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SNHG3/WISP2 Axis Promotes Hela Cell Migration and Invasion *via* Activating Wnt/β-Catenin Signaling

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Abstract. *Background/Aim: Cervical cancer (CC) poses a significant threat to women's health and has a relatively poor prognosis due to local invasion and metastasis. It is, therefore, crucial to elucidate the molecular mechanisms of CC metastasis. SNHG3 has been implicated in various tumor metastasis processes, but its involvement in CC has not been thoroughly studied. Our study aimed to investigate the role of SNHG3 in metastasis and elucidate its underlying mechanisms in CC. Materials and Methods: LncRNA SNHG3 expression in CC tissues was analyzed using TCGA and GSE27469 databases. Normal cervical epithelial cells and CC cell lines were used to detect mRNA expression of SNHG3 via quantitative reverse transcription polymerase chain reaction (qRT-PCR). With RNA interference (RNAi) technology, antisense oligonucleotides (ASO) can act on HeLa cells to knockdown target gene expression. The influence of SNHG3 on cell migration and invasion were determined by wound healing and transwell assays. Transcriptome sequencing (RNA-seq) was used to seek abnormally expressed genes between SNHG3 knockdown cells and control cells. The expressions of epithelial-mesenchymal transition (EMT) and Wnt/β-catenin signaling related proteins were detected using western blot. Results: SNHG3 was obviously up-regulated in CC tissues and cell lines, and ectopic expression of SNHG3 was associated with lymph node metastasis of CC. Knockdown of SNHG3 significantly inhibited cell migration and invasion in CC. Further molecular mechanism studies showed that SNHG3 knockdown could down-regulate the expression of WNT1 Inducible Signaling Pathway Protein 2 (WISP2) so as to inhibit the activation of the Wnt/β-catenin signaling pathway, and regulated the expression of EMT-related markers, that promoted the protein expression of E-cadherin, as well as decreased the expression of N-cadherin and vimentin. Conclusion: SNHG3 appears to exert a pro-metastatic effect in CC, as evidenced by inhibition of cell migration and invasion upon SNHG3 knockdown. EMT also appears to be attenuated. Of interest is the down-regulation of WISP2 following SNHG3 knockdown leads to the inactivation of the Wnt/β-catenin signaling pathway.*

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Cervical cancer (CC), a prevalent gynecologic malignancy worldwide, poses a significant threat to female health due to its high mortality rate (1). The number of new cases and deaths in 2020 reached a staggering 604,127 and 341,831 respectively (2). Local invasion and metastasis are the primary culprits behind advanced clinical staging of CC patients (3), necessitating an exploration into their underlying mechanisms. As such, it is imperative to identify novel therapeutic strategies that can effectively impede CC metastasis and improve patient prognosis.

Small nucleolar host gene 3 (*SNHG3*) is a long non-coding RNA located on Chromosome 1p36.1, which was initially implicated in the pathogenesis of Alzheimer's disease (AD) (4). Subsequently, it was found to be an oncogene and biomarker for malignancy or poor prognosis in various cancers, such as colorectal, ovarian, lung, prostate, clear cell renal cell carcinoma and hepatocellular carcinoma (5-11). Similar effects have been demonstrated in non-small-cell lung cancer (NSCLC), where *SNHG3* knockdown attenuated cell migration, invasion, and proliferation by inhibiting the *TGF-β* pathway as well as the *IL-6/JAK2/STAT3* pathway (12). *SNHG3* has recently been discovered to act as an oncogene and promote malignant phenotypes of CC cells (13, 14). However, the precise mechanisms underlying its role in cancer metastasis remain to be elucidated in detail.

In the current study, we investigated the detailed mechanism by which *SNHG3* regulates the migration and invasion of Hela cells. Firstly, we observed a remarkable up-regulation of *SNHG3* expression in HeLa cells. Secondly, knockdown of *SNHG3* impeded the migratory and invasive potential of HeLa cells. Additionally, knockdown of *SNHG3* enhanced the protein level of E-cadherin while reducing the protein expression of N-cadherin and vimentin. Furthermore, *SNHG3* up-regulated *WISP2* expression, leading to activation of the Wnt/β-catenin signaling pathway and promoting metastasis in HeLa cells. Overall, our study confirms the inhibitory effect of SNHG3 knockdown on cell migration and invasion and provides insights into the detailed mechanism by which SNHG3 promotes metastasis in Hela cells. These findings contribute to a better understanding of the functional role of SNHG3 in Hela cell biology and may have implications for the development of targeted therapies for treating and preventing local invasion and metastasis in CC.

Materials and Methods

Database analysis. The analysis of the expression of lncRNA *SNHG3* and the relationship between *SNHG3* and lymph node metastasis in CC was completed on Biomarker Exploration of Solid Tumors (BEST, https://rookieutopia.com/app_direct/BEST/).

As a web tool, BEST contains TCGA and GEO databases and provides visual analysis results. With support from BEST, the expression data of *SNHG3* in CESC tissues or normal tissues and in CC patients with or without lymph node metastasis were obtained from the TCGA database and GSE27469 database, respectively. All *SNHG3* expression data was normalized in the form of z-score after applying the calculation formula z=(expression value – mean)/standard deviation. Differential analysis was performed using a student's *t*-test.

Cell lines and cell culture. The human normal cervical epithelial cell line HUCEC and two CC cell lines (SiHa and CaSki) were kind gifts from Dr. Guo's research group (Department of Gynecology, People's Hospital of Zhengzhou University). HeLa cell line was obtained from Shanghai QuiCell Biotechnology Co. LTD. These cells cultured in RPMI-1640 medium (Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS, Gibco, Waltham, MA, USA), 5% carbon dioxide, at 37˚C.

Table I. *Primer sequences of quantitative real-time PCR.*

Gene	Sequences 5'-3'
SNHG3	TGCACTTCGCATTTTGGCAT
	GCACCTCAATCTTTTGCTCCA
WISP2	GGGGTCGCAGTCCACAAAACAG
	GCACGGACCATCTTCCATCAGC
GAPDH	GTCTCCTCTGACTTCAACAGCG
	ACCACCCTGTTGCTGTAGCCAA

Table II. *Target sequences of knock-down genes.*

Gene	Target sequence 5'-3'
SNHG3	CCAGCCCTCATACCTCTTTT (ASO-1)
	CTGTTCTCAACTCCTGACCT (ASO-2)
WISP2	GGGGTCGCAGTCCACAAAACAG (siWISP2-1)
	ACCACCCTGTTGCTGTAGCCAA (siWISP2-2)

RNA extraction and quantitative Real-Time PCR (qRT–PCR) assay. Total RNA was isolated with TRIZOL reagent (Invitrogen) according to the product description. One microliter total of RNA was reverse-transcribed into cDNA using Prime script RT reagent kit (Vazyme Biotech, Nanjing, PR China). cDNA was quantified by qRT-PCR and the data were acquired with FS Universal SYBR Green Master (Roche, Basel, Switzerland) using Applied Biosystems 7500 instrument. GAPDH was used as an internal control. The primers are listed in Table I.

Cell transfection. Antisense oligonucleotides (ASOs) or Small interfering RNA (siRNA) were obtained from Ribobio (Guangzhou, PR China) and transfected using the lipofectamine 3000 Transfection Kit (Invitrogen). Two ASOs or siRNAs and their respective Negative Controls (ASO-NC or siRNA-NC) were designed to prevent the off-target effects and ensure the efficiency of interference. The sequences of ASOs or siRNAs are presented in Table II. ASO-NC and siRNA-NC sequences provided by Ribobio was not opened to the public.

Cell invasion and migration assays. Sterile Transwell polycarbonate membrane chamber (catalog: 3422, CORNING, Inc.) with or without Matrigel (BD, San Jose, CA, USA) pre-coating were used for the Transwell invasion or migration assay respectively. The Transfected cells were collected and seeded at 1×10^5 cells/well (for invasion assay) or 5×10^4 cells/well (for migration assay) and were re-suspended into serum-free medium. Cells were inoculated into the upper chamber of Transwell inserts. The lower compartment was supplemented with 700 μl medium supplemented with 10% FBS as an attractant. After 24 h of incubation at 37˚C, the invaded or migrated cells were fixed with 4% paraformaldehyde (Servicebio, Wuhan, PR China) for 40 min and stained with 0.5% crystal violet

(Beyotime, Beijing, PR China) for 8 min, and cells free in the upper chamber were wiped off with cotton swab gently. Finally, images of invaded or migratory cells in five random fields per chamber were captured under a microscope. The relative invaded or migrated cells were counted by VisionWorks software.

Wound healing assay. The wound healing assay used culture-insert 2 well for self-insertion manufactured by iBiDi Company (CatLog: 190718/6, Germany). The culture-insert is placed on the surface of the 24-well plate and provides two cell culture chambers with walls 500 μm thick between the two inserts. Laying cells in two small chambers and removing the culture-inserts with clean sterile tweezers left cells at a distance on the cell culture plate with a wall thickness of 500 μm between them. After unplugging the culture-inserts, the residual floating cells were washed with PBS, gently washed twice, and fresh medium containing 2% serum was added for scratch experiment. The surface of ibiTreat provided excellent growth conditions for cells in the area without cell coverage, and the wound area was photographed and recorded in the same field of vision during a specific time.

RNA-seq. Total RNA from the knockdown *SNHG3* cells or control cells from three independent experiments was sent to OE Biotech Co., Ltd (Shanghai, PR China) for RNA-seq analysis. Total RNA was extracted using the mirVana miRNA Isolation Kit (Ambion) following the manufacturer's protocol. RNA integrity was evaluated using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The samples with RNA Integrity Number (RIN) ≥7 were subjected to the subsequent analysis. The libraries were constructed using TruSeq Stranded mRNA LTSample Prep Kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. Then these libraries were sequenced on the Illumina sequencing platform (Illumina HiSeq X10) and 125bp/150bp paired-end reads were generated. The transcriptome sequencing and analysis were conducted by OE biotech Co., Ltd. (Shanghai, PR China). Raw data (raw reads) were processed using Trimmomatic. Then the clean reads were mapped to the human genome (hg38) with default parameters using hisat2. Fragments per kilobase per million reads sequenced (FPKM) value of each gene was calculated using cufflinks, and the read counts of each gene were obtained by htseq-count. Differentially expressed genes (DEGs) were identified using the DESeq (2012) R package functions estimateSizeFactors and nbinomTest, and the significant DEGs were selected according to the following criteria: foldChange ≥2 or ≤0.5 and false discovery rate (FDR)-corrected $p<0.05$ ($q<0.05$). What's more, for up-regulated genes, the FPKM value of *SNHG3* knockdown group were all greater than or equal to 1, and for down-regulated genes, the FPKM value of NC group were all greater than or equal to 1.

Western blot assay. The related proteins in this study were extracted using radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, PR China) for 30 min on ice. The protein concentration was quantified by BCA Protein Assay Kit (Thermo Scientific) and denatured at 95˚C for 10 min. Equal quantities of proteins were loaded on 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) to electrophorese and subsequently transferred onto polyvinylidene difluoride (PVDF) membranes (Merck, Darmstadt, Germany). The membranes were incubated with primary antibodies, at 4˚C for 16 h after blocking with 5% nonfat milk for 1 h at RT, then fully washed three times for 15 min at RT; Subsequently, the membrane was incubated using horseradish peroxidase (HRP)-conjugated IgG for 1 h at RT and washed three times again. Finally, proteins were scanned using a Pierce SuperSignal West Picochemiluminescent substrate (Merck) with the Chemi-Doc XRS system (Bio-Rad, Hercules, CA, USA).

Statistical analysis. Quantitative data were presented as mean±standard deviation (SD) and analyzed using the SPSS 23.0 statistical software (SPSS Inc., Chicago, IL, USA). The differences between groups were evaluated using Student's *t*-test (for variables with normal distribution) or Mann-Whitney *U*-test (for variables without normal distribution). Differences in rates were analyzed by χ^2 or Fisher exact test. A *p*-value less than 0.05 ($p<0.05$) was considered statistically significant.

Results

SNHG3 expression was significantly up-regulated in CC and correlated with metastasis of CC. Analysis of The Cancer Genome Atlas (TCGA) datasets showed that the expression of lncRNA *SNHG3* was significantly overexpressed in CC (Figure 1A). Further analysis of GEO datasets (GSEA27469) showed that *SNHG3* was highly correlated with lymph node metastasis of cervical cancer, that is, *SNHG3* expression levels were increased in lymph node metastasis tissues compared with non-lymph node metastasis tissues in CC (Figure 1B). Moreover, *SNHG3* was significantly overexpressed on the further validation in CC cell lines, compared to corresponding normal epithelial cell (HUCEC) by qRT-PCR (Figure 1C). These data suggested that *SNHG3* plays vital roles in metastasis of CC and may play an oncogenic role in progression of human cervical cancer.

SNHG3 silencing inhibits migration and invasion of HeLa cells. Metastasis is a complex multistep process and leads to relapse or a poor prognosis in CC. As both the migration and invasion of tumor cells contribute to the metastasis, so we investigated whether *SNHG3* could affect the migration and invasion ability of CC cells. As shown in Figure 2A, an evident decrease in the wound closure rate was observed after *SNHG3* knockdown with ASO transfection compared to control group cells following NC transfection. Quantitative analysis further demonstrated that the wound areas were significantly larger in the *SNHG3* knockdown group compared to the control group in HeLa cells (Figure 2A). The result of transwell assay also confirmed the results of wound healing assay (Figure 2B). Furthermore, silencing of *SNHG3* significantly attenuated invasive ability through a Matrigel-reconstituted basement membrane matrix towards the bottom chamber (Figure 2C). Collectively, the above results indicated that *SNHG3* knockdown inhibited the migration and invasion of human CC cells.

SNHG3 silencing attenuated epithelial-mesenchymal transition in HeLa cells. Epithelial-mesenchymal transition (EMT) plays a critical role during tumor metastasis, which is

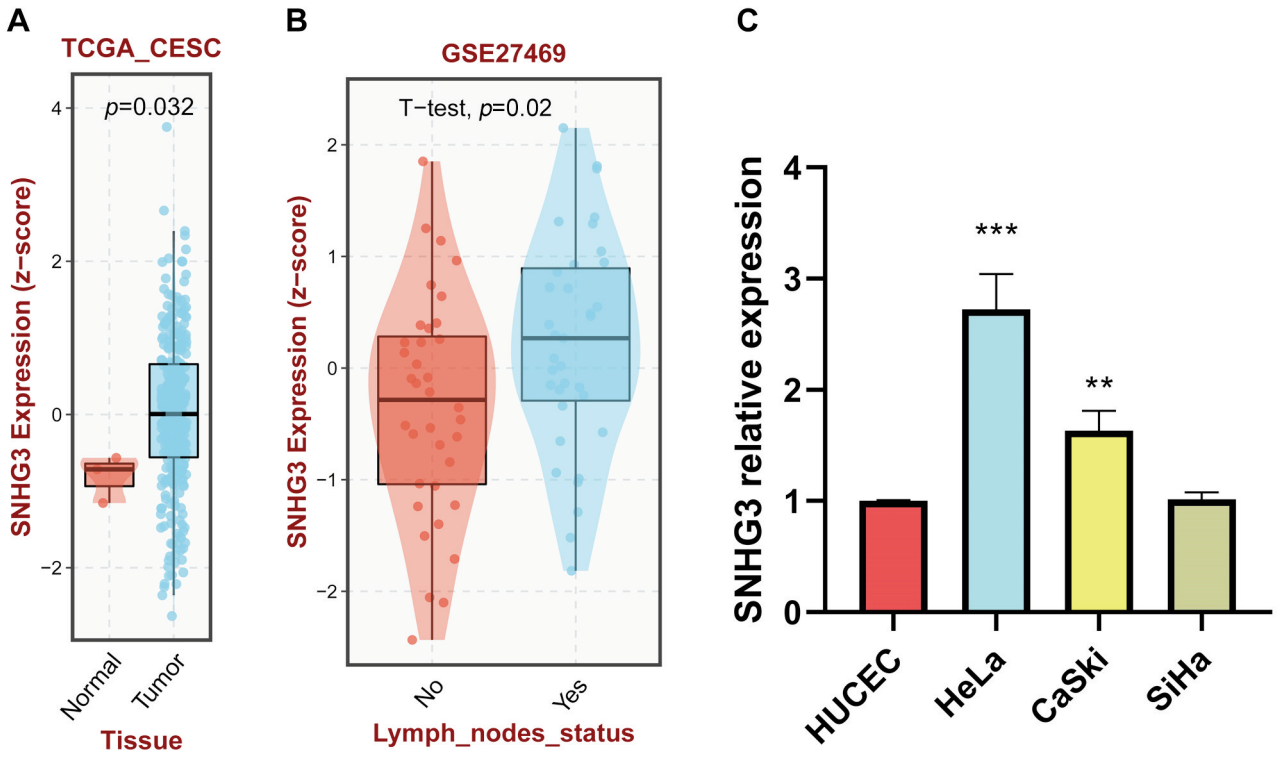

Figure 1. *SNHG3 exhibited high expression levels in cervical cancer tissues, lymph node metastasis tissues, and cervical cancer cells. (A) SNHG3 expression in cancer tissues and normal cervical tissues was analyzed using the TCGA database. CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma. (B) Expression of SNHG3 in lymph nodes with and without metastasis in cervical cancer utilizing the GSE27469 dataset. (C) SNHG3 expression in normal cervical endometrial epithelial cells HUCEC cells and CC cells (SiHa, HeLa and CaSki). Data are mean±sd; n=3 independent experiments, two-tailed Student's t-test. *p<0.05, **p<0.01, ***p<0.001.*

also a crucial factor in various cancers (15, 16). As described above, *SNHG3* could promote the migration and invasion of HeLa cells. To investigate the mechanism of metastasis, we conducted western blotting assays to evaluate the expression of EMT-related proteins (E-cadherin, N-cadherin, and Vimentin) in *SNHG3* knockdown and control cells. Our findings indicate that knockdown of *SNHG3* results in the up-regulation of E-cadherin and down-regulation of N-cadherin and vimentin, thereby attenuating the process of epithelial-mesenchymal transition (EMT) (Figure 3A, B).

SNHG3 silencing inactivated the Wnt/β-catenin signaling pathway in HeLa cells. To further explore the mechanism of *SNHG3* on metastasis of CC cells, we performed transcriptome sequencing on *SNHG3*-konckdown HeLa cells and its negative control cells. As was shown in volcano plot, the significant differentially expressed genes (DEGs) between the two groups were identified with Fold Changes (FC) of greater than 2.0 or less than 1/2 and *q* value less than 0.05 (Figure 4A). From the total 105 DEGs, 85 mRNAs were significant down-regulated, and 20 mRNAs were significant up-regulated (Figure 4B). We resorted down-regulated genes in ascending order according to FC and *q* value, *WISP2* is the most down-regulated gene in response to *SNHG3* knockdown in HeLa cells. Consistent with the result from transcriptomics, qRT-PCR and western blot showed that *WISP2* was down-regulated significantly in mRNA and protein level (Figure 4C, D).

WISP2, also known as WNT1-inducible-signaling pathway protein 2, is capable of activating the canonical WNT pathway and maintaining cells in an undifferentiated state. It is frequently utilized as a marker for canonical WNT activation or as a downstream effector of Wnt signaling (17, 18). It has been suggested that *WISP2* is positively associated with β-catenin in gastric cancer (19). Given that the transcriptome sequencing results indicated down-regulation of *WISP2* upon *SNHG3* knockdown, we focused on elucidating the molecular mechanism underlying canonical Wnt signaling pathway. As shown in Figure 4E, protein levels of β-catenin were significantly decreased after *SNHG3* knockdown, suggesting that *SNHG3* might influence the protein level of β-catenin *via* promoting its degradation. In the absence of Wnt signaling, cytosolic β-catenin is phosphorylated at specific serine and threonine residues by

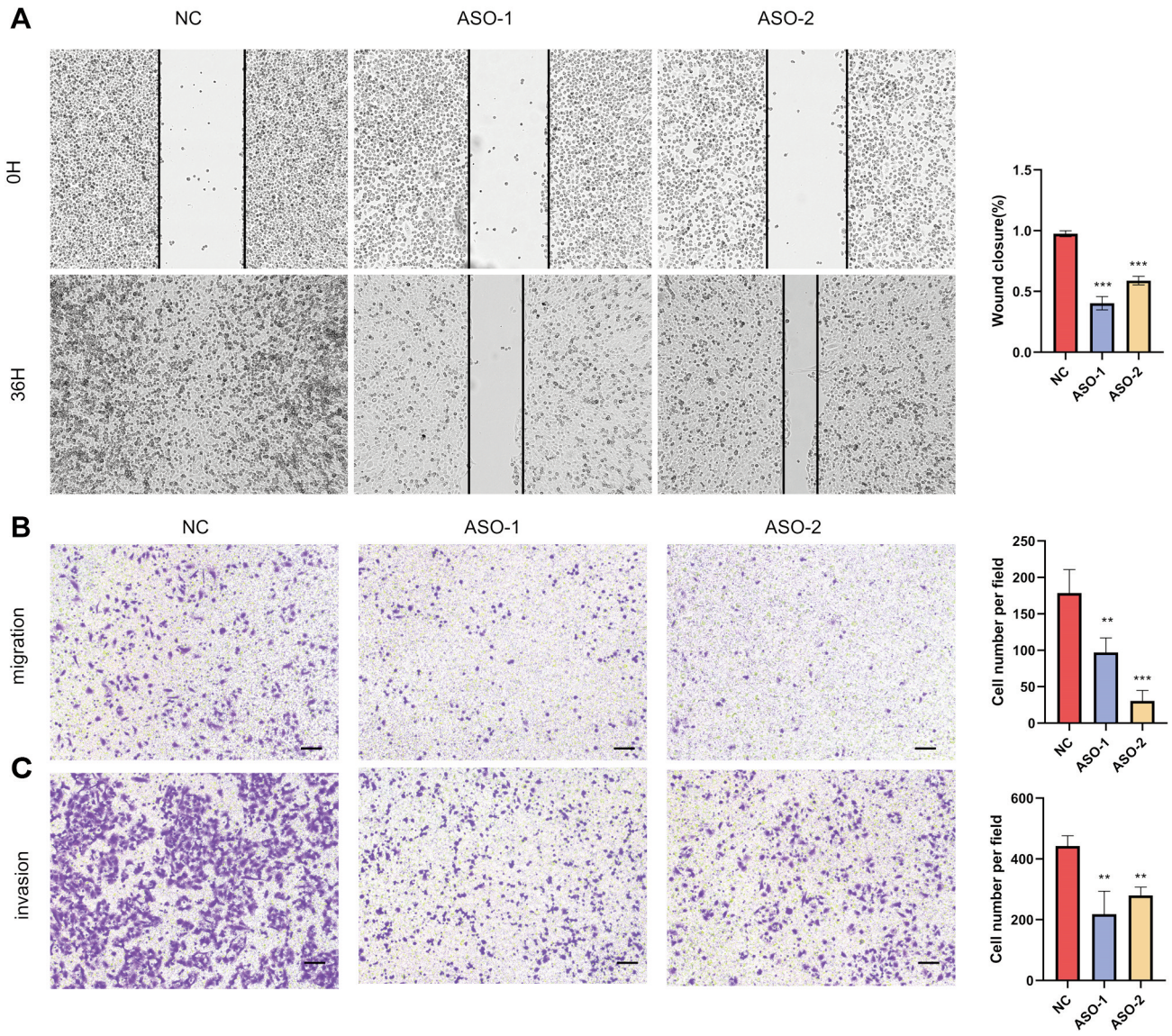

Figure 2. *Knockdown of SNHG3 inhibited the migration and invasion of HeLa cells. (A) Wound healing assay was performed in HeLa cells after SNHG3 knockdown by transfection with two ASOs (ASO-1 and ASO-2, NC as negative control). (B-C) Cell invasion and migration assays were performed in SNHG3-knockdown Hela cells and negative control Hela cells. ASO: Antisense oligonucleotides. NC: Negative controls. Above data are mean±sd; n=3 independent experiments, two-tailed Student's t-test. Scale bar: 100 μm. *p<0.05, **p<0.01, ***p<0.001.*

GSK-3β, leading to its degradation (20, 21). Since the proteasomal degradation of β-catenin is mostly controlled by GSK-3β phosphorylation, we then examined the phosphorylation state of GSK-3β and found that *SNHG3* knockdown caused a decreased level of GSK-3β Ser-9 phosphorylation (Figure 4E). These results suggested *SNHG3* could activate the Wnt signaling pathway in HeLa cells.

SNHG3 mediated HeLa cell migration and invasion by promoting the expression of WISP2. Then, we further investigated the role of *WISP2* in HeLa cell migration and invasion. Both mRNA and protein levels of *WISP2* were significantly down-regulated by small interfering RNA technique. Transwell assay showed that *WISP2* knockdown significantly inhibited the migration and invasion ability of HeLa cells, suggesting that *WISP2* is involved in the regulation of tumor metastasis (Figure 5A). Moreover, rescue experiments were used to study *WISP2* involvement in *SNHG3*-induced contributions to HeLa cell migration and invasion. Therefore, we performed co-transfection in HeLa

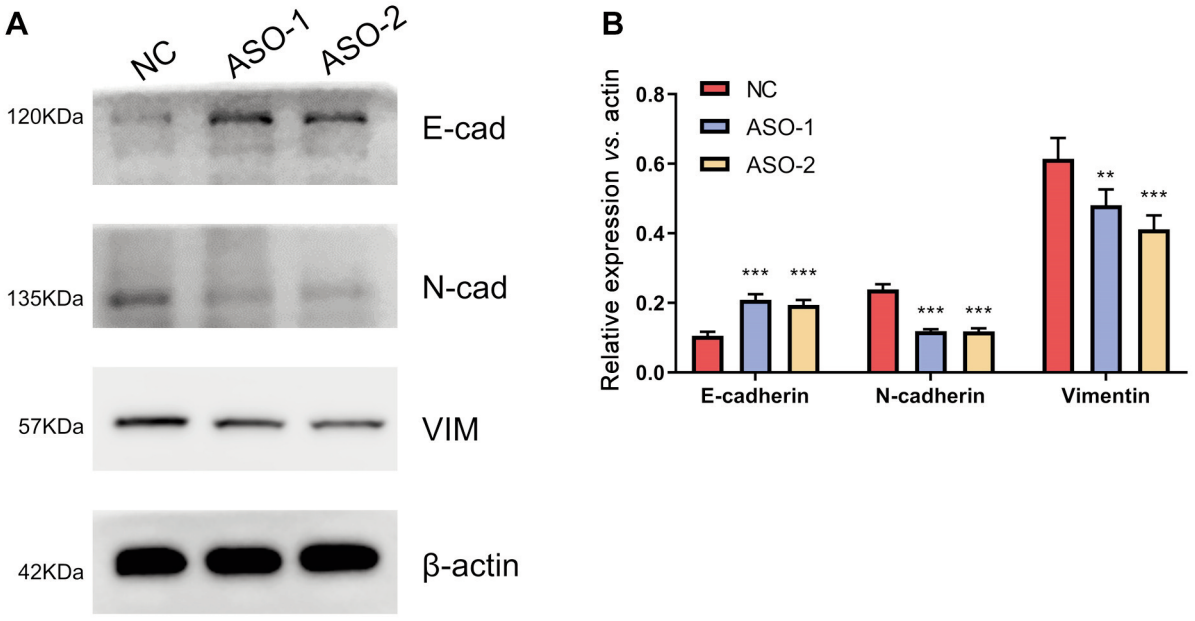

Figure 3. *SNHG3 silencing attenuated epithelial-mesenchymal transition in HeLa cells. (A) Western blot assay to observe the expression of E-cadherin, N-cadherin, and Vimentin protein level in SNHG3-knockdown Hela cells and negative control Hela cells. E-cad: E-cadherin; N-cad: N-cadherin. VIM: Vimentin. (B) Quantitative analysis of the result of western blotting assay. Above data are mean±sd; n=3 independent experiments, two-tailed Student's t-test. Scale bar: 100 μm. *p<0.05, **p<0.01, ***p<0.001.*

cells to reach the purpose of down-regulating *SNHG3* and up-regulating *WISP2*. Transwell assays showed that the co-transfection could partially reverse *SNHG3*-reduced migration and invasion (Figure 5B). These findings indicated that *SNHG3* accelerated migration and invasion of HeLa cells by up-regulating *WISP2*.

Discussion

SNHG3, Small nucleolar host gene 3, is a well-known oncogenic long non-coding RNA (lncRNA) that is located on chromosome 1p35.3. At present, the ectopic overexpression of *SNHG3* has been observed and certified to be an oncogenic factor and a biomarker of poor prognosis in various cancers (5, 6, 22-31). Additionally, *SNHG3* has been also demonstrated it can be involved in the process of tumor metastasis including migration, invasion, and EMT. For example, *SNHG3* promotes the migration, invasion and EMT process of prostate cancer through the epigenetic regulation of TRIM25 (30), and *SNHG3* facilitates the migration and invasion of cholangiocarcinoma cells *via* the miR-3173-5p/ERG axis (31). Although Zhu *et al.* reported that *SNHG3* regulates the migration and invasion of Hela cells by associating with YAP1 (13), the underlying regulatory mechanism of *SNHG3* in HeLa cells need to be illustrated in depth.

In the present study, we uncovered that *SNHG3* was ectopic expression in CC tissues and cell lines. The GSEA data demonstrated an important association between *SNHG3* and CC metastasis, which was consistent with Zhu *et al.* (13). We further demonstrated that knockdown of *SNHG3* significantly suppressed the migratory and invasive phenotypes in HeLa cells. Similar findings have been observed in studies on prostate cancer (30), gastric cancer (24), bladder carcinoma (32), and non-small cell lung cancer (25). These results suggest that *SNHG3* functions as an oncogenic long non-coding RNA, promoting metastasis in HeLa cells.

Small nucleolar RNAs (snoRNAs) are primarily transcribed from gene clusters encoding proteins or other non-coding RNAs. Although some snoRNA genes lack coding ability, they still contain introns and exons in their sequences; however, only the intronic regions produce snoRNAs. If full-length transcripts that include exons are retained, they function as long non-coding RNAs known as small nucleolar RNA host genes (SNHGs). *SNHG3* is an oncogenic long non-coding RNA that generates *SNORD17*. Research has demonstrated the close correlation between *SNHG3* and the EMT process in various types of cancers (6, 24, 25, 30, 32-34). Therefore, we hypothesized that *SNHG3* may be involved in EMT of HeLa cells. As anticipated, our findings revealed that knockdown of *SNHG3* suppressed EMT progression in HeLa cells. Further investigation revealed that it is associated with the regulation of EMT-related proteins, including the up-regulation of E-cadherin

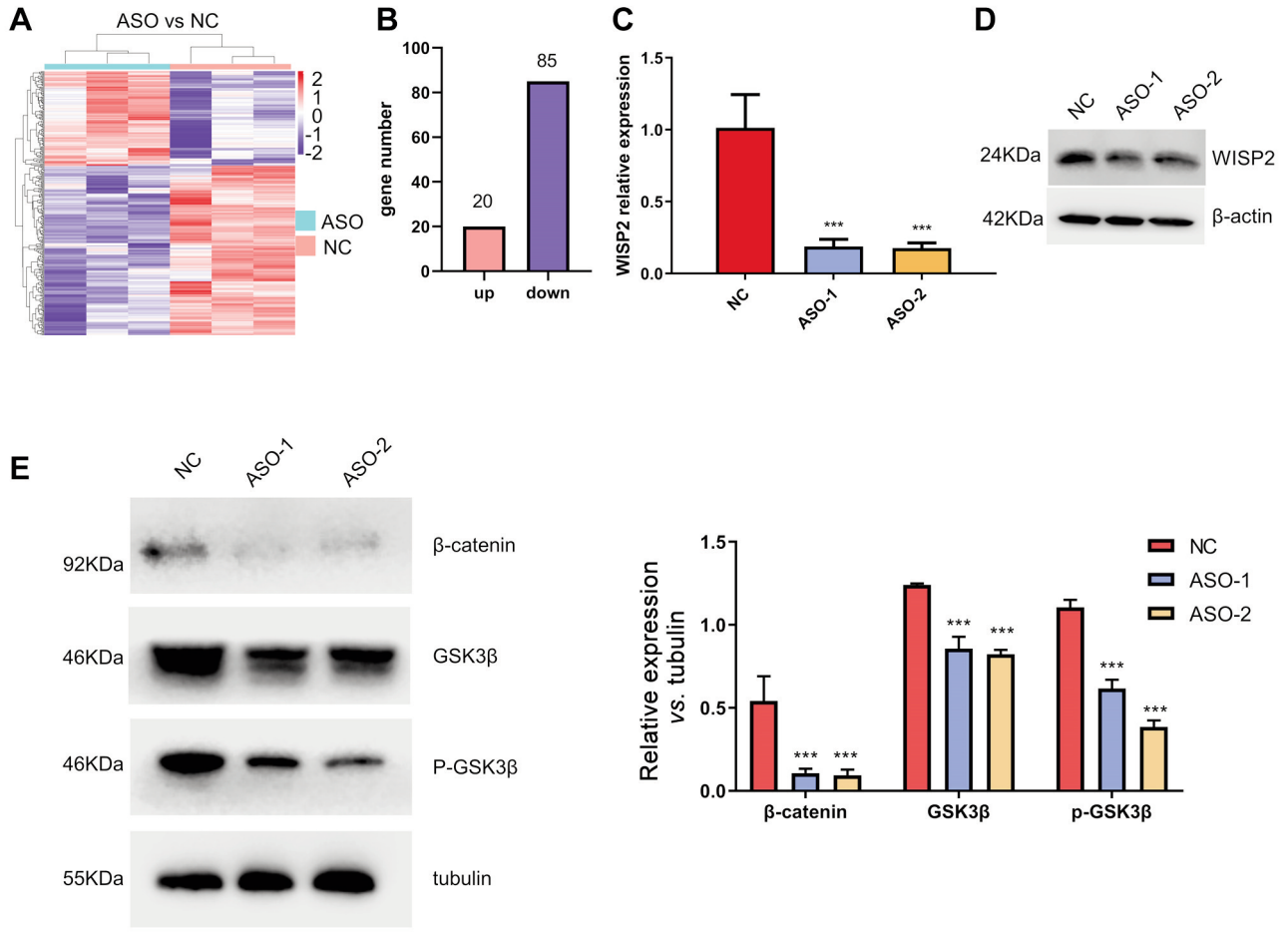

Figure 4. *Knockdown of SNHG3 down-regulated WISP2 expression and inhibited the activation of Wnt/β-catenin signaling pathway. (A-B) The significant differentially expressed genes (DEGs) between the SNHG3-knockdown cells and negative control cells. (C-D). The mRNA and protein level of WISP2 in Hela cells after SNHG3-knockdown were analyzed through qRT-PCR and western blot. (E) Change of the key proteins (β-catenin, GSK-3β and p-GSK-3β) of Wnt signaling pathway after SNHG3-knockdown in HeLa cells. Above data are mean±s.d.; n=3 independent experiments, two-tailed Student's t-test. Scale bar: 100 μm. *p<0.05, **p<0.01, ***p<0.001.*

expression and the down-regulation of vimentin and N-cadherin. These crucial proteins are involved in the mechanism leading to cancer metastasis.

The Wnt/β-catenin signaling pathway plays a pivotal role in the development of cancer (35-37). *WISP2*, a member of the *CCN* family of cysteine-rich and glycosylated signaling proteins, is involved in activating canonical WNT signaling (17). In the current investigation, transcriptome sequencing results revealed that knockdown of *SNHG3* led to a significant reduction in *WISP2* levels, thereby contributing to the deactivation of Wnt/β-catenin signaling. The absence of Wnt signaling resulted in decreased phosphorylation of GSK3β at serine 9, leading to β-catenin degradation and subsequent transcriptional down-regulation of its target genes responsible for EMT and cancer progression. Liu *et al*. discovered that the long non-coding RNA *UCA1* may up-regulate WISP2 expression by directly interacting with and inhibiting miR-185-5p, thereby activating the *WISP2/β-catenin* signaling pathway (38). Li *et al*. also found a positive correlation between *WISP2* and β-catenin in GC (18). These similar observations were made in our current study, indicating that *SNGH3* promotes migration, invasion and EMT in HeLa cells by activating the Wnt/β-catenin signaling pathway through up-regulating *WISP2* expression, which is closely associated with these cellular processes (39).

Our study demonstrated that *SNHG3* plays a tumor-promoting role in the progression of HeLa cells by facilitating migration and invasion. Mechanistically, we observed that *SNHG3* induces EMT through down-regulating E-cadherin and up-regulating N-cadherin and vimentin. Furthermore, our findings suggest that *SNHG3* activates the Wnt/β-catenin signaling pathway by up-regulating *WISP2*

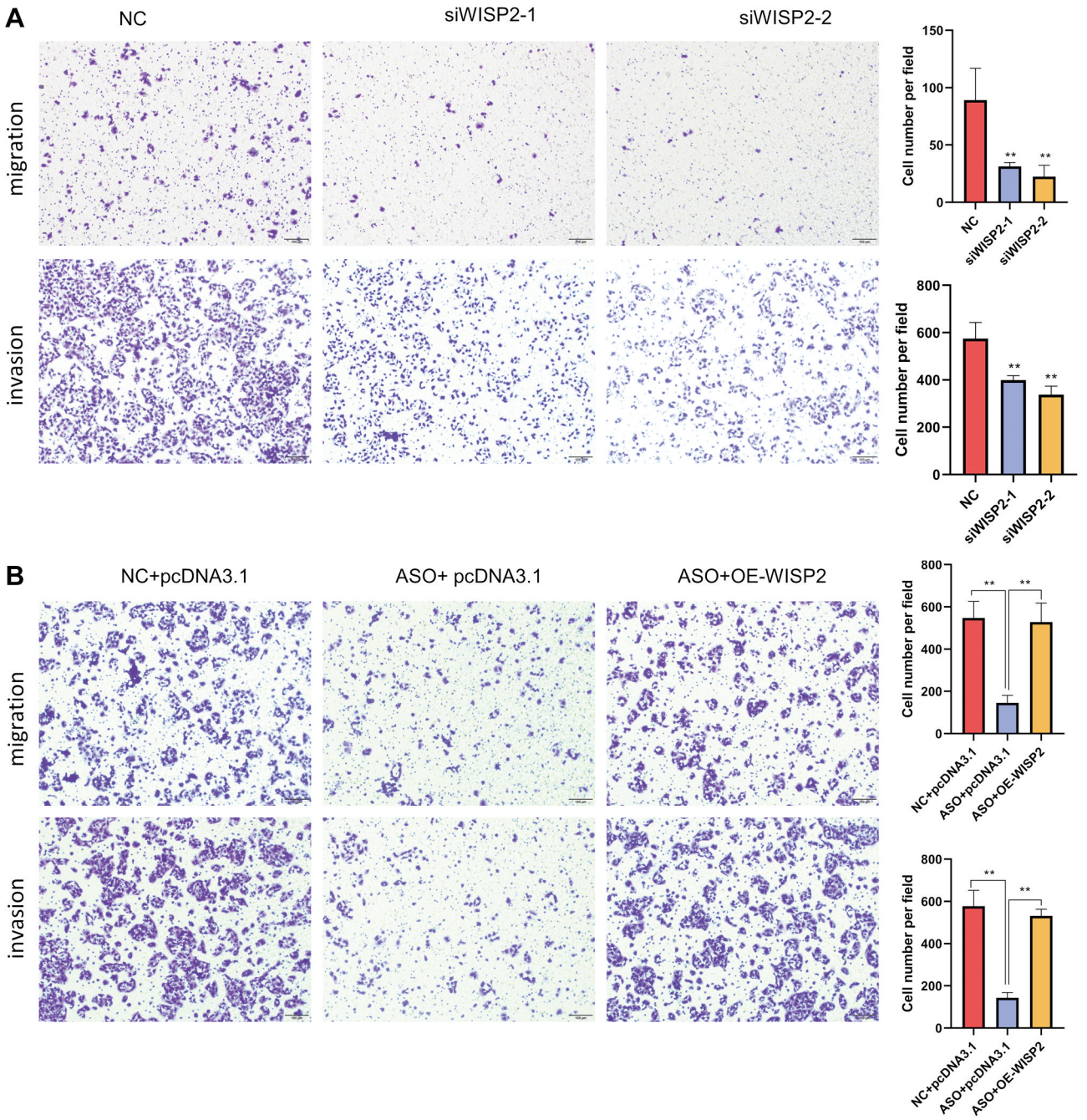

Figure 5. *SNHG3 promoted HeLa cell migration and invasion via WISP2. (A) Cell invasion and migration assays were performed in HeLa cells after WISP2 knockdown by transfection with two siRNAs (siWISP2-1 and siWISP2-2, siNC as negative control). (B) The rescue experiment with transwell assays was performed in Hela cells co-transfected with siNC or ASO-1 and pcDNA3.1 or pcWISP2. Above data are mean±s.d.; n=3 independent experiments, two-tailed Student's t-test. Scale bar: 100 μm. *p<0.05, **p<0.01, ***p<0.001.*

expression. Collectively, these results indicate an oncogene-like function for *SNHG3* in HeLa cells.

However, there are certain limitations in this study. For instance, an *in vivo* experiment was not conducted to verify whether *SNHG3* promotes the metastasis of CC; the research only focused on HeLa cells and more cell line experiments are needed to confirm these findings. Additionally, a more detailed analysis is required to demonstrate the signaling

cascade through which the *SNHG3/WISP2* axis regulates the Wnt/β-catenin signaling pathway in CC. Overall, our research findings suggest that the *SNHG3/WISP2* axis plays a pivotal role in promoting metastatic behaviors of HeLa cells, highlighting its potential as a therapeutic target for CC.

Conflicts of Interest

The Authors have declared that they have no conflicts of interest.

Authors' Contributions

All Authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Hao Feng, Zirui Ren, and Xiang Li. The first draft of the manuscript was written by Dengfei Xu and all Authors commented on previous versions of the manuscript. All Authors read and approved the final manuscript.

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