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MicroRNA-377 Targets Zinc Finger E-box-Binding Homeobox 2 to Inhibit Cell Proliferation and Invasion of Cervical Cancer

Cong Ye,* Yubo Hu,† and Junrong Wang*

*Department of Obstetrics and Gynecology, China–Japan Union Hospital of Jilin University, Changchun, Jilin, P.R. China
†Department of Anesthesiology, China–Japan Union Hospital of Jilin University, Changchun, Jilin, P.R. China

A large number of microRNAs (miRNAs) are aberrantly expressed in cervical cancer and play crucial roles in the onset and progression of cervical cancer by acting as either an oncogene or a tumor suppressor. Therefore, investigation of the expression, biological roles, and underlying mechanisms of miRNAs in cervical cancer might provide valuable therapeutic targets in the treatment for patients with this disease. In this study, miRNA-377 (miR-377) was downregulated in cervical cancer tissues and cell lines. Decreased miR-377 expression was strongly correlated with the International Federation of Gynecology and Obstetrics (FIGO) stage, lymph node metastasis, and distant metastasis in patients with cervical cancer. Enhanced expression of miR-377 prohibited cell proliferation and invasion in cervical cancer. Bioinformatics analysis predicted that zinc finger E-box-binding homeobox 2 (ZEB2) was a potential target of miR-377. Subsequent experiments confirmed that ZEB2 is a direct target gene of miR-377 in cervical cancer. In addition, ZEB2 was overexpressed in cervical cancer tissues and was inversely related with miR-377 levels. Furthermore, the suppressive effects of miR-377 on cervical cancer proliferation and invasion were rescued by restored ZEB2 expression. Overall, our findings indicated that miR-377 decreases proliferation and invasion of cervical cancer cells by directly targeting ZEB2 and provides novel evidence of miR-377 as a novel therapeutic strategy for the therapy of patients with this malignancy.

Key words: Cervical cancer; MicroRNA-377; Zinc finger E-box-binding homeobox 2 (ZEB2); Proliferation; Invasion

INTRODUCTION

Cervical cancer is recognized as the third most common cancer and the fourth leading cause of cancer-related deaths among women globally[1]. Approximately 500,000 novel cases and 260,000 mortalities are predicted annually worldwide as a result of cervical cancer[2]. Currently, the predominant treatments for patients with cervical cancer include surgery resection, radiotherapy, and chemotherapy[3]. Despite substantial developments in diagnostic technology and clinical therapeutics, the overall survival rate of patients with cervical cancer remains low, especially for those diagnosed at the advanced stages[4]. Sustained high-risk human papillomavirus (HPV) infection is identified as a major risk factor for cervical cancer[5]. However, emerging literature demonstrated that HPV infection alone is inadequate to cause cervical cancer, and other molecular mechanisms underlying the genesis and progression of this malignancy may exist[6–8]. Therefore, a complete understanding of the mechanisms underlying the cervical carcinogenesis and development is crucial to the diagnosis, therapy, and prognosis for patients with this disease.

MicroRNAs (miRNAs) are a large group of evolutionarily conserved, non-protein-coding and short RNAs of between 18 and 25 nucleotides in length[9]. miRNAs have significant gene expression regulatory roles mainly through complementary binding to the miRNA recognition elements, predominantly in the 3′-untranslated regions (3′-UTRs) of their targets, resulting in transcriptional repression and mRNA degradation[10]. More than 1,000 miRNAs have been identified in the human genome, which mediate the expression of approximately two thirds of all protein-coding genes[11]. miRNAs can regulate a wide range of biological processes, including cell proliferation, cell cycle, apoptosis, metabolism, development, and metastasis[12,13]. In recent years, miRNAs have been found to be dysregulated in almost all types of human cancers, including cervical cancer[14]. For instance, miR-1297

Address correspondence to Junrong Wang, Department of Obstetrics and Gynecology, China–Japan Union Hospital of Jilin University, No. 126 Xiantai Road, Changchun, Jilin 130033, P.R. China. E-mail: junrongwang1982@163.com

was underexpressed in cervical cancer, and this downregulation exhibited significant association with lymph node metastasis and lymphovascular space invasion[15]. Furthermore, aberrantly expressed miRNAs may serve as oncogenes or tumor suppressors, mainly depending on the functional roles of their target genes[16]. From these findings, miRNAs may become potential therapeutic targets or candidates in the diagnosis, treatment, and prevention of human cancers.

miR-377 plays tumor-suppressive roles in a variety of human cancers, such as esophageal cancer[17], oral squamous cell carcinoma[18], pancreatic cancer[19,20], and clear cell renal cell carcinoma[21]. However, the expression, roles, and underlying molecular mechanism of miR-377 in cervical cancer have not been elucidated. Therefore, the aims of this study were to detect miR-377 levels, investigate the clinical significance of miR-377 expression in cervical cancer, and explore its biological roles and underlying mechanisms in cervical cancer. The results of this study may be helpful in understanding the pathogenesis of cervical cancer and provide a novel therapeutic target for the treatment of patients with cervical cancer.

MATERIALS AND METHODS

Tissue Collection

A total of 53 primary human cervical cancer tissues and corresponding adjacent normal cervical tissues were collected from patients who had been diagnosed with cervical cancer and underwent surgical resection at the China–Japan Union Hospital of Jilin University (Jilin, P.R. China) between June 2014 and August 2016. Prior to surgical resection, no patients had been treated with radiotherapy or chemotherapy. All tissues were immediately snap frozen in liquid nitrogen following surgical resection and then kept in −80°C until further use. This study was approved by the Ethics Committee of the China–Japan Union Hospital of Jilin University, and written informed consent was provided by all patients enrolled in this research.

Cell Culture and Transfection

A total of four human cervical cancer cell lines (C-33A, CaSki, HeLa, and SiHa) and a normal human cervix epithelial cell line (Ect1/E6E7) were ordered from the American Type Culture Collection (Manassas, VA, USA) and cultured in Dulbecco's modified Eagle's medium (DMEM; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Medium was supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin (all from Thermo Fisher Scientific, Inc.). These cell lines were grown at 37°C in a humidified air atmosphere containing 5% CO_2. The culture medium was changed every 2 days.

miR-377 mimic and miRNA mimic negative control (NC) were purchased from GenePharma (Shanghai, P.R. China). Zinc finger E-box-binding homeobox 2 (ZEB2) overexpression plasmid pcDNA3.1-ZEB2 and empty pcDNA3.1 plasmid were chemically synthesized by RiboBio (Guangzhou, P.R. China). Cells were inoculated into six-well cell culture plates at a density of 6×10^5 cells/well and maintained in a culture medium without antibiotics. Following overnight incubation, miRNA mimic or plasmid was transfected into cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. Total RNA and protein were prepared at 48 and 72 h posttransfection and subjected to analysis by reverse transcription quantitative polymerase chain reaction (RT-qPCR) and Western blot analysis, respectively.

RT-qPCR

RT-qPCR was applied to detect miR-377 and ZEB2 mRNA expression levels. Total RNA was extracted from tissue samples or cells using TRIzol reagent (Invitrogen). For the analysis of miR-377 expression, complementary DNA (cDNA) was synthesized using a TaqMan miRNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA, USA). A TaqMan miRNA PCR Kit (Applied Biosystems) was used to perform qPCR with U6 small nuclear RNA (snRNA) as an internal control. To quantify the mRNA level of ZEB2, we converted total RNA into cDNA using a PrimeScript RT Reagent Kit (Takara Biotechnology, Co., Ltd., Dalian, P.R. China). ZEB2 mRNA was quantified with a SYBR Premix Ex Taq™ (Takara Biotechnology, Co., Ltd.). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) served as an internal control for ZEB2 mRNA expression. Data were analyzed with the $2^{-\Delta\Delta CT}$ method[22].

Cell Counting Kit-8 (CCK-8) Assay

At 24 h posttransfection, cells were plated into 96-well plates at a density of 4,000 cells per well. Cell proliferative ability was examined using the CCK-8 assay (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) after incubation for 0, 24, 48, and 72 h. A 10-μl volume of CCK-8 solution was added into each well, and the cells were incubated at 37°C for another 2 h. The optical density (OD) value was detected at a wavelength of 450 nm using an automatic multiwell spectrophotometer (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Transwell Invasion Assay

Transfected cells were incubated at 37°C for 24 h and suspended in FBS-free DMEM. A total of 1×10^5 transfected cells were seeded into the upper part of Transwell chambers precoated with Matrigel (all from BD Biosciences, Franklin Lakes, NJ, USA). The lower

part of the chambers was filled with 500 μl of DMEM containing 10% FBS. After 24 h of culture at 37°C, non-invasive cells that remained on the surface of the upper chamber were scraped off with cotton swabs. The invasive cells were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. The invasive cells were counted in at least five randomly selected fields under an inverted microscope (Olympus Corporation, Tokyo, Japan), and images were photographed at 200× magnification.

Bioinformatics Analysis

Two online software programs TargetScan (http://www.targetscan.org/) and miRanda (http://www.microrna.org) were utilized to predict the potential targets of miR-377.

Dual-Luciferase Reporter Assay

The human ZEB2 3′-UTR containing the wild-type (Wt) or mutant (Mut) miR-377 binding sites was chemically synthesized by GenePharma, inserted downstream of the luciferase gene in the psiCHECK-2 reporter vector (Promega, Madison, WI, USA), and named psiCHECK-ZEB2-3′-UTR Wt, or psiCHECK-ZEB2-3′-UTR Mut, respectively. Cells were inoculated into 24-well cell culture plates. For the reporter assay, miR-377 mimic or NC was cotransfected with psiCHECK-ZEB2-3′-UTR Wt or psiCHECK-ZEB2-3′-UTR Mut into cells using Lipofectamine 2000, in accordance with the manufacturer's protocol. After transfection for 48 h, a dual-luciferase reporter assay system (Promega) was employed to determine the luciferase activity. Expression was normalized against *Renilla* luciferase activity.

Western Blot Assay

Protein was isolated using radioimmunoprecipitation assay lysis buffer (Beyotime, Shanghai, P.R. China) from tissue samples or cells. A bicinchoninic acid protein assay kit (Beyotime) was used to detect the protein concentration. Equal amounts of protein were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes (Millipore, Billerica, MA, USA). The membranes were then blocked at room temperature for 1 h with Tris-buffered saline with Tween 20 (TBST) containing 5% nonfat milk and incubated with primary antibodies overnight at 4°C. Subsequent to washing thrice with TBST, the membranes were further incubated with horseradish peroxidase-conjugated goat anti-mouse secondary antibody (sc-2005; 1:5,000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA) at room temperature for 2 h. We visualized the protein blots using an enhanced chemiluminescence detection system (Pierce, Rockford, IL, USA) and analyzed the band intensities with Quantity One software version 4.62 (Bio-Rad Laboratories, Inc.). The primary antibodies used in this study included mouse anti-human ZEB2 monoclonal antibody (sc-271984; 1:1,000 dilution; Santa Cruz Biotechnology) and mouse anti-human GAPDH (sc-47724; 1:1,000 dilution; Santa Cruz Biotechnology) antibody.

Statistical Analysis

Data are expressed as the mean ± standard deviation and analyzed with SPSS software (version 21.0; IBM SPSS, Armonk, NY, USA). We analyzed the difference between groups using Student's *t*-tests or one-way analysis of variance followed by the Student–Newman–Keuls multiple comparison test. We used Spearman's correlation analysis to explore the association between miR-377 and ZEB2 mRNA in cervical cancer tissues. A value of $p < 0.05$ was considered statistically significant.

RESULTS

miR-377 Is Downregulated in Cervical Cancer Tissues and Cell Lines

To explore the expression pattern and clinical significance of miR-377 in cervical cancer, we detected its expression in 53 paired cervical cancer tissues and corresponding adjacent normal cervical tissues. The data from RT-qPCR analysis revealed that miR-377 was obviously underexpressed in cervical cancer tissues compared with that in adjacent normal cervical tissues ($p < 0.05$) (Fig. 1A). A total of 53 patients with cervical cancer were divided into two groups based on the median expression

Table 1. The Association Between miR-377 Expression and Clinicopathologic Features of Cervical Cancer

	miR-377 Expression		
Clinicopathologic Features	Low	High	*p* Value
Age (years)			0.465
<50	12	9	
≥50	15	17	
Tumor size (cm)			0.501
<4	10	12	
≥4	17	14	
Family history of cancer			0.697
No	19	17	
Yes	8	9	
FIGO stage			0.001
I–II	5	16	
III–IV	22	10	
Lymph node metastasis			0.019
Negative	7	15	
Positive	20	11	
Distant metastasis			0.039
Negative	8	15	
Positive	19	11	

level of miR-377, namely, the low miR-377 expression group ($n=27$) and high miR-377 expression group ($n=26$). The association between miR-377 expression and clinicopathological features of patients with cervical cancer is shown in Table 1. Decreased miR-377 expression was strongly correlated with the International Federation of Gynecology and Obstetrics (FIGO) stage ($p=0.001$), lymph node metastasis ($p=0.019$), and distant metastasis ($p=0.039$). However, no significant association was found with other clinicopathological factors, including age ($p=0.465$), tumor size ($p=0.501$), and family history of cancer ($p=0.697$). Additionally, we performed RT-qPCR to determine miR-377 expression in four cervical cancer cell lines (C-33A, CaSki, HeLa, and SiHa) and a normal human cervix epithelial cell line (Ect1/E6E7). Compared with the Ect1/E6E7 cell line, the expression level of miR-377 was reduced in all examined cervical cancer cell lines ($p<0.05$) (Fig. 1B). These findings suggested that miR-377 may exert tumor-suppressive activity in cervical cancer.

miR-377 Overexpression Inhibits the Proliferation and Invasion Ability of Cervical Cancer Cells

As miR-377 was underexpressed in cervical cancer, it was hypothesized that it may play tumor-suppressive roles in the progression of cervical cancer. To confirm this hypothesis, miR-377 mimics were transfected into CaSki and HeLa cells, which exhibited relatively lower miR-377 levels among these four cervical cancer cell lines. We conducted RT-qPCR analysis to determine transfection efficiency and found that miR-377 was markedly overexpressed in CaSki and HeLa cells after transfection with miR-377 mimics ($p<0.05$) (Fig. 2A). To examine the effect of miR-377 overexpression on cellular proliferative ability, we used CCK-8 assays to detect cell proliferation of CaSki and HeLa cells after modification of miR-377 expression. The results showed that upregulation of miR-377 reduced CaSki and HeLa cell proliferation compared with that of NC-transfected cells ($p<0.05$) (Fig. 2B). Furthermore, we utilized Transwell invasion assays to analyze the effect of miR-377 on the cell invasion capacity of cervical cancer. Restoration of the expression of miR-377 resulted in a reduced number of invasive CaSki and HeLa cells compared with the NC group ($p<0.05$) (Fig. 2C). These results suggested that miR-377 may serve an inhibitory role in cervical cancer growth and metastasis.

ZEB2 Is the Direct Target of miR-377 in Cervical Cancer

The biological roles of miRNAs in human cancer are mainly dependent on their target genes. Thus, we conducted bioinformatics analysis to search for the potential targets of miR-377. As shown in Figure 3A, a highly conserved miR-377 targeting site was predicted to occur in the 3′-UTR of ZEB2. It was selected for subsequent confirmation because it has been shown to be involved in cancer formation and progression[23–25]. We then performed dual-luciferase reporter assays to investigate whether miR-377 could directly interact with the 3′-UTR of ZEB2. As illustrated in Figure 3B, miR-377 overexpression significantly suppressed the luciferase activity of psiCHECK-ZEB2-3′-UTR Wt ($p<0.05$). However, altering the miR-377 level did not affect the luciferase activity of psiCHECK-ZEB2-3′-UTR Mut, suggesting that the 3′-UTR of ZEB2 could be directly targeted by miR-377. We further examined whether miR-377 could regulate endogenous ZEB2 expression in cervical cancer. The data of RT-qPCR and Western blot analysis demonstrated that resumption of the expression of miR-377

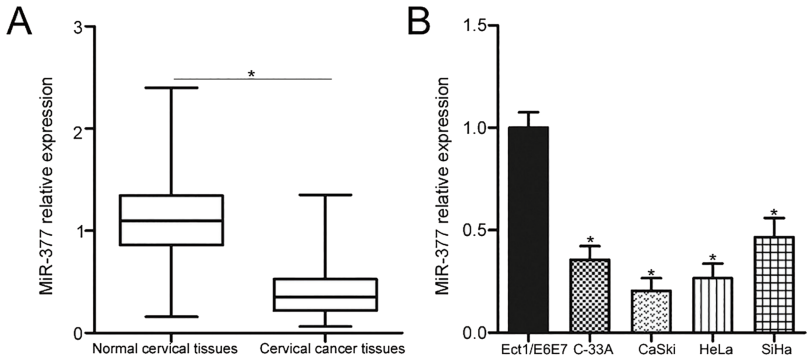

Figure 1. MicroRNA-377 (miR-377) expression is downregulated in cervical cancer tissues and cell lines. (A) Expression levels of miR-377 in 53 paired cervical cancer tissues and corresponding adjacent normal cervical tissues were detected by reverse transcription quantitative polymerase chain reaction (RT-qPCR). *$p<0.05$ compared with normal cervical tissues. (B) miR-377 expression in four cervical cancer cell lines (C-33A, CaSki, HeLa, and SiHa) and a normal human cervix epithelial cell line (Ect1/E6E7) was examined by RT-qPCR. *$p<0.05$ compared with normal Ect1/E6E7 cell line.

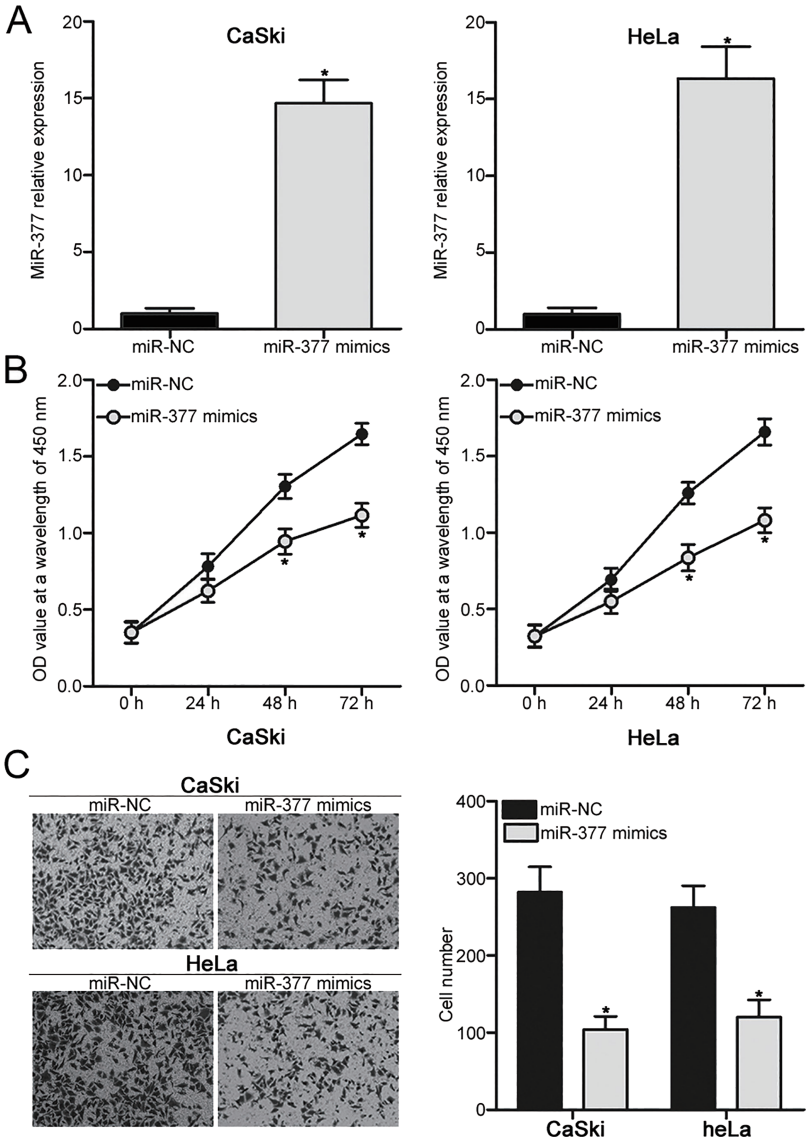

Figure 2. miR-377 suppresses proliferation and invasion of CaSki and HeLa cells. (A) miR-377 mimic or negative control (NC) was transfected into CaSki and HeLa cells, and RT-qPCR analysis was conducted to determine miR-377 expression after transfection. *$p<0.05$ compared with NC. (B) Cell counting kit-8 (CCK-8) assays were performed to detect proliferation of CaSki and HeLa cells either transfected with miR-377 mimic or NC. *$p<0.05$ compared with NC. (C) CaSki and HeLa cells were transfected with miR-377 mimic or NC. Cell invasion ability was determined using the Transwell invasion assay at 48 h posttransfection. *$p<0.05$ compared with NC.

decreased ZEB2 expression in CaSki and HeLa cells at the mRNA ($p<0.05$) (Fig. 3C) and protein ($p<0.05$) (Fig. 3D) levels. Overall, these data suggested that ZEB2 is a direct target gene of miR-377 in cervical cancer.

miR-377 Expression Is Inversely Correlated With ZEB2 Expression in Cervical Cancer Tissues

To further elucidate the association between miR-377 and ZEB2 in cervical cancer, we measured ZEB2 mRNA and protein expression in cervical cancer tissues and corresponding adjacent normal cervical tissues. Our data indicated that both ZEB2 mRNA ($p<0.05$) (Fig. 4A) and protein ($p<0.05$) (Fig. 4B and C) expression was upregulated compared with that in adjacent normal cervical tissues. In addition, the expression of ZEB2 mRNA exhibited a significantly negative correlation with those of miR-377 expression in cervical cancer tissues ($r=-0.5843$, $p<0.0001$) (Fig. 4D). These results further suggested that ZEB2 is a direct downstream target of miR-377 in cervical cancer.

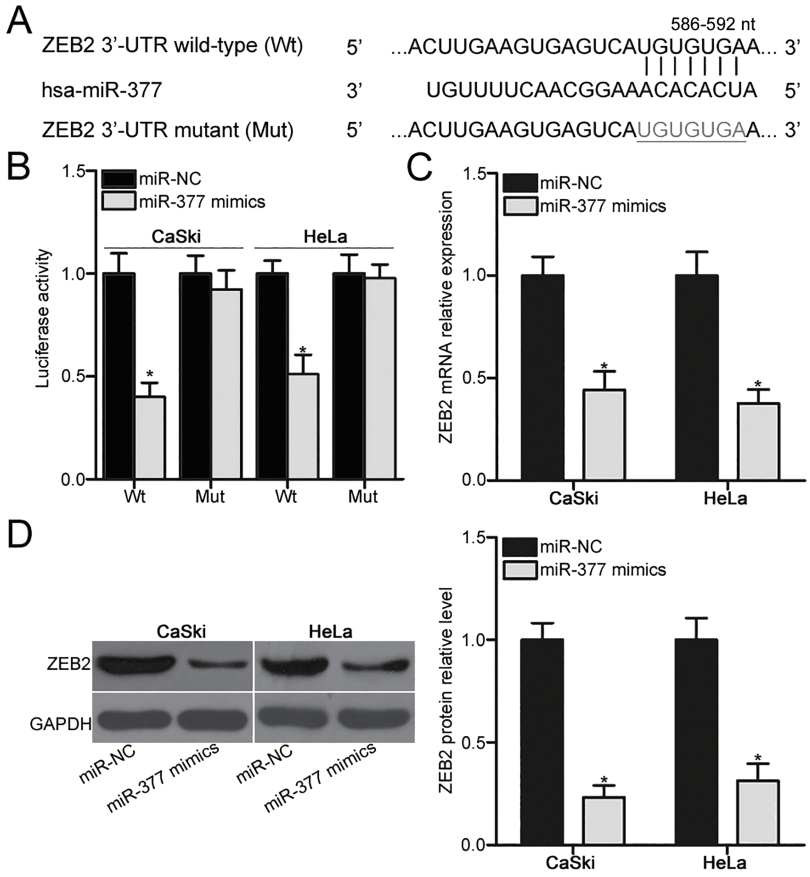

Figure 3. ZEB2 is a direct target of miR-377 in cervical cancer. (A) Putative wild-type miR-377-binding sequences in the 3′-UTR of ZEB2 and the corresponding mutant binding sequences. (B) Dual-luciferase reporter assays were carried out to detect the relative luciferase activity of luciferase reporter plasmid containing wild-type (Wt) or mutant (Mut) ZEB2-3′-UTR in CaSki and HeLa cells, which were cotransfected with the miR-377 mimic or NC. *$p<0.05$ compared with NC. (C) The mRNA and (D) protein levels of ZEB2 were determined by RT-qPCR and Western blot analysis in CaSki and HeLa cells transfected with miR-377 mimics or NC. *$p<0.05$ compared with NC.

Recovered ZEB2 Expression Is Able to Counteract the Tumor-Suppressing Effects of miR-377 Overexpression in Cervical Cancer Cells

To further validate that the effects of miR-377 overexpression on cervical cancer cells is dependent on the regulation of ZEB2 expression, we chemically synthesized ZEB2 overexpression plasmid pcDNA3.1-ZEB2 without the 3′-UTR of ZEB2, which was not modulated by miR-377, to carry out a series of rescue experiments. CaSki and HeLa cells were cotransfected with miR-377 mimics and pcDNA3.1-ZEB2 or pcDNA3.1. Transfection of CaSki and HeLa cells with pcDNA3.1-ZEB2 rescued the ZEB2 protein expression, which was decreased by miR-377 overexpression ($p<0.05$) (Fig. 5A). CCK-8 and Transwell invasion assays revealed that restored ZEB2 expression eliminated the inhibitory effects on cell proliferation ($p<0.05$) (Fig. 5B) and invasion ($p<0.05$) (Fig. 5C) induced by miR-377 overexpression. These results demonstrated that miR-377 exerts tumor-suppressive roles in cervical cancer at least partially by inhibiting ZEB2 expression.

DISCUSSION

A large number of miRNAs are aberrantly expressed in cervical cancer and play crucial roles in the onset and progression of cervical cancer by acting as either an oncogene or a tumor suppressor[26–28]. Therefore, investigation of the expression, biological roles, and underlying mechanisms of miRNAs in cervical cancer might provide valuable therapeutic targets in the treatment of patients with this disease. For the first time, in our current study, we demonstrated that miR-377 expression is reduced in cervical cancer tissues and cell lines. Downregulation of miR-377 is associated with FIGO stage, lymph node metastasis, and distant metastasis of patients with cervical

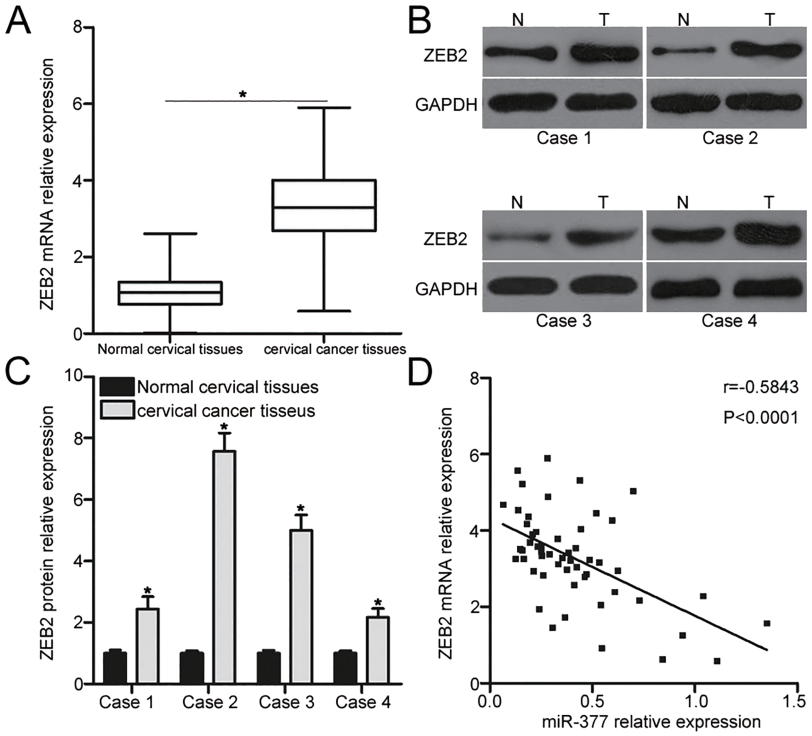

Figure 4. Expression of ZEB2 is overexpressed in cervical cancer tissues and negatively expressed related with miR-377 levels. (A) Expression of ZEB2 mRNA and (B, C) protein was detected in cervical cancer tissues and corresponding adjacent normal cervical tissues. *$p<0.05$ compared with normal cervical tissues. (D) Significant inverse association between miR-377 and ZEB2 mRNA was observed in cervical cancer tissues using Spearman's correlation analysis. $r=-0.5843$, $p<0.0001$.

cancer. Functional experiments showed that restoration of the expression of miR-377 prohibits cell proliferation and invasion in cervical cancer cell lines. Notably, ZEB2 was identified as a direct target gene of miR-377 in cervical cancer. ZEB2 is overexpressed in cervical cancer tissues and inversely related with the miR-377 level. Moreover, restoration of the expression of ZEB2 counteracts the inhibitory effects of miR-377 overexpression in cervical cancer cells. These results suggest that miR-377 has a critical role on the suppression of cervical cancer initiation and progression.

An increasing number of studies have indicated that miR-377 is dysregulated in various cancer types. For example, miR-377 is underexpressed in esophageal cancer, and this dysregulation is associated with pathologic tumor stage, distant metastasis, residual tumor status, and chemoradiotherapy resistance. Patients with esophageal cancer with low miR-377 level have shorter survival periods than patients with high miR-377 expression[17]. Decreased miR-377 levels are also reported in oral squamous cell carcinoma[18], pancreatic cancer[19,20], clear cell renal cell carcinoma[21], glioblastoma[29], osteosarcoma[30], non-small cell lung cancer[31,32], and hepatocellular carcinoma[33,34]. Nevertheless, miR-377 expression is upregulated in gastric cancer tissues and cell lines. The increased miR-377 level was significantly associated with distant metastasis; tumor, node, and metastasis stage; and early recurrence of patients with gastric cancer. Patients with gastric cancer with a high miR-377 level exhibited poorer overall survival and a shorter time to recurrence compared with patients with a low miR-377 level. miR-377 overexpression has been validated as an independent prognostic factor for gastric cancer[35]. These conflicting findings demonstrate that the expression pattern of miR-377 in human cancers has tissue specificity and suggests that miR-377 could potentially be developed as a novel biomarker of diagnosis and prognosis in these specific cancer types.

Accumulating evidence has revealed that the aberrant expression of miR-377 plays a vital role in human cancer onset and development. For instance, miR-377 upregulation decreases cell proliferation, angiogenesis, and metastatic colonization in esophageal cancer[17]. Rastogi et al. revealed that resumption of the expression of miR-377 restricts cell growth and metastasis and promotes apoptosis in oral squamous cell carcinoma[18]. Chang et al. found that miR-377 overexpression suppresses cell growth and migration and increases cell cycle arrest and apoptosis

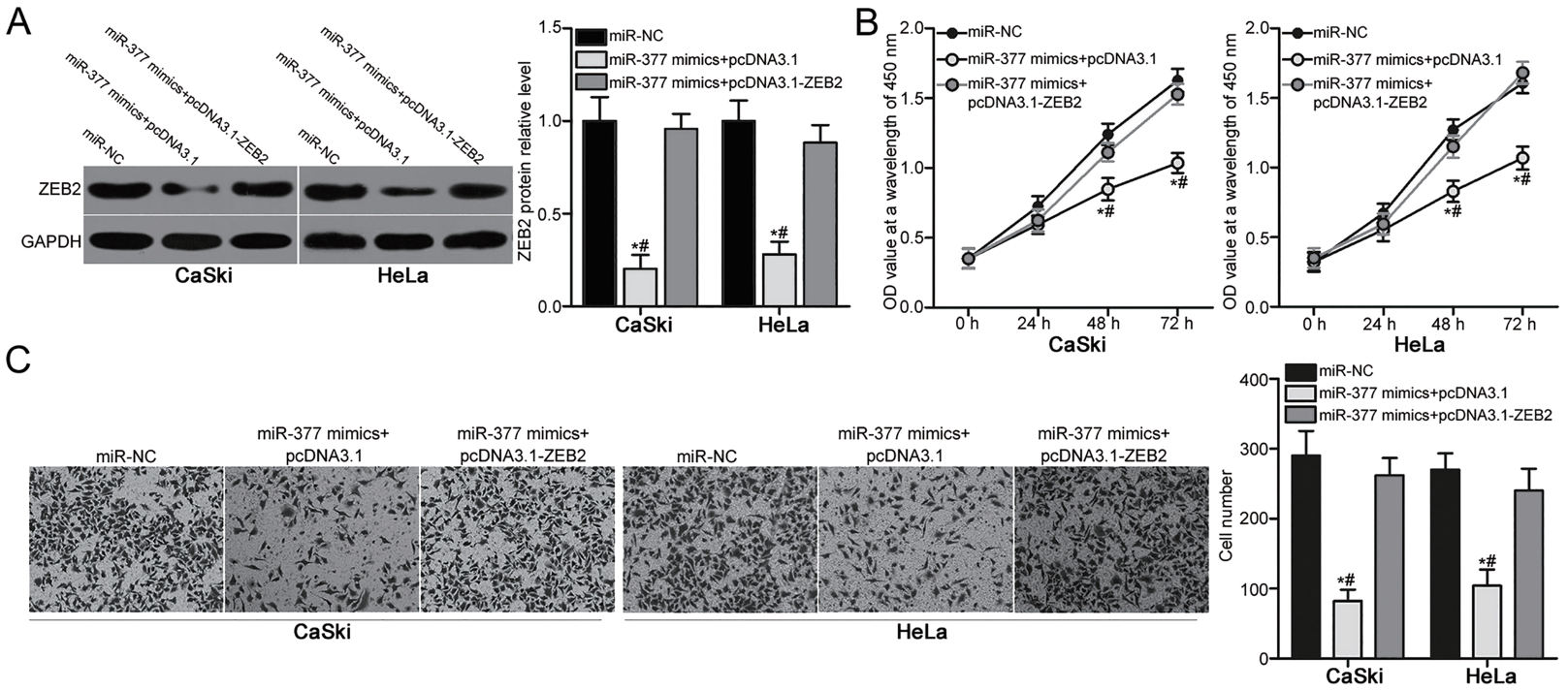

Figure 5. ZEB2 is able to abolish the suppressive effects of miR-377 overexpression in CaSki and HeLa cells. (A) CaSki and HeLa cells were cotransfected with miR-377 mimic and pcDNA3.1-ZEB2 or pcDNA3.1. Western blot analysis was used to determine ZEB2 protein level at 72 h posttransfection. *$p<0.05$ compared with NC. #$p<0.05$ compared with miR-377 mimics+pcDNA3.1-ZEB2. (B) CCK-8 and (C) Transwell invasion assays were applied in the abovementioned cells to determine cell proliferative and invasion abilities, respectively. *$p<0.05$ compared with NC. #$p<0.05$ compared with miR-377 mimic+pcDNA3.1-ZEB2.

in pancreatic cancer[19]. Wang et al. reported that miR-377 reexpression decreases cell proliferative, metastatic, and invasive abilities in clear cell renal cell carcinoma[21]. Zhang et al. demonstrated that restoration of the expression of miR-377 attenuates glioblastoma cell proliferation and invasion[29]. A study by Wang et al. showed that enforced expression of miR-377 inhibits cell proliferation and invasion in osteosarcoma[30]. Zhang et al. indicated that miR-377 represses cell growth and metastasis and induces apoptosis in non-small cell lung cancer[31]. Ge et al. revealed that restoration of the expression of miR-377 reduces cell growth and motility and promotes the apoptosis of hepatocellular carcinoma[33]. However, miR-377 serves oncogenic roles in gastric cancer by expediting cell proliferation and colony formation[35]. These findings provide novel insights into tumorigenesis and tumor development and imply that miR-377 may consequently serve as an effective therapeutic target for cancer treatment.

Numerous targets of miR-377, including CD133, vascular endothelial growth factor in esophageal cancer[17], histone deacetylase in oral squamous cell carcinoma[18], DNA methyltransferase 1 and Pim-3 in pancreatic cancer[19,20], ETS1 in clear cell renal cell carcinoma[21], SP1 in glioblastoma[29], CDK6 in osteosarcoma[30], AEG-1 in non-small cell lung cancer[32], Bcl-xL and IRX3 in hepatocellular carcinoma[33,34], and p53, PTEN, and TIMP1 in gastric cancer[35], have been validated. In this study, ZEB2, a member of the zinc finger family, is identified as a novel target of miR-377 in cervical cancer. ZEB2 is also overexpressed in several types of human cancer, such as gastric cancer[36], bladder cancer[37], ovarian cancer[38], colorectal cancer[25], and lung cancer[39]. ZEB2 dysregulation plays crucial roles in cancer occurrence and development by regulating various pathological processes, such as cell proliferation, cell cycle, apoptosis, angiogenesis, metastasis, epithelial–mesenchymal transition, and chemoresistance[23–25]. Our current study demonstrated that miR-377 targets ZEB2 to inhibit cell proliferation and invasion of cervical cancer. Thus, the miR-377/ZEB2 axis is a potential and valuable therapeutic target to treat patients with cervical cancer.

In conclusion, miR-377 is significantly decreased in cervical cancer tissues and cell lines, and its downregulation is associated with FIGO stage, lymph node metastasis, and distant metastasis. Ectopic expression of miR-377 restricts cervical cancer cell proliferation and invasion in vitro. Mechanistically, ZEB2 is a direct and functional target of miR-377 in cervical cancer. These results suggest that miR-377 might serve as a potential target for cervical cancer therapy.

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