European Review for Medical and Pharmacological Sciences 2019; 23: 5699-5704

Long noncoding RNA SNHG7 represses the expression of RBM5 to strengthen metastasis of hepatocellular carcinoma

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Abstract. – OBJECTIVE: Long noncoding RNAs (lncRNAs) have been reported to be vital in tumor progression. Hepatocellular carcinoma (HCC) is a common type of fatal primary liver cancers worldwide. This study aims to determine whether lncRNA SNHG7 (small nucleolar RNA host gene 7) functions in the metastasis of HCC.

PATIENTS AND METHODS: Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was conducted to detect the SNHG7 expression in HCC cells and tissue samples. Mo function assays were performed in vitro tify the role of SNHG7 in metastasis o cells. Western blot assay was used to ex the possible mechanism.

RESULTS: SNHG7 expression was rema ably higher in HCC tissues tha adjacen tissues. Moreover, HCC mig nvasion were suppressed after sile of SNH in HCC cells. Moreover, after sil of SN RBM5 was upregulated in H lls. expression of RBM5 tu was neg atively correlated t expre of SNHG7.

CONCLUSIONS: study sug that SNHG7 could prom ll invasion a gration in HCC cells ownregulatin RBM5, which may o a new peutic intervention for HCC pa ents.

Key Wor
Lon ncodin NA, SNHG7, Hepatocellular carcino BM5.

oduction

r carcinoma (HCC) is one of the ary liver cancers and accounts for nearly 90% l primary liver neoplasms. It has become one o ost common cancers among which HCC has second highest cancer-related mortality[1]. Moreov CC-r mortality is significantly higher in developing ies, especially in China. epatitis B vi BV) is particularly p ent among people[2,3]. Despite that advances e been made i he diagnosis and management HCC in the pa ears, the morbidity and mor- of HCC re high, and the 5-year overall s l rate is than 20%[4,5]. Therefore, understan rlying molecular mechanism of HCC is urgent and could improve the diagnosis, anagement, and prognosis of HCC patients.

echnology of human genome sequenc- g de ops, it is widely known that more than 90% of human DNA is converted into noncoding RNAs (ncRNAs). Long noncoding RNAs (lncRNAs), one subtype of ncRNAs, have caught much attention for its important role in the development and progression of cancers. For example, lncRNA HOTAIR promotes the proliferation and invasion of cervical cancer cells through targeting the Notch pathway[6]. LncRNA LINC00092 acts as an important driver of metastatic progression in the progression of ovarian cancer and is mediated by cancer-associated fibroblasts[7]. Repression of lncRNA NEAT1 promotes the development of prostate cancer by disturbing the cell cycle and inhibiting the proliferation of prostate cancer cells[8]. LncRNA NR_036575.1 acts as an oncogene in thyroid cancer by contributing to the cell proliferation and cell migration and could be applied as a potential biomarker and therapeutic target[9]. In addition, lncRNA SNHG5 serves as an important anti-oncogene in the progression of gastric cancer through trapping MTA2 in the cytosol[10]. However, the function of SNHG7 (small nucleolar RNA host gene 7) in HCC and the potential molecular mechanism haven't been studied so far.

In the present study, lncRNA SNHG7 expression was significantly upregulated in HCC sam-

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ples. Moreover, silence of SNHG7 repressed the invasion and migration of HCC cells. Furthermore, we discovered that lncRNA SNHG7 repressed RBM5 expression and promoted metastasis of HCC cells.

Patients and Methods

Patients and Sample Collection

A total of 55 paired HCC tissues and adjacent non-cancer samples were obtained sequentially from the patients who undergo hepatectomy in China-Japan Union Hospital of Jilin University in 2011-2017. All tissues were kept at –80°C. The written informed consent was obtained from every participant before the surgery. The protocol of the study was approved by the Ethics Committee of China-Japan Union Hospital of Jilin University.

Cell Culture

HepG2 and Bel-7402 HCC cell lines, and a normal liver epithelial cell L02 (Chinese Type Culture Collection, Chinese Academy of Sciences; Shanghai, China) were used in this study. Culture medium consisted of 100U/mL of penicillin, Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Rockville, MD, USA) and 10% fetal bovine serum (Gibco, Life Technologies, Gaithersburg, MD, USA). Besides, cells were cultured at 37°C in a humidified incubator containing 5% CO_2.

Quantitative Real Time Polymerase Chain Reaction (qRT-PCR)

According to the manufacturer's protocol, total RNA, extracted from tissue samples and cells with TRIzol reagent (Invitrogen, Carlsbad, CA, USA), was reverse transcribed into complementary deoxyribose nucleic acids (cDNAs) using reverse Transcription kit (TaKaRa, Dalian, China). The performance of qRT-PCR was conducted on the ABI 7500 system (Thermo Fisher Scientific, Waltham, MA, USA). And SYBR Green real-time PCR (TaKaRa, Dalian, China) was applied following the primers using for qRT-PCR: SNHG7 primers forward 5′-GTTCGCTGGGA-3′, reverse 5′-GGCCATCTGCTTTATTCC-3′; β-actin forward 5′-GATGGAAATCGTCAGAGGCT-3′ and reverse 5′-GGCACTTAGTTGGAAATGC-3′. The thermal cycle was as follows: 30 sec at 95°C, 5 sec at 95°C for 40 cycles, 35 sec at 60°C. The relative expression was calculated by performing the $2^{-\Delta\Delta CT}$ method.

Cell Transfection

We purchased lentivirus expressing short hairpin RNA (shRNA) against SNHG7 from GeneChem Pharma (Shanghai, China). Another, Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) was then used for the transfection of HCC cells. These treated cells were divided into three groups, empty vector group and SNHG7 shRNA group. The transfection efficiency was detected by qRT-PCR.

Wound Healing Assay

Empty vector and SNHG7 shRNA were transfected into HCC cells. The treated cells were cultured in 6-well plates and grown to about 90% confluent. The cells were scratched by a sterile 10 μL pipette tip and incubated at 37°C in a humidified incubator containing 5% CO_2. The wound closure was determined at 0 and 24 h. The experiments were performed for three times.

Transwell Assay

1×10^5 transfected cells in 100 μL serum-free DMEM were cultured in 8 μm pore size culture inserts (Corning, NY, USA) which were previously added with Matrigel (50 μg; BD, Bedford, MA, USA). In the bottom chamber, DMEM (500 μL) were added. 48 h later, a cotton swab was used to wipe the top surface of chambers and immersed for 10 min with precooling methanol. Following were stained in crystal violet for 30 min. The count for the invasion was counted in three fields *via* an inverted microscope (×20).

Western Blot Analysis

Anti-β-actin and anti-RBM5 were obtained from Abcam (Cambridge, MA, USA). After separated with 12% Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE), the protein samples were transferred onto polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). The primary antibodies were utilized to incubate the membranes at 4°C for the whole night. Furthermore, after washed, membranes were incubated with goat anti-rabbit secondary antibody (ProSci, Poway, CA, USA) for 2 h. Enhanced chemiluminescence (ECL) Western blotting detection reagents (Pierce antibodies; Rockford, IL, USA) was then used. Image J software (NIH, Bethesda, MD, USA) was applied for assessment of protein expression.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 19.0 (SPSS, Chicago, IL, USA) was uti-

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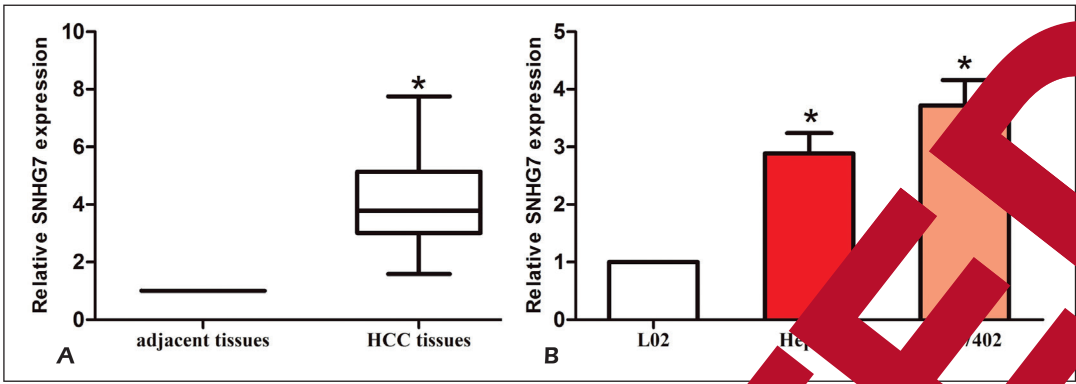


Figure 1. Expression levels of SNHG7 were increased in HCC tissues and cell lin[illegible], SNHG7 ex[illegible]significantly increased in the HCC tissues compared with adjacent tissues. **B**, Expression l[illegible]f SNHG7 relati[illegible]ctin were determined in the human HCC cell lines and normal liver epithelial cell (L02) [illegible] Data are prese[illegible]d as the mean ± standard error of the mean. *$p<0.05$.

lized to perform statistical analysis. The independent-sample test was used to compare continuous data. It was considered statistically significant when $p<0.05$.

Results

Expression Level of SNHG7 in HCC Tissues and Cells

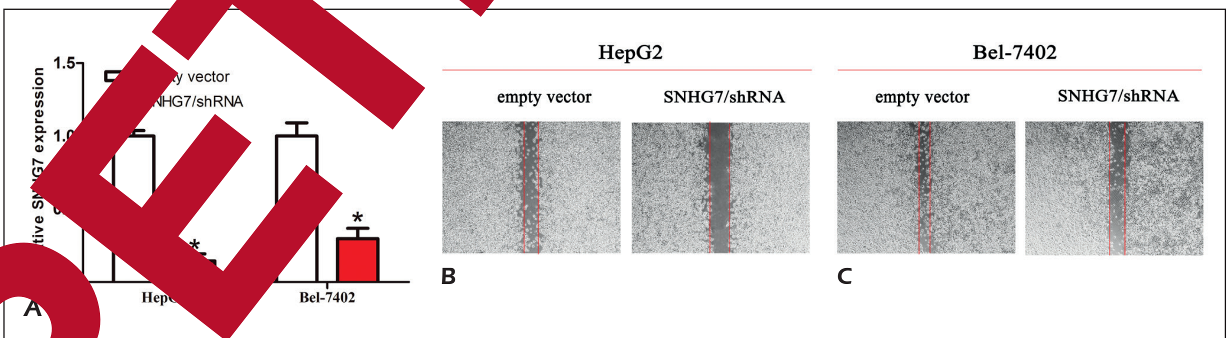
First, we performed qRT-PCR to de[illegible] SNHG7 expression in 55 paired patients' ti[illegible]sues and 5 HCC cells. The re[illegible]aled that SNHG7 was significantly [illegible]gulat[illegible]n HCC tissue samples than that i[illegible]jacent t[illegible]es (Figure 1A). Compared wit[illegible]xpre[illegible] SNHG7 expression le[illegible] wa[illegible]ntly higher in HCC cells (Figur[illegible]).

S[illegible]ce of SNHG7 Inhib[illegible]ted Cell [illegible]gration in H[illegible]C Cells

The SNHG7 s[illegible]NA and the empty vector were [illegible]hetized and [illegible]sfected into HepG2 and Bel-7[illegible]CC cell[illegible]en, the transfection efficiency was [illegible]y qRT-PCR (Figure 2A). We performed wo[illegible] healing assay and found that silence [illegible] SNHG7 inhibited HepG2 HCC cell migration [illegible]). Similarly, the silence of SNHG7 inhibit[illegible] cell migration in Bel-7402 HCC cells (Figure 2C).

Silence of SNHG7 Inhibited Cell Invasion in HCC Cells

Transwell assay results showed that number of invaded cells was decreased *via* silence of SNHG7 in HepG2 HCC cell (Figure 3A). Similarly, the number of invaded cells was decreased *via* silence of SNHG7 in Bel-7402 HCC cells (Figure 3B).



Fi[illegible] SNHG7 inhibited HCC cell migration. **A**, SNHG7 expression in HCC cells transduced with empty vector [illegible]HG7 sh[illegible] was detected by qRT-PCR. β-actin was used as an internal control. **B**, Wound healing assay showed that [illegible]migration in SNHG7 shRNA group was markedly inhibited compared with empty vector group in HepG2 HCC cells. **C**, [illegible] healing assay showed that cell migration in SNHG7 shRNA group was significantly inhibited compared with empty ve[illegible]up in Bel-7402 HCC cells. The results represent the average of three independent experiments (mean ± standard error [illegible]e mean). *$p<0.05$.

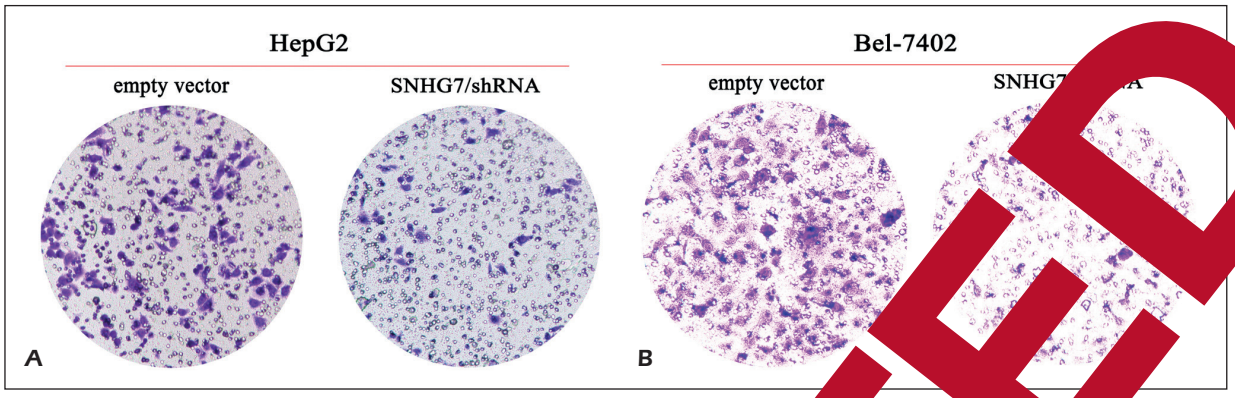


Figure 3. Silence of SNHG7 inhibited HCC cell invasion. **A**, Transwell assay sho[w]ed that number of [in]vaded cells in SNHG7 shRNA group was significantly decreased compared with empty vector group [in] HCC cells (ma[g]nification: 40×). **B**, Transwell assay showed that number of invaded cells in SNHG7 shRNA g[r]oup was [s]ignificantly decreased compared with empty vector group in Bel-7402 HCC cells (magnification: 40×). The results [p]resent th[e] mean of three independent experiments (mean ± standard error of the mean). *$p<0.05$.

Silence of SNHG7 Inhibited HCC Tumorigenesis Via Regulating RBM5

RBM5 has been identified to play a vital role in the metastasis of HCC. To explore the interaction between RBM5 and SNHG7, we performed qRT-PCR and found that the RBM5 mRNA expression was upregulated in HCC cells transfected [w]ith SNHG7 shRNA (Figure 4A). Western b[l]ot analysis results also revealed that the protein [l]evel of RBM5 was upregulated in HCC cells tran[s]fected with SNHG7 shRNA (Figure 4B). Moreo[v]er, RBM5 expression of HCC tissues [w]as marked[l]y lower in HCC tissues compar[e]d [t]hat of adjacent tissues (Figure 4C). T[h]e mean[i]ng relation analysis further revealed [t]hat the RB[M5] expression negatively correlate[d] [w]ith SNHG7 in HCC tissues (Figur[e] 4D).

Discussion

In recent years, numero[u]s studies have revealed that lncR[N]As function as im[p]ortant regulators of HCC a[n]d participate in the mo[l]ecular processes of HC[C] development. For instance, lncRNA CD-KN[2]B-AS1 pro[m]otes cell [g]rowth and cell migration [t]hrough [m]iR-153-5p/ARHGAP18 signaling [p]athway[11]. [T]hrough modulating the [E]RK-MAP[K] pathway, downregulation of lncRNA P7 fa[c]ilitates cell proliferation in HCC and [i]s associated with unfavorable prognosis[12]. As a [t]umor-149, lncRNA SNHG8 enhances [t]umorigenesis and metastasis in HCC and may be [a] potential biomarker and therapeutic strategy for [H]CC patients[13]. By sponging miR-206, lncRNA LINC00707 functions as an oncogene in the progression of HCC[14]. In addition, through regulating miR-202-5p, lncRNA NORAD enhances the [p]rogression of HCC *via* modulation of TGF-beta pathway[15].

SNHG7, 217[4] bp in length, is located on chromosome [1]. Chen et al[16] have indicated that SNHG7 is upregulated in many cancers. For example, the silence of SNHG7 inhibits proliferation and migration of bladder cancer cells *via* inactivation of Wnt/β-catenin pathway. Targeted by microRNA-186, SNHG7 facilitates cell proliferation and cell invasion in breast cancer and is associated with the malignant progression of breast cancer[17]. As a sponge of miR-503, SNHG7 enhances cell proliferation and cycle progression in prostate cancer through cyclin D1[18]. In addition, knockdown of SNHG7 significantly inhibits cell proliferation and cell migration in glioblastoma through inhibition of miR-5095[19]. In the present study, SNHG7 was found to be upregulated in both HCC tissue and cells. Furthermore, after SNHG7 was silenced, the ability of cell migration and invasion was inhibited. These data indicated that SNHG7 functioned as an oncogene and enhances the metastasis of HCC.

The RNA binding protein, RBM5, which resides within the 3p21.3 region, is significantly downregulated in many cancers and participates in tumor progressions. For example, the expression level of RBM5 mRNA and protein is significantly downregulated in lung adenocarcinoma and non-small cell lung cancer and is a diagnostic marker for patients with lung cancer[20,21]. Overexpression of RBM5 inhibits cell growth and cell invasion in prostate cancer by depressing the function of miR- 483-5p[22]. In addition, RBM5 is

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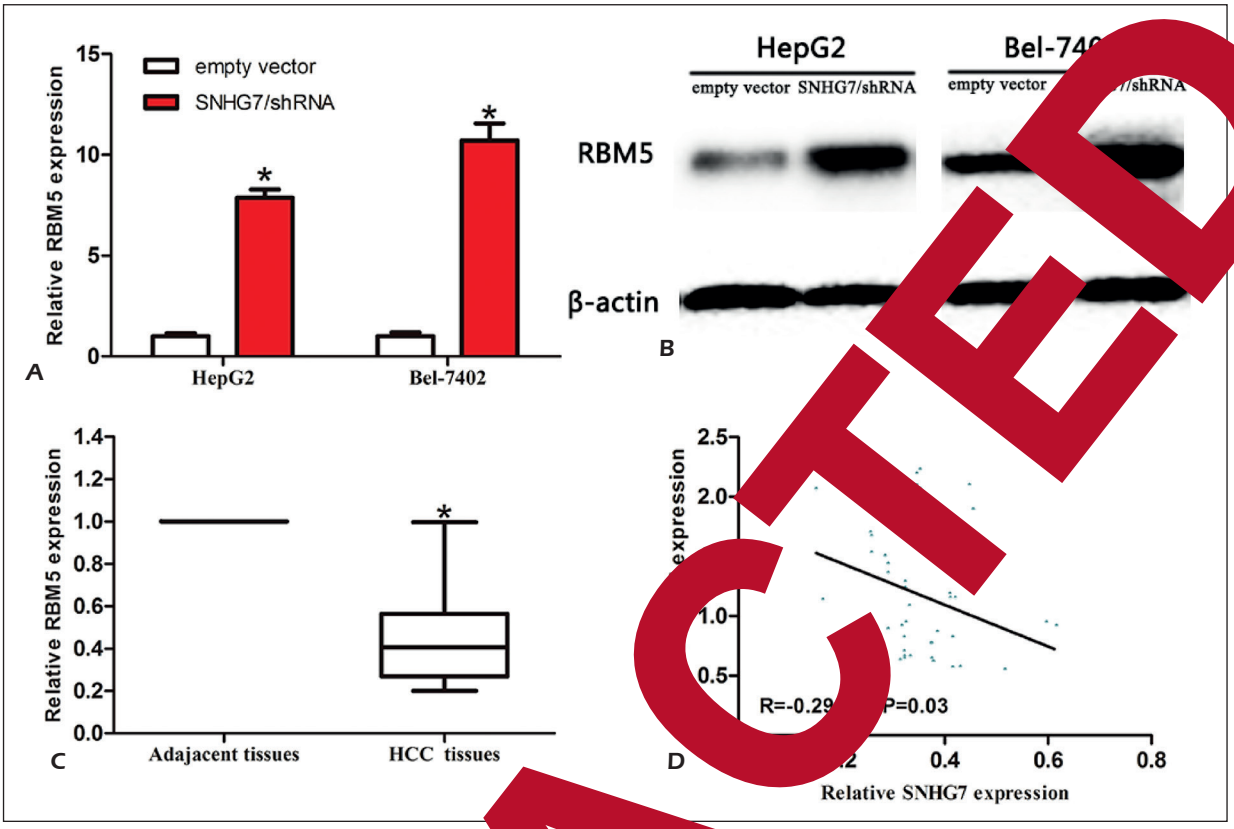

Figure 4. Interaction between RBM5 and SNHG7 in HCC. **A**, Expression level of RBM5 in SNHG7 shRNA group was significantly increased compared with empty vector group in HCC cells. **B**, Protein expression of RBM5 was increased after silence of SNHG7 in HCC cells. **C**, RBM5 was significantly downregulated in HCC tissues compared with adjacent tissues. **D**, The linear correlation between the expression level of RBM5 and SNHG7 in HCC tissues. The results represent the average of three independent experiments. Data are presented as the mean ± standard error of the mean. *p<0.05.

significantly down-expressed in pancreatic ductal adenocarcinoma and is related to advanced pathological characteristics of patients[23]. In our study, the result of western blot analysis indicated that RBM5 was upregulated when SNHG7 was overexpressed in vitro. What's more, positive correlation between RBM5 and SNHG7 expression was discovered in tumor tissues. The results above revealed that SNHG7 plays its function in HCC through repressing RBM5.

Conclusions

In conclusion, lncRNA SNHG7 acts as an oncogene in the carcinogenesis of HCC through suppressing RBM5 and can be served as a promising therapeutic target for HCC patients.

Conflict of Interests

The authors declare that they have no conflict of interest.

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