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MicroRNA-23b suppresses cervical cancer biological progression by directly targeting six1 and affecting epithelial-to-mesenchymal transition and AKT/mTOR signaling pathway

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Abstract. – OBJECTIVE: To elucidate the effects and mechanism of microRNA-23b (miR-23b) in cervical cancer (CC) progression.

PATIENTS AND METHODS: Fifty-six pairs of CC tissue samples and matched para-carcinoma tissue samples were collected. Meanwhile, human normal cervical epithelial cell and CC cell lines were cultured. The abilities of cell proliferation and migration were detected by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assays and transwell assays. The correlation between sine oculis homeobox 1 (six1) and miR-23b was clarified by dual-luciferase reporter assay. The relative protein and mRNA expression were detected by quantitative real-time polymerase chain reaction (qRT-PCR), immunohistochemistry (IHC) and Western blot. In addition, Xenograft tumor formation assay was performed in this study.

RESULTS: MiR-23b was remarkably down-regulated in CC and the low miR-23b expressions were associated with the poor prognosis and worse OS of CC patients. Additionally, the functional assays demonstrated that miR-23b overexpression obviously repressed CC cell proliferation, invasion and migration abilities through the regulation of the AKT/mTOR pathway and the epithelial-to-mesenchymal transition (EMT) progress. Moreover, the luciferase reporter assay indicated that six1 was one functional target for miR-23b in CC cells, indicating that the inhibitory functions of miR-23b in CC cells were partially regulated by six1. Moreover, miR-23b restoration could prominently repress tumor growth *in vivo*.

CONCLUSIONS: MiR-23b suppressed CC progression *via* directly targeting six1 and affecting AKT/mTOR signaling pathway as well as EMT progress. Therefore, miR-23b/six1 may be promising biomarkers for CC diagnosis and therapy.

Key Word:
Cervical cancer, microRNA-23b, six1, Epithelial-to-mesenchymal transition (EMT), AKT/mTOR.

Introduction

Cervical cancer (CC) is one common malignant tumor in gynecology with the second highest incidence among all female malignancies[1]. Currently, CC remains a huge threat to women's health due to limited medical technologies. CC patients can receive related treatments such as chemotherapy, radiotherapy, and surgical operations; however, metastasis and relapse are the main reasons for treatment failures[2,3]. Additionally, the survival rate for CC patients remains awful attributed to the difficulty of early diagnosis[4]. Therefore, further investigation of the underlying molecular mechanisms about CC progression is imperative to develop effective treatment approaches for improvement of CC prognosis.

MiRNAs have been confirmed to target the 3'-UTRs of relevant genes, thereby directly and indirectly modulating their expressions. Increasing research has demonstrated that miRNAs are aberrantly expressed in numerous tumors, being associated with tumorigenesis and tumor progression *via* regulation of certain tumor suppressors or oncogenes. For instance, Xie et al[5] found that miR-124 suppressed non-small cell lung cancer cell proliferation *via* downregulating SOX8 expressions; Xu et al[6] demonstrated that miR-532-5p promoted human gastric cancer progression by regulating RUNX3, serving as an oncogenic miRNA; Liu et al[7] revealed that miR-155 facilitated the colorectal cancer cell invasion through modulating the Wnt/beta-catenin. However, the effects of miR-23b on regulating CC remain unexplored. Therefore, it is worthwhile to elucidate the clinical significance of miR-23b in CC.

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Epithelial-to-mesenchymal transition (EMT) is a conserved process featured by converting polarized immotile epithelial cells into motile mesenchymal cells[8]. In this process, mesenchymal markers (such as vimentin) are upregulated whereas cell adhesion molecules (such as E-cadherin) are downregulated[9]. Growing studies have demonstrated that the EMT process is implicated in the metastasis and invasion of multiple tumors, including prostate cancer[10], gallbladder cancer[11], and gastric cancer[12]. The Akt/mTOR signaling pathway, an important oncogenic pathway which is frequently activated in carcinogenesis, is reportedly an important regulator of cell apoptosis, growth and survival[13]. Therefore, the molecular details of EMT and AKT/mTOR pathway should be determined to improve the diagnosis and therapies of CC patients.

The sine oculis homeobox 1 (six1) is a development-related transcription factor which belonged to the six family[14]. Overexpression of six1 has been found in various tumors, being associated with malignant tumor progression such as metastasis and poor survival[15]. For example, Yu et al[16] found that six1 promoted osteosarcoma cell tumorigenesis and proliferation *via* reducing PTEN expressions and activating PI3K/AKT signal; He et al[17] reported that six1 overexpression indicated poor prognosis of esophageal squamous cell cancer and induced radioresistance through AKT signaling; Zeng et al[18] found that increased six1 expressions were correlated with prostate cancer progression and prognosis. These findings suggested that six1 overexpression may promote tumor progression. Therefore, we further investigated the functions of six1 in CC.

Patients and Methods

Clinical Tissue Specimens

Fifty-six pairs of CC tissue samples and matched para-carcinoma tissue samples were collected from the CC patient who suffered from surgical resection in Binzhou People's Hospital from May 2015 to September 2017. No patients received any treatment before tissue collection. The written informed consent has been obtained from each enrolled patient. All the tissue samples were snap-frozen in liquid nitrogen immediately and stored at -80°C before usage. This study was approved by the Ethics Committee of Binzhou People's Hospital.

Cell Cultures

Human normal cervical epithelial cell (Ect1/E6E7) and CC cell lines (C4-1, SiHa, Ca-Ski and HeLa) were purchased from the Committee on Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Above cell lines were maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, Rockville, MD, USA) with 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA) at 37°C in a humidified incubator containing 5% CO_2.

Cell Transfections

MiR-23b mimics, inhibitor as well as negative control (NC), was purchased from RiboBio Co., Ltd. (Guangzhou, China). Cell transfections were carried out using Lipofectamine™ 2000 reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturers' instructions.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was utilized to extract the total RNA from the CC tissues and cells. Then, a Prime Script RT reagent kit (TaKaRa, Otsu, Shiga, Japan) was utilized to reversely transcribe RNA into complementary deoxyribose nucleic acid (cDNA). qRT-PCR reactions were carried out with ABI 7900 Real-Time PCR Detection System (Applied Biosystems; Carlsbad, CA, USA) by SYBR Green (TaKaRa Bio, Otsu, Shiga, Japan). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was an internal reference for six1 while U6 was an endogenous control for miR-23b, and relative expressions were detected by $2^{-\Delta\Delta Ct}$ method. The sequences of the primers were described in Table I.

Immunohistochemistry (IHC)

IHC assays were performed to determine the expressions of six1 in CC tissues. Tissues were formalin-fixed and paraffin-embedded. Then, slides were deparaffinized and rehydrated using xylene and descending alcohol series. Next, endogenous peroxidase activities were blocked by incubation in 3% hydrogen peroxidase and antigen retrieval was conducted in a microwave oven with citrate buffer. Subsequently, tissues were then incubated with primary six1 antibody (1:500, ab211359, Abcam, Cambridge, MA, USA) at 4°C overnight. And then, the slides were incubated with goat anti-rabbit IgG (1:2,000, ab205718, Abcam, Cambridge, MA, USA) labeled by horseradish peroxidase (HRP) for 30

Table I. Primer sequences for qRT-PCR.

Primer	Sequence
miR-23b forward	5'-GGTGCTCTGGCTGCTTGG-3'
miR-23b reverse	5'-GCCAAGGTCGTGGTTGCG-3'
U6 forward	5'-CTCGCTTCGGCAGCACA-3'
U6 reverse	5'-AACGCTTCACGAATTTGCGT-3'
six1 forward	5'-GTCAGCAACTGGTTCAAGA-3'
six1 reverse	5'-AGGAGGACCGAGTTCTGAT-3'
GAPDH forward	5'-CACAATTGGGACCACAAGGG-3'
GAPDH reverse	5'-AACTCATCACAGCACGTCACACC-3'

U6: small nuclear RNA, snRNA; six1: sine oculis homeobox 1; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

min at room temperature. The slides were stained with diaminobenzidine (DAB) as the chromogen and counterstained with hematoxylin. The sections were then photographed with a BX53F microscope (Olympus, Tokyo, Japan). The double-blind method was utilized to randomly select 5 visual fields from each section. The expression level was detected following the ratio of positive cells: stained cells/all cells < 25% was negative (-), while the ratio >25% was considered as positive (+)[19,20].

Cell Proliferation Assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assays (Sigma, St. Louis, MO, USA) were performed to detect the proliferation capacities of CC cells treated with miR-23b mimics or inhibitor. The transfected cells were seeded into 96-well plate and incubated at 37°C for 0 h, 24 h, 48 h or 72 h respectively. Subsequently, MTT (10 μL, 5 mg/mL) was added into each well, followed by an incubation for 4 h. Then, dimethyl sulfoxide (DMSO) (Sigma, St. Louis, MO, USA) was added to solubilize the crystals. Finally, the optical density $(OD)_{490}$ was examined using a microplate reader instrument (Bio-Rad, Hercules, CA, USA).

Transwell Assays

Cell migration and migration capacities of CC cells with transfection of miR-23b mimics or inhibitor were determined by performing transwell assays using transwell chamber (8.0 μm pore size, Corning Incorporated, Corning, NY, USA) pre-coated with or without Matrigel (BD Biosciences, Franklin Lakes, NJ, USA). Briefly, transfected cells were placed into the top chamber and incubated at 37°C with serum-free medium. In the meantime, medium with 10% FBS was added into the bottom chambers as a chemoattractant, followed by an incubation at 37°C with 5% CO_2 for 48 h. After that, cells remaining on the top surface were removed and while those adhered to the bottom surface were fixed with methanol and stained with crystal violet. Finally, cells were photographed and counted with a microscope (Olympus, Tokyo, Japan) from five randomly selected visual fields.

Western Blots

Ice-cold lysis buffer containing the protease inhibitor cocktail (Sigma Chemical, St. Louis, MO, USA) was utilized to lyse the cultured CC cells. A bicinchoninic acid (BCA) protein assay kit (Beyotime Institute of Biotechnology, Shanghai, China) was used to measure the protein concentrations according to the manufacturer's proposals. Subsequently, proteins were separated with 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). After that, the proteins were subjected to being transferred onto polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA) which was blocked in Tris-Buffered Saline and Tween 20 (TBST) containing 5% skim milk for 2 h at room temperature, and then were incubated with the primary antibodies: six1 (1:500, ab211359, Abcam, Cambridge, MA, USA), E-cadherin (1:1000, ab15148, Abcam, Cambridge, MA, USA), Vimentin (1:1000, ab137321, Abcam, Cambridge, MA, USA), mTOR (1:1000, ab32028, Abcam, Cambridge, MA, USA), Akt (1:1000, sc-56878, Santa Cruz Biotechnology, Santa Cruz, CA, USA), p-mTOR (1:1000, ab109268, Abcam, Cambridge, MA, USA), p-Akt (1:1000, sc-81433, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and (1:2000, ab70699, Abcam, Cambridge, MA, USA) at 4°C overnight. Next, the membrane was incubated with HRP-conjugated goat anti-rabbit second-

ary antibody (1:2000, ab205718, Abcam, Cambridge, MA, USA) for 2 h at room temperature. The proteins were visualized using enhanced chemiluminescence (ECL) reagents (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Dual-Luciferase Reporter Assay

The wildtype (WT) or mutant (MUT) six13'UTR containing the putative binding sequences of miR-23b were purchased from GenePharma, Co., Ltd. (Shanghai, China). For the luciferase reporter assays, CC cells were co-transfected with miR-23b mimics and six13'UTR-WT or MUT by Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The dual luciferase reporter assay system (Promega, Madison, WI, USA) was utilized to detect the luciferase activities at 48 h after transfections in strict line with the manufacturer's instructions.

Xenograft Tumor Formation Assay

The animal study was approved by the Institutional Animal Care and Use Committees of Binzhou People's Hospital. Six-week-old female nude mice were randomly assigned into two groups. The HeLa cells were stably transfected with lentiviral miR-23b (lenti-miR-23b) or the negative lentiviral miR-control (lenti-control) and injected subcutaneously into the flank of the mice in different groups. The size of tumors was measured every 3 days (volume = longest diameter × shortest diameter^2/2). After 4 weeks, mice were sacrificed and their tumors were dissected and trimmed.

Statistical Analysis

Statistical analysis was performed with Statistical Product and Service Solutions (SPSS) software version 17.0 (SPSS Inc., Chicago, IL, USA). To determine the significance of two groups and multiple groups, Student's *t*-test and one-way ANOVA followed by Tukey's post-hoc test were conducted respectively. The Kaplan-Meier curve and log-rank test were utilized to analyze the overall survival of CC patients. It was considered statistically significant when $p<0.05$.

Results

Down-Regulation of miR-23b was Associated with Poor Prognosis of CC Patients

To assess the functions of miR-23b in CC, we measured the expressions and clinical significance of miR-23b. qRT-PCR was conducted to measure the miR-23b expression levels in CC tissues. Results showed that miR-23b was prominently down-regulated in CC tissues (Figure 1A). In addition, the CC patients involved in the current study were assigned into low and high miR-23b expression group based on the median miR-23b expression level. Then, the association between miR-23b expressions and the clinicopathological characteristics of CC patients were analyzed. As shown in Table II, the declined miR-23b expression was found to be related to the malignant phenotypes of CC patients. Furthermore, we carried out the

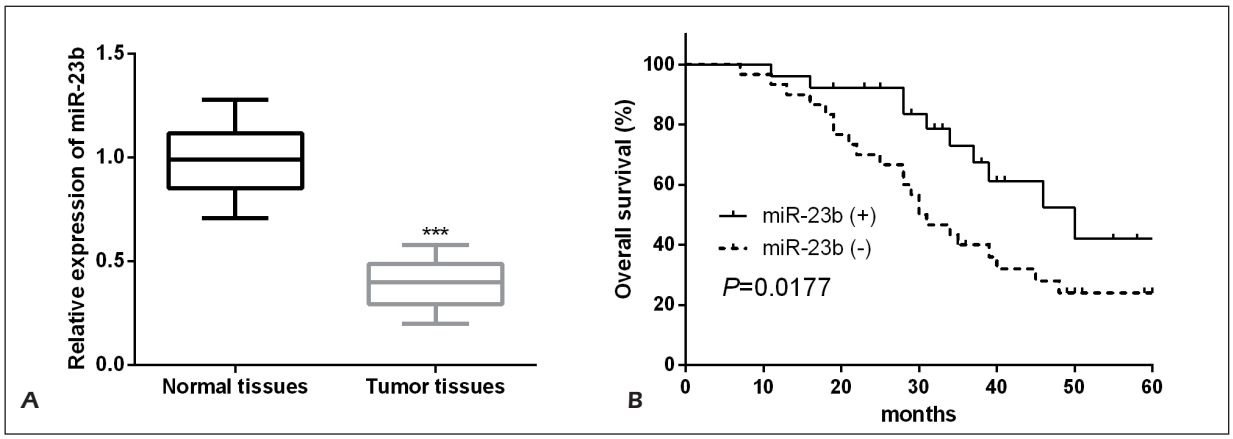

Figure 1. MiR-23b downregulation in CC tissues predicted the poorer prognosis of CC patients. **A**, MiR-23b expressions in CC tissue samples and matched normal tissue samples were examined by qRT-PCR. **B**, Low expressions of miR-23b demonstrated poor OS of CC patients. ***$p<0.001$.

Table II. Correlation of miR-23b expression with the clinicopathological characteristics of the cervical cancer patients.

Clinicopathological features	Cases (n=56)	SNHG20 expression		*p*-value
		High (n=20)	Low (n=36)	
Age (years)				0.3128
> 60	29	9	20	
≤ 60	27	11	16	
Family history of cancer				0.4317
Yes	27	8	19	
No	29	12	17	
Tumor size (cm)				0.0731
≥ 5.0	26	8	18	
< 5.0	30	12	18	
TNM stage				0.0028*
I-II	26	16	10	
III	30	4	26	
Lymph-node metastasis				0.0016*
Yes	16	5	11	
No	40	15	25	
Pausimenia				0.2348
Yes	25	9	16	
No	31	11	20	
FIGO stage				0.0041*
I-II	25	16	9	
III-IV	31	4	27	
Distant metastasis				0.3796
Yes	28	7	21	
No	28	13	15	

TNM: tumor-node-metastasis; FIGO: International Federation of Gynecology and Obstetrics. [a]The mean expression level of miR-23b was used as the cutoff. *Statistically significant.

Kaplan-Meier analysis to analyze the prognostic values of miR-23b in CC patients. Data demonstrated that CC patients with lower miR-23b expressions had notably shorter OS than the high miR-23b expression group (Figure 1B), indicating that low miR-23b expressions predicted poor prognosis of CC patients.

MiR-23b Upregulation Inhibited CC Cell Proliferation

To further elucidate that miR-23b was implicated in the CC progression, we next investigated the biofunctions of miR-23b in CC cell proliferation. Firstly, we examined miR-23b expression levels in normal cervical cells and CC cells by qRT-PCR. The results showed that the miR-23b expressions were remarkably declined in all CC cell lines in comparison to Ect1/E6E7 (Figure 2A). SiHa and Ca-Ski cells which expressed relatively low and high miR-23b expressions were treated with miR-23b mimics or inhibitor for further assays. The transfection efficiencies were confirmed by qRT-PCR and the results demonstrated that miR-23b was significantly upregulated in SiHa cells and downregulated in Ca-Ski cells (Figure 2B and 2C). The function of miR-23b in CC cell proliferation was analyzed by MTT assays. We found that restoration of miR-23b markedly inhibited CC cell proliferation whereas miR-23b inhibition promoted the proliferation ability (Figure 2D and 2E).

Overexpression of miR-23b Suppressed CC Cell Migration and Invasion

We further investigated the functions of miR-23b in CC cell invasion and migration by transwell assays. As presented in Figure 3A and 3B, miR-23b overexpression in SiHa cells remarkably repressed the invasion and migration abilities compared to the NC group. On the other hand, the migratory and invasive capacities of Ca-Ski cells were significantly promoted by a miR-23b inhibitor (Figure 3C and 3D). All the data re-

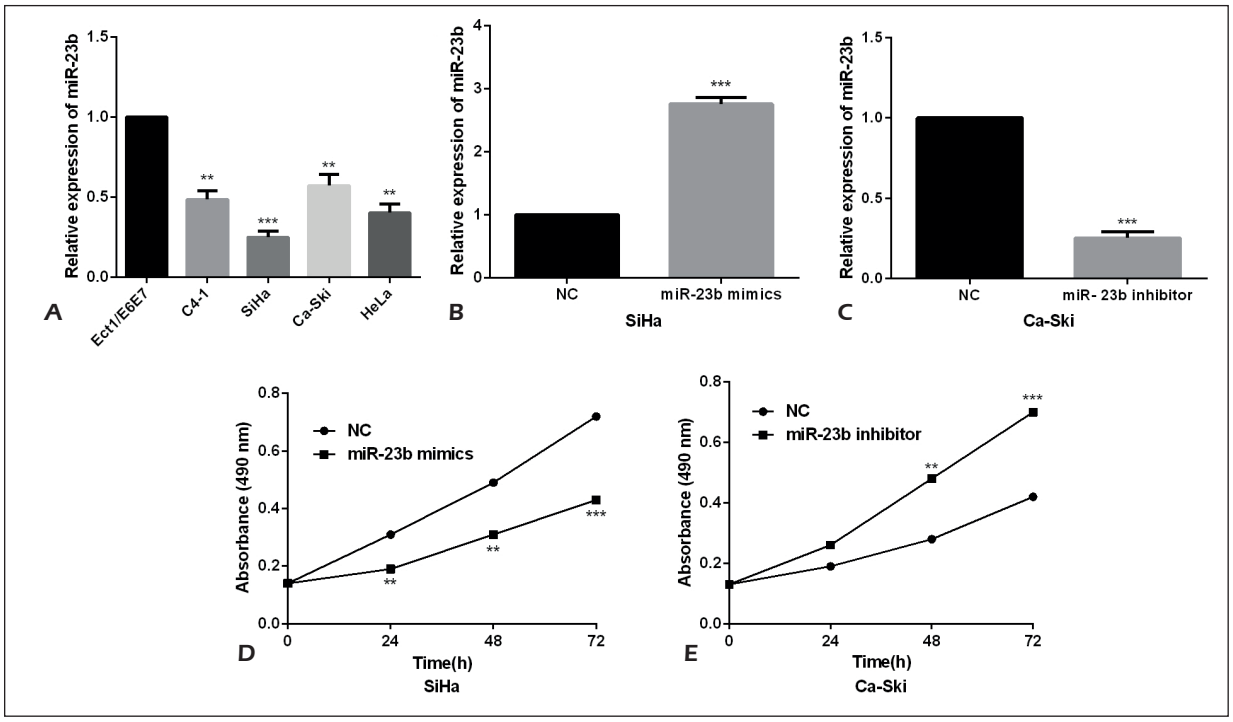

Figure 2. MiR-23b restoration significantly repressed CC cell proliferation. **A**, MiR-23b expressions in CC cells were measured by qRT-PCR. **B**, Overexpression of miR-23b in SiHa cells was confirmed using qRT-PCR. **C**, qRT-PCR was carried out to confirm the inhibition of miR-23b in Ca-Ski cells. **D**-**E**, MTT assays were carried out to detect the proliferation abilities of CC cells treated with miR-23b mimics or inhibitor. $^{**}p<0.01$, $^{***}p<0.001$.

vealed that miR-23b exerted anti-tumor functions in CC progression.

Six1 was a Functional Target of miR-23b in CC Cells

We conducted bioinformatics analysis to explore the potential targets of miR-23b. As shown in Figure 4A, highly-conserved miR-23b targeting sites were predicted in six1 3′-UTR. To verify the combination between miR-23b and six1 3'-UTR, luciferase reporter assays were carried out by co-transfecting miR-23b mimics and six1-3'UTR-WT or six1-3'UTR-MUT into CC cells. The results indicated that miR-23b mimics markedly inhibited the luciferase activities of CC cells with cotransfection of six1-3'UTR-WT. On the other hand, the luciferase activities of CC cells with cotransfection of six1-3'UTR-MUT and miR-23b mimics had no significant change (Figure 4B). Then, we further explored whether miR-23b exerted regulatory effects on endogenous six1 expressions in CC. Results showed that restored miR-23b expressions significantly declined the six1 expressions and miR-23b (Figure 4C). In addition, we found that six1 expressions were remarkably upregulated by miR-23b inhibition (Figure 4D). Collectively, six1 was a direct target of miR-23b.

MiR-23b Regulated AKT/mTOR Signaling Pathway and EMT in CC Cells

We next explored the potential mechanism by which miR-23b inhibited CC progression. Firstly, we measured the six1 expressions in CC tissues by performing IHC assays. Results demonstrated that six1 mainly localized at the nucleus and significantly overexpressed in CC tissues (Figure 5A and 5B). Moreover, we found that overexpressed six1 was related to poorer OS of CC patients (Figure 5C). Subsequently, we performed western blot analysis to explore the underlying mechanisms. Briefly, proliferation-related AKT/mTOR signaling pathway was investigated. Results demonstrated that expressions of p-AKT and p-mTOR were markedly suppressed in SiHa cells transfected with miR-23b mimics whereas notably enhanced by miR-23b inhibition in Ca-Ski cells; however, the AKT and mTOR expressions were not altered by miR-23b overexpression or inhibition (Figure 5D). Moreover, EMT served

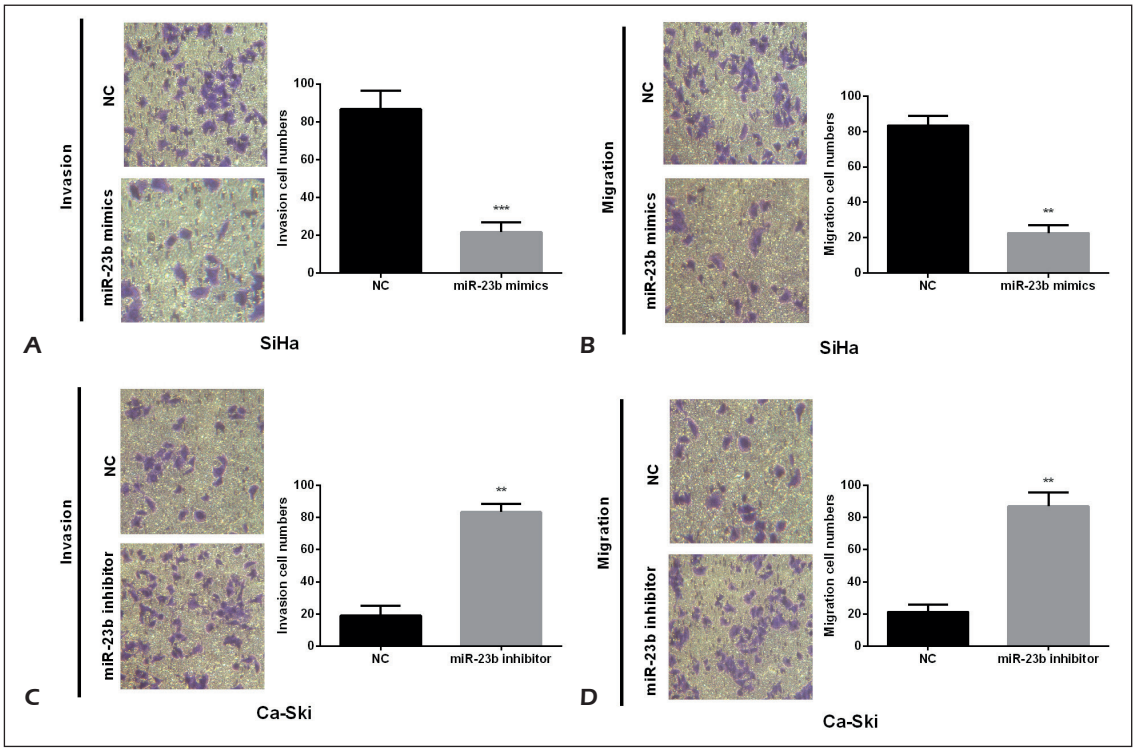

Figure 3. Overexpression of miR-23b prominently inhibited CC cell migration and invasion capacities. **A-B**, Transwell assay was conducted to assess the invasion and migration abilities of SiHa cells treated with miR-23b mimics. **C-D**, The invasion and migration capacities of Ca-Ski cells treated with miR-23b inhibitor were observed by transwell assays. **p<0.01, ***p<0.001.

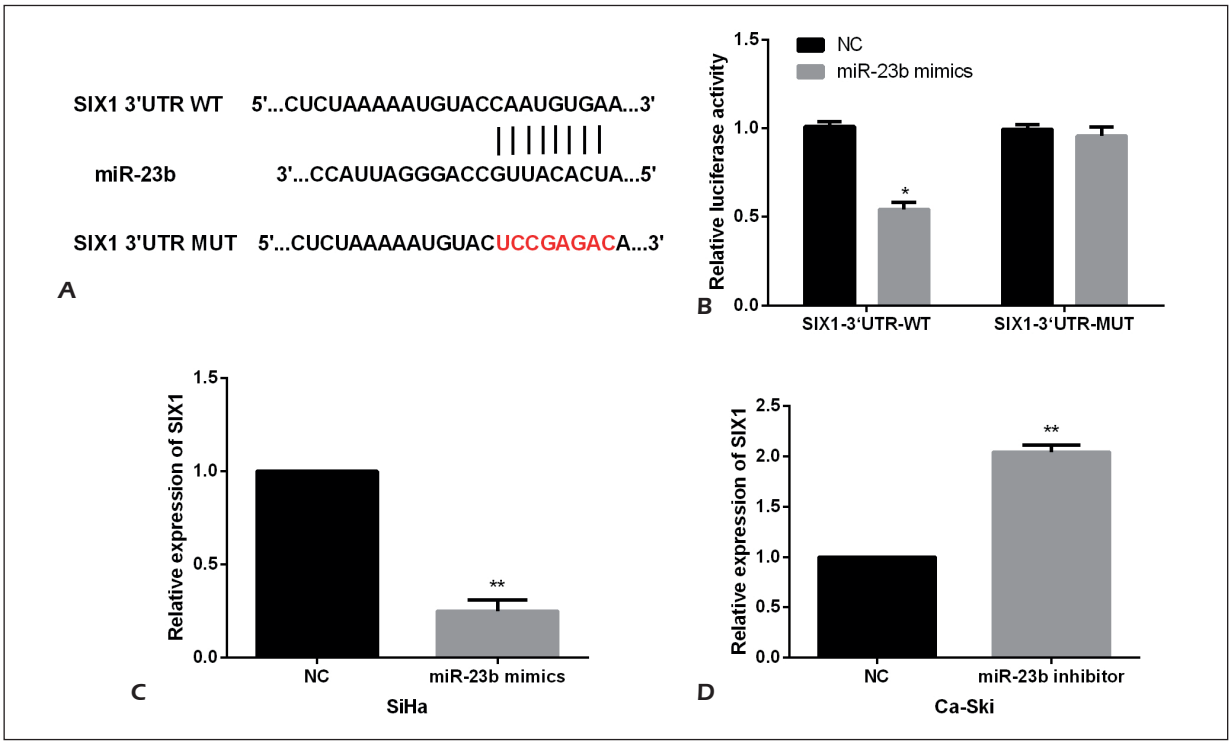

Figure 4. SIX1 was a functional target of miR-23b in CC cells. **A**, Predicted binding sequences of miR-23b in six1 3'-UTR. **B**, Luciferase reporter analysis was conducted to confirm the correlation between SIX1 and miR-23b. **C-D**, SIX1 expressions in SiHa or Ca-Ski cells transfected with miR-23b mimics or inhibitor. *p<0.05, **p<0.01.

important roles in regulating cell metastasis. We further investigated whether miR-23b could suppress CC cell migration and invasion by inhibiting EMT phenotype. Results revealed that miR-23b overexpression evidently decreased the expressions of N-cadherin and vimentin whereas enhanced the expressions of E-cadherin in SiHa cells. On the other hand, E-cadherin expressions were prominently reduced and N-cadherin and vimentin expressions were significantly increased by miR-23b inhibition (Figure 5D).

MiR-23b Suppressed CC Tumor Growth In Vivo

We stably transfected HeLa cells with lenti-miR-23b or the negative control and established xenograft tumor formation for CC. The results indicated that miR-23b overexpression had a significantly slower growth rate and reduced tumor volume, compared to the control group (Figure 6A and 6B).

Discussion

CC is one prevalent gynecological malignancy, the morbidities of which has increased year by year, seriously threatening the lives of women globally[21]. Therefore, exploring the underlying mechanism of CC tumorigenesis is conducive to identify novel therapeutics for CC[22]. The discovery of miRNA has provided novel insights into the tumor mechanisms and treatments, and

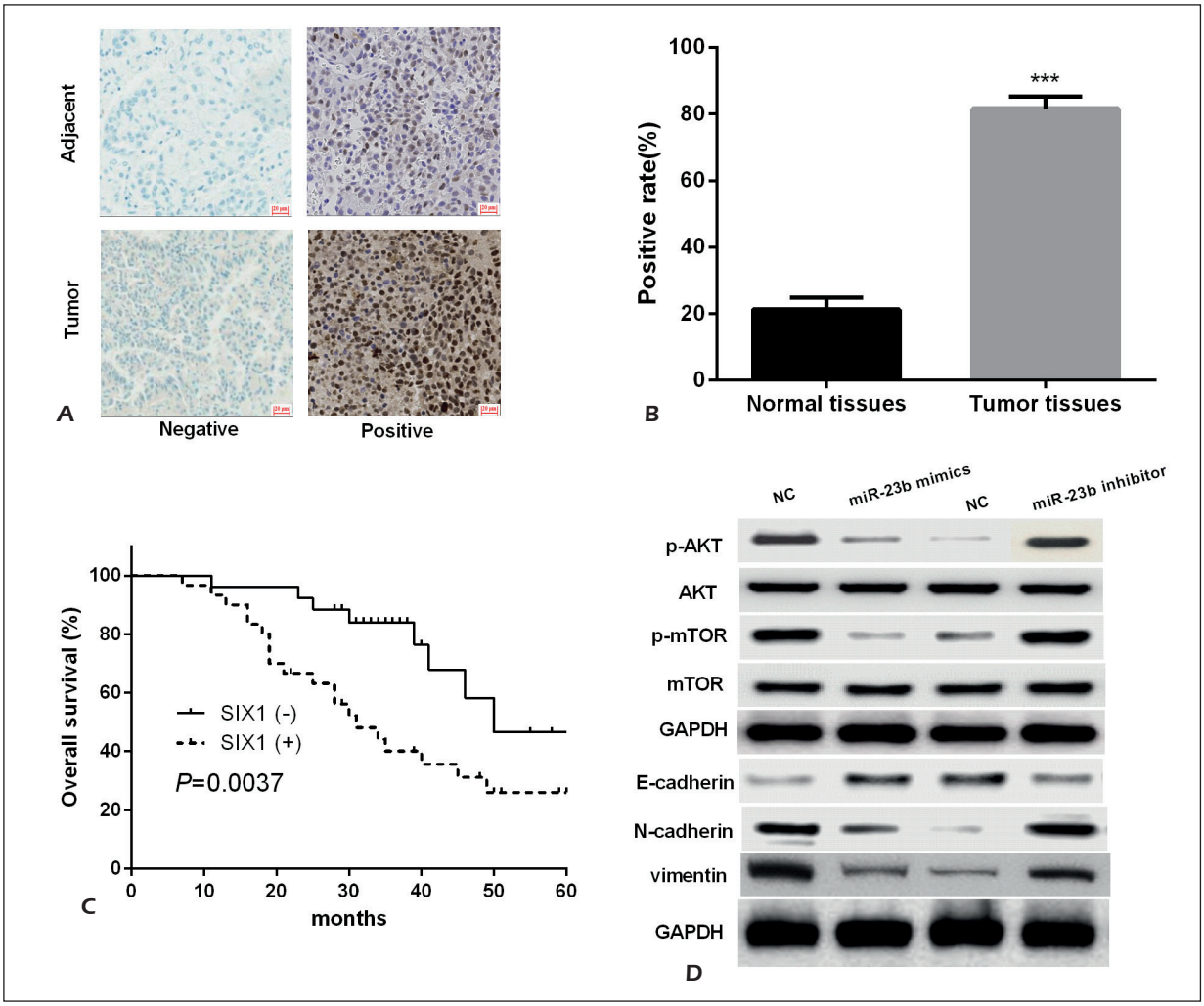

Figure 5. MiR-23b regulated the AKT/mTOR signaling pathway and EMT in CC cells. **A-B**, IHC assays were performed to determine the six1 expressions in CC tissues (magnification: 100×). **C**, Kaplan-Meier analysis showed that high six1 expressions were associated with poor OS of CC patients. **D**, Effects of miR-23b on AKT/mTOR signaling pathway and EMT in CC cells. ***$p<0.001$.

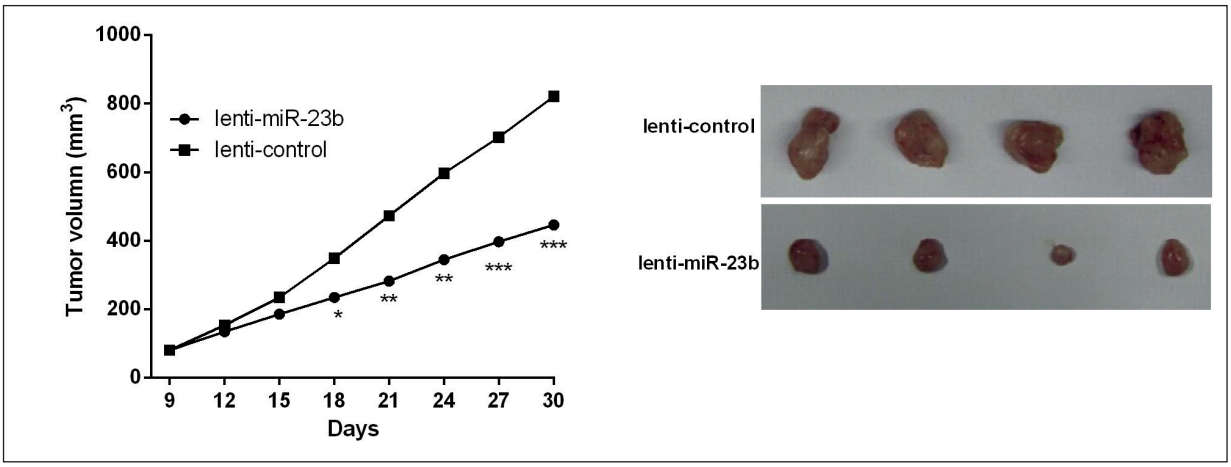

Figure 6. MiR-23b overexpression significantly inhibited CC tumorigenesis in vivo. **A**, Representative images of CC xenograft tissues in different groups. **B**, The tumor volumes were detected every 3 days from day 9 to 30. *p<0.05, **p<0.01, ***p<0.001.

the functions of miRNA in tumorigenesis have received increasing attention[23,24]. Previous studies have demonstrated that aberrant expressions of multiple miRNAs played crucial roles in CC progression[25]. For instance, Wang et al[26] found that miR-328 inhibited CC cell tumorigenesis and proliferation *via* targeting TCF7L2, Jiang et al[27] reported that miR-142 inhibited CC development *via* regulating HMGB1. Therefore, study of miRs has far-reaching significance for mechanism of tumor pathogenesis, which may further promote the progress of developing novel therapeutic biomarkers for CC. Recently, miR-23b has attracted increasing attention owing to its involvement in multiple tumors. For instance, Kou et al[28] indicated that miR-23b downregulation in plasma indicated poor prognosis of colorectal cancer patients; Yan et al[29] proposed that miR-23b suppressed tumorigenesis and progression in ovarian cancer *via* targeting cyclin G1; Zhuang et al[30] claimed that upregulation of plasma miR-23b predicted poor prognosis of gastric cancer. In accordance with these studies, the present data demonstrated the miR-23b was notably downregulated in CC, which was associated with the poor prognosis and worse OS of CC patients. In the meantime, we found that miR-23b restoration dramatically repressed CC cell proliferation, invasion and migration abilities by modulating AKT/mTOR signaling pathway and EMT. Moreover, we verified that miR-23b overexpression prominently represses tumor growth *in vivo*. Taken together, the above results showed that miR-23b exerted anti-tumor functions in CC progression.

Six1 is a critical transcription factor implicated in many diseases, particularly in tumorigenesis. Previous studies revealed that upregulated six1 in CC might contribute to CC occurrence and progression[31]. Consistent with these findings, we confirmed that six1 expressions were greatly increased in CC tissues and associated with poor OS of CC patients. Furthermore, six1 was proven to be a functional target of miR-23b and implicated in the functions of miR-23b in CC cells. Hence, our findings suggested that miR-23b suppressed CC progression, at least partially, *via* the regulation of six1.

Conclusions

We demonstrated that miR-23b exerted tumor suppressive functions in CC by repressing CC cell proliferation, invasion and migration. Briefly, miR-23b was found to be down-regulated in CC tissues and cells, which was associated with poor prognosis of CC patients. Additionally, miR-23b overexpression remarkably inhibited CC cell proliferation, invasion and metastasis by regulating the AKT/mTOR pathway and EMT. Moreover, six1 was confirmed as a functional target for miR-23b in CC cells and regulated the functional effects of miR-23b. Furthermore, miR-23b restoration could suppress tumor growth *in vivo*. The findings in this current study suggested that miR23b may provide novel insights into the molecular mechanism of the pathology and therapeutic targets in CC patients.

Conflict of Interests

The authors declare that they have no conflict of interest.

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