

MicroRNA-411 Inhibits Cervical Cancer Progression by Directly Targeting STAT3

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Cervical cancer is the third most common gynecological cancer and the fourth leading cause of cancer-related deaths in women around the world. Substantial evidence has demonstrated that microRNA (miRNA) expression is disordered in many malignant tumors. The dysregulation of miRNAs has been suggested to be involved in the tumorigenesis and tumor development of cervical cancer. Therefore, identification of miRNAs and their biological roles and targets involved in tumor pathology would provide valuable insight into the diagnosis and treatment of patients with cervical cancer. MicroRNA-411 (miR-411) has been reported to play an important role in several types of human cancer. However, the expression level, role, and underlying molecular mechanisms of miR-411 in cervical cancer remain unclear. Therefore, the objectives of this study were to investigate the expression pattern and clinical significance of miR-411 in cervical cancer and to evaluate its role and underlying mechanisms in this disease. In this study, we confirmed that the expression of miR-411 was significantly downregulated in both cervical cancer tissues and cell lines. Low expression of miR-411 was associated with tumor size, FIGO stage, lymph node metastasis, and distant metastasis. Additionally, miR-411 overexpression inhibited cell proliferation and invasion in cervical cancer. Furthermore, signal transducer and activator of transcription 3 (STAT3) was identified as a direct target of miR-411 in this disease. In clinical samples, miR-411 expression levels were inversely correlated with STAT3, which was significantly upregulated in cervical cancer. Restored STAT3 expression abolished the tumor-suppressing effects of miR-411 overexpression on the proliferation and invasion of cervical cancer cells. In conclusion, our data demonstrated that miR-411 inhibited cervical cancer progression by directly targeting STAT3 and may represent a novel potential therapeutic target and prognostic marker for patients with this disease.

Key words: Cervical cancer; MicroRNA-411; Proliferation; Invasion; Signal transducer and activator of transcription 3 (STAT3)

INTRODUCTION

Cervical cancer is the third most common gynecological cancer and the fourth leading cause of cancer-related deaths in women around the world¹. Over 500,000 novel cases and approximately 274,000 deaths due to cervical cancer are estimated to occur each year worldwide². A characteristic of cervical cancer is the transformation of normal cervical epithelium to a preneoplastic cervical intraepithelial neoplasia (CIN) that is subsequently transformed into cervical cancer³. At present, several therapeutic treatments, including surgery, chemotherapy, and radiotherapy, are employed to treat patients with cervical cancer^{4,5}. Despite considerable advancements in therapy, the prognosis of patients with cervical cancer remains unsatisfactory, especially for those at an advanced stage of the disease⁶. Recurrence and metastasis are major causes of treatment failure⁷. Therefore, fully understanding the

mechanisms underlying cervical cancer occurrence and development is necessary to develop novel therapeutic strategies for patients with this disease.

MicroRNAs (miRNAs) are a group of endogenous, noncoding and short RNA molecules of 19–24 nucleotides⁸. miRNAs negatively modulate the expression of their target genes by directly binding to the 3'-untranslated regions (3'-UTRs) of mature mRNAs at sequence-specific sites, thereby causing mRNA destabilization and protein downregulation⁹. A single miRNA may regulate the expression of numerous target genes simultaneously; therefore, miRNAs play important roles in a number of physiological and pathological processes, including cell proliferation, cell cycle progression, apoptosis, metabolism, metastasis, angiogenesis, epithelial–mesenchymal transition, and differentiation^{10–13}. Dysregulation of miRNA expression is commonly observed in various types of

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malignancies, such as cervical cancer¹⁴, glioma¹⁵, lung cancer¹⁶, and renal cell cancer¹⁷. miRNAs may serve as either tumor suppressors or oncogenes in tumorigenesis and tumor development depending on their target genes¹⁸. Many miRNAs are downregulated in cancers and act as tumor suppressors that inhibit carcinogenesis and progression through negative regulation of oncogenes. By contrast, some miRNAs are upregulated in tumors and generally participate in tumor suppressor overexpression^{19,20}. Therefore, miRNAs may be investigated as therapeutic targets for cancer treatment.

miR-411, located on chromosome 14q32, has been reported to play an important role in several types of human cancer^{17,21,22}. However, the expression level, role, and underlying molecular mechanisms of miR-411 in cervical cancer remain unclear. Therefore, the objectives of this study were to investigate the expression pattern and clinical significance of miR-411 in cervical cancer and to evaluate its role and underlying mechanisms in this disease.

MATERIALS AND METHODS

Clinical Tissues and Cell Lines

This study was approved by the Ethics Committee of Tianjin Hospital. Written informed consent was also obtained from each patient who participated in this research. A total of 45 paired cervical cancer tissues and corresponding adjacent normal tissues were obtained from cervical cancer patients who had undergone surgery between October 2014 and August 2016 in the Department of Obstetrics and Gynecology, Tianjin Hospital. None of the patients received chemotherapy, radiotherapy, or other treatments before surgery. Tissues were immediately frozen in liquid nitrogen and stored at -80°C until further use.

Four human cervical cancer cell lines (HeLa, SiHa, Ca-Ski, and C-33A) were purchased from the Shanghai Institute of Biochemistry and Cell Biology (Shanghai, P.R. China). Ect1/E6E7, a human normal cervical epithelial cell line, was acquired from the American Type Culture Collection (ATCC; Manassas, VA, USA). All cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 mg/ml streptomycin (all from Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA). All cell lines were grown in a humidified atmosphere at 37°C with 5% CO_2 .

Cell Transfection

Mature miR-411 mimic and miRNA mimic negative control (miR-NC) were synthesized by Guangzhou RiboBio Co., Ltd. (Guangzhou, P.R. China). Signal

transducer and activator of transcription 3 (STAT3) overexpression plasmid (pcDNA3.1-STAT3) and blank plasmid (pcDNA3.1) were obtained from the Chinese Academy of Sciences (Shanghai, P.R. China). Cells were plated in six-well plates at a density of 6×10^5 cells per well and cultured in DMEM with 10% FBS for 24 h. Cell transfection was performed using Lipofectamine™ 2000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR)

Total RNA was extracted from tissues or cells using TRIzol® (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. To quantify miR-411 expression, complementary DNA (cDNA) was synthesized using TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems; Thermo Fisher Scientific, Inc.), followed by qPCR with a TaqMan MicroRNA Assay Kit (Applied Biosystems; Thermo Fisher Scientific, Inc.). For STAT3 mRNA detection, reverse transcription was carried out using PrimeScript RT reagent kit (Takara Biotechnology Co., Ltd., Dalian, P.R. China). SYBR Premix Ex Taq (Takara Biotechnology Co., Ltd.) was then used to detect the mRNA expression of STAT3. U6 snRNA and GAPDH were used as internal controls for miR-411 and STAT3 mRNA, respectively. The relative expression level was calculated using the $2^{-\text{Ct}}$ method²³.

Cell Counting Kit-8 (CCK-8) Assay

CCK-8 assays were utilized to determine cervical cancer cell proliferation. Transfected cells were collected at 24 h posttransfection and seeded in 96-well plates at a density of 3×10^3 cells per well. Cells were then incubated at 37°C with 5% CO_2 for 0, 24, 48, and 72 h. The CCK-8 assay was performed at each time point according to the manufacturer's protocol. A volume of 10 μl of CCK-8 solution (Dojindo Molecular Technologies, Inc., Rockville, MD, USA) was added to each well prior to incubation at 37°C for an additional 2 h. Absorbance was subsequently detected at a wavelength of 450 nm on a microplate reader (BioTek Synergy HT; BioTek Instruments, Inc., Winooski, VT, USA). Each assay was performed in triplicate and repeated three times.

Transwell Invasion Assay

Transwell invasion assays were conducted to evaluate cell invasion ability using Transwell chambers (pore size, 8 μm ; Costar; Corning Incorporated, Corning, NY, USA) coated with Matrigel (BD Biosciences, San Jose, CA, USA). Transfected cells (5×10^4) suspended in 200 μl of FBS-free DMEM were seeded into the upper chamber. A total of 500 μl of DMEM containing 20% FBS was added

to the lower chambers as a chemoattractant. Following incubation at 37°C with 5% CO₂ for 24 h, cells that had not invaded through the pores on the polycarbonate membranes were carefully removed using a cotton swab. The invasive cells were fixed with 90% alcohol, stained with 0.5% crystal violet, and washed with PBS. Images of five randomly selected fields of the invasive cells were photographed, and the cells were counted under an inverted microscope (CKX41; Olympus Corporation, Tokyo, Japan). Each assay was performed in triplicate and repeated three times.

Bioinformatics Analysis

Bioinformatics analysis was performed to predict the putative targets of miR-411 using TargetScan (<http://www.targetscan.org/>) and miRanda (<http://www.microrna.org/microrna/>).

Luciferase Reporter Assay

For luciferase reporter assay, psiCHECKTM-2-STAT3-3'-UTR wild type (Wt) and psiCHECKTM-2-STAT3-3'-UTR mutant (Mut) were synthesized by GenePharma (Shanghai, P.R. China). Cells were seeded in 24-well plates at a density of 1.5×10^5 cells each well. After incubation overnight, cells were cotransfected with miR-411 mimic or miR-NC, and psiCHECKTM-2-STAT3-3'-UTR Wt or psiCHECKTM-2-STAT3-3'-UTR Mut, using LipofectamineTM 2000 reagent, in accordance with the manufacturer's guidance. At 24 h after transfection, luciferase activities were examined using the Dual-Luciferase Reporter Assay System (Promega Corporation, Madison, WI, USA), according to the manufacturer's protocol. *Renilla* luciferase activities were normalized to firefly luciferase activities.

Western Blotting Analysis

Total protein was extracted from tissues or cells using ice-cold radioimmunoprecipitation assay lysis buffer (Beyotime Institute of Biotechnology, Haimen, P.R. China). Total concentration was determined using a BCA protein assay kit (Beyotime Institute of Biotechnology) according to the manufacturer's protocol. Equal quantities of protein were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electroblotted onto a polyvinylidene fluoride membrane (EMD Millipore, Billerica, MA, USA). Subsequently, the membranes were blocked with 5% nonfat dry milk in TBS containing 0.05% Tween 20 (TBST) at room temperature for 2 h and incubated overnight at 4°C with primary antibodies: mouse anti-human monoclonal STAT3 (1:1,000; Cat. No. sc-293151; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) and mouse anti-human monoclonal GAPDH (1:1,000; Cat. No. sc-365062; Santa Cruz Biotechnology,

Inc.). After washing with TBST three times, the membranes were probed with goat anti-mouse horseradish peroxidase-conjugated secondary antibody (1:5,000; sc-2005; Santa Cruz Biotechnology, Inc.) for 2 h at room temperature. Finally, the protein bands were visualized using PierceTM ECL Plus Western Blotting Substrate (Pierce; Thermo Fisher Scientific, Inc.). GAPDH was used as a loading control. The densitometry of the signals was analyzed with ImageJ v1.49 software (National Institutes of Health, Bethesda, MD, USA).

Statistical Analysis

Data were expressed as the mean \pm standard deviation (SD). SPSS 17.0 (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis with Student's *t*-tests or one-way ANOVA. SNK (Student–Newman–Keuls) test was used to compare between two groups in the multiple group studies. Spearman's correlation analysis was adopted to assess the association between miR-411 and STAT3 mRNA expression in cervical cancer tissues. A value of $p < 0.05$ was considered statistically significant.

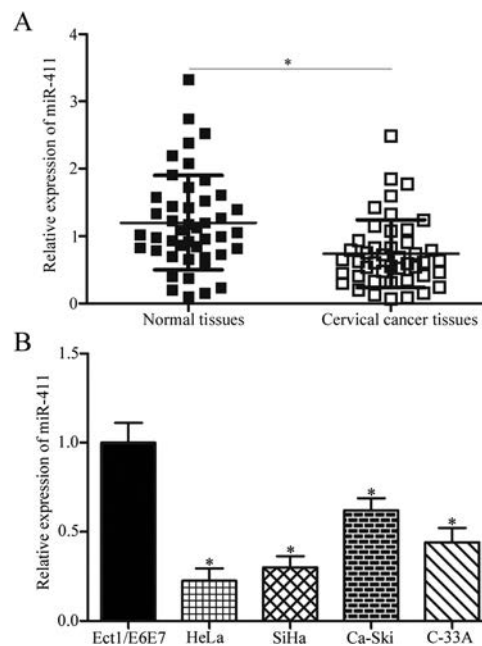


Figure 1. Expression level of microRNA-411 (miR-411) was decreased in cervical cancer tissues and cell lines. (A) miR-411 expression was detected in 45 paired cervical cancer tissues and corresponding adjacent normal tissues by reverse transcription quantitative polymerase chain reaction (RT-qPCR). $*p < 0.05$ compared with normal tissues. (B) RT-qPCR was performed to determine the miR-411 expression levels in four cervical cancer cell lines (HeLa, SiHa, Ca-Ski, and C-33A) and a human normal cervical epithelial cell line (Ect1/E6E7). $*p < 0.05$ compared with Ect1/E6E7.

RESULTS

miR-411 Is Downregulated in Cervical Cancer Tissue Specimens and Cell Lines

In the first stage of the study, miR-411 expression in 45 paired cervical cancer tissues and corresponding adjacent normal tissues was examined. RT-qPCR showed that miR-411 was significantly lower in cervical cancer tissues compared with that in corresponding adjacent normal tissues ($p < 0.05$) (Fig. 1A). The association between miR-411 expression levels and clinicopathological features of cervical cancer patients was analyzed. Median miR-411 expression level in cervical cancer tissues was regarded as the cutoff, and all cervical cancer patients were divided into either the miR-411 low-expression group ($n = 23$) or the miR-411 high-expression group ($n = 22$). As shown in Table 1, low miR-411 expression level was significantly correlated with tumor size ($p = 0.025$), International Federation of Gynecology and Obstetrics (FIGO) stage ($p = 0.023$), lymph node metastasis ($p = 0.004$), and distant metastasis ($p = 0.017$). However, no significant correlations were noted between miR-411 expression and age ($p = 0.608$) or HPV infection ($p = 0.163$).

The expression levels of miR-411 in a panel of human cervical cancer cell lines (HeLa, SiHa, Ca-Ski, and C-33A) and a human normal cervical epithelial cell line

(Ect1/E6E7) were studied. Compared with the Ect1/E6E7 cells, all cervical cancer cells had a significantly decreased miR-411 level ($p < 0.05$) (Fig. 1B). Additionally, HeLa and SiHa cells expressed relatively lower miR-411 expression levels; thus, HeLa and SiHa cells were selected for the rest of the study. These results suggested that miR-411 may be involved in the progression of cervical cancer.

miR-411 Inhibits Proliferation and Invasion of Cervical Cancer Cells

To elucidate whether miR-411 was involved in the initiation and progression of cervical cancer, miR-411 was overexpressed in HeLa and SiHa cells by transfection with miR-411 mimic. At 48 h following transfection, RT-qPCR analysis confirmed that miR-411 was markedly upregulated in HeLa and SiHa cells after transfection with miR-411 mimic ($p < 0.05$) (Fig. 2A). The CCK-8 assay was conducted to investigate the effect of miR-411 overexpression on cervical cancer cell proliferation. Compared with the miR-NC groups, upregulation of miR-411 inhibited the proliferation of HeLa and SiHa cells ($p < 0.05$) (Fig. 2B). Transwell invasion assays revealed that enforced expression of miR-411 decreased the invasion capacities in HeLa and SiHa cells ($p < 0.05$) (Fig. 2C). These results suggested that miR-411 may play tumor-suppressing roles in cervical cancer.

Table 1. Association Between MicroRNA-411 Expression and Clinicopathologic Features of Cervical Cancer Patients

Clinicopathological Features	No. of Patients	miR-411 Expression		<i>p</i> Value
		Low	High	
Age (years)				0.608
<50	16	9	7	
50	29	14	15	
HPV infection				0.279
Positive	32	18	14	
Negative	13	5	8	
Tumor size (cm)				0.025*
<4	19	6	13	
4	26	17	9	
FIGO stage				0.023*
I–II	17	5	12	
III–IV	28	18	10	
Lymph node metastasis				0.004*
Positive	20	15	5	
Negative	25	8	17	
Distant metastasis				0.017*
Positive	16	12	4	
Negative	29	11	18	

HPV, human papillomavirus; FIGO stage, International Federation of Gynecology and Obstetrics stage.

* $p < 0.05$.

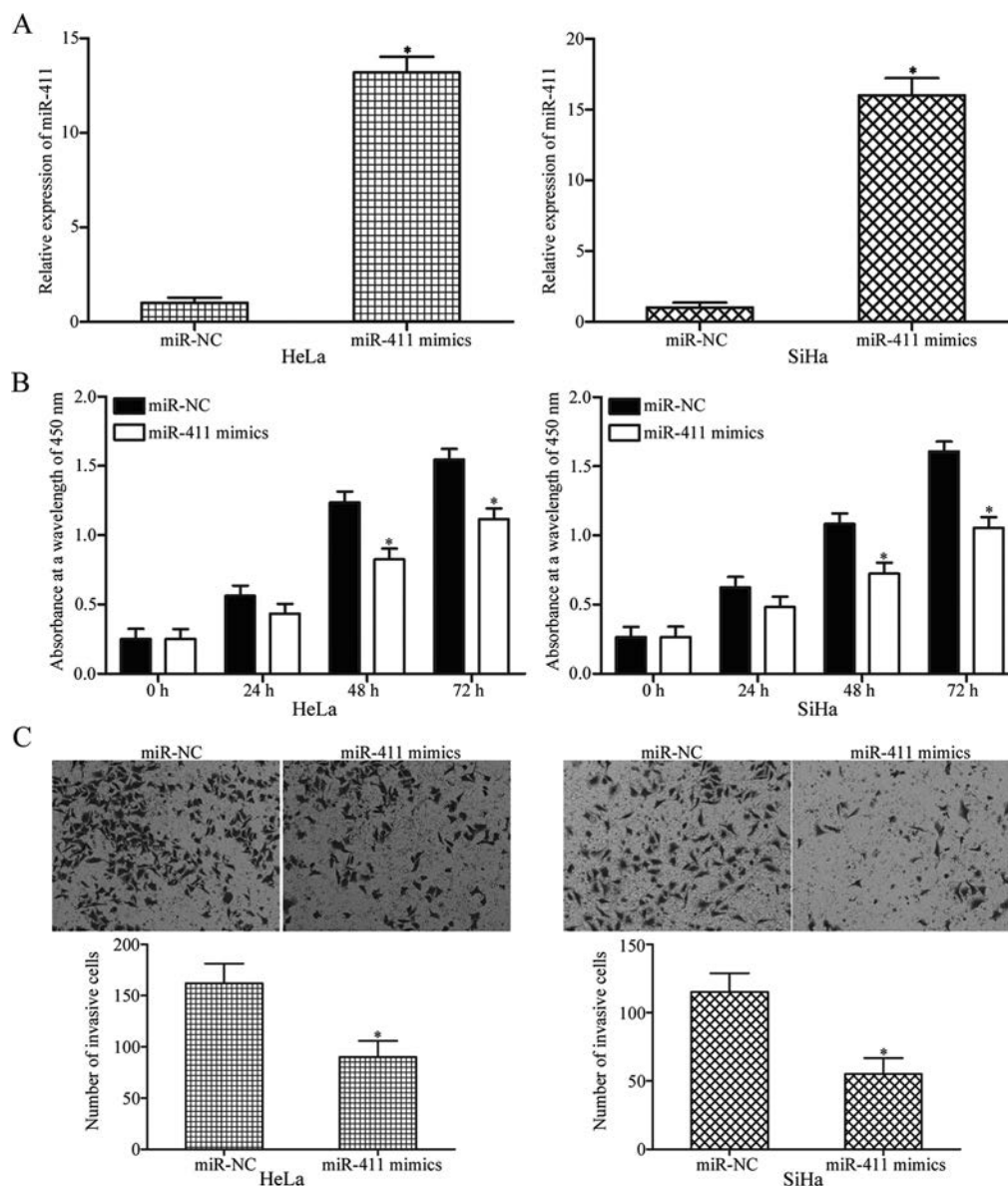


Figure 2. miR-411 overexpression attenuates cell proliferation and invasion in cervical cancer. (A) Forty-eight hours after transfection with miR-411 mimic or miR-NC (negative control) in HeLa and SiHa cells, RT-qPCR analysis was performed to evaluate the transfection efficiency. * $p < 0.05$ compared with miR-NC. (B) The cell counting kit-8 (CCK-8) assay was adopted to determine cell proliferation in HeLa and SiHa cells transfected with miR-411 mimic or miR-NC. * $p < 0.05$ compared with miR-NC. (C) Transwell invasion assay was conducted to investigate the effect of miR-411 overexpression on HeLa and SiHa cells. * $p < 0.05$ compared with miR-NC.

STAT3 Is a Direct Target of miR-411 in Cervical Cancer

To explore the molecular mechanisms underlying the functional roles of miR-411 in cervical cancer, bioinformatics analysis was carried out to predict the potential targets of miR-411. STAT3 (Fig. 3A), which was highly expressed in cervical cancer and contributed to the formation and progression of cervical cancer²⁴⁻²⁶,

was predicted as a candidate target of miR-411 and selected for further confirmation. Luciferase reporter assay was performed to confirm whether miR-411 could directly target the 3'-UTR of STAT3. miR-411 mimic or miR-NC was transfected into HeLa and SiHa cells, together with luciferase reporter plasmid carrying the Wt or Mut STAT3 3'-UTR. The results showed that restored miR-411 expression significantly reduced the luciferase activities of psiCHECK™-2-STAT3-3'-UTR

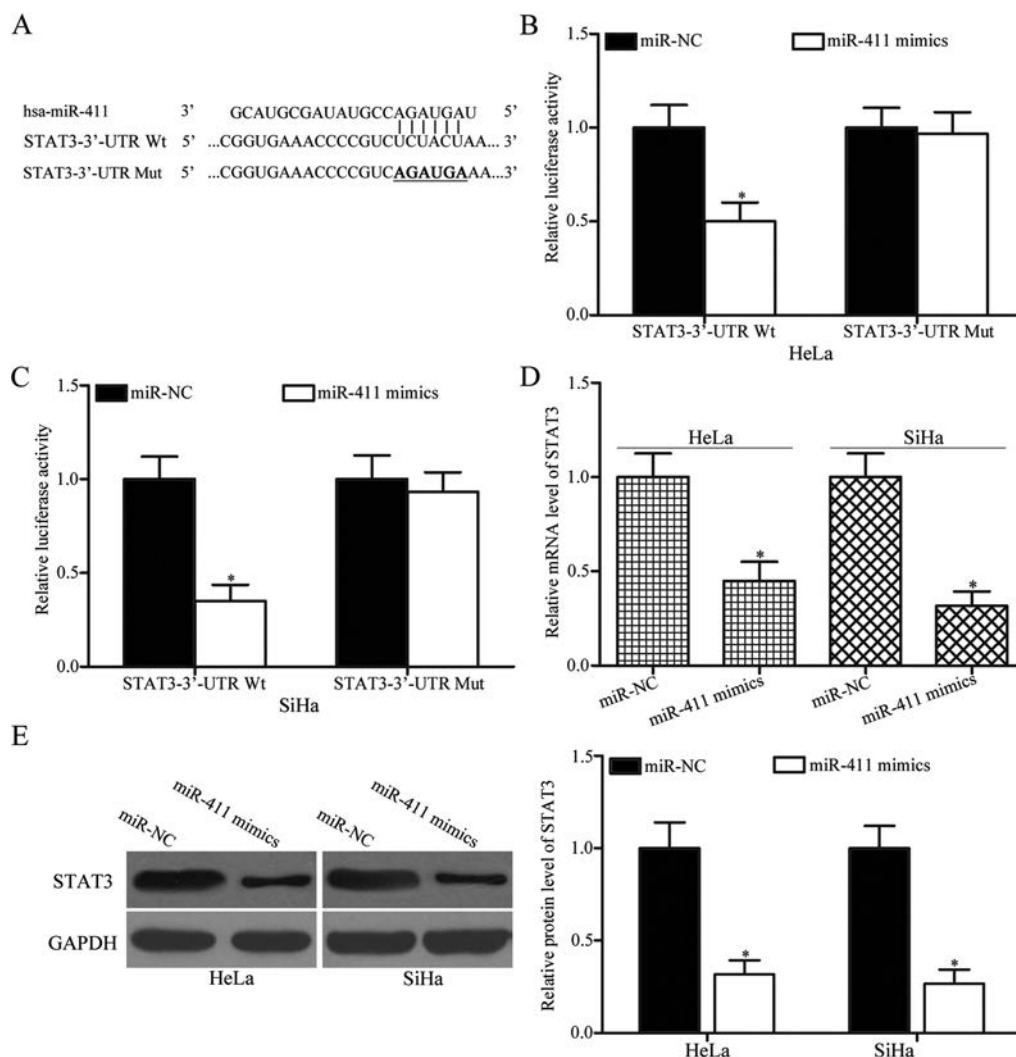


Figure 3. Signal transducer and activator of transcription 3 (STAT3) is a novel target of miR-411 in cervical cancer. (A) Predicted binding sites for miR-411 in the 3'-untranslated region (3'-UTR) of wild type (Wt) STAT3 and the mutations (Mut) in the binding sites are shown. Dual-luciferase reporter assays were conducted in (B) HeLa and (C) SiHa cells cotransfected with luciferase reporter plasmid carrying the Wt or Mut STAT3 3'-UTR, along with miR-411 mimic or miR-NC. * $p < 0.05$ compared with miR-NC. (D) STAT3 mRNA and (E) protein expression levels in HeLa and SiHa cells transfected with miR-411 mimic or miR-NC were detected by RT-qPCR and Western blotting analysis, respectively. * $p < 0.05$ compared with miR-NC.

Wt ($p < 0.05$) (Fig. 3B and C) and had no influence on that of the psiCHECKTM-2-STAT3-3'-UTR Mut in both HeLa and SiHa cells, thereby indicating that miR-411 directly targets the 3'-UTR of STAT3 in cervical cancer.

To further confirm the regulatory roles of miR-411 on endogenous STAT3 expression, RT-qPCR and Western blotting analysis were performed to detect STAT3 expression in HeLa and SiHa cells following transfection with miR-411 mimic or miR-NC. As shown in Figure 3D and E, resumption expression of miR-411 suppressed STAT3 mRNA ($p < 0.05$) (Fig. 3D) and protein ($p < 0.05$) (Fig. 3E) expression levels in HeLa and SiHa cells.

Overall, these results suggested that STAT3 is a direct target gene of miR-411 in cervical cancer.

STAT3 Is Upregulated in Cervical Cancer Tissues and Negatively Correlated With miR-411 Expression Level

To further elucidate the association between miR-411 and STAT3 in cervical cancer, RT-qPCR was used to measure STAT3 mRNA expression in 45 paired cervical cancer tissues and corresponding adjacent normal tissues. The results indicated that STAT3 mRNA was significantly upregulated in cervical cancer tissues compared with that in adjacent normal tissues ($p < 0.05$) (Fig. 4A).

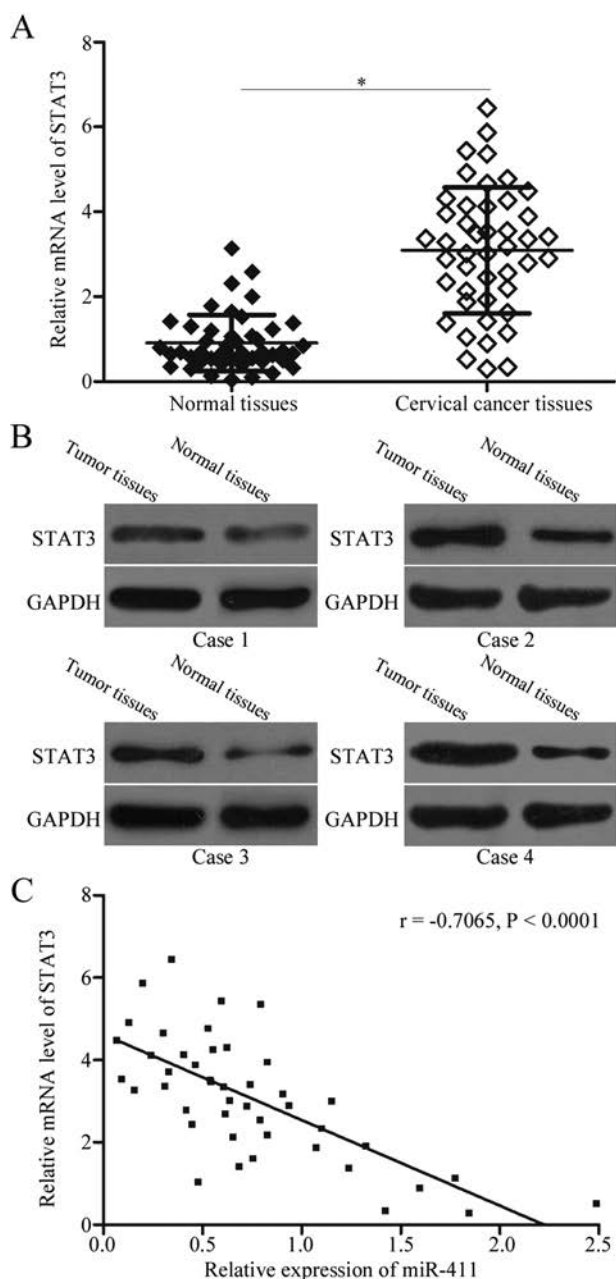


Figure 4. Association between the expression of miR-411 and mRNA levels of STAT3 in cervical cancer tissues. The expression of STAT3 (A) mRNA and (B) protein in cervical cancer tissues and corresponding adjacent normal tissues was detected using RT-qPCR and Western blotting analysis, respectively. * $p < 0.05$ compared with normal tissues. (C) Spearman's correlation analysis was conducted to analyze the relationship between the expression of miR-411 and STAT3 mRNA in cervical cancer tissues. $r = -0.7065$, $p < 0.0001$.

Additionally, Western blotting analysis revealed that expression levels of STAT3 protein were higher in cervical cancer tissues than those in corresponding adjacent normal tissues (Fig. 4B). Furthermore, an inverse association was observed between the expressions of miR-411 and

STAT3 mRNA in cervical cancer tissues ($r = -0.7065$, $p < 0.0001$) (Fig. 4C). These results suggested that increased levels of STAT3 in cervical cancer may be partly attributed to the downregulation of miR-411.

STAT3 Is Able to Rescue the Tumor-Suppressing Roles Induced by miR-411 Overexpression in Cervical Cancer

To further determine whether STAT3 mediated the tumor-suppressing roles of miR-411 on the cervical cancer cells, rescue experiments were performed on HeLa and SiHa cells transfected with miR-411 mimic, along with or without STAT3 overexpression plasmids (pcDNA3.1-STAT3). Western blotting analysis demonstrated that STAT3 overexpression is able to restore the STAT3 protein level decreased by miR-411 mimics ($p < 0.05$) (Fig. 5A). Furthermore, CCK-8 and Transwell invasion assays indicated that restoration of the expression of STAT3 rescued the suppressive effects of miR-411 overexpression on the proliferation ($p < 0.05$) (Fig. 5B) and invasion ($p < 0.05$) (Fig. 5C) in HeLa and SiHa cells. These results suggested that miR-411 exerted its tumor-suppressive role in cervical cancer at least by downregulation of STAT3.

DISCUSSION

Substantial evidence has demonstrated that miRNA expression is disordered in many malignant tumors²⁷⁻²⁹. The dysregulation of miRNAs has been suggested to be involved in the tumorigenesis and tumor development of cervical cancer³⁰⁻³². Therefore, identification of miRNAs and their biological roles and targets involved in tumor pathology would provide valuable insight into the diagnosis and treatment of patients with cervical cancer. In this study, we found that the expression of miR-411 was significantly downregulated in cervical cancer specimens and cell lines. Low miR-411 expression level was significantly correlated with tumor size, FIGO stage, lymph node metastasis, and distant metastasis. We also confirmed that upregulation of miR-411 inhibited the proliferation and invasion of cervical cancer cells. More importantly, STAT3 was identified and confirmed to be a direct and functional target of miR-411 in cervical cancer. The results suggested that miR-411 should be investigated as a potential therapeutic agent in the treatment of patients with cervical cancer.

A recent study reported that the aberrant expression of miR-411 is a characteristic of human malignancies. For example, miR-411 was lowly expressed in breast cancer tissues and cell lines, and decreased miR-411 expression was associated with lymph node metastasis and the histological grade of breast cancer patients³³. miR-411 expression was also observed to be downregulated in renal cell carcinoma tissues and cell lines when compared with

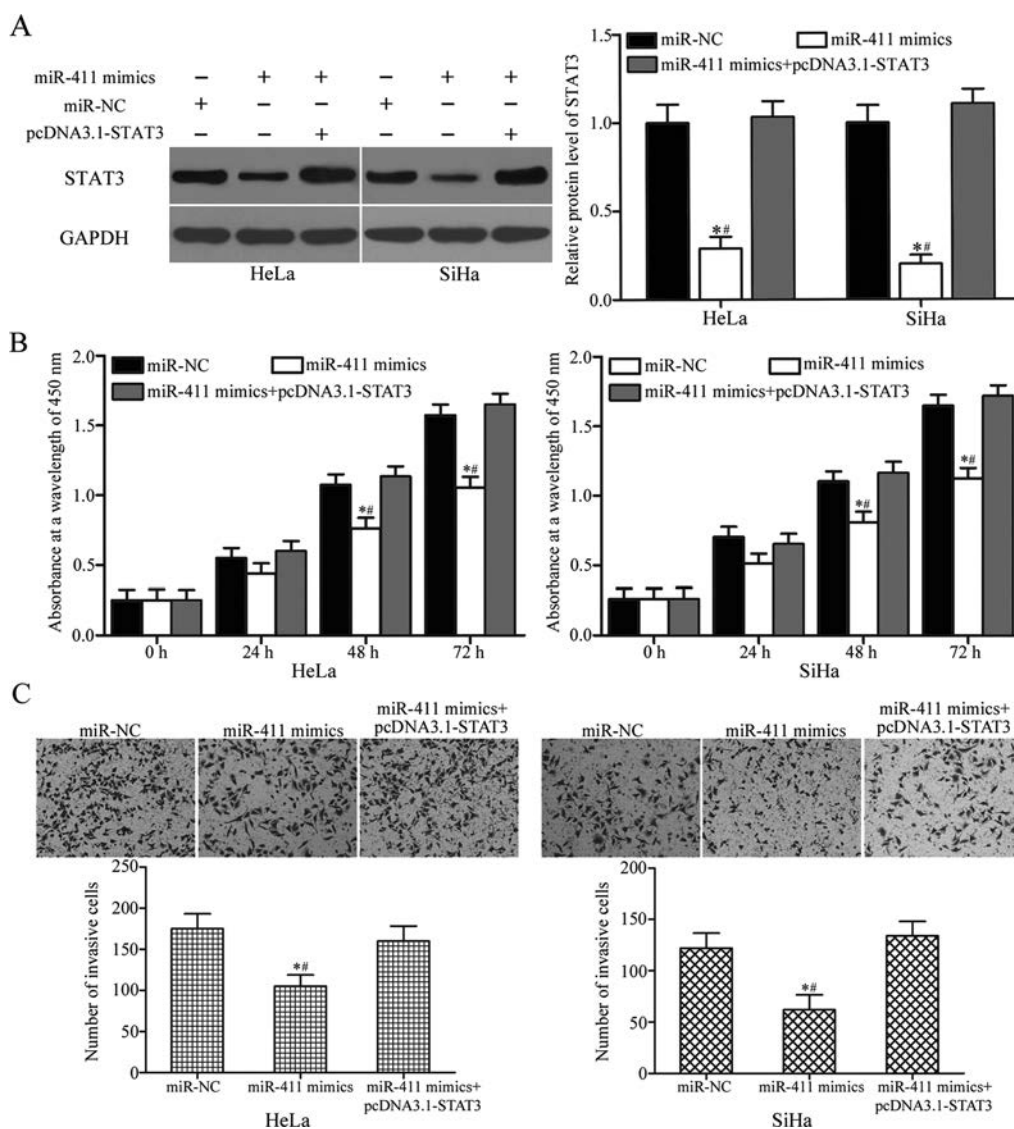


Figure 5. Upregulation of STAT3 partially rescues cell proliferation and invasion inhibited by miR-411 overexpression in cervical cancer cells. miR-411 mimic was transfected into HeLa and SiHa cells, either with or without pcDNA3.1-STAT3. (A) The protein expression level of STAT3 was determined by Western blotting analysis. * $p < 0.05$ compared with miR-NC. # $p < 0.05$ compared with miR-411 mimics + pcDNA3.1-STAT3. (B) CCK-8 and (C) Transwell invasion assays were conducted to examine cell proliferation and invasion in indicated cells. * $p < 0.05$ compared with miR-NC. # $p < 0.05$ compared with miR-411 mimics + pcDNA3.1-STAT3.

adjacent normal tissues and a normal renal cell line¹⁷. However, miR-411 expression was upregulated in hepatocellular carcinoma²¹ and lung cancer²². These conflicting studies indicated that miR-411 expression has tissue specificity and may serve as a potential diagnostic and prognosis marker for these human cancers.

miR-411 played tumor-suppressing roles in the formation and progression of several types of human cancers. For instance, Zhang et al.³⁴ found that upregulation of miR-411 suppressed cell proliferation, migration, and invasion of breast cancer. Sun et al.³⁵ reported that increased expression of miR-411 inhibited rhabdomyosarcoma cell

growth in vitro and in vivo. A study by Zhang et al.¹⁷ revealed that miR-411 overexpression attenuated cell growth and metastasis and promoted apoptosis in renal cell carcinoma. However, in hepatocellular carcinoma, miR-411 served in an oncogenic role by promoting cell proliferation and anchorage-independent growth²¹. Zhao et al.²² showed that ectopic expression of miR-411 increased anchorage-dependent and anchorage-independent growths in lung cancer. These findings suggested that the biological roles of miR-411 have tissue specificity and may be an attractive candidate therapeutic target in anti-tumor therapy.

Several direct targets have been identified, including SP1³³ and GRB2³⁴ in breast cancer, SPRY4³⁵ in rhabdomyosarcoma, ITCH²¹ in hepatocellular carcinoma, and FOXO1²² in lung cancer. In our current study, STAT3 was validated as a direct and functional target of miR-411 in cervical cancer. STAT3, a central member of the STAT family, was first verified to be an interleukin-6-activated acute phase response factor in 1994^{36,37}. It has been found that STAT3 was upregulated in a variety of human cancer types, such as cervical cancer³⁸, laryngeal squamous cell carcinoma³⁹, colorectal cancer⁴⁰, non-small cell lung cancer⁴¹, glioma⁴², and ovarian cancer⁴³. Further studies have demonstrated that STAT3 plays an important role in regulating cell proliferation, migration, invasion, apoptosis, angiogenesis, and epithelial–mesenchymal transition in human malignancies^{44–48}. Previous studies strongly demonstrated that STAT3 activation is a promising molecular target for cancer therapies^{49–51}. Multiple STAT3 inhibitors have been developed, and several STAT3 inhibitors have completed phase I/II clinical trials^{52–54}. Combined with the findings in this research, the miR-411/STAT3 axis may be developed as a clinically useful drug for cervical cancer treatment.

In conclusion, to the best of our knowledge, our study is the first of its kind to show that miR-411 was frequently downregulated in cervical cancer tissues and cell lines. Low miR-411 expression level was significantly correlated with tumor size, FIGO stage, lymph node metastasis, and distant metastasis. miR-411 played a tumor-suppressing role in cervical cancer progression by directly targeting STAT3. These findings suggest that miR-411 may serve as a diagnostic and prognosis marker and is a promising new target for patients with cervical cancer.

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