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RESEARCH ARTICLE

Long noncoding RNA *SNHG12* suppresses esophageal squamous cell carcinoma progression through competing endogenous RNA networks

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Abstract

Purpose Esophageal squamous cell cancer (ESCC) has high rates of recurrence and mortality. Small nucleolar RNA host gene 12 (*SNHG12*) is known to promote the progression of several cancers. Therefore, we aimed to investigate the expression and role of *SNHG12* in ESCC.

Methods The expression and clinical value of *SNHG12* in esophageal cancer were explored using data from The Cancer Genome Atlas (TCGA) and the online server GEPIA. Real-time quantitative polymerase chain reaction (qRT-PCR) was used to verify the expression levels of *SNHG12* in ESCC tissues and cell lines. Furthermore, loss-of-function assays were performed to examine the effect of *SNHG12* on ESCC cells in vitro and in vivo. The potential competing endogenous RNA networks of *SNHG12* in ESCC were explored.

Results *SNHG12* was downregulated in human ESCA tissues compared to control tissues. The expression of *SNHG12* was strongly associated with T stage, N stage, and TNM stage. Low *SNHG12* expression in esophageal tumor tissues was significantly correlated with poor prognosis. Furthermore, knockdown of *SNHG12* not only promoted proliferation, colony formation, migration, and invasion and inhibited apoptosis in ESCC cells in vitro, but also increased tumor growth in vivo. Additionally, this proves that the *SNHG12*/miRNA-195-5p/BCL9 network might be involved in ESCC.

Conclusion This is the first study to reveal that *SNHG12* is downregulated in ESCC tissues and could be used as a prognostic tool. *SNHG12* suppressed tumor progression in ESCC cells, serving as a potential biomarker. The *SNHG12*/miRNA-195-5p/BCL9 network is proposed to be the mechanism leading to ESCC progression.

Keywords lncRNAs · *SNHG12* · Esophageal squamous cell carcinoma · Prognosis · Competing endogenous networks

Abbreviations

lncRNAs	Long non-coding RNAs
SNHG12	Small nucleolar RNA host gene 12
ESCA	Esophageal cancer
ESCC	Esophageal squamous cell cancer
TCGA	The Cancer Genome Atlas
GEPIA	Gene Expression Profiling Interactive Analysis
qRT-PCR	Real-time quantitative polymerase chain reaction
CCK-8	Cell counting kit-8
OS	Overall survival
ceRNA	Competing endogenous RNA

M. Liang, Z. Pan and F. Yu contributed equally to this study.

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Introduction

Esophageal cancer (ESCA) is the seventh most common type of cancer and the sixth most common cause of cancer-related deaths worldwide [1]. Esophageal squamous cell carcinoma (ESCC) is the main subtype of ESCA in eastern countries, including China [2]. Although the therapeutic efficacy of

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early treatment against ESCC has been improving [3], the 5-year overall survival (OS) is still approximately 20–30% [4]. It is, therefore, crucial explore the molecular mechanisms underlying this disease and to seek novel therapeutic approaches for ESCC.

Long non-coding RNAs (lncRNAs) are a class of non-coding RNAs, which have a length of over 200 nucleotides and which are transcribed by RNA polymerase II [5]. They are located in the nucleus or in the cytoplasm and lack protein-coding function [6–8]. Although it was originally believed that lncRNAs were only transcriptional noise, a large number of studies have confirmed that lncRNAs are involved in tumor invasion, metastasis, autophagy, differentiation, and other biological processes and that they play an important role in the development and progression of certain diseases in humans, especially cancer [9, 10]. For example, the lncRNA HOX Transcript Antisense RNA (*HOTAIR*) is upregulated in hepatocellular carcinoma cells; knockdown of *HOTAIR* inhibits cancer cell migration and invasion [11]. Furthermore, the expression of the lncRNA metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*) was found to be upregulated in several types of cancer cells. Moreover, *MALAT1* may control cell proliferation and inhibit the proliferation of cancer cells by regulating the expression of the transcription factor B-Myb [12]. In addition, the lncRNA *XLOC_010588* is downregulated in cervical cancer and modulates cell proliferation by regulating the expression of c-Myc [13].

LncRNA small nucleolar RNA host gene 12 (*SNHG12*) is located on chromosome 1, in the 1p35.3 region, and has a length of 963 bp. *SNHG12* was first reported to be significantly upregulated in endometrial cancer in human [14]. Similarly, in osteosarcoma [15, 16], cervical cancer [17, 18], glioma [19], and colon cancer [20] in humans, the overexpression of *SNHG12* suggests that it acts as an oncogene, promoting the development of tumors. However, the expression levels and biological effects of *SNHG12* in ESCC have not yet been reported. Therefore, in this study, we aimed to analyze the expression and role of *SNHG12* in ESCC.

Materials and methods

Gene Expression Profiling Interactive Analysis (GEPIA)

GEPIA is an innovative new interactive web server developed to analyze RNA sequencing data from 9,736 tumors and 8,587 healthy samples. GEPIA provides customizable functions such as tumor/normal differential expression analysis, profiling according to cancer type or pathological stage, patient survival analysis, genetic similarity detection, correlation analysis, and dimensionality reduction analysis [21]. We used this open-access website to explore the expression and prognostic effect of *SNHG12* in ESCA.

ESCC tissue samples

Samples of cancerous tissues and healthy cells adjacent to these were resected from 85 patients with ESCC at Fujian Medical University Union Hospital between July 2014 and December 2015. The status of all samples was confirmed by pathological diagnosis. The patients did not receive chemotherapy, radiotherapy, immunotherapy, or other treatments for ESCC before the operation. Ethical approval for this study and patient consent were obtained.

Cell culture and transfection

ESCC cell lines (KYSE140, KYSE510, Eca9706, and Ec109) and one normal esophageal cell line (Het-1A) were purchased from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. Cells were cultured in 1640 medium (GIBCO-BRL) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 mg/mL streptomycin (Invitrogen, Carlsbad, CA, USA), and were maintained in 5% CO_2, at 37 °C. All cell lines tested negative for the presence of mycoplasma.

Small interfering RNA sequences against *SNHG12* (si-SNHG12) and nonspecific negative control sequences (si-NC) were obtained from Sangon Biotech (Shanghai, China). The sense and antisense strands of the siRNAs are shown in Table S1. Cells were cultured and transfected with si-SNHG12 or si-NC using Lipofectamine 3000 (Invitrogen), following the manufacturer's instructions.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA was extracted using the TRIzol reagent (Invitrogen), according to the manufacturer's instructions. cDNA was synthesized from 1000 μg of total RNA, using the Prime-Script™ One Step RT-PCR Kit (TaKaRa, Dalian, China). *SNHG12* levels were determined using the SYBR Premix Ex Taq II Kit (TaKaRa) and the Applied Biosystems 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). The relative expression level of the target gene was presented as $2^{-\Delta\Delta Ct}$, using the relative quantification method. The expression levels of *SNHG12* were normalized to GAPDH.

The following primer sequences were used for *SNHG12* and *GAPDH*:

SNHG12 (forward): 5′-TCTGGTGATCGAGGACTTCC-3′;

SNHG12 (reverse): 5′-ACCTCCTCAGTATCACACACT-3′;

GAPDH (forward): 5′-ACACCCACTCCTCCACCTTT-3′;

GAPDH (reverse): 5′-TTACTCCTTGGAGGCCATGT- 3′.

The above sequences were synthesized by Sangon Biotech (Shanghai, China).

Cell Counting Kit-8 (CCK-8) assay

SNHG12-silenced cells and control cells were inoculated onto a 96-well plate (3×10^3 cells/well), and 100 µL culture medium was added to each well. After 0 h, 24 h, 48 h and 72 h in culture, 10 µL CCK-8 solution was added to each well. After 2 h of culturing, the absorbance of each well was measured at the wavelength of 450 nm using the microplate reader.

Colony formation assay

For the colony formation assay, cells were trypsinized into single-cell suspensions. Then, 5×10^2 cells were plated into each plate and maintained in complete culture medium. The culture medium was refreshed every 3 days. After 2 weeks, colonies were fixed with formaldehyde and stained with 0.1% crystal violet respectively, for 20 min. Finally, visible colonies were photographed and manually counted. Each experiment was conducted in triplicate.

Apoptosis via flow cytometry

After transfection for 48 h, cells from the two groups were collected and placed in the dark for 15 min. The apoptotic rate of each group was detected using flow cytometry after double staining with Annexin V and propidium iodide.

Transwell assay

Cell migration or invasion assay was carried out respectively, using a 24-well transwell chamber (Corning, Kennebunk, ME, USA), coated with or without Matrigel (BD Biosciences, Bedford, MA, USA). The interference sequences and control sequences were transiently transfected into ESCC cells. After 48 h, a single-cell suspension was prepared and the density was adjusted to 1×10^5 cells/mL. Then, 200 µL of the cell suspension was added into the upper chamber of the transwell plate, and 600 µL of 1640 medium containing 20% FBS was added into the lower chamber. The cells were then incubated for 36 h under conventional conditions. We performed three technical replicates for each group, after which cells were fixed in formaldehyde and stained with 0.1% crystal violet.

Xenograft studies

The study was approved by Medical Laboratory Animal Welfare and Ethics Committee of Fujian Medical University. Five- to six-week-old male BALB/c nude mice were used, with each experimental group consisting of five mice. 2×10^6 cells were suspended with 200 µl PBS, and injected subcutaneously into the right armpit of each mouse. The volume and weight of the tumors were measured every 3 days with calipers and an electronic scale. The volume of the tumors was calculated using the formula: length × width^2/2. The mice were euthanized 3 weeks after injection, and the tumors were harvested. The methods were performed according to the National Guidelines for the Care and Maintenance of Laboratory Animals.

RNA interactome analysis

starBase v3.0 is an open-source platform for studying miRNA-ncRNA, miRNA-mRNA, ncRNA-RNA, RNA-RNA, RBP-ncRNA, and RBP-mRNA interactions from CLIP-seq, degradome-seq, and RNA-RNA interactome data [22]. Using this database, we explored the potential competing endogenous RNAs (ceRNA) of *SNHG12* in ESCC. We first investigated candidate targeted mRNAs of *SNHG12*. We then identified candidate miRNAs of *SNHG12* and targeted mRNA. The interactions between the mRNAs and miRNAs were then detected. Finally, the exact binding sites of *SHNG12*, miRNAs, and mRNAs were analyzed.

Statistical analysis

All experiments were performed in triplicate and were analyzed using SPSS version 23.0 (IBM Corporation, Armonk, NY, USA). Data were expressed as mean ± standard deviation, and the Student's *t* test was used to assess statistical significance. Clinicopathological factors were compared between the groups using either the Pearson's chi-square test or the Fisher's exact test. The Kaplan–Meier method and log-rank tests were conducted for survival curve analysis and statistical differences, using GraphPad Prism 7.0 (GraphPad Software Inc., San Diego, CA, USA). Two-tailed *p* values of <0.05 were considered to indicate statistical significance.

Results

Downregulated expression of *SNHG12* in ESCA is correlated with poor prognosis in data from the TCGA database

The expression of *SNHG12* in ESCA in TCGA was explored through GEPIA. *SNHG12* expression was significantly

downregulated in ESCA tissues compared with healthy tissues (Fig. 1a). Moreover, low expression of *SNHG12* was significantly associated with the poor OS of patients with ESCA (hazards rate = 0.6, $p = 0.045$, Fig. 1b).

Downregulated expression of *SNHG12* in ESCC from our data

To verify the previous results, tissue samples from 85 cases of ESCC and adjacent healthy tissues were collected. The relative expression level of *SNHG12* in ESCC tissues was detected via qRT-PCR. The results showed that *SNHG12* expression was downregulated in 85 cases of ESCC tissues compared to adjacent healthy tissues (Fig. 1c). Besides, low expression of *SNHG12* was found in 60.0% specimens from ESCC patients (51 of 85 patients). The correlations between *SNHG12* expression and clinicopathological features are shown in Table 1. *SNHG12* expression was strongly associated with T stage ($p = 0.024$), *N* stage ($p = 0.033$), and TNM stage ($p = 0.016$). Furthermore, the 3-year OS rate of patients with low *SNGH12* expression was significantly lower than that of patients with high *SNHG12* expression ($p = 0.0467$, Fig. 1d).

SNHG12 knockdown *SNHG12* in Ec109 cells

We selected four different ESCC cell lines (KYSE140, KYSE510, Eca9706, and Ec109) and one normal esophageal cell line (Het-1A) and analyzed the relative expression levels of *SNHG12* in these cells by qRT-PCR. The results showed that the relative expression levels of *SNHG12* were lower in the four ESCC cell lines (Fig. 2a). Ec109 cells were used for follow-up experiments. The *SNHG12* interference sequences were designed and synthesized, and transiently transfected into Ec109 cells. After 48 h, the transfection efficiency was analyzed via qRT-PCR (Fig. 2b).

Knockdown of SNHG12 promotes cell proliferation and colony formation

The CCK-8 assay was used to detect the effect of *SNHG12* on the proliferation capacity of ESCC cells. The results showed

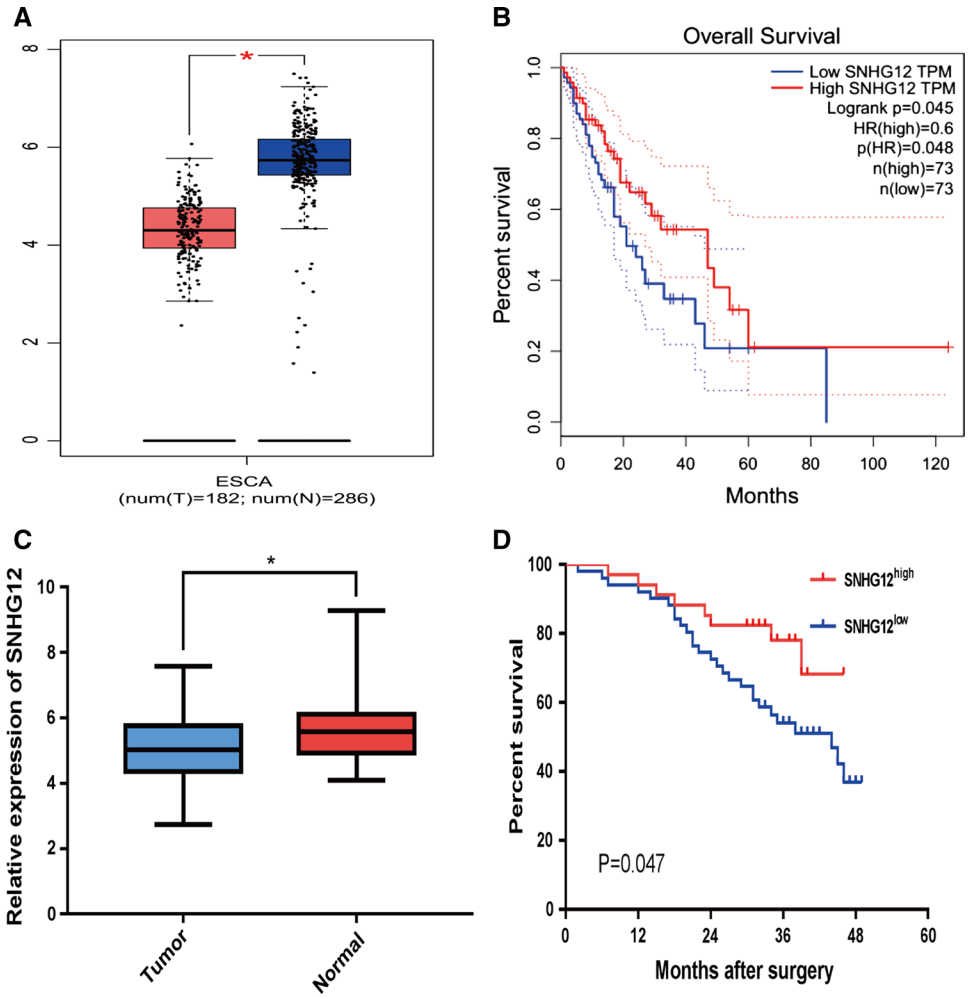


Fig. 1 *SNHG12* expression was significantly decreased in esophageal cancer tissues and low expression levels were correlated with poor prognosis. **a** Downregulation of *SNHG12* in ESCA tissues ($n = 182$) compared with normal tissues ($n = 286$) from the TCGA database (*$= p < 0.05$). **b** Kaplan–Meier curve of the overall survival rate of patients with ESCA from the TCGA database shows that the prognosis of patients with low *SNHG12* levels ($n = 73$) is poorer than that of patients with high *SNHG12* levels ($n = 73$, $p = 0.045$). **c** Downregulation of *SNHG12* in ESCC tissues ($n = 85$) compared with normal tissues ($n = 85$, *$= p < 0.05$). **d** Kaplan–Meier curve of the overall survival rate of patients with ESCC with low *SNHG12* levels ($n = 51$) was poorer than that of those with high *SNHG12* levels ($n = 34$) in this study ($p = 0.0467$)

Table 1 Association between *SNHG12* and clinicopathogical features in 85 ESCC patients

Variables	No. of patients	SNHG12 expression		*P* value
		Low ($n=51$)	High ($n=34$)	
Age				
≤60	26	15	11	0.773
>60	59	36	23	
Gender				
Female	47	33	14	0.117
Male	38	18	20	
Tumor size				
≤4 cm	58	33	25	0.392
>4 cm	27	18	9	
Tumor location				
Upper/middle	58	32	26	0.183
Lower	27	19	8	
Tumor grading				
G1	28	16	12	0.706
G2/3	57	35	22	
T stage				
T1	28	12	16	**0.024***
T2/3	57	39	18	
N stage				
N-	47	33	14	**0.033***
N+	38	18	20	
TNM stage				
≤II	49	24	25	**0.016***
>II	36	27	9	

* indicates statistical significance ($p < 0.05$)

that the cell proliferation capacity in the experimental group was significantly increased compared to that of the control group (Fig. 2c). Further, the colony formation assay was performed to study the effect of *SNHG12* on the proliferation capacity of ESCC cells. The number of colony foci in experimental group was significantly more than that of the control group (Fig. 2d, e).

Knockdown of *SNHG12* inhibits cell apoptosis

Furthermore, we examined the effect of *SNHG12* on apoptosis in ESCC cells using flow cytometry. Cells were collected after being transfected for 48 h. Flow cytometry showed that the apoptotic rate of Ec109 cells in the experimental group was decreased compared to that of the control group (Fig. 2f, g).

Knockdown of *SNHG12* promotes cell migration and invasion

A transwell assay was used to detect the changes in the migration and invasion capacity of ESCC cells after *SNHG12* silencing. After being transfected for 48 h, cells passing through the chamber were stained with crystal violet. Our results show that both the cell migration capacity (Fig. 3a, b) and the cell invasion capacity (Fig. 3c, d) in the experimental group were markedly increased compared with that in the control group.

Knockdown *SNHG12* significantly boosts cellular growth in vivo

To investigate the effects of *SNGH12* expression on ESCC cell growth in vivo, the Ec109 cell line was chosen to assess tumorigenicity. Cell lines of *SNHG12*-shRNA and *SNHG12*-shNC were thus xenografted into nude mice. Tumors harvested from nude mice with knockdown of SNHG12 were much larger than those in the control group. This suggested that the tumors grew at a much faster rate in the mice with SNHG12 knockdown (Fig. 4, all $P < 0.001$).

Identifying potential ceRNA networks involving SNHG12/miRNA-195-5p/BCL9 in ESCC

First, we identified 15 potential mRNAs of *SNHG12* (Fig. 5a). BCL9 [23], a part of the Wnt pathway, was chosen as the target mRNA. Second, we searched for candidate miRNAs of *SNHG12* and BCL9 (Table **S2**) and found 43 interactions of miRNAs (Fig. 5b). miRNA-195-5p, which had been identified in a previous study, was set as the target miRNA in ESCC. Third, we carefully explored the binding site between *SNHG12* and miRNA-195-5p and the binding site between miRNA-195-5p and BCL9 (Fig. 5c).

Discussion

Accumulating evidence has suggested that multiple oncogenes and tumor suppressor genes, including lncRNAs, a subgroup of non-coding RNAs that are over 200 nucleotides in length, participate in the molecular pathogenesis of tumors [24]. *SNHG12* is a novel lncRNA found to be upregulated and to function as an oncogene in several cancers. For instance, *SNHG12* is significantly upregulated in endometrial cancer. Knockdown of *SNHG12* suppresses proliferation and increases cell apoptosis and G1 phase arrest in endometrial cancer cells [14]. *SNHG12* has also been reported to be upregulated in triple-negative breast cancer tissues compared to normal tissues. Enforced expression of

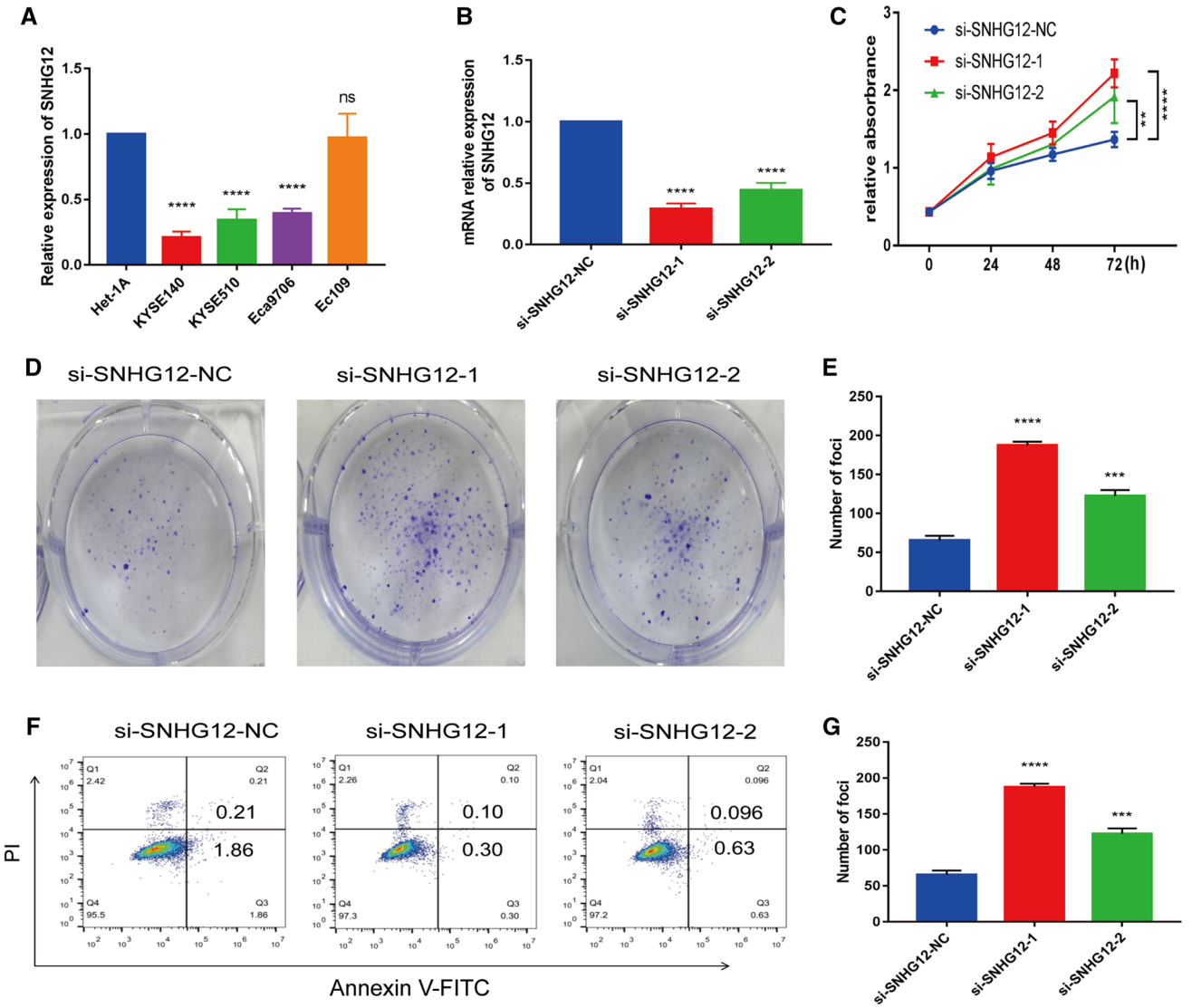


Fig. 2 Knockdown of *SNHG12* promotes ESCC cell proliferation and inhibits ESCC cell apoptosis. **a** mRNA expression levels of *SNHG12* in KYSE140, KYSE510, Eca9706, Ec109, and Het-1A cells. **b** qRT-PCR was used to analyze the expression of *SNHG12* in Ec109 cells transfected with *SNHG12* siRNA and scramble negative control (NC) siRNA. **c** CCK-8 assay was used to detect the proliferation rates. **d**, **e** A colony formation assay was used to detect the colony formation efficiency. Images of the colony formation of each group have been presented. The average colony formation number among different experimental groups are shown. **f**, **g** Cell death was monitored by flow cytometry after staining with Annexin V-fluorescein isothiocyanate and propidium iodide (FITC/PI). Quadrant statistics: early apoptosis cells on the lower right side (LR), late apoptosis on the upper right side (UR), and viable cells and necrotic cells separately on the lower left (LL) and upper left side (UL). Flow cytometry analysis of Ec109 cells in the different experimental groups is shown. The percentage of total apoptotic cells in each group has been presented. Experiments were performed in triplicate and data were expressed as mean ± SD (**=$p<0.01$, ***=$p<0.001$, ****=$p<0.0001$)

SNHG12 enhanced proliferation and migration, and reduced apoptosis of triple-negative breast cancer cells [25]. Similarly, *SNHG12* knockdown inhibits cell growth and induces cell apoptosis by upregulating miR-138 in non-small cell lung cancer [26]. However, the expression and the effect of *SNHG12* in esophageal cancer has not yet been reported, which is why we found this important to investigate.

In this study, we first examined the expression of *SNHG12* in TCGA data, and our results showed that *SNHG12* is underexpressed in ESCA tissues. Moreover, low expression of *SNHG12* is a poor prognostic factor. These results were inconsistent with previous studies, suggesting that *SNHG12* might play a different role in esophageal cancer than in other cancers. We then performed further experiments on our own set of samples. These also showed that *SNHG12* is downregulated in ESCC tissues compared to adjacent healthy tissue. Moreover, expression of *SNHG12* was strongly associated

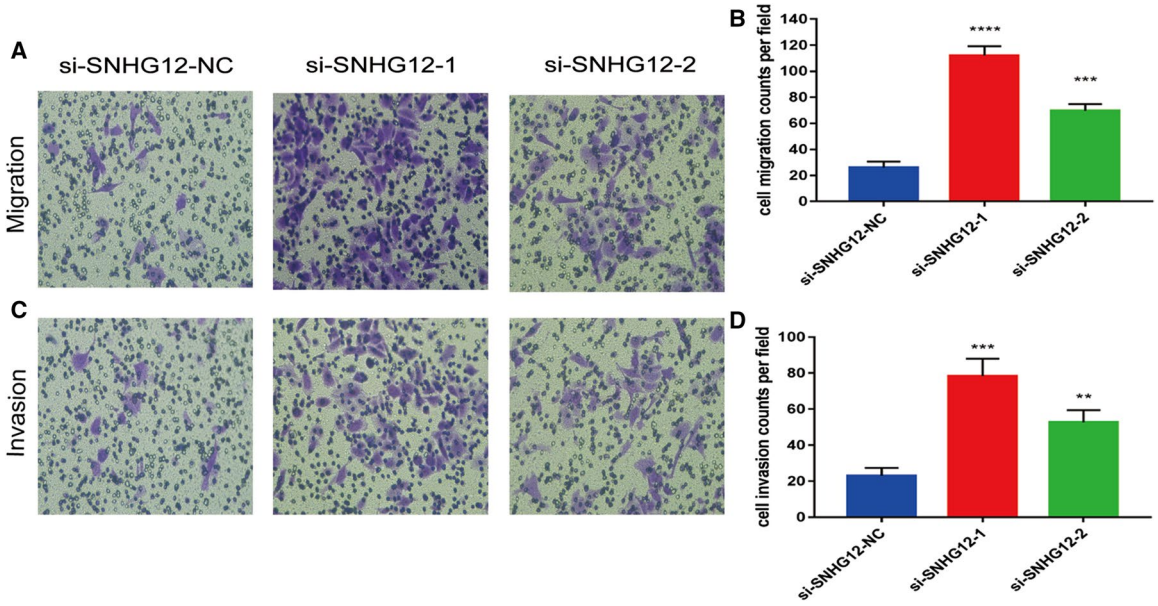


Fig.3 Knockdown of *SNHG12* promotes ESCC cell migration and invasion. **a** A transwell assay was used to detect cell migration (×20 magnification). Images of the migration ability of each group are presented. **b** The average migration cell number per field among different experimental groups. **c** A transwell assay was used to detect cell invasion (×20 magnification). Images of the invasion ability of each group are presented. **d** The average invasion cell number per field among different experimental groups. Data were expressed as mean ± SD from three independent experiments (**=$p<0.01$, ***=$p<0.001$, ****=$p<0.0001$)

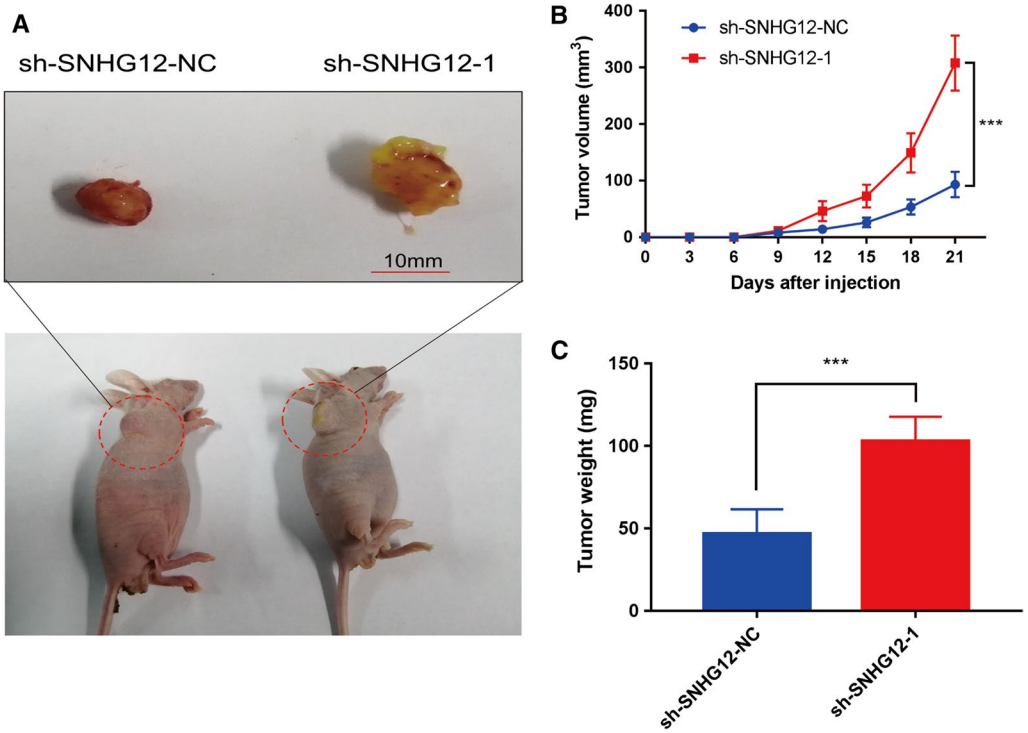


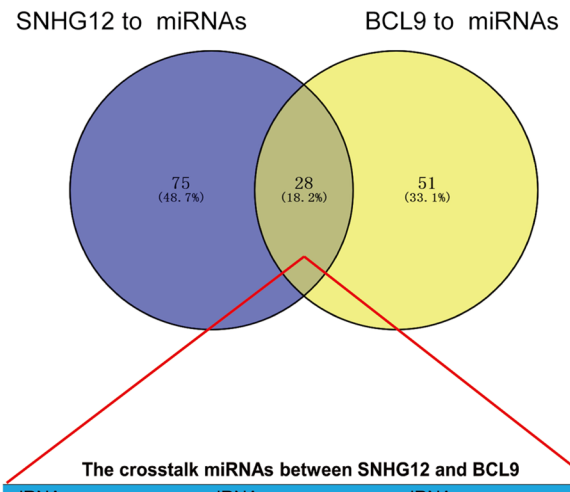
Fig.4 Knockdown of *SHNG12* significantly boosted cellular growth in vivo. **a** Representative images of the nude mice and bearing subcutaneous tumors. **b** The tumor volume curves were summarized at the indicated number of days after mice were injected with tumor cells. **c** The tumor weight was measured for each group. Data were collected from experiments and expressed as mean ± SD (***=$p<0.001$)

A

The key ceRNAs of SNHG12

ceRNA ID	ceRNA Name	P-Value
ENSG00000155275	TRMT44	1.03E-05
ENSG00000128928	IVD	2.55E-05
ENSG00000145819	ARHGAP26	6.03E-05
ENSG00000168924	LETM1	7.20E-05
ENSG00000163933	RFT1	7.55E-05
ENSG00000066136	NFYC	9.99E-05
ENSG00000116128	BCL9	1.05E-04
ENSG00000073008	PVR	1.76E-04
ENSG00000141564	RPTOR	1.90E-04
ENSG00000125818	PSMF1	2.51E-04
ENSG00000144579	CTDSP1	2.78E-04
ENSG00000118707	TGIF2	3.78E-04
ENSG00000114988	LMAN2L	3.90E-04
ENSG00000070159	PTPN3	6.61E-04
ENSG00000071246	VASH1	1.17E-03

B



The crosstalk miRNAs between SNHG12 and BCL9

miRNAname	miRNAname	miRNAname
hsa-let-7a-5p	hsa-let-7i-5p	hsa-miR-129-2-3p
hsa-let-7b-5p	hsa-miR-15b-5p	hsa-miR-361-3p
hsa-let-7d-5p	hsa-miR-138-5p	hsa-miR-330-5p
hsa-let-7e-5p	hsa-miR-195-5p	hsa-miR-488-3p
hsa-let-7f-5p	hsa-miR-326	hsa-miR-516a-5p
hsa-miR-15a-5p	hsa-miR-424-5p	hsa-miR-1301-3p
hsa-miR-16-5p	hsa-miR-494-3p	hsa-miR-4262
hsa-miR-197-3p	hsa-miR-495-3p	hsa-miR-378g
hsa-miR-199a-5p	hsa-miR-497-5p	hsa-miR-4458
hsa-miR-181a-5p	hsa-miR-181d-5p	hsa-miR-2467-3p
hsa-let-7a-6p	hsa-miR-515-5p	hsa-miR-5047
hsa-let-7b-6p	hsa-miR-519e-5p	hsa-miR-5688
hsa-let-7d-6p	hsa-miR-503-5p	hsa-miR-514a-5p
hsa-let-7e-6p	hsa-miR-514a-3p	
hsa-let-7f-6p	hsa-miR-140-3p	

C

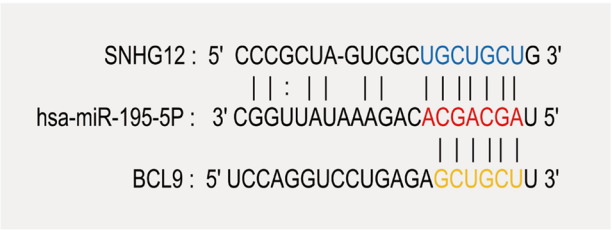


Fig.5 The potential ceRNA networks involving *SNHG12*/miRNA-195-5p/BCL9 in ESCC. **a** The potential 15 mRNAs of *SNHG12*. **b** The interaction of candidate miRNAs of *SNHG12* and BCL9. **c** The binding site between *SNHG12* and miRNA-195-5p, and the binding site between miRNA-195-5p and BCL9

with T stage, N stage, and TNM stage. Here as well, low *SNHG12* expression was associated with poor prognosis.

To verify these clinical findings, we designed and performed several in vitro and in vivo experiments. The expression of *SNHG12* was lower in four ESCC cell lines compared to normal esophageal cells. Knocking down *SNHG12* in ESCC cells promoted proliferation and inhibited apoptosis, suggesting *SNHG12* is correlated with T stage. Moreover, *SNHG12* knockdown promoted migration, suggesting that *SNHG12* is associated with N stage. Taken together, these indicate that *SNHG12* acts as a tumor suppressor in ESCA.

Based on these results, we believe that SNHG12 could serve as a prognostic biomarker that is able to monitor the post-operative recurrence or metastasis of ESCA. However, this needs to be confirmed by additional data from the peripheral blood of patients with ESCA in future studies.

The limitation of this study is that the underlying molecular mechanisms of *SNHG12* in ESCC are unclear. Previous studies have revealed that aberrant overexpression of *SNHG12* in several cancers occurs through distinct mechanisms. For example, *SNHG12* promotes tumorigenesis and metastasis by targeting miR-199a/b-5p in hepatocellular carcinoma [27]. Furthermore, c-Myc-mediated upregulation of long non-coding RNA *SNHG12* regulates proliferation and drug sensitivity in natural killer/T-cell lymphoma [28]. In addition, *SNHG12* promotes tumor progression in papillary thyroid carcinoma [5] and prostate cancer [29] by regulating the Wnt/β-catenin signaling pathway. Hence, we suggest that the potential mechanism of *SNHG12* in ESCC might involve competitive endogenous RNA networks and the Wnt/β-catenin pathway. This, together with our informatics analysis, suggest that *SNHG12*/miRNA-195-5p/BCL9 may be a candidate pathway in ESCC, which will need to be validated in the future.

In conclusion, this is the first study to reveal that *SNHG12* is downregulated in ESCC tissues. *SNHG12* was significantly associated with excellent prognosis. *SNHG12* suppressed tumor progression in ESCC cells and can serve as a prognostic biomarker. The *SNHG12*/miRNA-195-5p/BCL9 network as potentially the mechanism involved in ESCC progression.

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Authors' contributions The study was carried out in collaboration between all authors. Mingqiang Liang and Chun Chen defined the research theme, discussed analyses, and were involved in the interpretation and presentation of the data. Mingqiang Liang performed the statistical analysis. Mingqiang Liang and Zhipeng Pan drafted the manuscript, recorded the clinical data, analyzed the experimental data, and interpreted the results. Fengqiang Yu participated in reviewing all clinical records and assisted with data collection. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors have declared that no competing interest exists.

Ethical approval The Ethics approval and consent to participate in this study was approved by the Ethics Committee of Fujian Medical University Union Hospital.

Informed consent Informed consent was obtained from all individual participants included in the study.

Reference:s

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424.
2. Chen W, Zheng R, Zeng H, Zhang S. The updated incidences and mortalities of major cancers in China, 2011. Chin J cancer. 2015;34(11):502–7.
3. Ohashi S, Miyamoto S, Kikuchi O, Goto T, Amanuma Y, Muto M. Recent advances from basic and clinical studies of esophageal squamous cell carcinoma. Gastroenterology. 2015;149(7):1700–15.
4. Straatman J, Joosten PJ, Terwee CB, Cuesta MA, Jansma EP, van der Peet DL. Systematic review of patient-reported outcome measures in the surgical treatment of patients with esophageal cancer. Dis Esophagus. 2016;29(7):760–72.
5. Ding S, Qu W, Jiao Y, Zhang J, Zhang C, Dang S. LncRNA SNHG12 promotes the proliferation and metastasis of papillary thyroid carcinoma cells through regulating wnt/beta-catenin signaling pathway. Cancer biomarkers: section A of Disease markers. 2018;22(2):217–26.
6. Guttman M, Amit I, Garber M, French C, Lin MF, Feldser D, et al. Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature. 2009;458(7235):223–7.
7. Gutschner T, Diederichs S. The hallmarks of cancer: a long non-coding RNA point of view. RNA Biol. 2012;9(6):703–19.
8. Djebali S, Davis CA, Merkel A, Dobin A, Lassmann T, Mortazavi A, et al. Landscape of transcription in human cells. Nature. 2012;489(7414):101–8.
9. Saxena A, Carninci P. Long non-coding RNA modifies chromatin: epigenetic silencing by long non-coding RNAs. BioEssays: news and reviews in molecular, cellular and developmental biology. 2011;33(11):830–9.
10. Wang KC, Chang HY. Molecular mechanisms of long noncoding RNAs. Mol Cell. 2011;43(6):904–14.
11. Ding C, Cheng S, Yang Z, Lv Z, Xiao H, Du C, et al. Long non-coding RNA HOTAIR promotes cell migration and invasion via down-regulation of RNA binding motif protein 38 in hepatocellular carcinoma cells. Int J Mol Sci. 2014;15(3):4060–76.
12. Tripathi V, Shen Z, Chakraborty A, Giri S, Freier SM, Wu X, et al. Long noncoding RNA MALAT1 controls cell cycle progression by regulating the expression of oncogenic transcription factor B-MYB. PLoS Genet. 2013;9(3):e1003368.
13. Liao LM, Sun XY, Liu AW, Wu JB, Cheng XL, Lin JX, et al. Low expression of long noncoding XLOC_010588 indicates a poor prognosis and promotes proliferation through upregulation of c-Myc in cervical cancer. Gynecol Oncol. 2014;133(3):616–23.
14. Zhai W, Li X, Wu S, Zhang Y, Pang H, Chen W. Microarray expression profile of lncRNAs and the upregulated ASLNC04080 lncRNA in human endometrial carcinoma. Int J Oncol. 2015;46(5):2125–37.
15. Zhou S, Yu L, Xiong M, Dai G. LncRNA SNHG12 promotes tumorigenesis and metastasis in osteosarcoma by upregulating Notch2 by sponging miR-195-5p. Biochem Biophys Res Commun. 2018;495(2):1822–32.
16. Ruan W, Wang P, Feng S, Xue Y, Li Y. Long non-coding RNA small nucleolar RNA host gene 12 (SNHG12) promotes cell proliferation and migration by upregulating angiomotin gene expression in human osteosarcoma cells. Tumour Biol. 2016;37(3):4065–73.
17. Jin XJ, Chen XJ, Zhang ZF, Hu WS, Ou RY, Li S, et al. Long noncoding RNA SNHG12 promotes the progression of cervical cancer via modulating miR-125b/STAT3 axis. J Cell Physiol. 2019;234(5):6624–32.
18. Dong J, Wang Q, Li L, 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(5):2086–94.
19. Sun Y, Liu J, Chu L, Yang W, Liu H, Li C, et al. Long non-coding RNA SNHG12 facilitates the tumorigenesis of glioma through miR-101-3p/FOXP1 axis. Gene. 2018;676:315–21.
20. Wang JZ, Xu CL, Wu H, Shen SJ. LncRNA SNHG12 promotes cell growth and inhibits cell apoptosis in colorectal cancer cells. Braz J Med Biol Res. 2017;50(3):e6079.
21. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res. 2017;45(W1):W98–W102.
22. Li JH, Liu S, Zhou H, Qu LH, Yang JH. starBase v2.0. decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. Nucleic acids research. 2014, 42(Database issue): D92–97.
23. Brown TC, Nicolson NG, Korah R, Carling T. BCL9 upregulation in adrenocortical carcinoma: a novel Wnt/β-catenin activating event driving adrenocortical malignancy. J Am Coll Surg. 2018;226(6):988–95.

24. Guil S, Esteller M. RNA-RNA interactions in gene regulation: the coding and noncoding players. Trends Biochem Sci. 2015;40(5):248–56.
25. Wang O, Yang F, Liu Y, Lv L, Ma R, Chen C, et al. C-MYC-induced upregulation of lncRNA SNHG12 regulates cell proliferation, apoptosis and migration in triple-negative breast cancer. Am J Transl Res. 2017;9(2):533–45.
26. Wang X, Qi G, Zhang J, Wu J, Zhou N, Li L, et al. Knockdown of long noncoding RNA small nucleolar RNA host gene 12 inhibits cell growth and induces apoptosis by upregulating miR-138 in nonsmall cell lung cancer. DNA Cell Biol. 2017;36(11):892–900.
27. Lan T, Ma W, Hong Z, Wu L, Chen X, 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(1):11.
28. Zhu L, Zhang X, Fu X, Li Z, Sun Z, Wu J, et al. c-Myc mediated upregulation of long noncoding RNA SNHG12 regulates proliferation and drug sensitivity in natural killer/T-cell lymphoma. J Cell Biochem. 2019;120(8):12628–377.
29. Song J, Wu X, Ma R, Miao L, Xiong L, Zhao W. Long noncoding RNA SNHG12 promotes cell proliferation and activates Wnt/beta-catenin signaling in prostate cancer through sponging microRNA-195. J Cell Biochem. 2019;120(8):13066–75.