Original Paper

lncRNA SNHG8 Promotes the Tumorigenesis and Metastasis by Sponging miR-149-5p and Predicts Tumor Recurrence in Hepatocellular Carcinoma

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Key Words

Hepatocellular carcinoma • SNHG8 • miR-149-5p • PPM1F • Metastasis

Abstract

Background/Aims: Long noncoding RNAs (lncRNAs) are aberrantly expressed in multiple malignant tumors involved in tumor growth and metastasis. Accumulating data show that small nucleolar RNA host gene (SNHG) 1/12/20 plays a key role in the progression of hepatocellular carcinoma (HCC). However, the molecular mechanisms by which SNHG8 contributes to HCC remain elusive and merit exploration. **Methods:** The association between SNHG8 expression and the clinicopathological characteristics and prognoses in HCC patients was analysed by using qRT-PCR analysis and the data from The Cancer Genome Atlas. Cell growth and metastatic potential were determined by MTT, colony formation, Transwell assays, and the mouse xenograft tumor model and lung metastasis model. Epithelial-mesenchymal transition markers were detected by western blot analysis. The binding capacity of SNHG8 with miRNAs was evidenced by bioinformatic analysis and a luciferase reporter assay. In addition, the rescue experiments were performed based on co-transfection with sh-SNHG8 and a miR-149 inhibitor in HCC cells. **Results:** The expression levels of lncRNA SNHG8 were dramatically increased in HCC tissues and cell lines as compared with the adjacent normal tissues, and SNHG8 expression was an independent prognostic factor of tumor recurrence in HCC patients. Furthermore, knockdown of SNHG8 inhibited cell proliferation, invasion, and lung metastasis *in vitro* and *in vivo*, whereas overexpression of SNHG8 reversed these effects. SNHG8 acted as a sponge of miR-149 and counteracted the tumor suppressive effects of miR-149 in HCC cells. Expression of phosphatase, Mg²⁺/Mn²⁺ dependent 1F, a target of miR-149, displayed a negative correlation with miR-149 expression but a positive correlation

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with SNHG8 expression in HCC specimens. **Conclusion:** As lncRNA SNHG8 may promote HCC tumorigenesis and metastasis by sponging miR-149, it is a potential candidate marker and therapeutic target for HCC.

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide and is the second leading cause of cancer-related death [1]. Although numerous efforts have been taken to understand the molecular basis and pathogenesis of HCC, it is often diagnosed at an advanced stage, and the prognosis of HCC patients is very poor owing to distant metastases and postsurgical recurrences [2]. Therefore, it is necessary to identify novel biomarkers related to hepatocarcinogenesis and provide strategies for early diagnosis and treatment of HCC.

Long non-coding RNAs (lncRNAs) belong to a subgroup of noncoding RNAs that are more than 200 nucleotides in length and have no protein-coding capability [3]. Increasing evidence shows that lncRNAs participate in transcriptional regulation, epigenetic gene regulation, and the pathogenesis of multiple diseases including cancer [3, 4]. Deregulated lncRNAs have been reported to be associated with the tumorigenesis and metastasis of HCC [5]. For example, lnc-EGFR accelerates T-regulatory cell differentiation leading to immune evasion of HCC [6], and a variety of lncRNAs, such as MALT1 [7], TP73-AS1 [8], HULC [9], XIST [10], and HOTAIR [11], function as oncogenes, promoting HCC growth and metastasis by regulating miRNA or proteins. However, lncRNA uc.134 and lincRNA-p21 inhibit HCC progression by repressing CUL4A-mediated ubiquitination of LATS1 [12] or activating endoplasmic reticulum stress [13]. These findings indicate lncRNAs are potential prognostic factors for HCC.

Intriguingly, lncRNA small nucleolar RNA host genes (SNHG8) have been reported to have close associations with tumor growth and metastasis, and SNHG1/5/12 specifically enhances tumorigenesis and metastasis in non-small cell lung cancer [14], gastric cancer (GC) [15], colorectal cancer (CRC) [16], and HCC [17] by targeting miR-101-3p [14], miR-32 [15], and miR-199a/b [17]. However, SNHG5 suppresses GC progression by trapping MTA2 in the cytosol [18], suggesting that SNHG8 may also represent novel therapeutic targets for cancer.

In this study, we found that the expression levels of SNHG8 were dramatically increased in HCC tissues and cell lines as compared with the adjacent normal tissues and acted as an independent prognostic factor of tumor recurrence in HCC patients. As lncRNA SNHG8 promoted growth and invasion of HCC cells via sponging miR-149, it represents a potential target for the treatment of HCC.

Materials and Methods

Materials

The HCC cell lines used in our study (LO2, Huh6, Huh7, SK-hep1, HepG2, and PLC5) were purchased from the Institute of Chemistry and Cell Biology (Shanghai, China). Lentivirus-mediated sh-SNHG8 or negative control (sh-NC) vectors, virion-packaging elements, and a miR-149 mimic and inhibitor were purchased from Genechem (Shanghai, China). E-cadherin, N-cadherin, Vimentin, and phosphatase, Mg2+/Mn2+ dependent 1F (PPM1F) antibodies used in this study were purchased from Cell Signaling Technology (Danvers, MA). The Cancer Genome Atlas (TCGA) RNA sequencing data for HCC patients and SNHG8, miR-149, and PPM1F expression levels were downloaded from the TCGA website (<https://genome-cancer.ucsc.edu/>). The clinicopathologic data from HCC patients are summarized in Supplementary Table S1 (For all supplemental material see www.karger.com/10.1159/000495871/).

Plasmid construction

The miR-149-5p mimic and inhibitor were commercially synthesized by Genechem (Shanghai). The wild-type SNHG8 vector, which contained miR-149 binding sites, and the mutant fragment, which contained the mutant binding sites of miR-149, were constructed by annealing the sequences with double-stranded DNA and inserting them into the pmirGLO vector at the *Bam*HI and *Eco*RI sites. In addition, the sh-SNHG8 plasmid, which expresses a siRNA that targets SNHG8 transcription (ATTACGATGGATGATGGAAACATA) and the sh-NC sequence (CATAGCGGT GTAGTAAAGCATAATA), were constructed by annealing single-stranded hairpin cDNA.

Cell culture and transfection

HCC cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml of penicillin, and 100 µg/ml of streptomycin. Cells in this medium were placed in a humidified atmosphere containing 5% CO₂ at 37°C. When cells reached 60–80% confluence, HCC cells were transfected with sh-SNHG8 or SNHG8 overexpression vector and cultured at 37°C in 5% CO₂ for 4 h. Then, the supernatant was discarded, and serum-containing growth medium was added. Positive and stable transfectants were selected for further study.

RNA extraction and qRT-PCR analysis

To verify the mRNA expression level of SNHG8 in HCC cell lines, qRT-PCR analysis was performed. Total RNA was extracted from each clone using TRIzol reagent (Invitrogen, Karlsruhe, Germany) according to the manufacturer's protocol. Reverse transcription and cDNA amplification were performed using the PrimeScript RT Reagent Kit and SYBR Premix Ex Taq (TaKaRa, Dalian, China), respectively, according to the manufacturer's guidelines. The β-actin or U6 genes were used as endogenous controls. A miScript Primer Assay (QIAGEN, Hilden, Germany) was used to assay miR-149 and U6. The primers used are listed in Supplementary Table S2.

Western blot analysis

HCC cell lines were harvested and extracted using lysis buffer (Tris-HCl, sodium dodecyl sulfate, mercaptoethanol, glycerol). Cell extracts were boiled for 5 min in loading buffer and then equal amounts of cell extracts were separated on 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis gels. Separated protein bands were transferred onto polyvinylidene fluoride membranes. The primary antibodies against E-cadherin, N-cadherin, Vimentin, and PPM1F were diluted according to the instructions for each of the antibodies and incubated overnight at 4°C. Then, horseradish peroxidase-linked secondary antibodies were added to a dilution ratio of 1:1000, and samples were incubated at room temperature for 2 h. The membranes were washed with phosphate-buffered saline (PBS), and the immune-reactive bands were visualized using an ECL-PLUS/Kit (GE Healthcare, Chicago, IL) according to the manufacturer's instructions. The relative protein levels of the different groups were normalized to GAPDH concentrations. Three separate experiments were performed for each clone.

Cell viability assay

Cell proliferation was analyzed by MTT assay. HCC cells were incubated in 96-well-plates at a density of 2×10^3 cells per well with DMEM supplemented with 10% FBS. Cells were treated with 20 µl of MTT and subsequently incubated with 150 µl of dimethyl sulfoxide for 15 min. The color reaction was measured at 570 nm using an Enzyme Immunoassay Analyzer (Bio-Rad Laboratories, Hercules, CA).

Colony formation assay

For the colony formation assay, $2 \times$ DMEM containing 20% FBS and 2×10^3 cells was mixed with an equal volume of 0.7% agarose and immediately plated into 6-well plates containing an underlayer of 0.5% agarose made in $1 \times$ DMEM supplemented with 10% FBS. The plates were cultured at 37°C under 5% CO₂ for 10 days.

Transwell migration and invasion assays

For migration and invasion assays, Transwell migration chambers and Matrigel-coated chambers (Becton Dickinson, Waltham, MA) were used. Briefly, 5×10^4 HepG2 and LO2 cells were seeded into the upper chamber in serum-free culture medium. The lower chamber was filled with completed medium with 10% FBS. After 16 h for the migration assay and 24 h for the invasion assay, cells that have migrated or invaded through the membrane were stained with crystal violet and counted.

Dual-luciferase reporter assay

HCC cells were seeded into 24-well plates. After a 24-h incubation, a pmirGLO reporter vector carrying wild-type (WT) or mutant (Mut) SNHG8 was co-transfected with the miR-149 mimic or miR-NC into the HepG2 and PLC5 cells. After transfection for 48 h, luciferase activities were examined with a Dual-luciferase Reporter System (Promega, Fitchburg, WI).

Animal experiments

Six-week-old female immune-deficient nude mice (BALB/c-nu) were bred at the laboratory animal facility. Mice were injected subcutaneously with 5×10^6 sh-NC or sh-SNHG8 stably transfected HepG2 cells. Mice were monitored daily and developed subcutaneous tumors. Every three days, the tumor volume was measured with calipers using the following formula: volume = length $\times$ width²/2. All animal studies were performed in accordance with guidelines approved by the Animal Experimentation Ethics Committee of Shanghai Changzheng Hospital.

In vivo metastasis assays

For mouse tail vein injections, 1×10^6 HepG2 cells in 100 μ l of PBS were injected into the tail veins of nude mice. The mice were sacrificed on day 30. Lung tissues were resected, fixed with 4% paraformaldehyde, and then stained with hematoxylin and eosin (HE).

Statistical analyses

SPSS 20.0 (IBM Corp., Armonk, NY) was used for the statistical analyses. Each value was recorded as the mean $\pm$ standard error of the mean (SEM) from at least three independent experiments. Two-tailed Student's *t*-tests, chi-square tests, and analysis of variance were used to evaluate the differences among groups. Pearson correlation analysis was used to analyze the expression correlations of miR-149 with PPM1F or SNHG8 in HCC tissues. Survival and recurrence curves were plotted using the Kaplan–Meier method and assessed for statistical significance using a log-rank test. Statistical significance was considered at $P < 0.05$.

Results

lncRNA SNHG8 expression was upregulated in human HCC tissues

Previous research indicated that lncRNA SNHG8 is highly expressed in EBV-associated GC [19]. To elucidate the expression levels of SNHG8 in HCC tissues, we found that SNHG8 expression was noticeably increased in HCC tissues ($n = 23$) as compared with the adjacent normal tissues ($n = 23$; Fig. 1A). We further applied 2015 TCGA RNA sequencing data to analyze SNHG8 expression levels in HCC samples and found that SNHG8 expression was significantly increased in paired HCC samples ($n = 49$) as well as in unpaired HCC tissues ($n = 376$) as compared with the adjacent normal tissues ($n = 49$; Fig. 1B). These results demonstrated that increased expression of SNHG8 was common in HCC patients.

SNHG8 expression was positively associated with tumor recurrence in HCC patients

To analyze the relationship between SNHG8 expression and clinicopathological features and prognosis in HCC patients, according to SNHG8 expression level, overall survival (OS) time, and survival status, we obtained a cutoff value (2.032), area under the curve (0.55), specificity (78.5%), and sensitivity (33.3%) of SNHG8 in HCC patients (Fig. 1C), inferring that SNHG8 might be a potential biomarker for HCC. Based on its cutoff value, HCC patients

Fig. 1. Upregulation of SNHG8 expression was associated with tumor recurrence in HCC patients. (A) qRT-PCR analysis of the expression levels of SNHG8 in HCC tissues ($n = 23$). (B) TCGA dataset analysis of the expression of SNHG8 in pair-matched HCC samples ($n = 49$) as well as in unpaired HCC samples ($n = 376$). (C) Receiver operating characteristic curve analysis of the cutoff value, area under the curve, specificity, and sensitivity of SNHG8 in HCC samples. (D) According to the cutoff value of SNHG8, HCC patients were divided into high expression ($n = 84$) and low expression groups ($n = 231$). (E) The association of high SNHG8 expression or low SNHG8 expression with tumor recurrence in HCC patients. (F) TCGA cohort analysis of the association of SNHG8 expression with OS in HCC patients. (G) TCGA cohort analysis of the association of SNHG8 expression with tumor recurrence in HCC patients in the early stages (stage I+II) or late stages (stage III+IV).

were divided into high SNHG8 expression and low SNHG8 expression groups (Fig. 1D). As shown in supplementary Table S3, high SNHG8 expression had no relationship with age, gender, virus infection, pathological stage, and TNM stage in HCC patients (each $P > 0.05$). Kaplan-Meier analysis showed that HCC patients with high SNHG8 expression had higher recurrence rates in comparison with those with low SNHG8 expression (Fig. 1E), but had no association with OS in HCC patients (Fig. 1F). The patients in the early stages (stage I/II) or late stages (stage III/IV) with high SNHG8 expression also had no association with tumor recurrence as compared with those with low SNHG8 expression (Fig. 1G).

Univariate Cox regression analysis illustrated that high SNHG8 expression was associated with an increased risk of recurrence (relative risk 1.459, 95% confidence interval 1.022 to 2.082; $P = 0.038$; Table 1). Given the potential confounding factors, multivariate Cox

regression revealed that SNHG8 expression and T stage were independent prognostic factor of tumor recurrence in HCC patients (Table 1).

lncRNA SNHG8 promoted HCC cell growth

Increased SNHG8 expression in HCC tissues displayed a tumor-promoting role in HCC. To

confirm our hypothesis, we assayed the expression levels of SNHG8 in HCC cell lines and found that SNHG8 was expressed at a low level in the LO2 cell line but was highly expressed in HepG2 and PLC5 cell lines as compared with normal liver tissues (Fig. 2A). The knockdown efficiency of sh-SNHG8 in HepG2 and PLC5 cell lines (Fig. 2B) or overexpression of SNHG8 in the LO2 cell line (Fig. 2C) was verified by qRT-PCR analysis. Knockdown of SNHG8 reduced cell viability of HepG2 and PLC5 cells (Fig. 2D), but overexpression of SNHG8 accelerated cell proliferation in LO2 cells (Fig. 2E). The number of colonies in sh-SNHG8-transfected HepG2 and PLC5 cell lines was remarkably decreased (Fig. 2F), but it increased in SNHG8-transfected LO2 cells compared with the control group (Fig. 2G).

lncRNA SNHG8 promoted HCC cell migration and invasion

To investigate the effects of SNHG8 on HCC cell migration and invasion, we performed Transwell migration and invasion assays, which indicated that knockdown of SNHG8 attenuated cell migration (Fig. 3A) and invasive ability (Fig. 3B) of HepG2 and PLC5 cells, but overexpression of SNHG8 enhanced these effects in LO2 cells (Fig. 3C). Then, the expression levels of epithelial-mesenchymal transition-related markers, such as E-cadherin, N-cadherin, and Vimentin, were examined by western blot analysis (Fig. 3D), indicating that knockdown of SNHG8 upregulated the expression of E-cadherin, but downregulated the expression of N-cadherin and Vimentin in HepG2 and PLC5 cell lines. However, overexpression of SNHG8 had the opposite effects in LO2 cells.

lncRNA SNHG8 acted as a sponge of miR-149 in HCC cells

To clarify the molecular mechanism by which SNHG8 results in HCC, we used microRNA.org (<http://www.microRNA.org/microRNA/home.do>) to identify five miRNAs that could bind to SNHG8 (Supplementary Table S4). According to the mirSVR and PhastCons scores, we found that miR-149-5p (miR-149) had the highest binding affinity with SNHG8. Furthermore, TCGA data indicated that miR-149 was downregulated in 49 paired HCC tissues as well as in unpaired HCC tissues ($n = 263$) as compared with the adjacent normal tissues ($n = 49$; Fig. 4A), and SNHG8 expression had a negative correlation with miR-149 expression in HCC tissues ($r = -0.46$, $P = 0.0009$; Fig. 4B). It was demonstrated that the miR-149 mimic decreased the expression of SNHG8 in HepG2 and PLC5 cell lines, but the miR-149 inhibitor increased the expression of SNHG8 in LO2 cells (Fig. 4C). The binding sites of miR-149 with the WT or Mut SNHG8 are shown in Fig. 4D. To further confirm whether SNHG8 was a target of miR-149, we co-transfected the HepG2 and PLC5 cell lines with the WT or Mut SNHG8 reporter and the miR-149 mimic, and the LO2 cell line with the WT or Mut SNHG8 reporter and the miR-149 inhibitor. From these assays, we inferred that the miR-149 mimic decreased the luciferase activity of WT SNHG8 in HepG2 and PLC5 cell lines (Fig. 4E), while the miR-149 inhibitor increased it in the LO2 cell line (Fig. 4F). They had no effects on the luciferase activity of Mut SNHG8 compared with the control group (Fig. 4E, F).

Table 1. Cox regression analysis of SNHG81 expression as recurrence predictor in HCC patients. NA: not analyzed

Variables	Univariate Cox regression analysis RR (95% CI)	P value	Multivariate Cox regression analysis RR (95% CI)	P value
Age (years)				
≥60 vs. <60	1.137 (0.812 to 1.593)	0.455	NA	NA
HBV/HCV				
Positive vs. Negative	1.475 (1.055 to 2.063)	0.023	1.401 (0.990 to 1.982)	0.057
Pathological stage				
III/IV vs. I / II	2.770 (1.945 to 3.945)	<0.0001	0.989 (0.405 to 2.415)	0.981
T stage				
T3+T4 vs. T1+T2	2.957 (2.087 to 4.190)	<0.0001	2.938 (1.233 to 7.000)	0.015
N staging				
Positive vs. Negative	1.073 (0.737 to 1.563)	0.713	NA	NA
M stage				
Positive vs. Negative	0.939 (0.632 to 1.396)	0.755	NA	NA
SNHG8 expression				
High VS. Low	1.459 (1.022 to 2.082)	0.038	1.498 (1.045 to 2.148)	0.028

Fig. 2. LncRNA SNHG8 promoted HCC cell growth. (A) qRT-PCR analysis of the expression levels of SNHG8 in different HCC cell lines. (B, C) qRT-PCR analysis of the knockdown or overexpression efficiency after transfection with sh-SNHG8 in the HepG2 and PLC5 cell lines or SNHG8 vector in the LO2 cell line. (D, E) MTT analysis of cell viability after transfection with sh-SNHG8 in HepG2 and PLC5 cells or SNHG8 in LO2 cells. (F, G) Effects of SNHG8 knockdown or overexpression on cell colony formation in HCC cells. Data are the mean $\pm$ SEM of three experiments. * $P < 0.05$; ** $P < 0.01$.

Silencing SNHG8 counteracted the tumor-promoting effects of the miR-149 inhibitor in HCC cells

To reveal the molecular mechanism by which SNHG8 mediates the functions of miR-149 in HCC cells, the miR-149 inhibitor and sh-SNHG8 vector were co-transfected into the HepG2 cell line, and the miR-149 mimic and SNHG8 vector were co-transfected into the LO2 cell line. These experiments indicated that the miR-149 inhibitor accelerated cell activity and invasive potential, while the miR-149 mimic reversed these effects. Rescue experiments showed that silencing SNHG8 attenuated the tumor-promoting effects of the miR-149 inhibitor, but SNHG8 weakened the antitumor effects of miR-149 (Fig. 5A, B). Previous studies showed that PPM1F was a direct target of miR-149 in HCC [20]. We also identified PPM1F as a potential target of miR-149 based on its mirSVR score (-1.277) and PhastCons score (0.602) by using microRNA.org (<http://www.microrna.org/>). TCGA data showed that PPM1F expression was remarkably increased in 49 paired HCC tissues as well as in 353 HCC tissues compared with the adjacent normal tissues (Fig. 5C) and had a negative correlation with miR-149 expression but a positive correlation with SNHG8 expression in HCC tissues (Fig. 5D). Additionally, qRT-PCR (Fig. 5E) and western blot analysis (Fig. 5F) showed that the miR-149 inhibitor increased the expression of PPM1F, but the miR-149 mimic decreased its expression, and silencing SNHG8 reversed the increased expression of PPM1F induced by the miR-149 inhibitor in HepG2 cells. However, SNHG8 attenuated the decreased expression of PPM1F induced by the miR-149 mimic in LO2 cells.

Fig. 3. LncRNA SNHG8 promoted HCC cell migration and invasion. (A, B) Transwell analysis of the effects of SNHG8 knockdown on cell migration and invasion in HepG2 and PLC5 cell lines. (C) Effects of SNHG8 overexpression on cell migration and invasion were evaluated by Transwell assay in the LO2 cell line. (D) Expression levels of E-cadherin, N-cadherin, and Vimentin were detected by western blot analysis after transfection with sh-SNHG8 in the HepG2 and PLC5 cell lines or SNHG8 overexpression in the LO2 cell line. Data are the mean $\pm$ SEM of three experiments. * $P < 0.05$; ** $P < 0.01$.

Fig. 4. LncRNA SNHG8 acted as a sponge of miR-149 in HCC cells. (A) TCGA analysis of the expression levels of miR-149 in paired or unpaired HCC tissues. (B) TCGA analysis of the correlation of SNHG8 with miR-149 expression in HCC tissues. (C) qRT-PCR analysis of the expression levels of SNHG8 after transfection with the miR-149 mimic in the HepG2 and PLC5 cell lines and the miR-149 inhibitor in the LO2 cell line. (D) Binding sites of miR-149 with WT or Mut SNHG8. (E, F) Luciferase activity of WT or Mut SNHG8 after co-transfection with the miR-149 mimic or inhibitor and WT or Mut SNHG8 vector. Data are the mean $\pm$ SEM of three experiments. * $P < 0.05$; ** $P < 0.01$.

Fig. 5. Silencing SNHG8 reversed the tumor-promoting effects of the miR-149 inhibitor in HCC cells. (A, B) MTT and Transwell assays were performed to investigate the effects of co-transfection with sh-SNHG8 and the miR-149 inhibitor or SHNG8 and the miR-149 mimic on cell proliferation and invasive potential in HepG2 or LO2 cells. (C) TCGA analysis of the expression levels of PPM1F in paired and unpaired HCC tissues. (D) TCGA analysis of the correlation of PPM1F with miR-149 and SNHG8 expression in HCC samples. (E, F) qRT-PCR and western blot analysis of PPM1F expression after co-transfection with sh-SNHG8 and the miR-149 inhibitor in HepG2 cells or SHNG8 and the miR-149 mimic in LO2 cells. Data are the mean $\pm$ SEM of three experiments. * $P < 0.05$; ** $P < 0.01$.

Knockdown of SNHG8 inhibited xenograft tumor growth and lung metastasis

Having confirmed the tumor-promoting effects of SNHG8 *in vitro*, we further validated these effects *in vivo*. A xenograft tumor model was constructed to investigate tumor growth ability after subcutaneous inoculation with sh-SNHG8 stably transfected HepG2 cells. During the growth period of xenograft tumors, the length and weight of tumors were measured every other day. The proliferative activity of the tumors was lower in the sh-SNHG8 group (Fig. 6A, B). After tumor tissues were harvested, the average volumes and weight in the sh-SNHG8 group were decreased as compared with the sh-NC group (Fig. 6C, D).

Furthermore, we established the liver cancer with lung metastasis model. After the mouse tail vein was injected with sh-SNHG8-transfected HepG2 cells, they were sacrificed and metastatic lung tissues were sampled on 30th day. We found that the body weight of mice (Fig. 7A) and the lung weight (Fig. 7B) were lower in the sh-SNHG8 group as compared with the sh-NC group. Anatomical observation and HE staining showed that the number of lung metastases in the sh-SNHG8 group was decreased as compared with the sh-NC group (Fig. 7C).

Fig. 6. Knockdown of SNHG8 inhibited xenograft tumor growth. (A) Representative photographs of xenograft tumors in sh-SNHG8 and sh-NC groups. (B) Growth curves of tumor proliferation in sh-SNHG8 and sh-NC groups. (C, D) Average volumes and weight were assessed in sh-SNHG8 and sh-NC groups. (E) SNHG8 sponged miR-149 and upregulated PPM1F expression, leading to HCC tumorigenesis. Data are the mean $\pm$ SEM of three experiments. ** $P < 0.01$.

Discussion

lncRNAs have been reported to be dysregulated in many human diseases including cancer [4]. SNHG8 are closely associated with the prognosis and progression of multiple types of cancer including HCC. For example, upregulation of SNHG1/3/6/20 is used to predict malignancy and poor prognoses in patients with HCC [21-23], GC [24], and CRC [25]. In the present study, we found that SNHG8 expression was significantly elevated in HCC tissues compared with adjacent normal tissues and was positively associated with tumor recurrence, but displayed no correlation with other features. Multivariate Cox regression analysis revealed SNHG8 as an independent prognostic factor of tumor recurrence in HCC patients. Our results indicate that SNHG8 is a novel potential biomarker for predicting tumor recurrence in HCC patients.

Fig. 7. Knockdown of SNHG8 inhibited the lung metastasis of liver cancer in mice. (A, B) Average body weight and lung weight were assessed in sh-SNHG8 and sh-NC groups. (C) Representative photographs of metastatic lung tissues in sh-SNHG8 and sh-NC groups and HE staining analysis of the lung metastasis rate in sh-SNHG8 and sh-NC groups. Data are the mean $\pm$ SEM of three experiments. * $P < 0.05$.

Functionally, lncRNA SNHG8 has critical roles in regulating tumor progression. SNHG1/5/6/12/18 promotes cell proliferation, metastasis, and radio-resistance in a variety of malignancies [14-17, 21, 24, 26], and SNHG6 inhibits cell apoptosis and accelerates cycle progression in HCC [27]. However, SNHG5 functions as a tumor suppressor in GC [18]. In our study, the knockdown of SNHG8 suppressed HCC cell proliferation, invasion, and lung metastasis, and overexpression of SNHG8 reversed these effects, suggesting a tumor-promoting role of SNHG8 in HCC.

Mechanically, lncRNA SNHG8 can act as miRNA sponges to participate in tumor development. For example, SNHG1/5/6/12 is implicated in tumor progression by sponging different miRNAs such as miR-101-3p, miR-32, miR-199a/b [14-17], and miR-101 [24, 27]. Likewise, we found that SNHG8 could bind to miR-149, and miR-149 was downregulated and negatively correlated with SNHG8 expression in HCC tissues. Moreover, miR-149 decreased SNHG8 expression, whereas the miR-149 inhibitor increased its expression. Luciferase gene reporter assays showed miR-149 could bind to the 3'UTR of SNHG8 and decrease its luciferase activity. Moreover, we found that miR-149 inhibited cell viability and invasion, but its inhibitor reversed these effects. SNHG8 counteracted the inhibitory effects of miR-149 on cell viability and invasion, but knockdown of SNHG8 reversed the tumor-promoting effects induced by the miR-149 inhibitor. These results indicated that SNHG8 might act as a sponge of miR-149 to promote HCC cell viability and invasion.

Previously, miR-149 has been demonstrated to function as a tumor suppressor in multiple malignant tumors [20, 28-30]. It suppresses cell proliferation and invasion by targeting FOXM1 in CRC [28], GIT1 in breast cancer [29], and ZBTB2 in GC [30]. Intriguingly, PPM1F has been verified as a potential target of miR-149 in HCC [20]. In the present study, we found that miR-149 was downregulated in HCC tissues and had a negative correlation with PPM1F expression but a positive correlation with SNHG8 expression. Then, the miR-149 inhibitor increased cell proliferation and invasion and upregulated PPM1F expression, while the miR-149 mimic inhibited these effects and downregulated PPM1F expression in HCC. Moreover, silencing SNHG8 reversed the tumor-promoting effects, and PPM1F expression was induced by the miR-149 inhibitor. However, overexpression of SNHG8 attenuated the antitumor effects and low PPM1F expression caused by the miR-149 mimic. Our results suggest that SNHG8 might act as a sponge of miR-149, thereby resulting in increased expression of PPM1F and promoting HCC tumorigenesis (Fig. 6E).

Conclusion

Our findings demonstrated that lncRNA SNHG8 promotes HCC tumorigenesis and invasion via sponging miR-149 and acts as an independent prognostic factor of tumor recurrence in HCC patients. Our study has identified SNHG8 as a promising biomarker for HCC therapy.

Abbreviations

CRC (colorectal cancer); DMEM (Dulbecco's modified Eagle's medium); FBS (fetal bovine serum); GC (gastric cancer); HCC (hepatocellular carcinoma); HE (hematoxylin and eosin); lncRNA (long noncoding RNA); Mut (mutant); OS (overall survival); PBS (phosphate-buffered solution); PPM1F (phosphatase Mg²⁺/Mn²⁺ dependent 1F); qRT-PCR (quantitative real-time polymerase chain reaction); SEM (standard error of the mean); SNHG (small nucleolar RNA host gene); TCGA (The Cancer Genome Atlas); WT (wild type).

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ZF and GD conceived of the project and designed this study. JD, FT and WG contributed equally to this article. JD, FT and WG performed all the experiments. JD drafted the manuscript. JY was responsible for the data analysis. ZF and GD ensured the quality of the article. All authors read and approved the final manuscript. This work was supported by the Shanghai Sailing Program (grant no. 1425500), National Natural Science Foundation of China (grant no. 81470900, 81471585), the Foundation of Shanghai Science and Technology Commission (grant no. 15ZR1414100, 15411950403), and the Initiation Foundation for Young Scientist of Changzheng Hospital (grant no. 2013CZQN05). This study was approved by the Institutional Ethical Board of the Shanghai Chang Zheng Hospital, Second Military Medical University. Patients or the relatives were informed of the proper usage of the human samples and signed informed consents.

Disclosure Statement

The authors declare that they have no competing interests.

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