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ORIGINAL ARTICLE

LncRNA SNHG16 sponges miR-98-5p to regulate cellular processes in osteosarcoma

Shian Liao[1] · **Shuxing Xing**[2] · **Yanhui Ma**[3]

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Abstract

Background As has been illustrated that long noncoding RNAs (lncRNAs) and microRNAs (miRNAs) are potential regulators in the occurrence and progression of human cancers. LncRNA SNHG16 has been identified as an oncogene involved in the progression of human cancers. However, neither the function nor the underlying molecular mechanism of SNHG16 in osteosarcoma has been discovered.

Purpose The aim of the study is to explore the role and molecular regulation mechanism of SNHG16 in osteosarcoma.

Methods The expression of SNHG16 in HNSCC tissues and cells was detected by RT-qPCR assay. The biological function of SNHG16 in osteosarcoma was measured by CCK-8, cell cycle, cell apoptosis and transwell assays. The interaction between SNHG16 and miR-98-5p was studied by luciferase reporter and RIP assays.

Results The ectopic expression of SNHG16 was found in osteosarcoma tissues and cell lines, which indicated poor prognosis and lower overall survival rate of osteosarcoma patients. Knockdown of SNHG16 inhibited cell proliferation, migration, invasion, cell cycle and promoted apoptosis in osteosarcoma. It was demonstrated that SNHG16 directly interacts with miR-98-5p. What's more, we found a significantly negative correlation between SNHG16 and miR-98-5p expression. Finally, rescue experiments revealed that inhibition of miR-98-5p attenuated SNHG16 knockdown-mediated effects on cellular processes in osteosarcoma.

Conclusions LncRNA SNHG16 regulated cellular processes in osteosarcoma by sponging miR-98-5p, and SNHG16 may be a new and effective molecular therapeutic target for osteosarcoma.

Keywords SNHG16 · miR-98-5p · Cellular processes · Osteosarcoma

Introduction

Globally, osteosarcoma is one of the most common primary malignancies of bone, and with a high mortality in children and adolescents [1–3]. During the last decades, diagnostic and therapeutic regimes for osteosarcoma have made great advances, whereas the overall survival rate of osteosarcoma patients remains unsatisfactory [4]. The underlying molecular mechanisms for the tumorigenesis and progression of osteosarcoma still remain poorly understood. Therefore, it is particularly urgent to search for the valuable molecular therapeutic targets for osteosarcoma.

Long noncoding RNAs (lncRNAs), a class of by-products of genetic transcription, are longer than 200 nucleotides and without protein coding capacity [5, 6]. Many studies have observed and confirmed the critical roles of lncRNAs in the occurrence and development of cancers. In addition, they can participate in many cancer cellular processes, such as cell differentiation, cell cycle, metastasis and apoptosis [7–10]. It has been verified by mounting evidences that lncRNAs could function as oncogenes or tumor suppressor genes in multiple human cancers. For example, lncRNA PVT1 promotes ovarian cancer progression by silencing

Shian Liao and Shuxing Xing are co-first authors.

✉ Yanhui Ma
YanhuiMa_546@163.com

[1] Department of Bone and Soft Tissue Surgery, Affiliated Tumor Hospital of Guangxi Medical University, Nanning 530021, Guangxi, China

[2] Department of Orthopedics, Fifth People's Hospital of Chengdu, Chengdu 611130, Sichuan, China

[3] Department of Orthopedics, Affiliated Hospital, Department Orthoped and Traumatol, Yanan University, No. 43 North Street, Baota District, Yanan 716000, Shaanxi, China

miR-214 [11]. Long noncoding RNA PURPL suppresses basal p53 levels and promotes tumorigenicity in colorectal cancer [12]. The role of lncRNAs also has been investigated in osteosarcoma progression. For example, long noncoding RNA GAS5 suppresses cell growth and epithelial–mesenchymal transition in osteosarcoma by regulating the miR-221/ARHI pathway [13]. Long noncoding RNA miR210HG sponges miR-503 to facilitate osteosarcoma cell invasion and metastasis [14]. Long noncoding RNA EWSAT1 promotes osteosarcoma cell growth and metastasis through suppression of MEG3 expression [15]. Moreover, lncRNA SNHG16 contributes to the progression of many cancers [16–18]. The recent study also has revealed that SNHG16 promotes cell proliferation by sponging microRNA-205 and upregulating ZEB1 expression in osteosarcoma [19]. Long noncoding RNA SNHG16 promotes osteosarcoma cells migration and invasion via sponging miRNA-340 [20].

In this work, we identify the role of SNHG16 in modulating miR-98-5p to regulate cellular processes in osteosarcoma. We have studied the expression of SNHG16, its correlation with clinical characteristics as well as prognosis, and its biological effects on cellular processes of osteosarcoma. Further investigation revealed that SNHG16 could sponge miR-98-5p in osteosarcoma to promote cell proliferation, migration, invasion, cell cycle and inhibited apoptosis.

Materials and methods

Clinical samples

Ninety-six paired osteosarcoma tissues and adjacent normal tissues were obtained from patients at Affiliated Tumor Hospital of Guangxi Medical University. Patients had not suffered from radiotherapy or chemotherapy prior to surgery. All samples were immediately frozen at − 80 °C and stored for use. All patients signed the written informed consents. This work gained the approval of the Ethics Committee of Affiliated Tumor Hospital of Guangxi Medical University.

Cell culture and transfection

Human osteosarcoma cell lines (U2OS, Saos-2, HOS, MG-63) and the normal osteoblast cell line (hFOB 1.19) were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA), and cultured in Dulbecco's modified Eagle's medium (DMEM, Hyclone, Logan, UT, USA) which containing 10% fetal bovine serum (FBS; Gibco, Gran Island, NY, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin. These cells were cultured in a humidified incubator with 5% CO_2 at 37 °C.

Short hairpin RNA (shRNA) targeting SNHG16 (sh-SNHG16#1/2/3) and the negative control (sh-NC) were designed and synthesized by Genepharm (Shanghai, China). MiR-98-5p mimic, inhibitor and their control (miR-NC) were all purchased from GenePharma. Transfection for these plasmids was performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. RT-qPCR was used to assess the transfection efficacy.

The sequences of shRNAs:

sh-SNHG16#1: 5′- AAA GGC CTG AGA CTG CTC G-3′;
sh-SNHG16#2: 5′- GAA CGC ATG GCG ATT ACT T -3′;
sh-SNHG16#3: 5′- GAC AAA GCC TCT CAG CTC G -3′.

RNA extraction and quantitative real-time PCR

Total RNA was extracted from osteosarcoma tissues and cell lines using Trizol reagent (Takara, Dalian, China). First-strand cDNA was produced using the TaqMan™ Advanced miRNA cDNA Synthesis Kit (Waltham, MA, USA) or First Strand cDNA Synthesis Kit (Takara, Otsu, Japan). RT-qPCR was performed with the standard SYBR-Green PCR kit (Roche, America) on Applied Biosystems Step One Plus Real-Time PCR System (Applied Biosystems, Foster city, USA). The relative expression level of RNA was calculated by the $2^{-\Delta\Delta Ct}$ method. The internal controls were GAPDH and U6. The primer sequences used in this study were as follows:

SNHG16: 5′-GCAGAATGCCATGGTTTCCC-3′ (forward) and 5′-GGACAGCTGGCAAGAGACTT-3′ (reverse);
miR-98-5p: 5′-TGAGGTAGTAGTTTGTGCTGTT-3′ (forward) and 5′-GCGAGCACAGAATTAATACGAC-3′ (reverse);
GAPDH: 5′-GAA GGT GAA GGT CGG AGT C-3′ (forward) and 5′-GAA GAT GGT GAT GGG ATT TC-3′ (reverse);
U6: 5′-ATT GGA ACG ATA CAG AGA AGA TT-3′ (forward) and 5′-GGA ACG CTT CAC GAA TTT G-3′ (reverse).

CCK-8 assay

Cell Counting Kit-8 (CCK-8, Dojindo) was used to detect cell viability. Briefly, a total of 1×10^3 cells were seeded in each well of 96-well plates. After incubation for 0, 24, 48, 72 and 96 h, each well was added with CCK-8 solution. Finally, the absorbance was detected by a microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 450 nm for each well.

Cell cycle assay

Cell cycle assay was performed with a cell cycle detection kit (Fcmacs, Jiangsu, China). The transfected cells were collected and fixed in 95% ethanol overnight. Afterwards, the cells were stained with 50 μg/ml propidium iodide (PI) for 30 min in a dark chamber. The cell cycle distribution was measured on a flow cytometer (BD, FACS Calibur) using CellQuest software.

Cell apoptosis assay

Cell apoptosis assay was performed using the Annexin V-FITC kit (Biosea Biotechnology Co., Beijing, China) following the manufacturer's instructions. Transfected cells were seeded in 96-well plates for 48 h. Next, cells were harvested and washed with PBS, and then double stained with fluorescein isothiocyanate-labeled annexin V and propidium iodide (PI). Finally, the apoptotic cells were measured using a FACS Calibur flow cytometer (Becton–Dickinson, San Jose, CA, USA).

Transwell assay

Transwell assay was performed to detect the invasion and migration of osteosarcoma cells using chambers with or without Matrigel (BD Biosciences, Bedford, MA, USA). Transfected cells (1×10^4) suspended in 100 μl of serum-free DMEM medium were seeded in the upper chamber. DMEM medium (600 μl) supplemented with 20% FBS was added to the lower chamber. After 48 h, the cells migrated or invaded to the lower membrane were fixed using methanol, stained using crystal violet, and then counted under a microscope.

RIP assay

EZ-Magna RIP RNA-binding protein immunoprecipitation kit (Millipore, Billerica, MA, USA) was applied for RIP assay. Cells were lysed with RIP lysis buffer, then the lysate was incubated in RIP immunoprecipitation buffer supplemented with magnetic beads coated with human anti-argonaute2 (Ago2) antibody. Input and IgG were served as controls. Finally, the RNA was extracted and purified, and then detected by RT-qPCR.

Luciferase reporter assay

The SNHG16 3′UTR with binding sites for miR-98-5p or mutant sites was synthesized and cloned into pmirGLO plasmid (Promega), namely wild-type SNHG16 (Wt-SNG16) or mutant-type SNHG16 (Mut-SNHG16). U2OS and Saos-2 cells were seeded in 24-well plates and then were incubated for 24 h. MiR-98-5p mimic, miR-98-5p inhibitor or miR-NC was co-transfected with pmirGLO- SNHG16-Wt or pmirGLO- SNHG16-Mut into U2OS and Saos-2 cells using Lipofectamine 2000 reagent. After transfection for 48 h, the relative luciferase activity was measured using luciferase kits (Promega, Madison, WI, USA).

Statistical analysis

All data were displayed as the mean ± standard deviation (SD) and statistically analyzed using SPSS 20.0 software (SPSS, Chicago, IL, USA). All experiments were repeated in triplicate. Correlation between miR-98-5p and SNHG16 expression was compared using Pearson's correlation method. Kaplan–Meier and log-rank test were used to conduct survival analysis. Differences between groups were evaluated using Student's *t* test or the one-way ANOVA. Differences were considered significant at $p < 0.05$.

Results

SNHG16 is upregulated in osteosarcoma tissues and cell lines, and associated with the poor overall survival of patients

First, to explore the implication of SNHG16 in osteosarcoma, we detected the relative expression of SNHG16 in osteosarcoma tissues and cell lines via RT-qPCR assay. The results showed that compared with adjacent normal tissues and cell lines, SNHG16 expression was remarkably upregulated in osteosarcoma tissues and cell lines (Fig. 1a, b). To further explore the clinical significance of SNHG16 in osteosarcoma, all the patients were divided into two groups according to the mean level of SNHG16 expression: the SNHG16 low expression group and the SNHG16 high expression group. SNHG16 expression was significantly associated with tumor size ($p = 0.010$), TNM stage ($p = 0.011$) and metastasis ($p = 0.0001$) in osteosarcoma patients (Table 1). Additionally, we found that osteosarcoma tissues at advanced stage (stage III–IV) exhibited higher expression of SNHG16 (Fig. 1c). Multivariate analysis demonstrated that SNHG16 expression ($p = 0.025$) and TNM stage ($p = 0.026$) were independent prognostic factors for osteosarcoma patients (Table 2, $p < 0.05$). Kaplan–Meier analysis showed that osteosarcoma patients with higher SNHG16 expression had worse overall survival (Fig. 1d). In a word, SNHG16 may be an oncogene and involved in the progression of osteosarcoma.

Knockdown of SNHG16 inhibits cell proliferation, migration, invasion, cell cycle and promotes apoptosis in osteosarcoma

To evaluate the biological effect of SNHG16 on osteosarcoma progression, sh-SNHG16#1 and its control (sh-NC)

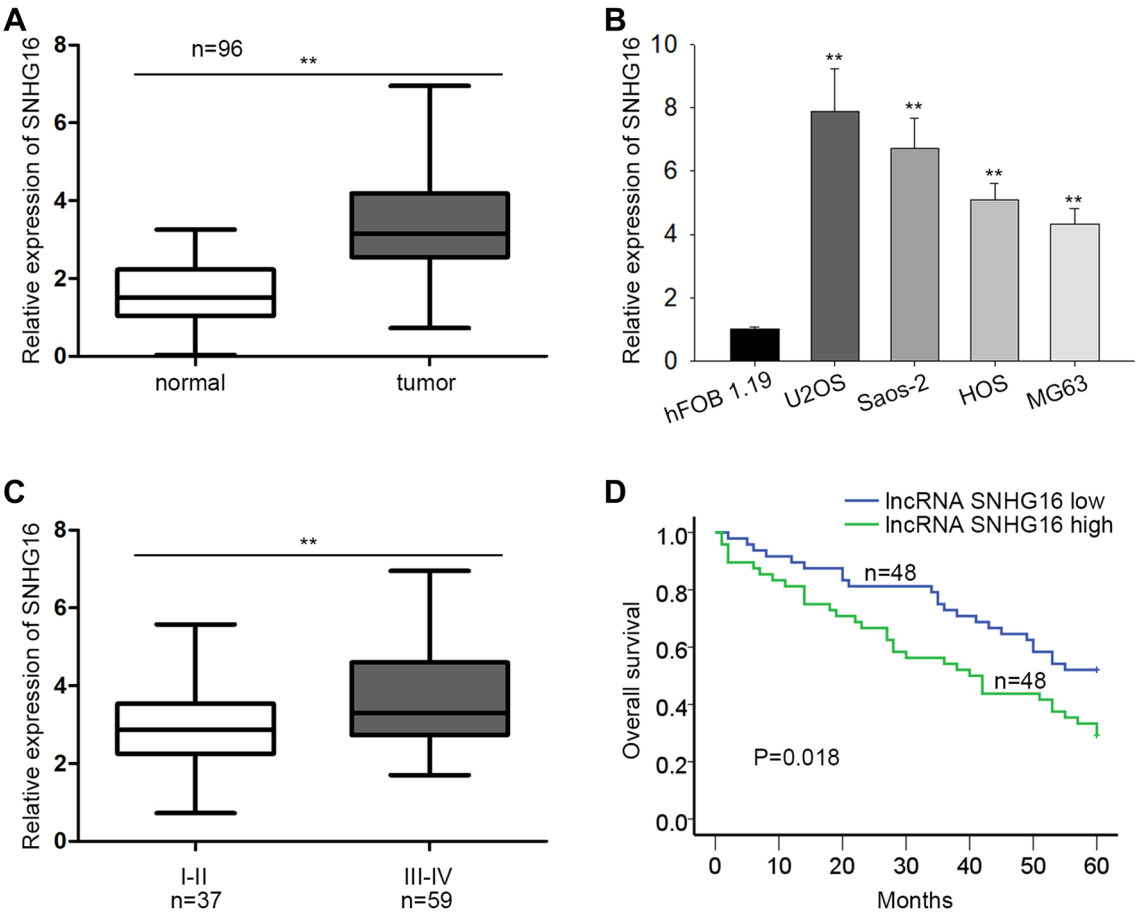

Fig. 1 SNHG16 is upregulated in osteosarcoma tissues and cell lines. **a** SNHG16 expression in osteosarcoma tissues and adjacent non-tumor tissues was determined by RT-qPCR. **b** SNHG16 expression in human osteosarcoma cell lines (U2OS, Saos-2, HOS, MG-63) and the normal osteoblast cell line (hFOB 1.19) was determined by RT-qPCR. **c** SNHG16 expression in different clinical stages was determined by RT-qPCR. **d** The correlation of overall survival and SNHG16 expression in osteosarcoma patients. $^{*}p < 0.05$, $^{**}p < 0.01$

were transfected into U2OS or Saos-2 cells. Then, the knockdown efficiency was confirmed by RT-qPCR (Fig. 2a). CCK-8 assay revealed that knockdown of SNHG16 notably inhibited osteosarcoma cell proliferation (Fig. 2b). Flow cytometry cell cycle assay indicated that upon SNHG16 downregulation, the proportion of U2OS and Saos-2 cells arrested at G0/G1 phase increased while the proportion at S and G2/M phase decreased, implying that SNHG16 induced cell cycle and then promoted cell proliferation (Fig. 2c). Furthermore, we also observed via flow cytometry cell apoptosis assay that knockdown of SNHG16 promoted osteosarcoma cell apoptosis (Fig. 2d). To determine whether SNHG16 regulated the migration and invasion of osteosarcoma cells, the transwell assay was performed. Consequently, results showed that knockdown of SNHG16 inhibited the migration and invasion of U2OS and Saos-2 cells (Fig. 2e, f). Overall, knockdown of SNHG16 inhibits cell proliferation, migration, invasion, cell cycle and promotes apoptosis in osteosarcoma.

SNHG16 acts as a sponge for miR-98-5p

Recent studies have pointed out that lncRNAs functioned as important regulators in many cancers by sponging miRNAs [21–23]. Through the Starbase online software, we searched for the potential target miRNAs of SNHG16, and observed that miR-98-5p had a putative binding site with SNHG16 (Fig. 3a). MiR-98-5p has been reported to play a critical role in the progression of multiple cancers [24–26]. To confirm our hypothesis that miR-98-5p was targeted by SNHG16, luciferase reporter assay was performed. As a result, miR-98-5p over-expression markedly decreased the luciferase activities of pmirGLO-SNHG16-Wt, rather than pmirGLO-SNHG16-Mut, and miR-98-5p inhibition presented opposite effects (Fig. 3b). RIP assay confirmed that SNHG16 and miR-98-5p were both immunoprecipitated by anti-Ago2, validating that SNHG16 could interact with miR-98-5p (Fig. 3c). Then we studied the correlation between SNHG16 and miR-98-5p. As a results, RT-qPCR assay

Table 1 Clinical characteristic and SNHG16 expression of patients with osteosarcoma ($n=96$)

Variable	SNHG16 expression		p value
	low	high	
Age			
<40	36	34	0.819
≥40	12	14	
Gender			
Male	31	33	0.829
Female	17	15	
Tumor size			
<5 cm	24	11	0.010*
>=5 cm	24	37	
TNM stage			
I–II	25	12	0.011*
III–IV	23	36	
Metastasis			
No	32	13	0.0001*
Yes	16	35	
Enneking stage			
I-A/I-B	37	40	0.609
II/III	11	8	
Tumor position			
Femur/tibia	16	19	0.672
Humerus/fibulars	32	29	

Low/high by the sample median. Pearson χ^2 test

Pearson χ^2 test was used to analyze the correlation between SNHG16 expression and clinical characteristics, *$p<0.05$, **$p<0.01$

*$p<0.05$ was considered statistically significant

Table 2 Multivariate analysis of prognostic parameters in patients with osteosarcoma by Cox regression analysis

Variable	Category	p value
Age		
	<40	0.472
	≥40	
Gender		
	Male	0.597
	Female	
Tumor size		
	<5 cm	0.567
	≥5 cm	
TNM stage		
	I–II	0.026*
	III–IV	
Metastasis		
	No	0.278
	Yes	
Enneking stage		
	I-A/I-B	0.972
	II/III	
Tumor position		
	Femur/tibia	0.975
	Humerus/fibulars	
SNHG16 level		
	Low	0.025*
	High	

Proportional hazards method analysis showed a positive, independent prognostic importance of SNHG16 expression ($p=0.025$)

*$p<0.05$ was considered statistically significant

demonstrated that knockdown of SNHG16 significantly increased the expression of miR-98-5p. Additionally, overexpression of miR-98-5p could obviously decrease SNHG16 expression in osteosarcoma cell lines and knockdown of miR-98-5p had opposite effects (Fig. 3d). We also found the downregulation of miR-98-5p in osteosarcoma tissues and a remarkably negative correlation between the expression of SNHG16 and miR-98-5p in osteosarcoma tissues (Fig. 3e, f). Taken together, SNHG16 acts as a sponge for miR-98-5p and negatively regulates the expression of miR-98-5p in osteosarcoma.

MiR-98-5p rescues the role of SNHG16 in osteosarcoma cells

Finally, we tested whether overexpression of miR-98-5p could rescue the effect of SNHG16 on cellular processes on osteosarcoma cells by carrying out rescue assays in U2OS cells. RT-qPCR assay showed that miR-98-5p expression in miR-98-5p inhibitor group was significantly decreased compared with NC inhibitor group (Fig. 4a). In CCK-8 assay, the proliferation of U2OS cells was remarkably decreased in sh-SNHG16#1+NC inhibitor group and increased in sh-NC+miR-98-5p inhibitor group compared with sh-NC group, and the declined cell proliferation in sh-SNHG16#1+NC inhibitor group was apparently rescued in sh-SNHG16+miR-98-5p inhibitor group (Fig. 4b). In addition, miR-98-5p inhibitor also rescued the inhibitory influences of downregulating SNHG16 on cell cycle (Fig. 4c). Moreover, SNHG16 knockdown (or miR-98-5p suppression) promoted (or impeded) cell apoptosis in U2OS, whereas inhibition of miR-98-5p countervailed the facilitative effect of SNHG16 knockdown on cell apoptosis (Fig. 4d). Transwell assay revealed that suppression of miR-98-5p reversed the hampered migration and invasion upon the knockdown of SNHG16 in U2OS cells (Fig. 4e, f). As shown in Fig. 4g, the miR-98-5p targets (ZEB1, E2F5 and STAT3) identified by previous studies were detected by western blot. Results showed that the expression of ZEB1, E2F5 and STAT3 were reduced by SNHG16 knockdown and enhanced by miR-98-5p suppression, whereas inhibition of miR-98-5p

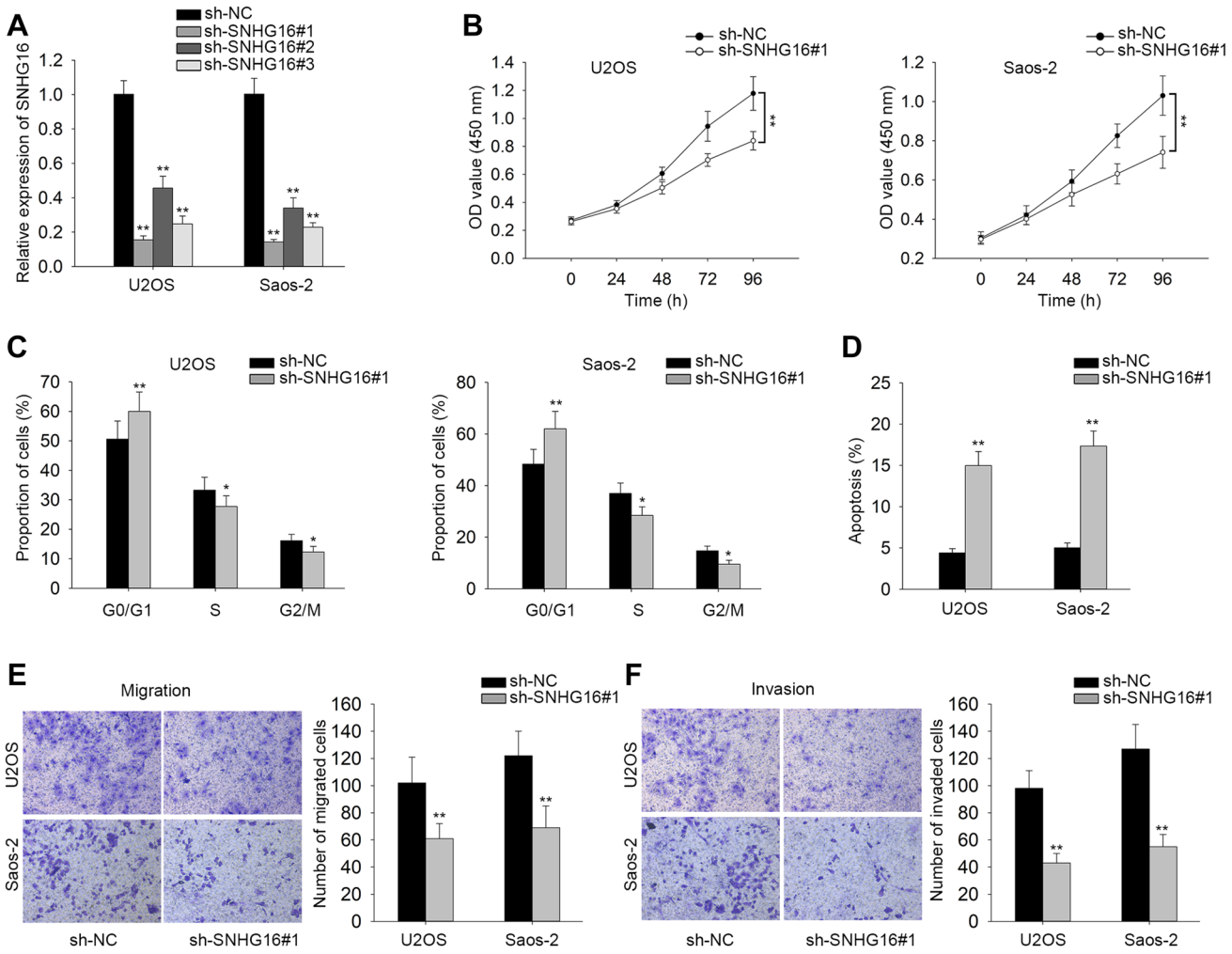

Fig. 2 Knockdown of SNHG16 inhibited cell proliferation, migration, invasion, cell cycle and promoted apoptosis in osteosarcoma cells. **a** SNHG16 expression in U2OS and Saos-2 cells transfected with sh-SNHG16#1/2/3 or sh-NC was determined by RT-qPCR. **b** Effects of sh- SNHG16#1 on the growth of U2OS and Saos-2 cells. **c** Effects of sh- SNHG16#1 on the cell cycle of U2OS and Saos-2 cells. **d** Effects of sh- SNHG16#1 on the apoptosis of U2OS and Saos-2 cells. **e, f** Effects of sh-SNHG16#1 on the migration and invasion abilities of U2OS and Saos-2 cells. $*p < 0.05$, $**p < 0.01$

attenuated the effects of SNHG16 knockdown on reducing ZEB1, E2F5 and STAT3 expression. Collectively, SNHG16 regulates cellular processes in osteosarcoma by sponging miR-98-5p.

Discussion

Osteosarcoma is a highly malignant bone tumor burdening patients with poor overall survival rates, and characterized by high metastasis, rapid progression, and poor prognosis [27, 28]. Although researches are increasing about the carcinogenic mechanisms and therapeutic strategies of osteosarcoma, the molecular regulation mechanisms involved in osteosarcoma have not yet been fully elucidated. Therefore, it is necessary to further investigate the molecular mechanism of osteosarcoma to find new molecular therapeutic strategies in osteosarcoma.

Increasing evidences have validated that lncRNAs could function as either oncogenes or tumor suppressor genes in multiple human cancers [11, 12]. The regulation of lncRNAs on osteosarcoma progression has also been investigated [13–15]. Moreover, current studies have revealed that lncRNA SNHG16 contributes to the progression of many cancers. For example, lncRNA SNHG16 drives proliferation, migration, and invasion of hemangioma endothelial cell through modulation of miR-520d-3p/STAT3 axis [16]. LncRNA SNHG16 functions as an oncogene by sponging MiR-4518 and upregulating PRMT5 expression in glioma [17]. LncRNA-SNHG16 predicts poor prognosis and promotes tumor proliferation through epigenetically silencing p21 in bladder cancer [18]. In consistency, we found that

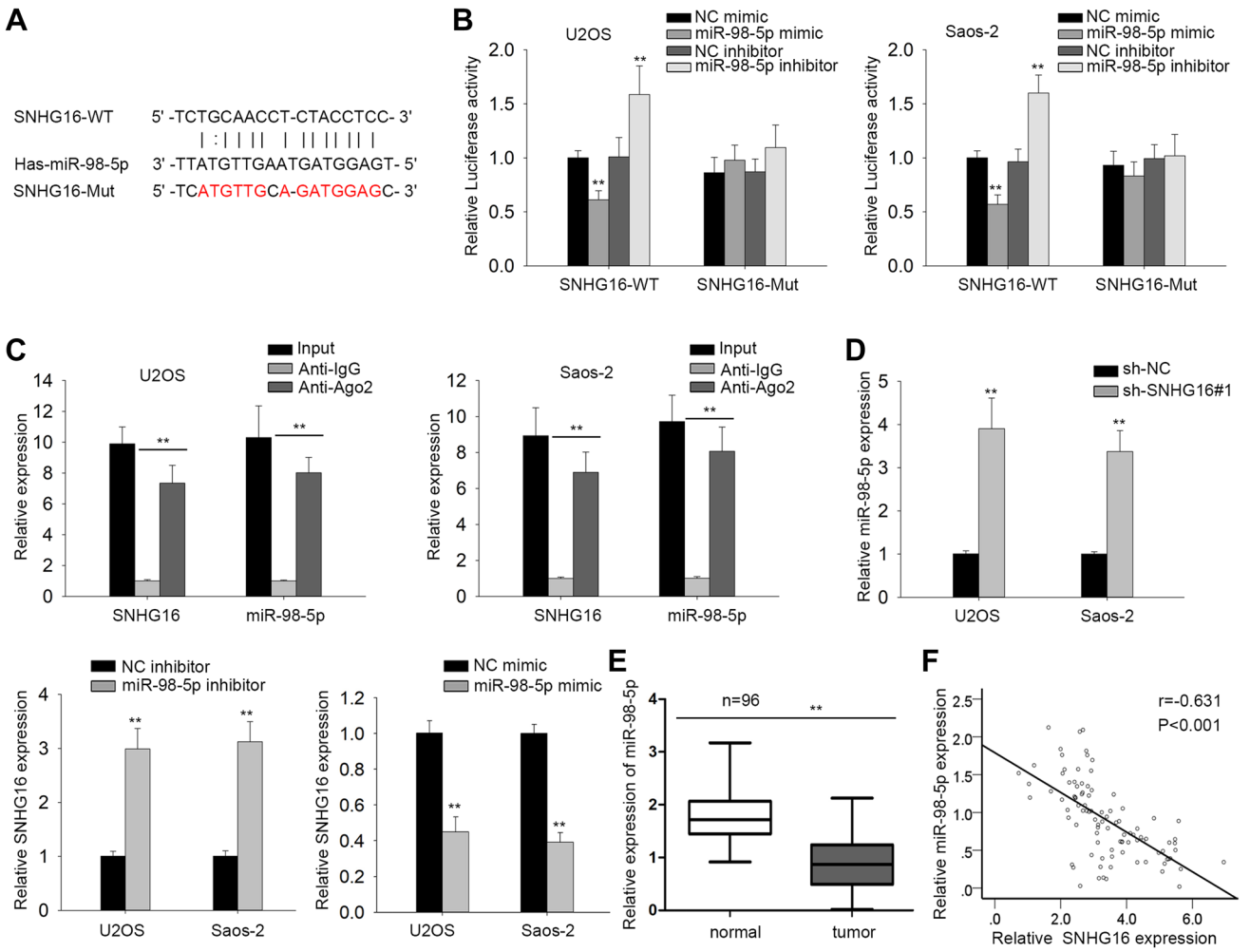

Fig. 3 SNHG16 directly targets miR-98-5p and is negatively correlated to miR-98-5p expression. **a** Binding region between miR-98-5p and SNHG16. StarBase was used to predict the target microRNAs which were regulated by SNHG16. **b, c** Luciferase reporter and RIP assays were used to test the interaction between miR-98-5p and SNHG16. **d** The interaction between miR-98-5p and SNHG16 in U2OS and Saos-2 cells was measured by RT-qPCR. **e** MiR-98-5p expression in osteosarcoma tissues and adjacent non-tumor tissues was determined by RT-qPCR. **f** The correlation between miR-98-5p and SNHG16 expression was measured by Spearman's correlation analysis. $*p < 0.05$, $**p < 0.01$

SNHG16 was significantly upregulated in osteosarcoma tissues and cell lines, and high expression of SNHG16 indicated poor prognosis and lower overall survival rate of osteosarcoma patients. Loss-of-function experiments demonstrated that knockdown of SNHG16 inhibited cell proliferation, migration, invasion, cell cycle and promoted apoptosis in osteosarcoma cells. In a word, our results suggested that SNHG16 acted as an oncogene in osteosarcoma.

MicroRNAs (miRNAs) are a class of small noncoding RNA with the length of 19–22 nucleotides [29]. It has been reported that miRNAs exert oncogenic or antitumor functions and regulate many cellular processes in cancers [30–32]. Recently, many studies have reported that miRNAs play vital roles in osteosarcoma. For example, miR-18a-5p promotes cell invasion and migration of osteosarcoma by directly targeting IRF2 [33]. MiR-203 acts as a tumor suppressor gene in osteosarcoma by regulating RAB22A [34]. Through the use of bioinformatics online prediction program (Starbase), we found that miR-98-5p was predicted to have a binding site for SNHG16. MiR-98-5p has been reported to act as antioncogene in human cancers. For example, NEAT1/has-mir-98-5p/MAPK6 axis is involved in non-small cell lung cancer (NSCLC) development [24]. Downregulated miR-98-5p promotes PDAC proliferation and metastasis by reversely regulating MAP4K4 [25]. The interaction between SNHG16 and miR-98 in other cancers has been reported in previous researches. Long noncoding RNA SNHG16 contributes to the development of bladder cancer via regulating miR-98/STAT3/Wnt/β-catenin pathway axis [35].

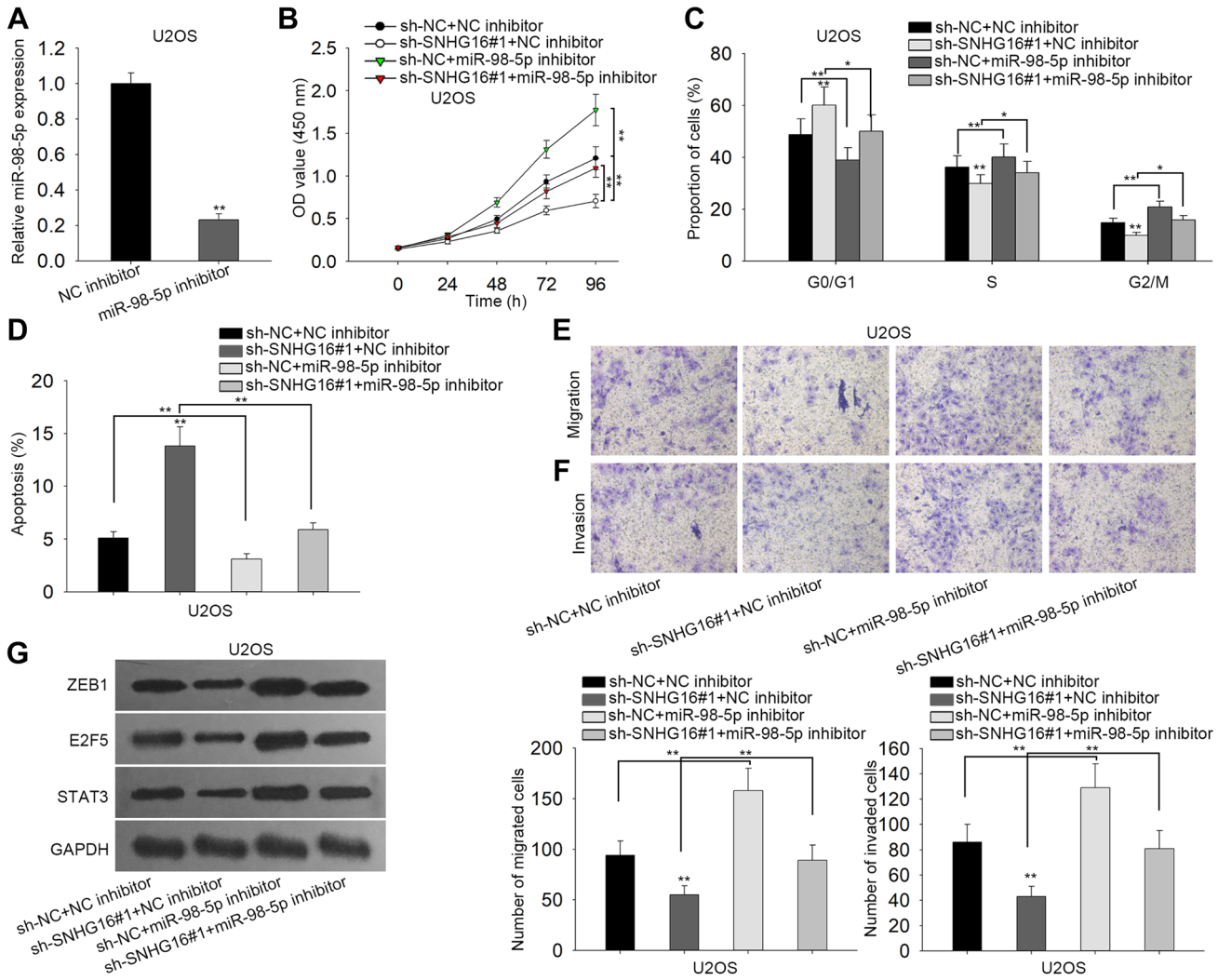

Fig. 4 miR-98-5p could reverse the role of SNHG16 on cellular processes in osteosarcoma. **a** miR-98-5p expression in U2OS cells transfected with NC inhibitor and miR-98-5p inhibitor. **b** CCK-8 assay was used to detect the proliferation ability of U2OS cells in different groups. **c, d** Flow cytometry assay was used to detect cell cycle and apoptosis of U2OS cells in different groups. **e, f** Transwell assay was used to detect the migration and invasion of U2OS cells in different groups. **g** The expression of ZEB1, E2F5 and STAT3 in different groups was detected by western blot. $^{*}p < 0.05$, $^{**}p < 0.01$

SNHG16 contributes to breast cancer cell migration by competitively binding miR-98 with E2F5 [36]. Therefore, we further explored that whether lncRNA SNHG16 sponged miR-98-5p to promote osteosarcoma progression. Luciferase reporter and RIP assays confirmed that SNHG16 directly interact with miR-98-5p. Moreover, we found that SNHG16 could negatively regulate the expression of miR-98-5p. Rescue experiments implied that miR-98-5p suppression could reverse the effects brought by SNHG16 inhibition on cell proliferation, migration, invasion, cell cycle and apoptosis in osteosarcoma. To draw a conclusion, lncRNA SNHG16 regulates cellular processes in osteosarcoma by sponging miR-98-5p. Thus, SNHG16/miR-98-5p axis may serve as a new and effective molecular therapeutic target for osteosarcoma.

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Compliance with ethical standards

Conflict of interest It is declared by the author that no conflict of interest exists in this paper.

Research involving human participants and/or animals Our research protocol was approved by affiliated Tumor Hospital of Guangxi Medical University.

Informed consent Standard written consent was obtained from each patient.

References

1. Benjamin RS (2015) Osteosarcoma: better treatment through better trial design. Lancet Oncol 16(1):12–13. https://doi.org/10.1016/S1470-2045(14)71186-6
2. Clark JC, Dass CR, Choong PF (2008) A review of clinical and molecular prognostic factors in osteosarcoma. J Cancer Res Clin Oncol 134(3):281–297. https://doi.org/10.1007/s00432-007-0330-x
3. Durfee RA, Mohammed M, Luu HH (2016) Review of osteosarcoma and current management. Rheumatol Ther 3(2):221–243. https://doi.org/10.1007/s40744-016-0046-y
4. Ando K, Heymann M-F, Stresing V, Mori K, Rédini F, Heymann D (2013) Current therapeutic strategies and novel approaches in osteosarcoma. Cancers 5(4):591–616. https://doi.org/10.3390/cancers5020591
5. Ponting CP, Oliver PL, Reik W (2009) Evolution and functions of long noncoding RNAs. Cell 136(4):629–641. https://doi.org/10.1016/j.cell.2009.02.006
6. Ulitsky I (2016) Evolution to the rescue: using comparative genomics to understand long non-coding RNAs. Nat Rev Genet 17(10):601–614. https://doi.org/10.1038/nrg.2016.85
7. Fatica A, Bozzoni I (2014) Long non-coding RNAs: new players in cell differentiation and development. Nat Rev Genet 15(1):7–21. https://doi.org/10.1038/nrg3606
8. Slaby O, Laga R, Sedlacek O (2017) Therapeutic targeting of non-coding RNAs in cancer. Biochem J 474(24):4219–4251. https://doi.org/10.1042/BCJ20170079
9. Kondo Y, Shinjo K, Katsushima K (2017) Long non-coding RNAs as an epigenetic regulator in human cancers. Cancer Sci 108(10):1927–1933. https://doi.org/10.1111/cas.13342
10. DiStefano JK (2018) The emerging role of long noncoding RNAs in human disease. Methods Mol Biol 1706:91–110. https://doi.org/10.1007/978-1-4939-7471-9_6
11. Chen Y, Du H, Bao L, Liu W (2018) LncRNA PVT1 promotes ovarian cancer progression by silencing miR-214. Cancer Biol Med 15(3):238–250. https://doi.org/10.20892/j.issn.2095-3941.2017.0174
12. Li XL, Subramanian M, Jones MF, Chaudhary R, Singh DK, Zong X, Gryder B, Sindri S, Mo M, Schetter A, Wen X, Parvathaneni S, Kazandjian D, Jenkins LM, Tang W, Elloumi F, Martindale JL, Huarte M, Zhu Y, Robles AI, Frier SM, Rigo F, Cam M, Ambs S, Sharma S, Harris CC, Dasso M, Prasanth KV, Lal A (2017) Long noncoding RNA PURPL suppresses Basal p53 levels and promotes tumorigenicity in colorectal cancer. Cell Rep 20(10):2408–2423. https://doi.org/10.1016/j.celrep.2017.08.041
13. Ye K, Wang S, Zhang H, Han H, Ma B, Nan W (2017) Long NONCODING RNA GAS5 suppresses cell growth and epithelial-mesenchymal transition in osteosarcoma by regulating the miR-221/ARHI pathway. J Cell Biochem 118(12):4772–4781. https://doi.org/10.1002/jcb.26145
14. Li J, Wu QM, Wang XQ, Zhang CQ (2017) Long noncoding RNA miR210HG sponges miR-503 to facilitate osteosarcoma cell invasion and metastasis. DNA Cell Biol 36(12):1117–1125. https://doi.org/10.1089/dna.2017.3888
15. Sun L, Yang C, Xu J, Feng Y, Wang L, Cui T (2016) Long noncoding RNA EWSAT1 promotes osteosarcoma cell growth and metastasis through suppression of MEG3 expression. DNA Cell Biol 35(12):812–818. https://doi.org/10.1089/dna.2016.3467
16. Zhao W, Fu H, Zhang S, Sun S, Liu Y (2018) LncRNA SNHG16 drives proliferation, migration, and invasion of hemangioma endothelial cell through modulation of miR-520d-3p/STAT3 axis. Cancer Med. https://doi.org/10.1002/cam4.1562
17. Lu YF, Cai XL, Li ZZ, Lv J, Xiang YA, Chen JJ, Chen WJ, Sun WY, Liu XM, Chen JB (2018) LncRNA SNHG16 functions as an oncogene by sponging MiR-4518 and up-regulating PRMT5 expression in glioma. Cell Physiol Biochem 45(5):1975–1985. https://doi.org/10.1159/000487974
18. Cao X, Xu J, Yue D (2018) LncRNA-SNHG16 predicts poor prognosis and promotes tumor proliferation through epigenetically silencing p21 in bladder cancer. Cancer Gene Ther 25(1–2):10–17. https://doi.org/10.1038/s41417-017-0006-x
19. Zhu C, Cheng D, Qiu X, Zhuang M, Liu Z (2018) Long non-coding RNA SNHG16 promotes cell proliferation by sponging microRNA-205 and upregulating ZEB1 expression in osteosarcoma. Cell Physiol Biochem 51(1):429–440. https://doi.org/10.1159/000495239
20. Su P, Mu S, Wang Z (2019) Long noncoding RNA SNHG16 promotes osteosarcoma cells migration and invasion via sponging miRNA-340. DNA Cell Biol 38(2):170–175. https://doi.org/10.1089/dna.2018.4424
21. Ba Z, Gu L, Hao S, Wang X, Cheng Z, Nie G (2018) Downregulation of lncRNA CASC2 facilitates osteosarcoma growth and invasion through miR-181a. Cell Prolif https://doi.org/10.1111/cpr.12409
22. Sui CJ, Zhou YM, Shen WF, Dai BH, Lu JJ, Zhang MF, Yang JM (2016) Long noncoding RNA GIHCG promotes hepatocellular carcinoma progression through epigenetically regulating miR-200b/a/429. J Mol Med (Berl) 94(11):1281–1296. https://doi.org/10.1007/s00109-016-1442-z
23. Hirata H, Hinoda Y, Shahryari V, Deng G, Nakajima K, Tabatabai ZL, Ishii N, Dahiya R (2015) Long noncoding RNA MALAT1 promotes aggressive renal cell carcinoma through Ezh2 and interacts with miR-205. Cancer Res 75(7):1322–1331. https://doi.org/10.1158/0008-5472.CAN-14-2931
24. Wu F, Mo Q, Wan X, Dan J, Hu H (2017) NEAT1/has-mir-98-5p/MAPK6 axis is involved in non-small-cell lung cancer (NSCLC) development. J Cell Biochem. https://doi.org/10.1002/jcb.26442
25. Fu Y, Liu X, Chen Q, Liu T, Lu C, Yu J, Miao Y, Wei J (2018) Downregulated miR-98-5p promotes PDAC proliferation and metastasis by reversely regulating MAP4K4. J Exp Clin Cancer Res 37(1):130. https://doi.org/10.1186/s13046-018-0807-2
26. Wang Y, Bao W, Liu Y, Wang S, Xu S, Li X, Li Y, Wu S (2018) miR-98-5p contributes to cisplatin resistance in epithelial ovarian cancer by suppressing miR-152 biogenesis via targeting Dicer1. Cell Death Dis 9(5):447. https://doi.org/10.1038/s41419-018-0390-7
27. Ye H, Lin J, Yao X, Li Y, Lin X, Lu H (2018) Overexpression of long non-coding RNA NNT-AS1 correlates with tumor progression and poor prognosis in osteosarcoma. Cell Physiol Biochem 45(5):1904–1914. https://doi.org/10.1159/000487966
28. Zeng SX, Cai QC, Guo CH, Zhi LQ, Dai X, Zhang DF, Ma W (2017) High expression of TRIM29 (ATDC) contributes to poor prognosis and tumor metastasis by inducing epithelial mesenchymal transition in osteosarcoma. Oncol Rep 38(3):1645–1654. https://doi.org/10.3892/or.2017.5842
29. D'Angelo B, Benedetti E, Cimini A, Giordano A (2016) MicroRNAs: a puzzling tool in cancer diagnostics and therapy. Anticancer Res 36(11):5571–5575. https://doi.org/10.21873/anticanres.11142
30. Setijono SR, Park M, Kim G, Kim Y, Cho KW, Song SJ (2018) miR-218 and miR-129 regulate breast cancer progression by targeting Lamins. Biochem Biophys Res Commun 496(3):826–833. https://doi.org/10.1016/j.bbrc.2018.01.146
31. Zhou W, He L, Dai Y, Zhang Y, Wang J, Liu B (2018) MicroRNA-124 inhibits cell proliferation, invasion and migration by targeting CAV1 in bladder cancer. Exp Ther Med 16(4):2811–2820. https://doi.org/10.3892/etm.2018.6537
32. Wang Y, Tian Y (2018) miR-206 inhibits cell proliferation, migration, and invasion by targeting BAG3 in human cervical cancer. Oncol Res. https://doi.org/10.3727/096504017X15143731031009

33. Lu C, Peng K, Guo H, Ren X, Hu S, Cai Y, Han Y, Ma L, Xu P (2018) miR-18a-5p promotes cell invasion and migration of osteosarcoma by directly targeting IRF2. Oncol Lett 16(3):3150–3156. https://doi.org/10.3892/ol.2018.9032
34. Yang D, Liu G, Wang K (2015) miR-203 acts as a tumor suppressor gene in osteosarcoma by regulating RAB22A. PLoS One 10(9):e0132225. https://doi.org/10.1371/journal.pone.0132225
35. Feng F, Chen A, Huang J, Xia Q, Chen Y, Jin X (2018) Long noncoding RNA SNHG16 contributes to the development of bladder cancer via regulating miR-98/STAT3/Wnt/beta-catenin pathway axis. J Cell Biochem 119(11):9408–9418. https://doi.org/10.1002/jcb.27257
36. Cai C, Huo Q, Wang X, Chen B, Yang Q (2017) SNHG16 contributes to breast cancer cell migration by competitively binding miR-98 with E2F5. Biochem Biophys Res Commun 485(2):272–278. https://doi.org/10.1016/j.bbrc.2017.02.094

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