

High expression of lncRNA-SNHG7 is associated with poor prognosis in hepatocellular carcinoma

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Abstract. Expression of long non-coding RNA SNHG7 (lncRNA-SNHG7) and its clinical significance in hepatocellular carcinoma (HCC) were explored. Quantitative real-time polymerase chain reaction (qRT-PCR) was used to detect the expression level of lncRNA-SNHG7 in cancer tissues. Kaplan-Meier curves and multivariate Cox proportional models were used to study the impact on clinical outcome. Expression of lncRNA-SNHG7 was much higher in cancer tissues than that in para-cancer tissues. The lncRNA-SNHG7 expression was correlated with tumor number, lymph node metastasis and clinical stage ($P < 0.05$). In addition, HCC patients with higher lncRNA-SNHG7 expression had significantly poorer progression-free survival time and overall survival time ($P < 0.001$). Both univariate analysis and multivariate analysis indicated that high expression of lncRNA-SNHG7 was an independent predictor of poor prognosis in HCC. lncRNA-SNHG7 might contribute to the development of HCC and serve as a clinical biomarker and a therapeutic target for HCC patients.

Introduction

Hepatocellular carcinoma (HCC) is a common malignant tumor worldwide, with increasing incidence rate and mortality rate. More than 700,000 new cases emerge each year, and its mortality rate ranks third among malignant tumors (1,2). There is often no specific clinical manifestation in the early stage, so the vast majority of HCC patients tend to be diagnosed in the late stage. Once distant metastasis occurs, its prognosis deteriorates significantly, and the survival rate after surgery

will sharply decline. To date, metastasis and recurrence have constituted major causes of death for HCC patients (3,4).

Long non-coding ribonucleic acids (lncRNAs) are RNAs that cannot encode proteins, with approximately 200 nucleotides in length (5,6). Originally considered as 'background noise' or 'junk DNA', lncRNAs were deemed as useless RNAs, which exert no influence on the transcription and translation of proteins (7,8). However, recent years have seen increasing number of studies that have pointed out that lncRNAs play an important role in the biological process as necessary regulators to promote or inhibit the growth of transcribed tumor cells or to spur tumor metastasis (9-12). Long non-coding RNA SNHG7 (lncRNA-SNHG7), which is 2176 bp-long and located on chromosome 9q34.3, is a recognized bidirectional lncRNA. It was reported that it could guide snoRNA to undergo post-translational modification (13). Furthermore, it was a ribosomal RNA involved in modifying the growth and proliferation of cells. Abnormal expression of snoRNA is a potential factor for carcinogenesis. It was reported that lncRNA-SNHG7 was able to affect the regulation of proliferation, metastasis and invasion and suppress apoptosis of malignant tumor cells including lung cancer (14), pancreatic cancer (15), esophageal cancer (16) and gastric cancer (17). However, its role in HCC has remained unclear.

The present study explored the expression of lncRNA-SNHG7 and its clinical significance in HCC with the aim of contributing to the clarification of the biological mechanism of the tumor as well as providing valuable targets for corresponding diagnosis and treatment.

Patients and methods

Collection of tissue specimens. This study was approved by the Ethics Committee of Affiliated Hospital of Weifang Medical University (Weifang, China). Signed informed consents were obtained from all participants before entry to the study. The HCC tissues and para-carcinoma tissues (>5 cm away from cancer tissues) were harvested from 100 patients undergoing radical or palliative resection from April 2016 to December 2018, and the personal information and detailed clinical data of patients, including patient's sex, age, AFP levels, HBsAg levels, tumor size, tumor number, lymph node

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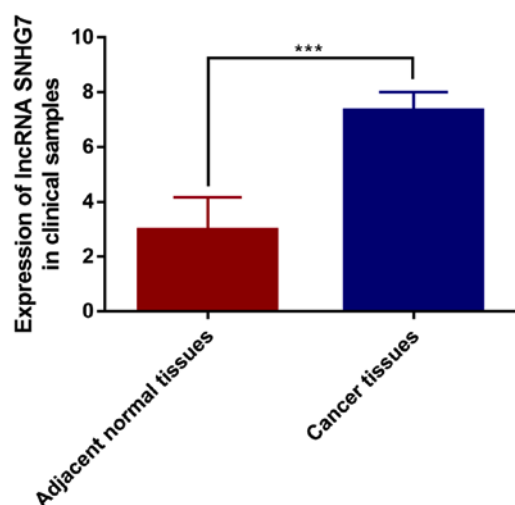


Figure 1. Expression level of lncRNA SNHG7 was evaluated in cancer tissues and para-cancer tissues by qRT-PCR. lncRNA-SNHG7, long non-coding RNA SNHG7; qRT-PCR, quantitative real-time polymerase chain reaction (***) $P < 0.001$).

metastasis and clinical stage were collected intact. Patients treated by radiotherapy and chemotherapy were excluded. The tissues were pathologically diagnosed with HCC, and the freshly-resected specimens were immediately cryopreserved in liquid nitrogen until quantitative real-time polymerase chain reaction (qRT-PCR) was performed.

QRT-PCR analysis. RNAs in primary HCC tissues and normal adjacent tissues were extracted according to the operation steps given in the specification of TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.), and lncRNA-SNHG7 gene was subjected to PCR based on the specification of reverse transcription kit. The conditions of PCR were as follows: pre-denaturation at 95°C for 10 min, followed by a total of 40 cycles for 15 sec at 95°C, 1 min at 60°C and 30 sec at 72°C. The relative expression level of lncRNA-SNHG7 was calculated by $2^{-\Delta\Delta C_t}$ method. Primer sequences used in the study are shown below: SNHG7 F: 5'-GTGTGTCCCTTGGTGGAGAG-3'; R: TCCCAGATACCAGCGAAGGA-3'. GAPDH F: 5'-AGAAGCTGGGGCTCATTG-3'; R: 5'-AGGGGCCATCCACAGTCTTC-3'.

Patient follow-up. The follow-up was conducted by phone or outpatient visit to record the survival of patients. The deadline for follow-up was 7 years. The total survival period was from the date of onset to the date of the final follow-up or death, in months.

Statistical analysis. Statistical Product and Service Solutions (SPSS) 19.0 software (IBM Corp.) was selected for the processing of result data, and measurement data were expressed as mean \pm standard deviation (mean \pm SD) or median. Paired t-test was used to compare the expression level of lncRNA-SNHG7 in primary HCC tissues and normal adjacent tissues. Group χ^2 test was adopted to analyze the associations of the expression of lncRNA-SNHG7 in HCC tissues with the clinicopathological features of patients. Overall survival (OS) and progression-free survival (PFS) of the patients was evaluated via Kaplan-Meier survival

Table I. lncRNA-SNHG7 expression and clinical features of patients with HCC.

Features	No.	lncRNA-SNHG7		P-value
		High	Low	
No.	100	53	47	
Sex				0.930
Male	66	34	32	
Female	34	19	15	
Age (years)				0.815
<60	43	23	20	
≥ 60	57	30	27	
AFP				0.894
<20	28	16	12	
≥ 20	72	37	35	
HBsAg				0.922
Positive	60	31	29	
Negative	40	22	18	
Tumor size (cm)				0.886
<5	39	20	19	
≥ 5	61	33	28	
Tumor number				0.001
Solitary	69	25	44	
Multiple	31	28	3	
Lymph node metastasis				0.008
Absence	62	24	38	
Presence	38	29	9	
Clinical stage				0.012
I + II	67	30	37	
III + IV	33	23	10	

lncRNA-SNHG7, long non-coding RNA SNHG7; HCC, hepatocellular carcinoma.

analysis and the intergroup differences were analyzed by log rank test. Cox proportional hazard regression model was, respectively, chosen for single factor analysis and multiple factor analysis of survival. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Expression of lncRNA-SNHG7 in HCC tissues. Expression of lncRNA-SNHG7 in 100 pairs of cancer tissues and para-normal tissues was detected. The results showed that the expression of lncRNA-SNHG7 in HCC tissues was much higher than that of para-normal tissues, the difference was statistically significant ($P < 0.001$) (Fig. 1). These results are consistent with lncRNA-SNHG7 described in literature.

The HCC samples were divided into SNHG7-high expression group ($n=53$) and SNHG7-low expression group ($n=47$) based on the mean expression level of lncRNA-SNHG7 from

Table II. Univariate and multivariate analyses of postoperative prognosis in patients with gastric cancer.

Features	Univariate analysis		Multivariate analysis	
	Hazard ratio/CI (95%)	P-value	Hazard ratio/CI (95%)	P-value
Sex	0.983/0.754-1.248	0.868		
Age	1.103/0.813-1.199	0.913		
Tumor size	1.627/0.917-2.425	0.071		
Tumor position	0.955/0.600-1.136	0.492		
Tumor differentiation	2.120/1.138-3.869	0.033	2.006/1.091-3.106	0.049
T stage	1.455/0.920-2.861	0.195		
N stage	3.374/1.821-5.429	0.007	3.173/1.720-4.851	0.030
M stage	3.987/2.349-4.780	0.012	3.294/2.322-4.234	0.027
TNM stage	4.102/3.076-5.371	0.003	3.722/2.926-5.110	0.008
lncRNACADM1-AS1 expression level	2.994/1.670-4.051	0.009	2.584/1.621-3.880	0.015

TNM, tumor-node-metastasis; lncRNA, long non-coding ribonucleic acid.

qRT-PCR analysis. The relationship between lncRNA-SNHG7 expression and clinicopathological features of patients was further analyzed, and it was found that there was no statistical difference between lncRNA-SNHG7 expression and patient's sex, age, AFP levels, HBsAg levels or tumor size ($P>0.05$). However, statistical difference was found between lncRNA-SNHG7 expression and tumor number, lymph node metastasis and clinical stage (Table I).

Effect of lncRNA-SNHG7 on the prognosis of patients with HCC. Correlation between lncRNA-SNHG7 expression and survival time of patients was evaluated using the Kaplan-Meier method. The results revealed that the patients with high expression of lncRNA-SNHG7 had worse progression-free survival (PFS) and overall survival (OS) time compared to SNHG7-low expression patients. The results were statistically significant ($P<0.001$) (Fig. 2), indicating high expression of lncRNA-SNHG7 in HCC patients predicted poor prognosis.

Univariate and multivariate analyses of lncRNA-SNHG7 expression and HCC clinicopathological data. Univariate Cox proportional hazards regression model analysis was used to analyze pathological parameters of HCC. The results revealed that tumor number, lymph node metastasis, clinical stage and lncRNA-SNHG7 expression level were statistically significant risk factors.

The above four factors with significant influence were incorporated into the multivariate Cox proportional hazard model for further analysis (Table II). Similarly to tumor number, lymph node metastasis and clinical stage. Expression of lncRNA-SNHG7 was an independent prognosis index of patients with HCC ($P<0.05$). These findings suggested that lncRNA-SNHG7 might play an important role in the progression of HCC.

Discussion

HCC is characterized by insidious onset and rapid progress. The vast majority of patients are diagnosed in the middle

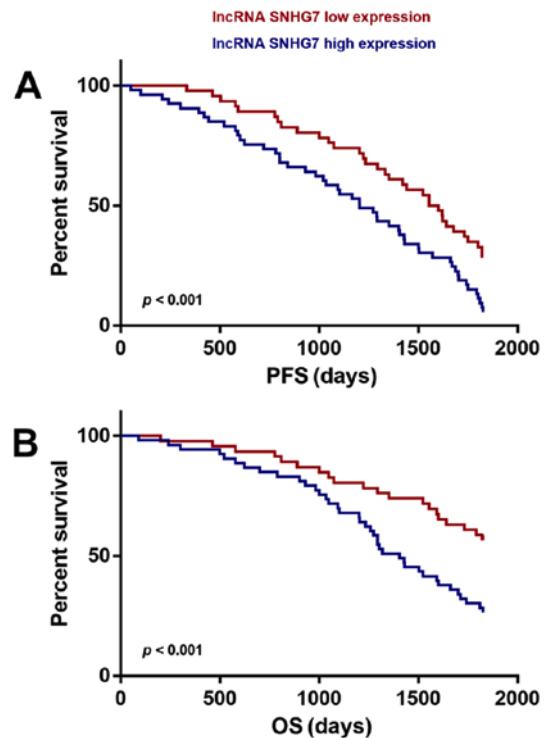


Figure 2. Relationship of lncRNA SNHG7 expression with progression-free survival (PFS) (A) and overall survival (OS) (B) of HCC patients. lncRNA-SNHG7, long non-coding RNA SNHG7; HCC, hepatocellular carcinoma.

and late stages, thus missing the best time for surgery. Even with radical resection, the postoperative recurrence and metastasis still significantly impair the long-term survival of patients (18,19). In order to realize early detection of HCC and improve the prognosis of patients, the cellular and molecular biological mechanisms leading to HCC invasion and metastasis have attracted extensive attention and study.

Sequencing of the human genome indicated that protein-encoding genes account for <3% of all sequences in the human genome, and >80% of the sequences are frequently

transcribed into RNAs without protein-encoding functions, called non-coding RNAs. They are a complex network that regulates gene expression and a key to the regulation of many essential biological functions of tumor cells (10,20,21). They are categorized into short non-coding RNAs and lncRNAs based on different sequence lengths (12). lncRNAs cannot be translated into proteins, but they could affect gene transcription, protein modification after translation and epigenetic regulation (22,23). A growing number of studies have shown that lncRNAs are abnormally expressed in various types of tumors, which imply their potential value in the early diagnosis of certain tumors and were considered as markers for early diagnosis. lncRNA-JADE was found with a key functional link that connects the DNA damage response (DDR) to histone H4 acetylation, and that dysregulation of lncRNA-JADE might contribute to breast tumorigenesis (24). The positive lncRNA-HOTAIR/HER2 correlation was associated with gastric cancer development. High expression of HOTAIR was associated with shorter overall survival of gastric cancer patients (25). lncRNA MALAT-1 was positively related to Bcl-2 expression in lung cancer and weak MALAT-1 expression patients with resected lung cancer demonstrated adverse prognosis (26).

Various studies have pointed out that lncRNA-SNHG7 is highly expressed in various malignant tumors and is a potential molecular marker. Qi *et al* (27) indicated that through miR-503/cyclin D1 pathway, lncRNA-SNHG7 promoted the development of prostate cancer. Colorectal cancer patients with high expression of lncRNA-SNHG7 had a significantly poor prognosis (28). Further results indicated that lncRNA-SNHG7 facilitated the proliferation and metastasis of tumor cells by regulating GALNT7 expression and PI3K/Akt/mTOR pathway. She *et al* (14) found that lncRNA-SNHG7 was highly expressed in lung cancer tissues, and the inhibition of lncRNA-SNHG7 expression could promote apoptosis of lung cancer cells. The role of lncRNA-SNHG7 in lung cancer was through the regulation of FAIM2 gene. The above studies indicated that lncRNA-SNHG7 might become a potential target for the treatment of tumors. However, so far, studies on the expression and biological functions of lncRNA-SNHG7 in tumor tissues are relatively few, and there are no relevant reports involving the field of HCC.

It was discovered in this study that the expression level of lncRNA-SNHG7 in HCC tissues was much higher than that in normal adjacent tissues, suggesting that the inhibition of lncRNA-SNHG7 expression might be related to the occurrence and procession of HCC. The correlation of the expression of lncRNA-SNHG7 with clinicopathological features of HCC patients found that the high expression of lncRNA-SNHG7 in HCC tissues was related to tumor number, lymph node metastasis and clinical stage but not associated with patient's sex, age, AFP levels, HBsAg levels and tumor size.

Multidisciplinary team (MDT) diagnosis and treatment of HCC patients by various treatment methods has gradually become a characteristic trend of HCC treatment (29). In clinical practice, certain indicators that were capable of accurately predicting the prognosis would promote the identification of people with strong recurrence tendency and provide the corresponding basis for prevention and treatment, thus preventing recurrence as early as possible. This study

analyzed the clinicopathological features of 100 HCC patients, and revealed that high expression of lncRNA-SNHG7 in HCC patients indicated a poor prognosis. Moreover, the results of univariate analysis and multivariate analysis model showed that lncRNA-SNHG7 expression was an influential factor for the prognosis of HCC patients. Clinical detection of lncRNA-SNHG7 expression level might assist in making the prognosis of HCC patients.

In conclusion, lncRNA-SNHG7 might contribute to the development of HCC and serve as a clinical biomarker and a therapeutic target for HCC patients.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

AS and XC designed the study and performed the experiments, AS and JM collected the data, XC and XH analyzed the data, AS and XC prepared the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Affiliated Hospital of Weifang Medical University (Weifang, China). Signed informed consents were obtained from the patients and/or the guardians.

Patient consent for publications

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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