

# The Mechanism of Long Non-Coding RNA *SNHG7* in Cholangiocarcinoma Cell Proliferation, Migration, and Epithelial-Mesenchymal Transition

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**Abstract:** *Objective:* To investigate the mechanism of long non-coding RNA *SNHG7* and its regulatory effect on the proliferation, migration, and epithelial-mesenchymal transition of cholangiocarcinoma cells. *Methods:* A total of 20 pairs of cholangiocarcinoma and adjacent non-tumor bile duct tissues were collected from patients with cholangiocarcinoma who underwent surgery in the Affiliated Hospital of Hebei University (Hebei, China). Cholangiocarcinoma cell lines CCLP-1, QBC939, RBE, and HCC-9810 as well as normal human biliary epithelial cell line HIBEC were purchased for cell culture. We performed cell transfection, quantitative real-time polymerase chain reaction (qRT-PCR) to detect gene expression, Cell Counting Kit-8 (CCK-8) experiment to determine cell proliferation ability, scratch test to determine cell migration ability, and Transwell test to detect cell invasion ability. *Results:* The expression of lncRNA *SNHG7* in cholangiocarcinoma cell lines CCLP-1, QBC939, RBE, and HCC-9810 was  $3.21 \pm 1.01$ ,  $3.03 \pm 1.02$ ,  $2.98 \pm 1.21$ , and  $3.12 \pm 1.14$ , respectively, while its expression in normal cell line HIBEC was  $3.21 \pm 1.21$ ; the expression of lncRNA *SNHG7* in CCLP-1 was the highest; compared with HIBEC, the p values were all less than 0.05, indicating that the difference was statistically significant. The expression of miR-520f-3p in CCLP-1, QBC939, RBE, and HCC-9810 was  $1.45 \pm 0.75$ ,  $1.55 \pm 0.71$ ,  $1.54 \pm 0.73$ , and  $1.61 \pm 0.72$ , respectively, while its expression in normal cell line HIBEC was  $3.21 \pm 1.21$ ; the expression of miR-520f-3p in CCLP-1 was the lowest, and compared with HIBEC, the p values were all less than 0.05, indicating that the difference was statistically significant. In qRT-PCR, the expression of lncRNA *SNHG7* of si-NC ( $3.21 \pm 1.11$ ) was significantly higher than that of si-*SNHG7* ( $1.14 \pm 0.76$ ), and the p value was less than 0.05, indicating that the difference was statistically significant. In the CCK-8 experiment, the proliferation ability of CCLP-1 cells of the si-NC group at 24 h, 48 h, and 72 h was  $0.61 \pm 0.59$ ,  $0.75 \pm 0.68$ , and  $1.36 \pm 0.71$ , respectively; the proliferation ability of CCLP-1 cells of the si-*SNHG7* group at 24 h, 48 h, and 72 h was  $0.51 \pm 0.64$ ,  $0.59 \pm 0.59$ , and  $0.63 \pm 0.61$ , respectively; there was a significant decrease in the proliferation ability, and the p value was less than 0.05, indicating a statistically significant difference. After 24 h of scratch treatment, compared with the si-NC group, the migration ability of CCLP-1 cells of the si-*SNHG7* group was reduced ( $t = 6.356$ ,  $P = 0.026$ ). The results of Transwell test showed that the cell invasion ability of CCLP-1 in the si-*SNHG7* group was significantly reduced compared with the si-NC group ( $t = 7.845$ ,  $P = 0.032$ ). *Conclusion:* Exploring the gene expression mechanism in relation to the occurrence and development of cholangiocarcinoma is beneficial to future clinical work in terms of diagnosis, treatment, and prognosis. The knockdown of lncRNA *SNHG7* can effectively inhibit the proliferation, migration, and invasion of cholangiocarcinoma.

**Keywords:** RNA *SNHG7*; Cholangiocarcinoma; Cell proliferation; Migration

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## 1. Introduction

Cholangiocarcinoma (CCA) is a highly malignant tumor originating from bile duct epithelial cells. The incidence and mortality of CCA have been rising globally in recent years. Early invasion and metastasis are the distinguishing features of CCA, and the average survival time after diagnosis is less than 24 months. Although chemotherapy has improved the treatment status of CCA to a certain extent, the 5-year survival rate of CCA patients is only about 30%. Therefore, there is an urgent need to understand the molecular mechanisms of CCA occurrence and metastasis and seek new diagnostic and therapeutic approaches. Long non-coding RNAs (lncRNAs) are a group of transcripts longer than 200 nucleotides with little or no protein-coding capacity<sup>[1,2]</sup>. It has been reported that lncRNA plays an important role in many biological functions, such as regulating DNA transcription, regulating gene activity, and remodeling chromosome structure<sup>[3]</sup>. *SNHG7* is a conserved lncRNA that has important biological functions in different cancers. For example, the knockdown of lncRNA *SNHG7* inhibits the proliferation and migration of cholangiocarcinoma cells by activating the Wnt/ $\beta$ -catenin pathway, and lncRNA *SNHG7* sponges miR-449a to promote the progression of pituitary adenoma<sup>[4-7]</sup>. At present, there are several studies on cholangiocarcinoma cells. This study mainly investigates the role of lncRNA *SNHG7* in the proliferation and migration of cholangiocarcinoma cells.

## 2. Materials and methods

### 2.1. General information

A total of 20 pairs of cholangiocarcinoma and adjacent non-tumor bile duct tissues were collected from patients with cholangiocarcinoma who underwent surgery in the Affiliated Hospital of Hebei University (Hebei, China) with approval from the Ethics Committee of Hebei University People's Hospital and informed consent from the patients' families. The cholangiocarcinoma cell lines CCLP-1, QBC939, RBE, and HCCC-9810 as well as the normal human biliary epithelial cell line HIBEC were purchased.

### 2.2. Methods

#### 2.2.1. Cell Culture

CCLP-1, QBC939, RBE, HCCC-9810, and HIBEC cells were all cultured in Dulbecco's Modified Eagle Medium. All mediums were supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin double antibody. They were then placed in an incubator at 37°C with 5% volume fraction carbon dioxide (CO<sub>2</sub>).

#### 2.2.2. Cell transfection

The CCLP-1 cells were inoculated in 6-well plates before transfection. When the cell confluency reached 40–50%, *si-SNHG7*, *si-NC*, *miR-520f-3p* mimics, *miR-NC*, *miR-520f-3p* inhibitor, and *inh-NC* were transfected into CCLP-1 cells according to the instructions on LipofectamineTM3000 transfection reagent. After transfection, the cells were cultured for 24 h, and the cells in the logarithmic growth phase were taken for subsequent experiments.

#### 2.2.3. qRT-PCR detection of gene expression

TRIzol Kit was used, and the RNA was reverse transcribed into cDNA using a reverse transcription kit. SYBR Green Mastermix Kit and C1000 thermal cycler were used for qRT-PCR experiments, and *GAPDH* and *U6* were selected as internal controls for *SNHG7* and *miR-520f-3p*, respectively.

#### 2.2.4. CCK-8 experiment to detect cell proliferation ability

The cells in the logarithmic growth phase following transfection were digested with trypsin and seeded in

96-well plates at 1,000/well, with 5 multiple wells in each group. 10  $\mu\text{L}$  of CCK-8 solution was added to each well at 0 h, 24 h, 48 h, and 72 h, respectively, and it was cultured for 4 h. Then, the absorbance (A) value at a wavelength of 450 nm was detected by a microplate analyzer. The proliferation ability of the cells was evaluated.

### 2.2.5. Scratch test to detect cell migration ability

$5 \times 10^5$  CCLP-1 cells were transfected with the corresponding vector into a 6-well plate. After the cell confluence reached 90%, a ruler and a pipette tip were used to draw a line in the plate. Subsequently, phosphate-buffered saline (PBS) was used to wash three times, and serum-free culture medium was added. The scratch distance was observed with a fluorescent inverted microscope at 0 h and 24 h.

### 2.2.6. Transwell test to detect cell invasion ability

A layer of Matrigel matrix membrane was spread on the upper layer of the Transwell chamber, 200  $\mu\text{L}$  of cell suspension ( $1 \times 10^5/\text{mL}$ ) after transfection was seeded into the upper chamber of the Transwell chamber, and 800  $\mu\text{L}$  of medium containing 10% serum was added into the lower chamber. The cells were cultured for another 24 h; those remaining in the upper chamber were gently wiped off with a cotton swab. The cells were fixed with formaldehyde for 30 min and stained with 0.1% crystal violet for another 30 min. The invaded cells were observed with an inverted microscope ( $\times 100$ ).

## 2.3. Observation indicators

lncRNA *SNHG7* gene expression and *miR-520f-3p* expression.

## 2.4. Statistical analysis

All experiments were repeated at least three times. The results were reported as mean  $\pm$  standard deviation (SD) based on at least three replicates. GraphPad Prism 8.4.0 and SPSS 24.0 were used to perform the statistical analysis. Independent sample t-test was used to compare the means of two independent samples. The measurement data were expressed as mean  $\pm$  standard deviation ( $\bar{x} \pm s$ ). Paired t-tests were performed to assess the significant differences between the groups. The differences were statistically significant when the p value was less than 0.05.

## 3. Results

### 3.1. Expression of lncRNA *SNHG7* and *miR-520f-3p* in cell lines

The expression of lncRNA *SNHG7* in cholangiocarcinoma cell lines CCLP-1, QBC939, RBE, and HCCC-9810 was  $3.21 \pm 1.01$ ,  $3.03 \pm 1.02$ ,  $2.98 \pm 1.21$ , and  $3.12 \pm 1.14$ , respectively, while its expression in normal cell line HIBEC was  $3.21 \pm 1.21$ . The expression of lncRNA *SNHG7* in CCLP-1 was the highest, and compared with HIBEC, the p values were all less than 0.05, indicating that the difference was statistically significant. The expression of *miR-520f-3p* in cholangiocarcinoma cell lines CCLP-1, QBC939, RBE, and HCCC-9810 was  $1.45 \pm 0.75$ ,  $1.55 \pm 0.71$ ,  $1.54 \pm 0.73$ , and  $1.61 \pm 0.72$ , respectively, while its expression in normal cell line HIBEC was  $3.21 \pm 1.21$ . The expression of *Mir-520F-3p* in CCLP-1 was the lowest, and compared with HIBEC, the p values were all less than 0.05, indicating that the difference was statistically significant (Table 1).

**Table 1.** Expression of lncRNA *SNHG7* and *miR-520f-3p* in cell lines

Items (n=20)	lncRNA <i>SNHG7</i>	<i>miR-520f-3p</i>	t	P
HIBEC	1.54 ± 0.83	3.21 ± 1.21	5.0899	< 0.05
CCLP-1	3.21 ± 1.01	1.45 ± 0.75	6.2567	< 0.05
QBC939	3.03 ± 1.02	1.55 ± 0.71	5.3258	< 0.05
RBE	2.98 ± 1.21	1.54 ± 0.73	4.5571	< 0.05
HCCC-9810	3.12 ± 1.14	1.61 ± 0.72	5.0084	< 0.05

P: compared with HIBEC

### 3.2. Knockdown of *SNHG7* can inhibit the proliferation of cholangiocarcinoma CCLP-1 cells

In qRT-PCR, the lncRNA *SNHG7* expression of si-NC ( $3.21 \pm 1.11$ ) was significantly higher than that of si-*SNHG7* ( $1.14 \pm 0.76$ ), and the p value was less than 0.05, indicating that the difference was statistically significant. In the CCK-8 experiment, the proliferation ability of CCLP-1 cells in the si-NC group at 24 h, 48 h, and 72 h was  $0.61 \pm 0.59$ ,  $0.75 \pm 0.68$ , and  $1.36 \pm 0.71$ , respectively, while that of the si-*SNHG7* group was  $0.51 \pm 0.64$ ,  $0.59 \pm 0.59$ , and  $0.63 \pm 0.61$ , respectively. There was a significant reduction in the proliferation ability, and the p value was less than 0.05, thus indicating that the difference was statistically significant (Table 2).

**Table 2.** Analysis of the proliferation ability of CCLP-1 cells

Group (n = 20)	qRT-PCR detection	CCK-8 experiment		
	lncRNA <i>SNHG7</i>	24 h	48 h	72 h
si-NC	$3.21 \pm 1.11$	$0.61 \pm 0.59$	$0.75 \pm 0.28$	$1.36 \pm 0.71$
si- <i>SNHG7</i>	$1.14 \pm 0.76$	$0.51 \pm 0.64$	$0.59 \pm 0.19$	$0.63 \pm 0.61$
t	6.8812	0.0007	2.1146	3.4877
P	< 0.05	> 0.05	> 0.05	< 0.05

### 3.3. Knockdown of *SNHG7* can inhibit the migration and invasion of cholangiocarcinoma CCLP-1 cells

After 24 h of scratch treatment, the migration ability of CCLP-1 cells in the si-*SNHG7* group was reduced compared with the si-NC group ( $t = 6.356$ ,  $P = 0.026$ ). The results of Transwell experiment showed that the invasive ability of CCLP-1 cells in the si-*SNHG7* group was significantly reduced compared with the si-NC group ( $t = 7.845$ ,  $P = 0.032$ ).

## 4. Discussion

CCA is a malignant tumor originating from the epithelial cells in intrahepatic and extrahepatic bile ducts. CCA, which is the second most common primary hepatocellular carcinoma malignancy, is the most common biliary tract malignancy worldwide. CCA is resistant to conventional chemotherapy and radiotherapy; in addition, there is a lack of available methods for early diagnosis and treatment of CCA. The majority of patients who are diagnosed with CCA are in advanced stages. Radical surgery is recommended only for patients in an early stage.

Its poor sensitivity to chemotherapy and radiotherapy is an important reason for the poor prognosis of patients [8-10]. Although many scholars have made huge efforts in improving the treatment of CCA, cholangiocarcinoma is still one of the deadliest diseases. Therefore, it is important to identify effective biomarkers and therapeutic targets for early diagnosis and treatment so as to improve the therapeutic effect.

The occurrence and development of CCA are associated with abnormal cell cycle regulation and imbalance of cell proliferation and apoptosis, resulting in the indefinite proliferation of tumor cells. Excessive cell proliferation is not only a characteristic of tumors, but also the impetus for various malignant biological behaviors of tumor cells. Apoptosis is a key process initiated in cancer cells, but it involves various complex pathways and regulators that cancer cells use to escape cell death. The abnormal proliferation and apoptosis of tumor cells, which result from the joint action of multiple factors and the abnormal expression of multiple signaling pathways, are known to be associated with the abnormal expression of multiple genes <sup>[11-14]</sup>.

Tumors develop as a result of the joint action of multiple factors, genes, and signaling pathways. The balance between cell proliferation and apoptosis is disrupted, there is abnormal cell cycle regulation, and cells begin to proliferate indefinitely. Excessive cell proliferation is not only a characteristic of tumors, but also an impetus for carcinogenesis. Studies have shown abnormally high expression of *SNHG7* in various tumor tissues, and downregulating or inhibiting its expression can promote apoptosis and inhibit tumor cell proliferation. In bladder cancer, the expression of lncRNA *SNHG7* in cancer tissues was significantly higher than that in adjacent normal tissues, and the high expression of *SNHG7* in vitro could significantly promote the proliferation of cholangiocarcinoma cells. Further gain-of-function and loss-of-function experiments have confirmed that the high expression of *SNHG7* promotes the proliferation of cancer cells by upregulating the expression of inhibitor of DNA binding/differentiation 2 (ID2).

Tumor invasion and metastasis are the hallmarks of malignant tumors. They involve the shedding of tumor cells from the primary lesion, followed by the infiltration of these cells into tissues, forming new metastases in adjacent or distant tissues and organs that are consistent with the nature of the primary tumor through tissue infiltration or circulating blood and lymph. H19 was originally proposed as a tumor suppressor gene. It plays an inhibitory role in tumor invasion and metastasis. In hepatocellular carcinoma, the downregulation of lncRNA *SNHG7* expression can promote the invasion and metastasis of tumor cells. This process is accompanied by the upregulation of Akt and cell division cycle (Cdc25) as well as the downregulation of glycogen synthase kinase-3 beta (GSK-3 $\beta$ ). Studies have found that a low expression of lncRNA *SNHG7* activates P13K/AKT signaling, which in turn inhibits the expression of the downstream target gene GSK-3 $\beta$  and the phosphorylation of Cdc25A by GSK-3 $\beta$ , and upregulates the expression of cell division cycle 25a (Cdc25a) in the cell cycle pathway. The disorder in the regulation of cell cycle promotes the invasion and metastasis of liver cancer cells <sup>[15-17]</sup>.

This research explores the gene expression mechanism in the occurrence and development of cholangiocarcinoma. The results from this research may provide reference for future clinical work in terms of diagnosis, treatment, and prognosis. The knockdown of lncRNA *SNHG7* can effectively inhibit the proliferation, migration, and invasion of cholangiocarcinoma.

## Disclosure statement

The authors declare no conflict of interest.

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