

Long Noncoding RNA FBXL19-AS1 Expedites Cell Growth, Migration and Invasion in Cervical Cancer by miR-193a-5p/PIN1 Signaling

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Background: Cervical cancer is one of the most prevalent malignancies in gynecology with increasing incidence in recent years. Long noncoding RNAs (lncRNAs) have been reported to regulate human cancers including cervical cancer. F-box and leucine-rich repeat protein 19 antisense RNA 1 (*FBXL19-AS1*) have been unmasked to exert carcinogenic functions in several cancers except cervical cancer.

Aim: Present study hammered at investigating the function and mechanism of *FBXL19-AS1* in cervical cancer.

Methods: RT-qPCR was utilized to test gene expression. EdU staining, colony formation, transwell, flow cytometry and TUNEL assays were applied for measuring the impact of *FBXL19-AS1* on cervical cancer cell functions. Moreover, RIP, RNA pull-down and luciferase reporter assays were utilized for detecting the correlations among *FBXL19-AS1*, *miR-193a-5p* and *PIN1* (peptidylprolyl cis/trans isomerase, NIMA-interacting 1).

Results: *FBXL19-AS1* exhibited elevated expression in cervical cancer tissues and cells. Silencing *FBXL19-AS1* repressed cell proliferation through arresting cell cycle and stimulating apoptosis, and losing *FBXL19-AS1* also restrained cell migration and invasion. Also, we discovered *FBXL19-AS1* as a *miR-193a-5p* sponge, while *miR-193a-5p* was a tumor inhibitor in cervical cancer. Further, *PIN1* was proved as the *miR-193a-5p* target, and *FBXL19-AS1* augmented *PIN1* expression in cervical cancer via sequestering *miR-193a-5p*. Of note, *PIN1* accelerated the progression of cervical cancer, and its upregulation counteracted the impacts of depleted *FBXL19-AS1* on cervical cancer cell functions.

Conclusion: *FBXL19-AS1* contributes to malignant phenotypes in cervical cancer by sponging *miR-193a-5p* and regulating *PIN1*.

Keywords: *FBXL19-AS1*, *miR-193a-5p*, *PIN1*, cervical cancer

Introduction

Cervical cancer is one of the most prevalent malignancies in gynecology with serious endangerment to women's health.¹ In recent years, the incidence of cervical cancer is increasing with a younger trend.² With the development of early screening technology and treatment, the survival rate of early patients is also increasing.³⁻⁵ However, the prognosis of those developed into advanced stages is still not good.⁶ Thus, developing new biomarkers for cervical cancer are urgently demanded.

As a subclass of noncoding RNAs, long noncoding RNAs (lncRNAs) possess a length with longer than 200 nucleotides. lncRNAs cannot translate into proteins, but they can modulate gene expression through diverse manners including

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post-transcriptional regulation.^{7,8} An increasing number of reports have indicated that lncRNAs take part in different cellular processes to regulate the development of assorted diseases, even human cancers.^{9,10} Dysfunctional lncRNAs are always discovered in cancers and they are considered to be associated with cancer development. For example, *TUG1* was upregulated and promoted cell proliferation in epithelial ovarian cancer via modulating *AURKA*.¹¹ A former research also indicated that upregulated *PVT1* was associated with low survival rate and it could accelerate the progression of gallbladder cancer via *miR-143/HK2* axis.¹² *F-box* and leucine-rich repeat protein 19 antisense RNA 1 (*FBXL19-ASI*) is a kind of lncRNA which has been reported to regulate cancer development by some researches. For examples, *FBXL19-ASI* expedited oncogenic cellular behaviors via absorbing *miR-346* in osteosarcoma;¹³ *FBXL19-ASI* facilitated cell growth and epithelial-mesenchymal transition in non-small cell lung cancer;¹⁴ *FBXL19-ASI* acted as a *miR-178* sponge to accelerate breast cancer cell proliferation.¹⁵ Nonetheless, the specific role and mechanism of *FBXL19-ASI* in cervical cancer are still unclear.

In the current work, we hammered at investigating the function and latent mechanism of *FBXL19-ASI* in cervical cancer, which may conduce to improving cervical cancer treatment.

Materials and Methods

Human Cervical Cancer Samples

The paired tumor and non-tumor samples were surgically acquired from 100 cervical cancer patients in the Yijishan Hospital of Wannan Medical College. The patients with no any treatment before operation signed the written informed consents prior to this research. The work was supported by the Ethics Committee of Yijishan Hospital of Wannan Medical College. Specimens were all subjected to quick-frozen via liquid nitrogen and preservation at -80°C .

Cell Culture

Human normal cervical cell line (Ect1/E6E7) and cervical cancer cell lines (C-4-I, SiHa, C-33A and HeLa) were selected for our research. Ect1/E6E7, SiHa, C-33A and HeLa cells were obtained from ATCC (Manassas, VA, USA). C-4-I cell was purchased from CoBioer Biosciences Co., Ltd (Nanjing, China). Keratinocyte Serum-Free Medium (K-SFM; Gibco Laboratories, Grand Island, NY, USA) was utilized to

cultivated Ect1/E6E7 cell. Eagle's Minimum Essential Medium (EMEM) was applied for cultivating SiHa, C-33A and HeLa cells. C-4-I was cultured in DMEM medium with 10% FBS (Gibco). Each kind of culture medium was added with 10% fetal bovine serum (FBS; Gibco). All cells were cultivated under the condition with 5% CO_2 at 37°C .

Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR)

The TRIzol reagent was implemented for separating total RNA from indicated tissues or cells. Then, the reverse transcription kit (Takara, Dalian, China) was utilized for reverse transcription of RNA to cDNA. Following, RT-qPCR was conducted via the ABI7500 System (Applied Biosystems, CA, USA). Finally, gene expression relative to GAPDH/U6 was calculated via the $2^{-\Delta\Delta\text{Ct}}$ method.

Cell Transfection

For inhibiting *FBXL19-ASI* and *PIN1*, specific short-hairpin RNAs (sh-RNAs) were devised and synthesized through GenePharma (Shanghai, China), with a nonsense sequence as the negative control (sh-NC). For upregulating the expression of *PIN1*, the sequence of *PIN1* cDNA was cloned into pcDNA3.1 vector (Invitrogen, Carlsbad, CA, USA) for acquiring pcDNA3.1/PIN1, with empty vector as the negative control. Also, NC mimics/inhibitor and *miR-193a-5p* mimics/inhibitor were also devised and synthesized through GenePharma. Cell transfection with indicated plasmids for 48 hours was achieved by the utilization of Lipofectamine 3000 (Invitrogen).

5-Ethynyl-2'-Deoxyuridine (EdU) Assay

In accordance with the protocol of EdU labeling/detection kit (RiboBio, Guangzhou, China), EDU experiment was employed for evaluating cell proliferation capability. Cells (1×10^4) were placed in 96-well plates, followed by the addition of 50 μM of EdU diluent for cultivating cells for 2 hours under the condition of 5% CO_2 and 37°C . After fixation by 4% paraformaldehyde and rinsing via PBS, cells were processed with Apollo 567 working solution for 2 hours. Following, DAPI was utilized to counterstain the nuclei for 5 minutes. In the end, cells were observed by fluorescent microscopy (Thermo Fisher Scientific). Bio-repeats were run in triplicate.

Colony Formation Assay

Transfected cells were planted in the 6-well plates (500 cells per well) for two-week incubation. After that, cells

were fixated with 4% paraformaldehyde and stained by 0.1% crystal violet. Finally, the number of colonies was monitored manually. Bio-repeats were run in triplicate.

TUNEL Assay

TUNEL assay was operated for assessing cell apoptosis with the in situ Cell Apoptosis Detection Kit (Roche, Basel, Switzerland). Simply put, 1×10^5 cells were added into 24-well plates. After that, cells were processed consecutively with 4% paraformaldehyde, 0.2% Triton X-100, and DAPI staining solution. Subsequently, the images were captured with a light microscope (CX23, Olympus, Japan). Bio-repeats were run in triplicate.

Flow Cytometry Analysis

As for cell apoptosis detection, cells were subjected to double-staining via FITC-Annexin V Apoptosis Detection Kit (BD Biosciences) in accordance with protocol of supplier. With respect to cell cycle evaluation, cells were digested and fixed with 75% ethanol, followed by discard of the supernatant and cultivation with PI-contained RNA enzyme (Sigma-Aldrich). Finally, cell apoptotic rate and cell cycle distribution were both determined via flow cytometry (FACScan; BD Biosciences. Bio-repeats were run in triplicate).

Transwell Assay

The capabilities of C-4-I and C-33A cells to invade or migrate were measured by using the transwell chamber with or without Matrigel (Corning Incorporated, Corning, NY), respectively. 2×10^4 cells in serum-free medium were seeded to the upper chamber, while the medium supplementing 10% FBS was poured into the lower chamber. After 24 hours, cells in the lower chamber were subjected to fixation by 4% paraformaldehyde and staining with crystal violet. Afterwards, we observed cells by the microscope (magnification, $\times 200$; Olympus, Tokyo, Japan). Bio-repeats were run in triplicate.

Fluorescent in situ Hybridization (FISH)

RiboTM Fluorescent in situ Hybridization Kit (Ribobio) was applied for conducted this assay based on the user guides. In short, the *FBXL19-AS1* probes labeled with fluorescent dye were incubated with indicated cells. Finally, the signals of fluorescence were analyzed by a confocal laser-scanning microscope (Leica). Bio-repeats were run in triplicate.

Subcellular Fractionation

The nuclear or cytoplasmic Isolation Kit (Biovision, San Francisco, CA, USA) was utilized for the isolation of cytosolic and nuclear fractions in C-4-I or C-33A cells in line with the user guide. The RNA molecules in the cytoplasm and nucleus were then detected via RT-qPCR. U6 and GAPDH served as the controls. Bio-repeats were run in triplicate.

Luciferase Reporter Assay

The synthesized sequences of *FBXL19-AS1* or *PIN1* 3'UTR covering wild-type (WT) or mutated-type (Mut) of *miR-193a-5p* interacting sites were inserted into pmirGLO vectors (Promega, Madison, WI, USA) for constructing *FBXL19-AS1*-WT or *PIN1*-WT/Mut. Then, cells were subjected to co-transfection with the constructed vectors and *miR-193a-5p* mimics (or NC mimics) using LipofectamineTM3000 (Invitrogen) for 48 hours. In the end, dual-luciferase reporter gene assay system (Promega) was employed for measuring the luciferase activity. Bio-repeats were run in triplicate.

RNA Immunoprecipitation (RIP) Assay

Magna RIPTM RNA Binding Protein Immunoprecipitation Kit (Millipore) was utilized for conducting this assay. Simply put, cells were lysed via RIP lysis buffer (Solarbio) and then the obtained lysates were cultivated for a whole night in RIP buffer with magnetic beads coating anti-Ago2 (Millipore) or anti-IgG. After that, the precipitated RNAs was isolated and analyzed through RT-qPCR. Bio-repeats were run in triplicate.

RNA Pull-Down Assay

The lysates acquired from C-4-I and C-33A cells via RIPA lysis buffer were cultivated for 1 hour at 4°C with biotinylated *miR-193a-5p* sequences with wild-type or mutated seed region (Bio-*miR-193a-5p*-WT/Mut), with the biotin-labelled nonsense sequence as the negative control (Bio-NC). Then, the streptavidin agarose magnetic beads were supplemented into above mixtures, and the enrichment of RNAs captured by beads were analyzed via RT-qPCR. Bio-repeats were run in triplicate.

Statistical Analysis

The data from all three bio-repeats were expressed as mean \pm standard deviation (SD) after analysis via SPSS 22.0 software (IBM, Armonk, NY, USA). The group

differences were measured via Student's *t*-test or one-way ANOVA, with $P < 0.05$ as statistically significant.

Results

Knockdown of FBXL19-ASI Restrains Cell Proliferation, Migration and Invasion in Cervical Cancer

First of all, in order to examine the function of *FBXL19-ASI* in cervical cancer, we tested its expression under malignant conditions. Intriguingly, *FBXL19-ASI* presented high expression in cervical cancer tissues relative to matched non-cancerous ones (Supplementary Figure 1A). Consistently, we observed that *FBXL19-ASI* was highly expressed in cervical cancer cell lines (C-4-I, SiHa, C-33A and HeLa) in contrast with the normal cell line (Ect1/E6E7), especially in C-4-I and C-33A cells (Figure 1A). Then, for conducting loss-of-function assays, we interfered *FBXL19-ASI* expression in C-4-I and C-33A cells using sh-*FBXL19-ASI*#1/2 (Figure 1B). Subsequently, we discovered that cell proliferative capability was restrained through the lack of *FBXL19-ASI*, because the quantity of colonies and the rate of EdU positive cells were both reduced after *FBXL19-ASI* was knocked down (Figure 1C-D). After that, we measured cell migration and invasion through transwell experiments. Results displayed that the number of migrated and invaded cells was dramatically declined when *FBXL19-ASI* was inhibited (Figure 1E), indicating the repressive impact of depleted *FBXL19-ASI* on cervical cancer cell migration and invasion. In the end, to further uncover the in-depth influence of *FBXL19-ASI* on cervical cancer cell proliferation, we then investigated its impact on cell cycle progression and cell apoptosis. It manifested that the absence of *FBXL19-ASI* led to increased percent of cells at G0/G1 phase and decreased proportion of cells at S and G2/M phases (Supplementary Figure 1B), disclosing that *FBXL19-ASI* inhibition induced cell cycle arrest in cervical cancer cells. Meanwhile, the outcomes of flow cytometry analysis and TUNEL experiment revealed that both the rates of apoptotic cells and TUNEL positive cells were elevated under *FBXL19-ASI* deficiency (Figure 1F-G), which demonstrated that cell apoptotic ability could be expedited by *FBXL19-ASI* shortage. In a word, the expression of *FBXL19-ASI* is extremely high and knockdown of *FBXL19-ASI* restrains cell proliferation, migration and invasion in cervical cancer.

FBXL19-ASI Sponges miR-193a-5p and Overexpression of miR-193a-5p Inhibits the Progression of Cervical Cancer

For the sake of exploring the regulatory mechanism of *FBXL19-ASI* in cervical cancer, we conducted subcellular fractionation assay and FISH experiment to figure out the distribution of *FBXL19-ASI* in cervical cancer cells. The outcomes indicated the major location of *FBXL19-ASI* in C-4-I and C-33A cell cytoplasm (Figure 2A-B), which signified that *FBXL19-ASI* might serve as a miRNA sponge to exert its function at post-transcription level. Thus, we utilized the ENCORI database (<http://starbase.sysu.edu.cn/index.php>) to search for the latent miRNAs binding to *FBXL19-ASI*. On the basis of specific conditions (CLIP-Data ≥ 2 ; Degradome-Data ≥ 0 ; pan-Cancer ≥ 4), we found seven miRNA candidates (including *miR-331-3p*, *miR-193a-5p*, *miR-378a-3p*, *miR-378c*, *miR-378d*, *miR-5586-5p* and *miR-6512-3p*). Then, we implemented RT-qPCR experiments to examine the expression of the aforementioned miRNAs in cervical cancer cells. As a result, we discovered that only *miR-193a-5p* was lowly expressed in cervical cancer cells, while the levels of other miRNAs were relatively high (Figure 2C). Of importance, cervical cancer samples tended to possess higher *miR-193a-5p* level than paired controls (Supplementary Figure 1C). Hence, we selected *miR-193a-5p* for further experiments. Through RNA pull-down experiments, we discovered that the enrichment of *FBXL19-ASI* was extremely high in biotinylated wild-type *miR-193a-5p* group rather than the other two groups (Figure 2D). Following, we elevated *miR-193a-5p* expression in these two cells (Figure 2E), and also acquired the predicted binding sites between *miR-193a-5p* and *FBXL19-ASI* by ENCORI (Figure 2F). As expected, the results of luciferase reporter experiments displayed that overexpressed *miR-193a-5p* caused a notable reduction in the luciferase activity of *FBXL19-ASI*-WT, while the luciferase activity of *FBXL19-ASI*-Mut emerged no visible change (Figure 2G). Furthermore, we examined the role of *miR-193a-5p* in cervical cancer. Through colony formation and EdU experiments, we discovered that upregulation of *miR-193a-5p* inhibited cell proliferation (Figure 2H-I). In the same way, cell migration and invasion were also suppressed by *miR-193a-5p* overexpression according to the results of transwell experiments (Figure 2J). Further, we discovered that *miR-193a-5p* elevation resulted in cell cycle arrest in both the two cervical cancer cells (Supplementary Figure 1D). Nevertheless, the outcomes of flow cytometry analysis and TUNEL experiments indicated that cell apoptotic ability was

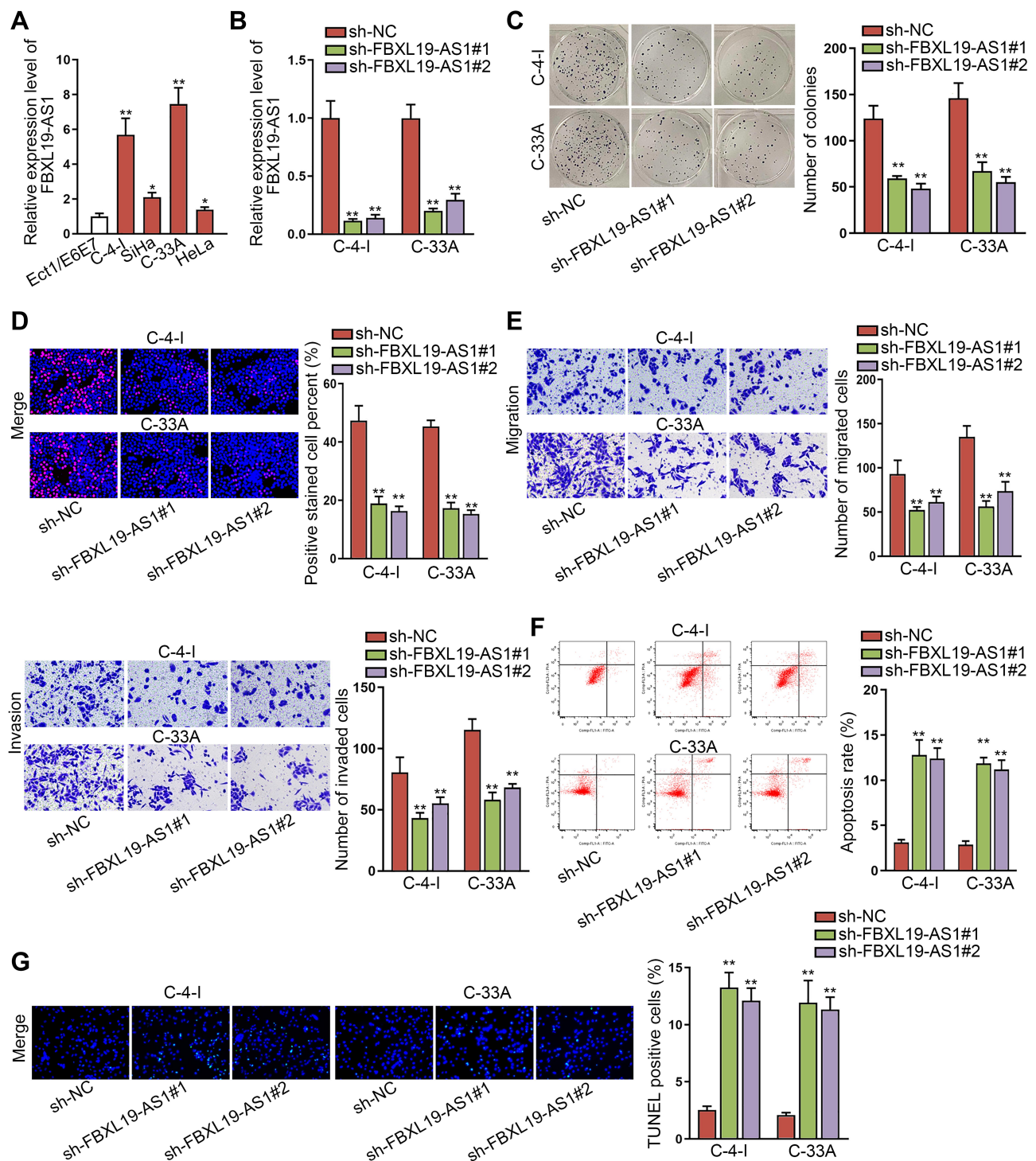


Figure 1 Knockdown of *FBXL19-AS1* restrains cell proliferation, migration and invasion in cervical cancer. **(A)** The expression of *FBXL19-AS1* in cervical cancer cells was tested through RT-qPCR. **(B)** Knockdown efficiency of *FBXL19-AS1* in C-4-I and C-33A cells was detected via RT-qPCR. **(C-D)** Cell proliferative capability was estimated by colony formation and EdU staining experiments when *FBXL19-AS1* was inhibited. **(E)** Transwell experiments were carried out to evaluate cell migratory and invasive capabilities after silencing *FBXL19-AS1*. **(F-G)** Flow cytometry and TUNEL experiments were adopted to evaluate the influence of knocking down *FBXL19-AS1* on cell apoptosis. *P<0.05, **P<0.01.

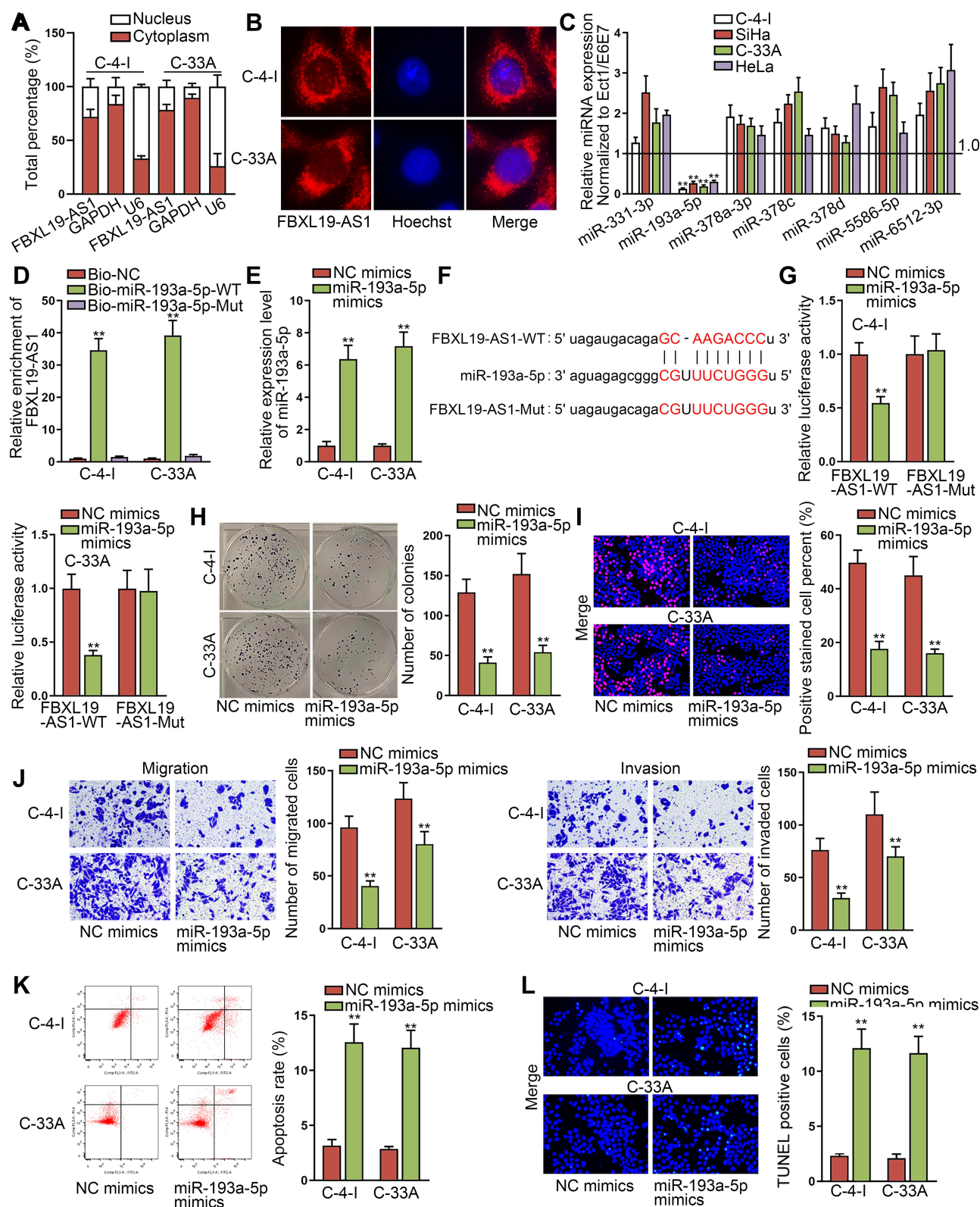


Figure 2 *FBXL19-AS1* sponges *miR-193a-5p* and overexpression of *miR-193a-5p* inhibits the progression of cervical cancer. (A-B) The subcellular fractionation and FISH experiments were utilized to detect the location of *FBXL19-AS1* in C-4-I and C-33A cells. (C) The expression of possible miRNAs in cervical cancer cells was tested through RT-qPCR. (D) RNA pull-down assay was conducted to verify the interaction between *miR-193a-5p* and *FBXL19-AS1*. (E) The overexpression efficiency of *miR-193a-5p* was detected by RT-qPCR. (F) The binding site between *miR-193a-5p* and *FBXL19-AS1* was predicted by ENCORI. (G) Luciferase reporter experiments were conducted to detect the correlation between *miR-193a-5p* and *FBXL19-AS1*. (H-I) Colony formation and EdU experiments were implemented to measure cell proliferation when *miR-193a-5p* was overexpressed. (J) Transwell assays were conducted to estimate cell migration and invasion after overexpressing *miR-193a-5p*. (K-L) Cell apoptosis was measured by flow cytometry analysis and TUNEL assay when *miR-193a-5p* was subjected to upregulation. ** $P < 0.01$.

accelerated after overexpressing *miR-193a-5p* (Figure 2K-L). Overall, *FBXL19-AS1* sponges *miR-193a-5p*, a progression-repressor in cervical cancer.

PIN1 is the Target of miR-193a-5p in Cervical Cancer

In order to further investigate the downstream molecules, we began to find the target genes of *miR-193a-5p*. After searching on ENCORI database based on following conditions: CLIP-Data ≥ 2 , Degradome-Data ≥ 1 , pan-Cancer ≥ 10 and programNum ≥ 1 , we discovered 5 potential genes (*CARD19*, *FDFT1*, *PIN1*, *INO80E* and *REPIN1*) targeted by *miR-193a-5p*. Thereafter, RT-qPCR was conducted to measure the influence of upregulated *miR-193a-5p* on the expression of above targets. The results presented that the expression of *PIN1* (peptidylprolyl cis/trans isomerase, NIMA-interacting 1) was visibly inhibited by *miR-193a-5p* enhancement, while that of others almost unchanged (Figure 3A). Then, we intended to inhibit *miR-193a-5p* expression in the two cells by transfecting with *miR-193a-5p* inhibitor, and examined the interference efficiency using RT-qPCR. The results indicated that *miR-193a-5p* expression was evidently declined by an *miR-193a-5p* inhibitor (Figure 3B). Further, we discovered that the expression of *PIN1* was ascended in response to *miR-193a-5p* deficiency (Figure 3C), but declined in face of *FBXL19-AS1* knockdown (Figure 3D). In addition, it was unveiled that the expression of *PIN1* was heightened in cervical cancer specimens compared to adjacent non-tumor ones (Supplementary Figure 1E), as well as in cervical cancer cells relative to normal controls (Figure 3E). Following, RIP experiments were implemented and the outcomes displayed that *FBXL19-AS1*, *miR-193a-5p* and *PIN1* were co-precipitated by anti-Ago2, proving their existence in RNA-induced silencing complexes (RISCs) (Figure 3F). In Figure 3G, we displayed the binding sites between *miR-193a-5p* and *PIN1* which were predicted by ENCORI. Furthermore, luciferase reporter experiment results showed that only the luciferase activity of *PIN1* 3'UTR-WT was evidently declined when *miR-193a-5p* was upregulated (Figure 3H). Moreover, the outcomes of RNA pull-down experiments also proved the combining relationship between *miR-193a-5p* and *PIN1* (Figure 3I). Subsequently, we evaluated the function of *PIN1* in cervical cancer cellular behaviors after validating the successful decline of *PIN1* expression by sh-*PIN1*#1/2 (Figure 3J). As anticipated, we found that loss of *PIN1* could hinder cell proliferation, migration and invasion (Figure 3K-M). In depth, knockdown of *PIN1* in cervical cancer cells could

induce cell cycle arrest (Supplementary Figure 1F), and boost apoptosis (Figure 3N-O). In sum, *PIN1* that serves as a tumor-accelerator is the downstream of *FBXL19-AS1*/*miR-193a-5p* signaling in cervical cancer.

FBXL19-AS1 Accelerates Cervical Cancer Progression via Regulating PIN1

In order to examine whether *FBXL19-AS1* accelerated cervical cancer progression via regulating *PIN1*, we conducted following rescue experiments. Firstly, we overexpressed *PIN1* in C-4-I and C-33A cells and tested the overexpression efficiency of *PIN1* through RT-qPCR (Figure 4A). Then, we implemented colony formation and EdU experiments to evaluate the influence of overexpressed *PIN1* on the proliferation of *FBXL19-AS1*-silenced cells. The outcomes indicated that cell proliferation restrained by silenced *FBXL19-AS1* was then reversed by overexpression of *PIN1* (Figure 4B-C). Also, transwell experiments were carried out and the outcomes demonstrated that the inhibited cell migration and invasion caused by the lack of *FBXL19-AS1* could be recovered when *PIN1* was upregulated (Figure 4D). Additionally, we revealed that cell cycle arrested by depleted *FBXL19-AS1* was propelled under *PIN1* elevation (Supplementary Figure 1G). Furthermore, it was proved that the promoting influence of *FBXL19-AS1* suppression on cell apoptosis was offset when augmenting *PIN1* (Figure 4E-F). Taken together, *FBXL19-AS1* accelerates cervical cancer progression via regulating *PIN1*.

Discussion

Cervical cancer is one of the most common gynecological malignant tumors, and it is a major hidden danger to women's health in the world. So far, a large number of researches have confirmed that assorted cancers are associated with the maladjusted lncRNAs, which means that the variation on the expression of lncRNAs influences the process of tumor formation. Similarly, cervical cancer, as a cancer with high incidence, has been discovered to be tightly associated with lncRNAs by a crowd of scholars. For instance, *GAS5* restrains the development of cervical cancer through sponging *miR-196a* and *miR-205*.¹⁶ *PVT1* epigenetically silences *miR-195* and regulates EMT in cervical cancer.¹⁷ Also, *SNHG20* strengthens the proliferative capability of cervical cancer cells by *miR-140-5p*/*ADAM10* axis.¹⁸ Although lots of lncRNAs have been searched in cervical cancer, the functions of many other lncRNAs in cervical cancer still need to be further

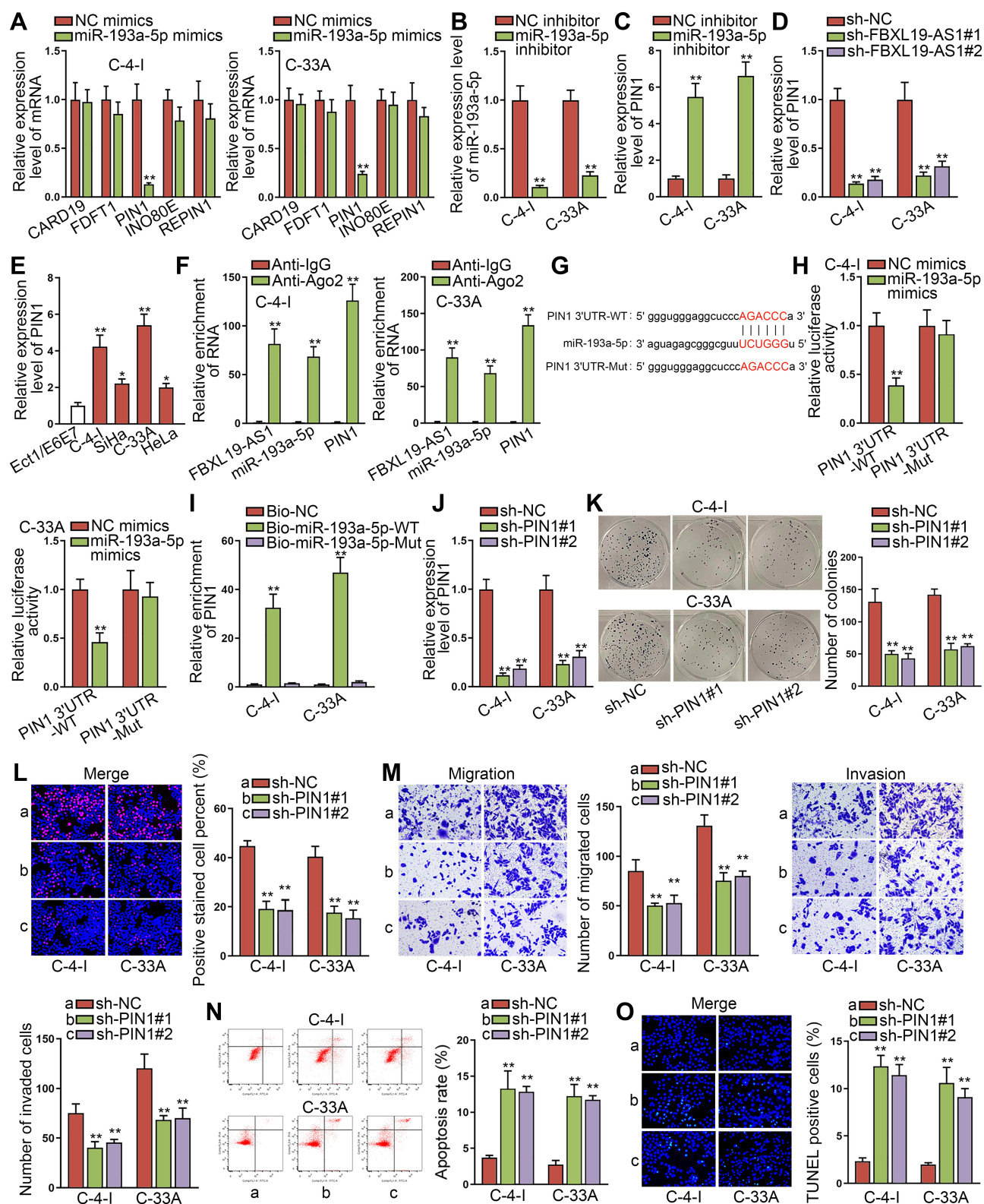


Figure 3 *PIN1* is the target of *miR-193a-5p* in cervical cancer. (A) The expression of indicated genes was detected via RT-qPCR when *miR-193a-5p* was overexpressed. (B) Interference efficiency of *miR-193a-5p* was detected by RT-qPCR in C-4-I and C-33A cells. (C-D) The expression of *PIN1* was tested via RT-qPCR after *miR-193a-5p* or *FBXL19-AS1* were silenced. (E) The expression of *PIN1* in cervical cancer cells was tested through RT-qPCR. (F) RIP experiment was utilized to detect the co-existence of *FBXL19-AS1*, *miR-193a-5p* and *PIN1* in RISCs. (G) The binding sites between *miR-193a-5p* and *PIN1* predicted by ENCORI. (H-I) Luciferase reporter and RNA pull-down experiments were implemented to prove the interplay between *miR-193a-5p* and *PIN1*. (J) The knockdown efficiency of *PIN1* was tested through RT-qPCR. (K-L) Colony formation and EdU experiments were carried out to test cell proliferation when *PIN1* was inhibited. (M) Transwell experiments were implemented to test cell migration and invasion after silencing *PIN1*. (N-O) Flow cytometry and TUNEL experiments were utilized to test cell apoptosis when *PIN1* was subjected to inhibition. * $P < 0.05$, ** $P < 0.01$.

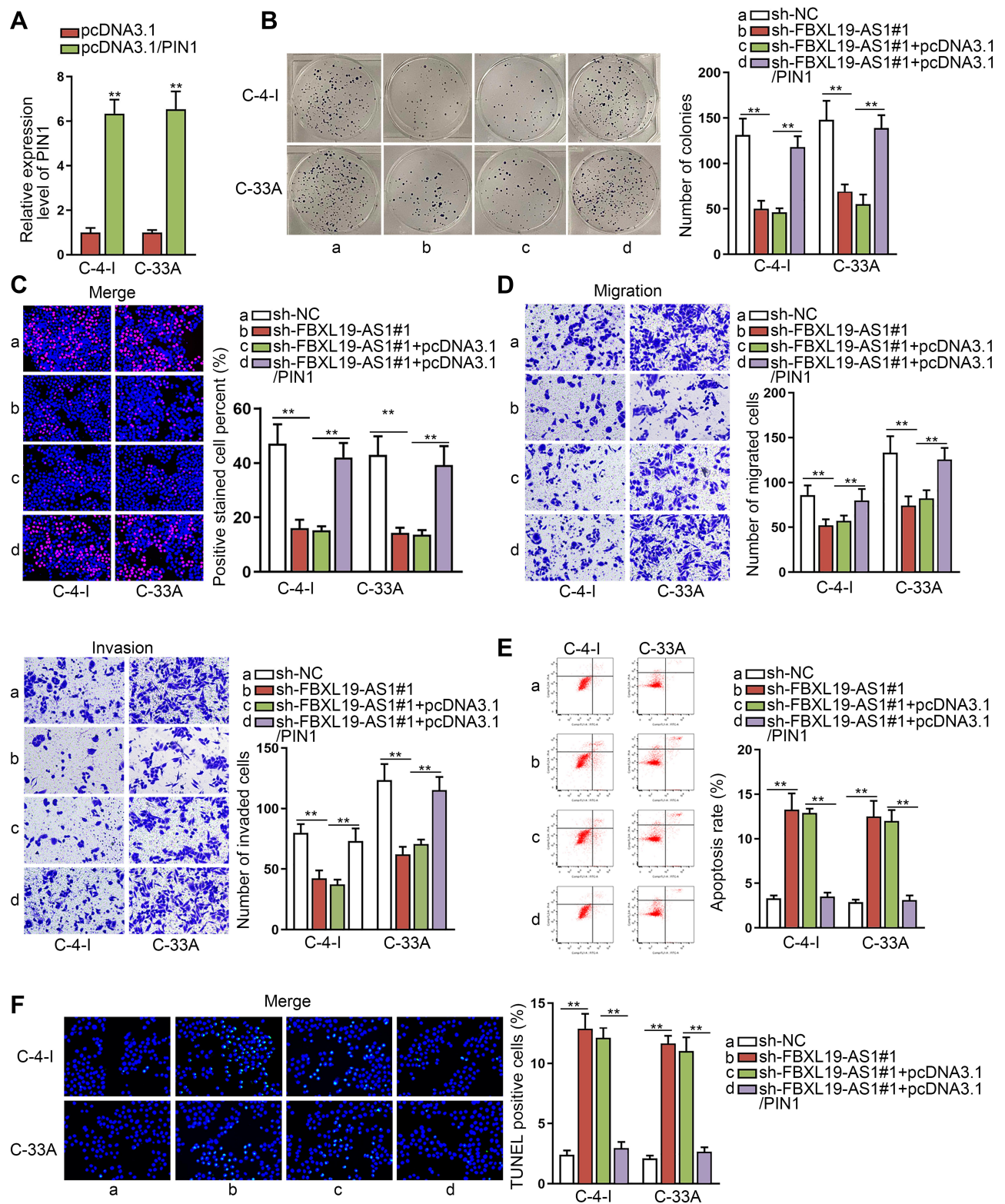


Figure 4 *FBXL19-AS1* accelerates cervical cancer progression via regulating *PIN1*. **(A)** The overexpression efficiency of *PIN1* in C-4-I and C-33A cells was tested by RT-qPCR. **(B-C)** Colony formation and EdU experiments were implemented to evaluate the influence of silencing *FBXL19-AS1* or together with overexpressing *PIN1* on cell proliferation. **(D)** Transwell experiments were utilized to measure the influence of overexpressing *PIN1* on the migration and invasion of *FBXL19-AS1*-inhibited cells. **(E-F)** Flow cytometry analysis and TUNEL assay were conducted to detect cell apoptosis in different groups. ** $P < 0.01$.

unearthed. *FBXL19-AS1* has been unveiled to be highly expressed in osteosarcoma cells and to facilitate malignancy in this cancer.¹³ Nevertheless, the specific function and mechanism of *FBXL19-AS1* in cervical cancer are still in an unknown state. In our research, we discovered that the expression of *FBXL19-AS1* was singularly strong in cervical cancer tissues and cells. In recent years, lncRNAs have been reported to be regulated at transcriptional and post-transcriptional levels.¹⁹ Further, *FBXL19-AS1* was proved to be post-transcriptionally stabilized by LIN28A in breast cancer.²⁰ Nevertheless, the mechanism whereby *FBXL19-AS1* was upregulated in cervical cancer remains unknown, and this needs to be further focused on in future studies.

Thereupon we took the functional experiments to search the influence of *FBXL19-AS1* silence on the behaviors of cervical cancer cells. Interestingly, we discovered that cervical cancer cell proliferation could be restrained by the lack of *FBXL19-AS1*, which was proved to be attributed to arrested cell cycle and accelerated cell apoptosis under the context of *FBXL19-AS1* inhibition. Meanwhile, we also elucidated that cell migration and invasion capabilities were suppressed when *FBXL19-AS1* was subjected to inhibition. Consequently, we deduced that *FBXL19-AS1* exerted a carcinogenic function in cervical cancer.

In recent years, lncRNAs have been proposed to serve as the sponge of miRNAs, so as to release the controlled mRNAs.²¹ For example, *HOXD-AS1* acts as a ceRNA for *SOX4* to accelerate the development of liver cancer.²² Besides, *UICLM* expedites colorectal cancer progression through serving as a sponge for *miR-215* to boost *ZEB2* expression.²³ Importantly, whether certain lncRNA could function as a ceRNA primarily depends on its cellular position. In other words, nuclear lncRNAs always regulate pre-transcription or transcription, while cytoplasmic lncRNAs often function as a ceRNA by sponging miRNAs to indirectly regulate target mRNA expression at the post-transcriptional level. In our research, we found that the distribution of *FBXL19-AS1* was mostly in the cytoplasm of cervical cancer cells. Consequently, we conjectured that *FBXL19-AS1* worked by the ceRNA mechanism. Hence, we discovered *miR-193a-5p* as the downstream sponged by *FBXL19-AS1* in cervical cancer. Further, *miR-193a-5p* was indicated to hamper cell proliferation, migration and invasion in cervical cancer, consistently as it did in other cancer types like colon cancer,²⁴ osteosarcoma,²⁵ and gastric cancer.²⁶

Subsequently, we discovered that *PIN1* was the target of *miR-193a-5p*. *PIN1* has been reported to expedite the progression of hepatocellular carcinoma,²⁷ esophageal squamous-cell carcinoma²⁸ and prostate cancer.²⁹ In our study, we proved that *PIN1* was the downstream of *FBXL19-AS1/miR-193a-5p* pathway in cervical cancer. Besides, silencing *PIN1* mitigated the oncogenic behaviors of cervical cancer cells, and the findings supporting *PIN1* as a cancer-promoter in this tumor was consistent with a former study.³⁰ Moreover, rescue experiments demonstrated that overexpressing *PIN1* could reverse the influences of silenced *FBXL19-AS1* on cervical cancer cellular functions. Previously, *PIN1* has been suggested to have roles in cell cycle control,³¹ regulation of PI3K/AKT and Wnt/ β -catenin pathways,³² or modulation of Hippo signaling.³³ More importantly, several reports revealed that *PIN1* also affects cyclin D1 expression and Wnt/ β -catenin pathway in cervical cancer,^{34,35} which might be the downstream mechanism underlying the contribution of *FBXL19-AS1/miR-193a-5p/PIN1* axis to cervical cancer development.

Conclusions

All in all, our research unmasked that *FBXL19-AS1* accelerates cell growth, migration and invasion in cervical cancer by sponging *miR-193a-5p* to augment *PIN1*, which provides a new direction for treating patients with cervical cancer.

Data Sharing Statement

Research data have been presented within this manuscript and the [Additional file](#).

Ethics Approval and Informed Consent

This work was conducted under the support of the Ethics Committee of Yijishan Hospital of Wannan Medical College. All cervical cancer patients enrolled in signed the informed consents before this research.

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Author Contributions

Conceptualization: Su Wan

Methodology: Guantai Ni

Software: Yuansheng Huang
 Validation: Jin Ding
 Formal Analysis: Su Wan
 Investigation: Jin Ding
 Data Curation: Guantai Ni
 Writing – Original Draft Preparation: Su Wan
 Writing – Review & Editing: Yuansheng Huang
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Disclosure

The authors declare that they have no competing interests in this work.

References

- Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin*. 2011;61(2):69–90.
- Small W Jr., Bacon MA, Bajaj A, et al. Cervical cancer: a global health crisis. *Cancer*. 2017;123(13):2404–2412. doi:10.1002/cncr.30667
- Galic V, Herzog TJ, Lewin SN, et al. Prognostic significance of adenocarcinoma histology in women with cervical cancer. *Gynecol Oncol*. 2012;125(2):287–291. doi:10.1016/j.ygyno.2012.01.012
- Lee YY, Choi CH, Kim TJ, et al. A comparison of pure adenocarcinoma and squamous cell carcinoma of the cervix after radical hysterectomy in stage IB-IIA. *Gynecol Oncol*. 2011;120(3):439–443. doi:10.1016/j.ygyno.2010.11.022
- Saei Ghare Naz M, Kariman N, Ebadi A, Ozgoli G, Ghasemi V, Rashidi Fakari F. Educational interventions for cervical cancer screening behavior of women: a systematic review. *Asian Pac J Cancer Prev*. 2018;19(4):875–884.
- Park JY, Kim DY, Kim JH, Kim YM, Kim YT, Nam JH. Outcomes after radical hysterectomy in patients with early-stage adenocarcinoma of uterine cervix. *Br J Cancer*. 2010;102(12):1692–1698. doi:10.1038/sj.bjc.6605705
- Guttman M, Rinn JL. Modular regulatory principles of large non-coding RNAs. *Nature*. 2012;482(7385):339–346. doi:10.1038/nature10887
- Khalil AM, Guttman M, Huarte M, et al. Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. *Proc Natl Acad Sci U S A*. 2009;106(28):11667–11672. doi:10.1073/pnas.0904715106
- Bhan A, Soleimani M, Mandal SS. Long noncoding RNA and cancer: a new paradigm. *Cancer Res*. 2017;77(15):3965–3981. doi:10.1158/0008-5472.CAN-16-2634
- Enfield KS, Pikor LA, Martinez VD, Lam WL. Mechanistic roles of noncoding RNAs in lung cancer biology and their clinical implications. *Genet Res Int*. 2012;2012:737416. doi:10.1155/2012/737416
- Li T, Chen Y, Zhang J, Liu S. LncRNA TUG1 promotes cells proliferation and inhibits cells apoptosis through regulating AURKA in epithelial ovarian cancer cells. *Medicine*. 2018;97(36):e12131. doi:10.1097/MD.00000000000012131
- Chen J, Yu Y, Li H, et al. Long non-coding RNA PVT1 promotes tumor progression by regulating the miR-143/HK2 axis in gallbladder cancer. *Mol Cancer*. 2019;18(1):33. doi:10.1186/s12943-019-0947-9
- Pan R, He Z, Ruan W, et al. LncRNA FBXL19-AS1 regulates osteosarcoma cell proliferation, migration and invasion by sponging miR-346. *Onco Targets Ther*. 2018;11:8409–8420. doi:10.2147/OTT.S160963
- Yu DJ, Li YH, Zhong M. LncRNA FBXL19-AS1 promotes proliferation and metastasis via regulating epithelial-mesenchymal transition in non-small cell lung cancer. *Eur Rev Med Pharmacol Sci*. 2019;23(11):4800–4806.
- Ding Z, Ye P, Yang X, Cai H. LncRNA FBXL19-AS1 promotes breast cancer cells proliferation and invasion via acting as a molecular sponge to miR-718. *Biosci Rep*. 2019;39:4. doi:10.1042/BSR20182018
- Yang W, Hong L, Xu X, Wang Q, Huang J, Jiang L. LncRNA GAS5 suppresses the tumorigenesis of cervical cancer by downregulating miR-196a and miR-205. *Tumour Biol*. 2017;39(7):1010428317711315. doi:10.1177/1010428317711315
- Shen CJ, Cheng YM, Wang CL. LncRNA PVT1 epigenetically silences miR-195 and modulates EMT and chemoresistance in cervical cancer cells. *J Drug Target*. 2017;25(7):637–644. doi:10.1080/1061186X.2017.1307379
- Guo H, Yang S, Li S, Yan M, Li L, Zhang H. LncRNA SNHG20 promotes cell proliferation and invasion via miR-140-5p-ADAM10 axis in cervical cancer. *Biomed Pharmacother*. 2018;102:749–757. doi:10.1016/j.biopha.2018.03.024
- Wu Z, Liu X, Liu L, et al. Regulation of lncRNA expression. *Cell Mol Biol Lett*. 2014;19(4):561–575. doi:10.2478/s11658-014-0212-6
- Zhang Y, Xiao X, Zhou W, Hu J, Zhou D. LIN28A-stabilized FBXL19-AS1 promotes breast cancer migration, invasion and EMT by regulating WDR66. *In Vitro Cell Dev Biol Anim*. 2019;55(6):426–435. doi:10.1007/s11626-019-00361-4
- Qu J, Li M, Zhong W, Hu C. Competing endogenous RNA in cancer: a new pattern of gene expression regulation. *Int J Clin Exp Med*. 2015;8(10):17110–17116.
- Wang H, Huo X, Yang XR, et al. STAT3-mediated upregulation of lncRNA HOXD-AS1 as a ceRNA facilitates liver cancer metastasis by regulating SOX4. *Mol Cancer*. 2017;16(1):136. doi:10.1186/s12943-017-0680-1
- Chen DL, Lu YX, Zhang JX, et al. Long non-coding RNA UICLM promotes colorectal cancer liver metastasis by acting as a ceRNA for microRNA-215 to regulate ZEB2 expression. *Theranostics*. 2017;7(19):4836–4849.
- Shirafkan N, Shomali N, Kazemi T, et al. microRNA-193a-5p inhibits migration of human HT-29 colon cancer cells via suppression of metastasis pathway. *J Cell Biochem*. 2018.
- Pu Y, Zhao F, Cai W, Meng X, Li Y, Cai S. MiR-193a-3p and miR-193a-5p suppress the metastasis of human osteosarcoma cells by down-regulating Rab27B and SRR, respectively. *Clin Exp Metastasis*. 2016;33(4):359–372. doi:10.1007/s10585-016-9783-0
- Chou NH, Lo YH, Wang KC, Kang CH, Tsai CY, Tsai KW. MiR-193a-5p and -3p play a distinct role in gastric cancer: miR-193a-3p suppresses gastric cancer cell growth by targeting ETS1 and CCND1. *Anticancer Res*. 2018;38(6):3309–3318. doi:10.21873/anticancer.12596
- Leong KW, Cheng CW, Wong CM, Ng IO, Kwong YL, Tse E. miR-874-3p is down-regulated in hepatocellular carcinoma and negatively regulates PIN1 expression. *Oncotarget*. 2017;8(7):11343–11355. doi:10.18632/oncotarget.14526
- Chen M, Xia Y, Tan Y, Jiang G, Jin H, Chen Y. Downregulation of microRNA-370 in esophageal squamous-cell carcinoma is associated with cancer progression and promotes cancer cell proliferation via upregulating PIN1. *Gene*. 2018;661:68–77.
- Lee KH, Lin FC, Hsu TI, et al. MicroRNA-296-5p (miR-296-5p) functions as a tumor suppressor in prostate cancer by directly targeting Pin1. *Biochim Biophys Acta*. 2014;1843(9):2055–2066. doi:10.1016/j.bbamcr.2014.06.001

30. Ma JQ, Yang Y, Juan J, et al. Over-expression of prolyl isomerase Pin1 promotes cervical tumorigenesis and metastasis. *Int J Clin Exp Pathol.* **2018**;11(2):664–674.
31. Cheng C-W, Tse E. PIN1 in cell cycle control and cancer. *Front Pharmacol.* **2018**;9:1367. doi:10.3389/fphar.2018.01367
32. Zhang Z, Yu W, Zheng M, et al. Pin1 inhibition potently suppresses gastric cancer growth and blocks PI3K/AKT and Wnt/ β -catenin oncogenic pathways. *Mol Carcinog.* **2019**;58(8):1450–1464. doi:10.1002/mc.23027
33. Khanal P, Yeung B, Zhao Y, Yang X. Identification of Prolyl isomerase Pin1 as a novel positive regulator of YAP/TAZ in breast cancer cells. *Sci Rep.* **2019**;9(1):6394.
34. Li H, Wang S, Zhu T, et al. Pin1 contributes to cervical tumorigenesis by regulating cyclin D1 expression. *Oncol Rep.* **2006**;16(3):491–496.
35. Wang T, Liu Z, Shi F, Pin WJ. 1 modulates chemo-resistance by up-regulating FoxM1 and the involvements of Wnt/ β -catenin signaling pathway in cervical cancer. *Mol Cell Biochem.* **2016**;413(1):179–187. doi:10.1007/s11010-015-2651-4

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