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ORIGINAL ARTICLE

# Clinical significance of C/D box small nucleolar RNA U76 as an oncogene and a prognostic biomarker in hepatocellular carcinoma

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**KEYWORDS**
Small nucleolar RNA;
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**Summary**
*Background:* Recent evidence has suggested novel roles of small nucleolar RNAs (snoRNAs) in tumorigenicity. However, the roles of C/D box snoRNA U76 (SNORD76) in the development of hepatocellular carcinoma (HCC) remain unknown. Herein, we systematically evaluated dysregulation of snoRNAs in HCC and clarified the biomarker potential and biological significance of SNORD76 in HCC.
*Methods:* We performed quantitative analyses of the expression of SNORD76 in 66 HCC specimens to compare its expression pattern between tumor tissue and matched non-tumor tissue. The effects of SNORD76 on HCC tumorigenicity were investigated in SK-Hep1 and Huh7 cells as well as in a xenograft nude mouse model.
*Results:* SNORD76 expression was significantly upregulated in HCC tissues compared to corresponding non-tumor tissues. This upregulation of SNORD76 in HCC tumors was significantly associated with poorer patient survival. Furthermore, inhibiting SNORD76 expression suppressed cell proliferation by inducing G0/G1 cell cycle arrest and apoptosis. Low SNORD76 expression also resulted in decreased HCC growth in an animal model. Conversely, overexpressing SNORD76

*Abbreviations:* snoRNAs, small nucleolar RNAs; SNORD76, C/D box snoRNA U76; HCC, hepatocellular carcinoma; EMT, epithelial-mesenchymal transition; ssnoRNPs, small nucleolar RNP; RNP, ribonucleoprotein particles; NSCLC, non-small cell lung cancer; BCLC, Barcelona Clinic Liver Cancer Stage; PVTT, portal vein tumor thrombus; GAS5, growth arrest-specific transcript 5; OS, overall survival; qRT-PCR, quantitative real-time polymerase chain reaction; ROC, receiver operating characteristic; ncRNA, snoncoding RNAs.

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promoted cell proliferation. SNORD76 increased HCC cell invasion by inducing epithelial-mesenchymal transition (EMT). Finally, we found that SNORD76 promoted HCC tumorigenicity through activation of the Wnt/β-catenin pathway.
*Conclusions:* Therefore, we demonstrated for the first time that SNORD76 may function as a novel tumor promoter in HCC and may serve as a promising prognostic biomarker in patients with HCC.


## Introduction

Hepatocellular carcinoma (HCC) is one of the most common fatal cancers worldwide [1,2], representing the third leading cause of cancer death globally and the second leading cause of cancer death in China and Southeast Asia [3]. The poor prognosis of this disease is mainly attributable to the high rate of tumor recurrence or metastasis, accounting for approximately 90% of HCC-related mortality [4,5]. Consequently, it is of urgent need to identify novel biomarkers of HCC and to reveal the precise molecular mechanism underlying the development of HCC.

Small nucleolar RNAs (snoRNAs) are a massive group of noncoding RNAs (ncRNAs) 60 to 300 nucleotides in length [6]. snoRNAs interact with a set of proteins to form small nucleolar RNPs (snoRNPs), which function as guide RNAs in the post-transcriptional synthesis of 2-O-methylated nucleotides and pseudouridines in rRNAs, small nuclear RNAs (snRNAs) and probably other cellular RNAs, including even mRNAs [7]. C/D box snoRNA U76 (SNORD76) is located at 1q25 locus and is excised from the intron of growth arrest-specific transcript 5 (GAS5) [8]. snoRNAs were formerly recognized to possess housekeeping functions due to their critical roles in rRNA maturation, whereas snoRNAs were found to have a relatively small impact on cellular homeostasis [9].

However, accumulating research has demonstrated that dysregulated snoRNAs may play a role in human malignancies. Higher plasma levels of SNORD33, SNORD66, SNORD76 were found in NSCLC tissue than in non-tumor tissue with high sensitivity and specificity [10]. SNORA42 also was frequently overexpressed during lung tumorigenicity [11,12] and in colorectal cancer [13], suggesting that this snoRNA may be a relevant diagnostic and therapeutic marker of these forms of cancer. Despite increasing evidence that snoRNAs might have a crucial role in controlling cell behavior and although snoRNA dysfunction might significantly contribute to carcinogenesis, few studies have investigated the clinical significance and functional roles of snoRNAs in HCC progression.

In the present study, we first demonstrated that SNORD76 was significantly upregulated in HCC tissues compared with adjacent non-tumor tissues and that this upregulation of SNORD76 was associated with decreased survival of HCC patients. Furthermore, functional studies in which SNORD76 expression was altered in HCC cells in vitro and in vivo consistently indicated that SNORD76 promoted HCC cell growth and tumorigenicity.

## Materials and methods

### Patient and clinical sample collection

A total of 66 pairs of human HCC and adjacent non-tumor tissues were obtained from surgical specimens immediately after resection from patients undergoing primary surgical treatment for HCC in Zhongnan Hospital of Wuhan University. The diagnosis of HCC was histopathologically confirmed. Written consent for tissue donation was obtained from the patients prior to tissue collection, and the study protocol was approved by the Protection of Human Subjects Committee of Zhongnan Hospital. Clinical and pathological information was extracted from the patients' medical charts and their pathology reports. Overall survival (OS) was defined as the interval between resection and death or the last follow-up visit.

### Cell culture

The human HCC cell lines Hep3B, SK-Hep1, Huh7, HepG2, and HCC-LM3 and the normal liver cell line HL-7702 were purchased from the Cell Bank of Type Culture Collection (CBTCC, Chinese Academy of Sciences, Shanghai, China) and cultured in high-glucose DMEM (Gibco, USA) supplemented with 10% FBS (Gibco, USA). Cells were maintained in a humidified incubator at 37 in the presence of 5% CO2.

### RNA extraction and quantitative real-time PCR analysis

Total RNA was isolated from tumor tissues and cell lines using TRIzol reagent (Life Technologies, USA). Reverse transcription was performed using a miRNA cDNA Synthesis Kit, with Poly (A) Polymerase Tailing (ABM, Canada). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using SYBR qPCR Mix (Toyobo, Osaka, Japan) in a CFX Connect Real-Time PCR Detection System (Bio-Rad, USA). The U6 level was measured as an internal control. The relative fold changes in the expression of snoRNAs were calculated using the $2^{-\Delta\Delta Ct}$ method. The primer sequences were as follows: SNORD76: forward 5'-TGCCACAATGATGACAGTTTATTTG-3', reverse 5'-GCCTCAGTTAAGATAATGGTGGTT-3'; U6: forward 5'-GCTTCGGCAGCACATATACTAAAAT-3', reverse 5'-CGCTTCACGAATTTGCGTGTCAT-3'.

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