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LncRNA SNHG14 promotes the progression of cervical cancer by regulating miR-206/YWHAZ

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ABSTRACT

Accumulating evidence suggests that lncRNAs play key roles in many cancers. It has been reported that long non-coding RNA SNHG14 promotes cell proliferation and metastasis in multiple cancers. However, the role and underlying molecular mechanism of SNHG14 in cervical cancer (CC) remain largely unclear. In this study, we discovered that the relative expression of SNHG14 was significantly upregulated in CC tissues and cells, and associated with the overall survival of CC patients. Moreover, knockdown of SNHG14 significantly inhibited cell proliferation, migration and invasion, and promoted cell apoptosis in CC. Molecular mechanism explorations revealed that SNHG14 acted as a sponge of miR-206 and that YWHAZ was a downstream target gene of miR-206 in CC. Spearman's correlation analysis uncovered a significantly negative correlation between SNHG14 (or YWHAZ) and miR-206 expression, while a significantly positive correlation between SNHG14 and YWHAZ expression in CC tissues. We also found that the effect of SNHG14 knockdown on the CC progression could be partly rescued by overexpression of YWHAZ at the same time. Our findings revealed that SNHG14 acted as a sponge of miR-206 to regulate the expression of YWHAZ in CC, hinting the promising therapeutic target role of SNHG4 for CC patients.

1. Introduction

It is known that cervical cancer (CC) is the second common malignant tumor, and accounts for a large proportion of cancer-associated mortality in females [17,19]. Great advancements have been made in the treatment of CC including surgery, chemotherapy and radiotherapy, but the long-term survival rate of CC patients is still unsatisfactory [16,26]. Therefore, it is urgent to explore the potential molecular mechanism of CC tumorigenesis and development, and search for new molecular therapeutic targets for CC.

Long non-coding RNAs (lncRNAs), a group of non-coding transcripts with more than 200 nucleotides in length, and have no function of protein coding [20]. It has been reported that lncRNAs play crucial roles in the regulation of cell proliferation, migration, invasion and even stem-cell biology [2,7,10]. A number of lncRNAs act as important regulators in the initiation and development of CC. For instance, long non-coding RNA TTN-AS1 promotes cell growth and metastasis in cervical cancer via miR-573/E2F3 [1]. Long non-coding RNA NORAD upregulate SIP1 expression to promote cell proliferation and invasion in cervical cancer [9]. Recent studies indicated that long non-coding RNA SNHG14 induces trastuzumab resistance of breast cancer via regulating PABPC1 expression through H3K27 acetylation [3]. Long non-coding RNA SNHG14 contributes to gastric cancer development through targeting miR-145/SOX9 axis [13]. SP1-induced up-regulation of lncRNA SNHG14 as a ceRNA promotes migration and invasion of clear cell renal cell carcinoma by regulating N-WASP [12]. Nevertheless, the function and molecular mechanism of lncRNA SNHG14 in CC remain unclear.

In the present study, we planned to explore the function and potential underlying mechanism of SNHG14 in CC. Loss- and gain-of-function experiments were performed to prove the biological function of SNHG14 in CC progression. Finally, our study discovered that lncRNA SNHG14 promoted cell proliferation, migration, invasion, and inhibited cell apoptosis via miR-206/ YWHAZ axis in CC.

2. Materials and methods

2.1. Clinical samples

A total of 80 paired CC tumor tissues and adjacent non-tumor tissues were provided by patients at Xinjiang Municipal People's Hospital. All

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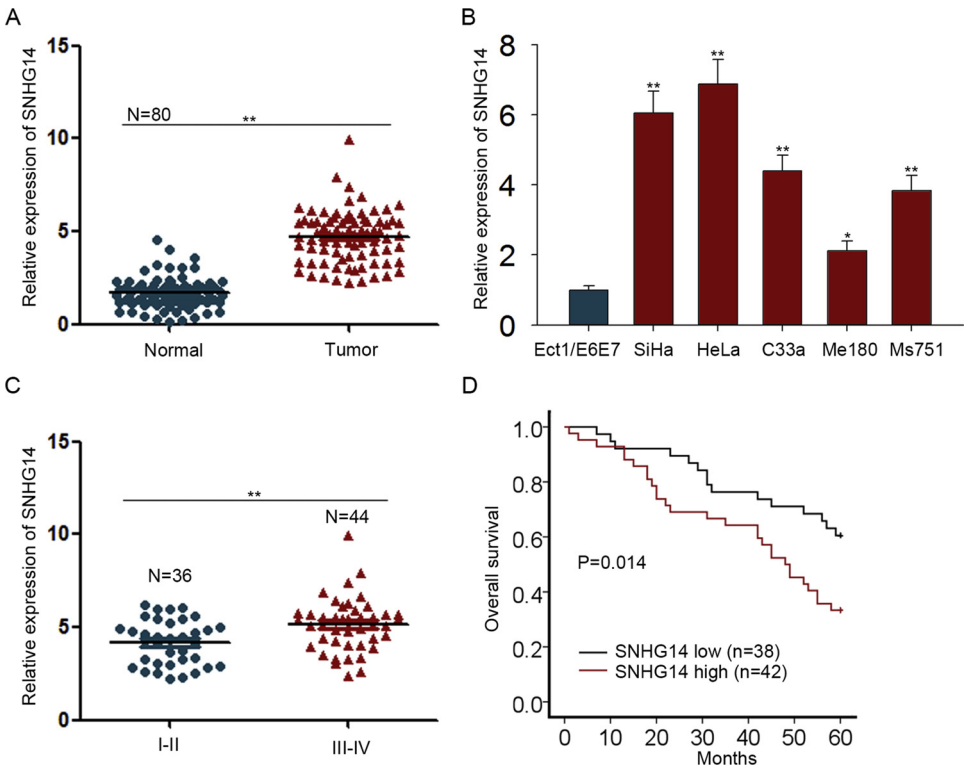

Fig. 1. SNHG14 is upregulated in CC tissues and cell lines, and associated with the prognosis of CC patients.
(A) RT-qPCR showed expression of SNHG14 in tumor tissues and adjacent non-tumor tissues (n = 80). (B) RT-qPCR tested the expression of SNHG14 in CC cell lines (SiHa, HeLa, C33a, Me180 and Ms751) and human normal cervical cell lines (Ect1/E6E7). (C) Relative SNHG14 expression levels in different clinical stages (I-II and III-IV). (D) Kaplan-Meier curve and the log-rank test showed that the expression of SNHG14 was associated with overall survival in patients with CC. All results were presented as the mean ± SD of at least 3 independent experiments. $^{*}P < 0.05$, and $^{**}P < 0.01$.

Table 1
Clinical characteristic and SNHG14 expression of patients with cervical cancer. (n = 80).

Variable	SNHG14 Expression		P-value
	low	high	
Age			
< 55	14	17	0.820
≥ 55	24	25	
Tumor Size			
< 4cm	27	27	0.634
> = 4 cm	11	15	
Histology			
Squamous	23	23	0.655
Adenocarcinoma	15	19	
FIGO Stage			
I/II	23	13	0.013*
III/IV	15	29	
Differentiation			
Well	20	10	0.011*
Moderate-poor	18	32	
Lymph node metastasis			
No	22	11	0.006*
Yes	16	31	

Low/high by the sample median. Pearson χ^2 test. $^{*}P < 0.05$ was considered statistically significant.

Table 2
Multivariate analysis of prognostic parameters in patients with cervical cancer by Cox regression analysis.

Variable	Category	P-value
Age		
	< 55	0.299
	≥ 55	
Tumor size		
	< 4cm	0.152
	> = 4 cm	
Histology		
	Squamous	0.761
	Adenocarcinoma	
FIGO Stage		
	I/II	0.030*
	III/IV	
Differentiation		
	Well	0.074
	Moderate-poor	
Lymph node metastasis		
	No	0.564
SNHG14 level	Yes	0.015*
	Low	
	High	

Proportional hazards method analysis showed a positive, independent prognostic importance of SNHG14 expression (P = 0.015). $^{*}P < 0.05$ was considered statistically significant.

tissues were immediately preserved at −80°C. None of patients received treatment. Written informed consents were signed by each patient. This work was approved by the Ethics Committee of Xinjiang Municipal People's Hospital

2.2. Cell culture and transfection

CC cell lines (SiHa, HeLa, C33a, Me180 and Ms751) and human normal cervical cell lines (Ect1/E6E7) were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 10% fetal bovine serum (FBS; Gibco/Invitrogen Inc., Carlsbad, CA, USA), 100 μg/ml phytomycin and 100 U/ml penicillin. All cells were cultured in a humidified incubator of 5% CO_2 at 37°C.

Short hairpin RNA (shRNA) targeting SNHG14 (sh-SNHG14) and the scrambled negative control (sh-NC) were designed and synthesized by GenePharma (Shanghai, China). MiR-206 mimic, miR-206 inhibitor,

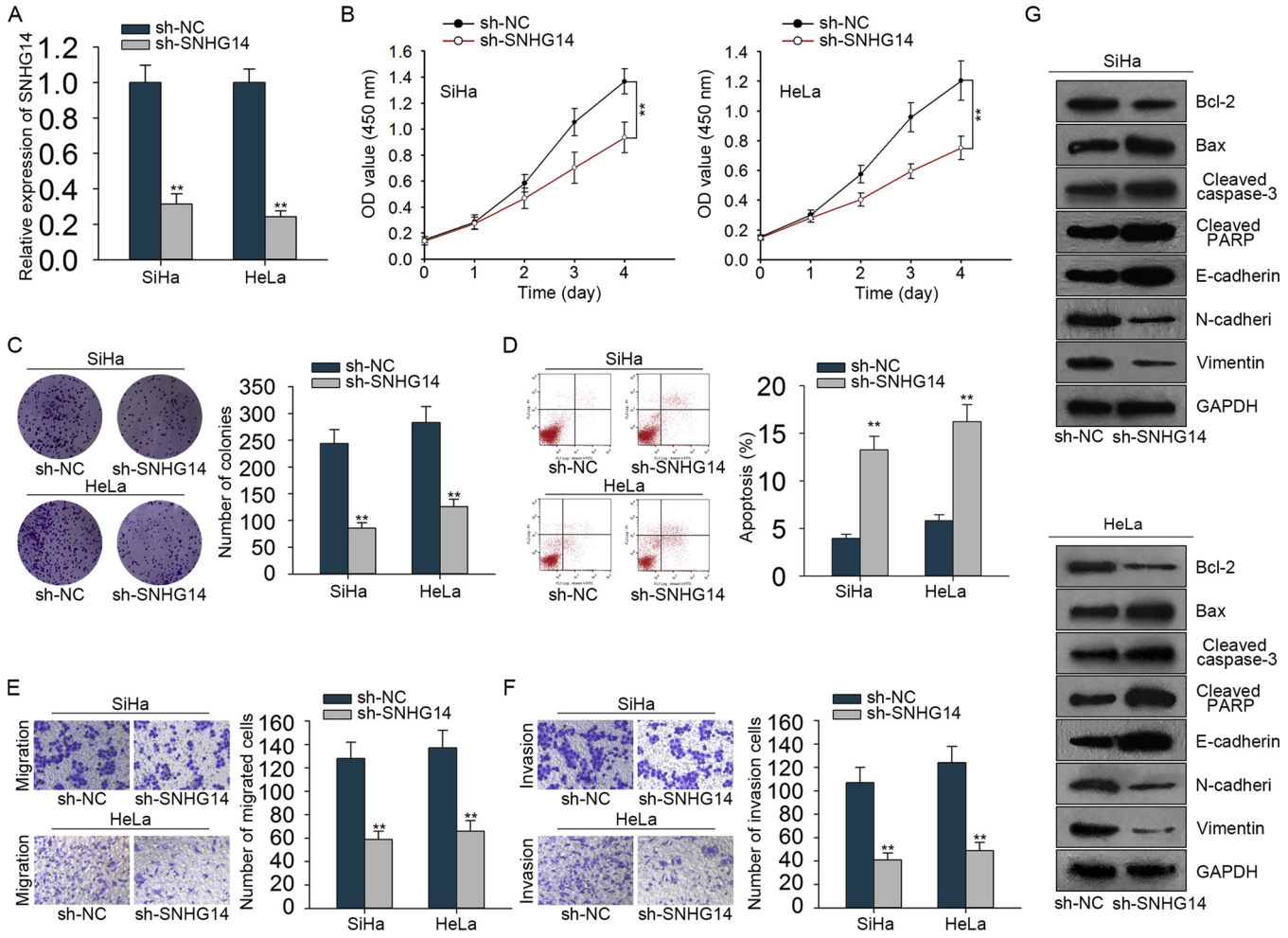

Fig. 2. Suppression of SNHG14 inhibits cell proliferation, migration, invasion, and promoted cell apoptosis in CC cells.
(A) RT-qPCR measured the knockdown efficiency of SNHG14 in SiHa and HeLa cells. (B) CCK-8 results of the proliferation of transfected cells at the indicated time points (24, 48, 72, 96 and 120 h). (C) Colony formation assay was also performed to detect the proliferation of transfected cells. (D) The apoptosis rate of SiHa and HeLa cells was analyzed by flow cytometry. (E–F) Transwell assay showed that SNHG14 suppression reduced the migration and invasion activities in SiHa and HeLa cells. (G) Western blot assay examined the expression of apoptosis associated proteins (Bax, Cleaved caspase-3, Cleaved PARP and Bcl-2) and EMT markers (E-cadherin, N-cadherin and vimentin) in SiHa and HeLa cells after different transfections. All results were presented as the mean ± SD of at least 3 independent experiments. $^{*}P < 0.05$, and $^{**}P < 0.01$.

and the corresponding negative control (miR-NC) were purchased from GenePharma. The full-length sequences of SNHG14 and YWHAZ were respectively synthesized and cloned into pcDNA3.1 (Invitrogen, Carlsbad, USA) plasmid to produce pcDNA3.1/SNHG14 and pcDNA3.1/YWHAZ. Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) was used to perform the transfection of these plasmids according to manufacturer's recommendations.

2.3. RNA extraction and quantitative real-time PCR

Total RNA was extracted from tissues and cells by the use of Trizol reagent (Takara, Otsu, Japan). TaqMan™ Advanced miRNA cDNA Synthesis Kit (Waltham, MA, USA) or the reverse transcription kit (Takara, Otsu, Japan) was applied for RNAs reverse transcription. The RT-qPCR was performed by the use of SYBR Green PCR Kit (Takara, Otsu, Japan). GAPDH and U6 acted as endogenous controls for mRNAs or miRNAs, respectively. RT-qPCR analysis was performed with Applied Biosystems Step One Plus Real-Time PCR System (Applied Biosystems, Foster city, USA), and relative quantification was performed by the $2^{-\Delta\Delta Ct}$ method. The primers for RT-qPCR were as follows: SNHG14: 5′-GGG TGT TTA CGT AGA CCA GAA CC-3′ (Forward) and 5′- CTT CCA AAA GCC TTC TGC CTT AG-3′ (Reverse); miR-206: 5′-ACA ACA AGG ACC GGT TGC AGA-3′ (Forward) and 5′- GGG CAT ACA TCG GCT AAT ACA-3′ (Reverse); YWHAZ: 5′-TGT TGT AGG AGC CCG TAG-3′ (Forward) and 5′-GCA ACC TCA GCC AAG TAA-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).

2.4. CCK-8 assay

The growth of CC cells was assessed by the use of Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies). The transfected cells (1×10^3 cells/well) were seeded in 96-well plates and then incubated for 24, 48, 72, 96 or 120 h. CCK-8 reagent was added to each well, and then incubated for another 4 h. The absorbance of each well was measured at 450 nm with a Multiskan Go spectrophotometer (Thermo Fisher Scientific, Inc.).

2.5. Colony formation assay

Transfected cells at a density of 1×10^3 cells/well were plated into 6-well plates and maintained in DMEM medium. Medium was replaced

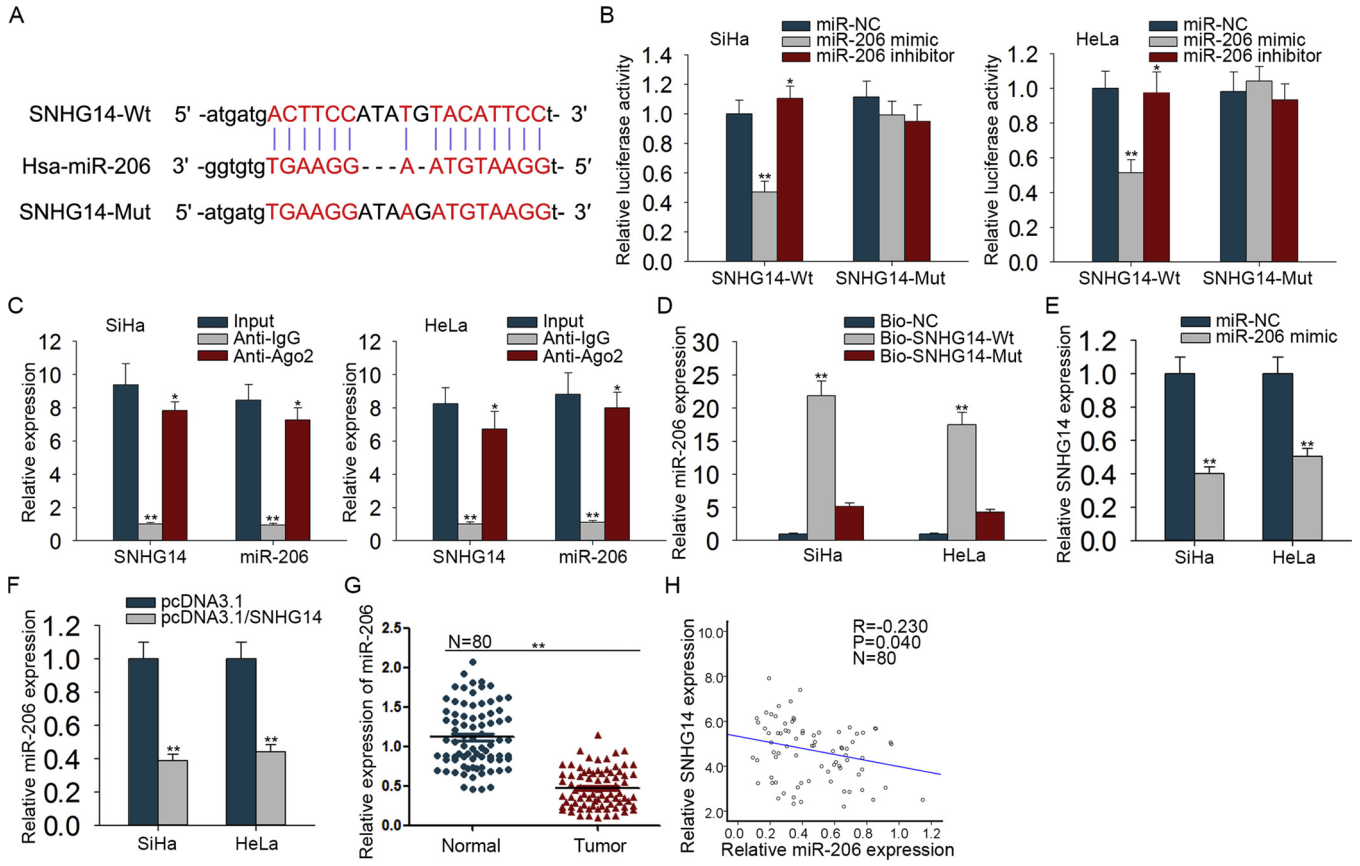

Fig. 3. SNHG14 acts as a sponge for miR-206 in CC cells.
(A) Bioinformatical predication showed the predicted binding site between SNHG14 and miR-206. (B) Luciferase reporter assay suggested that miR-206 mimics reduced the luciferase activity of SNHG14-Wt in SiHa and HeLa cells. (C) RIP and RT-qPCR assays were performed to determine the enrichment degrees of SNHG14 and miR-206 in IgG or Ago2 immunoprecipitate. (D) RNA pull down assay was carried out to further confirm the binding ability between SNHG14 and miR-206 in SiHa and HeLa cells. (E) RT-qPCR revealed that overexpression of miR-206 reduced the expression of SNHG14. (F) RT-qPCR demonstrated that overexpression of SNHG14 reduced the expression of miR-206. (G) RT-qPCR results of the expression of miR-206 in tumor tissues and adjacent non-tumor tissues. (H) Spearman's correlation analysis found the negative correlation between the expression of SNHG14 and miR-206. All results were presented as the mean ± SD of at least 3 independent experiments. $^{*}P < 0.05$, and $^{**}P < 0.01$.

every 3 days. After 2 weeks, colonies were fixed with methanol, and then stained with 0.1% crystal violet. Then, colonies that contained more than 50 cells were counted manually.

2.6. Transwell assay

Transwell assay was used to examine cell invasion and migration. The transwell membrane (Corning Incorporated, Corning, NY, USA) was coated with (invasion) or without (migration) matrigel (BD Biosciences, Bedford, MA, USA). 200 µl serum-free medium containing transfected cells (1×10^4 cells/well) was plated into the upper chambers, and 600 µl DMEM medium containing 10% FBS was added into the lower chambers. After incubation for 48 h, invaded or migrated cells were fixed with methanol and stained with crystal violet. Then, a light microscope (Olympus Corporation, Tokyo, Japan) were used to count these stained cells.

2.7. RIP assay

Magna RNA-binding protein immunoprecipitation kit (Millipore, Billerica, MA, USA) was used to perform RIP assay. Cell lysate (SiHa and HeLa) was incubated in RIP buffer containing magnetic beads conjugated with human anti-Ago2 antibody. Input and normal IgG were used as controls. Then immunoprecipitated RNAs were isolated by the use of Proteinase K. Finally, purified RNAs were determined by RT-qPCR.

2.8. RNA pull-down assay

SNHG14-Wt, SNHG14-Mut and NC were biotinylated to be Bio-SNHG14-Wt, Bio-SNHG14-Mut and Bio-NC by GenePharma Company (Shanghai, China). Bio-SNHG14-Wt, Bio-SNHG14-Mut and Bio-NC were transfected into SiHa or HeLa cells. After incubation for 48 h, cells were lysed with lysis buffer. Then, cell lysate was incubated with Dynabeads M-280 Streptavidin (Invitrogen, CA). RT-qPCR was used to measure purified RNA complex.

MiR-206-Wt, miR-206-Mut and miR-NC were transcribed using TranscriptAid T7 High Yield Transcription Kit (ThermoFisher Scientific, USA). Biotin RNA labeling mix (Roche Diagnostics, Indianapolis, IN, USA) was used to produce Bio-miR-206-Wt, Bio-miR-206-Mut and Bio-miR-NC. 50 pmol biotinylated RNA and 200 µg cell lysates (SiHa and HeLa) were mixed, and incubated with 50 µl streptavidin agarose beads (Invitrogen, Carlsbad, CA, USA) for 1 h at 4°C. After washing, RT-qPCR was used to measure the eluted proteins.

2.9. Luciferase reporter assay

The 3′-UTR sequences of YWHAZ containing the binding sites of miR-206 and full-length sequences of SNHG14 were cloned and constructed into the pGL3 vector (Promega, Madison, WI, USA) to generate the wild-type YWHAZ reporter (YWHAZ-Wt) and the wild-type SNHG14 reporter (SNHG14-Wt). GeneArt™ Site-Directed Mutagenesis System (Thermo Fisher Scientific) was used to generate the mutant-type

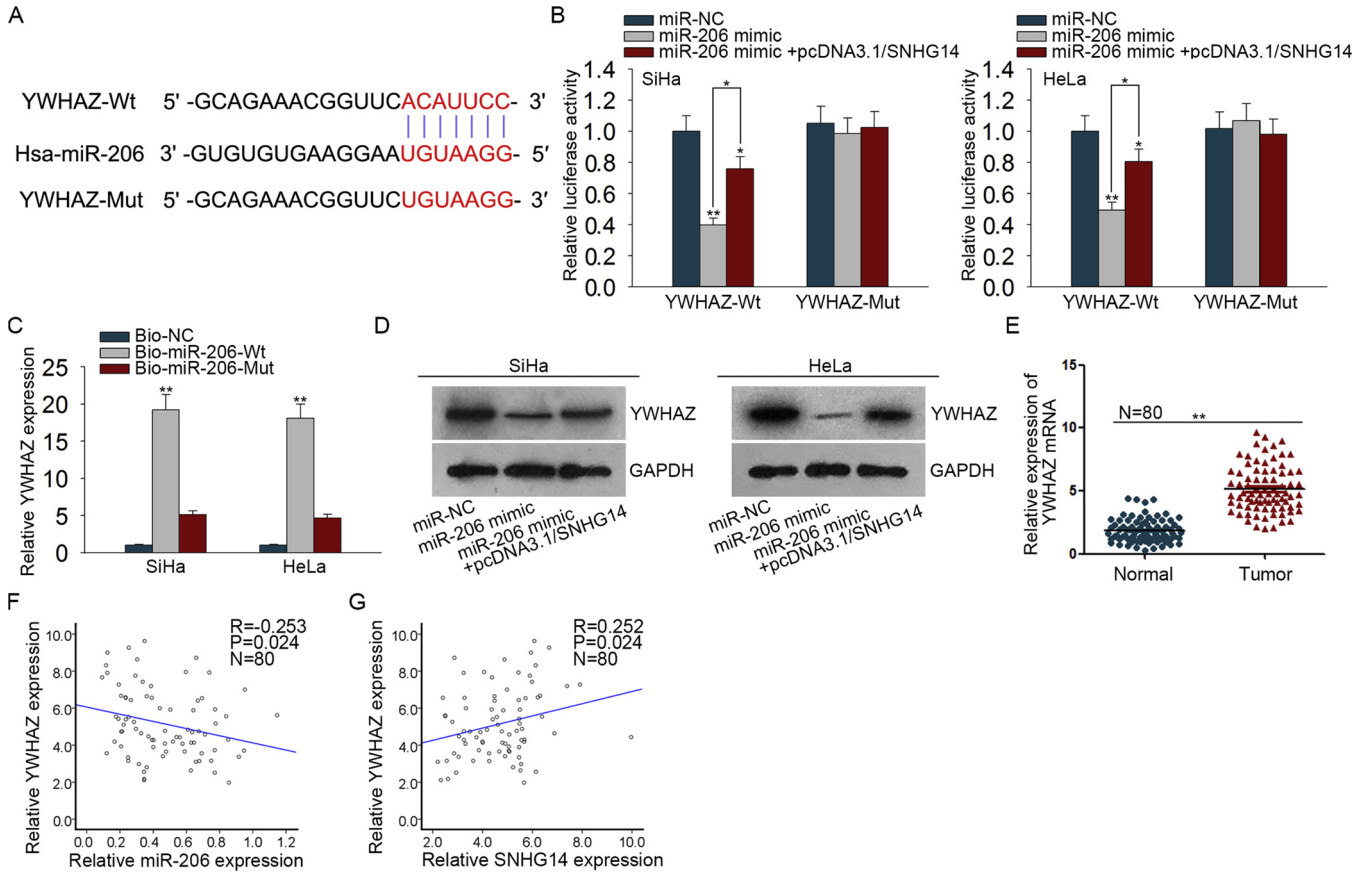

Fig. 4. MiR-206 directly targets YWHAZ and is negatively correlated to YWHAZ expression.
(A) Bioinformatic predication showed the predicted binding sites of YWHAZ and miR-206. (B) Luciferase reporter assay elucidated that the luciferase activity of wild-type YWHAZ was significantly repressed in miR-206 mimic transfected cells, but rescued under co-transfection of pcDNA3.1/SNHG14 (C) RNA pull down assay was conducted to further confirm the binding ability between YWHAZ and miR-206 in SiHa and HeLa cells. (D) Western blot assay displayed that overexpression of miR-206 reduced the expression of YWHAZ, which could be rescued after co-transfection of miR-206 mimic + pcDNA3.1/SNHG14. (E) RT-qPCR results of YWHAZ expression in tumor tissues in comparison to adjacent non-tumor tissues. (F) Spearman's correlation analysis indicated the negative correlation between expression of YWHAZ and miR-206. (G) Spearman's correlation analysis also disclosed the positive correlation between expression of SNHG14 and YWHAZ. All results were presented as the mean ± SD of at least 3 independent experiments. $^{*}P < 0.05$, and $^{**}P < 0.01$.

SNHG14 reporter (SNHG14-Mut) and the mutant-type YWHAZ reporter (YWHAZ-Mut). SNHG14-Wt or SNHG14-Mut were co-transfected with miR-206 mimic, miR-206 inhibitor or miR-NC into SiHa or HeLa cells with Lipofectamine 2000. YWHAZ-Wt or YWHAZ-Mut were also co-transfected with miR-NC, miR-206 mimic or miR-206 mimic + pcDNA3.1/SNHG14 into SiHa or HeLa cells. After transfection for 48 h, the luciferase activity was measured by luciferase reporter assay system (Promega, Madison WI, USA).

2.10. Cell apoptosis assay

The Annexin V-FITC kit (Biosea Biotechnology Co., Beijing, China) was used to detect cell apoptosis. After transfection for 24 h, SiHa or HeLa cells were harvested through trypsinization and then resuspended with PBS buffer. Subsequently, cells were double stained with Annexin V-Alexa Fluor 647 and propidium iodide (PI). Finally, the apoptotic rate was then analyzed using flow cytometer (BD Biosciences, USA).

2.11. Western blot

RIPA lysis buffer (Beyotime Biotechnology, China) with protease inhibitors (Roche, China) was used to extract total proteins. The protein extracts were then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto the polyvinylidene difluoride (PVDF) membranes. Followed by blockade with skim milk, membranes were incubated with the primary antibodies at 4°C overnight. The primary antibodies were anti-YWHAZ, anti-Bcl-2, anti-Bax, anti-cleaved caspase-3, anti-cleaved PARP, anti-E-cadherin, anti-*N*-cadherin, anti-Vimentin and anti-GAPDH (Abcam, Cambridge, UK). Then, membranes were incubated with the appropriate secondary antibody at room temperature for another 2 h. Chemiluminescent detection system was applied to capture the signals. GADPH served as internal control.

2.12. Statistical analysis

Data were presented as the mean ± standard deviation (SD). Each experiment was conducted for three times. SPSS 20.0 software (SPSS, Chicago, IL, USA) was applied for statistical analysis. Differences among groups were compared by the one-way ANOVA or Student's t-test. Correlation examination was determined by Spearman's correlation analysis. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. LncRNA SNHG14 is upregulated in CC tissues and cells

To detect whether lncRNA SNHG14 played a key role in CC progression, we firstly measured the expression of lncRNA SNHG14 in tissues and cells of CC and non-tumor groups, individually. The results

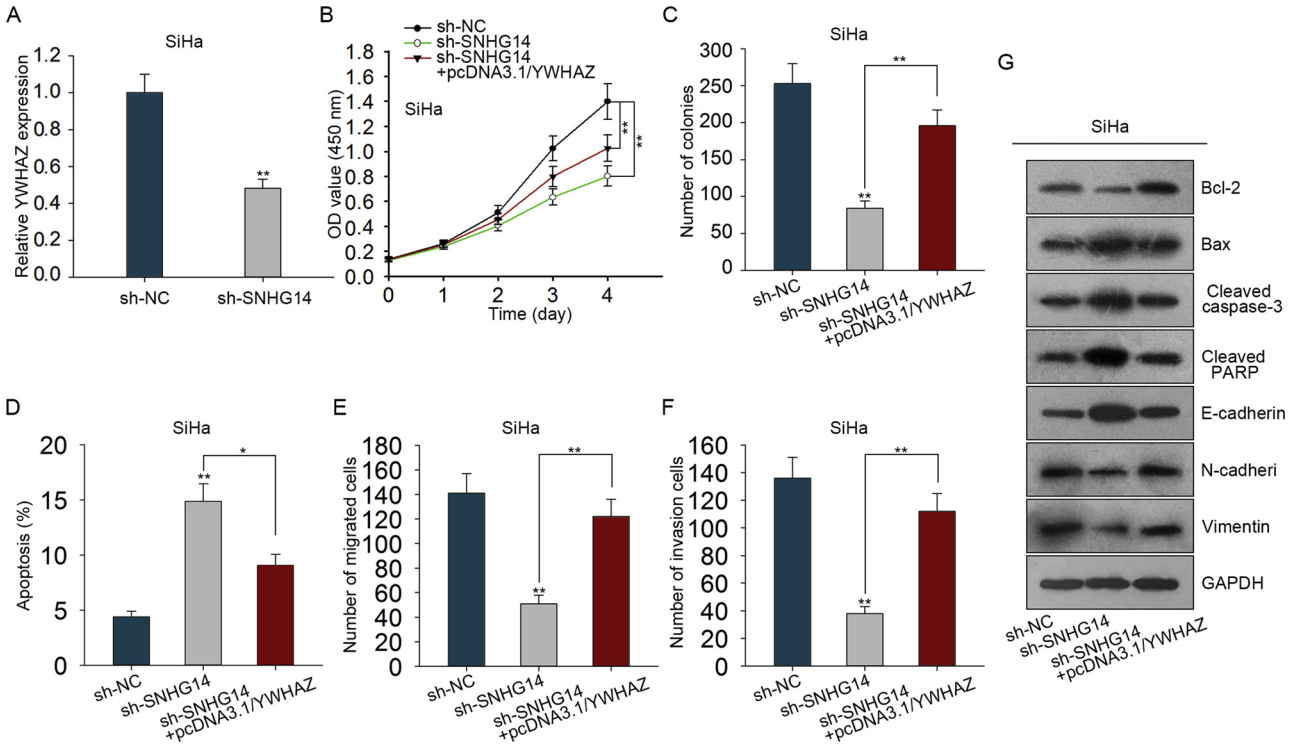

Fig. 5. YWHAZ rescues SNHG14 knockdown-mediated effects on CC cells.
(A) RT-qPCR uncovered that the silencing of SNHG14 reduced the expression of YWHAZ. (B) CCK-8 assay indicated that knockdown of SNHG14 inhibited cell proliferation in SiHa and HeLa cells. However, overexpression of YWHAZ weakened SNHG14 knockdown-mediated inhibition of cell proliferation. (C) Colony formation assay further found that knockdown of SNHG14 repressed cell proliferation in SiHa and HeLa cells. However, overexpression of YWHAZ abrogated SNHG14 down-regulation-mediated inhibition of cell proliferation. (D) Cell apoptosis assay illustrated that knockdown of SNHG14 promoted cells apoptosis in SiHa and HeLa cells. However, overexpression of YWHAZ abolished SNHG14 knockdown-mediated promotion on cell apoptosis. (E–F) Transwell assay indicated that knockdown of SNHG14 restrained cell migration and invasion in SiHa and HeLa cells. However, overexpression of YWHAZ alleviated SNHG14 down-regulation-mediated inhibition of cell migration and invasion. (G) Western blot assay exhibited that overexpression of YWHAZ restored SNHG14 knockdown-mediated effects on the expression of apoptosis associated proteins and EMT markers. All results were presented as the mean ± SD of at least 3 independent experiments. $^{*}P < 0.05$, and $^{**}P < 0.01$.

showed that lncRNA SNHG14 expression was strinkingly upregulated in CC tissues (n = 80) in comparison with that in adjacent non-tumor tissues (n = 80) (Fig. 1A). Moreover, a remarkable increase of SNHG14 was discovered in CC cells (SiHa, HeLa, C33a, Me180 and Ms751) relative to that in human normal cervical cell lines (Ect1/E6E7) (Fig. 1B). As shown in Fig. 1B, the expression of SNHG14 was higher in SiHa and HeLa cells than other CC cells, thus, SiHa and HeLa cells were chosen for subsequent experiments. Then, we explored the correlation between the expression of SNHG14 and the clinicopathologic features of CC patients. The results showed that the high expression of SNHG14 was associated with advanced FIGO stage, differentiation, and lymph node metastasis (Table 1, $P < 0.05$). Furthermore, we discovered that the expression of SNHG14 in patients with advanced tumor stage was dramatically increased (Fig. 1C). As shown in Fig. 1D, the patients with high SNHG14 expression had notably poorer overall survival than those with low SNHG14 expression. Multivariate analysis revealed that only lncRNA SNHG14 expression (P = 0.015) and FIGO stage (P = 0.03) were independent prognostic factors for CC patients (Table 2). These results above indicated that SNHG14 may be an oncogene in CC.

3.2. SNHG14 knockdown inhibits cell proliferation, migration, invasion, and promotes cell apoptosis in CC

In order to study the biological function of SNHG14 in CC, sh-SNHG14 and its scramble control (sh-NC) were respectively transfected into SiHa and HeLa cells. RT-qPCR assay was used to confirm the knockdown efficiency of sh-SNHG14 in SiHa and HeLa cells. Compared with scramble control, the introduction of sh-SNHG14 in SiHa and HeLa cells triggered an obvious abatement of SNHG14 expression (Fig. 2A). CCK-8 assay and colony formation assays indicated that SNHG14 knockdown inhibited the proliferation of SiHa and HeLa cells (Fig. 2B–C). Flow cytometry cell apoptosis assay elucidated that knockdown of SNHG14 promoted cell apoptosis in SiHa and HeLa cells (Fig. 2D). Transwell assay further demonstrated that the migration and invasion abilities of SiHa and HeLa cells were visibly suppressed in cells transfected with sh-SNHG14 (Fig. 2E–F). It is known that epithelial-mesenchymal transition (EMT) process is related to cell invasive capability. Next, the expression of apoptosis-related proteins and EMT markers were determined by western blot, and the results showed that SNHG14 knockdown reduced the expression of anti-apoptotic protein (Bcl-2) and mesenchymal markers (N-cadherin and Vimentin), but increased the expression of pro-apoptosis proteins (Bax, Cleaved caspase-3 and Cleaved PARP) and epithelial marker (E-cadherin) in SiHa and HeLa cells (Fig. 2G). All these data illustrated that SNHG14 knockdown inhibits cell proliferation, migration, invasion, and promotes cell apoptosis in CC.

3.3. SNHG14 functions as a sponge for miR-206 in CC cells

We then studied the regulatory mechanism of SNHG14 in CC. It has been reported that lncRNAs could function as ceRNAs by sponging miRNAs [23]. Firstly, starBase online website was used to predict the potential miRNAs which might be sponged by SNHG14. We discovered that SNHG14 had a binding site for miR-206 (Fig. 3A). MiR-206 has been declared to play an anti-tumor role in multiple cancers [14,24]. To evaluate the interaction of miR-206 and SNHG14, luciferase assay was

performed and the results displayed that the luciferase activity of mutant-type SNHG14 was significantly decreased in cells co-transfected with SNHG14-Wt and miR-206 mimic, while significantly increased in cells co-transfected of SNHG14-Wt and miR-206 inhibitor (Fig. 3B). However, there was no distinct difference in luciferase activity of mutant-type SNHG14 between miR-206 mimic (or miR-206 inhibitor) group and control group. RIP assay indicated that SNHG14 and miR-206 expression were strikingly abundant in Ago2 pellet in comparison to IgG control (Fig. 3C). RNA pull-down assay further validated that miR-206 could directly bind with SNHG14 (Fig. 3D). Then we further explored the correlation between SNHG14 and miR-206. RT-qPCR results indicated that SNHG14 expression was decreased in cells transfected with miR-206 mimic, and miR-206 expression was also decreased in cells transfected with pcDNA3.1/SNHG14 (Fig. 3E–F). Subsequently, we discovered that miR-206 expression in tumor tissues was markedly downregulated in comparison to adjacent non-tumor tissues (Fig. 3G). Moreover, spearman's correlation analysis indicated thatSNHG14 expression was in negative correlation with miR-206 expression in tumor tissues ($r = -0.23$, $P < 0.05$, Fig. 3H). These findings revealed that SNHG14 functions as a sponge of miR-206 in CC cells.

3.4. YWHAZ is a downstream target gene of miR-206

In order to probe the regulatory mechanism of miR-206 in CC, the potential target genes of miR-206 were then predicted by Targetscan. And then we discovered that YWHAZ had a binding site for miR-206 (Fig. 4A). It has been reported that YWHAZ is a critical regulator in human cancers [6,15,22]. To determine the interaction of miR-206 and YWHAZ, the luciferase reporter assay was performed, which exhibited that luciferase activity of wild-type YWHAZ was significantly reduced in cells transfected with miR-206 mimic, but rescued under co-transfection of miR-206 mimic + pcDNA3.1/SNHG14 (Fig. 4B). However, there was no notable difference in luciferase activity of mutant-type YWHAZ between miR-206 mimic (or miR-206 mimic + pcDNA3.1/SNHG14) group and control group. RNA pull-down assay further indicated that miR-206 could directly bind with YWHAZ (Fig. 4C). To further study the correlation of YWHAZ and miR-206, we detected that YWHAZ expression was decreased by miR-206 mimic in CC cells, but restored by the addition of pcDNA3.1/SNHG14 (Fig. 4D). Moreover, YWHAZ expression in CC tissues was visibly upregulated in comparison to non-tumor tissues (Fig. 4E). Moreover, spearman's correlation analysis demonstrated a negative correlation between YWHAZ and miR-206 expression, and a positive correlation between SNHG14 and YWHAZ expression in tumor tissues ($r = -0.253$, $r = 0.252$, $P < 0.05$, Fig. 4F–G). All these results suggested that YWHAZ is a downstream target gene of miR-206, and SNHG14 could regulate YWHAZ expression by targeting miR-206.

3.5. SNHG14 promotes the CC progression by regulating YWHAZ

To explore whether SNHG14 promoted the CC progression by regulating YWHAZ, we performed rescue experiments using pcDNA3.1/YWHAZ. As presented in Fig. 5A, RT-qPCR exhibited that YWHAZ expression was decreased in cells transfected with sh-SNHG14. The co-transfection of pcDNA3.1/YWHAZ rescued the inhibitive role of sh-SNHG14 in the proliferation of CC cells (Fig. 5B–C). The co-transfection of pcDNA3.1/YWHAZ abolished the promotive role of sh-SNHG14 in cell apoptosis in CC (Fig. 5D). The co-transfection of pcDNA3.1/YWHAZ also rescued the inhibitive effect of sh-SNHG14 on the migration and invasion abilities of CC cells (Fig. 5E–F). Furthermore, compared with the expression of those in cells transfected with sh-SNHG14, the expression of apoptosis associated proteins (Bax, Cleaved caspase-3, Cleaved PARP and Bcl-2) and EMT markers (E-cadherin, N-cadherin and Vimentin) were also rescued in cells co-transfected with sh-SNHG14 and pcDNA3.1/YWHAZ (Fig. 5G). Overexpression of YWHAZ partially abrogated SNHG14 knockdown-mediated effect on the CC progression. Based on the results, we concluded that lncRNA SNHG14 promotes the CC progression by miR-206/YWHAZ axis.

4. Discussion

Mounting evidence reported that lncRNAs are of great importance for the progression of many cancers, such as bladder cancer, breast cancer and cervical cancer [4,11,21]. In human cancers, the regulatory mechanism of some lncRNAs have been defined, but most lncRNAs have not been widely studied. Recent studies demonstrated that long non-coding RNA SNHG14 promotes cell proliferation and metastasis in many cancers [3,12,13]. Nevertheless, the molecular mechanism and biological function of lncRNA SNHG14 in CC remain largely unknown. In the present study, we found that the expression of SNHG14 was significantly upregulated in CC tissues and cells. Moreover, knockdown of SNHG14 inhibited the proliferation and metastasis, and promoted apoptosis in SiHa and HeLa cell lines. We also found that knockdown of SNHG14 decreased the expression of anti-apoptotic protein (Bcl-2) and mesenchymal marker (N-cadherin and Vimentin), but increased the expression of pro-apoptosis proteins (Bax, Cleaved caspase-3 and Cleaved PARP) and epithelial marker (E-cadherin). All these data indicated that SNHG14 promoted the progression of CC.

MicroRNAs (miRNAs) are short non-coding RNA molecules with 20–24 nucleotides, and could regulate many physiological and pathological processes in cancers [5,18]. LncRNAs could function as ceRNAs by sponging miRNAs to regulate the progression of cancers. For example, the long noncoding RNA, TINCR, functions as a competing endogenous RNA to regulate PDK1 expression by sponging miR-375 in gastric cancer [8]. LncRNA Gas5 acts as a ceRNA to regulate PTEN expression by sponging miR-222-3p in papillary thyroid carcinoma [25]. MiR-206, a cancer-related miRNA, was abnormally expressed in many cancers. It has been reported that microRNA-206 suppresses PGE2-induced colorectal cancer cell proliferation, migration, and invasion by targeting TM4SF1 [14]. MicroRNA-206 suppresses TGF-β signaling to limit tumor growth and metastasis in lung adenocarcinoma [24]. In present study, it was predicted that miR-206 had a binding site for SNHG14 from starBase website. Luciferase, RIP, RNA pull down and RT-qPCR assays further affirmed that SNHG14 could bind to miR-206 and the expression of miR-206 can be negatively regulated by SNHG14. All these data suggested that SNHG14 regulated CC progression by sponging miR-206.

In present study, it was predicted that YWHAZ had the binding sites for miR-206 via Targetscan website. YWHAZ has been reported to have carcinogenic effect in human cancers [6,15,22]. For example, YWHAZ expression has significant prognostic significance in localized prostate cancer [15]. Inhibition of tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta (YWHAZ) overcomes drug resistance and tumorigenicity in ovarian cancer [6]. In our work, we found that overexpression of miR-206 as well as knockdown of SNHG14 can reduce YWHAZ expression. Data delineated that overexpression of YWHAZ partially rescued SNHG14 knockdown-mediated effects on CC proliferation, migration, invasion, apoptosis and the expression of apoptosis associated proteins and EMT markers. In conclusion, SNHG14 silencing inhibited CC progression by targeting miR-206/YWHAZ. Therefore, these findings indicated that SNHG14 may provide a diagnostic/therapeutic target for CC.

Conflict of interest

The authors suggest that there is no conflicts for interest exists in this study.

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