Am J Transl Res 2021;13(9):10233-10247
www.ajtr.org /ISSN:1943-8141/AJTR0133412

Original Article

LncRNA SNHG12 promotes the development and progression of colon cancer by regulating the miR-15a/PDK4 axis

Kun Guo[1], Yun Zhang[1], Lizhi Liu[2], Hua Meng[1]

[1]*Department of Gastroenterology, Caoxian People's Hospital, Heze 274400, Shandong Province, China;*
[2]*Department of General Surgery, Linyi People's Hospital, Dezhou 251500, Shandong Province, China*

Received March 23, 2021; Accepted July 20, 2021; Epub September 15, 2021; Published September 30, 2021

Abstract: Colon cancer is a common gastrointestinal tumor with complex pathological process. Recently, the relationship between long non-coding RNA (lncRNA) and colon cancer has attracted more and more attention, whereas the underlying molecular mechanism is still poorly understood. Here, we found that the expression of lncRNA small nucleolar RNA host gene 12 (SNHG12) was markedly upregulated in colon cancer samples compared to normal adjacent tissues. Notably, patients with low expression of SNHG12 displayed higher survival rate than those with high expression of SNHG12. Further researches revealed that knockdown of SNHG12 suppressed the malignant phenotype of colon cancer cells. Interestingly, SNHG12 could function as a sponge to specifically bind to microRNA-15a (miR-15a). Moreover, we confirmed that pyruvate dehydrogenase kinase 4 (PDK4) is a direct target gene of miR-15a. Finally, inhibiting miR-15a expression largely abolished the effect of SNHG12 silencing on colon cancer cells. In conclusion, our data uncovered the critical role of SNHG12 in the development and progression of colon cancer through regulating the miR-15a/PDK4 axis, therefore providing a promising target for treating this disease.

Keywords: SNHG12, miR-15a, PDK4, colon cancer, progression

Introduction

Colon cancer is recognized as one of the most common gastrointestinal malignancies in human beings [1, 2]. With the increase of morbidity and mortality of colon cancer, it has become a serious public health problem worldwide, ranking fourth among cancer deaths [3, 4]. Although current strategies for treating colon cancer have been greatly improved, the five-year survival rate of patients in China remains only about 31% because of early metastasis and high recurrence rates [5]. Hence, it is required to clarify the underlying mechanism and explore new strategies for the treatment of colon cancer.

It is well known that the pathogenesis of colon cancer is extremely complicated, which is the consequence of the accumulation of multigene mutations. Although many critical genes have been identified in colon cancer [6, 7], the underlying molecular mechanism has not been completely uncovered. In recent years, the relationship between long non-coding RNAs (lncRNAs) and colon cancer has been paid more and more attention. LncRNA is defined as a type of non-protein coding RNA with more than 200 nucleotides [8-10]. Numerous researches show that lncRNA plays a variety of biological functions in the development and progression of various human diseases, including tumors [11, 12]. In fact, several lncRNAs, such as LINC01234, SNHG15 and MALAT1 [2, 13, 14], have critical regulatory functions in pathological process of colon cancer. However, due to the diversity of species and functions, a large number of lncRNAs have not been fully studied.

Small nucleolar RNA host gene 12 (SNHG12) located on human chromosome 1p35.3 is a newly identified lncRNA [15]. In recent years, researches have shown that SNHG12 participates in regulating the progression of many types of tumors. For example, SNHG12 contrib-

utes to the occurrence and metastasis of cervical cancer through sponging miR-424-5p [16]. Meanwhile, SNHG12 plays a crucial role in osteosarcoma development via the miR-195-5p/Notch2 signaling pathway [17]. In addition, SNHG12 modulates cell proliferation and apoptosis by negative regulation of miR-199a/b-5p expression in hepatocellular carcinoma [18]. Nevertheless, the exact role of SNHG12 in colon cancer, as well as underlying mechanism, has not been revealed.

In this study, we first used a public database and observed that SNHG12 is highly expressed in colon cancer. Consistently, similar results were obtained from colon cancer specimens and multiple colon cancer cell lines. Of note, increased SNHG12 expression was positively related to the poor prognosis of colon cancer patients. Mechanistically, SNHG12 promoted colon cancer progression by targeting the microRNA-15a (miR-15a)/pyruvate dehydrogenase kinase 4 (PDK4) axis. Collectively, the present study demonstrates that SNHG12 functions as an oncogenic gene in colon cancer and may be used as a novel target for developing anti-tumor drug.

Materials and methods

Tissue samples

Clinical colon cancer tissues as well as tumor-adjacent tissues were obtained from 134 colon cancer patients who had undergone surgical treatment. Patients were enrolled between January 2012 and December 2016 (Caoxian People's Hospital, Heze, Shandong Province, China). The informed consents were signed by all participants. This study was approved by the Ethics Committee of Caoxian People's Hospital (Approval number: 2018121001).

Cell culture and transfection

Human colon cancer cell lines (Lovo, HCT116, SW480 and HT29) and normal human intestinal epithelial cell line (HIEC) (Shanghai Institutes for Biological Science, Shanghai, China) were cultured in DMEM/High Glucose (HyClone, Logan, UT, USA) in the presence of 10% fetal bovine serum (FBS; Gibco, Waltham, MA, USA) and grown in 37°C incubator containing 5% CO_2.

Lentivirus containing small hairpin RNA (shRNA) against SNHG12 (sh-SNHG12, 5'-CCCGGCGTACTTAAGCAGATGAAGA-3') or shRNA-negative control (sh-NC, 5'-CCGGTTTCTCCGAACGTGTC-3') were generated at the GenePharma Co., Ltd (Shanghai, China). The transduction of lentivirus into colon cancer cell lines was conducted in the presence of polybrene (GenePharma Co., Ltd). In addition, the transfections of miR-15a-inhibitor, miR-15a-mimic or corresponding negative controls (Ribobio Co., Ltd, Guangzhou, China) were performed in the presence of Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA).

Animal experiments

BALB/c nude mice (six-week-old; male; weighing 18±2 g) were purchased from Hua-Fu-Kang Biosciences (Beijing, China). The mice were kept in the specific pathogen free (SPF) laboratory animal room (Laboratory Animal Center) with the following conditions: temperature 22-24°C, 12/12 hours day/night cycle and eating and drinking freely. SW480 cells (5×10^5) transfected with lentivirus containing sh-NC or sh-SNHG12 were implanted into BALB/c nude mice via subcutaneous injection. The tumor volume in each group was detected every 5 days. The formula is as follows: $V = (L\times W^2)/2$. L presents the length of each tumor and W presents the width of each tumor. All mice were euthanized 30 days after implantation via intraperitoneal injection of pentobarbital sodium (100 mg/kg). Then, tumors were isolated from mice and their weight was measured. The animal experiments were authorized by the Animal Ethics Committee of Caoxian People's Hospital.

MTT assay

This assay was carried out using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) kit (Sigma, St. Louis, MO, USA). Briefly, colon cancer cells were grown in 96-well plates (3×10^3/per well) for 24 hours. Then, each well was added with 0.5 mg/ml MTT and incubated at 37°C for 4 hours. After adding 200 μl of dimethyl sulfoxide (DMSO; Sigma), the absorbance of samples at 570 nm was measured using a microplate reader (Bio-Rad, Hercules, CA, USA).

Table 1. Primer sequences for qRT-PCR analysis

Names	Sequences (5'-3')
SNHG12: F	TCTGGTGATCGAGGACTTCC-3
SNHG12: R	ACCTCCTCAGTATCACACACT
GAPDH: F	TGTTCGTCATGGGTGTGAAC
GAPDH: R	ATGGCATGGACTGTGGTCAT
miR-15a: F	TCCAGCTGGCAGCATG
miR-15a: R	GTCGTGGAGTCACTCG
U6 snRNA: F	ATTGGAACGATACAGAGAAGATT
U6 snRNA: R	GGAACGCTTCACGAATTTG

Colony formation assay

Cells (5×10^3) were seeded into 6-well plates. After cultured for 2 weeks, cell fixation was conducted using 4% paraformaldehyde (Beyotime Biotechnology, Shanghai, China). Subsequently, 0.1% crystal violet (Beyotime Biotechnology) was used to stain the cells. Finally, colony numbers were determined under light microscopy.

Cell invasion/migration assay

Cells were seeded on the upper chamber with (invasion) or without (migration) containing Matrigel (Corning, NY, USA). The lower compartment of chamber was filled with medium containing 10% FBS. After cultured at 37°C for 24 hours, invaded or migrated cells were fixed with 4% paraformaldehyde (Beyotime Biotechnology), stained with 0.2% crystal violet (Beyotime Biotechnology), and washed with PBS. Finally, cell imaging and counting were performed under an inverted microscope.

Cell apoptosis analysis

Cultured cells were collected and stained with Annexin V-FITC and 7-AAD (BD Biosciences, San Diego, CA, USA) at room temperature for 30 min. The apoptosis rate was detected using the FACSCanto flow cytometer (BD Biosciences) and then analyzed by the FlowJo10.0 software (TreeStar, San Carlos, CA, USA).

Luciferase reporter assay

In brief, wild-type or mutant SNHG12 or PDK4 (Sangon Biotech, Shanghai, China) was transfected into 293T cells in the presence of Lipofectamine 3000 (Invitrogen). Forty-eight hours later, detection of luciferase activity was performed using a dual-luciferase reporter assay kit (Promega Biotech Co., Ltd, Madison, Wisconsin, USA) following the manufacturer's instructions.

Quantitative RT-PCR (qRT-PCR)

Total RNA was isolated from cells or tumor tissues using TRIzol reagent (Invitrogen) following the instructions. For detection of mRNA expression, cDNA was converted with a PrimeScript RT kit (Takara, Tokyo, Japan). Then, quantitation was performed using a SYBR Premix Ex Taq kit (Takara). The detection of microRNA (miRNA) expression was performed using a mirVana qRT-PCR miRNA detection kit (Invitrogen). The following reaction procedure was used: pre-denaturation at 95°C for 3 min, 40 cycles of denaturation at 95°C for 15 sec, and annealing and extension at 60°C for 30 sec. $2^{-\Delta\Delta Ct}$ method was used to determine the relative expression of lncRNA or miRNA (GAPDH and small RNA U6 was used as internal reference, respectively). All primer sequences are provided in **Table 1**.

Immunohistochemical staining

Paraffin-embedded tissues were sliced, dewaxed and rehydrated, and were then stained with anti-Ki-67 antibody (dilution 1:500; #ab15580; Abcam, Cambridge, UK). Subsequently, tissue sections were stained with the goat anti-rabbit IgG secondary antibody (Beyotime Biotechnology). After counterstaining with hematoxylin, the color development was carried out using 3,3'-diaminobenzidine (Beyotime Biotechnology). Image J software (version. 1.53i, National Institutes of Health) was used to analyze the percentage of Ki-67$^+$ cells in each section.

Western blotting

Western blotting was carried out as previously reported [19]. The following antibodies were used in the present study: Caspase-3 (dilution 1:1000; #9962S; Cell Signaling Technology, Danvers, MA, USA), Clvaved caspase-3 (dilution 1:1000; #9961S; Cell Signaling Technology), Caspase-9 (dilution 1:1000; #9502S; Cell Signaling Technology), Clvaved caspase-9 (dilution 1:1000; #9507S; Cell Signaling Technology), PDK4 (dilution 1:1000; #ab110336;

Abcam), HIF1α (dilution 1:500; #ab216842; Abcam), VEGFA (dilution 1:2000; #ab52917; Abcam), and GAPDH (dilution 1:5000; #AF11-86; Beyotime Biotechnology). Quantification of band intensities was performed using the Image J software (version. 1.53i, National Institutes of Health, Bethesda, MD, USA).

Statistical analysis

All experiments were independently performed at least three times and shown as mean ± standard deviation (SD). Experimental data analysis was carried out using SPSS 19.0 (SPSS Inc., Chicago, IL, USA) and Graph Prism 6.0 (GraphPad Software, Inc., La Jolla, CA, USA). The difference between two groups was determined by Student's t-test. The difference among multiple groups was determined by One-way ANOVA followed by Tukey's post-hoc test. The overall survival rates of patients were compared by long-rank analysis and shown as Kaplan-Meier curve. $^{*}P<0.05$ and $^{**}P<0.01$ were considered as significant.

Results

SNHG12 was upregulated in colon cancer samples and various colon cancer cell lines

In order to assess the potential role of SNHG12 in colon cancer, we first screened its expression using the Starbase 3.0 database. It was noticed that SNHG12 expression was increased in patients with colon cancer compared with that in healthy controls (**Figure 1A**). Then, the strikingly elevated expression of SNHG12 in colon cancer specimens was verified by qRT-PCR (**Figure 1B**). Similarly, it was exhibited that colon cancer cell lines (Lovo, HCT116, SW480 and HT29) expressed higher level of SNHG12 than normal human intestinal epithelial cell line (HIEC) (**Figure 1C**). Of note, patients with low expression of SNHG12 displayed higher survival rate than those with high SNHG12 expression (**Figure 1D**). These data suggest that SNHG12 may participate in the modulation of the development and progression of colon cancer.

SNHG12 knockdown inhibited the malignant phenotype of colon cancer cells

To determine whether SNHG12 modulates pathological process of colon cancer, SNHG12 expression were knocked down in HCT116 and SW480 cells by lentivirus transduction (**Figure 2A**). The results showed that inhibition of SNHG12 evidently impaired the *in vitro* colony formation and proliferation of colon cancer cells (**Figure 2B** and **2C**). In addition, we observed that the expression levels of cleaved caspase-3 and cleaved caspase-9, which are crucial proteins to mediate cell apoptosis, were upregulated in colon cancer cells following silence of SNHG12 (**Figure 2D**). As anticipated, flow cytometric results exhibited that the apoptosis rate was substantially increased in SNHG12-knockdown colon cancer cells (**Figure 2E**). Moreover, knockdown of SNHG12 resulted in a markedly reduced number of invasive and migratory colon cancer cells, as determined by Transwell assays (**Figure 2F** and **2G**). These results indicate that SNHG12 may contribute to the malignant phenotype of colon cancer cells.

Knockdown the expression of SNHG12 suppressed the colon cancer progression in a xenotransplants mouse model

To further confirm if knockdown of SNHG12 has a negative effect on *in vivo* tumorigenesis, HCT116 cells following sh-NC or sh-SNHG12 transfection were implanted into BALB/c nude mice via subcutaneous injection. As shown in **Figure 3A** and **3B**, tumor volume was substantially lower in sh-SNHG12 group than that in sh-NC group. Meanwhile, compared with sh-NC group, sh-SNHG12 group displayed evidently reduced tumor weight (**Figure 3C** and **3D**). Consistent with above data, immunohistochemical analysis showed that Ki-67 expression was evidently reduced in tumor tissues obtained from mice inoculated with sh-SNHG12-transfected HCT116 cells (**Figure 3E**). These findings illustrate that decreasing the expression of SNHG12 can suppress the proliferation of colon cancer cells *in vivo*.

SNHG12 could negatively regulate the expression of its target gene miR-15a

It has been well established that lncRNAs can bind to specific miRNAs like sponges [20, 21]. We then used bioinformatics analysis to predict candidate target miRNAs and noticed that miR-15a, a critical tumor suppressor, has the potential binding site in SNHG12. In line with this finding, the expression level of miR-15a was elevated in SNHG12-knockdown colon

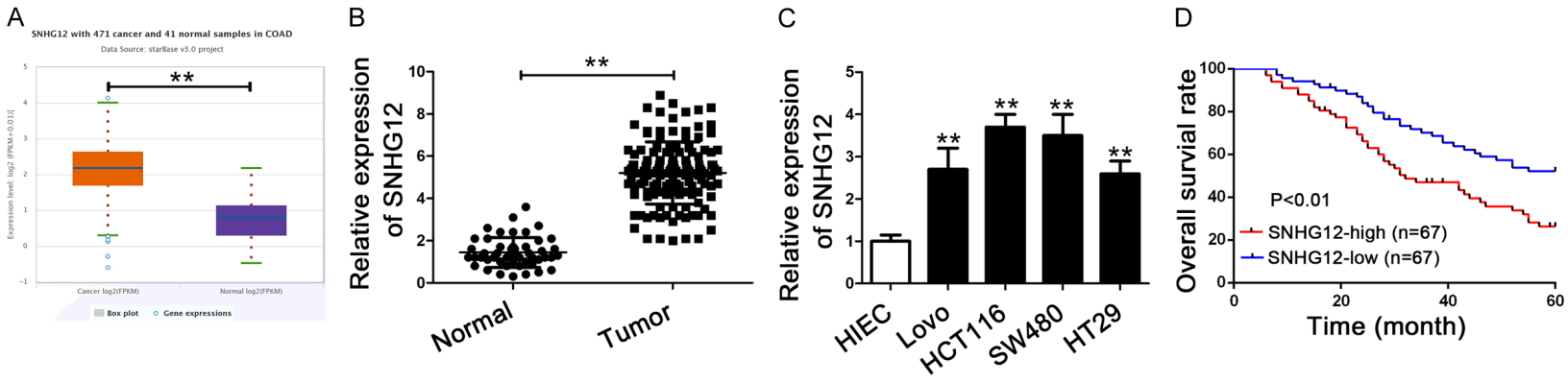
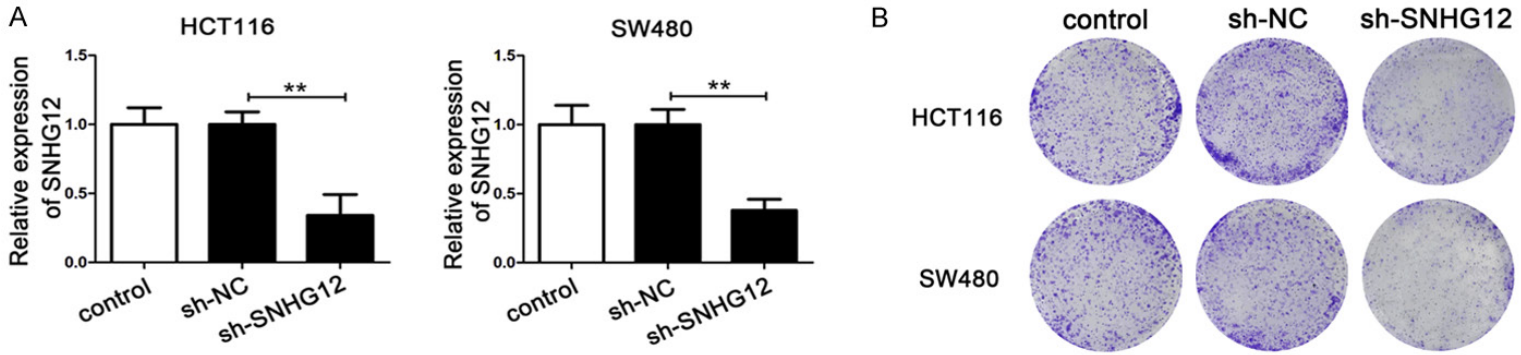
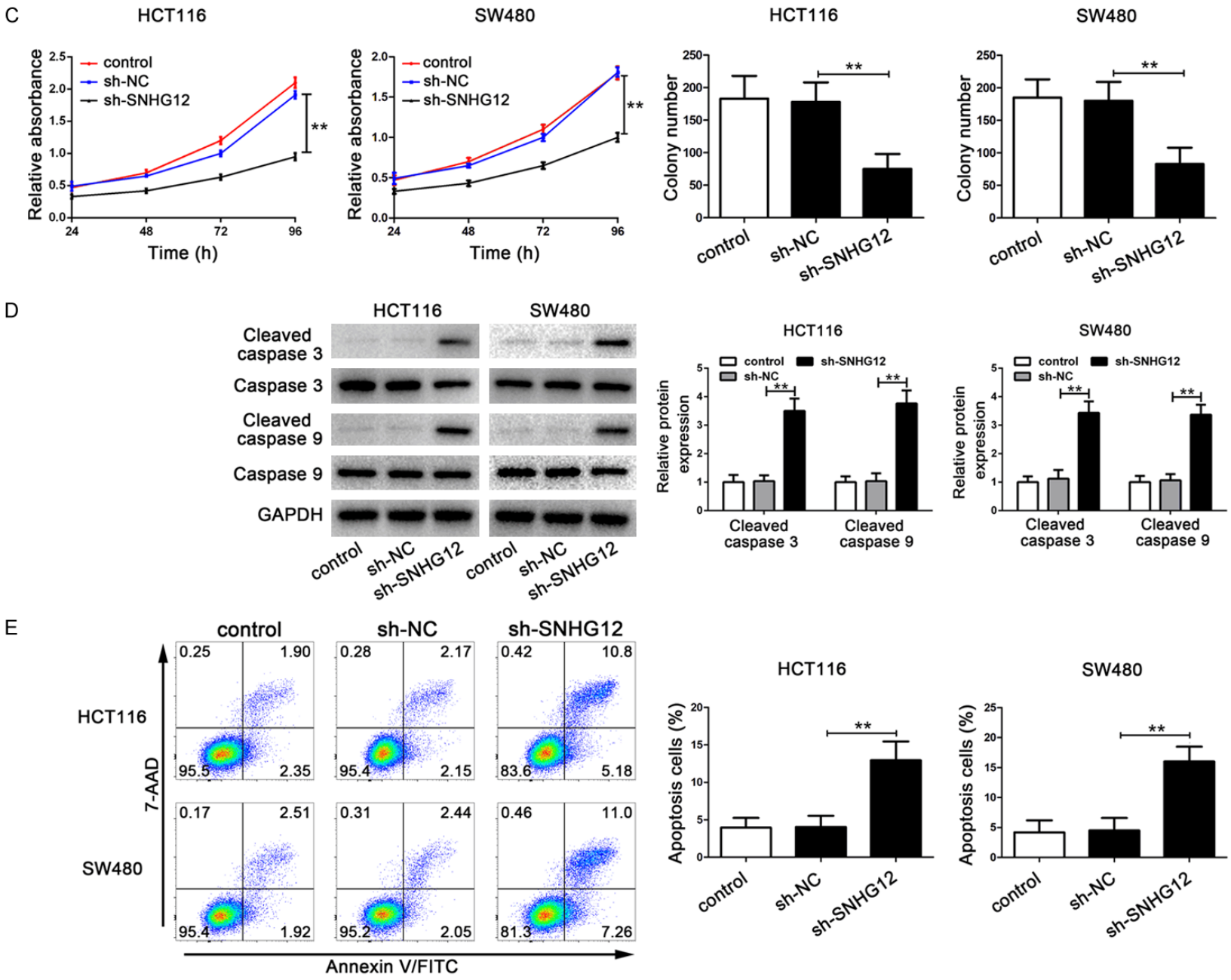


Figure 1. SNHG12 was upregulated in colon cancer tissues and various colon cancer cell lines. A. The expression level of SNHG12 in 471 colon cancer samples and 41 normal samples. Data was obtained from the Starbase 3.0 database (http://starbase.sysu.edu.cn). B. Quantification of the relative SNHG12 expression in colon cancer samples and corresponding adjacent tissues obtained from 134 patients by qRT-PCR. C. Quantification of the relative SNHG12 expression in HIEC, Lovo, HCT116, SW480 and HT29 cell lines by qRT-PCR (n=4). D. The overall survival rates of 134 colon cancer patients undergoing surgery, which were divided into two groups based on the median SNHG12 expression levels. Data are shown as Kaplan-Meier curve (long-rank analysis). **P<0.01.




C
HCT116
SW480
HCT116
SW480
control
sh-NC
sh-SNHG12
Relative absorbance
Time (h)
Colony number
**
D
HCT116
SW480
Cleaved caspase 3
Caspase 3
Cleaved caspase 9
Caspase 9
GAPDH
control
sh-NC
sh-SNHG12
Relative protein expression
Cleaved caspase 3
Cleaved caspase 9
E
control
sh-NC
sh-SNHG12
HCT116
SW480
7-AAD
Annexin V/FITC
0.25
1.90
95.5
2.35
0.28
2.17
95.4
2.15
0.42
10.8
83.6
5.18
0.17
2.51
95.4
1.92
0.31
2.44
95.2
2.05
0.46
11.0
81.3
7.26
Apoptosis cells (%)

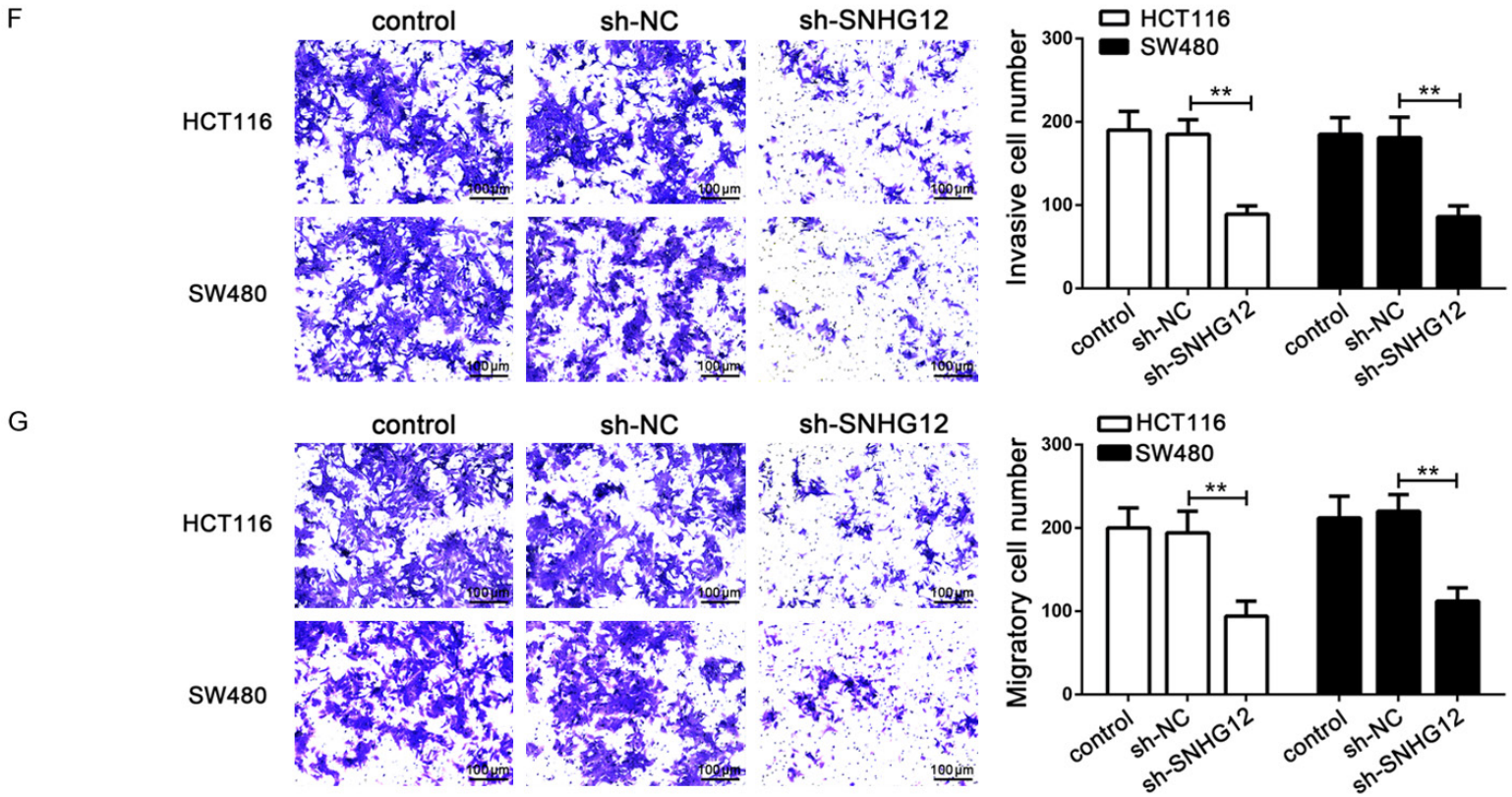


Figure 2. SNHG12 knockdown inhibited the malignant phenotype of colon cancer cells. (A) Quantification of the relative SNHG12 expression in HCT116 and SW480 cells after silence of SNHG12 by qRT-PCR (n=4). (B) The number of colonies formed from HCT116 and SW480 cells after silence of SNHG12 (n=5). (C) MTT analysis of the proliferation of HCT116 and SW480 cells following silence of SNHG12 (n=5). (D) The protein levels of cleaved caspase-3 and cleaved caspase-9 in HCT116 and SW480 cells following silence of SNHG12 were measured by Western blot (n=3). (E) The apoptosis rates of HCT116 and SW480 cells following silence of SNHG12 were measured by flow cytometry (n=5). (F, G) Transwell assays showing the (F) invasion and (G) migration of HCT116 and SW480 cells after silence of SNHG12 (n=5). $^{**}P<0.01$.

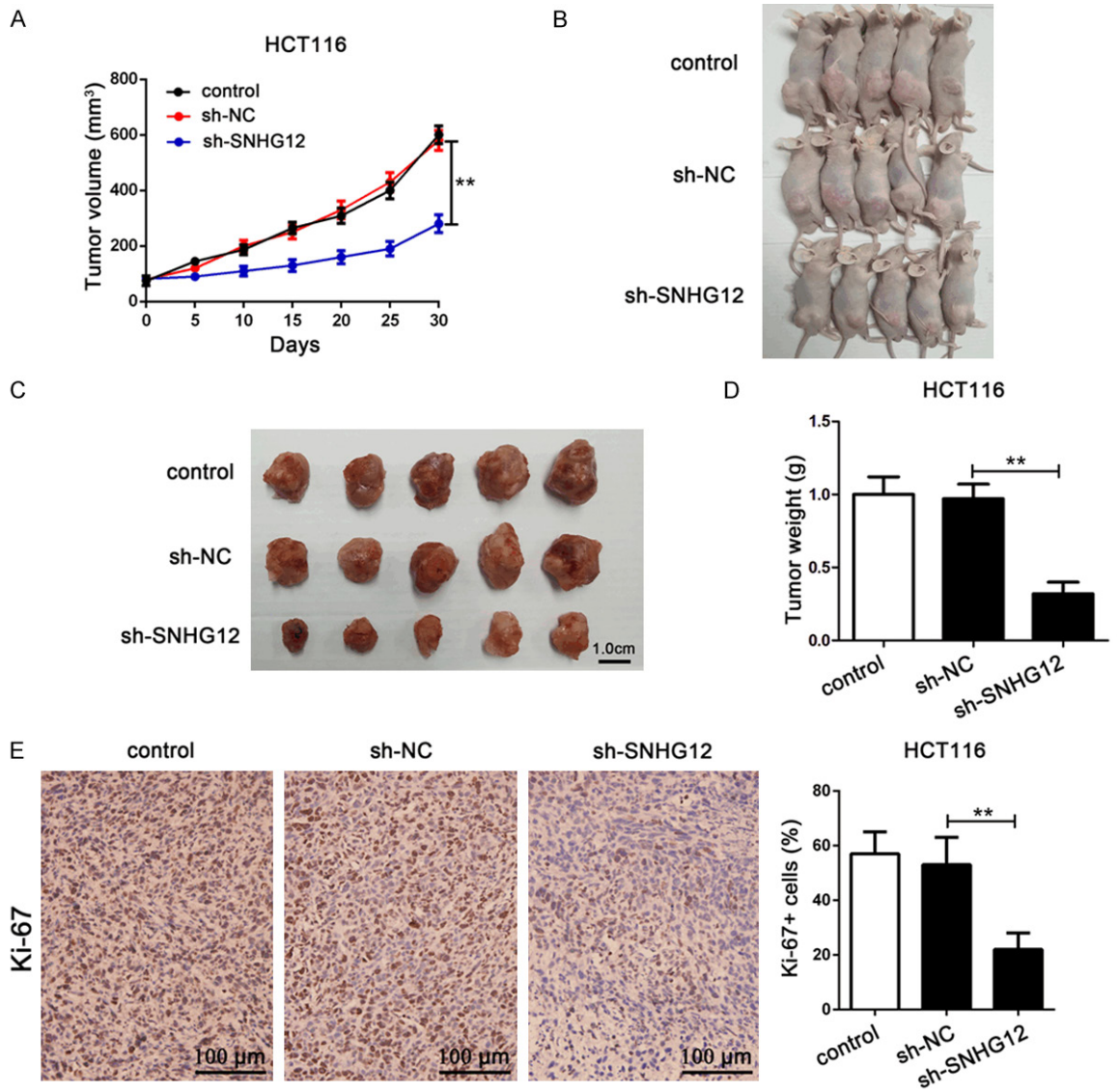


Figure 3. Knockdown of SNHG12 suppressed colon cancer progression in a xenotransplants mouse model. A-D. BALB/c nude mice were subcutaneously injected with saline (control), sh-NC-transducted HCT116 cells or sh-SNHG12-transducted CT116 cells (n=5 mice per group). A. The tumor volume detected every 5 days. B. The mice images 30 days after injection. C. The tumor images 30 days after injection. The scale bar indicates 1.0 cm. D. The tumor weight 30 days after injection. E. Immunohistochemical analysis of Ki-67 expression in tumor tissues obtained from these groups. $^{**}P<0.01$.

cancer cells (**Figure 4A**). Meanwhile, compared with adjacent normal tissues, miR-15a was significantly downregulated in colon cancer samples (**Figure 4B**), consistent with a previous finding [22]. As expected, the expression level of miR-15a was also lower in colon cancer cell lines (**Figure 4C**). In particular, we found that miR-15a level was negatively associated with SNHG12 expression in colon cancer samples (**Figure 4D**). To further confirm this finding, we conducted a luciferase reporter assay. It was observed that SNHG12 could directly bind to miR-15a (**Figure 4E**). Thus, our data indicate that SNHG12 may facilitate colon cancer progression by targeting miR-15a.

SNHG12/miR-15a/PDK4 axis modulated colon cancer progression

miRNA is well-known for its role in inhibiting gene expression via post-transcriptional regulatory mechanisms. Based on bioinformatics

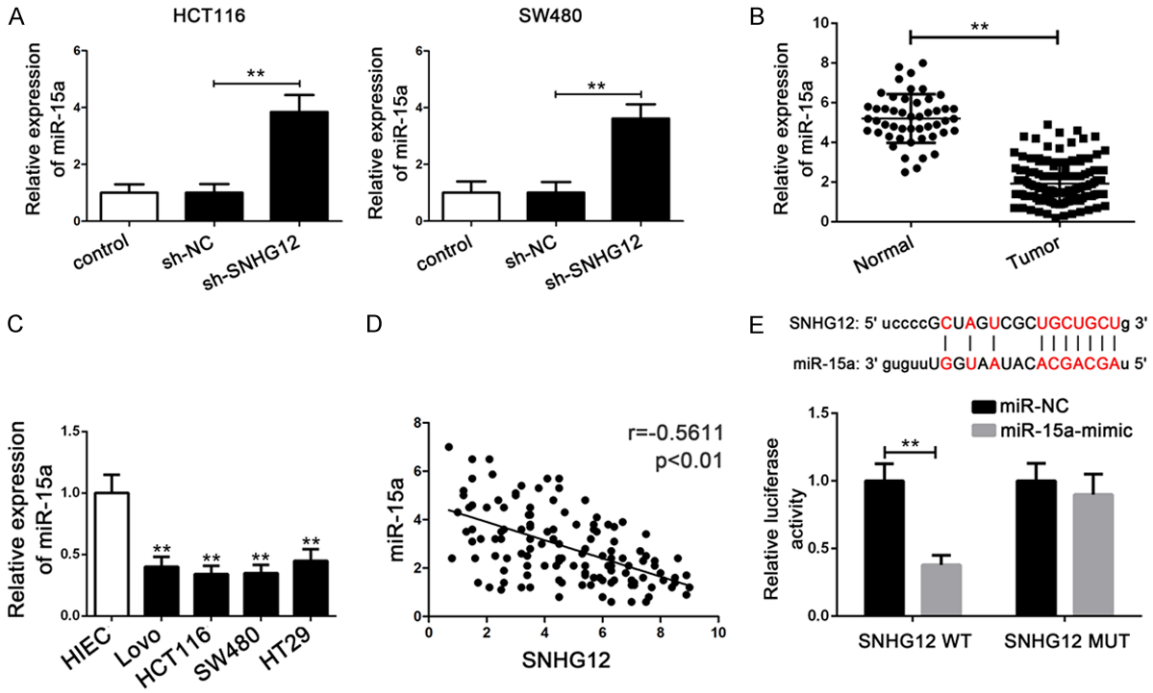
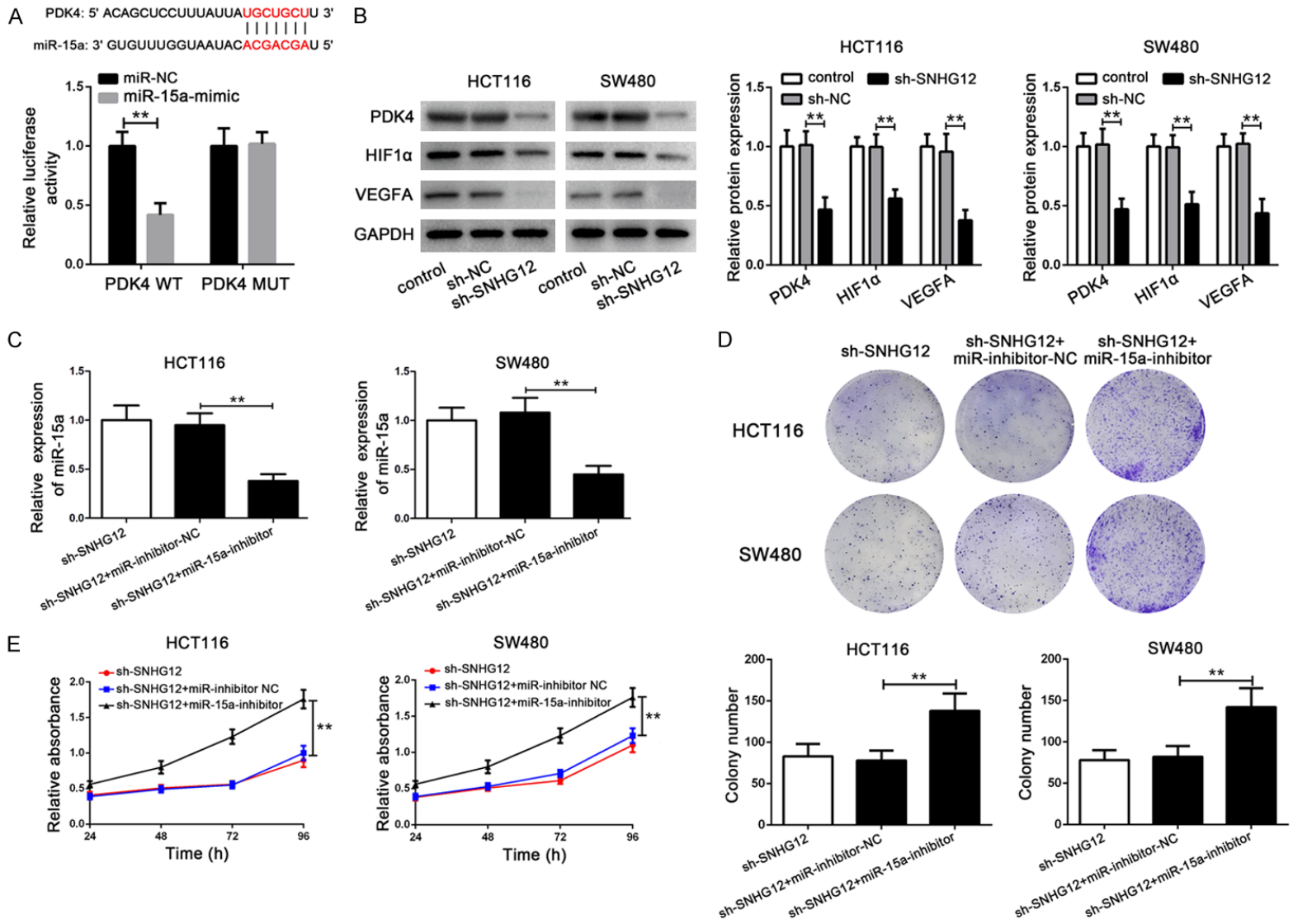


Figure 4. SNHG12 could negatively regulate the expression of its target gene miR-15a. A. Quantification of the relative miR-15a expression in HCT116 and SW480 cells after SNHG12 knockdown by qRT-PCR (n=4). B. Quantification of the relative miR-15a expression in 134 colon cancer samples and adjacent tissues by qRT-PCR. C. Quantification of the relative miR-15a expression in HIEC, Lovo, HCT116, SW480 and HT29 cell lines by qRT-PCR (n=4). D. The negative association between SNHG12 and miR-15a expression in colon cancer patient samples, as determined by Pearson correlation analysis (r=-0.5611, P<0.01). E. The binding detail of SNHG12 to miR-15a. Data was obtained from StarBase 3.0 database. 293T cells were co-transfected with WT-SNHG12 or MUT-SNHG12 and miR-15a-mimic or miR-NC, followed by luciferase activity measurement (n=4). WT-SNHG12, wild-type-SNHG12; MUT-SNHG12, mutant-SNHG12; miR-NC, miRNA-mimic negative control. **P<0.01.

analysis by TargetScan database, we observed that PDK4 was a direct target gene of miR-15a (**Figure 5A**). Subsequent luciferase reporter assay further verified this finding (**Figure 5A**). It was reported that the PDK4 facilitated colon cancer development by upregulating HIF1α expression [23]. Consistent with these results, we found a reduced PDK4 expression in colon cancer cells after knockdown of SNHG12, accompanied by decreased expression of HIF1α and its target VEGFA (**Figure 5B**). Finally, we sought to validate whether SNHG12 facilitates colon cancer progression through modulating the miR-15a/PDK4 axis. We then used a miR-15a-inhibitor that markedly decreased miR-15a expression in colon cancer cells transducted with sh-SNHG12 (**Figure 5C**). Importantly, miR-15a inhibition significantly rescued the colony formation, proliferation, apoptosis, invasion as well as migration of colon cancer cells with SNHG12 knockdown (**Figure 5D-H**). Furthermore, miR-15a suppression largely restored the expression of PDK4, HIF1α and VEGFA in SNHG12-knockdown colon cancer cells (**Figure 5I**). Collectively, our findings demonstrate that upregulation of SNHG12 is responsible for the progression of colon cancer by regulating the miR-15a/PDK4 axis.

Discussion

In recent years, many non-coding RNAs (ncRNAs), such as lncRNA, miRNA and circular RNA (circRNA), have been discovered due to the rapid development of high-throughput sequencing technology [24, 25]. These ncRNAs were initially considered to be genomic "junk" with no biological function, whereas recent researches have shown that they are involved in the tumorigenesis and progression of various cancers [10, 26]. In this paper, we demonstrate for the first time that SNHG12 plays a critical role in regulating colon cancer progression by targeting the miR-15a/PDK4 pathway.


A
PDK4: 5' ACAGCUCCUUUAUUAUGCUGCUU 3'
miR-15a: 3' GUGUUUGGUAAUACACGACGAU 5'
miR-NC
miR-15a-mimic
Relative luciferase activity
PDK4 WT
PDK4 MUT
B
HCT116
SW480
PDK4
HIF1α
VEGFA
GAPDH
control
sh-NC
sh-SNHG12
Relative protein expression
C
Relative expression of miR-15a
sh-SNHG12
sh-SNHG12+miR-inhibitor-NC
sh-SNHG12+miR-15a-inhibitor
D
sh-SNHG12
sh-SNHG12+ miR-inhibitor-NC
sh-SNHG12+ miR-15a-inhibitor
Colony number
E
sh-SNHG12+miR-inhibitor NC
Relative absorbance
Time (h)

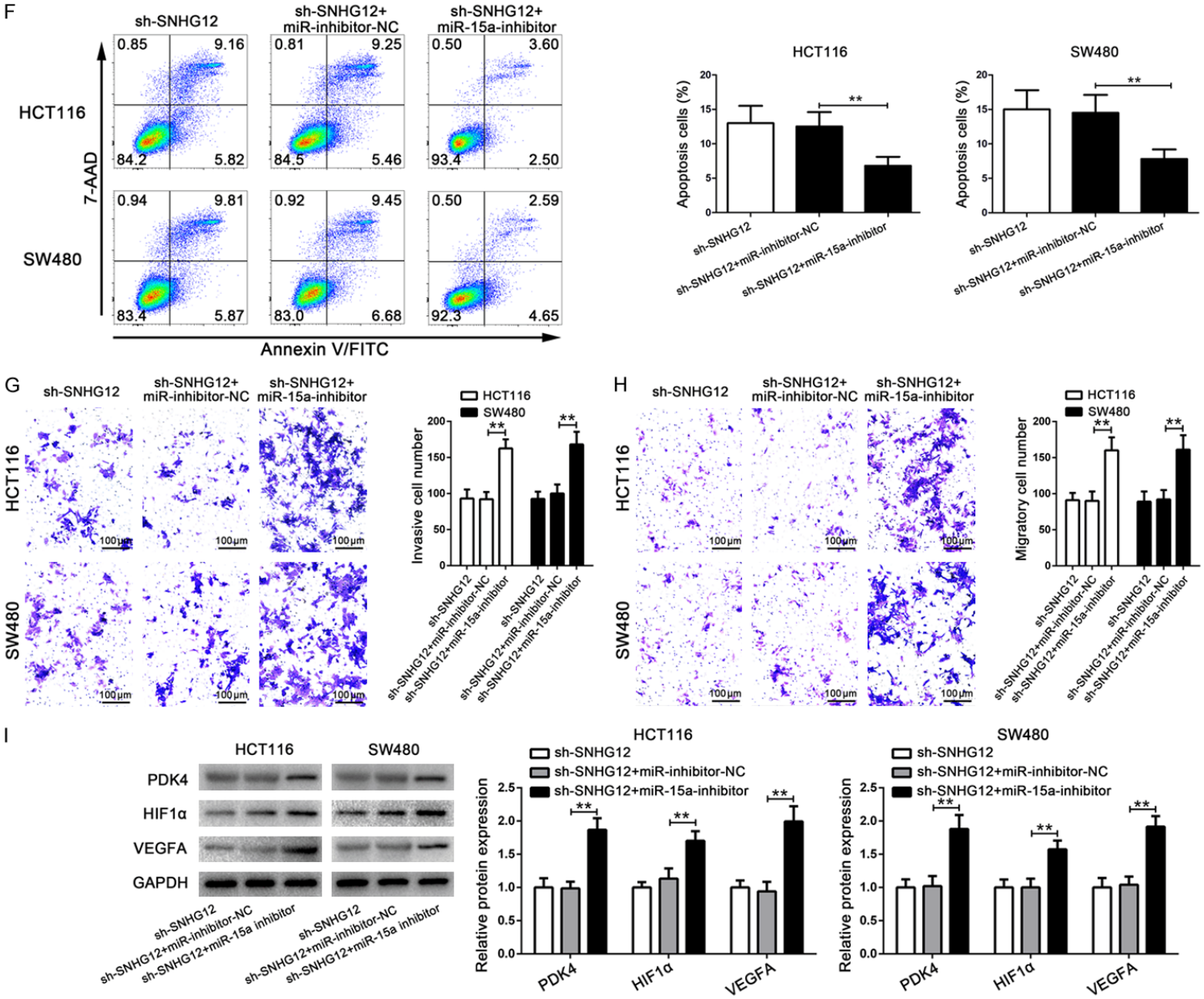

F
sh-SNHG12
sh-SNHG12+ miR-inhibitor-NC
sh-SNHG12+ miR-15a-inhibitor
HCT116
SW480
7-AAD
Annexin V/FITC
0.85
9.16
84.2
5.82
0.81
9.25
84.5
5.46
0.50
3.60
93.4
2.50
0.94
9.81
83.4
5.87
0.92
9.45
83.0
6.68
0.50
2.59
92.3
4.65
HCT116
SW480
Apoptosis cells (%)
sh-SNHG12
sh-SNHG12+miR-inhibitor-NC
sh-SNHG12+miR-15a-inhibitor
G
H
Invasive cell number
Migratory cell number
100 μm
I
PDK4
HIF1α
VEGFA
GAPDH
Relative protein expression

Figure 5. SNHG12/miR-15a/PDK4 axis modulated colon cancer progression. (A) The binding detail of miR-15a to PDK4 mRNA. Data were obtained from TargetScan database (http://www.targetscan.org/vert_71/). 293T cells were co-transfected with WT-PDK4 or MUT-PDK4 and miR-15a-mimic or miR-NC, followed by luciferase activity measurement (n=4). (B) The protein levels of PDK4 and its specific downstream targets HIF1α and VEGFA in colon cancer cells following knockdown of SNHG12 were measured by Western blot (n=3). (C-I) SNHG12-knockdown HCT116 and SW480 cells were treated with miR-15a-inhibitor or miR-inhibitor-NC. (C) The expression level of miR-15a was then detected by qRT-PCR (n=4). (D) Colony number, (E) proliferation, (F) apoptosis, (G) invasion as well as (H) migration were subsequently determined (n=5). Furthermore, the expression levels of PDK4, HIF1α and VEGFA was detected by Western blot (I) (n=3). $^{**}P<0.01$.

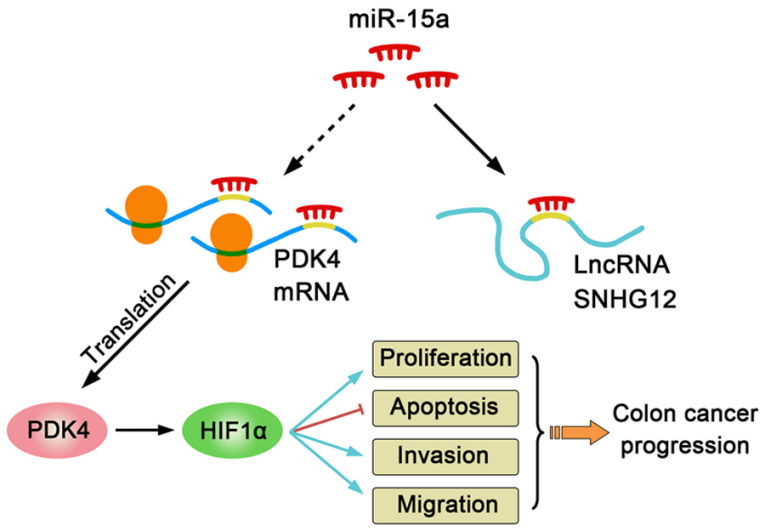


Figure 6. Schematic diagram of SNHG12 in promoting colon cancer progression via the miR-15a/PDK4 axis.

LncRNAs play different roles in various diseases due to the complexity of function and heterogeneity of expression pattern [27-29]. For instance, upregulation of H19 promotes the metastasis of lung cancer [30]. In addition, downregulation of GAS5 contributes to cell proliferation and predicts the adverse consequence of cervical cancer [31]. In our study, we used a public database and noticed that SNHG12 expression was significantly increased in colon cancer patients. Afterwards, qRT-PCR analysis confirmed that SNHG12 expression was evidently higher in colon cancer samples than that in normal tissues adjacent to tumors. On the other hand, a distinct increase in the expression of SNHG12 was observed in different colon cancer cell lines. It is worth noting that the patients with high SNHG12 expression had good prognosis. Therefore, these results hint that SNHG12 may be served as a potential prognostic marker of colon cancer.

The uncontrollable proliferation and metastasis and the decrease of apoptosis rate are the main reasons for the development and deterioration of cancers [32]. In this study, we found that silence of SNHG12 can inhibit the proliferation, colony formation, invasion as well as migration of colon cancer cells. Besides, SNHG-12 silencing induced colon cancer cell apoptosis. Additionally, upregulation of cleaved caspase-3 and -9 may be responsible for this phenomenon. More importantly, decreasing the expression of SNHG12 also compromised the proliferation of colon cancer tumors in BALB/c nude mice. Taken together, these data indicate that inhibiting SNHG12 can slow the progression of colon cancer. Indeed, previous studies have reported that SNHG12 regulates the progression of several types of cancers, such as diffuse large B-cell lymphoma, gastric and prostate cancer [33-35], which finding is consistent with our data.

As we know, lncRNA can interact with miRNA to participate in the pathogenesis of cancers [36]. In fact, it has been reported that SNHG12 is implicated in many kinds of cancers by regulating specific miRNAs, including miR-125b, miR-199a and miR-129 [18, 37, 38]. Interestingly, we observed that silence of SNHG12 caused a significantly increased expression of miR-15a. Meanwhile, a negative association was seen between the expression of SNHG12 and miR-15a in colon cancer samples. Subsequently, the binding of SNHG12 to miR-15a was verified by luciferase reporter gene experiment. Consistent with our results, a recent study reported that miR-15a, a critical tumor suppressor, is significantly decreased in colon cancer [39]. It has been well-accepted that miRNA

regulates cell biological function mainly by inhibiting the expression of target gene at the post-translational level [40, 41]. As a consequence, we identified a new target gene of miR-15a, PDK4, which plays an oncogenic role in colon cancer cells [23]. Specifically, the expression of PDK4 and its downstream molecule HIF1α was decreased in SNHG12-knockdown colon cancer cells. HIF-1α signaling has received a lot of attention regarding its role in facilitating colon cancer tumorigenesis [42]. Specifically, downregulation of HIF-1α target VEGFA was observed in colon cancer cells with SNHG12-knockdown. Furthermore, suppression of miR-15a expression largely recovered the malignant phenotype, as well as the expression of PDK4, HIF1α and VEGFA, in colon cancer cells with SNHG12-knockdown. These data indicate that SNHG12 may facilitate the progression of colon cancer via the miR-15a/PDK4 axis. On the other hand, it has been shown that lncRNA can modulate the biological functions of cells in multiple ways, including changing gene expression, altering protein location and affecting chromatin remodeling [43]. Thus, we cannot rule out the possibility that other mechanisms may also mediate the regulatory role of SNHG12 in colon cancer cells, which still needs further researches.

Conclusions

Our findings underscore the importance of SNHG12/miR-15a/PDK4 axis in the modulation of colon cancer progression (**Figure 6**). Hence, this research not only uncovers the intrinsic regulatory mechanism of colon cancer development but also provides a novel molecular target for treating this disease.

Acknowledgements

We sincerely thank all participants in the present study. This study did not receive specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Hua Meng, Department of Gastroenterology, Caoxian People's Hospital, East Qinghe Road, South Fumin Avenue, Caoxian Development Zone, Heze 274400, Shandong Province, China. Tel: +86-0530-3490800; E-mail: 1162175576@qq.com

References

[1] Taieb J, Andre T and Auclin E. Refining adjuvant therapy for non-metastatic colon cancer, new standards and perspectives. Cancer Treat Rev 2019; 75: 1-11.

[2] Wu Q, Meng WY, Jie Y and Zhao H. LncRNA MALAT1 induces colon cancer development by regulating miR-129-5p/HMGB1 axis. J Cell Physiol 2018; 233: 6750-6757.

[3] Aiello P, Sharghi M, Mansourkhani SM, Ardekan AP, Jouybari L, Daraei N, Peiro K, Mohamadian S, Rezaei M, Heidari M, Peluso I, Ghorat F, Bishayee A and Kooti W. Medicinal plants in the prevention and treatment of colon cancer. Oxid Med Cell Longev 2019; 2019: 2075614.

[4] Sun N, Zhang G and Liu Y. Long non-coding RNA XIST sponges miR-34a to promotes colon cancer progression via Wnt/beta-catenin signaling pathway. Gene 2018; 665: 141-148.

[5] Banerjee A, Pathak S, Subramanium VD, G D, Murugesan R and Verma RS. Strategies for targeted drug delivery in treatment of colon cancer: current trends and future perspectives. Drug Discov Today 2017; 22: 1224-1232.

[6] Gurkan AC, Arisan ED, Yerlikaya PO, Ilhan H and Unsal NP. Inhibition of autophagy enhances DENSpm-induced apoptosis in human colon cancer cells in a p53 independent manner. Cell Oncol (Dordr) 2018; 41: 297-317.

[7] Zykova T, Zhu F, Wang L, Li H, Lim DY, Yao K, Roh E, Yoon SP, Kim HG, Bae KB, Wen W, Shin SH, Nadas J, Li Y, Ma W, Bode AM and Dong Z. Targeting PRPK function blocks colon cancer metastasis. Mol Cancer Ther 2018; 17: 1101-1113.

[8] Li J, Li Z, Zheng W, Li X, Wang Z, Cui Y and Jiang X. LncRNA-ATB: an indispensable cancer-related long noncoding RNA. Cell Prolif 2017; 50: e12381.

[9] Tian M, Gong W and Guo J. Long non-coding RNA SNHG1 indicates poor prognosis and facilitates disease progression in acute myeloid leukemia. Biol Open 2019; 8: bio046417.

[10] Cao C, Zhang J, Yang C, Xiang L and Liu W. Silencing of long noncoding RNA UCA1 inhibits colon cancer invasion, migration and epithelial-mesenchymal transition and tumour formation by upregulating miR-185-5p in vitro and in vivo. Cell Biochem Funct 2020; 38: 176-184.

[11] Li J, Huang L, Li Z, Zhong X, Tai S, Jiang X and Cui Y. Functions and roles of long noncoding RNA in cholangiocarcinoma. J Cell Physiol 2019; 234: 17113-17126.

[12] Noh JH, Kim KM, McClusky WG, Abdelmohsen K and Gorospe M. Cytoplasmic functions of long noncoding RNAs. Wiley Interdiscip Rev RNA 2018; 9: e1471.
[13] Lin C, Zhang Y, Chen Y, Bai Y and Zhang Y. Long noncoding RNA LINC01234 promotes serine hydroxymethyltransferase 2 expression and proliferation by competitively binding miR-642a-5p in colon cancer. Cell Death Dis 2019; 10: 137.
[14] Jiang H, Li T, Qu Y, Wang X, Li B, Song J, Sun X, Tang Y, Wan J, Yu Y, Zhan J and Zhang H. Long non-coding RNA SNHG15 interacts with and stabilizes transcription factor slug and promotes colon cancer progression. Cancer Lett 2018; 425: 78-87.
[15] Liu Y, Cheng G, Huang Z, Bao L, Liu J, Wang C, Xiong Z, Zhou L, Xu T, Liu D, Yang H, Chen K and Zhang X. Long noncoding RNA SNHG12 promotes tumour progression and sunitinib resistance by upregulating CDCA3 in renal cell carcinoma. Cell Death Dis 2020; 11: 515.
[16] Dong J, Wang Q, Li L and Xiao-Jin Z. Upregulation of long non-coding RNA small nucleolar RNA host gene 12 contributes to cell growth and invasion in cervical cancer by acting as a sponge for MiR-424-5p. Cell Physiol Biochem 2018; 45: 2086-2094.
[17] Zhou S, Yu L, Xiong M and Dai G. LncRNA SNHG12 promotes tumorigenesis and metastasis in osteosarcoma by upregulating Notch2 by sponging miR-195-5p. Biochem Biophys Res Commun 2018; 495: 1822-1832.
[18] Lan T, Ma W, Hong Z, Wu L, Chen X and Yuan Y. Long non-coding RNA small nucleolar RNA host gene 12 (SNHG12) promotes tumorigenesis and metastasis by targeting miR-199a/b-5p in hepatocellular carcinoma. J Exp Clin Cancer Res 2017; 36: 11.
[19] Marchiori C, Scarpa M, Kotsafti A, Morgan S, Fassan M, Guzzardo V, Porzionato A, Angriman I, Ruffolo C, Sut S, Dall'Acqua S, Bardini R, De Caro R, Castoro C, Scarpa M and Castagliuolo I. Epithelial CD80 promotes immune surveillance of colonic preneoplastic lesions and its expression is increased by oxidative stress through STAT3 in colon cancer cells. J Exp Clin Cancer Res 2019; 38: 190.
[20] Wang M, Wei J, Shang F, Zang K and Zhang P. Down-regulation of lncRNA SNHG5 relieves sepsis-induced acute kidney injury by regulating the miR-374a-3p/TLR4/NF-kappaB pathway. J Biochem 2021; 169: 575-583.
[21] Lu J, Wu X, Wang L, Li T and Sun L. Long noncoding RNA LINC00467 facilitates the progression of acute myeloid leukemia by targeting the miR-339/SKI pathway. Leuk Lymphoma 2021; 62: 428-437.
[22] Dai L, Wang W, Zhang S, Jiang Q, Wang R, Dai L, Cheng L, Yang Y, Wei YQ and Deng HX. Vector-based miR-15a/16-1 plasmid inhibits colon cancer growth in vivo. Cell Biol Int 2012; 36: 765-770.
[23] Leclerc D, Pham DN, Levesque N, Truongcao M, Foulkes WD, Sapienza C and Rozen R. Oncogenic role of PDK4 in human colon cancer cells. Br J Cancer 2017; 116: 930-936.
[24] Prados J, Melguizo C, Roldan H, Alvarez PJ, Ortiz R, Arias JL and Aranega A. RNA interference in the treatment of colon cancer. BioDrugs 2013; 27: 317-327.
[25] Mishra J, Drummond J, Quazi SH, Karanki SS, Shaw JJ, Chen B and Kumar N. Prospective of colon cancer treatments and scope for combinatorial approach to enhanced cancer cell apoptosis. Crit Rev Oncol Hematol 2013; 86: 232-250.
[26] Anitha A, Maya S, Sivaram AJ, Mony U and Jayakumar R. Combinatorial nanomedicines for colon cancer therapy. Wiley Interdiscip Rev Nanomed Nanobiotechnol 2016; 8: 151-159.
[27] Wang D and Hu Y. Long non-coding RNA PVT1 competitively binds MicroRNA-424-5p to regulate CARM1 in radiosensitivity of non-small-cell lung cancer. Mol Ther Nucleic Acids 2019; 16: 130-140.
[28] Zhao X, Li D, Huang D, Song H, Mei H, Fang E, Wang X, Yang F, Zheng L, Huang K and Tong Q. Risk-associated long noncoding RNA FOXD3-AS1 inhibits neuroblastoma progression by repressing PARP1-mediated activation of CTCF. Mol Ther 2018; 26: 755-773.
[29] Zhang LG, Zhou XK, Zhou RJ, Lv HZ and Li WP. Long non-coding RNA LINC00673 promotes hepatocellular carcinoma progression and metastasis through negatively regulating miR-205. Am J Cancer Res 2017; 7: 2536-2544.
[30] Zhao Y, Feng C, Li Y, Ma Y and Cai R. LncRNA H19 promotes lung cancer proliferation and metastasis by inhibiting miR-200a function. Mol Cell Biochem 2019; 460: 1-8.
[31] Wang X, Zhang J and Wang Y. Long noncoding RNA GAS5-AS1 suppresses growth and metastasis of cervical cancer by increasing GAS5 stability. Am J Transl Res 2019; 11: 4909-4921.
[32] Wu J, Li L, Wu S and Xu B. CMTM family proteins 1-8: roles in cancer biological processes and potential clinical value. Cancer Biol Med 2020; 17: 528-542.
[33] Zhang R, Liu Y, Liu H, Chen W, Fan HN, Zhang J and Zhu JS. The long non-coding RNA SNHG12 promotes gastric cancer by activating the phosphatidylinositol 3-kinase/AKT pathway. Aging (Albany NY) 2019; 11: 10902-10922.
[34] Cheng G, Song Z, Liu Y, Xiao H, Ruan H, Cao Q, Wang K, Xiao W, Xiong Z, Liu D, Chen K and

Zhang X. Long noncoding RNA SNHG12 indicates the prognosis of prostate cancer and accelerates tumorigenesis via sponging miR-133b. J Cell Physiol 2020; 235: 1235-1246.
[35] Chen LY, Zhang XM, Han BQ and Dai HB. Long noncoding RNA SNHG12 indicates the prognosis and accelerates tumorigenesis of diffuse large B-cell lymphoma through sponging microR-195. Onco Targets Ther 2020; 13: 5563-5574.
[36] Wang G, Li X, Song L, Pan H, Jiang J and Sun L. Long noncoding RNA MIAT promotes the progression of acute myeloid leukemia by negatively regulating miR-495. Leuk Res 2019; 87: 106265.
[37] Jin XJ, Chen XJ, Zhang ZF, Hu WS, Ou RY, Li S, Xue JS, Chen LL, Hu Y and Zhu H. Long noncoding RNA SNHG12 promotes the progression of cervical cancer via modulating miR-125b/STAT3 axis. J Cell Physiol 2019; 234: 6624-6632.
[38] Sun D and Fan XH. LncRNA SNHG12 accelerates the progression of ovarian cancer via absorbing miRNA-129 to upregulate SOX4. Eur Rev Med Pharmacol Sci 2019; 23: 2345-2352.
[39] Xu M, Zhou B, Wang G, Wang G, Weng X, Cai J, Li P, Chen H, Jiang X and Zhang Y. miR-15a and miR-16 modulate drug resistance by targeting bcl-2 in human colon cancer cells. Zhonghua Zhong Liu Za Zhi 2014; 36: 897-902.
[40] Chen L, Heikkinen L, Wang C, Yang Y, Sun H and Wong G. Trends in the development of miRNA bioinformatics tools. Brief Bioinform 2019; 20: 1836-1852.
[41] Xu J, Shao T, Ding N, Li Y and Li X. miRNA-miRNA crosstalk: from genomics to phenomics. Brief Bioinform 2017; 18: 1002-1011.
[42] Kim DH, Sung B, Kang YJ, Hwang SY, Kim MJ, Yoon JH, Im E and Kim ND. Sulforaphane inhibits hypoxia-induced HIF-1alpha and VEGF expression and migration of human colon cancer cells. Int J Oncol 2015; 47: 2226-2232.
[43] Engreitz JM, Haines JE, Perez EM, Munson G, Chen J, Kane M, McDonel PE, Guttman M and Lander ES. Local regulation of gene expression by lncRNA promoters, transcription and splicing. Nature 2016; 539: 452-455.