Prognostic significance of overexpressed long non-coding RNA TUG1 in patients with clear cell renal cell carcinoma

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Abstract. – OBJECTIVE: The long non-coding RNAs (lncRNAs) study has gradually become one of the hot topics in the field of RNA biology. However, little is known about the pathological role of lncRNA TUG1 in clear cell renal cell carcinoma (ccRCC) patients. This study attempted to investigate the association of lncRNA TUG1 expression with progression and prognosis in ccRCC patients.

PATIENTS AND METHODS: Using qRT-PCR, the expression of TUG1 was measured in 203 ccRCC tissues and 45 adjacent non-cancerous tissues. Then, the relationships between TUG1 level and the clinicopathological factors of patients with ccRCC were analyzed. The prognostic significance was evaluated using Kaplan-Meier and Cox regression analyses.

RESULTS: The relative level of TUG1 was significantly higher in ccRCC tissues compared to the adjacent non-tumor tissues ($p < 0.01$). Furthermore, TUG1 was associated significantly with histological grade, tumor stage, lymph node metastasis and distant metastasis (all $p < 0.05$). Interestingly, Kaplan-Meier analysis showed that higher TUG1 expression levels were associated with a shorter overall survival ($p < 0.001$) in ccRCC patients. Cox proportional hazards analysis showed that high TUG1 expression was an independent prognostic marker of poor outcome.

CONCLUSIONS: These findings suggested that TUG1 may act as a tumor promoter in ccRCC and could serve as a potential therapeutic target for this tumor.

Key Words:
Long non-coding RNAs, TUG1, Clear cell renal cell carcinoma, Prognosis.

Introduction

It has been reported that renal cell cancer (RCC) accounts for approximately 2-3% of all human tumors, and the incidence is still increasing at a rate of 2% per year[1,2]. RCC can be histologically classified into several subtypes, among which Clear-cell renal cell carcinoma (ccRCC) is the most common, accounting for 70-80% of all[3]. Radical nephrectomy is effective to cure early and local ccRCC, but no effective for patients at advanced stages. ccRCC is generally resistant to standard chemotherapy and radiotherapy and median survival in a recent cohort was only 1.5 years with fewer than 10% of patients surviving to 5 years[4,5]. Therefore, it is critical to identify effective biomarkers which not only predict the progression and prognosis of ccRCC but also help to develop the new targeted therapies for ccRCC.

Long noncoding RNA (lncRNA) is a subset of noncoding RNA which exceed 200 nucleotides in length. Accumulating evidence confirmed that lncRNAs contribute to cancer initiation and progression[6,7] and more studies report that lncRNAs function as oncogene or anti-oncogene in several tumors. For instance, Yang et al[8] reported that lncRNA AK001796 suppresses colony formation *in vitro* and tumor growth *in vivo*. Gupta et al[9] showed lncRNA HOTAIR was increased in expression in primary breast tumors and metastases, and HOTAIR expression level in primary tumors was a powerful predictor of eventual metastasis and death. Yang et al[10] demonstrated the biological significance of lncRNA MALAT1 in cervical cancer progression and provided novel evidence that MALAT1 may serve as a therapeutic target in the prevention of human cervical cancer. Recently, Accumulating evidence showed that lncRNA TUG1 play an important role in tumor[11,12]. However, the clinical significance of TUG1 in ccRCC has not been evaluated. In the

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Table I. HCV genotypes distribution and diagnosis of liver disease according with age of patients.

Variables	Cases (No.=203)	TUG1 expression		*p*-value
		High	Low	
Gender				0.642
Male	117	56	61	
Female	86	44	42	
Age (years)				0.481
< 65	116	60	56	
≥ 65	87	40	47	
Tumor size (cm)				0.984
< 4 cm	126	62	64	
≥ 4 cm	77	38	39	
Histological grade				0.004
I-II	143	61	82	
III-IV	60	39	21	
Tumor stage				0.000
T_1-T_2	119	45	74	
T_3-T_4	84	55	29	
Lymph nodes metastasis				0.004
Absence	161	71	90	
Presence	42	29	13	
Distant metastasis				0.003
Absence	156	68	88	
Presence	47	32	15	

present work, we investigated TUG1 expression in tumor tissues and matched adjacent normal tissues from ccRCC patients to determine its clinicopathological and prognostic significance.

Patients and Methods

Patients and Tissue Samples

A total of 203 paired clear cell renal cell carcinoma and corresponding noncancerous tissues were obtained sequentially from patients undergoing radical nephrectomy Department of Critical Care Medicine, Linzi District People's Hospital between 2008 to 2011. None of the patients had received chemotherapy or radiotherapy before surgery. Fresh specimens were collected, snap frozen in liquid nitrogen immediately after resection, and directly stored at -80°C until RNA extraction was performed. Clinicopathological features in our study were shown in Table I. Survival time was calculated from the date of the initial surgery to death. Clinical sample cohorts used for this study were approved by the Institution Research Ethics Committee of Linzi District People's Hospital. Written informed consent was obtained from all patients before surgery.

RNA Extraction and Quantitative Real-time PCR

Total RNA was isolated using TRIzol reagent (Invitrogen Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. For qPCR, RNA reverse transcribed to complementary DNA (cDNA) from 1 μg of total RNA was reverse transcribed in a final volume of 10 μL using random primers and a Reverse Transcription Kit (Takara, Dalian, China), according to the manufacturer's instructions. The PCR amplification were performed for 40 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s, on a Applied Biosystems 7900HT (Applied Biosystems, Foster City, CA, USA) with 1.0 μl of cDNA and SYBR Green Real-time PCR Master Mix (Life Technologies, Carlsbad, CA, USA). Also, the sequence of TUG1 primer was as follows: sense: 5′-CTGA-AGAAAGGCAACATC-3′; antisense: 5′-GTAG-GCTACTACAG GATTTG-3′. All experiments were performed in triplicate. The relative expression of TUG1 was calculated and normalized using the $2^{-\Delta\Delta Ct}$ method relative to GAPDH.

Statistical Analysis

All statistical analyses were performed using SPSS 20.0 statistical software (SPSS, Inc., Chicago, IL, USA). Differences between groups were analyzed by the Student *t*-test or the chi-square test.

Table II. Univariate and multivariate analysis of overall survival in ccRCC patients.

Variable	Univariate analysis			Multivariate analysis		
	Risk ratio	95% CI	*p*	Risk ratio	95% CI	*p*
Gender						
Male vs. female	1.461	0.562-2.883	0.471			
Age (years)						
≥ 65 vs. < 65	2.631	0.492-3.811	0.363			
Tumor size						
≥ 4 cm vs. < 4 cm	3.367	0.731-6.541	0.213			
Histological grade						
III-IV vs. I-II	4.361	1.894-6.822	0.001	4.029	1.672-6.042	0.007
Tumor stage						
T_{3-4} vs. T_{1-2}	3.348	2.231-5.883	0.017	2.935	1.872-5.428	0.011
Lymph node						
Presence vs. absence	4.983	2.237-7.023	0.006	3.784	1.783-6.234	0.014
Distant metastasis						
Presence vs. absence	5.138	2.862-8.784	0.003	4.342	2.348-7.452	0.009
TUG1						
High vs. low	3.231	1.672-7.754	0.013	2.337	1.451-6.673	< 0.001

Overall survival was analyzed by the Kaplan-Meier method, and the differences between groups were estimated by the log-rank test. The variables were used in multivariate analysis on the basis of the Cox proportional hazards model. For all analyses, statistical significance was considered at $p < 0.05$.

Results

Differentially Expressed TUG1 Between ccRCC Tissues and Adjacent Non-Cancer Tissues

Relative expression levels of TUG1 were determined by qRT-PCR in a total of 203 patients with ccRCC. As shown in Figure 1, the expression level
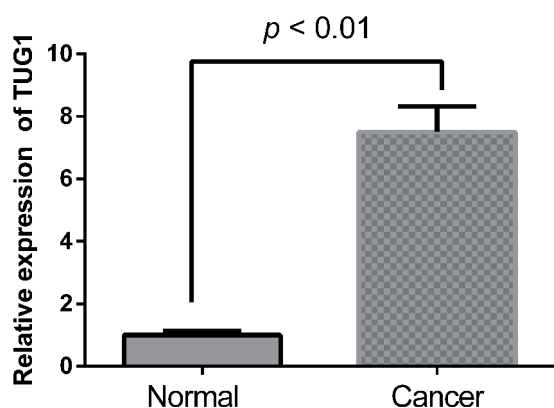

Figure 1. The expression level of TUG1 in ccRCC patients. The results showed that the TUG1 expression level in patients with ccRCC was significantly higher than that in healthy controls ($p < 0.01$).

of TUG1 in ccRCC tissues was significantly higher than that in adjacent non-tumor tissues ($p < 0.01$). The data indicated that abnormal TUG1 expression might be related to ccRCC pathogenesis.

Correlations Between Patients' Characteristics and TUG1 Expression

To assess the clinical relevance of TUG1 expression in ccRCC, we examined the correlation between TUG1 expression and clinicopathological parameters. As shown in Table I, a statistically significant association was observed between TUG1 expression and histological grade ($p = 0.004$), tumor stage ($p = 0.000$), lymph nodes metastasis ($p = 0.004$), and distant metastasis ($p = 0.003$). However, no significant differences about other characteristics of patients were found. Taken together, these observations indicated that increased TUG1 expression is associated with the progression and development of ccRCC.

Correlation of TUG1 Expression with Prognosis of ccRCC Patients after Surgery

The overall survival analysis using the Kaplan-Meier method revealed that the prognosis of ccRCC patients with high TUG1 expression was significantly poorer than those with low TUG1 expression (Figure 2; $p < 0.001$). Also, we performed univariate and multivariate analysis using the Cox proportional hazard regression model to determine whether TUG1 expression and other clinical parameters are independent factors for prognostic prediction in ccRCC patients. As shown in Table II, univariate propor-
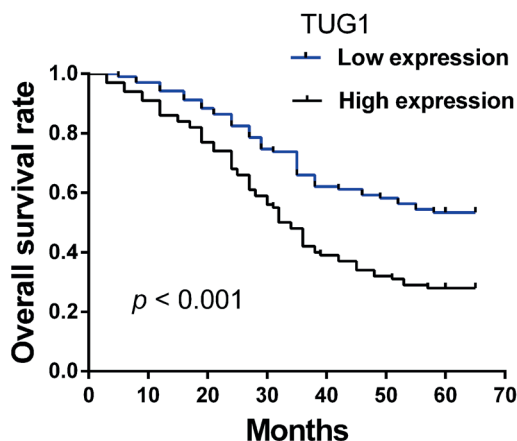
Figure 2. Overall survival curves for two groups defined as low and high ex¬pression of TUG1 in ccRCC patients. Higher TUG1 expression was signifi¬cantly associated with poorer outcome of patients ($p < 0.001$, log-rank test).

tional hazard model showed that histological grade ($p = 0.001$), tumor stage ($p = 0.017$), lymph nodes metastasis ($p = 0.006$), distant metastasis ($p = 0.003$) and TUG1 ($p = 0.013$) expression level were prognostic predictors. In addition, multivariate analyses indicated that high TUG1 expression was an independent poor prognostic factor for ccRCC patients.

Discussion

Up to now, more than 5600 separate reports focusing on ccRCC prognostic markers have been published. However, a little powerful prognostic indicator for human ccRCC has been identified. In recent years, more and more studies showed that dysregulation in lncRNAs are proved to contribute to the tumor development in many cancer types and can be used to develop as biomarkers and prognosis factors[13,14]. In the present study, our attention focused on the TUG1.

In this research, we firstly found that the expression level of TUG1 was significantly upregulated in ccRCC tissues versus corresponding non-tumor tissues and the high expression of TUG1 was significantly associated with histological grade, tumor stage, lymph node metastasis and distant metastasis, suggesting that TUG1 might be involved in the carcinogenesis and metastasis of ccRCC. According to the Kaplan-Meier survival analysis, the patients with high TUG1 expression exhibited evidently poorer overall survival rates than those with low TUG1 expression. In the mul-

tivariate Cox proportional hazards analysis, high TUG1expression was independently associated with poor survival.

LncRNA taurine upregulated gene 1 (TUG1) was initially detected in a genomic screen for genes upregulated in response to taurine treatment in developing mouse retinal cells[15]. However, increased evidence showed that TUG1 played an important role in a variety of tumors. For instance, Xu et al[16] found that silencing TUG1 via siRNA inhibited the proliferation and migration of esophageal squamous cell carcinoma cells and blocked the progression of cell cycle. Huang et al[17] revealed that up-regulation of Long non-coding RNA TUG1 promoted hepatocellular carcinoma cell growth and apoptosis by epigenetically silencing of KLF2. Zhang et al[18] showed that increased expression of long noncoding RNA TUG1 predicted a poor prognosis of gastric cancer and regulated cell proliferation by epigenetically silencing of p57. These findings illustrate that TUG1 are widely involved in the pathogenesis of cancer.

Conclusions

Our study is the first to demonstrate TUG1 expression as an independent prognostic factor in ccRCC. High expression of TUG1 is associated with shortened survival. Moreover, further experimental studies are also required to identify the detailed role of TUG1 in ccRCC.

Conflict of interest
The authors declare no conflicts of interest.

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