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Original Article

Increased expression of long non-coding RNA TUG1 associates with poor prognosis of hepatocellular carcinoma

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Abstract: Background: Previous studies found that lncRNA TUG1 expression was up-regulated in hepatocellular carcinoma (HCC) tissues. In the present study, we aimed to investigate the clinical significance and prognostic value of lncRNA TUG1 in HCC. Methods: Matched fresh specimens of HCC and paracarcinomatous liver tissues were obtained from 137 patients who underwent hepatic resection. qRT-PCR assay was performed to determine the expression level of lncRNA TUG1. Kaplan-Meier survival curves and Cox proportional hazards regression analysis were performed using SPSS software. Results: lncRNA TUG1 expression was significantly higher in HCC tissues compared with normal adjacent liver tissues (P<0.001). High lncRNA TUG1 expression level was observed to be closely correlated with liver cirrhosis (P=0.015), Child-Pugh score (P=0.004), tumor size (P=0.016), tumor number (P=0.001), TNM stage (P<0.001), Edmondson-Steiner grade (P<0.001), and vein invasion (P<0.001). A significant relationship was found between lncRNA TUG1 expression and 5-year overall survival (P=0.009, log-rank test). Furthermore, in a multivariate Cox model, we found that lncRNA TUG1 expression was an independent poor prognostic factor for 5-year overall survival in HCC (HR=2.966, CI=1.273-9.783, P=0.022). Conclusion: This study demonstrated that increased lncRNA TUG1 expression levels were correlated with poor prognosis of patients with HCC, indicating that lncRNA TUG1 may serve as a novel prognostic marker for HCC after hepatic resection.

Keywords: Hepatocellular carcinoma, long non-coding RNA, TUG1, prognosis, biomarker

Introduction

Hepatocellular carcinoma (HCC) is the third most common cause of death from cancer worldwide [1]. HCC is a complex malignant tumor, and its development and progression is influenced by various factors. Two important risk factors for developing HCC are cirrhosis and hepatitis B virus infection [2]. Currently, surgery is the most common treatment for HCC. However, the recurrence rate is high and surgery affects long-term recovery and survival in HCC patients after curative resection. For advanced HCC patients, several novel molecular targeted therapies can be applied but with limited therapeutic effects [3-5]. Therefore, studies should focus on biomarker development to distinguish patients with poor prognosis or at high risk of early recurrence and to serve as basis for molecular targeted therapy for HCC.

Long non-coding RNA (lncRNA) is an RNA molecule with a length of 200 bp-100 kbp that lacks protein-coding potential [6]. Recent studies revealed that lncRNAs play a pivotal role in the regulation of gene expression, such as chromatin modification, transcription and post-transcriptional processing [7-9]. Furthermore, more and more evidences revealed the contribution of lncRNAs as having oncogenic and tumor suppressor roles in tumorigenesis [10-13].

Taurine up-regulated gene 1 (TUG1) is a long noncoding RNA greater than 7.1 kb in length. It was originally reported to be upregulated in response to taurine treatment of developing mouse retinal cells [14]. Previous studies have shown that lncRNA TUG1 was aberrantly expressed and involved in carcinogenesis and progression. LncRNA TUG1 was found to be upregulated in urothelial carcinoma of the bladder, osteosarcoma and esophageal squamous

Table 1. Association between lncRNA TUG1 expression and patients' clinicopathologic features

Clinicopathologic variables	N	lncRNA TUG1 level		*P* value
		High (n=67)	Low (n=70)	
Gender				
Male	85	43	42	0.725
Female	52	24	28	
Age (years)				
≤65	71	37	34	0.495
>65	66	30	36	
Hepatitis history				
Yes	89	49	40	0.073
No	48	18	30	
Serum AFP level (ng/ml)				
≤25	36	17	19	0.848
>25	101	50	51	
Liver cirrhosis				
Presence	81	47	34	0.015
Absence	56	20	36	
Child-Pugh score				
A	75	28	47	0.004
B	62	39	23	
Tumor size (cm)				
≤5	76	30	46	0.016
>5	61	37	24	
Tumor number				
Multiple (rcm)	55	37	18	0.001
Solitary	82	30	52	
TNM stage				
I-II	64	14	50	<0.001
III-IV	73	53	20	
Edmondson-Steiner grade				
I-II	63	17	46	<0.001
III-IV	74	50	24	
Vein invasion				
Presence	33	30	3	<0.001
Absence	104	37	67	

cell carcinoma (ESCC) [15-17]. However, other study found that lncRNA TUG1 was downregulated in non-small cell lung cancer (NSCLC) [18]. This finding is probably because lncRNAs exhibit remarkably tissue-specific expression patterns compared with protein-coding genes and indicates that lncRNA TUG1 may have a tissue-specific expression pattern.

Previously, Huang et al found that lncRNA TUG1 expression was up-regulated in HCC tissues. Moreover, silencing of lncRNA TUG1 expression inhibited HCC cell proliferation, colony formation, tumorigenicity and induced apoptosis in HCC cell lines [19]. In the present study, we aimed to investigate the clinical significance and prognostic value of lncRNA TUG1 in HCC.

Materials and methods

Patients and tissue samples

A total of 137 patients with primary HCC who underwent a curative liver resection at the Department of General Surgery, Beijing Ditan Hospital, Capital Medical University, were included in this retrospective study. Tissues used in the study were retrieved from the tissue bank of the Department of Pathology in Beijing Ditan Hospital, Capital Medical University. These patients were diagnosed as HCC between April 2008 and March 2015. None of the patients recruited in this study had chemotherapy or radiotherapy before the surgery. HCC diagnosis was based on World Health Organization (WHO) criteria. Tumor differentiation was defined according to the Edmondson grading system. Tumor staging was determined according to the sixth edition of the tumor-node-metastasis (TNM) classification of the International Union against Cancer. The clinicopathological features of 137 patients are summarized in **Table 1**. The study was approved by the Research Ethics Committee of Beijing Ditan Hospital, Capital Medical University. Informed consent was obtained from all of the patients. All specimens were handled and made anonymous according to the ethical and legal standards. The original data was shown in Supplementary Table.

Evaluation of lncRNA TUG1 expression in HCC samples and normal adjacent liver tissues

Total RNA was extracted from tissues using TRIzol reagent (Invitrogen). For qRT-PCR, RNA was reverse transcribed to cDNA by using a Reverse Transcription Kit (Takara). Real-time

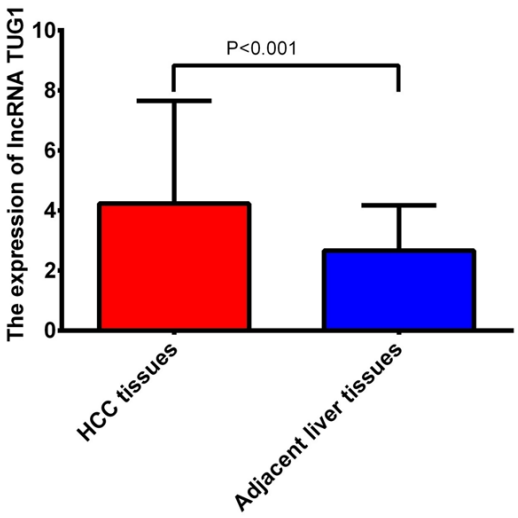

Figure 1. Comparison of LncRNA TUG1 expression level between tumor tissues and matched adjacent normal tissues.

PCR analyses were performed with Power SYBR Green (Takara). The sequences of primers used here were as follows: TUG1-F 5'-TAGCAGTTCCCCAATCCTTG-3', TUG1-R 5'-CACAAATTCCCATCATTCCC-3'; GAPDH-F 5'-GTGTCTGAGCGATGTGGCT-3', GAPDH-R 5'-GGATTTGGTCGTATTGGGC-3'. Data was collected and analyzed by SDS2.3 Software (Applied Biosystems). The expression level of lncRNA TUG1 was internally normalized against that of the GAPDH. The relative quantitative value was expressed by the $2^{-\Delta\Delta Ct}$ method. Each experiment was performed in triplicates and repeated three times.

Statistical analysis

All statistical analyses were performed using the software of SPSS version 18.0 for Windows (SPSS Inc, IL, USA). Student's t test was performed to evaluate the difference in lncRNA TUG1 expression between HCC and adjacent non-cancerous tissues. The relationships between lncRNA TUG1 expression and different clinicopathological characteristics were evaluated using the Chi-square test and Fisher's exact test, as appropriate. Survival analyses were performed using the Kaplan-Meier method, and differences between curves were compared by the log-rank test. Factors shown to be of prognostic significance in univariate models were further evaluated in a multivariate Cox regression model. All *P* values were two-sided, and a *P* value of <0.05 was considered statistically significant.

Results

LncRNA TUG1 expression in clinical HCC samples

We analyzed the expression levels of lncRNA TUG1 in 137 pairs of primary tumor and normal adjacent samples from 137 HCC patients. As revealed by quantitative RT-PCR analysis, lncRNA TUG1 expression was significantly higher in HCC tissues compared with normal adjacent liver tissues (P<0.001, shown in **Figure 1**). The 137 HCC patients were classified into two groups according to the median of lncRNA TUG1 expression level as determined by quantitative RT-PCR. 67 cases were placed in the high expression group and 70 in the low expression group.

Correlation between lncRNA TUG1 expression and clinical characteristics in HCC

The correlations of lncRNA TUG1 expression with clinicopathologic features of patients with HCC were statistically analyzed. As shown in **Table 1**, high lncRNA TUG1 expression level was observed to be closely correlated with liver cirrhosis (P=0.015), Child-Pugh score (P=0.004), tumor size (P=0.016), tumor number (P=0.001), TNM stage (P<0.001), Edmondson-Steiner grade (P<0.001), and vein invasion (P<0.001). However, there were no significant correlations between lncRNA TUG1 expression level and other clinicopathologic factors, including gender (P=0.725), age (P=0.495), hepatitis history (P=0.073), and serum AFP level (P=0.848).

Prognostic values of lncRNA TUG1 expression in HCC

To further investigate the clinical usefulness of lncRNA TUG1 expression in HCC, we compared five-year overall survival according to various clinicopathologic factors including the expression level of lncRNA TUG1. The Kaplan-Meier plot of 5-year overall survival curves stratified by lncRNA TUG1 expression was shown in **Figure 2**. A significant relationship was found between lncRNA TUG1 expression and 5-year overall survival (P=0.009, log-rank test, **Figure 2**). Furthermore, in a multivariate Cox model, including tumor size, tumor stage, tumor grading, presence of cirrhosis, gender, age, and lncRNA TUG1 expression, we found that lncRNA TUG1 expression was an independent poor

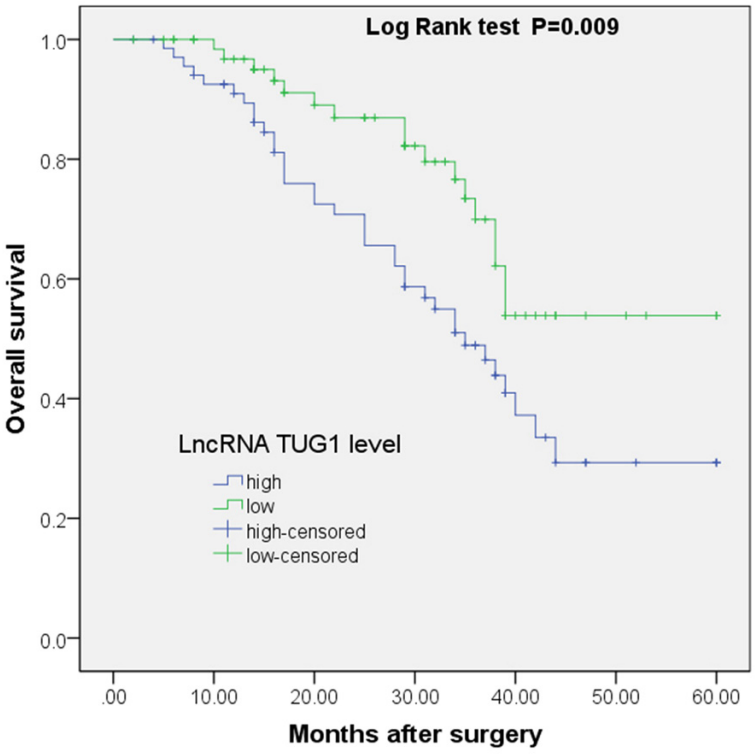

Figure 2. Kaplan-Meier survival curves based on LncRNA TUG1 expression level.

Table 2. Multivariate analyses of parameters associated with overall survival of 137 HCC patients

Variable	Hazard ratio	95% CI	*P*-value
Gender	0.782	0.557-2.839	0.757
Age (years)	1.555	0.829-3.192	0.281
Hepatitis history	2.263	0.872-7.133	0.091
Serum AFP level	2.174	0.699-4.173	0.132
Liver cirrhosis	3.159	0.823-3.222	0.084
Child-Pugh score	2.881	0.465-3.586	0.215
Tumor size (cm)	2.192	0.921-7.112	0.091
Tumor number	2.012	0.892-5.194	0.115
TNM stage	3.183	1.293-10.095	0.011
Edmondson-Steiner grade	4.792	1.723-10.928	0.008
Vein invasion	3.123	2.128-9.067	<0.001
lncRNA TUG1 expression level	2.966	1.273-9.783	0.022

prognostic factor for 5-year overall survival (HR=2.966, CI=1.273-9.783, P=0.022, **Table 2**) in HCC.

Discussion

The dysregulation of lncRNA is common in various carcinomas and plays an important role in cancer progression by altering normal gene expression. It has been noted that alterations in single lncRNA expression correlate highly with the progression and prognosis of human tumors. Thus, identification of lncRNA molecular profiles associated with the prognosis of patients with HCC may not only elucidate the underlying biological mechanisms involved in the development or progression of the disease but also provide the opportunity to identify novel targets for HCC therapy [20-23].

Previous studies have shown that lncRNA TUG1 was aberrantly expressed and involved in carcinogenesis and progression. LncRNA TUG1 was found to be upregulated in urothelial carcinoma of the bladder, osteosarcoma and ESCC [15-17]. However, other study found that lncRNA TUG1 was downregulated in NSCLC [18]. This finding is probably because lncRNAs exhibit remarkably tissue-specific expression patterns compared with protein-coding genes and indicates that lncRNA TUG1 may have a tissue-specific expression pattern. Furthermore, the clinical significance and prognostic value of lncRNA TUG1 have been investigated in several cancers. For example, Zhang et al found that the average level of lncRNA TUG1 in gastric cancer tissues was significantly higher than in corresponding nontumor tissues. The high expression level of lncRNA TUG1 in gastric cancer patients was positively correlated with invasion depth and TNM stage. Moreover, high lncRNA TUG1 expression in gastric cancer tissues was associated with a poor prognosis and could be an independent prognostic indicator. These results suggested that

lncRNA TUG1 may have an important role in gastric cancer progression [24]. Iliev R et al observed significantly increased levels of lncRNA TUG1 in bladder cancer tissue in comparison to adjacent non-tumor bladder tissue (P<0.0001). LncRNA TUG1 levels were significantly increased in metastatic tumors (P= 0.0147) and were associated with shorter overall survival of muscle-invasive bladder cancer (MIBC) patients (P=0.0241), suggesting an oncogenic role of lncRNA TUG1 and its potential usage as biomarker or therapeutic target in MIBC [25]. Ma et al found that lncRNA TUG1 was significantly overexpressed in the osteosarcoma tissues compared with matched adjacent normal tissues (P<0.01) and was closely correlated with tumor size, post-operative chemotherapy, and Enneking surgical stage. Upregulation of lncRNA TUG1 strongly correlated with poor prognosis and was an independent prognostic indicator for overall survival (HR=2.78, 95% CI=1.29-6.00, P=0.009) and progression-free survival (HR=1.81, 95% CI= 1.01-3.54, P=0.037) [26].

The study of TUG1 in HCC is very less. Previously, Huang et al found that lncRNA TUG1 expression was up-regulated in HCC tissues. Moreover, silencing of lncRNA TUG1 expression inhibited HCC cell proliferation, colony formation, tumorigenicity and induced apoptosis in HCC cell lines [19]. However, the clinical significance and prognostic value of lncRNA TUG1 in HCC have not been investigated. In the present study, we found that lncRNA TUG1 expression was significantly higher in HCC tissues compared with normal adjacent liver tissues. Then the correlations of lncRNA TUG1 expression with clinicopathologic features of patients with HCC were statistically analyzed. We found that high lncRNA TUG1 expression level was observed to be closely correlated with liver cirrhosis, Child-Pugh score, tumor size, tumor number, TNM stage, Edmondson-Steiner grade, and vein invasion. To further investigate the clinical usefulness of lncRNA TUG1 expression in HCC, we compared five-year overall survival according to various clinicopathologic factors including the expression level of lncRNA TUG1. A significant relationship was found between lncRNA TUG1 expression and 5-year overall survival. Furthermore, in a multivariate Cox model, including tumor size, tumor stage, tumor grading, presence of cirrhosis, gender, age, and lncRNA TUG1 expression, we found that lncRNA TUG1 expression was an independent poor prognostic factor for 5-year overall survival in HCC.

In conclusion, our study demonstrated that increased lncRNA TUG1 expression levels were correlated with poor prognosis of patients with HCC, indicating that lncRNA TUG1 may serve as a novel prognostic marker for HCC after hepatic resection.

Disclosure of conflict of interest

None.

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