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Long noncoding RNA SNHG15, a potential prognostic biomarker for hepatocellular carcinoma

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Abstract. – OBJECTIVE: The purpose of this study was to investigate the expression of lncRNA SNHG15 and its prognostic value in hepatocellular carcinoma (HCC).

PATIENTS AND METHODS: Expression levels of lncRNA SNHG15 in 152 pairs of HCC tissues and adjacent normal tissues were detected by quantitative real-time PCR. Associations between clinicopathological parameters and lncRNA SNHG15 expression were evaluated using chi-square tests. The overall survival was analyzed by log-rank test, and survival curves were plotted according to Kaplan-Meier. Univariate and multivariate Cox regression analyses were conducted to determine whether lncRNA SNHG15 was an independent predictor of survival.

RESULTS: The lncRNA SNHG15 expression was significantly upregulated in tumor tissues compared with that in adjacent non-tumor tissues ($p < 0.01$). It is also proved that lncRNA SNHG15 expression was associated with histological grade ($p = 0.000$), TNM stage ($p = 0.015$), and vein invasion ($p = 0.000$). In addition, Kaplan-Meier analysis showed that increased LncRNA SNHG15 expression was associated with poor overall survival of patients ($p = 0.0011$). Moreover, the results of the multivariate analysis showed that high lncRNA SNHG15 expression was a significant independent predictor of poor survival in HCC ($p < 0.05$).

CONCLUSIONS: Our findings suggest that lncRNA SNHG15 may serve as an efficient clinical biomarker and a therapeutic target for HCC patients.

Key Words:
LncRNA SNHG15, Hepatocellular carcinoma, Prognosis.

Introduction

Hepatocellular carcinoma (HCC) is one of the five most frequent cancers and the third leading cause of cancer death globally, posing a serious threat to human health[1,2]. The prognosis of HCC is poor and most patients are generally diagnosed at advanced stage[3]. So far, surgery remains the best prognostic tool for long-term survival of HCC patients[4]. However, the postoperative recurrence rate remains as high as 70%[5]. Therefore, it is of great clinical significance to identify prognostic factors which can improve the individual treatment.

Long non-coding RNAs (lncRNAs) are evolutionarily conserved non-coding RNAs that are longer than 200 nucleotides in length with no protein-coding capacity[6,7]. Increasing evidences have shown that lncRNAs play critical roles in many human key biologic processes including tissue differentiation, cell proliferation, and embryonic development[8,9]. For instance, Tuo et al[10] found that Long noncoding RNA UCA1 function as a tumor promoter in breast cancer by down-regulating miR-143. Shi et al[11] reported that GAS5 overexpression could induce apoptosis and growth arrest in non-small-cell lung carcinoma. Ding et al[12] showed that the relative expression levels of lncRNA PVT1 were significantly up-regulated in hepatocellular carcinoma tissues and predicted recurrence in hepatocellular carcinoma patients. However, the clinical relevance and the role in carcinogenesis of lncRNA SNHG15 in HCC remain to be elucidated. In the present study, we investigated the clinical significance and prognostic value of lncRNA SNHG15 in HCC.

Patients and Methods

Patients and Tissue Samples

152 HCC tissue samples and matched adjacent noncancerous tissue samples were collected from patients with HCC who had undergone surgery in

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Linyi People's Hospital, Linyi, Shandong, China (between 2007 and December 2010). All patients recruited in this study were not subjected to preoperative radiotherapy and/or chemotherapy. HCC diagnosis was based on World Health Organization (WHO) criteria. All tissue samples were immediately frozen in liquid nitrogen after surgical removal and stored at –80°C until further use. The present study was approved by the Research Ethics Committee of The Linyi People's Hospital. Informed consent was obtained from all the patients. The clinicopathological features of 152 patients were summarized in Table I.

RNA Extraction and Quantitative RT-PCR

Total RNA was extracted from tissue samples using the Trizol reagent (Invitrogen, Carlsbad, CA, USA). The purity and concentration of RNA were determined using NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Quantitative real-time PCR (qPCR) was performed using SYBR Premix EX TaqTM II (Takara, Dalian, China) on 7900HT Fast Real-time System (Applied Biosystems, Foster City, CA, USA). GAPDH was taken as the endogenous control. Each sample was examined in triplicate and analyzed by the comparative threshold cycle (Ct) method.

Statistical Analysis

All data were analyzed by SPSS 21.0 software (SPSS Inc., Chicago, IL, USA). The correlation between lncRNA SNHG15 expression and clinicopathological features were evaluated by the chi-square test. Overall survival curves were plotted using the Kaplan-Meier method and were evaluated for the statistical significance using a log-rank test. A Cox's regression model was used for univariate and multivariate analysis. $p < 0.05$ was considered to indicate a significant difference.

Results

LncRNA SNHG15 was Up-Regulated in HCC Tissues

The expression level of lncRNA SNHG15 in 152 pairs of HCC tissues and adjacent non-tumor tissues was examined by qRT-PCR. As shown in Figure 1, the results revealed that lncRNA SNHG15 expression levels were significantly higher in HCC tissues than in the corresponding noncancerous tissues ($p < 0.01$).

Association Between lncRNA SNHG15 Expression and the Clinicopathological Features of HCC

To evaluate the correlation between lncRNA SNHG15 expression and clinicopathological factors, the patients were divided into high and low expression groups by the median expression level of LncRNA SNHG15. As shown in Table I, the group with high SNHG15 expression was associated with an advanced histologic grade ($p = 0.000$), higher TNM stage ($p = 0.015$), and posi-

Table I. Association of lncRNA SNHG15 expression with clinicopathologic factors of HCC patients.

Parameters	Group	Total	lncRNA SNHG15		*p*-value
			Low	High	
Gender	Male	92	49	43	0.231
	Female	60	26	34	
Age (years)	< 50	68	31	37	0.405
	≥ 50	84	44	40	
Tumor size (cm)	< 5 cm	53	22	31	0.158
	≥ 5 cm	99	53	46	
Histologic grade	Low	76	49	29	0.000
	High	76	28	48	
TNM stage	I-II	66	40	26	0.015
	III-IV	86	35	51	
Vein invasion	Absence	64	43	21	0.000
	Presence	88	32	56	
Cirrhosis	Negative	107	52	55	0.777
	Positive	45	23	22	
Hepatitis B	Negative	55	29	26	0.530
	Positive	97	46	51	

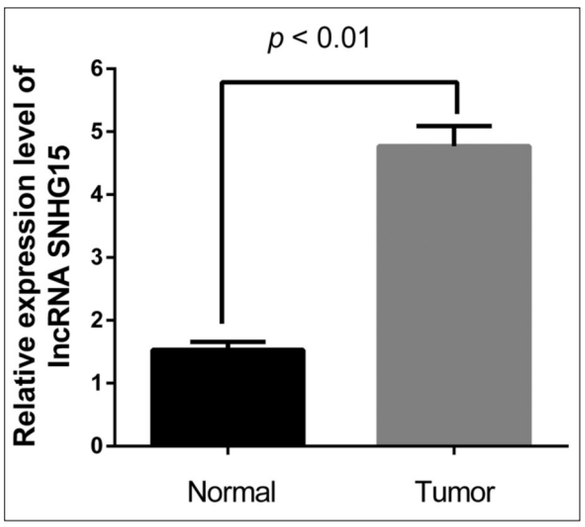

Figure 1. The relative expression level of lncRNA SNHG15 in human hepatocellular carcinoma (HCC) tissues (n = 152) and matched adjacent non-cancerous liver tissues (n =152). lncRNA SNHG15 expression was significantly higher in HCC tissues compared with normal adjacent liver tissues ($p < 0.01$).

tive vein invasion ($p = 0.000$) than the group with low SNHG15 expression. However, the expression status of lncRNA SNHG15 was not associated with gender, age, tumor size, cirrhosis and hepatitis B ($p > 0.05$).

LncRNA SNHG15 Expression is Associated With Overall Survival in HCC Patients

To explore the prognostic value of lncRNA SNHG151 expression in HCC patients, we performed Kaplan-Meier analysis with the log-rank test to assess the correlation between the levels of lncRNA SNHG151 expression and patients' survival. The results showed that higher lncRNA SNHG151 expression had significantly worse overall survival compared with patients with higher lncRNA SNHG151 expression (log-rank test, $p = 0.0011$) (Figure 2). Next, univariate analysis for OS indicated that histologic grade, TNM sncRNA SNHG151 expression were significantly associated with OS. Furthermore, multage, vein invasion and multivariate analysis revealed that lncRNA SNHG151 expression ($p = 0.001$) was independently associated with the overall survival (shown in Table II).

Discussion

HCC accounts for 70 to 90% of primary liver cancer of the world, and is the third major cause of cancer-related mortality worldwide[13,14]. Although a growing number of novel treatment strategies have been developed for HCC, such as molecular targeted therapy and gene therapy. However, the patients with HCC are still suffering from low 5-year survival rate[15]. Therefore, it is necessary to develop a novel and reliable biomarker to evaluate the prognosis and efficacy of therapeutic strategies for HCC.

A recent report suggested that lncRNAs expression was significantly altered between HCC tissues and non-cancerous tissues by cDNA microarray interrogating putative lncRNAs. For example, Zheng et al[16] suggested patients with higher expression of MALAT1 showed a significantly worse overall survival and disease-free survival than those patients with lower expression of MALAT1. Dimple et al[17] conducted the study that NEAT1 could act as one of the differentially regulated lncRNAs in prostate cancer by activating prostate cancer genes. Li et al[18] reported that LncRNA HOTAIR promotes tumorigenesis via downregulating SETD2 in liver cancer stem cells and also correlated with shorter overall survival of HCC. The human SNHG15 gene is a long intergenic noncoding RNA (lincRNA), is located on chromosome 7p13. As a newly found lncRNA, to our knowledge, only one study reported that the expression level of lncRNA SNHG15 was up-regulated in GC tissues. Furthermore, they found that lncRNA SNHG15 in-

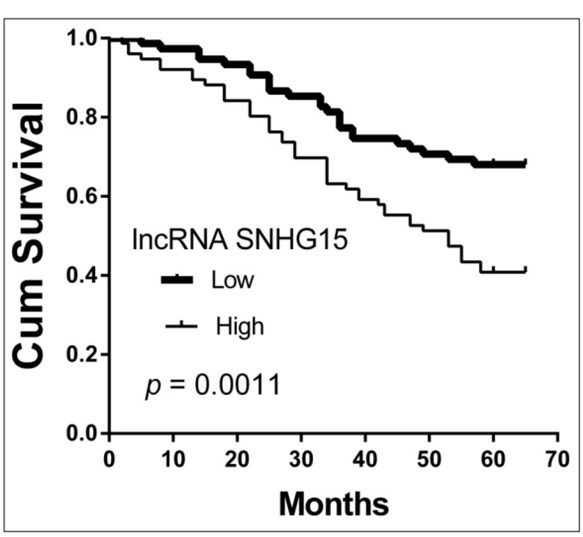

Figure 2. The association between lncRNA SNHG15 expression and OS. The survival analysis revealed that the HCC patients with higher expression level of lncRNA SNHG15 had worse 5-year OS ($p = 0.0011$).

Table II. Univariate and multivariate Cox regression analyses of overall survival in HCC patients.

Variable	Univariate analysis			Multivariate analysis		
	Risk ratio	95% CI	*p*-value	Risk ratio	95% CI	*p*-value
Gender						
Male *vs*. Female	1.335	0.819-1.726	0.541			
Age (years)						
≥ 50 *vs*. < 50	2.461	0.673-4.319	0.316			
Tumor size						
≥ 5 cm *vs*. < 5 cm	1.991	0.762-3.642	0.429			
Histologic grade						
High vs. Low	2.547	1.373-4.527	0.009	2.256	1.544-4.361	0.018
TNM stage						
III-IV *vs*. I-II	3.428	1.816-5.672	0.017	3.661	1.673-5.992	0.003
Vein invasion						
Presence *vs*. Absence	3.257	1.261-5.336	0.006	2.719	1.554-5.652	0.002
Cirrhosis						
Positive *vs*. Negative	1.432	0.549-2.671	0.816			
Hepatitis B						
Positive *vs*. Negative	1.225	0.569-2.571	0.374			
lncRNA SNHG15						
High *vs*. Low	3.017	1.448-6.221	0.018	2.247	1.331-6.255	0.001

hibited proliferation and invasion of human GC cells by directly targeting MMP2/MMP9[19]. Those results revealed that lncRNA SNHG15 may function as a tumor promoter in cancer. So, in the present study, we focus on the prognostic value of lncRNA SNHG15 in HCC.

In the present study, our results of PCR showed that the expression level of lncRNA SNHG15 in HCC tissues is higher than that in matched noncancerous liver tissues. Up-regulation of lncRNA SNHG15 was correlated with histologic grade, TNM stage, and vein invasion. Furthermore, the overall survival rate of the high lncRNA SNHG15 expression group was significantly lower than that of the low lncRNA SNHG15 expression group. In the multivariate analysis, high lncRNA SNHG15 expression was an independent prognostic factor for OS. All those results suggested that lncRNA SNHG15 might be a potential prognostic biomarker and therapeutic target for NSCLC.

Conclusions

To our knowledge, this is the first report to investigate the clinical significance of lncRNA SNHG15 in pediatric HCC patients. Our data informed that up-regulation of lncRNA SNHG15 expression was closely associated with HCC development. The results of the study may provide a new potential marker and therapy method for HCC treatment. However, further investigations are needed to illuminate the detailed molecular mechanism by which lncRNA SNHG15 plays a role in HCC.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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