Expression of Long Non-Coding RNA (lncRNA) Small Nucleolar RNA Host Gene 1 (SNHG1) Exacerbates Hepatocellular Carcinoma Through Suppressing miR-195

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Background: Aberrant expression of lncRNA has been suggested to have an association with tumorigenesis. Our study was designed to reveal the underlying connection between lncRNA SNHG1 and hepatocellular carcinoma (HCC) pathogenesis.

Material/Methods: A total of 122 pairs of HCC tissues (case group) and matched adjacent non-tumor liver tissues (control group) were collected for this study. RT-PCR and *in situ* hybridization were conducted to investigate differences in lncRNA SNHG1 expression between the case and control group. The expression levels of lncRNA SNHG1 and miR-195 in HepG2 cells transfected with SNHG1-mimic and SNHG1-inhibitor were measured by RT-PCR. The proliferation, invasion, and migration status of HepG2 cells after transfection were assessed through MTT assay, wound healing assay, and Transwell assay, respectively. Whether miR-195 is a direct downstream target of lncRNA SNHG1 was verified by both bioinformatics target gene prediction and dual-luciferase report assay.

Results: The expression level of lncRNA SNHG1 was remarkably upregulated in HCC tissues and cell lines compared with normal tissues and cell lines. High expression of lncRNA SNHG1 contributed to the downregulation of miR-195 in HepG2 cells. Also, lncRNA SNHG1 exacerbated HCC cell proliferation, invasion, and migration *in vitro* through the inhibition of miR-195. This suggests that miR-195 is a direct downstream target of lncRNA SNHG1.

Conclusions: lncRNA SNHG1 may contribute to the aggravation of HCC through the inhibition of miR-195.

MeSH Keywords: Carcinoma, Hepatocellular • Hep G2 Cells • *In Situ* Hybridization, Fluorescence • RNA, Long Noncoding

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Background

Hepatocellular carcinoma (HCC) is believed to be one of the most prevalent cancers, with high morbidity and mortality. HCC accounts for about 80%-90% of liver cancer cases and results in an annual death of 600,000 [1–3]. Previous studies have shown that contributors to HCC include liver cirrhosis, infection with hepatitis B/C virus (HBV/HCV), adiposity, aflatoxin contamination, excessive alcohol consumption, and environmental pollution [4]. Despite emerging therapies, including percutaneous ablation, liver transplantation, and the recently approved sorafenib (a systemic therapy for HCC), there is still no cure for HCC [1,5–8]. Therefore, the underlying molecular mechanisms involved in the development of HCC should be further explored to identify innovative therapeutic targets for HCC patients [9–11].

Long non-coding RNAs (lncRNAs) are transcriptional RNA molecules with more than 200 nucleotides; lncRNAs have limited or non-protein-coding capacity [12]. Functional lncRNA shares several specific sequence features, including fewer introns and low GC content, poor start codon, and open reading frame contexts. As suggested by previous studies, several important biological processes, including transcription, translation, and cellular differentiation, have been found to be regulated by lncRNAs at post-transcriptional levels. Also, lncRNAs are involved in chromatin modification and trafficking between nuclear and cytoplasmic structures [13]. Accumulating reports have suggested that lncRNAs are involved in the pathogenesis and progression of several cancers. For instance, a high expression level of metastasis triggered by lung adenocarcinoma transcript 1 (MALAT-1), a kind of lncRNA, has been suggested to be associated with the development of non-small-cell lung carcinomas (NSCLCs). In addition, MALAT-1 expression has been suggested to exhibit an upward trend in colorectal, prostate, pancreatic, and breast cancer tissues compared with normal tissues [14]. Therefore, it is hypothesized that lncRNA expression, which is aberrantly expressed in a variety of cancers, might have a significant impact on the development of HCC [13]. Previous reports have provided evidence that the small nucleolar RNA host gene (SNHG1) of lncRNA was significantly upregulated in NSCLC cell lines, and may act as a new potential therapeutic target for NSCLC interventions [15]. However, the association between lncRNAs and HCC has not yet been clarified.

The lncRNAs are able to bind to specific structures of microRNAs (miRNAs), which also participate in the pathogenesis and progression of a variety of cancers, and miRNA motif regulates protein-coding genes at expression levels [12]. Previous studies showed that miRNAs play a crucial role in the angiogenesis and metastasis process of HCC [16]. For example, miR-135, miR-21, and miR-17 exhibited abnormally high expressions in HCC tissues compared to normal liver tissues, while expression levels of miR-101, miR-122, miR-125, miR-150, miR-375, and miR-195 in HCC tissues were markedly decreased [17,18]. As suggested by an online database (*http://starbase.sysu.edu.cn/mirLncRNA.php*), miR-195 may interact with SNHG1. However, the synergic effects of miR-195 and SNHG1 in suppressing carcinoma angiogenesis or metastasis remain unclear [16]. Since both SNHG1 and miR-195 expressions levels are altered in NSCLC, we suspected that SNHG1 may regulate miR-195 expressions, thereby affecting the carcinogenesis process of HCC.

This research was conducted using both *in situ* hybridization and RT-PCR for the purpose of assessing whether lncRNA SNHG1 was overexpressed in HCC tissues and cell lines. More importantly, we aimed to investigate the association between SNHG1 and miR-195 with respect to HCC pathogenesis using bioinformatics target gene prediction, dual-luciferase report assay, RT-PCR, cell proliferation assay, wound healing assay, and Transwell assay.

Material and Methods

Ethical statement

This research project was supported by the Medical Ethical Committee of Fujian Provincial Cancer Hospital, Fujian Medical University Teaching Hospital. All 122 patients were informed about the project and gave written consent to participate in the research.

Human tissue samples and cell lines

We obtained a total of 122 pairs of HCC and matched adjacent non-tumor tissues from patients admitted in the Fujian Provincial Cancer Hospital, Fujian Medical University Teaching Hospital (Fuzhou, China) between March 2014 and March 2015. Patients had not received chemotherapy or radiotherapy prior to surgery. The collected tissues were immediately frozen at –80°C after surgery. Human HCC cell lines HepG2 and normal human liver cell line L02 were obtained from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM, Gibco, Shanghai) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco, Shanghai) was prepared to cultivate these cell lines. The moist culture atmosphere was set at 37°C with 5% CO_2.

Hybridization *in situ*

To detect lncRNA SNHG1 and miR-195 expression levels in liver tissues, hybridization *in situ* was conducted based on the manufacturer's instructions. Digoxin (DIG)-labeled lncRNA SNHG1 and miR-195 probe (1:400 and 1:400, respectively)

were added to tissue slices (embedded in paraffin) at 55°C for one hour. After washing, the tissue was sealed by reagents for one hour, the sealing reagent was removed, and Tris Buffered Saline Tween (TBST) containing anti-DIG antibody (1:200) was incubated with tissue slices at 37°C for one hour. Results were observed by microscope after staining with hematoxylin and eosin (H & E). We purchased the probes and reagent kit from Boster (Wuhan).

Quantitative real-time PCR

In order to quantify the expression levels of lncRNA SNHG1 and miR-195 in both tissues and cells, quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) was conducted. Total RNA was extracted by using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA). Then a reverse transcription kit (Bio-Rad, Hercules, California, USA) was used to reverse transcribe lncRNA SNHG1, miR-195, and an internal control U6a into cDNA. Subsequently, RT-PCR reactions were performed using a preheated ABI 7500 RT-PCR System (Applied Biosystems, Carlsbad, USA) along with SYBR Premix Ex Taq (Takara, Japan). Analysis of relative gene expressions was independently performed three times and each sample was verified in triplicate. The relative expression levels of RNA were calculated using Ct values and the level of target gene expression ($2^{-\Delta\Delta Ct}$) was normalized with respect to the U6 control reference.

Plasmid construction and transfection

Full-length cDNA of lncRNA SNHG1 and miR-195 was inserted into the pcDNA™ 6.2-GW/EmGFP-miR vector (National Center for Biotechnology Information; *http://www.ncbi.nlm.nih.gov/*). Then, 2'-O-methyl-anti-lncRNA SNHG1, presented as lncRNA SNHG1 inhibitor, was chemically synthesized by Genechem (Shanghai, China). HepG2 cells were transfected into different groups: mock, mimic control, inhibitor control, SNHG1-mimic, SNHG1-inhibitor, and SNHG1-mimic + miR-195-mimic. After cells were grown in growth medium to 60–70% confluency, HepG2 cells were transfected using Lipofectamine 2000 Reagent (Invitrogen Corp, CA, USA) following the manufacturer's instruction.

Dual-luciferase report assay

As suggested by the manufacturer's protocol, the miR-195 full length 5' lncRNA SNHG1-binding seed region was inserted into the pGL3 promoter vector (Genscript, Nanjing, China). The sequences in the binding site were mutated using GeneTailor Site-Directed Mutagenesis System (Invitrogen, USA), and then the mutant 3' UTR was cloned into the same vector. These two constructed vectors were named pGL3-miR-195 and pGL3-miR-195-mut, respectively. A total number of 1×10⁴ HEK293 cells

were transfected with 50nM lncRNA SNHG1 and pcDNA6.2-control, and either 100 ng pGL3-miR-195 or 100 ng pGL3-miR-195-mut. Afterwards, cells were seeded in 96-well plates and the Lipofectamine 2000 Reagent was addedfollowing the manufacturer's instructions. Data collected from the luminometer were normalized by dividing firefly luciferase activity with that of Renilla luciferase vector pRL-SV40, which was also cotransfected into HepG2 cells.

Cell proliferation assay

Briefly, 3-(4,5)-dimethyl thiahiazo-(-z-y1)-3,5-di-phenytetrazoliumromide (MTT) assay (Sigma, USA) was used to evaluate cell growth status. HepG2 cells were grouped and transfected respectively as mentioned earlier. After 1, 2, and 3 days of transfection, HepG2 cells were incubated with fresh medium containing 20 µL MTT for another four hours. Supernatant was removed and replaced with 150 µL dimethyl sulfoxide. Cells were incubated in a humidified incubator (37°C, 5% CO_2). Universal Microplate Spectrophotometer (Bio-Tek Instruments, Inc., Winooski, USA) was used to measure the absorbance value at a wavelength of 490 nm.

Transwell assay

In vitro, Transwell assay was used to investigate cell migration and invasion status. The experiments used Transwell chambers (24-well, 8 µm pore size, Costar, Corning, Switzerland), uncoated for migration assays, or Matrigel coated for invasion assays. After 48 hours, we trypsin-digested the post-transfected cells with 0.25% trypsin containing EDTA and resuspended the cells in serum-free RPMI 1640 medium. Subsequently, we added 200 µL of the cell suspension into the upper chamber, and 600 µL of medium containing 10% FBS as chemoattractant was added into the lower chamber. After 24-hour incubation in a moist atmosphere (37 °C, 5% CO_2), we used wet cotton swabs to wipe off non-migrated cells through the pores from the upper face of the filters, while cells adhered to the bottom surface of the inserts were fixed with cold methanol for 10 minutes and then stained with 0.01% crystal violet for 2 minutes. Finally, the entire filters were washed twice in water and observed under an inverted microscope (Olympus, Japan).

Wound healing assay

The transfected HepG2 cells were seeded in 6-well plates at a cell density of 8×10⁴ and were grown into monolayer cells overnight. A 10-µL micropipette tip was used to scratch the surface of plates to create a "wound" and the suspended cells were mildly washed with PBS. Cells in the plates were cultured in serum-free medium. Wound images were photographed using a phase-contrast microscope on Day 0, 1, and 2. The wound area was scored by the ImageJ software.

Table 1. Relationship between lncRNA SHNG1 expression and clinicopathological features of 122 HCC patients.

Clinicopathological features (n)	lncRNA SNHG1(%)		P value
	High expression (n=70)	Low expression (n=52)	
Age (yr)			
>55 (66)	40 (60.6)	26 (39.4)	0.434
≤55 (56)	30 (53.6)	26 (46.4)	
Gender			
Male (92)	54 (58.7)	38 (41.3)	0.606
Female (30)	16 (53.3)	14 (46.7)	
Alcohol consumption			
Low (90)	50 (55.5)	40 (44.5)	0.495
Excessive (32)	20 (62.5)	12 (37.5)	
Tumor size (cm)			
≤5 (82)	38 (46.3)	44 (53.7)	<0.001
>5 (40)	32 (80.0)	8 (20.0)	
Liver cirrhosis			
Yes (96)	56 (58.3)	40 (41.7)	0.682
No (26)	14 (53.8)	12 (46.2)	
HBsAg			
Positive (98)	56 (57.1)	42 (42.9)	0.916
Negative (24)	14 (58.3)	10 (41.7)	
HCV Ab			
Positive (22)	12 (54.6)	10 (45.4)	0.767
Negative (100)	58 (58.0)	42 (42.0)	
Metastasis			
With (20)	10 (50.0)	10 (50.0)	0.466
Without (102)	60 (58.9)	42 (41.2)	
TNM stage			
I–II (42)	16 (38.1)	26 (61.9)	0.002
III–IV (80)	54 (67.5)	26 (32.5)	

Statistical analysis

We performed statistical analysis using SPSS 17.0 statistical software, and data presented using Graph PAD prism software. The χ^2 test was applied to infer the relationship between lncRNA SNHG1 expression and clinicopathological characteristics of HCC patients. All the results obtained from the *in vitro* experiments were expressed as mean ± standard deviation, and continuous data were analyzed using the double-sided Student's *t*-test. A *p*-value of less than 0.05 was considered statistically significant.

Results

lncRNA SHNG1 expression correlated with several clinicopathological features

Clinicopathological features of 122 HCC patients are summarized in Table 1. The average age of patients was 55.5±10.2 years (56 patients ≤55 years of age and 66 patients >55 years of age). There were 92 males and 30 females in the patient group. As suggested by *in situ* hybridization, the expression levels of lncRNA SNHG1 were further classified (using mean
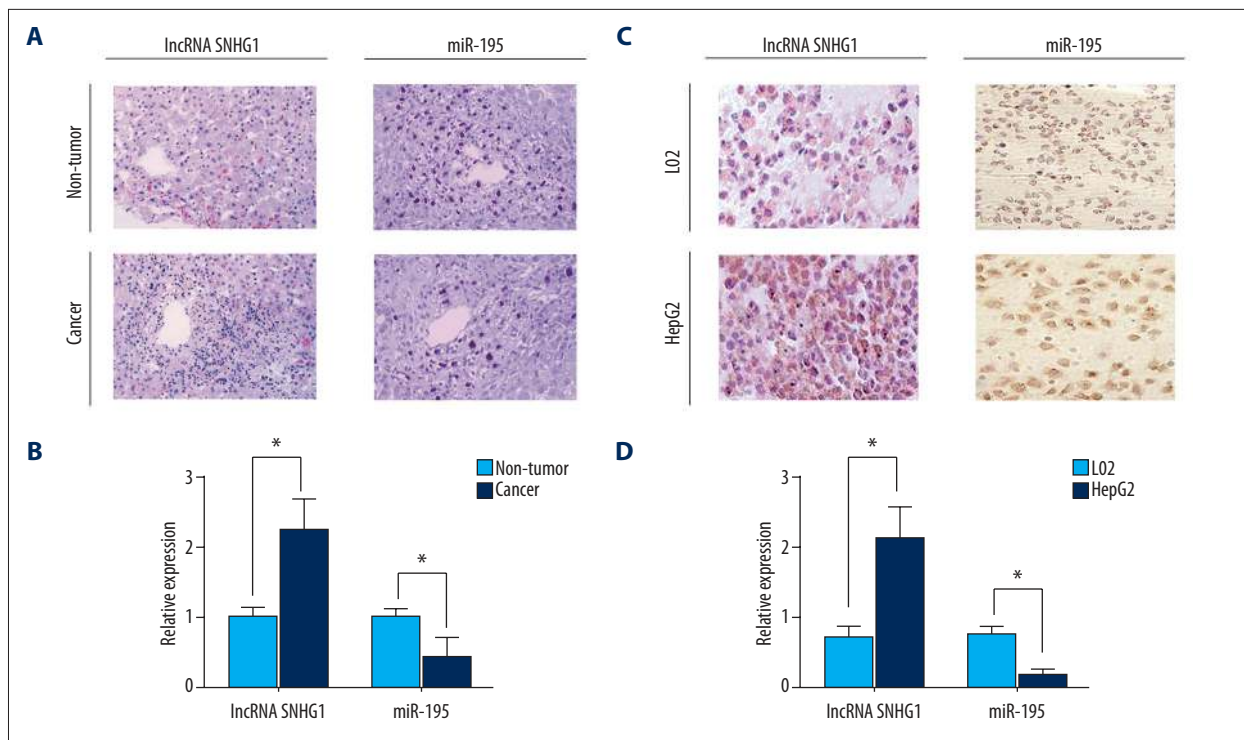
Figure 1. lncRNA SNHG1 exhibited high expression levels in both HCC tissues and HepG2 cell lines. (**A**) lncRNA SNHG1 and miR-195 expression was detected in HCC tissues and matched non-tumor tissues from HCC patients using hybridization *in situ*. (**B**) RT-PCR was used to measure lncRNA SNHG1 and miR-195 expression in HCC tissues and matched non-tumor tissues. (**C**) Relative expression levels of lncRNA and miR-195 were detected in HepG2 and L02 cells using hybridization *in situ*. (**D**) RT-PCR was used to measure lncRNA SNHG1 and miR-195 expression in HepG2 and L02 cells. lncRNA SNHG1 and miR-195 data were presented in the form of mean ± standard deviation (n=3). Similar results were obtained from three independent experiments. (* $p<0.05$).

area of positive staining as the cutoff value) into high expression with lncRNA SNHG1 ≥30% of the tumor section and low expression with lncRNA SNHG1 <30% of the tumor section. The expression of lncRNA SNHG1 in HCC tissues was closely related to several clinicopathological features, including tumor size ($p<0.001$) and TNM stage ($p=0.002$). HCC patients with larger tumor size or TNM stage III–IV exhibited relatively higher lncRNA SNHG1 expression levels. However, lncRNA SNHG1 expression was not associated with gender, age, or alcohol consumption (all $p>0.05$). These results indicated that high expression level of lncRNA SNHG1 might predict the aggravation of HCC.

lncRNA SNHG1 showed high-level expression in both HCC tissues and cell lines

As revealed by hybridization *in situ*, lncRNA SNHG1 expression levels were significantly upregulated in HCC tissues compared with matched non-tumor tissues, while miR-195 expression levels were significantly downregulated in HCC tissues ($p<0.05$; Figure 1A). These results were consistent with the results obtained from RT-PCR ($p<0.05$; Figure 1B). In addition,

lncRNA SNHG1 was significantly upregulated in HepG2 HCC cells compared with L02 normal hepatocyte cells, whereas the expression levels of miR-195 were significantly downregulated as shown in hybridization *in situ* and RT-PCR ($p<0.05$; Figure 1C, 1D). We concluded that lncRNA SNHG1 was highly expressed in both HCC tissues and cell lines. Results from HepG2 cell lines were consistent with those from HCC tissues, further indicating that the expression of lncRNA SNHG1 was increased in HCC tissues.

lncRNA SNHG1 inhibited miR-195 expression

To further explore the relationship between the expression of lncRNA SNHG1 and miR-195 in HepG2 cells, transfection experiments were performed using HepG2 cell lines as previously described. As demonstrated by RT-PCR, miR-195 expression of HepG2 cells transfected with SNHG1-mimic (0.0088±0.0030) was remarkably lower than that of other groups, including the mimic control group (0.2760±0.0562), indicating that overexpression of lncRNA SNHG1 could contribute to a decreased miR-195 expression. In addition, downregulation of lncRNA SNHG1 after transfection of SNHG1-inhibitor significantly increased the
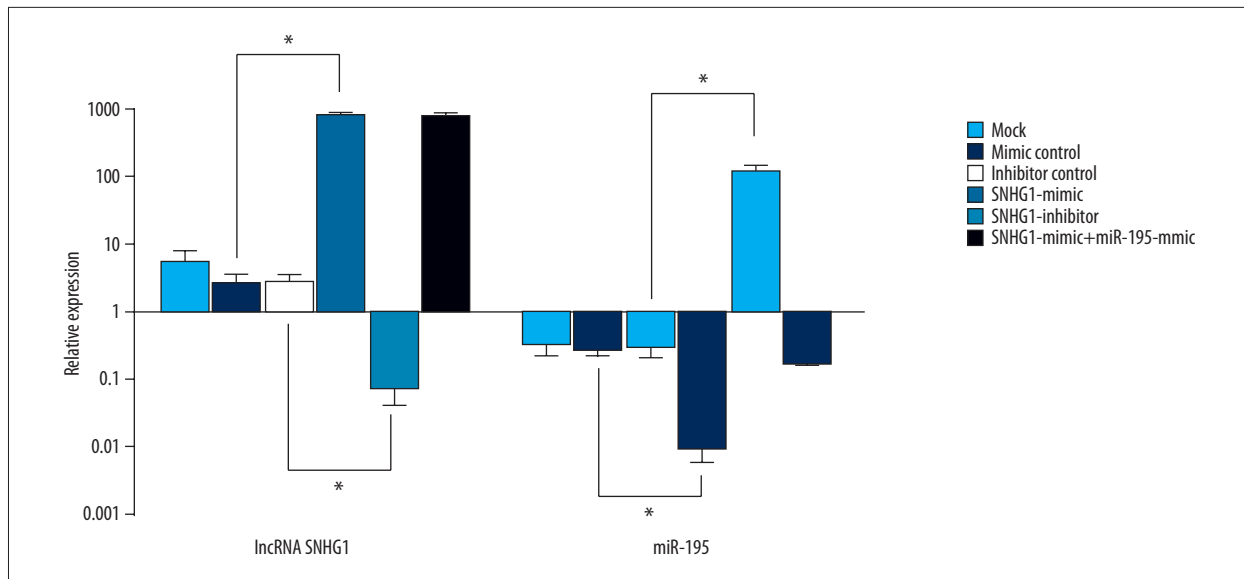
Figure 2. lncRNA SNHG1 inhibited miR-195 expression. Expression levels of lncRNA SNHG1 and miR-195 in HepG2 after transfection as mentioned above were detected by RT-PCR. lncRNA SNHG1 and miR-195 data were presented in the form of mean ± standard deviation (n=3). Similar results were obtained from three independent experiments (* $P<0.05$).

expression level of miR-195 (all $p<0.05$; Figure 2). Interestingly, we noticed that neither upregulation or downregulation of miR-195 had significant effect on lncRNA SNHG1 expression.

lncRNA SNHG1 exacerbated cell proliferation, invasion and migration

MTT growth assay was conducted over a 3-day period in order to discover the function of lncRNA SNHG1 in cell growth. As demonstrated in Figure 3A, MTT optical density (OD) values of HepG2 cells transfected with SNHG1-mimic on Day 1 (2.52±0.15), Day 2 (3.61±0.27) and Day 3 (4.27±0.30) were significantly higher than those of other groups ($p<0.05$), indicating that over-expression of lncRNA SNHG1 can significantly enhance cell proliferation. Cells transfected with SNHG1-inhibitor exhibited significantly decreased cell proliferation rates with relatively lower MTT OD values ($p<0.05$). These results

demonstrated that high level of lncRNA SNHG1 expressions were associated with HepG2 cell proliferation.

Transwell assay results demonstrated that both the migration cells (137.31±7.15) and the invasion cells (123.72±7.67) in the SNHG1-mimic group were significantly increased compared to other groups, whereas the migration cells (35.88±3.81) and invasion cells (31.71±3.63) in the SNHG1-inhibitor group were significantly reduced (all $p<0.05$; Figure 3B), suggesting that migration and invasion of HepG2 cells transfected with SNHG1-mimic were significantly enhanced as compared with other groups, while cells transfected with SNHG1-inhibitor exhibited significantly decreased cell migration and invasion. In addition, the wound healing assay showed that the relative wound area of the SNHG1-mimic group on Day 1 (0.183±0.032) and Day 2 (0.061±0.012) were decreased significantly compared to other groups, whereas the relative wound area of
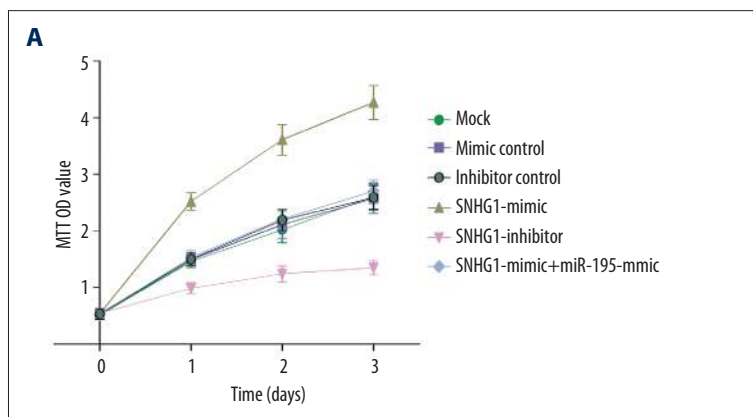

Figure 3. lncRNA SNHG1 regulated proliferation, migration and invasion of HCC cells *in vitro*. (**A**) HepG2 cell proliferation was measured by MTT assay. (**B**) HepG2 cell migration and invasion status were restored after transfection as indicated earlier (* $P<0.05$). (**C**) HepG2 cells after transfection were performed by wound healing assays with a recovery period of 1–2 days. Wound areas were measured using the Image J software (* $P<0.05$).
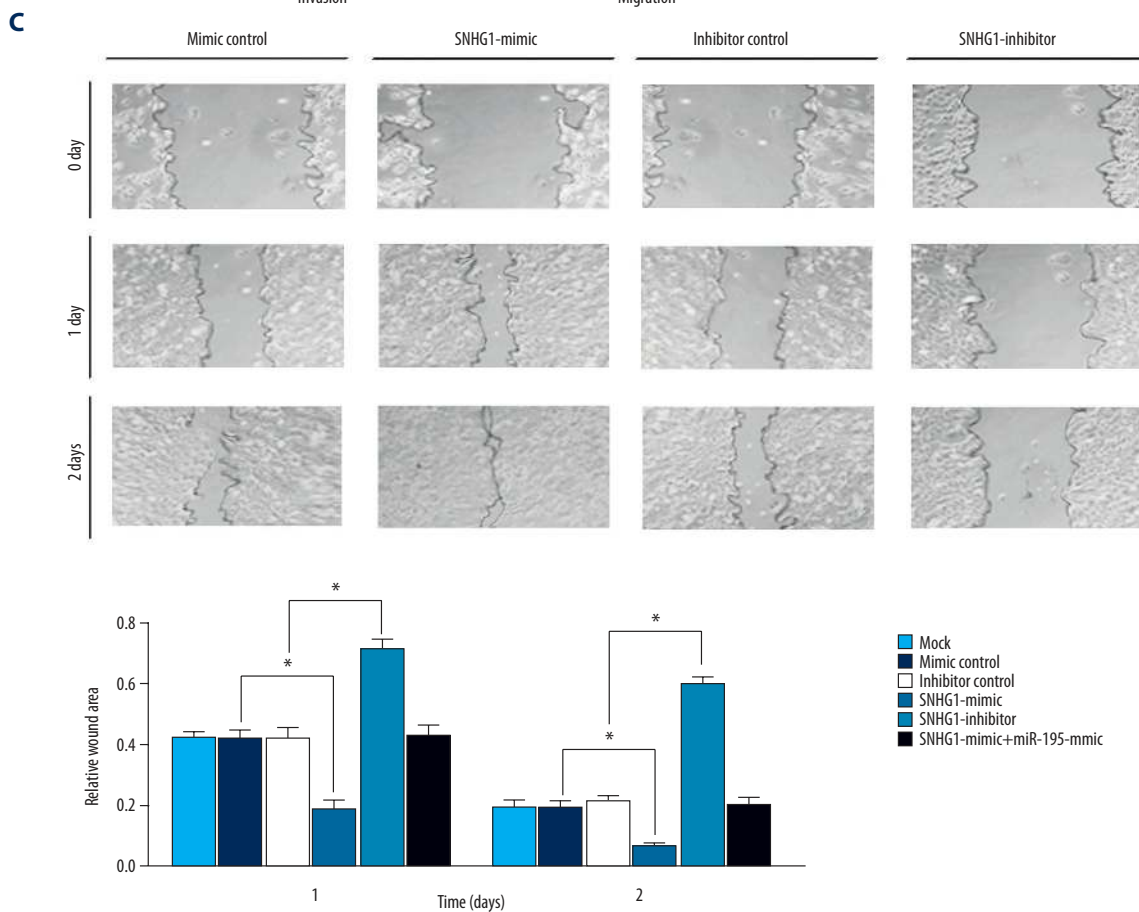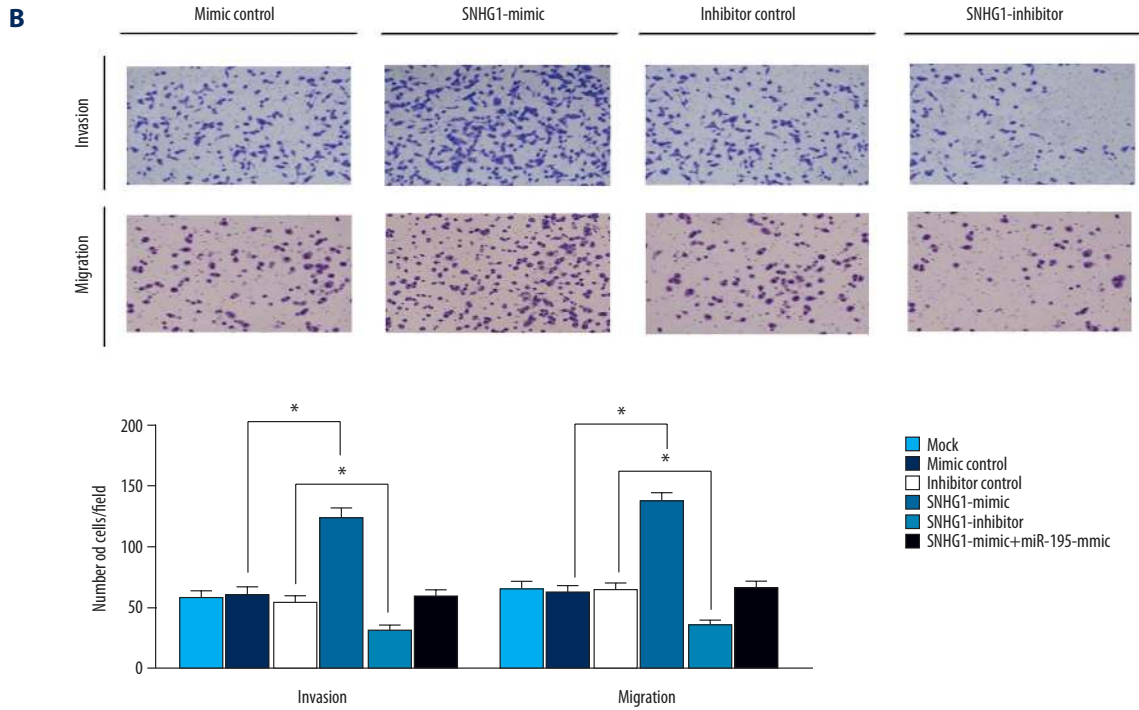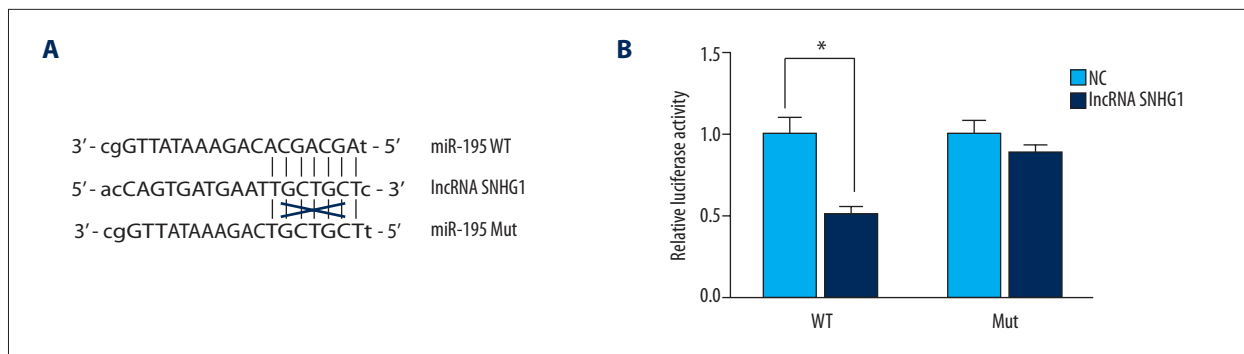
Figure 4. miR-195 directly targeted lncRNA SNHG1. (**A**) The lncRNA SNHG1-binding sequence in the 5' region of miR-195. A mutation was generated in the miR-195 5' region sequence in the complementary site for the seed of lncRNA SNHG1. (**B**) HEK293 cells seeded in 96-well plates were cotransfected with either the wild-type or mutant miR-195-5' lncRNA SNHG1-binding seed region, together with lncRNA SNHG1 or pcDNA6.2-control. The relative luciferase values were measured and normalized to the values of Renilla luciferase activity in 48 hours. All data were indicated as the mean ± standard deviation (n=3). Similar results were obtained from three independent experiments (* $P<0.05$).

the SNHG1-inhibitor group on Day 1 (0.712±0.031) and Day 2 (0.594±0.024) were reduced only slightly (all $p<0.05$; Figure 3C). These results suggest that high expression levels of lncRNA SNHG1 could facilitate HepG2 cell invasion and migration.

miR-195 acted as a direct downstream target of lncRNA SNHG1

We next investigated whether miR-195 was a direct functional target of lncRNA SNHG1. Since a complementary 5' binding site for the seed sequence of lncRNA SNHG1 (Figure 4A) was predicted through the online database (*http://starbase.sysu.edu.cn/mirLncRNA.php*), we suspected that miR-195 might act as a potential target of lncRNA SNHG1. Cotransfection with lncRNA SNHG1 and miR-195 wild-type 5'lncRNA SNHG1-binding seed region resulted in a significant reduction in luciferase activity compared with that of the control group ($p<0.05$; Figure 4B). However, cotransfection with lncRNA SNHG1 and miR-195-mut did not have significant effect on luciferase activity. All of these results indicated that lncRNA SNHG1 directly targeted miR-195.

Discussion

Unfavorable prognosis of HCC is associated with the invasion and metastasis of cancer cells, and HCC has become a major life-threatening disease in the world [19]. Recent studies have suggested that a number of long non-coding RNAs (lncRNAs) are involved in HCC pathogenesis and progression. Therefore, this study aimed to confirm that the upregulation of lncRNA SNHG1 can directly inhibit miR-195 expression, thereby providing an underlying molecular mechanism which may explain the detailed role of lncRNA SNHG1 in regulating human liver tumorigenesis.

Although lncRNAs cannot be translated into protein products, they may participate in a variety of pathobiological processes such as cell proliferation, apoptosis, invasion, and metastasis through regulating protein-coding mRNA expressions [20]. Over the past few years, a class of lncRNAs has been found to be aberrantly expressed in patients with HCC and to play a role in modulating malignant phenotypes, suggesting a new understanding of liver carcinogenesis [11]. Also, it has been shown that lncRNAs may be involved in almost every aspect of gene regulation, including transcription, mRNA splicing, translation, chromosome dosage-compensation, control of imprinting, chromatin modification, and intracellular trafficking [21,22]. Moreover, high expression levels of lncRNA NEAT1 possess a pivotal role in tumorigenesis and metastasis of HCC [23], and lncRNA AOC4P is a tumor suppressor for HCC through which vimentin degradation is promoted and epithelial-mesenchymal transition is suppressed [24]. In addition, J You et al. discovered that lncRNA SNHG1 expression was significantly upregulated in lung cancer cells when compared with normal bronchial epithelial cells [15].

However, to the best of our knowledge, there is limited research explaining the role of lncRNA SNHG1 in HCC. This retrospective study showed that lncRNA SNHG1 was overexpressed in HCC tissues compared with normal liver tissues. Similar results were obtained when we compared the expression of lncRNA SNHG1 between HCC cell lines HepG2 and normal liver cell lines L02. These results were consistent with the the reported function of lncRNA SNHG1 in lung cancer [15]. Furthermore, several clinicopathological features of HCC patients, including tumor size and TNM stage, were closely associated with higher lncRNA SNHG1 expression levels, further implying that lncRNA SNHG1 could contribute to HCC pathogenesis and progression.

There is also reported evidence that lncRNAs may regulate miR-NAs by acting as competing endogenous RNAs or by being processed into small RNAs [25]. Studies have reported that miR-195 plays an important role in inhibiting the development of various human cancers, including HCC, bladder, cervical, and breast cancers [26–29]. As suggested by previous studies, miR-195 targeted cyclin D1, CDK6, and E2F3 can suppress cell proliferation of human HCC cells [18]. Apart from that, miR-195 targets the TNF-α/NF-κB pathway and downregulates both IκB kinase alpha and TAB3 in HCC [30]. However, current studies have been unable to clarify the exact relationship between lncRNA and miR-195. After transfection with lncRNA SNHG1-mimic, HepG2 cells exhibited downregulated expression of miR-195, while transfection with miR-195 mimic did not significantly influence lncRNA SNHG1 expression. Therefore, we concluded that lncRNA SNHG1 inhibited miR-195 expression in HCC cells. Finally, bioinformatics target gene prediction enabled us to unfold the lncRNA SNHG1 binding sites in the 5' regions of miR-195, and dual-luciferase report assay was subsequently performed to further verify that miR-195 was a direct target of lncRNA SNHG1.

For the purpose of detecting whether lncRNA SNHG1 and miR-195 can influence HCC cell proliferation, invasion and migration status – which are closely related to HCC metastasis – *in vitro* experiments, including MTT assay, wound healing assay, and Transwell assays, were separately conducted using HepG2

HCC cells. HepG2 cells transfected with lncRNA SNHG1-mimic exhibited increased cell proliferation, invasion, and migration status, while miR-195 overexpression suppressed these cell activities. Therefore, lncRNA SNHG1 may play an important role in regulating human liver tumorigenesis through inhibiting miR-195 expression.

This study had several limitations. Despite patients being consecutively selected over a one year period (from 2014 to 2015), we had a small sample size (due to the lack of resources), and selection bias may have occurred. Therefore, mice xenograft models should be carried out in future studies and tumor growth should be observed *in vivo* to further confirm the relationship between lncRNA SNHG1 and miR-195 in HCC.

Conclusions

In summary, this study suggests that overexpression of lncRNA SNHG1 can directly suppress miR-195 expression, which contributes to the pathogenesis and progression of HCC. Since HCC has extremely poor prognosis due to the lack of available curative therapeutic approaches [31], we believe that this study contributes to the understanding of the roles of lncRNAs in liver tumorigenesis, and these findings may be important for developing a potential therapeutic candidate for curing HCC.

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