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Knockdown of long non-coding RNA LINC00518 inhibits cervical cancer proliferation and metastasis by modulating JAK/STAT3 signaling

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Abstract. – OBJECTIVE: Long intergenic non-protein coding RNA 518 (LINC00518) was reported to be implicated and aberrantly expressed in multiple cancers. However, the pathogenic implications of LINC00518 in cervical cancer (CC) are still unclear. In this study, we focused on LINC00518 and investigated its expression pattern, clinical significance, and biological function in CC.

PATIENTS AND METHODS: The expression levels of LINC00518 in CC tissues and cell lines were determined by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR), and its clinical significance was assessed by statistical analysis. Cell apoptosis was determined by flow cytometry. The cell proliferation was evaluated by MTT assay and colony forming assay, and the migration and invasion were evaluated by wound healing assays and transwell assay. Western blot was used to detect the expression of relative proteins, including EMT markers and the JAK/STAT3 signaling markers.

RESULTS: We found that LINC00518 was upregulated in CC tissues and associated with International Federation of Gynaecology and Obstetrics (FIGO) stage, lymph node metastasis, depth of cervical invasion and poor survival of CC patients. Univariate and multivariate Cox regression analysis showed that LINC00518 played a significant role of independent prognostic markers in overall survival rates. Furthermore, knocking down LINC00518 expression significantly suppressed CC cell proliferation, migration and invasion, and induced apoptosis in vitro. Mechanistically, the downregulation of LINC00518 suppressed JAK/STAT3 activation and subsequently decreased N-Cadherin and Vimentin.

CONCLUSIONS: The present work first suggests that LINC00518 acts as an oncogene in CC via regulation of the JAK/STAT3 signaling pathway. In the future, LINC00518 may serve as a predictive biomarker and potential therapeutic target for CC patients.

Key Words:
LncRNA, LINC00518, JAK/STAT3 signaling, Cervical cancer, EMT.

Introduction

Cervical cancer (CC) is the third most common gynecologic cancer in women worldwide, accounting for significant morbidity and mortality in females worldwide[1,2]. More than 85% of CC incidences and mortalities occur in developing countries, such as China, where diagnostic programs are still not well established[3,4]. In spite of current advances in the chemotherapy and molecular targeting therapy for CC, patients with advanced CC still have a very poor prognosis and significantly variable clinical outcomes due to tumor recurrence and metastasis[5,6]. Therefore, to develop more effective treatment methods, it is urgent to fully discover the genetic and molecular features of CC. Growing studies indicate that the molecular mechanisms of carcinogenesis and cancer progression involve not only to protein-coding genes, but also non-coding RNAs[7]. Long non-coding RNA (lncRNA) longer than 200 nucleotides are a class of messenger RNA (mRNA)-like transcripts lacking a significant reading frame[8]. Numerous studies have indicated that lncRNAs are critical biological regulators

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which participate in the various cellular biological processes, including proliferation, differentiation, apoptosis and metastasis[9,10]. With the improvement of technology and research in transcriptome profiles, emerging evidence indicates that lncRNAs may have complex and extensive functions in the development and progression of cancer[11,12]. For instance, lncRNA ABHD11-AS1 was reported to act as a carcinogenic lncRNA in epithelial ovarian cancer, which promoted ovarian cancer, cells proliferation, invasion and migration by targeted regulation of RhoC[13]. LncRNA BANCR, a well-studied lncRNA, was found to be highly expressed in endometrial cancer and to promote endometrial cancer cell proliferation and invasion[14]. In addition, several lncRNAs, such as lncRNA ANRIL[15], lncRNA LINC00473[16] and lncRNA MEG3[17], were also been identified in CC. However, the expression and function of a large number of lncRNAs remain to be elucidated. Long intergenic non-protein coding RNA 518 (LINC00518), mapped to chromosome 6, has been reported to be dysregulated in melanoma and triple-negative breast cancer[18,19]. Chang et al[20] showed that LINC00518 contributes to multidrug resistance by regulating the miR-199a/MRP1 Axis in breast cancer. However, little is known about the expression or functional roles of BDNF-AS in human CC. In this work, we first reported that LINC00518 was a potential prognostic marker and functioned as an oncogene in CC.

Patients and Methods

Patients and Tissue Samples

Human CC tissues and adjacent normal tissues were obtained from patients who received surgical resection in the Yantai Yuhuangding Hospital from 2009 to 2013. The study was approved by the Ethics Committee of the Yantai Yuhuangding Hospital. Written informed consent was obtained from each participant. None of these patients received any other treatment before the operation. The tissue specimens were immediately snap frozen in liquid nitrogen and stored at -80°C until required. The clinicopathological characteristics of these patients with CC were listed in Table I.

Cell Lines and Cell Culture

CC cell lines, HeLa, SiHa, C-4I, HT-3 and C-33A, were all obtained from the Chinese Academy of Sciences Cell Bank (Xuhui, Shanghai, China). A non-carcinoma cervical epithelial HPV-16 E6/E7 transformed cell line, Ect1/E6E7, was purchased from the BioVector Science Lab, Inc. (Beijing, China). The cells were cultured in Roswell Park Memorial Institute-1640 medium (RPMI-1640; Gibco, Grand Island, NY, USA) with 10% fetal bovine serum (FBS, Gibco, Grand Island, NY, USA), streptomycin (100 microg/mL) and penicillin (100 U/mL). All cells were maintained in a humidified atmosphere chamber containing 5% CO_2 at 37°C.

Table I. Clinicopathological features associated with LINC00518 expression in 133 CC patients.

Clinicopathological features	Total	LINC00518 expression: Low	LINC00518 expression: High	p-value
Age (years)				NS
< 45	53	28	25	
≥ 45	80	40	40	
Tumor size (cm)				NS
< 4.0	79	44	35	
≥ 4.0	54	24	30	
Histologic grade				NS
G1 + G2	85	47	38	
G3	48	21	27	
FIGO stage				0.002
Ib-IIa	61	50	31	
IIb-IIIa	52	18	34	
Lymph node metastasis				0.007
Absence	96	56	40	
Presence	37	12	25	
Depth of cervical invasion				0.010
<2/3	90	53	37	
≥2/3	43	15	28	

Table II. The primer sequences included in this study.

Name	primer sequences (5′–3′)
LINC00518: forward	GTGAAAATCTGGCTACTCGTCCC
LINC00518: reverse	CTGACTTTTGCCACAGACTCCTG
GAPDH: forward	CCACAGTCCATGCCATCAC
GAPDH: reverse	TCCACCACCCTGTTGCTGTA

Cell Transfection and Reagents

LINC00518 small interfering RNAs (siRNAs) were synthesized by Ribobio (Guangzhou, Guangdong, China), which were utilized to specifically silence the expression of LINC00518 in SiHa and HeLa cells. Cells were transfected with siRNAs using transfection reagent Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's advised procedure. In short, SiHa or HeLa cells (2×10^5 cells per well) were grown in medium to about 70% confluency in 6-well plates (NEST, Wuxi, Jiangsu, China). Afterward, the siRNAs (20 μM; 6 μl) and Lipofectamine 2000 reagents (10 μl) were mixed gently. The mixture was then added into the cells and continued to culture for 4-5 h at 37˚C with 5% CO_2. The medium was subsequently replaced with fresh medium containing 10% of FBS (Gibco, Grand Island, NY, USA) and antibiotics. The cells were then used for the following experiments.

Reverse Transcription-Quantitative Polymerase Chain Reaction (qRT-PCR)

Total RNA was isolated from CC tissues and cells using TRIzol (Solarbio, Beijing, China). Then, 2 μg of RNA were reversely transcribed into cDNA by the M-MLV Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's instructions. Afterward, the qRT-PCR assays were performed on a Fast Real Time-Polymerase Chain Reaction 7500 System (Applied Biosystems, Foster City, CA, USA) using SYBR-green PCR Master Mix kit (TIANGEN, Beijing, Haidian, China), according to the manufacturer's instructions. The results were standardized with the expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The primers used in this study were listed in Table II. Expression fold changes were calculated using the $2^{-\Delta\Delta Ct}$ method.

Western Blot Analysis

Cells were lysed in Radioimmunoprecipitation assay (RIPA; Beyotime, Shanghai, China) buffer, and the protein concentration was measured by BCA Protein Assay Kit (Beyotime, Shanghai, China). Afterward, total proteins were subjected to 8-15% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) electrophoresis and then transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After incubation with antibodies specific for E-Cadherin, N-Cadherin, Vimentin, Caspase 3, Caspase 9, JAK2, STAT3, p-JAK2, and p-STAT3, the membranes were washed three times. Then, the secondary antibodies were employed to incubate the PVDF membranes in Tris-Buffered Saline and Tween (TBST; Qiancheng Biotechnology, Pudong, Shanghai, China) for 1 h at room temperature. The proteins were then visualized using an enhanced chemiluminescence detection kit (Beyotime, Shanghai, China) and specific antibody binding was imaged by a BioSpectrum Gel Imaging System (Bio-Rad, Hercules, CA, USA). Image J software (version 1.46; Bethesda, MD, USA) was used to quantify the protein expression.

Cell Proliferation Assay

Cells viability was evaluated using 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Sigma-Aldrich, St. Louis, MO, USA). Approximately 2000 cells per well were seeded in the 96-well plates (NEST, Wuxi, Jiangsu, China). At the indicated time-points, 20 μl of MTT solution (0.5 mg/ml) was added to the plates (NEST, Wuxi, Jiangsu, China) and incubated at 37°C for another 4 h. Subsequently, 100 μl of dimethyl sulfoxide (DMSO; Aladdin, Pudong, Shanghai, China) was added into cells and optical density (OD) value was measured using a Microplate Reader (Bio-Rad, Hercules, CA, USA) at a wavelength of 490 nm.

Clonogenic Assay

For colony formation assays, SiHa or HeLa cells (500 cells per well) were transfected as indicated, and then seeded into 6-well plates (NEST,

Wuxi, Jiangsu, China) with medium containing 10% Fetal Bovine Serum (FBS; Gibco, Grand Island, NY, USA). The culture medium was changed every 2 days. The cells were fixed by 4% paraformaldehyde (Sigma-Aldrich, St. Louis, MO, USA) and subsequently stained with 0.5% crystal violet (Qiancheng Biotechnology, Pudong, Shanghai, China) after culturing for 2 weeks. After the plates were washed three times with Phosphate-Buffered Saline (PBS; Gibco, Grand Island, NY, USA), the colonies with more than 50 cells were counted by Image J software (version 1.46; Bethesda, MD, USA).

Flow Cytometry Analysis of Apoptosis

An Annexin-V-Fluorescein isothiocyanate (FITC) apoptosis detection kit (RiboBio, Guangzhou, Guangdong, China) was applied to determine the apoptosis of SiHa or HeLa cells under different experimental conditions. Briefly, SiHa or HeLa cells transfected with siRNAs (NC, si-LINC00518#1 and si-LINC00518#2) were harvested at 48 h after transfection. After collected, the cells were resuspended using the binding buffer in centrifuge tubes (Corning, Lowell, MA, USA). Afterward, Annexin V-FITC and propidium iodide (PI) were added into the centrifuge tubes and incubated in the dark at room temperature for 15-20 min. The apoptosis cells were analyzed using a fluorescence-activated cell sorting sorter (Applied Biosystems, Foster City, CA, USA). The data were then analyzed using ModFit software (Applied Biosystems, Foster City, CA, USA).

Wound Healing Assays

Cell motility was measured by wound healing assays using a 35 mm μ-Dish with culture insert consisting of two reservoirs separated by a 500-lm-thick wall. In Brief, cell suspensions (70 μl, 5×10^5 cells/ml) of SiHa or HeLa cells were seeded into each well. Twenty-four hours later, the insert was gently removed creating a gap of 500 μm. Thereafter, non-adherent cells were washed with PBS and the cells were maintained in medium to migrate at 37°C for 24 h. The pictures were captured using a Nikon Eclipse TE2000-S microscope (Nikon, Tokyo, Japan). Migration rates were quantified by the average distance that cells migrated towards the original wound field.

Transwell Assays

Cell invasive abilities were evaluated using transwell filters purchased from BD Biosciences (Franklin Lakes, NJ, USA). According to the protocols from the manufacturer, SiHa or HeLa cells (100 μl; 5×10^4 cells) in the medium without FBS were seeded into the upper chamber pre-coated with the Matrigel matrix (BD Biosciences, Franklin Lakes, NJ, USA) of a transwell insert. Afterward, the medium supplemented with 10% FBS (Gibco, Grand Island, NY, USA) was added into the lower chamber. Twenty-four hours later, cells on the upper side of the membrane were removed using a cotton swab, followed by washing with PBS three times. Then, the cells on the lower surface of the membrane were fixed by 4% paraformaldehyde (Sigma-Aldrich, St. Louis, MO, USA) and stained with 0.3% crystal violet (Qianchen Biotechnology, Pudong, Shanghai, China). Finally, inverted microscope (Nikon, Tokyo, Japan) was applied to capture the images.

Statistical Analysis

Statistical analyses were performed with the SPSS 16.0 statistics software (SPSS, Inc., Chicago, IL, USA). Data were expressed as mean ± SEM. The independent-samples *t*-test was applied to two-group analysis, while one-way ANOVA was used when analyzing multiple groups followed by a Post-Hoc Tukey's test. The chi-square test was used to examine the associations between LINC00518 expression and the clinicopathological characters. The Kaplan-Meier method was conducted to establish survival curves, and the survival differences were compared using the log-rank test. The significance of different variables was analyzed using the univariate and multivariate Cox regression analyses. $p < 0.05$ was considered statistically significant.

Results

LINC00518 is Upregulated Both in CC Tissues and Cell Lines

Previous studies showed that the expression levels of LINC00518 were significantly up-regulated in breast cancer and melanoma. Then, we analyzed RNA-Seq data (from TCGA: The Cancer Genome Atlas) of lncRNAs of CC by using the bioinformatics tools Cancer RNA-Seq Nexus (http://syslab4.nchu.edu.tw/) and GEPIA (http://gepia.cancer-pku.cn/). The results showed that LINC00518 expression was up-regulated in CC tissues compared to normal cervical tissues (Figure 1A and 1B). Then, we detected the expression of LINC00518 in CC patients by Real Ti-

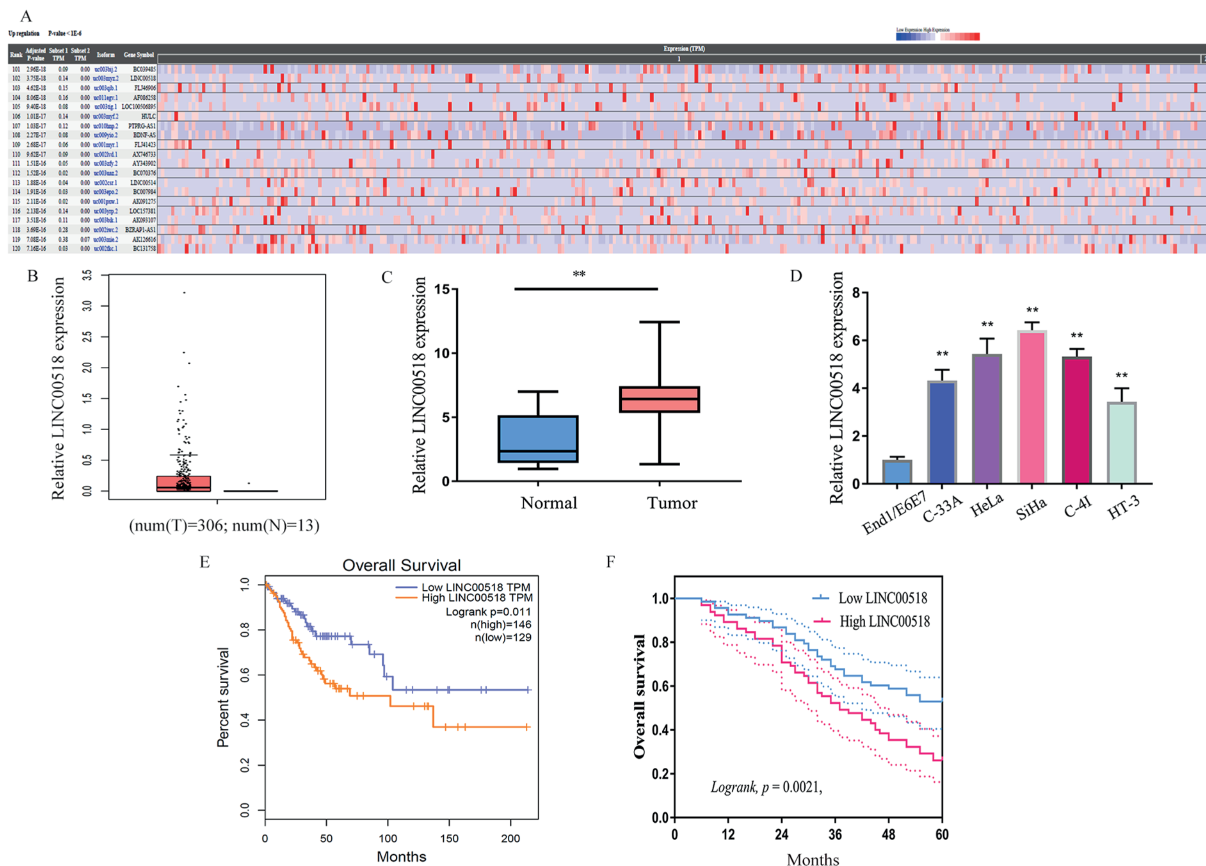

Figure 1. Expression levels of LINC00518 in CC and its clinical significance. ***A-B***, LINC00518 expression levels in CC tissues and normal cervical tissues from TCGA database. ***C,*** LINC00518 expression was examined by qRT-PCR in 133 paired CC tissues and adjacent non-tumor tissues. ***D,*** The expression level of LINC00518 was detected in four CC cell lines and normal cervical epithelial cells (End1/E6E7) using qRT-PCR. E, Kaplan-Meier survival plots demonstrated that higher LINC00518 abundance correlated with a poorer overall survival, using TCGA database. ***F,*** Kaplan-Meier analysis for the overall survival of CC patients with different expression of LINC00518. $^{*}p < 0.05$, $^{**}p < 0.01$.

me-Polymerase Chain Reaction, finding that CC tissues showed markedly higher expression than matched adjacent normal tissues ($p < 0.01$, Figure 1C). Moreover, the expression of LINC00518 was also detected in several CC cell lines and normal cervical cell line. As shown in Figure 1D, it showed that, as compared to End1/E6E7, LINC00518 were remarkably upregulated in all evaluated CC cell lines. These results indicated that abnormal expression of LINC00518 may be related to CC progression.

Association Between LINC00518 Expression and Prognosis of CC Patients

To explore the clinical significance of LINC00518 in CC, LINC00518 expression levels were classified as high or low in relation to the median value. As shown in Table I, we found that high expression of LINC00518 was significantly associated with International Federation of Gynaecology and Obstetrics (FIGO) stage ($p = 0.002$), lymph node metastasis ($p =0.007$) and depth of cervical invasion ($p = 0.010$). However, there was no association between LINC00518 expression and other clinical factors, such as age, tumor size, and Histologic grade ($p > 0.05$). Moreover, the data from TCGA dataset showed that CC patients with high LINC00518 showed poorer overall survival than those with low LINC00518 (Figure 1E, $p = 0.011$). At the same time, in our patient samples, Kaplan-Meier survival analysis and log-rank test showed that the high expressions of LINC00518 were correlated with the reduced overall survival of CC patients (log-rank test $p = 0.0021$; Figure 1F). More importantly, univariate analysis and multivariate analyses indicated that

Table III. Prognostic factors in Cox proportional hazards model.

	Univariate analysis			Multivariate analysis		
Variable	**RR**	**95% CI**	***p***	**RR**	**95% CI**	***p***
Age (years)	1.435	0.577-2.215	0.233	-	-	-
Tumor size (cm)	1.897	0.784-2.553	0.178	-	-	-
Histologic grade	1.345	0.689-2.346	0.366	-	-	-
FIGO stage	3.236	1.675-5.447	0.005	2.563	1.231-4.025	0.017
Lymph node metastasis	3.452	1.427-5.134	0.008	2.784	1.318-4.446	0.011
Depth of cervical invasion	3.045	1.357-4.452	0.015	2.553	1.126-3.987	0.023
LINC00518 expression	3.668	1.482-6.234	0.001	2.786	1.217-5.774	0.003

LINC00518 expression was an independent prognostic factor for CC patients (Table III).

Knockdown of LINC00518 Inhibited SiHa and HeLa Cells Proliferation and Promoted Cells Apoptosis

The frequent upregulation of LINC00518 in both CC tissues and cell lines indicated that LINC00518 might play a critical role in CC tumorigenesis. Thus, specific siRNAs against LINC00518 (si-LINC00518#1 and si-LINC00518#2) were first applied to suppress the expression of LINC00518 in SiHa and HeLa cells. As the data presented in Figure 2A, the CC cells (SiHa and HeLa) transfected with LINC00518 siRNAs showed lower LINC00518 expression levels than cells transfected with negative control siRNAs. To further evaluate the effects of LINC00518 on the development of CC, we next detected the cells proliferation of SiHa and HeLa cells using MTT assays. The results demonstrated that LINC00518 knockdown markedly impaired the proliferation of both SiHa and HeLa cells (Figure 2B and 2C). Analogously, cell colony forming assays clearly showed that decreased LINC00518 expression significantly reduced cell colony number in both SiHa and HeLa cells (Figure 2D and 2E). Furthermore, the apoptotic rates of SiHa and HeLa cells were evaluated by flow cytometry analysis, and increased apoptosis was observed after the downregulation of LINC00518 in SiHa and HeLa cells (Figure 2F). Besides, cell apoptosis-related molecules including caspase 3 and caspase 9 were also determined by Western blot assays. As shown in Figure 2G and 2H, the protein levels of caspase 3, as well as caspase 9, were dramatically decreased in SiHa and HeLa cells transfected with LINC00518 siRNAs. Overall, these results provided strong evidence that LINC00518 was a positive regulator of CC development and the knockdown of LINC00518 suppressed the tumorigeneses of CC.

Depression of LINC00518 Impaired the Migration and Invasion of SiHa and HeLa Cells

To investigate the potential effects of LINC00518 silencing on metastatic properties in CC cells, we next performed wound healing and transwell invasion assays. Wound healing assays revealed that, although SiHa and HeLa cells transfected with negative control siRNAs (NC) showed robust *in vitro* migration abilities, silencing LINC00518 with either of the two siRNAs (si-LINC00518#1 and si-LINC00518#2) remarkably impaired the wound closure potentials (Figure 3A and 3B). Moreover, transwell invasion assays suggested that the invasive abilities of SiHa and HeLa cells were dramatically reduced when LINC00518 was decreased (Figure 3C and 3D). Besides, Western blot analysis was carried out to evaluate the protein levels of E-cadherin, N-cadherin and Vimentin involved in epithelial-mesenchymal transition (EMT). According to the data, both SiHa and HeLa cells transfected LINC00518 siRNAs exhibited significant declines in the expression of N-cadherin and Vimentin, while the protein levels of epithelial marker E-cadherin was increased (Figure 3E and 3F). We demonstrated that LINC00518 played crucial roles in modulating the migration and invasion of CC cells, and LINC00518 could exert its inhibitory effects of CC metastasis by affecting the EMT pathway.

Silence of LINC00518 Suppressed the JAK/STAT3 Signaling in Cervical Carcinoma Cells

To further characterize the molecular mechanisms underlying the LINC00518 modulating development and progression of cervical carcinoma, we next evaluated the alteration of the proteins involved in the JAK/STAT3 signaling pathway by Western blot assays. As shown in Figure

4A, marked repression of phosphorylated JAK2 (p-JAK2), as well as the phosphorylated STAT3 (p-STAT3), was observed in LINC00518 siRNAs transfection SiHa cells compared with the controls, while the protein levels of JAK2 and STAT3 were not changed. Similarly, the protein levels of p-JAK2 and p-STAT3 were also decreased in HeLa cells after suppressing the expression of LINC00518 (Figure 4B). Our results suggested that LINC00518 knockdown impaired the activation of the JAK/STAT3 pathway.

Discussion

CC is a malignant tumor that damages the health of women. It is currently defined according to the International Federation of Gynaecology and Obstetrics (FIGO)[21]. Up to date, the long-term prognosis of CC is still poor, with expected 5-year survival rate less than 10%. The identification of specific and sensitive biomarkers for the prediction of CC may help improve the prognosis of CC patients[22]. Recently, more and more studies[23-25] suggested lncRNAs as an ideal diagnostic and prognostic biomarker for CC. In addition, several lncRNAs, such as lncRNA UCA1, lncRNA AGAP2-AS1 and lncRNA PVT1 have been reported to prognostic markers and potential therapeutic target for various tumors, including CC. In this work, we characterized the expression patter and clinical significances of LINC00518 in CC tissues by taking advantages of publicly available microarray datasets in TCGA database: we found that LINC00518 expression was significantly up-regulated in CC and associated with

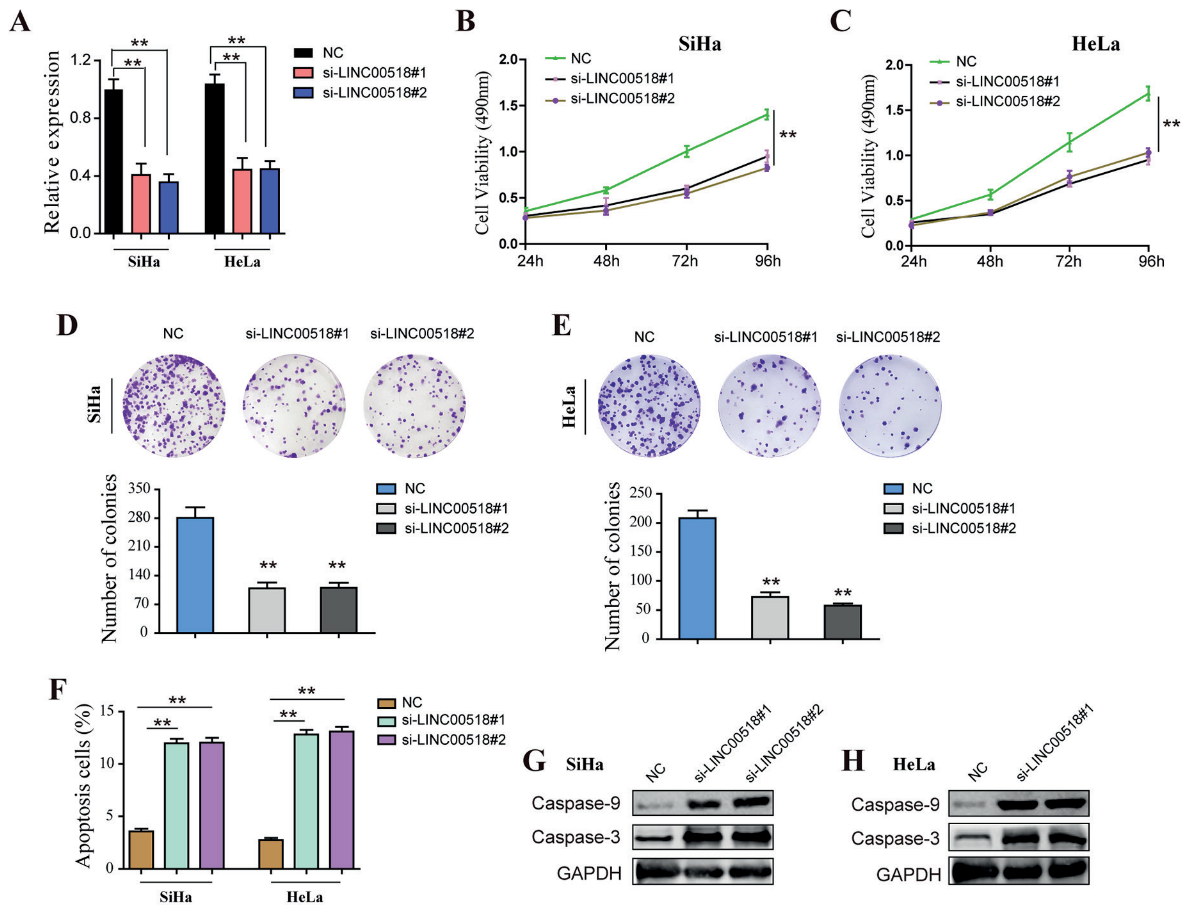

Figure 2. LINC00518 regulated CC cells proliferation and apoptosis. ***A,*** Relative mRNA expression levels of LINC00518 in Siha and Hela cells transfected with LINC00518 siRNAs (si-LINC00518#1 and si-LINC00518#2) or negative control siRNAs (NC). ***B,*** and ***C,*** LINC00518 knockdown inhibited the proliferation of Siha and Hela cells determined by MTT assays. ***D, E,*** Knockdown of LINC00518 inhibited colony formation ability of Siha and Hela cells. ***F,*** Flow cytometry analysis of Siha and Hela cells apoptosis. ***G-H,*** Western blot assays were applied to detect the protein levels of caspase 3 and caspase 9. $^{*}p < 0.05$, $^{**}p < 0.01$.

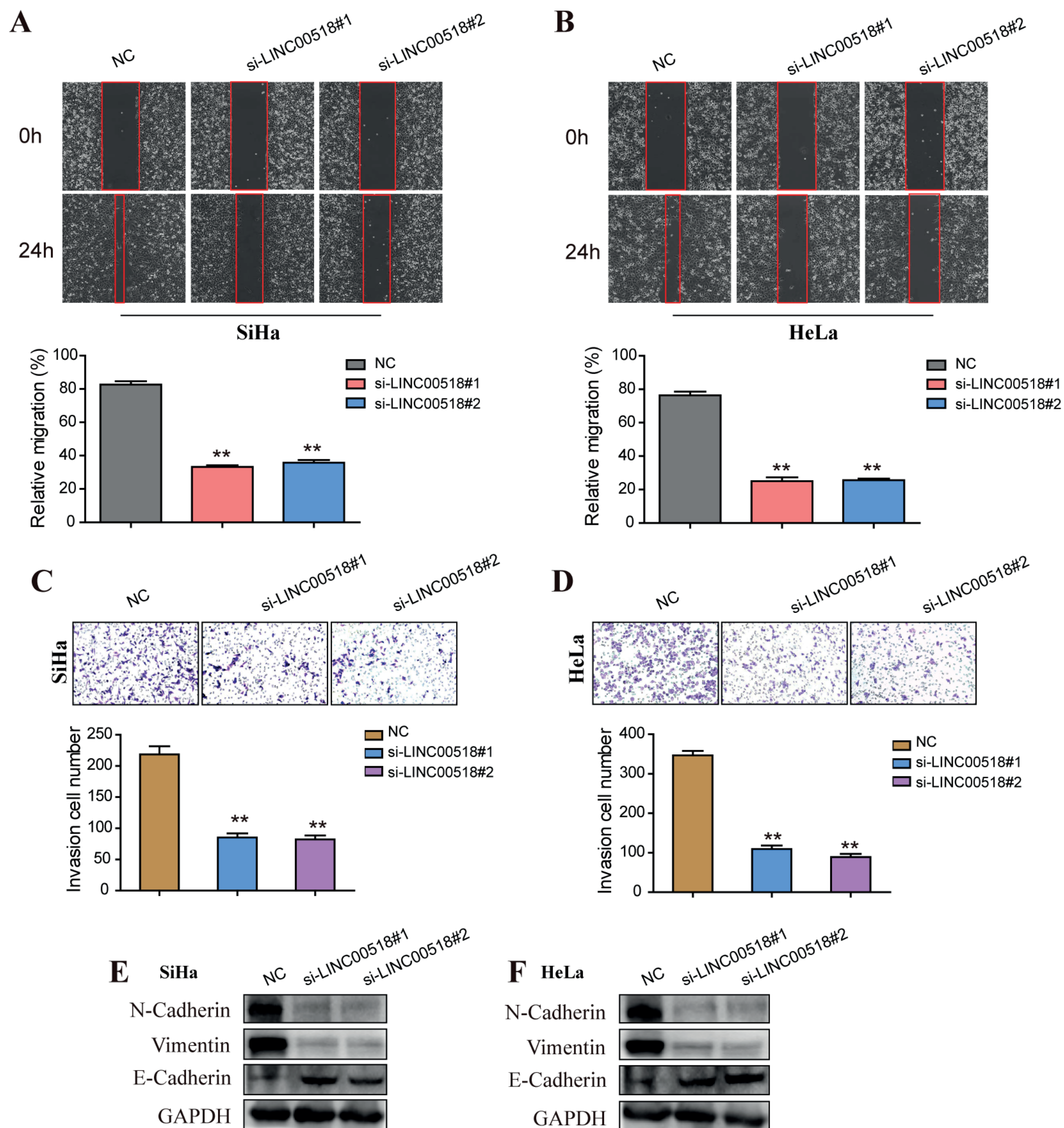

Figure 3. Effects of LINC00518 on migration and invasion of CC cells. ***A,*** Wound healing assays were carried out to assess the migration of Siha and Hela cells transfected with either NC or LINC00518 siRNAs. ***B,*** Transwell invasion assays were used to determine the invasive abilities of Siha and Hela cells. ***C-D***, Western blot assays were utilized to evaluated the protein expression levels of N-cadherin, Vimentin and E-cadherin in Siha and Hela cells. $*p < 0.05$, $**p < 0.01$.

poor overall survival in CC patients. Then, using the samples from our hospital, we further performed Real Time-Polymerase Chain Reaction (RT-PCR) and confirmed that LINC00518 was highly expressed in CC. Then, we found that high expression of LINC00518 was markedly associated with advanced International Federation of Gynaecology and Obstetrics (FIGO) stage, lymph node metastasis and depth of cervical invasion, which revealed that LINC00518 was implicated in the progress and development of CC. Moreover, the result of Kaplan-Meier curves indicated that the 5-year overall survival of high LINC00518 expression group was remarkably shorter than that of low LINC00518 expression group. Further multivariate survival analysis showed that LINC00518 is involved in CC and could be used as an independent potential prognostic biomarker for CC patients. LINC00518 was a newly identified lncRNA which has been reported to be up-regulated in melanoma and triple-negative breast cancer[18,19]. Chang et al[20] found that LINC00518 was highly expressed in breast cancer and associated with advanced

clinical stages. In their functional assay, it was found that LINC00518 contributes to multidrug resistance by regulating the miR-199a/MRP1 axis in breast cancer, indicating that LINC00518 acts as a positive regulator in breast cancer. However, the role of LINC00518 in CC has not been investigated. To investigate the biological significance of LINC00518 in CC, we explored the effects of LINC00518 on various aspects of CC cell biology. We found that the knockdown of LINC00518 significantly suppressed CC cells proliferation and induced apoptosis. In addition, we performed RT-PCR to detect the expression of Caspase-9 and Caspase-3, which were required for apoptosis. The previous clinical assay indicated that LINC00518 was associated with lymph node metastasis. Thus, we wondered whether LINC00518 could regulate the migration and invasion ability of CC cells. The results of wound healing and transwell invasion assays confirmed that the down-regulation of LINC00518 suppressed CC cells migration and invasion. In order to explore the potential mechanism of LINC00518 in metastasis of CC, we detected the expression of molecules of the EMT pathway: we found that the down-regulation of LINC00518 markedly suppressed the EMT pathway. Our findings, for the first time, indicated that LINC00518 may be an important contributor to CC development. STAT3 is a cytoplasmic transcription factor and its activation contributes to tumorigenesis via multiple cellular functions and biological processes, including proliferation, survival, angiogenesis and metastasis[26,27]. Janus kinases/signal transducer and activator of transcription 3 (JAK/STAT3) signaling is frequently presented in human cancer including CC and implicated in transformation, tumorigenicity, EMT and metastasis[28,29]. In addition, the JAK/STAT3 pathway can regulate another molecular signaling by cross-talking[30,31]. Given the important role of the JAK/STAT3 signaling in the progression of the tumor, we wondered whether LINC00518 exhibited its tumor promoter role by modulating JAK/STAT3 signaling. In this work, we performed Western blot to detect the expression of p-JAK and p-STAT3 in CC cells transfected with si-LINC00518 and we found the protein level of p-JAK and p-STAT3 remarkably reduced, indicating that the JAK/STAT3 signaling pathway was regulated by LINC00518 in CC cells. Further research is needed to elucidate the underlying mechanism.

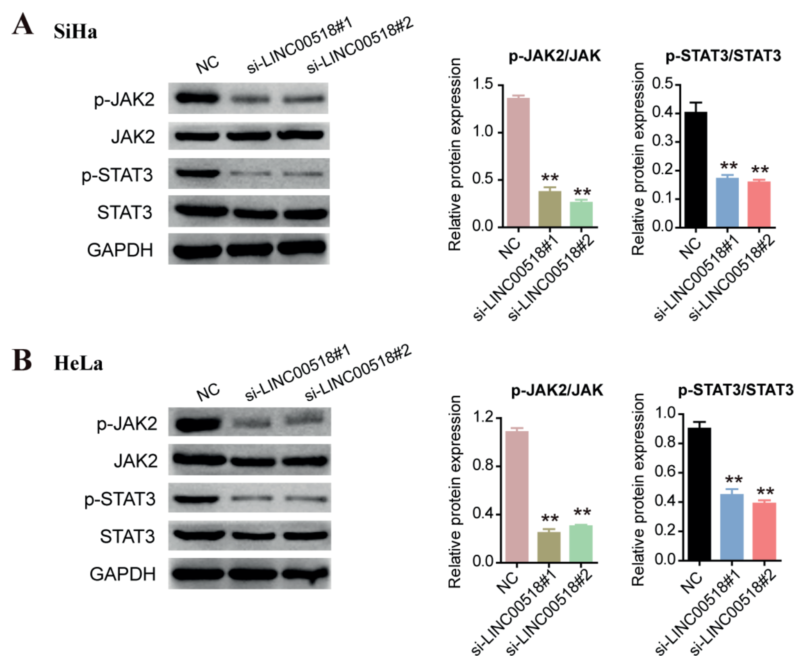

Figure 4. Effects of LINC00518 on JAK/STAT3 signaling in CC. ***A,*** The protein expression levels and optical density analysis of p-JAK2, JAK2, p-STAT3 and STAT3 in SiHa cells determined by Western blot assay. ***B,*** Western blot assays were applied to detect the protein expression levels of p-JAK2, JAK2, p-STAT3 and STAT3 in Hela cells. $*p < 0.05$, $**p < 0.01$.

Conclusions

We provided the first evidence that LINC00518 was overexpressed in CC tissues, and LINC00518 modulated JAK/STAT3, and then promoted cell proliferation, migration, invasion and EMT *in vitro*. In addition, the higher LINC00518 expression level was associated with poor prognosis in CC patients. These data highlight the significance of LINC00518 in CC progression, indicating that LINC00518 may be a crucial predictor for CC metastasis/poor prognosis and a potential therapeutic target.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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