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The lncRNA SNHG9, an oncogenic regulator, stabilizes PRMT7 by binding to PTBP1 in the progression of colorectal cancer

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Abstract. Long noncoding RNAs (lncRNAs), widely regarded as nonprotein-coding transcripts longer than 200 nucleotides in length, play active roles in tumorigenesis, including in colorectal cancer (CRC). The abnormally elevated lncRNA small nucleolar RNA host gene 9 (SNHG9) in CRC samples was observed in The Cancer Genome Atlas (TCGA) database. However, the biological role and potential mechanism of SNHG9 in CRC development remain elusive. Herein, 49 paired CRC tissues and matched adjacent normal tissues were obtained to examine SNHG9 levels. Biological functions related to cell proliferation, migration and invasion were evaluated using CCK-8, colony formation, and Transwell assays and western blot analysis. RNA pull-down and RNA immunoprecipitation (RIP) assays were used to verify the SNHG9/PTBP1/PRMT7 regulatory axis. Interestingly, our data revealed that increasing levels of SNHG9 in CRC tissues and cells were positively correlated with poor prognosis and tumour metastasis, while depletion of SNHG9 caused the suppression of cell proliferation, migration, and invasion. Moreover, cytoplasmic SNHG9 enhanced the mRNA stability of PRMT7 by directly binding to PTBP1 through the "lncRNA-RNA binding protein (RBP)" complex. The regulatory function of SNHG9 on PRMT7 was also validated *via* rescue functional assays. In summary, our data demonstrated that SNHG9 may play an oncogenic role in CRC tumorigenesis by stabilizing PRMT7 by recruiting PTBP1. This could be a prognostic biomarker and therapeutic target for CRC.

Key words: Colorectal cancer — SNHG9 — PTBP1 — PRMT7

Introduction

Colorectal cancer (CRC), derived from the gastrointestinal system, is recognized as one of the fourth leading causes of cancer-related death worldwide (Marmol et al. 2017; Siegel et al. 2017). Metastasis, especially hepatic metastasis, accounts for the high mortality and is a complex process involving various cell-intrinsic and extrinsic microenvironment factors (Lambert et al. 2017). Although several novel methods for the diagnosis and therapy of CRC have been discovered, a large number of CRC patients suffer from poor prognosis, displaying a low 5-year overall survival rate. Molecular and genetic alterations have attracted much attention for playing vital roles in CRC tumorigenesis and development. Hence, a better in-depth understanding of the molecular mechanisms should be obtained to discover novel effective therapeutic targets for CRC.

Noncoding RNAs have been widely identified to account for approximately 90% of total transcribed RNAs in the human genome (Patrushev and Kovalenko 2014). Additionally, long noncoding RNAs (lncRNAs) longer than 200 nucleotides in length, serving as RNA polymerase II transcripts, lack the capacity to encode proteins (Lee 2012), but their biogenesis and functions have been extensively elucidated. Nonetheless, the potential application of clinical therapy of specific lncRNAs remains a long way off. The subcellular localization of lncRNAs suggests

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their cellular process. On the one hand, the main nuclear lncRNAs can play a role in chromatin interactions, RNA processing, and transcriptional regulation of downstream effectors; on the other hand, lncRNAs mainly located in the cytoplasm can regulate mRNA stability or translation and become involved in cellular signalling cascades (Schmitt and Chang 2016). Increasing evidence has revealed that an increasing number of lncRNAs play a role *via* specific interactions with other cellular factors (such as DNA and proteins) by acting as miRNA sponges. These findings support the idea that finding lncRNA interacting partners could provide a strategy to gain insights into underlying mechanisms.

Small nucleolar RNA host gene 9 (SNHG9), located on chromosome 16p13.3, has been investigated in some types of human tumours. As previously reported, elevated SNHG9 can indicate the poor prognosis of patients with glioblastoma (Zhang et al. 2019). Ye et al. demonstrated that SNHG9 promoted the proliferation, migration and invasion of hepatocellular carcinoma cells by regulating GSTO1 methylation (Ye and Ni 2021). Moreover, SNHG9 has also been shown to serve as a papillary thyroid cancer exosome-enriched lncRNA, which represses autophagy and triggers apoptosis of normal thyroid epithelial cells by regulating the YBPX3/P21 pathway (Wen et al. 2021). In addition, depletion of SNHG9 in endometrial cancer cells was shown to effectively weaken cell proliferation and glycolysis (Wang L et al. 2021). A recent work from Li et al. (2021) based on bioinformatic analysis primarily implied a potential relationship between SNHG9 and diverse immune infiltration in prostate cancer. Taking these findings into consideration, dysregulation of SNHG9 seems to be common among multiple types of human cancers. However, the biological role and mechanism of SNHG9 in CRC tumorigenesis have not been investigated in depth.

Here, we aimed to investigate the role of SNHG9 in CRC and revealed that aberrantly upregulated SNHG9 was associated with a lower overall survival rate of CRC patients and with tumour metastasis. Mechanistically, SNHG9 facilitated the proliferation, migration, and invasion of CRC cells by enhancing the stabilization of PRMT7 by binding to PTBP1. Thus, our data may provide novel potential therapeutic targets for CRC treatment.

Materials and Methods

Collection of clinical specimens

First, the clinical aspect of the present study was approved by the ethics committee of The Second Xiangya Hospital of Central South University (No. SBQLL-2020-168) and conducted in accordance with the Declaration of Helsinki. A cohort of 49 paired CRC tissues and the corresponding adjacent normal tissues were gathered from patients diagnosed with CRC by professional pathologists during surgical operations at Hunan Cancer Hospital. Written informed consent was obtained from all patients, who had not received any chemotherapy, radiotherapy or other treatment before surgery. Samples were preserved in liquid nitrogen and stored at −80°C for subsequent examination. The patient information is listed in Table 1 and Table 2.

Table 1. Correlation of the expression of SNHG9 in CRC with clinicopathologic features

Parameter	SNHG9		*p* value
	low	high	
Age (Years)			
>58	10	13	0.121
≤58	13	13	
Gender			
male	11	14	0.689
female	12	12	
Location			
colon	11	13	0.268
rectum	12	13	
TNM stage			
I–II	13	10	0.022
III–IV	10	16	
Lymph node metastasis			
no	13	9	0.015
yes	10	17	

Table 2. Correlation of the expression of PRMT7 in CRC with clinicopathologic features

Parameter	PRMT7		*p* value
	low	high	
Age (Years)			
>58	10	17	0.098
≤58	11	11	
Gender			
male	9	16	0.441
female	12	12	
Location			
colon	11	16	0.462
rectum	10	12	
TNM stage			
I–II	12	12	0.021
III–IV	9	16	
Lymph node metastasis			
no	13	9	0.009
yes	8	19	

Cell lines

The normal human colonic epithelial cell line NCM460 (obtained from INCELL, San Antonio, USA) and seven CRC cell lines (HCT116, HT-29, SW620, SW480, RKO, LoVo, and DLD1), purchased from American Type Culture Collection (ATCC, Manassas, VA, USA), were used to determine the role of SNHG9. Cells were authenticated by short tandem repeat profiling before formal utilization. Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) and 1% penicillin/streptomycin was prepared for culturing these cells in a humidified atmosphere of 5% CO_2 at 37°C. In addition, all cell lines mentioned above were tested and determined to be mycoplasma-free.

Plasmids and transfection

For the loss- or gain-of-function assays of SNHG9, short hairpin RNAs (shRNAs) were synthesized and subcloned into the pGPU6/GFP/Neo vector (GenePharma, Shanghai, China) to specifically silence SNHG9 expression; similarly, full-length SNHG9 cloned into the expression vector pCVM (Vigene, Shandong, China) was constructed to overexpress SNHG9. In addition, PTBP1 and PRMT7 siRNAs were designed and synthesized by Ambion (USA). All plasmid vectors and siRNAs were transfected into CRC cells by Lipofectamine 3000 (Invitrogen, Thermo Fisher Scientific, USA) according to the manufacturer's instructions.

Evaluation of cell proliferation

Cell viability was estimated using a Cell Counting Kit-8 (CCK-8, Dojindo, Japan). CRC cells under the indicated transfection conditions were seeded on 96-well plates and incubated for 0 h, 24 h, 48 h, 72 h, and 96 h. Ten microliters of CCK-8 solution was then added to each well and incubated for another 2 h. The absorbance value at 450 nm was measured *via* a microplate reader.

Cell proliferation was examined using a colony formation assay. Transfected CRC cells were seeded on a 6-well plate with the corresponding medium for two weeks. Finally, the colonies were fixed with methanol, followed by staining with 0.1% crystal violet. The number of colonies was calculated by an inverted microscope.

Evaluation of cell migration and invasion

Twenty-four-well Transwell chambers with or without Matrigel (BD Bioscience, San Jose, CA, USA) were used to determine the invasive and migratory capacities of CRC cells. Cells were seeded in serum-free medium into the upper chamber, and medium with 10% FBS was added into the lower chamber. Twenty-four hours after incubation, the cells in the upper chamber were gradually removed with a cotton swab. In addition, 1% crystal violet was used to stain the migrated or invaded cells with prefixing. ImageJ software was used to calculate the cell numbers from five random fields, and the representative images were photographed by an inverted microscope. Each data point was collected from at least three triplicate experiments.

Table 3. The primers used in qRT-PCR

SNHG9	F: 5'-CCCGAAGAGTGGCTATAAACG-3'
	R: 5'-GGAGGACCAGTGTCCTAAGTGAA-3'
PTBP1	F: 5'-AGGTCACCAACCTCCTGATG-3'
	R: 5'-GGGTCACCGAGGTGTAGTAG-3'
PRMT7	F:5'-TGAAATCTTCCAGCGGGGTC-3'
	R: 5'-GGTTGGTGACCAGCTGTTTG-3'
GAPDH	F: 5'-AAATCCCATCACCATCTTCCAG-3'
	R: 5'-TGATGACCCTTTTGGCTCCC-3'
U6	F: 5'-CTCGCTTCGGCAGCACA-3'
	R: 5'-AACGCTTCACGAATTTGCGT-3'

F, forward; R, reverse.

Nuclear-cytoplasmic fractionation

A PARIS^{TM} Kit (Ambion, Austin, TX) was used to determine the nuclear-cytoplasmic fractionation of SNHG9 in CRC cells according to the manufacturer's instructions. GAPDH and U6 were used as the cytoplasmic and nuclear internal references, respectively.

Quantitative real-time PCR (qRT–PCR)

Total RNA from tissues and cell lines was isolated and extracted by using TRIzol solution (Takara, Dalian, China) and then transcribed into complementary DNA with the PrimerScript^{TM} II 1st Strand Synthesis Kit according to the manufacturer's instructions. Next, RT-PCR was conducted on an ABI PRISM 7500 real-time PCR system (Applied Biosystems) by using SYBR Green PCR Master Mix (Applied Biosystems). The relative mRNA levels of targeted genes were normalized to the internal reference gene GAPDH using the $2^{-\Delta\Delta CT}$ method. The specific primers used are listed in Table 3.

Western blot analysis

RIPA buffer (Sigma-Aldrich, St. Louis, MO) containing a proteinase inhibitor cocktail (Sigma-Aldrich) was prepared for extracting total protein. The protein concentration was quantified using a BCA Protein Assay (Pierce, Thermo Fisher Scientific). Then, equal amounts of proteins (15 μg) were subjected to SDS-PAGE and transferred onto PVDF mem-

branes (Millipore, Billerica, MA, USA). After blocking with 5% nonfat milk for 1 h at room temperature, the membranes were incubated with the indicated primary antibodies at 4°C overnight. After washing with TBST, the membranes were incubated with HRP-conjugated secondary antibody (Thermo Fisher Scientific) for 1 h. Finally, an Enhanced Chemiluminescence Detection Kit (ECL, Thermo Fisher Scientific, USA) was used to visualize the protein bands.

RNA pull-down assay

Protein lysates isolated from CRC cells were incubated with a biotinylated SNHG9-wild-type (WT) or mutant (MUT) probe, as well as with PRMT7-WT or PRMT7-MUT. Then, streptavidin agarose magnetic beads were added to isolate the RNA-protein complexes. Western blot analysis of the complexes was then used for further exploration.

RNA immunoprecipitation (RIP) assay

RIP assays were conducted using a Magna RIP RNA-binding protein immunoprecipitation kit (Millipore, Cambridge, MA, USA) in accordance with the manufacturer's protocol. Cells were lysed using RIP buffer and then incubated with protein A/G beads followed by incubation with antibodies against PTBP1 (Abcam, Cambridge, MA, USA) and immunoglobulin G (IgG, EMD, Millipore) at 4°C overnight. After RNA isolation and purification, RNAs were subjected to qRT-PCR analysis for verification.

Statistical analysis

All experiments in this study were performed at least in triplicate. Data are presented as the means ± standard deviations (SD). Normality analysis was only on the clinical tissue sample data. Variance was similar between the groups that were being statistically compared. Student's *t*-test was used for comparisons between two groups, and one-way analysis of variance (ANOVA) was used for comparisons among multiple groups. Statistical analyses were performed using GraphPad Prism (v5.0, San Diego, CA, USA). The difference was considered statistically significant when the p value < 0.05.

Results

Elevated SNHG9 in CRC tissues and cells is positively correlated with poor prognosis and metastasis

Most SNHGs have been demonstrated to be aberrantly dysregulated in multiple human cancers, including CRC. Here, we focused on SNHG9, which remains largely unstudied in the tumorigenesis of CRC. As originally indicated by The Cancer Genome Atlas (TCGA) database in CRC tissues, SNHG9 was observed to be highly expressed in CRC tissues compared with normal controls (Fig. 1D). Similarly, the extremely elevated expression levels of SNHG9 were also examined in the corresponding collected CRC tissue samples in comparison with matched adjacent normal controls, shown in Figure 1A, which provide a possible means for exploring the oncogenic role of SNHG9. In addition, the correlation analysis by qRT-PCR between SNHG9 expression and the clinical-pathological factors of CRC patients was also performed, and the results showed that enhanced SNHG9 expression was positively correlated with advanced TNM stage and a deeper CRC invasion depth (Fig. 1B,C). In addition, Kaplan-Meier curve analysis also showed that the overall survival rate of CRC patients with a higher SNHG9 expression was dramatically shorter than that of patients with a lower SNHG9 expression (Fig. 1E). Based on these results, the following exploration on seven CRC cell lines by using qRT-PCR was carried out. As expected, SNHG9 overexpression was widely observed in CRC cells compared to the normal human colonic epithelial cell line NCM460 (Fig. 1F).

SNHG9 overexpression promotes the proliferation, migration and invasion of CRC cells, while SNHG9 depletion has the opposite effects

Accordingly, to assess the functional role of SNHG9 in CRC cells, two CRC cell lines, HCT116 and SW480, were chosen for further study, with one showing relatively high SNHG9 expression and another showing relatively low SNHG9 expression, based on the baseline SNHG9 RNA levels. HCT116 and SW480 cells were used for loss- and gain-of-function studies, respectively. We found that SNHG9 silencing significantly inhibited SNHG9 expression, while SNHG9 overexpression promoted SNHG9 expression (Fig. 2A). The cell proliferation and colony forming capacities of CRC cells were reduced by SNHG9 depletion but enhanced by SNHG9 overexpression (Fig. 2B,C). Transwell assays also showed that SNHG9 knockdown exerted an inhibitory effect on cell migration and invasion, whereas SNHG9 overexpression induced increases in migratory and invasive cells (Fig. 2D). Consistent with these findings, Western blot analysis of epithelial-mesenchymal transition (EMT)-associated biomarkers, including E-cadherin, N-cadherin, and vimentin, also showed that SNHG9 inhibition significantly repressed the protein levels of N-cadherin and vimentin and increased the protein level of E-cadherin in HCT116 cells, while ectopic expression of SNHG9 exerted the opposite results, suggesting the promotive functions of SNHG9 on aggressive phenotypes of CRC cell lines (Fig. 2E). Collectively, these results demonstrated an oncogenic role of SNHG9 in CRC development.

SNHG9 in the cytoplasm of CRC cells positively regulates PRMT7 expression

Previously, lncRNAs have been identified as vital regulators in the cellular activities of various tumours by mediating the levels of mRNAs. Thus, we continued to browse CRC tissues in the TCGA dataset. PRMT7 was also proven to be highly expressed mRNA (Fig. 3A,C) and to have a positive expression correlation with SNHG9 in CRC samples (Fig. 3B,E). Additionally, survival analysis determined that CRC patients with high PRMT7 expression levels exhibited poorer overall survival than those with low PRMT7 expression levels (Fig. 3D). qRT-PCR analysis showed that PRMT7 was overtly augmented in CRC cell lines (Fig. 3F). In addition, the negative or positive regulation of SNHG9 knockdown or overexpression on the mRNA and protein levels of PRMT7 was validated using qRT-PCR and Western blot (Fig. 3G, H), which indicated some kind of internal connection. Subsequently, the exact interaction between SNHG9 and PRMT7 was identified. As shown in Figure 3I, subcellular fractionation revealed that SNHG9 was mainly distributed in the cytoplasm of CRC cells. Further validation through an RNA pull-down assay proved that SNHG9 was not immunoprecipitated by an anti-Ago2 antibody (Fig. 3J), and these data may exclude the classical competing endogenous RNA (crRNA) mechanism. Accordingly, the mRNA stability of PRMT7 was measured through qRT-PCR after Actinomycin (Act) D treatment, and the data revealed that the half-life of PRMT7 mRNA was shortened by SNHG9 knockdown, while ectopic expression of SNHG9 prolonged the half-life of PRMT7 mRNA and inhibited its degradation (Fig. 3K). Therefore, SNHG9 may play a role in PRMT7 expression at the post-transcriptional level.

SNHG9 enhances the stability of PRMT7 by recruiting PTBP1

Importantly, the significance of lncRNA–protein interactions and RNA-binding proteins (RBPs) in tumorigenicity has gained much attention in recent years. Based on this, an RNA pull-down assay was used, and PTBP1 was identified to be an SNHG9-interacting RBP (Fig. 4A), which was further validated using a RIP assay (Fig. 4B). These findings revealed

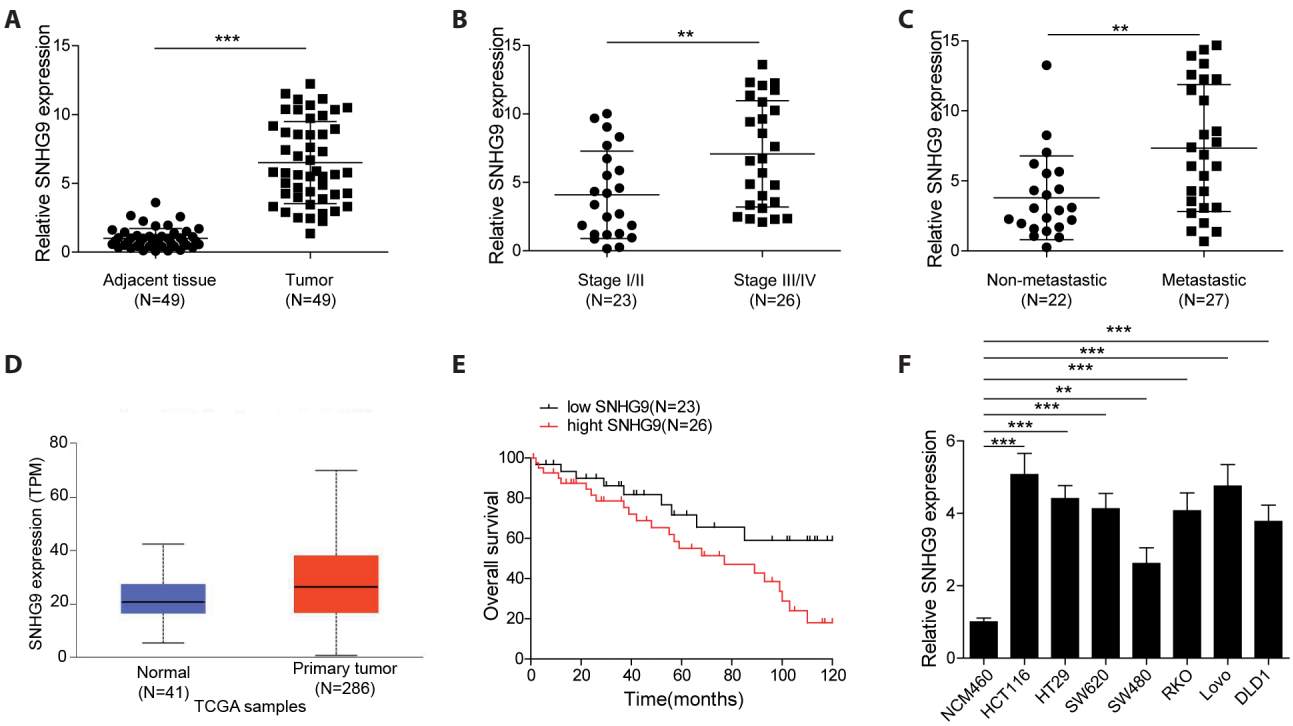


Figure 1. Aberrant overexpression of SNHG9 in CRC tissues and cells indicates poor prognosis and positively correlates with metastasis. **A.** Forty-nine paired CRC tissues and adjacent normal tissues. The expression patterns of SNHG9 were determined by qRT-PCR analysis at the clinical level. The relationships of SNHG9 with tumour TNM stage (**B**) and tumour metastasis (**C**) were further explored. **D.** The expression level of SNHG9 in the TCGA dataset. **E.** Survival was analysed and compared between patients with high and low SNHG9 expression in indicated CRC cohorts by Kaplan-Meier method. **F.** At the cellular level, SNHG9 expression in NCM460 cells and seven CRC cell lines was measured by qRT-PCR. The data are representatives and are expressed as the mean ± SD error of the mean from $n = 3$ experiments. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

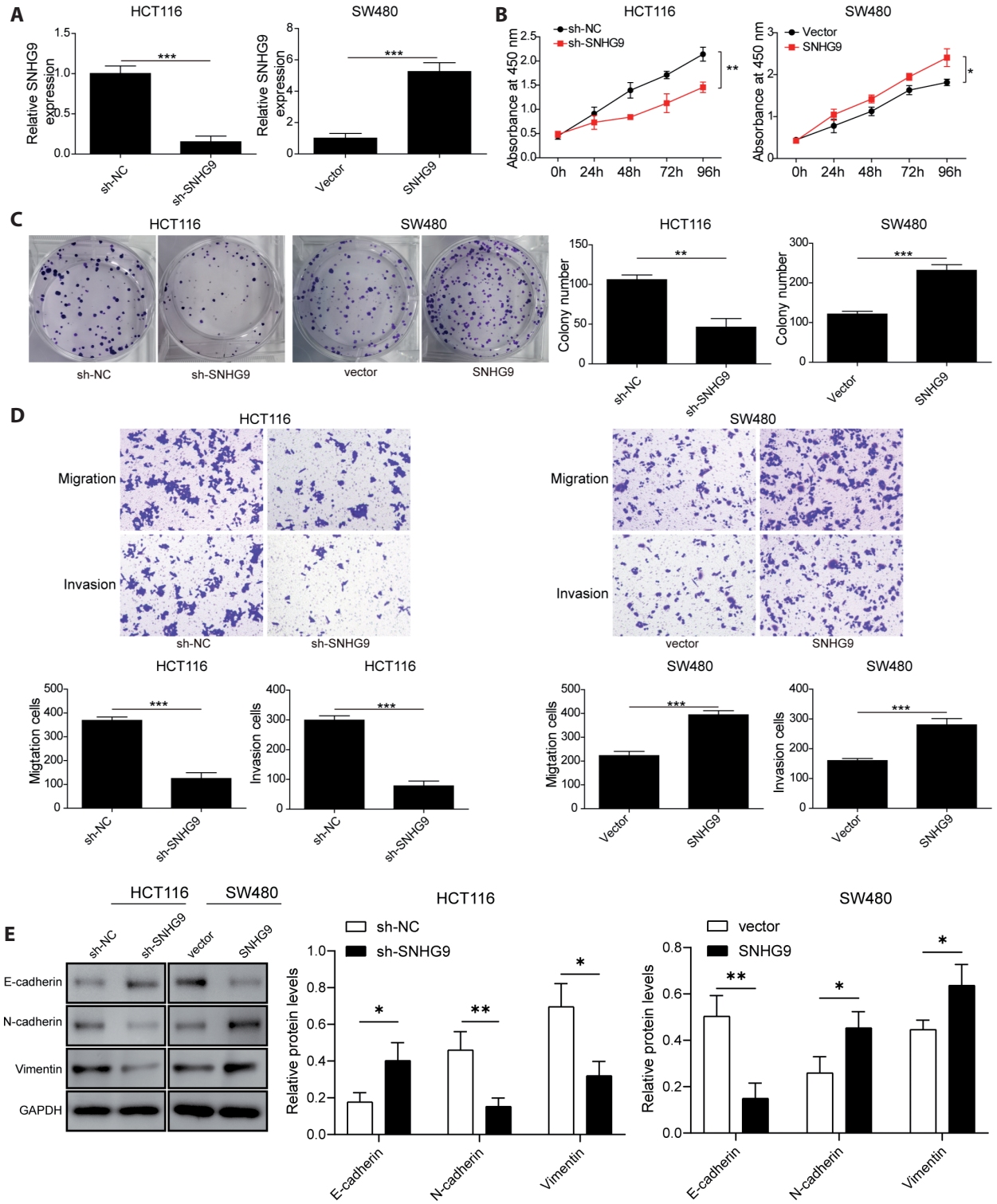


Figure 2. Effect of overexpression or knockdown of SNHG9 on CRC cells. **A.** qRT-PCR analysis was used to evaluate the transfection efficiency of SNHG9 overexpression or silencing plasmids. **B.** A CCK-8 assay was performed to assess the viability of SW480 and HCT116 cells. **C.** A colony formation assay to examine cell proliferation capacity. **D.** Transwell assays for cell migration and invasion were performed to determine the role of SNHG9 in metastatic characteristics. **E.** Western blot analysis examining the protein levels of epithelial-mesenchymal transition biomarkers (E-cadherin, N-cadherin, and vimentin). GAPDH was normalized as a loading control. Data are representative images or are expressed as the mean ± SD error of the mean from $n = 3$ experiments. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

the interplay of SNHG9 with PTBP1. In addition, qRT-PCR revealed that there was no obvious change in the expression of PTBP1 or SNHG9 after SNHG9 knockdown or PTBP1 silencing in either HCT116 or SW480 cells (Fig. 4C,D). Hence, we considered that PTBP1 might be recruited by SNHG9 to carry out its biological functions. Next, qRT-PCR analysis was performed to determine the regulatory effect of PTBP1 on PRMT7 mRNA levels and RNA stability after treatment

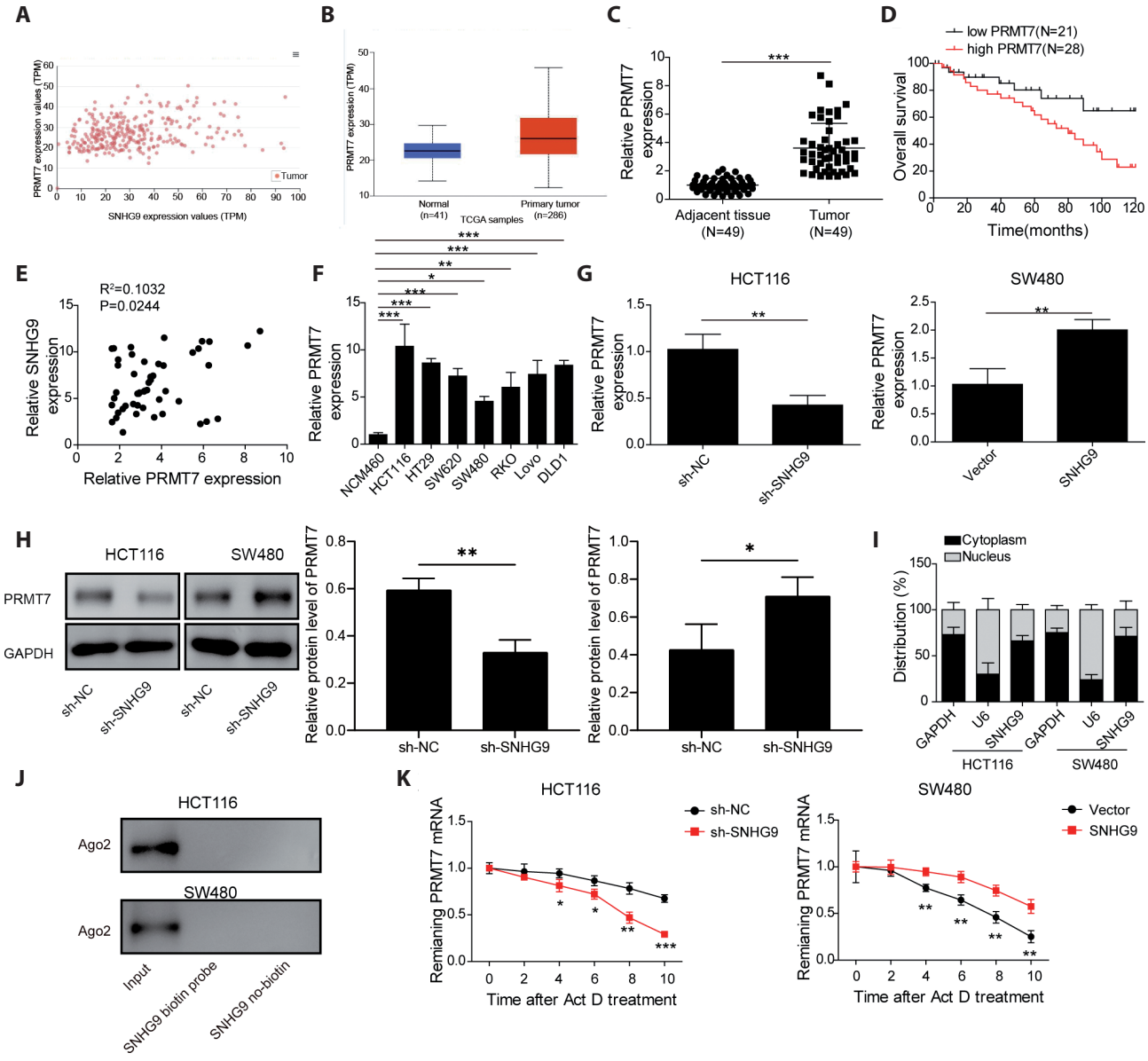
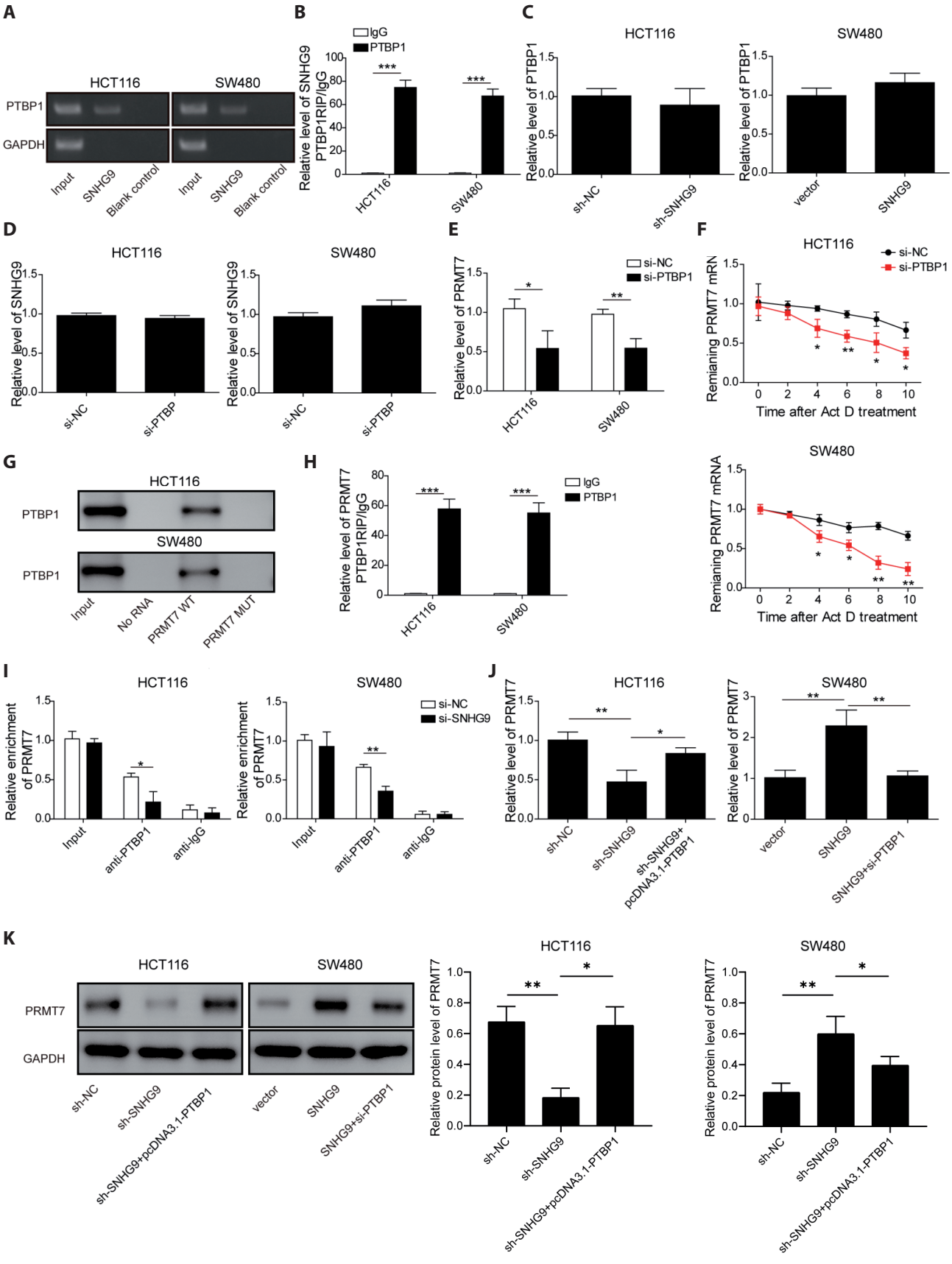


Figure 3. The regulation of SNHG9 on PRMT7 expression. **A.** The TCGA dataset revealed a positive correlation between SNHG9 and PRMT7. **B.** The TCGA dataset revealed elevated PRMT7 in CRC. **C.** PRMT7 expression levels in CRC tissues compared with normal adjacent tissues. **D.** Kaplan-Meier curves for overall survival were generated to evaluate the role of PRMT7 in prognosis. **E.** Spearman correlation analysis to determine the relationship between SNHG9 and PRMT7 in CRC. **F.** The levels of PRMT7 were measured in seven CRC cell lines and compared to those in normal NCM460 cells. qRT-PCR (**G**) and Western blot (**H**) analysis were performed to verify the positive regulation of PRMT7 mRNA and protein levels by SNHG9. **I.** The subcellular localization of SNHG9 in CRC cells was determined using subcellular fractionation. **J.** A RIP assay was used to test whether SNHG9 was precipitated with anti-Ago2 antibody in SW480 and HCT116 cells. **K.** qRT-PCR was used after Act D pretreatment to determine the half-life of PRMT7 with SNHG9 knockdown or overexpression. Data are representative images or are expressed as the mean ± SD error of the mean from $n = 3$ experiments. ** $p < 0.01$; *** $p < 0.001$.


A
HCT116
SW480
PTBP1
GAPDH
Input
SNHG9
Blank control
B
IgG
PTBP1
Relative level of SNHG9 PTBP1RIP/IgG
HCT116
SW480
C
HCT116
SW480
Relative level of PTBP1
sh-NC
sh-SNHG9
vector
SNHG9
D
HCT116
SW480
Relative level of SNHG9
si-NC
si-PTBP
E
si-NC
si-PTBP1
Relative level of PRMT7
F
HCT116
si-NC
si-PTBP1
Remianing PRMT7 mRN
Time after Act D treatment
G
HCT116
SW480
PTBP1
Input
No RNA
PRMT7 WT
PRMT7 MUT
H
IgG
PTBP1
Relative level of PRMT7 PTBP1RIP/IgG
SW480
Remianing PRMT7 mRNA
Time after Act D treatment
I
HCT116
SW480
si-NC
si-SNHG9
Relative enrichment of PRMT7
Input
anti-PTBP1
anti-IgG
J
HCT116
SW480
Relative level of PRMT7
sh-NC
sh-SNHG9
sh-SNHG9+ pcDNA3.1-PTBP1
vector
SNHG9
SNHG9+si-PTBP1
K
HCT116
SW480
PRMT7
GAPDH
sh-NC
sh-SNHG9
sh-SNHG9+pcDNA3.1-PTBP1
vector
SNHG9
SNHG9+si-PTBP1
Relative protein level of PRMT7

with Act D. As illustrated in Figure 4E and F, depletion of PTBP1 induced the downregulation of PRMT7 mRNA, and the half-life of PRMT7 mRNA was distinctly shortened by silencing PTBP1. Moreover, RNA pull-down and RIP assays also validated the interaction between PRMT7 and PTBP1. PTBP1 was markedly pulled down by the biotinylated PRMT7 wild-type probe (Fig. 4G), and PRMT7 was also abundantly precipitated in the anti-PTBP1 antibody group (Fig. 4H). Interestingly, the recruitment of PTBP1 to PRMT7 was obviously impaired by SNHG9 knockdown (Fig. 4I). Subsequently, PRMT7 levels at both the mRNA and protein levels were decreased by SNHG9 silencing or increased by SNHG9 overexpression, whereas these effects were abrogated by ectopic expression of PTBP1 or PTBP inhibition (Fig. 4J,K). In summary, SNHG9 regulated PRMT7 expression through recruitment of PTBP1.

Knockdown of PRMT7 reverses the promotive biological functions induced by SNHG9 overexpression in CRC cells

Finally, rescue experiments in SW480 cells transfected with SNHG9-overexpressing plasmids with or without si-PRMT7 were performed. As expected, the increasing cell proliferation induced by SNHG9 was remarkably abolished by PRMT7 inhibition (Fig. 5A), and cell migration and invasion capacities showed similar tendencies in the transwell assay (Fig. 5B). In addition, the transfection of si-PRMT7 also resulted in inhibitory effects on CRC cell proliferation, migration, and invasion (Fig. 5A,B), which implied the oncogenic role of PRMT7 in CRC malignant phenotypes. Additionally, Western blot analysis of PRMT7 and EMT-associated biomarkers further supported the above conclusion (Fig. 5C). Altogether, these results suggest that SNHG9 plays a tumour promotive role in CRC progression by positively regulating PRMT7.

Discussion

Clearly, an increasing population of humans has been demonstrated to suffer from different kinds of cancers. CRC is one of the most common malignant tumours in the digestive system. Although enhanced progression has been achieved in the treatment of CRC, recurrence and metastasis still seem to be insurmountable, leading to a poor prognosis for CRC patients. Currently, the essential regulation of lncRNAs has been investigated among various tumours, thereby providing several novel therapeutic targets. As host genes of small nucleolar RNAs, lncRNA SNHGs have also been widely proven to be abnormally expressed in human cancers and to regulate cell proliferation, apoptosis, chemoresistance and metastasis (Pei et al. 2018; Qin et al. 2020; Zou et al. 2021). Nonetheless, further investigation to elucidate how individual lncRNAs function should be carried out. Hence, our study focused on the role of SNHG9 and explored its exact biological functions in CRC progression.

Recently, accumulating evidence has partially revealed the role of SNHG9 in different human cancers, but it is not clear whether it functions as an oncogene or a tumour suppressor, which remains a controversial topic. On the one hand, some studies have shown that SNHG9 acts as an oncogene in prostate cancer (Li et al. 2021), non-small cell lung cancer (Wang R et al. 2020), and hepatocellular carcinoma (Ye and Ni 2021). On the other hand, Chen et al. (2021) also verified that SNHG9 could act as a tumour suppressor gene in ovarian cancer by targeting miR-214-5p/cryptochrome circadian regulator 2. Additionally, the precise regulation of SNHG9 in non-small cell lung cancer remains to be determined, as Wang et al. showed that SNHG9 could function as a sponge of miR-21 through methylation to suppress cell proliferation (Wang D et al. 2020). Overall, the broad roles of SNHG9 in tumorigenesis are still largely uncertain. Accordingly, our current study focused on discovering the role of SNHG9 in CRC development. On the basis of the TCGA dataset and multiple CRC cohorts, we confirmed that SNHG9 is significantly upregulated in CRC tissues. Functional experiments also revealed that SNHG9 depletion could inhibit cell proliferation, migration and invasion, thus indicating its tumour-promoting functions. Additionally, the population assessed in this study could not be representative of the whole population worldwide, and the precise expression pattern of SNHG9, as well as its correlation with the prognosis of survival in CRC, should be further validated with a larger number of CRC samples from different parts of the world.

◂ **Figure 4.** SNHG9 enhances the stability of PRMT7 by binding to PTBP1. **A.** RNA pull-down assay was performed, and the results showed that PTBP1 protein was directly enriched by the SNHG9 probe in CRC cells. **B.** RIP assay was performed with anti-PTBP1 and negative control IgG antibodies, followed by qRT-PCR analysis. **C.** The expression of PTBP1 after SNHG9 silencing or overexpression. **D.** The expression of SNHG9 after PTBP1 depletion assessed by qRT-PCR. **E.** The regulation of PTBP1 on the mRNA level of PRMT7 in CRC cells. **F.** qRT-PCR after Act D pretreatment was used to determine the half-life of PRMT7 with PTBP1 silencing. RNA pull-down (**G**) and RIP (**H**) assays were conducted to examine the interaction between PTBP1 and PRMT7. **I.** Endogenous PTBP1 binding to PRMT7 mRNA was influenced by SNHG9 silencing or overexpression, as shown by RIP experiments. qRT-PCR (**J**) and Western blot (**K**) analysis were used to further determine the mRNA and protein levels of PRMT7 by the regulation of the SNHG9/PTBP1 axis. Data are representative images or are expressed as the mean ± SD error of the mean from $n = 3$ experiments. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

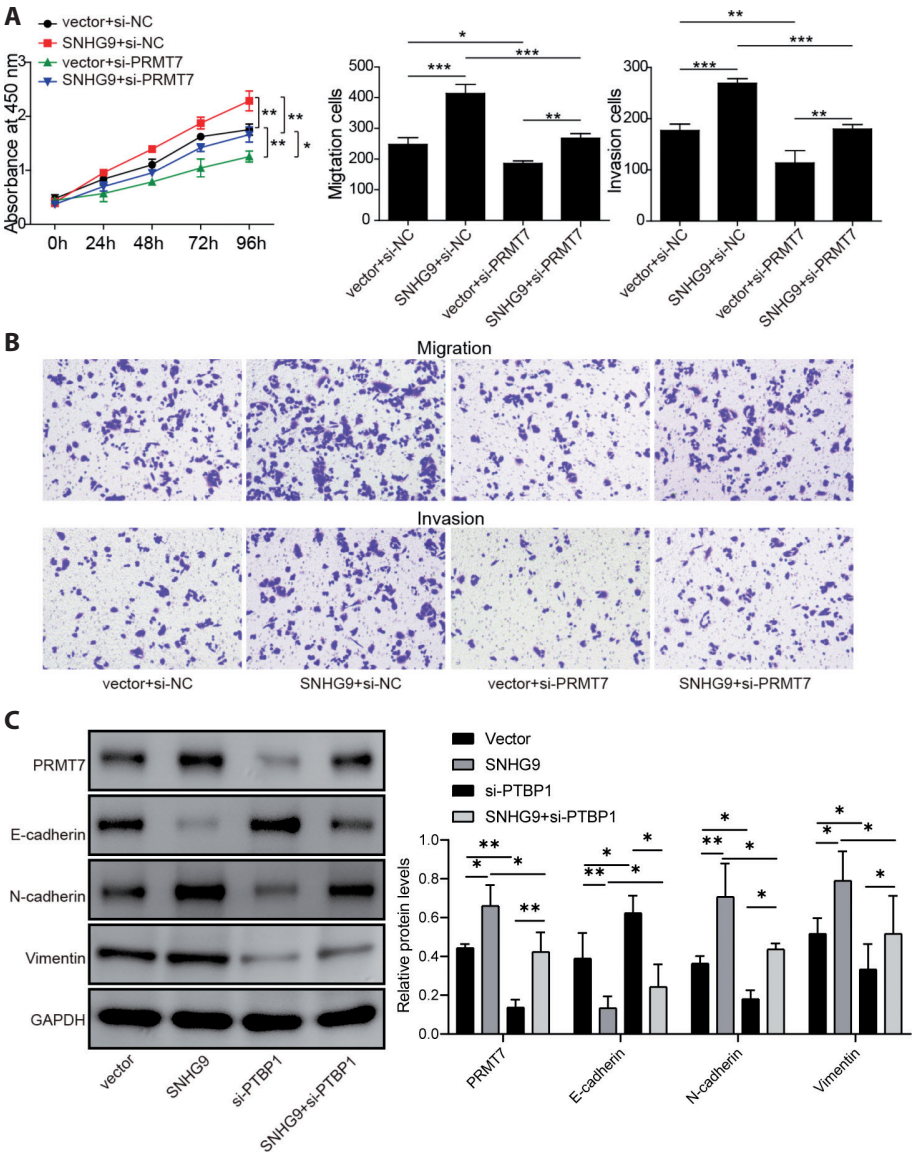


Figure 5. PRMT7 serves as a downstream effector in SNHG9-mediated biological effects in CRC cells. SW480 cells were transfected with an SNHG9-overexpressing vector with or without si-PRMT7. **A.** CCK-8 assay was performed to assess cell viability. **B.** Transwell assays were performed to evaluate cell migration and invasion capacities. **C.** Western blot analysis of PRMT7 and epithelial-mesenchymal transition biomarkers (E-cadherin, N-cadherin, and vimentin). GAPDH was normalized as a loading control. Data are representative images or are expressed as the mean ± SD error of the mean from $n = 3$ experiments. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

The classical mechanism of lncRNAs is to act as a sponge for microRNAs among human tumours. For example, SNHG9 has been demonstrated to function as a miR-199a-5p sponge to upregulate Wnt2 in the aerobic glycolysis of glioblastoma (Zhang et al. 2019). In addition, Feng et al. also found that SNHG9 exerted its oncogenic role by sponging iR-23a-5p to promote hepatoblastoma development (Feng et al. 2021). Interestingly, lncRNAs can also play their roles by interacting with RBPs (Ferre et al. 2016; Elguindy and Mendell 2021). According to the cytoplasmic and nuclear localization of SNHG9, we determined that SNHG9 was mainly distributed in the cytoplasm in CRC cells. Additionally, the data from the RNA pull-down assay excluded the possible interplay between SNHG9 and Ago2. Furthermore, subsequent studies using RNA pull-down and RIP assays identified PTBP1 as an RBP that interacts with SNHG9. As previously reported, PTBP1 has also been proven to interact with FIRRE to promote the stabilization of BECN1 mRNA (Wang et al. 2022). The lncRNA ZNF649-AS1 could enhance trastuzumab resistance in breast cancer by recruiting PTBP1 to contribute to ATG5 transcription (Han et al. 2020). Consistently, our study also revealed the interplay between SNHG9 and PTBP1, which may represent the underlying mechanism of SNHG9 in CRC malignancy.

Widely recognized as being functionally important in arginine methylation, protein arginine methyltransferases (PRMTs) are involved in the regulation of gene expression, mRNA processing and translation, and intracellular signalling at the transcriptional or posttranscriptional levels during disease progression (Guccione and Richard

2019). Several PRMTs have been observed to be aberrantly expressed in human diseases, especially in tumours, which may indicate a class of therapeutic targets for cancer treatment (Cheng et al. 2018; Wang B et al. 2021). Among the members of the PRMT family, PRMT7 has been found to be abnormally overexpressed in various cancers, such as breast cancer (Yao et al. 2014) and non-small cell lung cancer (Cheng et al. 2018). These findings reveal the oncogenic role of PRMT7 in maintaining cancer cell proliferation, pluripotency and metastasis. Similarly, in our current data based on the TCGA dataset, PRMT7 was highly expressed in CRC tissues, which also exhibited a positive correlation with SNHG9 expression. Furthermore, PRMT7 expression was reduced by SNHG9 knockdown but increased by SNHG9 overexpression. Based on all the above data, we hypothesized that SNHG9 might interact with PTBP1 to stabilize PRMT7 mRNA. Mechanistic studies also confirmed the interaction between PTBP1 and PRMT7, and this interaction could be disturbed by SNHG9 depletion. Furthermore, rescue assays confirmed that the promotive effect of SNHG9 on cell proliferation, migration and invasion could be blocked by PRMT7 silencing. However, a recent study deepened the understanding of PRMT7 in cancer development by showing that it could mediate arginine methylation of the splicing factor hnRNPA1, thereby exerting a functional role on RNA alternative splicing regulation (Wu et al. 2021). This points to some limitations of our current study, such as whether the downstream effector of PRMT7 is classical arginine methylation, which remains largely unclear.

In summary, our study first revealed a novel role of SNHG9 in CRC progression through recruiting PTBP1 to enhance the stability of PRMT7, providing a therapeutic marker in CRC. Unfortunately, the exact mechanism of the modulation of the SNHG9/PTBP1/PRMT7 regulatory axis is still unknown and should be further explored by *in vivo* experiments.

Ethics approval and consent to participate. This study was approved by the Medical Ethics Committee of the Second Xiangya Hospital of Central South University (No. SBQLL-2020-168) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients, who had not received any chemotherapy, radiotherapy or other treatment before surgery.

Data availability. The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflicts of interest. Authors declare no conflicts of interest.

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