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Long noncoding RNA SNHG1 predicts a poor prognosis and promotes hepatocellular carcinoma tumorigenesis

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ABSTRACT

Hepatocellular carcinoma (HCC) is the main cause of cancer mortality worldwide. Its poor prognosis is mainly ascribed to high recurrence rate. Identifying novel prognostic biomarkers and therapeutic targets would be vital for HCC management. Long noncoding RNA (lncRNA) is a class of RNA with various roles in tumorigenesis. The aim of this study was to investigate the clinical significance and functions of lncRNA-small nucleolar RNA host gene 1 (SNHG1) in HCC. In this study, we found SNHG1 was upregulated in HCC tissues in comparison with adjacent liver tissues in both publicly available microarray data and our own cohort. High SNHG1 expression was correlated with large tumor size, poor differentiation, and aggressive BCLC stage. Kaplan-Meier survival analysis demonstrated that high SNHG1 expression predicts poor prognosis of HCC patients. Gain-of-function and loss-of function experiments showed that SNHG1 promotes HCC cells proliferation, cell cycle progression, and inhibits HCC cells apoptosis. Further experiments revealed that SNHG1 promotes HCC cells proliferation through inhibiting p53 and p53-target genes expression. Collectively, our results demonstrated the clinical prognostic significance and roles of SNHG1 in HCC, and suggested that SNHG1 may be considered as a prognostic biomarker and therapeutic target for HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the second most common cause of cancer mortality worldwide, with nearly 600,000 deaths occurring each year [1,2]. Despite recent advances in diagnosis and therapies of HCC, the prognosis of HCC patients remains poor due to high tumor recurrence rate after surgery [3]. Thus, it is imperative to comprehend the molecular mechanisms underlying HCC tumorigenesis and to identify new prognosis evaluation biomarkers and therapeutic targets for HCC [4–6].

Long noncoding RNAs (lncRNAs) are a class of RNA transcripts, with over 200 nucleotides in length and no protein-coding potential [7,8]. Increasing numbers of reports have shown the complexity and diversity of their functions and mechanisms of action [9,10]. Aberrant expressions of certain lncRNAs have been confirmed in a variety of cancers, including HCC, lung cancer, gastric cancer, cervical cancer, and et al. [11–15]. More importantly, lncRNAs play pivotal roles in nearly all aspects of tumor biology [16,17]. Their dysregulation is an important cause of tumorigenesis and accelerates tumor progression [18,19]. However, the clinical prognosis significance and potential functions of lncRNAs are poorly understood in HCC tumorigenesis.

In the present study, we focus on an lncRNA termed small nucleolar RNA host gene 1 (SNHG1, GenBank accession ID: 23642). SNHG1 localized at 11q12.3 and has 11 exons. We examined the expression pattern of SNHG1 in human HCC tissues and cell lines, and explored its correlation with clinicopathological characteristics and patients' recurrence and survival. Furthermore, we

Abbreviations: lncRNA, long noncoding RNA; HCC, hepatocellular carcinoma; SNHG1, small nucleolar RNA host gene 1; qRT-PCR, quantitative real-time polymerase chain reaction; siRNA, small interfering RNA; CCK-8, Cell Counting Kit-8; BCLC, Barcelona Clinic Liver Cancer; HBsAg, hepatitis B surface antigen; AFP, alpha-fetoprotein.

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investigated the functions of SNHG1 on HCC tumorigenesis and the underlying molecular mechanisms. Our results suggest that SNHG1 may be used as a novel prognostic biomarker and a potential therapeutic target for HCC.

2. Materials and methods

2.1. Patients and tissues samples

This study was reviewed and approved by the Institutional Review Board of the First Affiliated Hospital of Liaoning Medical University. All patients signed informed consent. Fresh HCC tissues and paired adjacent non-tumor liver tissues from 82 patients were obtained from patients with HCC undergoing hepatectomy at the First Affiliated Hospital of Liaoning Medical University. All tissues specimens were confirmed by pathological examination. None of the patients recruited in this study received preoperative treatments. The clinicopathological characteristics of all patients are shown in Table 1.

2.2. Cell lines and cell culture

The human HCC cell lines SMMC-7721, MHCC97H, HCCLM3 and HepG2, and human normal hepatocyte cell lines QSG-7701 and L02, were obtained from Chinese Academy of Sciences (Shanghai, China). All the cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (Gibco, NY, USA) in an incubator at 37 °C containing 5% CO2.

2.3. RNA extraction, reverse transcription, and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from cells or tissues specimens using TRIzol reagent (Invitrogen, Carlsbad, USA) following the manufacturer's instruction. 2 μg of RNA were reversely transcribed into cDNA by the M-MLV Reverse Transcriptase (Invitrogen). The expressions of target genes were determined using a standard SYBR-Green method on ABI7300 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). All reactions were run in triplicate using genes specific primers. GAPDH was employed as an endogenous control. The primer sequences used were as follows: 5′-AGGCTGAAGTTACAGGTC-3′ (forward) and 5′-TTGGCTCCCAGTGTCTTA-3′ (reverse) for SNHG1; 5′-GGAGCGAGATCCCTCCAAAAT-3′ (forward) and 5′-GGCTGTTGTCATACTTCTCATGG-3′ (reverse) for GAPDH; 5′-TTGCTTCAGGGTTTCATCCAG-3′ (forward) and 5′-GACACTCGCTCAGCTTCTTG-3′ (reverse) for BAX; 5′-CATGGCTTAGAAGTGGAAAT-3′ (forward) and 5′-TTGGTGTTGCTGGTGAGT-3′ (reverse) for FAS; 5′-TGTCACTGTCTTGTACCCTTG-3′ (forward) and 5′-TTCCTGTGGGCGGATTAG-3′ (reverse) for CDKN1A. The relative expressions of target genes were calculated using the $2^{-\triangle\triangle Ct}$ method.

2.4. Vectors construction, small interfering RNA (siRNA) synthesis, and transfection

SNHG1 transcript was PCR amplified using the Phusion Flash High-Fidelity PCR Master Mix (Thermo Fisher, Waltham, MA, USA) and subcloned into the *Kpn* I and *Bam*H I sites of pcDNA ™ 3.1 (+) (Invitrogen). The primers used were as follows: 5′-GGGGTACCGTTCTCATTTTTCTACTGCTCGTG-3′ (forward) and 5′-CGGGATCCATGTAATCAATCATTTTATTATTTTCATC-3′ (reverse).

siRNAs specifically targeting SNHG1 and scrambled negative control were synthesized by GenePharma (Shanghai, China). The sequences of the siRNA for SNHG1 were CAGCAGTTGAGGGTTTGCTGTGTAT. Human HCC cells were transfected with vectors or siRNAs using Lipofectamine 3000 (Invitrogen) according to the manufacturer's instructions.

2.5. Cell proliferation assay

Equal number of transfected human HCC cells was plated in each well of a 96-well plate, and cell proliferation was measured using Cell Counting Kit-8 (CCK-8, Dojindo Laboratories, Kumamoto, Japan) every 24 h according to the manufacturer's protocols. Cell proliferation was also assessed by colony formation assay. Equal number of transfected human HCC cells was plated in each well of a 6-well plate, and cultured in DMEM with 10% FBS for 14 days. Then the cells were fixed and stained with 0.1% crystal violet. The number of stained colonies was counted.

2.6. Flow cytometric analysis of cell cycle and apoptosis

Transfected human HCC cells were harvested and stained with propidium iodide according to the Cell Cycle Analysis Kit (Biyuntian, Jiangsu, China), and then assessed by flow cytometer. The percentage of the cells in different phases was counted. For

Table 1
Correlation between SNHG1 expression and clinicopathological characteristics in HCC.

Characteristics	SNHG1 expression		*P*
	Low	High	
Gender			0.762
Male	34	35	
Female	7	6	
Age (years)			0.503
>55	16	19	
≤55	25	22	
HBsAg			0.364
Positive	33	36	
Negative	8	5	
Liver cirrhosis			0.494
With	24	27	
Without	17	14	
AFP (ng/ml)			0.240
>20	25	30	
≤20	16	11	
Tumor size (cm)			0.018
>5	8	18	
≤5	33	23	
Tumor number			0.577
Single	34	32	
Multiple	7	9	
Tumor differentiation			0.031
Well/moderate	33	24	
Poor	8	17	
Encapsulation			0.122
Complete	24	17	
No	17	24	
Vascular invasion			0.052
Present	8	16	
Absent	33	25	
BCLC stage			0.025
A	29	19	
B–C	12	22	

HBsAg, hepatitis B surface antigen; AFP, alpha-fetoprotein.