

## SHORT COMMUNICATION

# LncRNA SNHG7 is an Oncogenic Biomarker Interacting with MicroRNA-193b in Colon Carcinogenesis

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### SUMMARY

**Background:** The expression and significance of small nucleolar RNA host gene 7 (SNHG7) in early-stage colon carcinogenesis remains unclear.

**Methods:** The level of SNHG7 and microRNA-193b (miR-193b) was detected by qRT-PCR in colon tumor tissues and cells. The interaction of SNHG7 and miR-193b and the influence of SNHG7 silencing on colon tumor cells was evaluated.

**Results:** Stepwise upregulated SNHG7 in colon advanced adenomas and early-stage cancer negatively correlates with miR-193b level, the direct interaction was confirmed *in vitro*. SNHG7 silencing in HT29 cells decreased proliferation and promoted apoptosis by inhibiting K-ras/ERK/cyclinD1.

**Conclusions:** SNHG7 is an oncogenic biomarker in colon carcinogenesis. The effect may be mediated by interaction with miR-193b.

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### KEY WORDS

SNHG7, colon cancer, miR-193b, lncRNA

### INTRODUCTION

As the third most common cancer, colorectal cancer (CRC) is considered as one of the major causes of cancer-related death all over the world [1]. The theory of adenoma-adenocarcinoma sequence in colorectal carcinogenesis has been well known and identifying novel biomarkers in the adenoma-adenocarcinoma sequence is crucial for developing efficient strategies of early diagnosis and management [2].

Long noncoding RNAs (lncRNAs) are a group of small-molecule, noncoding RNAs without protein-coding capacity which play important roles in various diseases including cancer. Abnormal expression of lncRNAs is closely related to the progression and prognosis of multiple tumors [3].

lncRNA SNHG7 (small nucleolar RNA host gene 7) is located on chromosome 9q34.3 with a length of 2,157 bp. Recently, several studies have reported that SNHG7 acts as an oncogenic gene in multiple cancers,

including breast cancer, lung cancer, bladder cancer, prostate cancer, glioblastoma, osteosarcoma, esophageal cancer, gastric cancer, and pancreatic cancer, et al. [4-12]. In CRC, it is reported that high expression of SNHG7 correlates with cancer progression and metastasis [13,14]. However, the expression and significance of SNHG7 in early-stage colon cancer carcinogenesis remains unknown.

## MATERIALS AND METHODS

### Clinical specimens and cell line preparation

Thirty cases of advanced colon adenomas (all with high-grade intraepithelial neoplasia, HGIN) and 29 cases of early-stage colon cancer (AJCC stage I-II) were included in this study. The tumor tissues were sampled via surgery or colonoscopy resection at the Gastroenterology Department, Beijing Shijitan Hospital. In addition, 30 cases of tissue were collected as normal control during colonoscopy when only hyperplastic polyps were identified without any adenoma, cancer, or inflammation. Pathological diagnosis was confirmed by experienced pathologists. This study was approved by the Beijing Shijitan Hospital Medical Ethics Board. Informed consent was obtained from each participant. HT29 and 293T cells were preserved in the laboratory.

### Cell line transfections

The transfection was performed using Lipofectamine 2000 (Invitrogen, USA). Commercial small interfering RNAs (siRNAs) against SNHG7 (Silencer<sup>®</sup> Select) and negative controls were also purchased from Invitrogen. miR-193b mimics were constructed by GenePharma Technology (Shanghai, China).

### Real-Time polymerase chain reaction

Primer sequences of SNHG7, microRNA-193b (miR-193b), and GAPDH were as follows: SNHG7 (forward 5'-GCGTGGGGTGTGGGTA-3' and reverse

5'-GGGGCAGTGTCTGTTGAA-3'),

miR-193b (forward

5'-TAGATAACTGGCCCTCAAAGTCC-3' and reverse

5'-TATGGTTTTGACGACTGTGTGAT-3'),

GAPDH (forward

5'-CATGAGAAGTATGACAACAGCCT-3' and reverse

5'-AGTCCTTCCACGATACCAAAGT-3').

Relative expression level of the target gene was evaluated using  $2^{-\Delta\Delta CT}$  method.

### Dual-luciferase reporter assay

The wild-type SNHG7 3'UTR was cloned into the GP-miRGLO luciferase vector (GenePharma, Shanghai, China). The mutant SNHG7 3'UTR was generated by mutating 3 nucleotides recognized by miR-193b. The reporter plasmid was transiently transfected into 293T

cells in the presence of hsa-miR-193-3p mimics or control. The luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega, USA).

### Assays of cell proliferation and apoptosis

The proliferation viability was measured by Cell Counting Kit-8 (CCK-8, Japan). Colony formation capacity was evaluated using soft agar assay. Cell apoptosis was evaluated using two-color immunofluorescence staining for flow cytometric analysis.

### Western blot analysis

Protein extraction reagent (Pierce Biotechnology, Rockford, IL, USA) was used to extract proteins from total cell lysates. Primary antibodies include K-ras antibody (1:200, sc-30, SANTA CRUZ), cyclin D1 antibody (1:10,000, ab134175, Abcam), ERK antibody (1:1,000, 137F5, CST), and  $\beta$ -Actin antibody (1:10,000, A5441, Sigma).

### Statistical analysis

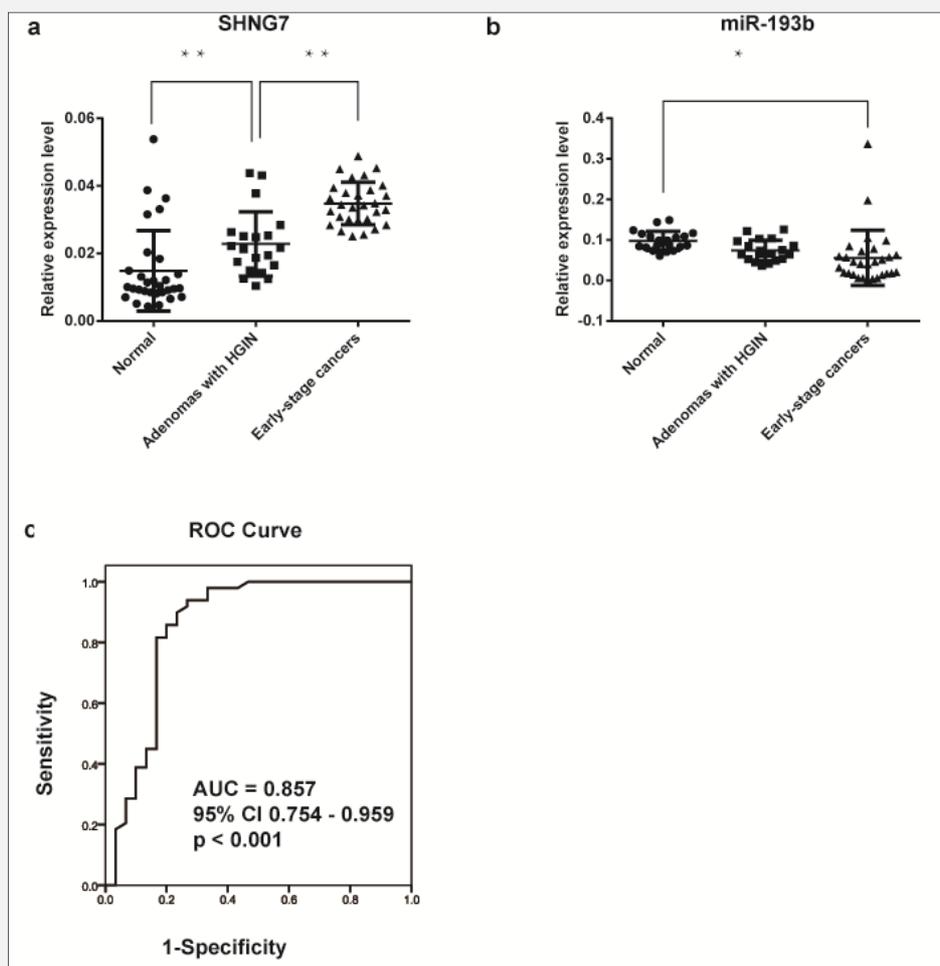
The SPSS 17.0 (Chicago, IL, USA) software was used. All data are reported as mean  $\pm$  standard deviation. Intergroup comparison was performed with Student's *t*-test or one-way ANOVA was used.  $p < 0.05$  was considered as statistically significant.

## RESULTS

### lncRNA SNHG7 and miR-193b was reciprocally expressed in colon tissue and tumor cells

Compared with normal tissues ( $n = 30$ ), SNHG7 was found to be stepwise up-regulated in colon adenomas with high grade intraepithelial neoplasia (HGIN,  $n = 20$ ) and colon cancers ( $n = 29$ ) ( $p < 0.001$ ). In contrast, the expression of miR-193b was stepwise down-regulated in normal tissues, colon adenomas with HGIN and colon cancer, respectively ( $p < 0.05$ ). In addition, a significant negative correlation between SNHG7 and miR-193b was found in Spearman's correlation analysis ( $r = -0.532$ ,  $p < 0.001$ ). Furthermore, ROC curve analysis showed that the expression level of SNHG7 distinguishes adenomas with HGIN ( $n = 20$ ) and early-stage colon cancer ( $n = 29$ ) from normal control ( $n = 30$ ) with an acceptable AUC (0.857, 95% CI 0.754 - 0.959,  $p < 0.001$ ) (Figure 1).

To further determine the expression relationship between SNHG7 and miR-193b *in vitro*, miR-193b mimic and siRNA against SNHG7 were transiently transfected into HT29. It was found that miR-193b mimic significantly decreased the expression level of SNHG7 in HT29 cells, which was similar to SNHG7 silencing. Reciprocally, miR-193b level was significantly increased after SNHG7 silencing. The sites for miR-193b to directly bind to SNHG7 were predicted using Starbase database. A luciferase reporter assay in 293T cells was performed to further confirm that SNHG7 was directly targeted by miR-193b. The luciferase activity was



**Figure 1.** The expression level of SNHG7 was increased stepwise in normal tissue, adenomas with HGIN and early-stage cancers ( $p < 0.001$ ) (a). In contrast, the expression level of miR-193b was decreased stepwise ( $p < 0.05$ ) (b). Negative correlation was observed in Spearman's correlation analysis ( $r = -0.532$ ,  $p < 0.001$ ). ROC curve analysis showed that SNHG7 expression level distinguishes adenomas with HGIN ( $n = 20$ ) and early-stage cancer ( $n = 29$ ) from normal control ( $n = 30$ ) with AUC = 0.857 (95% CI 0.754 - 0.959,  $p < 0.001$ ).

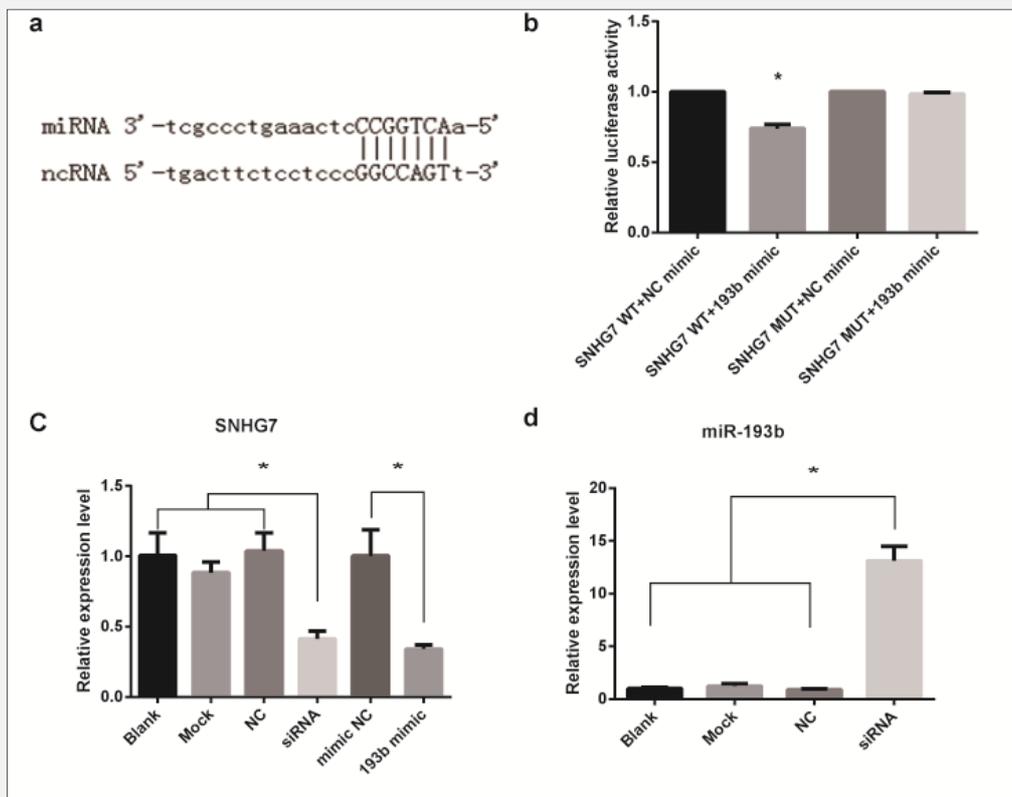
HGIN - High-grade intraepithelial neoplasia, \*\* -  $p < 0.001$ , \* -  $p < 0.01$ .

shown to be markedly reduced when wild-type SNHG7 3'-UTR and miR-193b mimic were cotransfected into 293T cells in comparison with the activity in 293T cells cotransfected with wild-type SNHG7 3'-UTR and miR-193b control. However, the luciferase activity of Mut-SNHG7 showed no obvious change (Figure 2). All these findings suggest that SNHG7 may interact with miR-193b reciprocally in colon tumor tissues.

#### **SNHG7 silencing inhibited proliferation and promoted apoptosis in HT29 cells**

To determine the effect of SNHG7 silencing on HT29 cells, proliferation and apoptosis assays were performed.

In CCK-8 assays, SNHG7 silencing significantly reduced the proliferation ability of HT29 cells. As well, in soft agar colony formation assays, SNHG7 silencing remarkably inhibited the colony forming ability of HT29 cells. Accordingly, in apoptosis assays, the early apoptosis of HT29 cells were significantly promoted after silencing SNHG7. In addition, WB showed that K-ras, ERK, and cyclinD1 were all significantly decreased after SNHG7 silencing (Figure 3).



**Figure 2.** (a) The predicted binding sites of miR-193b to SNHG7 sequence were shown, (b) in the luciferase activity assay, luciferase activity of 293T cells cotransfected with miR-193b mimic and luciferase reporters containing SNHG7-Wt was significantly inhibited compared with cells cotransfected with miR-193b mimic and luciferase reporters containing SNHG7-Mut transcript, (c) after SNHG7 silencing in HT29 cells, SNHG7 level was significantly inhibited, similar to the effect of miR-193b mimic transfection, (d) while miR193b level was significantly increased after SNHG7 silencing.

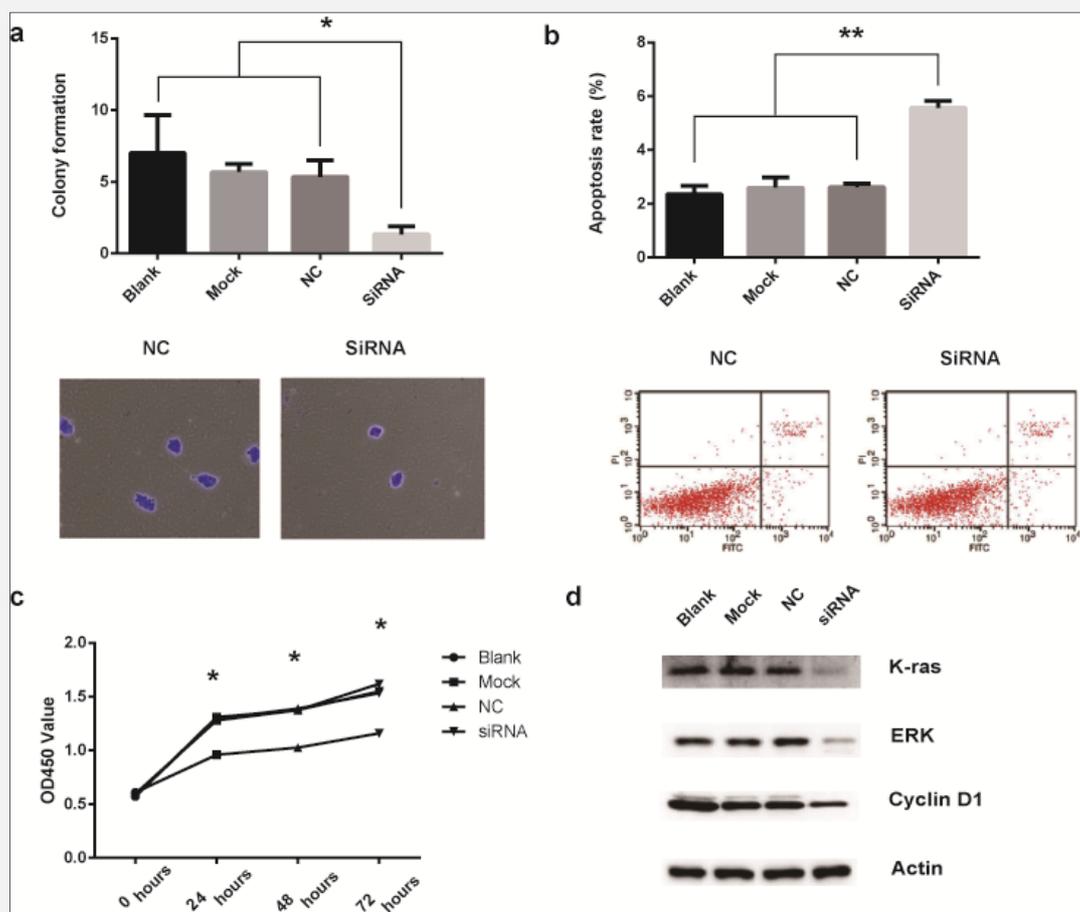
SNHG7 WT - luciferase reporters containing wild type SNHG7 sequence, SNHG7 MUT - luciferase reporters containing mutation type SNHG7 sequence, NC mimic - microRNA mimic as negative control, \* -  $p < 0.001$ , compared with other groups.

## DISCUSSION

SNHG7 is an emerging oncogenic biomarker in multiple cancers arising from breast, urinary tract, respiratory system, and central nervous system [4-10]. In digestive system, it was also reported elevated in pancreatic cancer, esophageal cancer, gastric cancer, and colorectal cancer [11-14]. The elevated expression of SNHG7 correlated with prognosis in prostate cancer and osteosarcoma [7,9]. In a study by Li et al., the expression of SNHG7 in 53 CRC tumor tissues was significantly correlated with tumor size, lymphatic metastasis, distant metastasis, and tumor stage; in addition, patients with higher SNHG7 expression in tumor had worse 5-year survival [13].

As far as we know, the alteration of SNHG7 in adenomas and early-stage colon cancer has not yet been eval-

uated. In this study, it is confirmed that SNHG7 is obviously increased in colon cancer, which is consistent with the findings of Li et al. In addition, increased SNHG7 is also seen in adenomas, suggesting that the SNHG7 level alteration is an early event in colon carcinogenesis. The ROC curve analysis achieved an acceptable AUC of tissue SNHG7 level in distinguishing early-stage cancer and advanced adenomas from normal tissue, which suggests that SNHG7 may be a promising candidate biomarker in screening advanced adenoma and early-stage colon cancer. However, circulating SNHG7 level in colon cancer patients should be investigated in future. The interaction among mammalian lncRNAs and miRNAs is an important mechanism for lncRNA to exert function in tumors. It was revealed in several studies that SNHG7 interacts with multiple miRNAs, including miR-34a, miR-342-3p, miR-503,



**Figure 3.** (a) After SNHG7 silencing in HT29 cells using siRNA against SNHG7, the colony formation ability was inhibited markedly in soft agar colony formation assay, ( $p < 0.001$ ); (b) the early apoptosis of HT29 cells was obviously promoted in apoptosis assay, ( $p < 0.01$ ); (c) the proliferation of HT29 cells was significantly inhibited at three time points in CCK8 assay (all  $p < 0.001$ ); (d) K-ras, ERK, Cyclin D1 were all significantly decreased in HT29 cells after SNHG7 silencing.

Blank - no treatment to cells, Mock - mock transfection with lipofectamine 2000, NC - transfection of siRNA as negative control, siRNA - transfection of siRNA against SNHG7. \* -  $p < 0.001$ , \*\* -  $p < 0.01$ .

miR-5095, etc. [7-9,12]. In CRC, it was elegantly demonstrated that SNHG7 interacts with miR-34a and miR-216b to increase GALNT7 and GALNT1 level to promote cancer progression and metastasis in the studies of Li et al. and Shan et al. [13,14]. In addition, She et al. reported that SNHG7 has the effect of antagonizing miR-193b availability to promote FAIM2-induced tumor progression in lung cancer.

In this study, consistent with the results of She et al. [5], the direct interaction between miR-193b and SNHG7 was confirmed. Interestingly, the reciprocal expression of miR-193b and SNHG7 was always present in the adenoma-adenocarcinoma sequence, as well as in HT29 cells, suggesting SNHG7/miR-193b axis is involved in

colorectal carcinogenesis. Further *in vitro* studies confirmed the oncogenic role of SNHG7 in HT29 cells, and it was demonstrated that K-ras, ERK, and cyclin D1 were all decreased after SNHG7 silencing. Considering K-ras and cyclinD1 are both direct targets of miR-193b [15], which is an intensely studied microRNA as a tumor suppressor gene [16]. It is reasonable to speculate that SNHG7 promotes colon carcinogenesis by sponging miR-193b.

## CONCLUSION

In summary, the alteration of SNHG7 in colon advanced adenomas and early-stage cancer was confirmed in this preliminary study. SNHG7 may work as an oncogenic gene by interacting with miR-193b in colon cancer. Further studies are needed to investigate the effects and mechanism of SNHG7 in colorectal carcinogenesis.

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### Declaration of Interest:

None.

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