

MiR-139-5p-ZEB1 is a Molecular Regulator of Growth, Invasion, and Epithelial-to-Mesenchymal Transition of Cervical Cancer

This article was published in the following Dove Press journal:
Cancer Management and Research

Jinrui Sun¹
Shanshan Wang²
Ping Liu¹
Yulan Liu³

¹Department of Gynecology, Shanxi Provincial People's Hospital, Taiyuan, Shanxi Province 030012, People's Republic of China; ²Department of Cardiology, Yidu Central Hospital of Weifang City, Weifang, Shandong, People's Republic of China; ³Department of Gynecology, Maternal and Child Health Hospital of Hubei Province, Wuhan, Hubei 430070, People's Republic of China

Objective: To verify that *miR-139-5p*-zinc finger *E*-box-binding homeobox 1 (*ZEB1*) is a molecular regulator of the biological function and epithelial-mesenchymal transition (EMT) of cervical cancer (CC) cells.

Methods: Cancerous tissues, corresponding paracancerous tissues, and serum were sampled from patients with CC. *MiR-139-5p* and *ZEB1* in tissue specimens, serum specimens, and purchased CC cell lines were quantified, and Pearson correlation coefficient was adopted for correlation analysis of *miR-139-5p* in clinical specimens. Receiver operating characteristic (ROC) curves were adopted to analyze the diagnostic value of *miR-139-5p* and *ZEB1* for CC. The expression of genes in CC cells was changed by transfection. The proliferation, colony formation, invasion, and apoptosis of cells were determined, and the protein level of EMT markers (N-cadherin, vimentin, and E-cadherin) was also quantified. Moreover, the targeting relationship between *miR-139-5p* and *ZEB1* was determined.

Results: Our data showed that the expression of *miR-139-5p* decreased greatly in CC tissues, and it also significantly decreased in the serum, while the expression of serum *ZEB1* was opposite. In addition, the *miR-139-5p* expression in CC tissues was positively correlated with that in serum, while serum *miR-139-5p* was negatively correlated with serum *ZEB1*. The areas under the curves (AUCs) of the two for identifying CC were 0.923 and 0.890, respectively. Both up-regulation of *miR-139-5p* and down-regulation of *ZEB1* suppressed the colony formation, proliferation, invasion, and EMT of CC cells, and intensified their apoptosis. Moreover, *miR-139-5p* negatively regulated the transcription of *ZEB1*, and down-regulation of the former could reverse the molecular regulatory effects of down-regulating *ZEB1* on the above biological behaviors of CC cells.

Conclusion: The above data imply that *miR-139-5p*-*ZEB1* axis may be the key to curbing the progression of CC.

Keywords: cervical cancer, *miR-139-5p*, *ZEB1*, growth, invasion, epithelial-mesenchymal transition

Introduction

Cervical cancer (CC) is a gynecologic tumor with poor prognosis, which may deteriorate to brain metastasis or bone metastasis.^{1,2} According to global data, there were 570,000 new cases with CC and up to 311,000 cases dying of the disease in 2018.³ At present, the exact mechanism of the progression of CC remains unclear, and biological indicators for the diagnosis of CC remain to be found,^{4,5} so it is crucial for the management of CC to study the mechanism of CC progression and find potential screening indicators.

Correspondence: Yulan Liu
Department of Gynecology, Maternal and Child Health Hospital of Hubei Province, NO. 745 Wuluo Road, Hongshan, Wuhan, Hubei 430070, People's Republic of China
Tel +86-15698784852
Email yulantang885@163.com

MicroRNA (miRNA), as a small non-coding RNA molecule, is a molecular regulator of the growth, invasion and epithelial–mesenchymal transition (EMT) of various tumors including CC.^{6–8} MiRNA is found to have regulatory effect in various gynecologic tumors. For example, in endometrial carcinoma, *miR-136* regulates the development of tumor cells by regulating NOTCH3 under the regulation of *Circ_pumilio homolog 1 (PUM1)*,⁹ and *miR-653-5p* accelerates the malignant growth of CC under the sponge of *DGUOK-AS1*.¹⁰ In the present study, we focused on the expression and role of *miR-139-5p* in CC, which reportedly serves as a tumor inhibitor in the disease.¹¹ It has been found that *miR-139-5p* can regulate the pathological processes of glioma such as metastasis, infiltration, and EMT.¹² We found a potential targeting relationship between *miR-139-5p* and *zinc finger E-box-binding homeobox 1 (ZEB1)* based on an online target gene prediction website. Moreover, it has been uncovered that both *miR-139-5p* and *ZEB1* have targeted regulatory effects on breast cancer (BC), colorectal cancer (CRC), as well as glioblastoma. For example, *miR-139-5p-ZEB1* axis affects the malignant biological behaviors of BC cells under the regulation of *TTN-AS1*, and it also affects the pathological changes of CRC cells under the regulation of *HLA complex P5 (HCP5)*.^{13–15} However, *ZEB1* is reported abnormally up-regulated in cases with CC, which affects the proliferation, metastatic behavior, and EMT of CC cells.^{16,17}

In the present study, we inferred that *miR-139-5p-ZEB1* was a molecular regulator of the growth, invasion, and EMT of CC, and thus we conducted relevant studies to verify it.

Materials and Methods

Sample Collection

This study was approved by the Ethics Committee of Shanxi Provincial People's Hospital, and written consent forms were obtained from all participants. Cancerous tissues and corresponding paracancerous tissues were sampled from 99 patients with CC (CC group) during operation from April 2016 to April 2020, and serum was also sampled from them before operation. All the patients were diagnosed with CC based on pathology.¹⁸ In addition, serum was sampled from 50 healthy individuals in physical examination during the same period as a healthy control group (HC group). All patients with CC had not received any surgery or drug treatment before sample

collection, and all sampled specimens were stored at –80°C for later analysis. Moreover, the clinical staging of the patients was evaluated according to the International Federation of Gynecology and Obstetrics (FIGO) criteria,¹⁹ and the histological grading of them is also evaluated in Table 1.

Cell Culturing

Human normal cervical epithelial cells (HUCEC) (FE1528, Qiming Biotechnology Co., Ltd., Shanghai, China) and human CC cells (HeLa 229, H1HeLa, C-33 A, and MS751) (YBCC102035, YBCC101654, YBCC101021, and YBCC100277, Yubo Biological Technology Co., Ltd., Shanghai, China) were incubated in dulbecco's modified eagle medium (DMEM; PM150220B, Yaji Biotechnology Co., Ltd., Shanghai, China) supplemented with 10% phosphate buffer saline (PBS) under 5% CO₂ at 37°C.

Cell Transfection

Transfectants used in this study included *miR-139-5p mimics (miR-139-5p)*, inhibition sequence (inhibitor), miR negative control (miR-NC), targetedly overexpressed sequence of *ZEB1 (ZEB1)*, targetedly inhibited sequence of *ZEB1 (si-ZEB1)*, and si-negative control (si-NC), which were mainly purchased from Shanghai Huishen Biological Technology Co., Ltd. CC cells were transfected with above transfectants, respectively using a cell transfection kit (YSRIBIO-C5838, Yansheng Industrial Co., Ltd., Shanghai, China) in strict accordance with the kit instructions. After 48 h, the transfected cells were harvested for later analysis.

Real-Time Polymerase Chain Reaction (PCR)

Total RNA was extracted from harvested tissues, serum, as well as cells by a TRIzol kit (R523-200, Spectral Experimental Equipment Technology Co., Ltd., Dongguan, China), followed by detection of its purity and concentration with an ultraviolet spectrophotometer (SPCC, Spectral Experimental Equipment Technology Co., Ltd., Dongguan, China). Subsequently, total RNA was reversely transcribed into cDNA and amplified. The data in this assay were analyzed using the $2^{-\Delta\Delta Ct}$, with β -Actin as internal reference for *ZEB1*, *Caspase-3*, *Bax*, and *Bcl-2*, and *U6* as internal reference for *miR-139-5p*.

Table I Relationship Between *MiR-139-5p* and Pathological Data of Patients with CC [Mean±SD]

Factor	n=76	<i>MiR-139-5p</i>	T-value	P-value
Menopause or not?			0.509	0.612
No	32	3.72±0.55		
Yes	44	3.65±0.62		
Age			0.892	0.376
<55 years old	38	3.75±0.61		
≥55 years old	38	3.62±0.66		
FIGO staging			6.204	<0.001
I/II	36	4.10±0.51		
III/IV	40	3.22±0.70		
Pathological differentiation			3.968	<0.001
Low differentiation	42	3.38±0.63		
Moderate and high differentiation	34	3.90±0.48		
Tumor diameter			0.887	0.378
<4cm	41	3.71±0.64		
≥4cm	35	3.59±0.52		
Histological type			0.925	0.358
Squamous cell carcinoma	48	3.73±0.65		
Adenocarcinoma	28	3.60±0.47		
SCC-Ag (ng/mL)			1.150	0.254
<1.5	26	3.81±0.41		
≥1.5	50	3.63±0.74		

Abbreviations: miR, microRNA; CC, cervical cancer; FIGO, Federation of Gynecology and Obstetrics; SCC-Ag, squamous cell carcinoma antigen.

Western Blot

Protein in tissues or cells was isolated by RIPA buffer (HLIT0050, Haling Biotechnology Co., Ltd., Shanghai, China), and then ionized and transferred to a membrane. Subsequently, the membrane was sealed with blocking solution (BH-DB6564, Bohu Biotechnology Co., Ltd., Shanghai, China) for 1 h, followed by incubation with primary antibodies (N-cadherin, vimentin, E-cadherin, *ZEB1*, and β -Actin) at 4°C overnight. Afterwards, the membrane was subjected to 1-h incubation with secondary antibody at 4°C. All antibodies were purchased from Shanghai Xuanya Biotechnology Co., Ltd. Finally, the protein strips were visualized and analyzed by Quantity One software (ECL-0013, EASYBIO Technology Co., Ltd., Beijing, China).

Cell Proliferation

Cell proliferation was determined using a MTT kit (RF(m) 11,473, Qiming Biotechnology Co., Ltd., Shanghai, China) as follows: The cells were seeded into a 96-well plate at 1000 cells/well, incubated at 37°C for 3 d, and then added

with 20μL MTT solution (2.5 mg/mL), followed by 3-h incubation. Subsequently, each well was added with 150 μL dimethyl sulfoxide, and the optical density of cells in each group at 570 nm was detected using an ELx808LBS microplate reader (ZEPING Bioscience & Technologies Co., Ltd., Beijing, China).

Colony Formation Assay

CC cells (2×10^3 cells) were seeded into a 6-well plate. After 14 d, the cells were immobilized with 4% paraformaldehyde (PFA; M002, Gefan Biotechnology Co., Ltd., Shanghai, China) at room temperature for 10–20 minutes, and then dyed with 0.05% crystal violet for 10–20 minutes. Finally, colonies were counted under a microscope.

Cell Invasion

Collected cells were transferred to a upper compartment coated with Matrigel (356,234, Haoyang Biotechnology Co., Ltd., Shanghai, China), and medium with 20% FBS was added into the lower compartment. The insert was incubated at 37°C for 24 h, and then the cells were immobilized with PFA for 10 min after being washed with PBS

three times. Then the cells were dyed with 0.5% crystal violet. Finally, invasive cells were counted under a microscope.

Cell Apoptosis

Cell apoptosis was detected by a cell apoptosis kit (BW3302, Biomiga Medical Technology Co., Ltd., Hangzhou, China) as follows: The transfected cells were trypsinized, and then the cells were washed with PBS twice, and then mixed with 100 μ L binding buffer to produce 1×10^6 cells/mL suspension. The suspension was mixed with 10 μ L AnnexinV-FITC and 10 μ L PI in sequence, cultured at room temperature in the dark for 5 min, and finally determined with a DxFLEX flow cytometer (Beckman Coulter Trading (China) Co., Ltd., Shanghai, China).

Luciferase Determination

Cells were transferred to a 24-well plate. After 24 h, *ZEB1* wild-type (*ZEB1*-Wt) and *ZEB1* mutant (*ZEB1*-Mut) were constructed by a dual luciferase reporter (DLR) gene assay kit (SLDL-100, Bei Nuo Biotechnology Co., Ltd., Shanghai, China) and transfected into cells. After 48 h, the luciferase activity of the cells was determined.

Xenotransplantation Tumor Model of Mouse

We purchased 12 female BALB/c nu/nu nude mice (4 weeks old and 18–22 g in weight) from Cavens Experimental Animal Co., Ltd., Changzhou, China, and raised them in specific pathogen-free conditions under 12 h light/12 h dark cycle and studied them. The animal study was approved by the Animal Ethics Committee of our hospital (Shanxi Provincial People's Hospital), and it was carried out in strict accordance with the Laboratory animal—Guideline for ethical review of animal welfare (Standardization Administration of the People's Republic of China) for protecting animals. First, the mice were fed for 1 week, and then randomly divided into two groups (MOCK group and *miR-139-5p* group). H1HeLa cells transfected with miR-NC or *miR-139-5p* mimics (107 cells per mouse) were injected subcutaneously into the left armpit of mice in the two groups. On the 21st day, mice were anesthetized with chloral hydrate (A4988, Shifeng Biological Technology Co., Ltd., Shanghai, China) and euthanized (decapitated), and their tumors were removed, measured and weighed.

Statistical Analyses

In the present study, all assays were repeated three times and the collected data were analyzed statistically and visualized into figures via GraphPad 6. Data differences were analyzed using the independent *t*-test, one-way ANOVA, LSD-*t*-test, and Bonferroni post hoc test. In addition, receiver operating characteristic (ROC) curves were drawn to analyze the area under the curve (AUC) of *miR-139-5p* in diagnosing CC, and Pearson correlation analysis was carried out to analyze the relationship between *miR-139-5p* expression in the serum of patients with CC and that in cancerous tissues of the patients, and the correlation between serum *miR-139-5p* and serum *ZEB1*. $P < 0.05$ indicates a remarkable difference.

Results

MiR-139-5p Was Down-Regulated in Cancerous Tissues and Serum of Patients with CC, While *ZEB1* Was Up-Regulated in Them

For the purpose of verifying our assumption, we quantified *miR-139-5p* in the cancerous tissues and serum specimens from patients with CC, finding that *miR-139-5p* was lowly expressed in both specimens. The results implied that *miR-139-5p* may serve as a cancer suppressor in CC. Further investigation revealed that the *miR-139-5p* expression in the two specimens was positively correlated, suggesting that *miR-139-5p* may have a certain clinical value as a non-invasive serum indicator. We also found that the expression of *ZEB1* in the two samples was significantly opposite to that of *miR-139-5p* in them, and the expression of *miR-139-5p* and *ZEB1* in serum was significantly negatively correlated. Then, we drew ROC curves of *miR-139-5p* and *ZEB1* in identifying CC as serum indicators, and found that the AUC of *miR-139-5p* for diagnosing CC was as high as 0.923, while that of *ZEB1* was 0.890, indicating that they may be used as auxiliary indicators for CC screening [Figure 1](#).

MiR-139-5p Was Linked to the FIGO Staging and Pathological Differentiation of Patients with CC

With the aim of exploring the potential correlation between *miR-139-5p* and pathological parameters of CC patients, we analyzed the relative expression of *miR-139-5p* in patients with different pathological parameters, finding that *miR-*

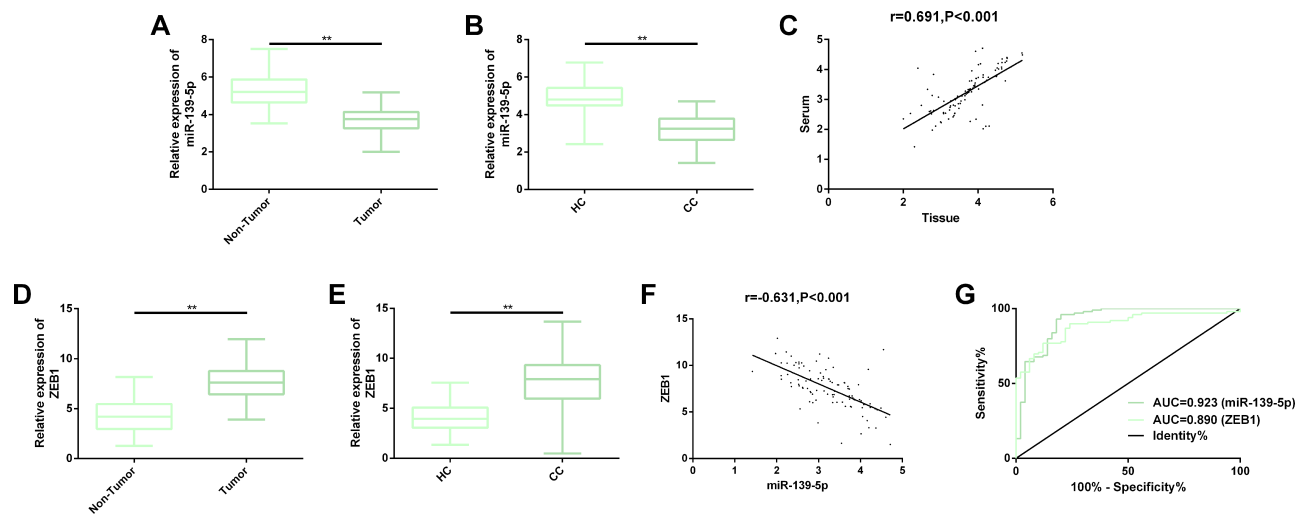


Figure 1 Expression of *miR-139-5p* and *ZEB1* in the cancerous tissues and serum of patients with CC. (A, B) *MiR-139-5p* was down-regulated in both cancerous tissues and serum of patients with CC. (C) The expression of *miR-139-5p* in cancerous tissues was strongly positively correlated with that in serum. (D, E) The expression of *ZEB1* was up-regulated in cancerous tissues and serum of patients with CC. (F) *MiR-139-5p* and *ZEB1* in serum were negatively correlated. (G) AUC of serum *miR-139-5p* in identifying CC exceeded 0.900, and that of serum *ZEB1* for identifying CC exceeded 0.850.

Note: **Indicates that in terms of inter-group comparison, $P < 0.01$.

Abbreviations: miR, microRNA; CC, cervical cancer; AUC, area under the curve; HC, healthy control; *ZEB1*, zinc finger E-box-binding homeobox 1.

139-5p was not strongly linked to menopause, age, tumor diameter, histological grading, as well as squamous cell carcinoma antigen (SCC-Ag) (all $P > 0.05$), but low *miR-139-5p* expression was significantly linked to high FIGO staging and low differentiation (both $P < 0.05$). The results imply that *miR-139-5p* may be helpful to predict FIGO staging and differentiation of patients in Table 1.

MiR-139-5p Could Inhibit the Growth, Invasion, and EMT of CC Cells

In order to explore the potential anticancer effect of *miR-139-5p* in CC, we analyzed its effect on the biological behaviors of CC cells. First of all, we found that *miR-139-5p* was generally expressed at low levels in CC cells, especially in H1HeLa and C-33 A cells, so we mainly studied H1HeLa and C-33A cells. We transfected *miR-139-5p* mimics and *miR-139-5p* inhibition sequence into H1HeLa and C-33 A cells to overexpress and underexpress *miR-139-5p* in them, respectively. The cellular function test revealed that down-regulating *miR-139-5p* accelerated the malignant proliferation, invasion, colony formation, and EMT of CC cells, inhibited the apoptosis rate, decreased the levels of pro-apoptotic factors (*Caspase-3* and *Bax*), and increased the level of anti-apoptotic factor (*Bcl-2*), but the above results were significantly reversed after *miR-139-5p* was up-regulated. The malignant behaviors of CC cells were strongly suppressed and the cell apoptosis was induced. The results

implied that regulating *miR-139-5p* could control the malignant development of CC cells, and *miR-139-5p* had certain cancer inhibiting effect in CC Figure 2.

MiR-139-5p Negatively Affected the Transcription and Protein Level of *ZEB1*

For the purpose of further understanding the mechanism of *miR-139-5p* in CC, we also analyzed its potential downstream targets. Based on TargetScan (http://www.targetscan.org/vert_72/), we found potential binding locus between *miR-139-5p* and *ZEB1*, so we carried out analysis for further verification. The DLR revealed that *miR-139-5p* mimics down-regulated only *ZEB1*-Wt (not *ZEB1*-Mut), and the Western blot assay showed that *miR-139-5p* negatively regulated the transcription and protein level of *ZEB1*, which suggested that *miR-139-5p* could regulate *ZEB1* negatively (Figure 3).

ZEB1 Was Up-Regulated in CC Cells and Could Promote the Growth, Invasion, and EMT of CC Cells

We also explored whether *ZEB1* had influence on the malignant function of CC cells. First of all, we quantified *ZEB1* in CC cells, and found that it was generally abnormally up-regulated, indicating that it may also be involved in the malignant development of CC cells. Further analysis of cell behaviors showed that up-regulation of *ZEB1*

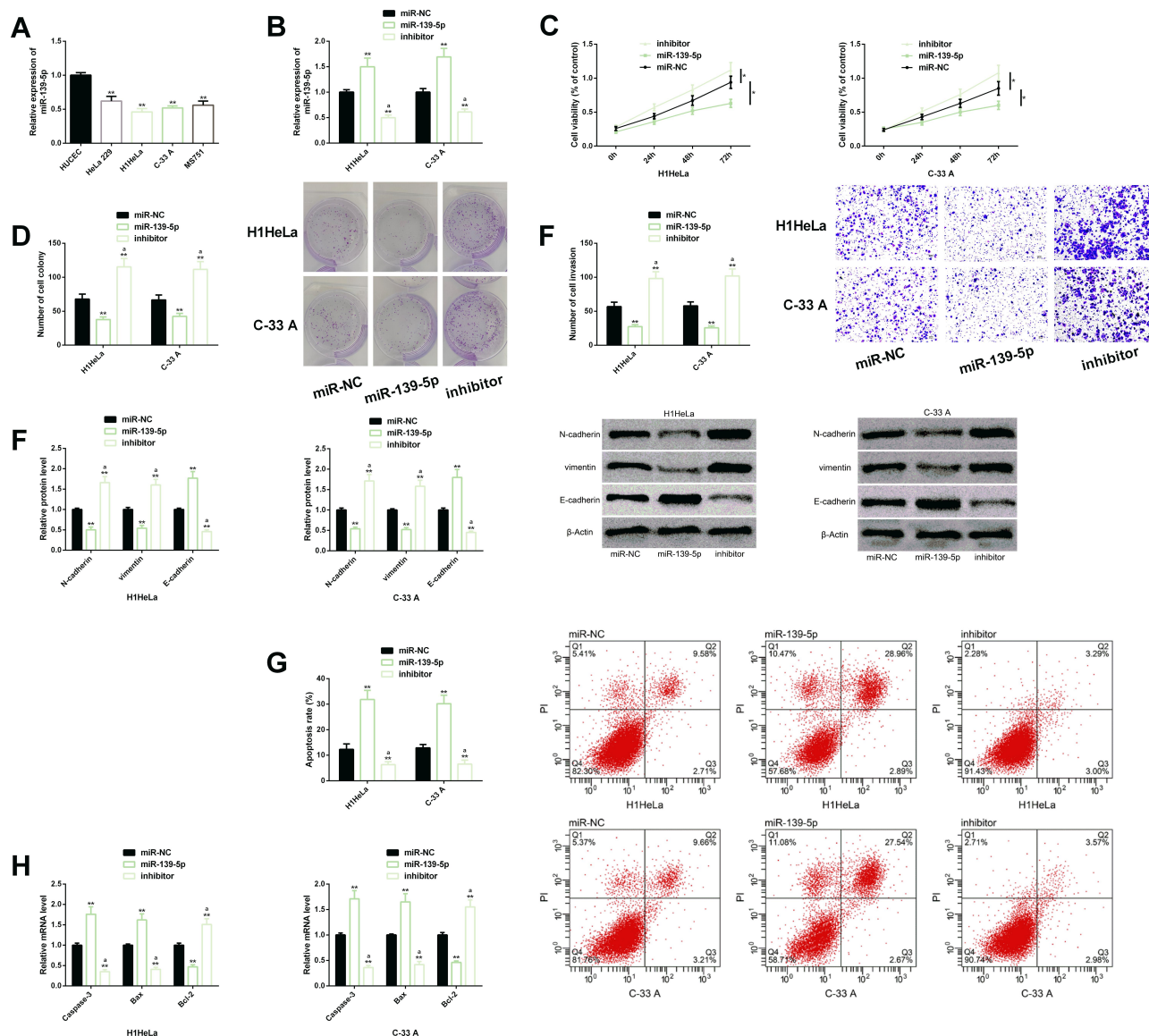


Figure 2 Effects of *miR-139-5p* on the biological function of CC cells. (A) *Mir-139-5p* was significantly down-regulated in CC cells. (B) Transfection efficiency of *miR-139-5p*. (C–F) Up-regulation of *miR-139-5p* significantly inhibited the malignant proliferation, colony formation, invasion, EMT of CC cells. Protein profiling of EMT-related factors of CC cells. (G) Up-regulation of *miR-139-5p* significantly promoted the apoptosis rate of CC cells. Its cell flow cytometry profiling. (H) Down-regulation of *ZEB1* increased the expression of *Caspase-3* and *Bax*, but inhibited the expression of *Bcl-2*.

Notes: In terms of inter-group comparison or comparison with *miR-NC*, *Indicates $P < 0.05$ and **Indicates $P < 0.01$; in terms of comparison with *miR-139-5p*, ^aIndicates $P < 0.01$ in terms of comparison with *miR-139-5p*.

Abbreviations: miR, microRNA; CC, cervical cancer; EMT, epithelial–mesenchymal transition; NC, negative control; PI, propidium iodide.

promoted the malignant proliferation, colony formation, invasion, and EMT of CC cells, strongly inhibited cell apoptosis, decreased the expression of *Caspase-3* and *Bax*, and increased the expression of *Bcl-2*, while knock-down of *ZEB1* significantly inhibited the malignant function of CC cells and intensified their apoptosis. The results implied that both down-regulating *ZEB1* and up-regulating *miR-139-5p* had significant anti-tumor effects on CC cells (Figure 4).

Down-Regulating *Mir-139-5p* Could Weaken the Anti-Tumor Activity of Transfecting Si-*ZEB1* in CC Cells

We carried out a co-transfection experiment about *miR-139-5p* and *ZEB1*, finding that down-regulating *miR-139-5p* could reverse the inhibiting effect of down-regulating *ZEB1* on the malignant proliferation, invasion, colony formation, as well as EMT of CC cells and induction of it on the cell apoptosis (including the effect on apoptotic factor),

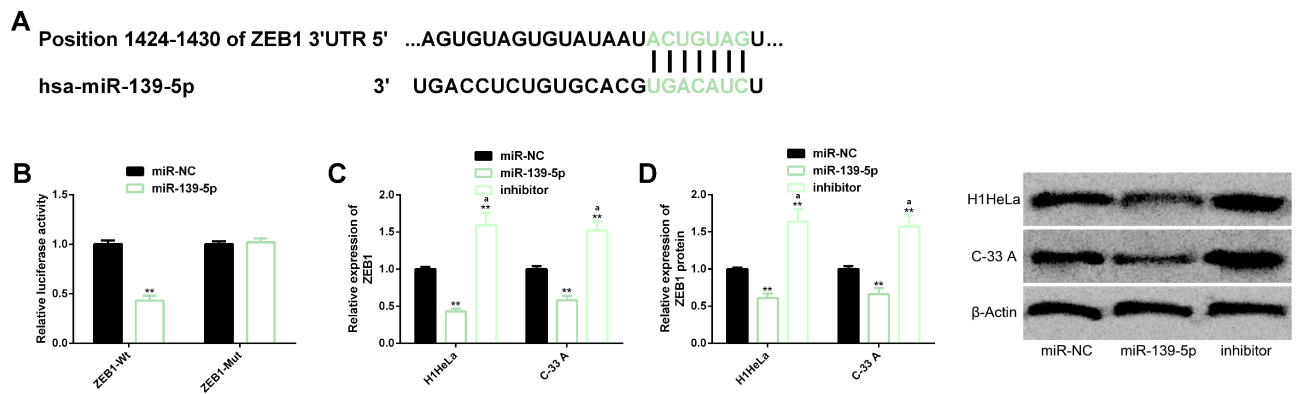


Figure 3 Targeting regulatory relationship between *miR-139-5p* and *ZEB1*. **(A)** Binding locus between *miR-139-5p* and *ZEB1*. **(B)** DLR result. **(C, D)** Up-regulation of *miR-139-5p* down-regulated the transcription and protein level of *ZEB1*. Its protein profiling.

Notes: **Indicates $P < 0.01$ in terms of comparison with the miR-NC group. ^aIndicates $P < 0.01$ in terms of comparison with the miR-139-5p group.

Abbreviations: miR, microRNA; *ZEB1*, zinc finger E-box-binding homeobox 1; DLR, dual luciferase reporter; NC, negative control; Wt, wild type; Mut, mutant.

which implied that *miR-139-5p* regulated the biological behaviors of CC cells through targeting *ZEB1* (Figure 5).

Up-Regulation of *MiR-139-5p* Can Inhibit Tumor Growth in CC Xenotransplantation Model Mice

To study the effect of *miR-139-5p* on the tumor growth of CC model in vivo, H1HeLa cells transfected with miR-NC or *miR-139-5p* mimetic were inoculated into the left armpit of nude mice. The results showed that at 4 weeks after inoculation, up-regulation of *miR-139-5p* significantly inhibited the tumor volume and mass in CC xenotransplantation model mice compared with the MOCK group (Figure 6).

Discussion

The etiology of CC is strongly linked to human papillomavirus (HPV) infection. As a fatal gynecologic tumor, CC shows a continuously increasing incidence in some countries, which results in great cancer burden to women.^{20,21} At present, CC is intractable, which is mainly manifested in drug resistance and lack of effective treatment strategies for advanced or recurrent CC.²² A large body of evidence has verified that the abnormal dysregulation of miRNA is linked to tumor progression,^{23,24} so analyzing the molecular mechanism of miRNA in CC is of great value for improving treatment schemes, prevention, and management of CC.

According to clinical analysis, *miR-139-5p* was expressed at low levels in cancerous tissues and serum of patients with CC, while *ZEB1* was expressed at high levels

in them, and both serum *miR-139-5p* and *ZEB1* had relatively high diagnostic value (AUC=0.923 and 0.890, respectively). Therefore, we believed that the two may have good diagnostic value in CC, and the former may inhibit cancer, while the latter may induce cancer. One study by Miyoshi et al²⁵ has pointed out that serum *miR-139-5p* in patients with CRC is helpful to predict tumor recurrence and metastasis. In this study, further analysis showed that low *miR-139-5p* level was strongly linked to high FIGO staging and low pathological differentiation, indicating that *miR-139-5p* level also has certain predictive value for pathological parameters of patients. According to one report by Wang et al,²⁶ low *miR-139-5p* level is linked to FIGO staging and lymph node metastasis of patients with ovarian cancer, which is different from the results of our study. Similarly, one study by Ji et al¹¹ has revealed that the low *miR-139-5p* level was strongly linked to lymph node metastasis of patients with CC. We inferred that *miR-139-5p* may also be adopted to predict the prognosis of patients, but we did not get data about it in this study, so we were unable to further verify it. In addition to cases with CC, *miR-139-5p* is also lowly expressed in cases with non-small cell lung cancer (NSCLC), low-grade glioma in children, as well as prostate cancer, implying that *miR-139-5p* may have relatively extensive cancer inhibition performance.^{27–29} For example, it exerts anti-cancer effect in NSCLC by inhibiting oncogenic gene, c-Met, in a targeted manner, and suppresses the survival of low-grade glioma cells in children by disabling oncogenic signaling pathway conduction (PI3K-AKT pathway) in them. In addition, it also hinders the progression of prostate cancer by lowering *SOX5*. On the other hand,

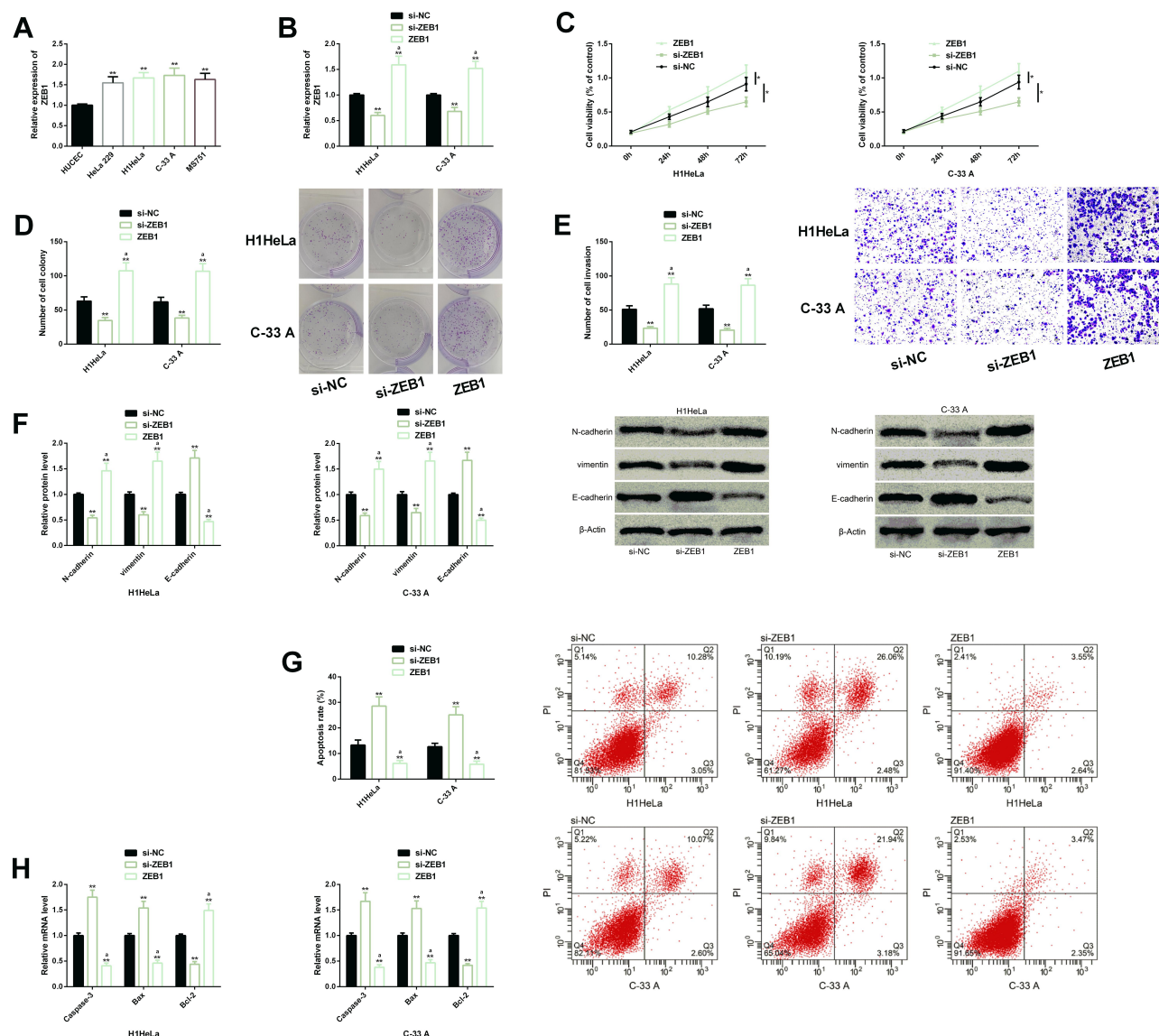


Figure 4 Effects of *ZEB1* on the biological function of CC cells. (A) *ZEB1* was generally highly expressed in CC cells. (B) Transfection efficiency of *ZEB1*. (C–F) Down-regulation of *ZEB1* significantly inhibited the malignant proliferation, colony formation, invasion, EMT of CC cells. Protein profiling of EMT-related factors. (G) Down-regulation of *ZEB1* significantly promoted the apoptosis rate of CC cells. Its cell flow cytometry profiling. (H) Down-regulation of *ZEB1* increased the expression of *Caspase-3* and *Bax*, but inhibited the expression of *Bcl-2*.

Notes: In terms of inter-group comparison or comparison with si-NC, *Indicates $P < 0.05$ and **Indicates $P < 0.01$; in terms of comparison with *ZEB1*, ^aIndicates $P < 0.01$.

Abbreviations: *ZEB1*, zinc finger E-box-binding homeobox 1; CC, cervical cancer; EMT, epithelial–mesenchymal transition; si, short interfering; NC, negative control; PI, propidium iodide; Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma-2.

ZEB1 is not only highly expressed in cases with CC, but also overexpressed in cases with diseases such as esophageal squamous cell carcinoma, liver cancer, and cutaneous squamous cell carcinoma, which suggests that it may have extensive carcinogenicity.^{30–32}

An increasing number of scholars have shown interest in studying the influence of *miR-139-5p* on gynecologic tumors, and have published many studies. For instance, Liu et al³³ have reported that *miR-139-5p* can suppress the viability and migration of endometrial carcinoma cells

through targeted inhibition on *HOXA10*. Liu et al³⁴ have pointed out that *miR-139-5p* can suppress the progression of ovarian cancer by regulating downstream target, *ROCK2*, under the targeted control of *TTN-AS1*. Furthermore, Ji et al¹¹ have verified that *miR-139-5p* can mediate *TCF4* and inactivate the *Wnt/β-catenin* signal, thereby exerting potential therapeutic effect on CC. In our study, *miR-139-5p* exerted its anticancer ability in CC by inhibiting the proliferation, colony formation, invasion, and EMT of CC cells and intensifying their apoptosis, increasing the

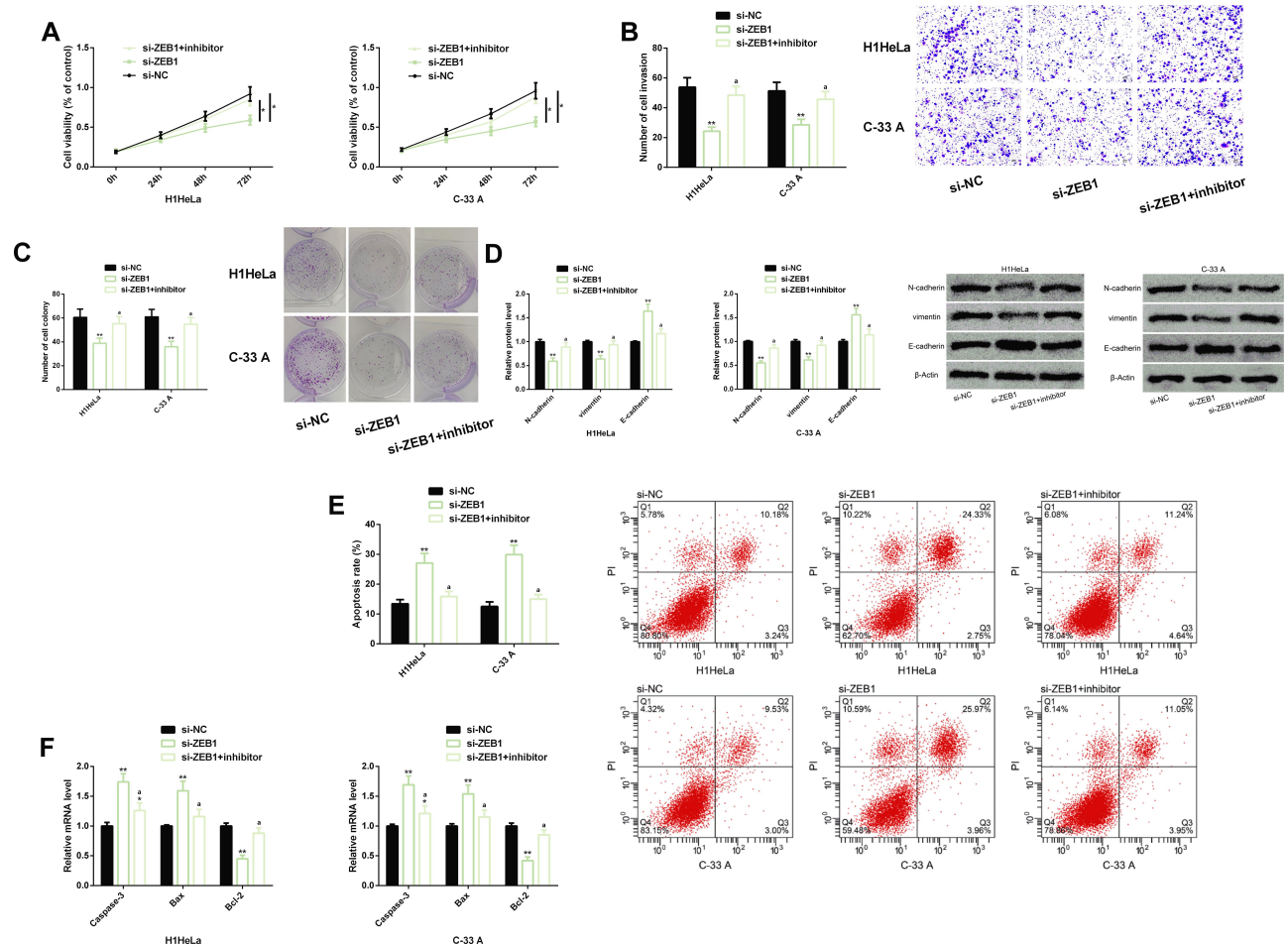


Figure 5 Effects of down-regulating *miR-139-5p* on the anti-tumor activity of *si-ZEB1* in CC cells. (A–D) Inhibiting *miR-139-5p* could reverse the inhibiting effect of down-regulating *ZEB1* on the malignant proliferation, invasion, and colony formation, and EMT of CC cells. Protein profiling of EMT-related factors. (E) Inhibiting *miR-139-5p* could eliminate the induction of *si-ZEB1* on the apoptosis of CC cells. Cell flow cytometry profiling. (F) Inhibiting *miR-139-5p* could eliminate the induction of *si-ZEB1* on pro-apoptotic factors (*Caspase-3* and *Bax*) and the inhibition of it on anti-apoptotic factor (*Bcl-2*).

Notes: In terms of inter-group comparison or comparison with *miR-NC*, *Indicates $P < 0.05$ and **Indicates $P < 0.01$; in terms of comparison with *miR-139-5p*, ^aIndicates $P < 0.01$.

Abbreviations: miR, microRNA; *ZEB1*, zinc finger E-box-binding homeobox 1; CC, cervical cancer; EMT, epithelial–mesenchymal transition; si, short interfering; NC, negative control; PI, propidium iodide; *Bax*, Bcl-2-associated X protein; *Bcl-2*, B-cell lymphoma-2.

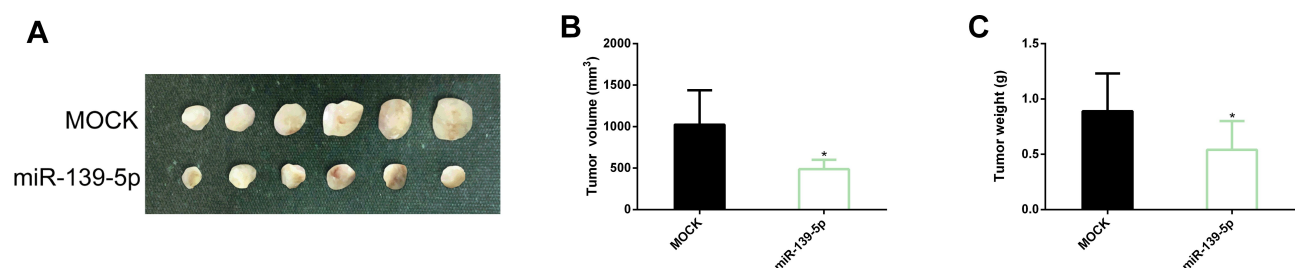


Figure 6 Effect of up-regulating *miR-139-5p* on tumor growth in CC xenotransplantation model mice. (A) Tumor experimental map of CC xenotransplantation model mice in the two groups. (B, C) Up-regulation of *miR-139-5p* could inhibit tumor volume and mass in CC xenotransplantation model mice.

Note: *Indicates $P < 0.05$ vs. the MOCK group.

Abbreviations: miR, microRNA; CC, cervical cancer.

expression of pro-apoptotic factors (*Caspase-3* and *Bax*) and decreasing the expression of anti-apoptotic factor *Bcl-2*. Further exploration on the mechanism of *miR-139-5p*

revealed that it had a targeted relationship with *ZEB1*, and could negatively regulate the level of *ZEB1*, and down-regulating *ZEB1* could suppress the aforementioned

malignant biological behaviors of CC cells and induce apoptosis. *ZEB1* has also been found to be carcinogenic in a variety of gynecological tumors. For instance, Chen et al³⁵ have reported that *ZEB1* can promote the accumulation of tumor-related macrophages in the hypoxic tumor microenvironment, thus aggravating the malignant progression of CC. Cui et al³⁶ have also reported that *ZEB1* is related to the resistance of ovarian cancer cells against chemotherapy, and targeted regulation of *ZEB1-SLC3A2* is conducive to strengthening cisplatin-related chemosensitivity. We down-regulated the two indexes meantime, and found that down-regulating *miR-139-5p* could strongly eliminate the anti-tumor activity after down-regulation of *ZEB1*. Namely, the inhibitory effect of transfecting si-*ZEB1* on the malignant progression of CC cells was greatly weakened. All data suggest that *miR-139-5p* can ameliorate the malignant progression of CC cells through targeted inhibition on *ZEB1*, and the *miR-139-5p-ZEB1* axis had molecular regulatory influence on the growth, invasion, as well as EMT of CC cells. Finally, we also found through xenotransplantation assay that up-regulation of *miR-139-5p* could inhibit the growth of tumor in CC mice, namely reducing the volume and mass of tumor, which indicated that over-expression of *miR-139-5p* had a positive effect on inhibiting the development of tumor in CC mice. Based on the above research results, we believe that the development of preparations for targetedly overexpressing *miR-139-5p* or *ZEB1* inhibitors may be helpful to the treatment of patients with CC.

Our study has confirmed the functional expression of *miR-139-5p-ZEB1* in CC, but it still has some room for improvement. First of all, we can supplement the relevant research on whether *miR-139-5p* has upstream factors in the regulatory mechanism of CC to further expand the molecular regulatory network. In addition, we can also explore the effect of *miR-139-5p* on *miR-139-5p-ZEB1* signal transduction pathway by analyzing whether the molecular action of the pathway has reverse influence on *miR-139-5p*. Furthermore, we can analyze the influence of *miR-139-5p* on the chemical sensitivity of CC and explore whether *miR-139-5p* is helpful to alleviate the drug resistance of patients. Finally, we can supplement the detection of inflammatory factors to further explore the effect of *miR-139-5p* on inflammation in pathological process of CC. In the future, we will gradually improve our research from the above points.

To sum up, we have proposed for the first time that *miR-139-5p-ZEB1* axis can exert molecular regulation on the growth, invasion, and EMT of CC, which may provide a new direction for treating CC.

Disclosure

The authors report no conflicts of interest for this work.

References

- Kim H, Lee KK, Heo MH, Kim JY. The prognostic factors influencing overall survival in uterine cervical cancer with brain metastasis. *Korean J Intern Med.* 2019;34(6):1324–1332.
- Zhang Y, Guo X, Wang G, et al. Real-world study of the incidence, risk factors, and prognostic factors associated with bone metastases in women with uterine cervical cancer using surveillance, Epidemiology, and End Results (SEER) data analysis. *Med Sci Monit.* 2018;24:6387–6397. doi:10.12659/MSM.912071
- Arbyn M, Weiderpass E, Bruni L, et al. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. *Lancet Glob Health.* 2020;8(2):e191–e203. doi:10.1016/S2214-109X(19)30482-6
- Yao S, Xu J, Zhao K, et al. Down-regulation of HPGD by miR-146b-3p promotes cervical cancer cell proliferation, migration and anchorage-independent growth through activation of STAT3 and AKT pathways. *Cell Death Dis.* 2018;9(11):1055. doi:10.1038/s41419-018-1059-y
- Park S, Eom K, Kim J, et al. MiR-9, miR-21, and miR-155 as potential biomarkers for HPV positive and negative cervical cancer. *BMC Cancer.* 2017;17(1):658. doi:10.1186/s12885-017-3642-5
- Liu S, Song L, Zeng S, Zhang L. MALAT1-miR-124-RBG2 axis is involved in growth and invasion of HR-HPV-positive cervical cancer cells. *Tumour Biol.* 2016;37(1):633–640. doi:10.1007/s13277-015-3732-4
- Xin M, Qiao Z, Li J, et al. miR-22 inhibits tumor growth and metastasis by targeting ATP citrate lyase: evidence in osteosarcoma, prostate cancer, cervical cancer and lung cancer. *Oncotarget.* 2016;7(28):44252–44265. doi:10.18632/oncotarget.10020
- Qu ZY, Cui GY, Shi PJ, Wang HQ. Potential suppressive functions of microRNA-504 in cervical cancer cells malignant process were achieved by targeting PAICS and regulating EMT. *Arch Gynecol Obstet.* 2020;302(1):173–182. doi:10.1007/s00404-020-05538-x
- Zong ZH, Liu Y, Chen S, Zhao Y. Circ PUM1 promotes the development of endometrial cancer by targeting the miR-136/NOTCH3 pathway. *J Cell Mol Med.* 2020;24(7):4127–4135. doi:10.1111/jcmm.15069
- Wu N, Song H, Ren Y, Tao S, Li S. DGUOK-AS1 promotes cell proliferation in cervical cancer via acting as a ceRNA of miR-653-5p. *Cell Biochem Funct.* 2020.
- Ji X, Guo H, Yin S, Du H. miR-139-5p functions as a tumor suppressor in cervical cancer by targeting TCF4 and inhibiting Wnt/beta-catenin signaling. *Onco Targets Ther.* 2019;12:7739–7748. doi:10.2147/OTT.S215796
- Li J, Li Q, Lin L, et al. Targeting the Notch1 oncogene by miR-139-5p inhibits glioma metastasis and epithelial-mesenchymal transition (EMT). *BMC Neurol.* 2018;18(1):133.
- Fang J, Huang C, Ke J, et al. lncRNA TTN-AS1 facilitates proliferation, invasion, and epithelial-mesenchymal transition of breast cancer cells by regulating miR-139-5p/ZEB1 axis. *J Cell Biochem.* 2020. doi:10.1002/jcb.29700
- Yang C, Sun J, Liu W, et al. Long noncoding RNA HCP5 contributes to epithelial-mesenchymal transition in colorectal cancer through ZEB1 activation and interacting with miR-139-5p. *Am J Transl Res.* 2019;11(2):953–963.
- Yue S, Wang L, Zhang H, et al. miR-139-5p suppresses cancer cell migration and invasion through targeting ZEB1 and ZEB2 in GBM. *Tumour Biol.* 2015;36(9):6741–6749. doi:10.1007/s13277-015-3372-8
- Xu J, Wang H, Wang H, et al. The inhibition of miR-126 in cell migration and invasion of cervical cancer through regulating ZEB1. *Hereditas.* 2019;156:11.

17. Gan L, Chen Y, Liu H, Ju WH. Long non-coding RNA ZEB1-antisense 1 affects cell migration and invasion of cervical cancer by regulating epithelial-mesenchymal transition via the p38MAPK signaling pathway. *Gynecol Obstet Invest.* **2019**;84(2):136–144. doi:10.1159/000493265
18. Kuhn L, Saidu R, Boa R, et al. Clinical evaluation of modifications to a human papillomavirus assay to optimise its utility for cervical cancer screening in low-resource settings: a diagnostic accuracy study. *Lancet Glob Health.* **2020**;8(2):e296–e304. doi:10.1016/S2214-109X(19)30527-3
19. Canaz E, Ozyurek ES, Erdem B, et al. Preoperatively assessable clinical and pathological risk factors for parametrial involvement in surgically treated FIGO stage IB-IIA cervical cancer. *Int J Gynecol Cancer.* **2017**;27(8):1722–1728. doi:10.1097/IGC.0000000000001060
20. Liu M, Wang Z, Liu Q, Zhu H, Xu N. Expression of micro-RNA-492 (MiR-492) in human cervical cancer cell lines is upregulated by transfection with wild-type P53, irradiation, and 5-fluorouracil treatment in vitro. *Med Sci Monit.* **2018**;24:7750–7758. doi:10.12659/MSM.911585
21. Msyamboza KP, Phiri T, Sichali W, Kwenda W, Kachale F. Cervical cancer screening uptake and challenges in Malawi from 2011 to 2015: retrospective cohort study. *BMC Public Health.* **2016**;16(1):806. doi:10.1186/s12889-016-3530-y
22. Rui X, Xu Y, Jiang X, Ye W, Huang Y, Jiang J. Long non-coding RNA C5orf66-AS1 promotes cell proliferation in cervical cancer by targeting miR-637/RING1 axis. *Cell Death Dis.* **2018**;9(12):1175. doi:10.1038/s41419-018-1228-z
23. Babion I, Jaspers A, van Splunter AP, van der Hoorn IAE, Wilting SM, Steenbergen RDM. miR-9-5p exerts a dual role in cervical cancer and targets transcription factor TWIST1. *Cells.* **2019**;9:1. doi:10.3390/cells9010065
24. Gu X, Dong M, Liu Z, Yang J, Shi Y. MiR-499a-5p inhibits proliferation, invasion, migration, and epithelial-mesenchymal transition, and enhances radiosensitivity of cervical cancer cells via targeting eIF4E. *Onco Targets Ther.* **2020**;13:2913–2924. doi:10.2147/OTT.S241631
25. Miyoshi J, Toden S, Yoshida K, et al. MiR-139-5p as a novel serum biomarker for recurrence and metastasis in colorectal cancer. *Sci Rep.* **2017**;7:43393. doi:10.1038/srep43393
26. Wang Y, Li J, Xu C, Zhang X. MicroRNA-139-5p inhibits cell proliferation and invasion by targeting RHO-associated coiled-coil-containing protein kinase 2 in ovarian cancer. *Oncol Res.* **2018**;26(3):411–420. doi:10.3727/096504017X14974343584989
27. Sun C, Sang M, Li S, et al. Hsa-miR-139-5p inhibits proliferation and causes apoptosis associated with down-regulation of c-Met. *Oncotarget.* **2015**;6(37):39756–39792. doi:10.18632/oncotarget.5476
28. Catanzaro G, Besharat ZM, Miele E, et al. The miR-139-5p regulates proliferation of supratentorial paediatric low-grade gliomas by targeting the PI3K/AKT/mTORC1 signalling. *Neuropathol Appl Neurobiol.* **2018**;44(7):687–706.
29. Yang B, Zhang W, Sun D, et al. Downregulation of miR-139-5p promotes prostate cancer progression through regulation of SOX5. *Biomed Pharmacother.* **2019**;109:2128–2135. doi:10.1016/j.biopha.2018.09.029
30. Zhao YL, Li JB, Li YJ, Li SJ, Zhou SH, Xia H. Capn4 promotes esophageal squamous cell carcinoma metastasis by regulating ZEB1 through the Wnt/beta-catenin signaling pathway. *Thorac Cancer.* **2019**;10(1):24–32. doi:10.1111/1759-7714.12893
31. Qin Y, Yu J, Zhang M, Qin F, Lan X. ZEB1 promotes tumorigenesis and metastasis in hepatocellular carcinoma by regulating the expression of vimentin. *Mol Med Rep.* **2019**;19(3):2297–2306.
32. Murata M, Ito T, Tanaka Y, Yamamura K, Furue K, Furue M. OVOL2-mediated ZEB1 downregulation may prevent promotion of actinic keratosis to cutaneous squamous cell carcinoma. *J Clin Med.* **2020**;9(3):618. doi:10.3390/jcm9030618
33. Liu J, Li C, Jiang Y, Wan Y, Zhou S, Cheng W. Tumor-suppressor role of miR-139-5p in endometrial cancer. *Cancer Cell Int.* **2018**;18:51. doi:10.1186/s12935-018-0545-8
34. Liu X, Li Y, Wen J, Qi T, Wang Y. Long non-coding RNA TTN-AS1 promotes tumorigenesis of ovarian cancer through modulating the miR-139-5p/ROCK2 axis. *Biomed Pharmacother.* **2020**;125:109882. doi:10.1016/j.biopha.2020.109882
35. Chen XJ, Deng YR, Wang ZC, et al. Hypoxia-induced ZEB1 promotes cervical cancer progression via CCL8-dependent tumour-associated macrophage recruitment. *Cell Death Dis.* **2019**;10(7):508. doi:10.1038/s41419-019-1748-1
36. Cui Y, Qin L, Tian D, et al. ZEB1 promotes chemoresistance to cisplatin in ovarian cancer cells by suppressing SLC3A2. *Chemotherapy.* **2018**;63(5):262–271. doi:10.1159/000493864

Cancer Management and Research

Publish your work in this journal

Cancer Management and Research is an international, peer-reviewed open access journal focusing on cancer research and the optimal use of preventative and integrated treatment interventions to achieve improved outcomes, enhanced survival and quality of life for the cancer patient.

Submit your manuscript here: <https://www.dovepress.com/cancer-management-and-research-journal>

Dovepress

The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.