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Expression of lncRNA TCONS_00027978 in hepatocellular carcinoma and its influence on prognosis and survival

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Abstract. – OBJECTIVE: Long noncoding RNAs (lncRNAs) have been reported to have crucial roles in the regulation of various tumors. Recently, lncRNA TCONS_00027978 (TCONS_00027978) was found to be downregulated in hepatocellular carcinoma (HCC). This study aims at analyzing the value of TCONS_00027978 in valuing the prognosis of HCC patients.

PATIENTS AND METHODS: The expression of TCONS_00027978 was detected using qRT-PCR in 241 hepatocellular specimens and matched adjacent normal tissues. The association between TCONS_00027978 expression levels and clinicopathological factors was examined. Survival and Cox proportional-hazards regression analyses were performed to explore the association between TCONS_00027978 expression levels and prognosis in HCC patients.

RESULTS: We found that the TCONS_00027978 expression level was significantly decreased in HCC tissues compared with matched normal tissues ($p < 0.01$). TCONS_00027978 was positively associated with TNM stage ($p = 0.002$), lymph node metastasis ($p = 0.001$) and histologic grade ($p = 0.002$) in HCC patients. In addition, Kaplan-Meier analysis indicated that low levels of TCONS_00027978 expression were associated with poorer overall survival ($p = 0.002$) and disease-free survival ($p = 0.001$). Subsequently, multivariate analyses indicated that low TCONS_00027978 expression was an independent poor prognostic factor for HCC patients.

CONCLUSIONS: Our study is the first to demonstrate the TCONS_00027978 expression as an independent prognostic factor in HCC.

Key Words:
Long noncoding RNAs, TCONS_00027978, Prognosis.

Introduction

Hepatocellular carcinoma (HCC), a predominant histological subtype of primary liver cancer, continues to be a leading cause of cancer-related deaths worldwide and one of the most common cancers diagnosed worldwide[1,2]. Although prognosis of patients with HCC has improved largely owing to the improvement of current treatment, long-term survival after surgical resection remains low due to the high rate of recurrence and metastasis[3,4]. Until now, there are few specific biomarkers available for diagnosis and prognosis use[5]. Thus, it is necessary to develop new biomarkers with the potential to predict the outcome of GC patients.

Long noncoding RNAs (lncRNAs) are RNA molecules that are longer than 200 nucleotides and lack significant protein-coding capacity[6]. LncRNAs have been implicated in several biological functions, such as cell proliferation, cell cycle progression, cell growth and cell apoptosis[7,8]. At present, only a relatively small number of lncRNAs have been identified. For instance, Fang et al[9] found that lncRNA XIST promoted non-small cell lung cancer cell proliferation, migration and invasion by epigenetically repressing KLF2 expression. The lncRNA LeXis, which is highly expressed in osteosarcoma, was found to function as a tumor promoter through upregulation of CTNNB1 expression[10]. Recently Yu et al[11] reported that forced expression of HOXA11-AS could inhibit the proliferation of HCC cells by regulating the expression of LATS1. Given the important role of lncNRAs in tumors, lncRNAs received a lot of attention for their potential to serve as prognostic and diagnostic biomarkers. Of note, several lncRNAs involved in HCC prognosis have been identified[12,13].

A novel lncRNA TCONS_00027978 was found to be down-regulated in HCC tissues by microarray assays[14]. Gao et al[15] further reported that up-regulation of TCONS_00027978 suppressed the

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migration and invasion of HCC cells by targeting R-cadherin pathway. Thus, we wondered whether TCONS_00027978 was associated with the prognosis of HCC patients. To our best knowledge, the present study was the first to report the prognostic significance of TCONS_00027978 in HCC.

Patients and Methods

Specimen Collection

Paired resected tumor and normal hepatocellular tissues were obtained from 241 HCC patients who underwent surgeries at the First People's Hospital of Jinan (Jinan, China) between 2007 and 2011. All patients had a definite diagnosis of HCC and complete medical records. None of the patients had received neoadjuvant chemotherapy before their operation. All specimens were frozen immediately in liquid nitrogen and stored at -80°C until required. TNM staging was determined by the criteria established by the International Union Against Cancer (UICC) in 2009. All patients' clinicopathological parameters are shown in Table I. Written informed consent was obtained from all of the patients. Study protocol was approved by the Ethics Committee of our hospital.

Quantitative Real-time PCR Assay

Total RNA was extracted with TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instruction. Expression level of TCONS_00027978 was analyzed using SYBR Green PCR Master Mix kit (Applied Biosystems, Foster City, CA, USA). GAPDH was used as a lncRNA internal control. qPCR was carried out with SYBR Green Master Mix (Life Technologies, Darmstadt, Germany). The relative gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method. The primer sequences for qPCR were designed and purchased by Thermo Fisher Scientific (Waltham, MA, USA).

Statistical Analysis

Statistical software SPSS version 13.0 (SPSS Inc., Chicago, IL, USA) was used for the assessment. The distinct expression of TCONS_00027978 between tumor tissues and matched normal tissues was examined by independent samples *t*-test. The association between TCONS_00027978 expression and HCC clinicopathological features of patients was analyzed using x^2 test. Survival curves were plotted by the Kaplan-Meier method; the significance was evaluated by the log-rank test. Multivariate Cox proportional hazards regression analysis was used to evaluate the significance of survival variables. Differences were defined as statistically significant for p-values < 0.05.

Results

Down-Regulation of TCONS_00027978 in HCC Tissues

To analyze the role of TCONS_00027978 in HCC, we performed Real-time PCR to detect the expression levels of HCC tissues and adjacent normal tissues. As shown in Figure 1, we found that the average level of TCONS_00027978 in HCC tissues was significantly downregulated when compared with the matched normal tissues ($p < 0.01$). Therefore, these results revealed that TCONS_00027978 might be involved in HCC development.

Correlation Between TCONS_00027978 Expression and Clinicopathological Characteristics of HCC

To further explore the association of TCONS_00027978 with clinicopathological features of HCC patients, we divided HCC patients into high expression group and low-expression group according to the median value of

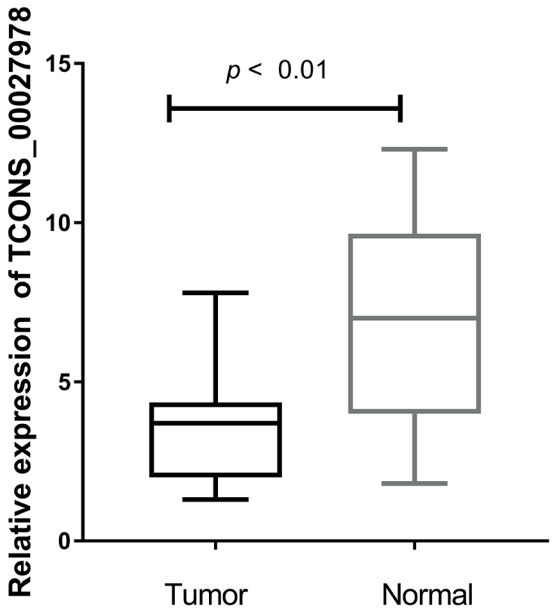

Figure 1. The expression level of TCONS_00027978 in 241 pairs of cancerous and matched normal tissue samples.

Table I. Association between the clinicopathologic characteristics and expression of TCONS_00027978 in HCC.

Characteristics	Number of patients	TCONS_00027978		*p*-value
		High-expression	Low-expression	
Age				0.293
<50	107	51	56	
≥50	134	73	61	
Gender				0.092
Male	145	81	64	
Female	96	43	53	
HBsAg				0.733
Positive	156	79	77	
Negative	85	45	40	
Vein invasion				0.121
Negative	160	88	72	
Positive	81	36	45	
Encapsulation				0.282
Complete	126	69	57	
No	115	55	60	
Tumor size (cm)				0.157
≤5	151	83	68	
>5	90	41	49	
AFP (ng/L)				0.201
≥400	75	34	41	
<400	166	90	76	
TNM stage				0.002
I + II	140	84	56	
III-IV	101	40	61	
Lymph node metastasis				0.001
No	146	88	58	
Yes	95	36	59	
Histologic grade				0.002
High	107	43	64	
Low	134	81	53	

TCONS_00027978. Results of statistical analysis indicated that TCONS_00027978 was positively associated with TNM stage (p = 0.002), lymph node metastasis (p = 0.001) and histologic grade (p = 0.002) in HCC patients (Table I). However, there was no relationship between TCONS_00027978 expression and other factors such as age, gender, HBsAg, vein invasion, encapsulation, tumor size and AFP (all $p > 0.05$).

Reduced Expression of TCONS_00027978 Predicts a Poor Prognosis

We next performed survival analysis to evaluate whether TCONS_00027978 was associated with prognosis of HCC patients. Our results indicated that overall survival time of low TCONS_00027978 expression group was significantly shorter than that of high TCONS_00027978 expression group (p = 0.002, Figure 2). On the other hand, we also found that patients with low TCONS_00027978 expression

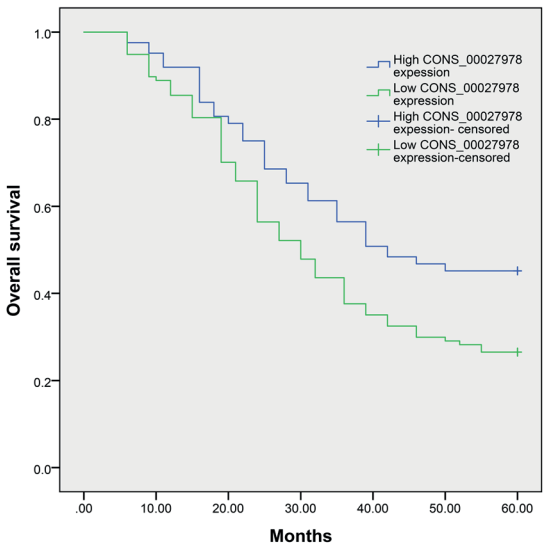

Figure 2. Overall survival curves for two groups defined by low and high expression of TCONS_00027978 in HCC patients. The patients with lower expression level of TCONS_00027978 had a poorer overall survival rate (p = 0.002).

Table II. Multivariate survival analysis of overall survival and disease-free survival in 241 patients with HCC.

Variables	Overall survival			Disease-free survival		
	HR	95% CI	*p*	HR	95% CI	*p*
TNM stage	4.321	1.534-7.673	0.005	5.313	1.831-9.323	0.002
Lymph node metastasis	5.268	1.733-9.241	0.001	5.762	1.928-11.621	0.001
Histologic grade	3.893	1.332-6.589	0.003	4.139	1.573-7.232	0.001
TCONS_00027978 expression	3.231	1.277-5.904	0.009	3.562	1.528-6.893	0.004

had poorer disease-free survival compared with high TCONS_00027978 group ($p = 0.001$, Figure 3). Moreover, multivariate analysis using the Cox proportional hazards model revealed that TCONS_00027978 expressions were independent prognostic factors for overall survival (HR = 3.231, 95% CI: 1.277-5.904; $p = 0.009$), as well as disease-free survival (HR = 3.562, 95% CI: 1.528-6.893; $p = 0.004$) of HCC patients (Table II).

Discussion

As different tumor therapies are effective in different subgroups of patients, it is very important to investigate prognostic markers to improve the prognosis of cancer patients[16]. HCC is still one of the main problems in health field worldwide. Clinicopathological features as predictors of poor prognosis in HCC patients include alpha-fetoprotein (AFP), vein invasion, tumor size, TNM stage and lymph node metastasis[17]. However, most of these features had not been proven to be sufficiently effective. In order to explore novel effective biomarkers, more and more studies focused on cancer-related genes and lncRNA. In the present study, we focused on a novel lncRNA. Growing evidence has revealed that lncRNAs are emerging tools for prediction of HCC patients because of their important regulatory effect on tumor-related genes[18,19]. Recently, several lncRNA has been identified to be involved in HCC carcinogenesis and patient prognosis. For instance, Wang et al[20] found that lncRNA PCAT-14 was an independent prognostic factor and served as an oncogene in HCC by inducing methylation of miR-372. Zhang et al[21] reported that lncRNA SNHG15 expression was associated with clinical features of HCC such as histologic grade, TNM stage and overall survival. Moreover, lncRNA SNHG15 was also proved to be an independent prognostic factor for HCC. Zhang et al[22] showed that increased lncRNA AFAP1-AS1 expression was a poor independent prognostic factor for HCC patients and downregulation of lncRNA AFAP1-AS1 significantly suppressed HCC cell proliferation and invasion via upregulation of RhoA/Rac2 signaling. Recently, a novel lncRNA (TCONS_00027978) was identified in HCC. Unlike some other well-studied lncRNAs such as lncRNA BANCR[23] and lncRNA ANRIL[24], the function of TCONS_00027978 remains largely unknown. Gao et al[15] firstly reported that TCONS_00027978 functioned as a tumor promoter in TCONS_00027978. Furthermore, they identified R-cadherin pathway as downstream target. However, the prognostic value of TCONS_00027978 has not been reported. In the present study, we showed that TCONS_00027978 levels were down-regulated in patients with HCC compared to those of normal hepatocellular tissues. Furthermore, we

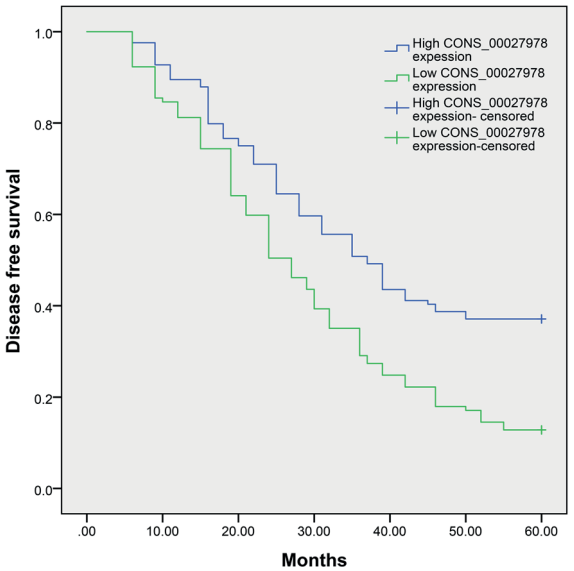

Figure 3. Disease-free survival curves for two groups defined by low and high expression of TCONS_00027978 in HCC patients. The patients with lower expression level of TCONS_00027978 had a poorer disease-free survival rate ($p = 0.001$)

found that TCONS_00027978 expression was closely related to TNM stage, lymph node metastasis and histologic grade. Subsequently, we performed the Kaplan-Meier method to explore the prognostic value of TCONS_00027978 in HCC patients. The results revealed that low levels of TCONS_00027978 expression were associated with poorer overall survival and disease-free survival. Finally, according to multivariate analysis, TCONS_00027978 was an independent poor prognostic factor for HCC patients.

Conclusions

Our findings demonstrate that the expression level of TCONS_00027978 has the potential to predict HCC prognosis. However, the mechanism by which TCONS_00027978 is downregulated in HCC is still unclear. Further studies should focus on the mechanism responsible for the tumor-suppressive role of TCONS_00027978 in HCC.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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