of HCC patients after HCC resection. OS and DFS curves were depicted in accordance to

GHET1 levels by the Kaplan-Meier analysis and log-rank test, the results were shown in **Figure 2A** and **2B**. HCC patients with high intratumoral GHET1 expression level had shorter OS and DFS than the low GHET1 expression group. Our data demonstrated that up-regulated expression of GHET1 in HCC tissues was closely correlated with patients' prognostic outcomes.

To further understand the clinical significance of multiple prognostic factors that might affect survival in HCC patients, univariate logistic regression analysis was conducted for OS and DFS. As demonstrated in **Tables 3** and **4**, AFP, tumor size, TNM stage, tumor differentiation, vascular invasion and GHET1 expression were

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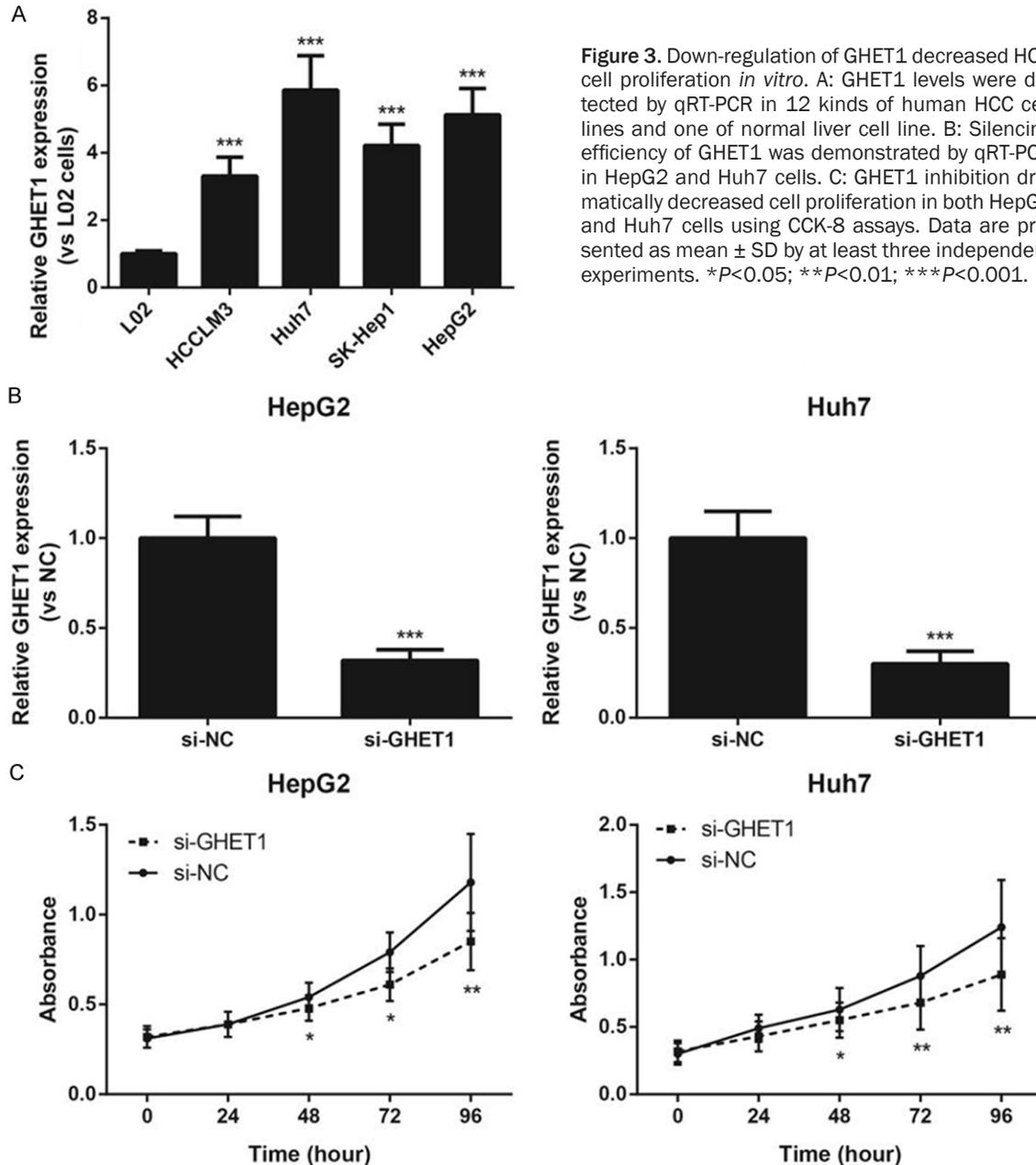


Figure 3. Down-regulation of GHET1 decreased HCC cell proliferation *in vitro*. **A:** GHET1 levels were detected by qRT-PCR in 12 kinds of human HCC cell lines and one of normal liver cell line. **B:** Silencing efficiency of GHET1 was demonstrated by qRT-PCR in HepG2 and Huh7 cells. **C:** GHET1 inhibition dramatically decreased cell proliferation in both HepG2 and Huh7 cells using CCK-8 assays. Data are presented as mean \pm SD by at least three independent experiments. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

statistically significant risk factors affecting OS and DFS of HCC patients. The other clinicopathological features, including age, gender, liver cirrhosis and tumor number were not statistically significant prognosis factors. To assess the robustness of the prognostic value of GHET1 expression, parameters with a value of $P < 0.05$ were chosen for multivariate logistic regression analysis. The results of multivariate logistic regression analysis showed that GHET1 expression and AFP were independent poor prognostic factors for HCC. Collectively, these

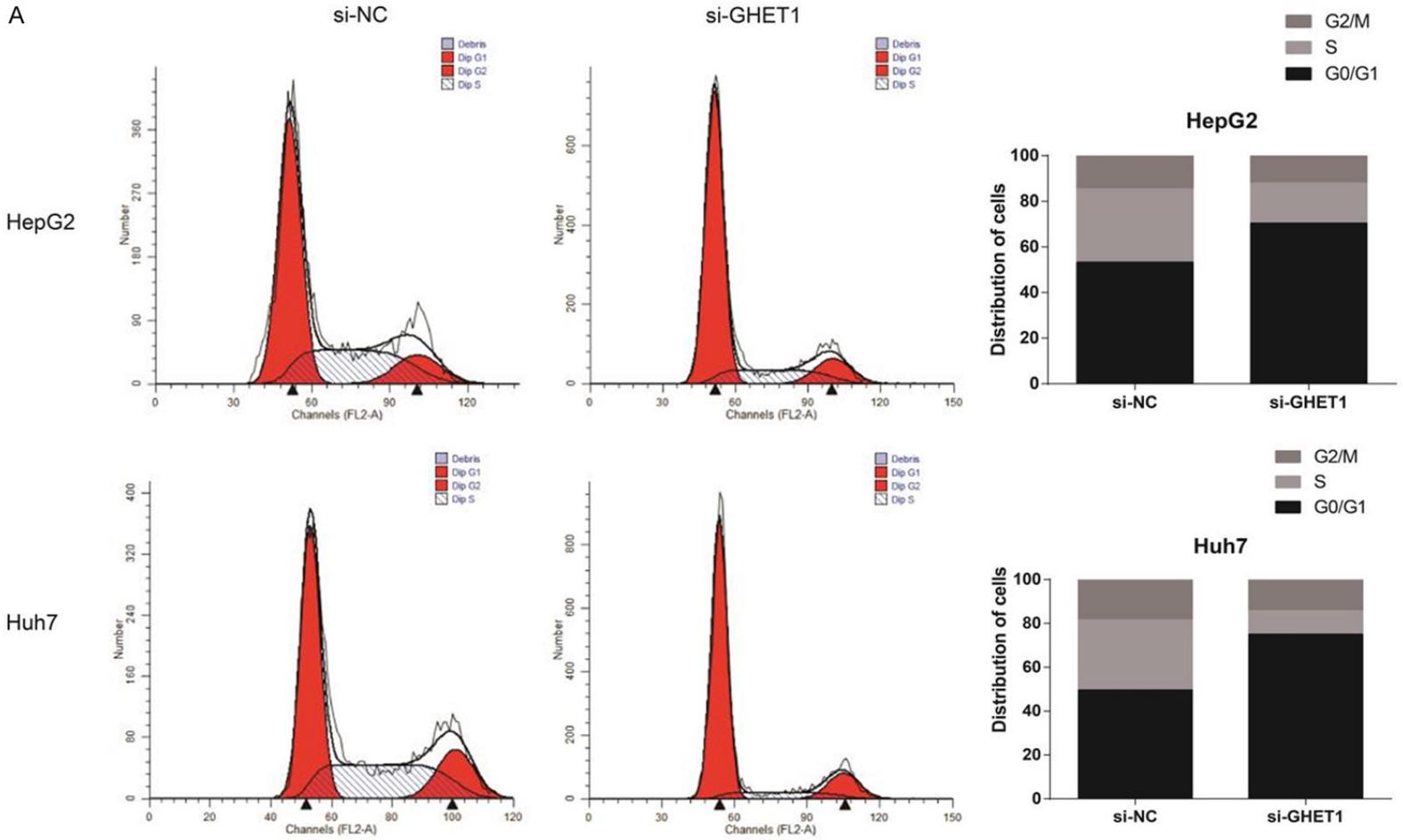
data revealed that high GHET1 level was an independent risk factor for HCC patients.

Silence of GHET1 by siRNA repressed HCC cell proliferation in vitro

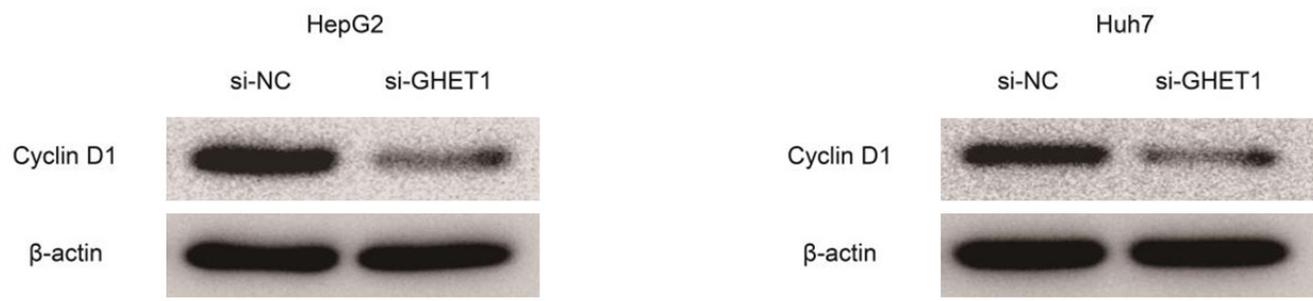
As above data demonstrated that GHET1 expression was markedly up-regulated in HCC tissues, we also detected GHET1 expression in human HCC cells through qRT-PCR. **Figure 3A** shown that the levels of GHET1 were remarkably increased in four kinds of human HCC cell

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A



B



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Figure 4. Down-regulation of GHET1 induced G0/G1 arrest. A: Cycle phase distributions (percentage) of HepG2 and Huh7 cells were determined by FACS analysis and plotted. Significant G1 phase arrest and S phase decrease were observed in both cell lines. B: Western blotting showed that the Cyclin D1 protein level was decreased after the silencing of GHET1 in HepG2 and Huh7 cells. Data were representative of at least three independent experiments.

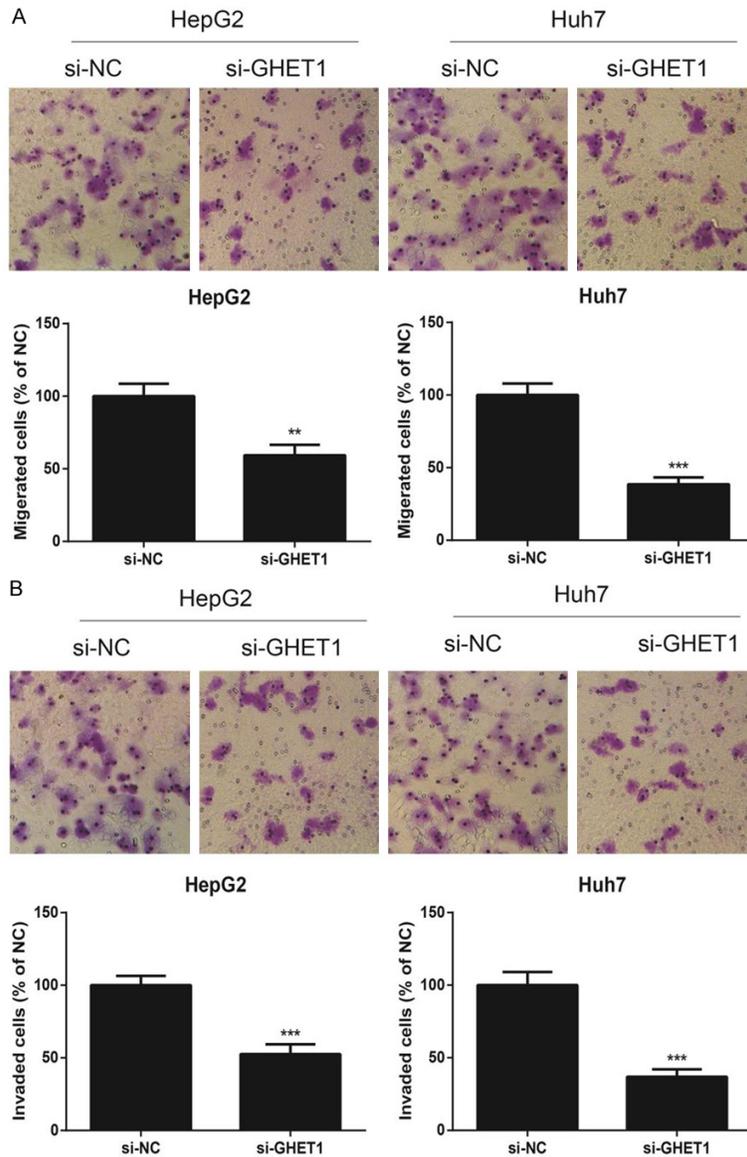


Figure 5. GHET1 inhibition affected HCC cell migration and invasion. A: GHET1 inhibition dramatically reduced cell migration in both HepG2 and Huh7 cells using transwell assays. B: GHET1 inhibition dramatically reduced cell invasion in both HepG2 and Huh7 cells using transwell assays. Data are presented as mean \pm SD by at least three independent experiments. ** $P < 0.01$; *** $P < 0.001$.

lines (HCCLM3, Huh7, SK-Hep1, and HepG2) compared to normal liver cell line (L02).

To investigate the biological functions of GHET1 on HCC cells, si-GHET1 was employed to inhibit

endogenous GHET1 in HepG2 and Huh7 cells. As shown in **Figure 3B**, after transfection 48 h, si-GHET1 knocked down GHET-1 effectively by approximately 70% when compared to the si-NC transfected cells. Next the growth curves determined by CCK-8 assays revealed that HepG2 and Huh7 cell proliferation was remarkably inhibited by si-GHET1 (**Figure 3C**), indicating that GHET-1 down-regulation could significantly suppress HCC cell proliferation *in vitro*.

Down-regulation of GHET1 induced G0/G1 arrest

To figure out the underlying mechanism of proliferation-facilitating function of GHET1 on HCC cells, we tested the influence of GHET1 knock-down on cell cycle. As shown in **Figure 4A**, compared to the control cells, knock-down of GHET1 led to a blockade of HepG2 and Huh7 cells in G0/G1 phase with a remarkable decrease in S phase. To further verify the flow cytometric data, western blotting analysis was performed and shown that Cyclin D1 was also obviously decreased after the silencing of GHET1 (**Figure 4B**). These findings indicated that knock-down of GHET1 might repress the proliferation of HCC cells through inducing G0/G1 arrest.

GHET1 promoted HCC cell migration and invasion in vitro

The observation that down-regulation of GHET1 could noticeably repress cell proliferation pro-

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mpted us to explore HCC cell migration and invasion affected by GHET1. Transwell assays confirmed that the migratory and invasive capacities of HepG2 and Huh7 cells transfected with si-GHET1 was dramatically reduced compared to that of control cells (all $P < 0.01$, **Figure 5**). Collectively, these results suggested that GHET1 was a positive mediator of HCC invasion.

Discussion

In the present research we have established a protocol to identify functional participation of GHET1 in HCC tumorigenesis. Our findings revealed that tissues and CRC cells expressed high level of GHET1 and this up-regulation correlated with HCC cell proliferation and invasion. Previous studies have been suggested that GHET1 serves as an onco-promotor for other types of cancers. This present research was the first to define GHET1 as a tumor promotor in HCC.

Up to now, genome-wide analysis has reported that the human genome contains ~20000 protein-coding genes and >98% of the total genome can be transcribed, generating numerous short or long noncoding RNAs (lncRNAs) with limited or no protein-coding capacities [19]. There are over 3000 human lncRNAs greater than 200 nt in length, but less than 1% of them have been well studied and characterized [20]. Emerging articles indicated that lncRNAs might perform complex and extensive roles in promoting the development and progression of various malignancies [21].

HCC is the leading cause of cancer-related death around the world, thus finding new biomarkers and therapeutic targets may be helpful for improving the clinical strategies and outcomes of this disease [22]. Dysregulated expression of several lncRNAs has been reported in HCC. For example, Yuan *et al.* have found that lncRNA-DANCR significantly elevated stemness features of HCC cells to promote tumorigenesis and intra-/extrahepatic tumor colonization [23]. Also, Li and his colleagues demonstrated that HCC patients with ZEB1-AS1 hypomethylation or with high ZEB1-AS1 expression have unsatisfactory recurrence-free survival [24]. These articles highlighted that lncRNAs

might exert critical functions in HCC initiation and development, and thus have a great potential for clinical application.

GHET1 is an lncRNA originally featured in gastric cancer [25]. Yang *et al.* revealed that GHET1 serves an oncogenic role in gastric cancer through increasing the stability of c-Myc mRNA and expression [15]. However, whether GHET1 exerts its oncogenic functions in human HCC remains to be explored. First we determined the expression profiles of GHET1 in HCC tissues and cell lines. GHET1 was found to be overexpressed in HCC tissues than in adjacent normal tissues. Statistical analyses revealed that high GHET1 expression was more frequently detected in HCC patients with aggressive clinicopathological characteristics, including larger tumor size, advanced TNM stage, and positive vascular invasion. Multivariate analysis using the Cox proportional hazard model identified high GHET1 expression as an independent indicator of unfavorable overall postoperative survival rates.

To further explore the effects of GHET1 expression on malignant phenotypes of HCC cells, we then performed loss-of function validations. Our findings demonstrated that down-regulation of GHET1 might suppress cell proliferation via inducing cell cycle arrest in G0/G1 stage, and inhibit cell migration and invasion abilities in two kinds of HCC cell lines. Accordingly, GHET1 could potentially serve as an oncogenic gene in HCC, consistent with the previous articles in other kinds of human malignancies [16, 17].

We are conscious of several limitations exist in our present research. First, the retrospective nature of this study is itself a limitation. Prospective studies with a larger sample size are needed for further confirmation of the clinical significance of GHET1 in HCC. Second, although we indicated the oncogenic function of GHET1 in HCC, its potential downstream mediators are still largely elusive. Third, the survival data are missing because of the fact that most patients were lost after they were discharged from our hospital. The prognostic value of GHET1 in HCC needs further verification.

In summary, despite of the above limitations, our study is the first to indicate that GHET1 was

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substantially overexpressed in HCC tissues and cell lines, and was correlated with HCC cell proliferation and invasion thereby contributes to HCC tumor growth and metastasis. Our findings provided new insights into the biological roles of lncRNAs in the etiology and pathogenesis of HCC and showed that GHET1 might represent a potential indicator of unsatisfactory prognosis and a novel therapeutic target in HCC.

Disclosure of conflict of interest

None.

Address correspondence to: Zhonghao Chen, Department of Surgery, Tongren Hospital, Shanghai Jiao Tong University School of Medicine, 1111 Xianxia Road, Shanghai 200336, China. E-mail: upinarm1@163.com

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