

MicroRNA-195 inhibits proliferation of cervical cancer cells by targeting cyclin D1a

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Abstract Cervical cancer is one of the most frequent gynecological malignancies in women worldwide. MicroRNA-195 (miR-195) was recently found highly expressed in cervical cancer. However, the role of miR-195 in the pathology of cervical cancer remains poorly understood. In this study, we first confirmed the downregulation of miR-195 in primary cervical cancer tissues. For the functional study, we introduced the sequences of miR-195 or miR-195 inhibitor into HeLa and SiHa cervical cancer cell lines. Overexpression of miR-195 inhibited the proliferation of both HeLa and SiHa cells. In contrast, reducing the endogenous miR-195 level by miR-195 inhibitor promoted the proliferation of cervical cancer cells. Flow cytometric assay showed that overexpression of miR-195 induced G1 phase arrest, whereas miR-195 inhibitor shortened G1 phase of cervical cancer cells. In addition, the suppressive role of miR-195 in cell cycle was also demonstrated by the western blot results of various cell cycle indicators, such as phosphorylated retinoblastoma (p-Rb) and proliferating cell nuclear antigen (PCNA), in the gain and loss of function experiments. Furthermore, Dual-Luciferase Reporter Assay revealed that miR-195 targeted the 3'-untranslated region of cyclin D1a transcript, such as to regulate cyclin D1 expression. In summary, our results suggest that miR-195 acts as a suppressor in the proliferation and cell cycle of cervical cancer cells by directly targeting cyclin D1a mRNA.

Keywords MicroRNA-195 · Cyclin D1a · Cervical cancer · Cell cycle · Proliferation

Background

Cervical cancer, the second most frequent gynecological malignancy worldwide, accounts for almost 12 % of all cancers in women [1]. In China, there are about 150,000 cases of newly diagnosed cervical cancer every year, accounting for 30 % global new cases of cervical cancer [2]. Although the pathogenesis of cervical cancer is still unclear, human papillomavirus (HPV) test has been established to be an effective diagnostic and prognostic test for cervical cancer since the crucial role of chronic HPV infection of cervical epithelium in the development of cervical cancer was characterized [3]. As a result of HPV screening, majority of cervical cancers can be identified early and cured with surgery. Furthermore, a significant reduction in the prevalence of cervical cancer is expected with the increasing use of HPV vaccines [4]. Despite the advances in the diagnosis and prevention of the disease, little improvement of the therapeutic regimen was achieved in the recent years and radical hysterectomy is still the standard treatment. Less radical approaches are demanded not only for patients who desire to preserve fertility but also for those with early-stage cervical cancer [5]. In this regard, the research on the molecular mechanisms underlying the oncology of cervical cancer is of clinical significance for developing new therapeutic targets and approaches for cervical cancer.

MicroRNAs (miRNAs) are recently identified noncoding small RNAs of 19–25 nucleotides. miRNAs are firstly transcribed into 70–90-nucleotide single-stranded RNA precursors with hairpin structure, and then processed by the Dicer enzyme to form the mature miRNA, which can complement perfectly or imperfectly with misparings to target messenger

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RNA (mRNA) with specific recognition on the 3'-untranslated region (3'-UTR) to induce either translational repression or RNA degradation [6]. MiRNAs have been demonstrated to play critical roles in the regulation of a variety of physiological and pathological processes including cell proliferation, differentiation, metabolism, cell death, and tumorigenesis [6–8]. MiR-195 is a member of the micro-15a/b/16/195/497 family miRNAs [9], and its aberrant expression was found to be associated with multiple cancers [10–13]. MiR-195 was previously demonstrated to suppress tumorigenesis of hepatocellular carcinoma and glioma cells by modulating the expression of a variety of cell cycle proteins [14–16]. Recently, miR-195 was reported to be downregulated in primary cervical cancer tissues [17]. However, whether such deregulated expression of miR-195 plays a role in the tumorigenesis of cervical cancer is unclear, and the potential of miR-195 as a therapeutic target of cervical cancer remains to be assessed.

In the present study, we investigated the role of miR-195 in cervical cancer cells by transfecting miR-195 expression construct or miR-195 inhibitor sequence into cervical cancer cell lines HeLa and SiHa. We further identified the targets of miR-195 in regulating the proliferation of cervical cancer cells.

Methods

Patients

All patients agreed to participate in this study and gave their written informed consent. Collection and experimentation of human tissues were approved by the Ethics Committee of China Medical University. Eighteen paired cervical tissue specimens from patients with cervical cancer and patients with benign uterine disease without history of cervical dysplasia or abnormal Pap smear were collected at Department of Obstetrics and Gynecology, Shengjing Hospital of China Medical University. The tissues that were obtained from biopsy or surgery were stored immediately in liquid nitrogen. Cancerous or noncancerous cervical specimens were further confirmed by pathological examination.

Real-time PCR

Total RNA was extracted with the RNAsimple Total RNA Extraction Kit (TIANGEN, Beijing, China) following the manufacturer's instructions. Reverse transcription of mature miR-195 was conducted using a stem-loop primer-based method as previously described [18]. Quantitative real-time PCR was performed using the SYBR Green PCR Master Mix (Solarbio, Beijing, China) in the Exicycler 96 Real-Time Thermal Block (Bioneer, Daejeon, Korea). The PCR primers for miR-195 were designed as one strand targeting on miR-195 (5'-GAGCGTAGCAGCACAGAAAT-3') and

the other strand on the stem-loop RT primer (5'-GTGCAGGGTCCGAGGTATTC-3'); U6 was used as the internal control with the following primers: U6 forward 5'-CTCGCTTCGGCAGCACACA-3' and U6 reverse 5'-AACGCTTACGAATTTGCGT-3'.

Oligonucleotides and plasmids

All primers and RNA oligonucleotides were synthesized by Sangon Biotech (Shanghai, China). The coding sequence (CDS) of miR-195 was amplified from cDNA library using the following primers: miRNA-195 CDS F, 5'-GCTGAAGCTTGAGACCCTGGGAGTAAGTTC-3' (HindIII site), and miRNA-195 CDS R, 5'-GCTGGATCCGACCTTCATCTGATGGACAT-3' (BamHI site). The amplified sequences were cloned into plasmid control DNA (pcDNA) 3.1 expression vector (Takara Clontech, Otsu, Japan) to make pcDNA3.1-miRNA-195. The sequence of miR-195 inhibitor is 5'-GCCAAUAAUUUCUGUG-CUGCUA-3'. The negative control (NC) miRNA, 5'-CAGUACUUUUGUGUAGUACAA-3', is not homologous to any human genome sequence.

The 3'-UTR of cyclin D1a mRNA was PCR-amplified using the following primers: cyclin D1a 3'-UTR F, 5'-ACAGCTAGCGAAGGGAGGTGGCAAGAGTG-3' (NheI site), and cyclin D1a 3'-UTR R, 5'-TCGGTTCGACGATGGCTAAGTGAAGCATGA GG-3' (Sall site), and cloned into pmirGLO Dual-Luciferase miRNA Target Expression Vector (Progenia, Madison, WI, USA). The construct miRNA-195 mutant was cloned by site-directed mutation using pcDNA3.1-miRNA-195 as the template and the following primers: miRNA-195 mutant F, 5'-TCTACGACGACAGAAATATTGGCAC-3', and miRNA-195 mutant R, 5'-CTGTCTCGTCTAGAGCCAGGGAA-3'.

Cell culture and transfection

Human cervical cancer cell lines HeLa and SiHa were purchased from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). HeLa and SiHa cells were cultured in DMEM (Gibco, Carlsbad, CA, USA) supplemented with 10 % fetal bovine serum (FBS; Hyclone, Logan, UT, USA) at 37 °C in an atmosphere consisting of 5 % CO₂. The cells were maintained in serum-free medium for 1 h prior to transfection and transfected with the indicated plasmid and/or oligonucleotides with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The medium was replaced with fresh culture medium 4 h later.

In vitro proliferation assay

Cell proliferation was analyzed by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich,

St. Louis, MO, USA) assay. HeLa and SiHa cells were seeded in 96-well plates, transfected with the indicated plasmid or oligonucleotides, and incubated at 37 °C for the indicated period of time. Thereafter, the cells were incubated with the medium containing 0.2 mg/ml MTT for 4 h at 37 °C. Subsequently, MTT solution was aspirated and 200 µl dimethyl sulphoxide (DMSO) (Sigma-Aldrich) was added into each well to dissolve the formazan crystals. Absorbance at 490 nm was measured with an ELX-800 Microplate Reader (BioTeke, Winooski, VT, USA).

Colony formation assay

To assess the clonogenicity of a single tumor cell, colony formation assay was performed based on a previously described method [19]. After transfection, the cells were sparsely seeded in 35-mm culture dishes at a density of 400 cells per dish. The cells were cultured at 37 °C in a 5 % CO₂ incubator for 14 days with the medium changed every 3 days. The cells were fixed with 4 % paraformaldehyde for 20 min at room temperature and stained with Wright's-Giemsa stain (Jiancheng, Nanjing, China). The stained cells were observed under a microscope, and the colonies of more than 50 cells were counted. The colony-forming ratio was calculated as (number of colonies/400) × 100 %.

Protein extraction and western blotting

Cells were lysed in NP-40 lysis buffer (Beyotime, Beijing, China) with 1 % phenylmethylsulfonyl fluoride (PMSF) (Beyotime). The protein concentration was determined with the BCA Protein Assay Kit (Beyotime). Forty-microgram protein samples were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and then transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membrane was blocked with 5 % nonfat milk and incubated with a specific primary antibody at 4 °C overnight. Anti-proliferating cell nuclear antigen (PCNA), anti-cyclin D1, anti-retinoblastoma tumor suppressor (Rb), and anti-β-actin antibodies were purchased from Cell Signaling (Danvers, MA, USA), and anti-phosphorylated Rb (p-Rb) antibody was purchased from Bioss (Beijing, China). Thereafter, the membrane was incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG secondary antibody (Beyotime) for 1 h at room temperature. The proteins of interest were visualized using the enhanced chemiluminescence (ECL) system (7Sea Biotech, Shanghai, China).

Luciferase reporter assay

MiR-195, miR-195 mutant, or pcDNA3.1 vector was co-transfected into HeLa or SiHa cells with the firefly (FL) luciferase reporter plasmid pmirGLO or pmirGLO-cyclin D1a

3'-UTR. Renilla (RL) luciferase reporter plasmid (pRL-TK) (Promega) was also co-transfected as the internal reference. Forty-eight hours after the transfection, the cell lysates were subjected to luciferase activity measurement by Dual-Luciferase[®] Reporter Assay System (Promega) according to the manufacturer's instructions.

Flow cytometric analysis

Cell cycle distribution was analyzed by flow cytometry. Forty-eight hours after the transfection, the cells were harvested and fixed in 70 % ethanol at 4 °C. After centrifugation at 1500g for 5 min, the supernatant was removed and the cells were stained with 10 µM propidium iodide (PI) solution (Beyotime). Following staining, the samples were analyzed by the FACSCalibur flow cytometer (BD Biosciences, Baltimore, MD, USA).

Statistical analysis

The data are expressed as the mean ± standard deviation (SD) of at least three independent experiments. The differences between two groups were analyzed by the Student's *t* test. Statistical analysis and data plotting were conducted with GraphPad Prism, version 5 (GraphPad Software, Inc., San Diego, CA). All tests were two-sided; *P* < 0.05 was considered statistically significant.

Results

MiR-195 was downregulated in cervical cancer tissues

To study the role of miR-195 in cervical cancer, we first examined the expression levels of miR-195 in the cervical tissues from cervical cancer patients and patients with benign uterine disease without history of cervical dysplasia or abnormal Pap smear. Quantitative real-time PCR results showed that miR-195 expression was significantly reduced in the cervical cancer tissues compared with the noncancerous cervical tissues (Fig. 1), implying that deregulated expression of miR-195 may play a role in the development of cervical cancer.

Overexpression of miR-195 inhibited growth of cervical cancer cells

To identify the role of miR-195 in cervical cancer cells, miR-195 expression construct was introduced into cervical cancer cell lines and the proliferation and clonogenicity of miR-195-overexpressing cells were assessed by MTT assay and colony formation assay, respectively. Transfection with miR-195 expression construct resulted in over threefold increase in the cellular miR-195 level in HeLa cells (*P* < 0.001; Fig. 2a).

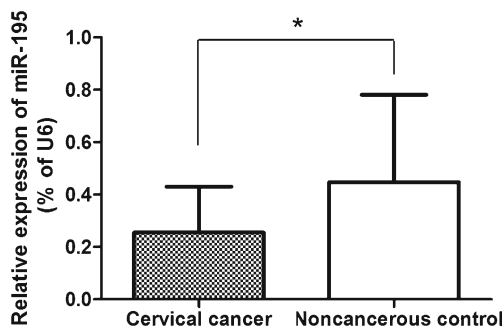


Fig. 1 Expression of miR-195 in cervical cancer and noncancerous cervical tissues. Eighteen pairs of cervical tissue specimens from patients with cervical cancer and patients with benign uterine disease without history of cervical dysplasia or abnormal Pap smear were obtained. The level of miR-195 was examined by real-time PCR using U6 as the internal control. The data are expressed as the mean \pm SD. * P <0.05

The MTT assay results revealed that overexpression of miR-195 significantly inhibited the proliferation of HeLa cells at 24, 48, and 72 h after transfection compared with control (P <0.05; Fig. 2b). In addition, the colony formation assay also showed that overexpression of miR-195 markedly

inhibited the clonogenicity of HeLa cells (P <0.01; Fig. 2c). Similarly, overexpression of miR-195 in SiHa cells resulted in compromised proliferation and clonogenicity of SiHa cells (Fig. 2d–f). Thus, these results demonstrated a suppressive role of miR-195 in the clonogenicity and proliferation of cervical cancer cells.

Underexpression of miR-195 promoted growth of cervical cancer cells

To verify the function of miR-195 in the growth of cervical cancer cells, HeLa and SiHa cells were transfected with miR-195 inhibitor sequence to reduce the level of endogenous miR-195. As shown in Fig. 3a, miR-195 inhibitor reduced the level of endogenous miR-195 by nearly half in HeLa cells. MTT assay revealed that HeLa cells with reduced miR-195 expression grew faster compared with the nontransfected control cells or the cells transfected with NC sequence (Fig. 3b). Moreover, compared with the control cells, HeLa cells that were transfected with miR-195 inhibitor showed increased clonogenicity (Fig. 3c). Consistently, reducing the level of miR-195 in SiHa cells by the inhibitor sequence resulted in

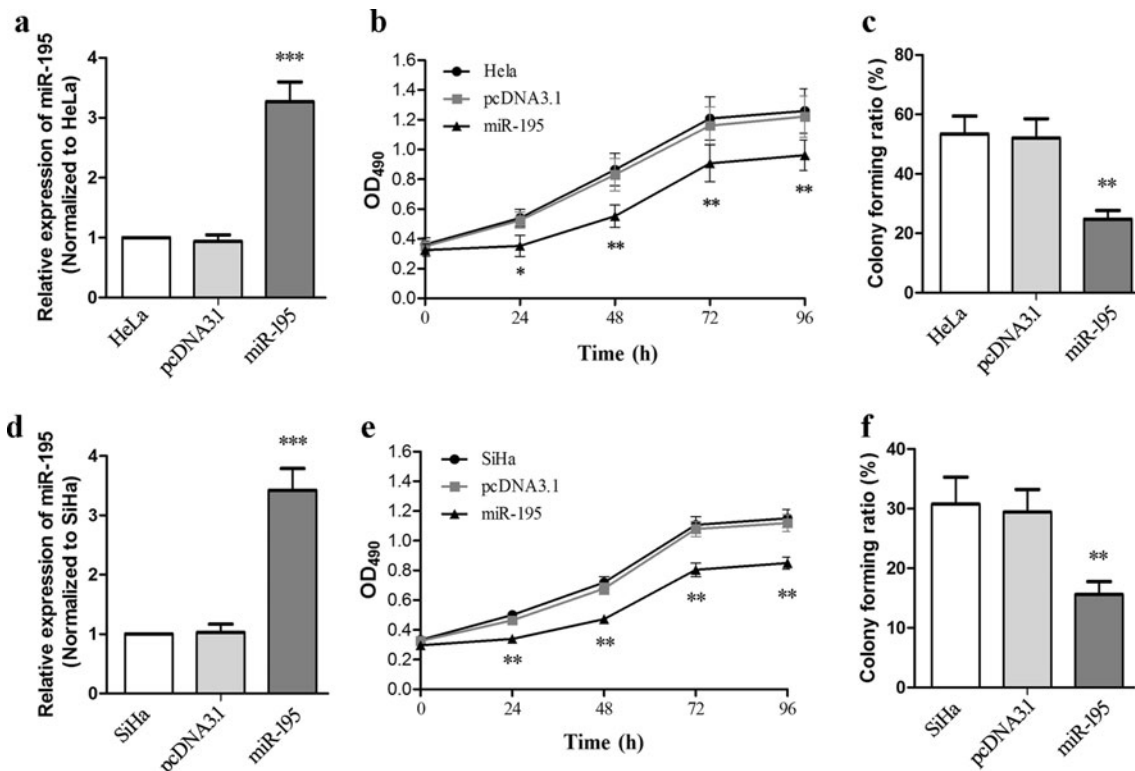


Fig. 2 Overexpression of miR-195 suppressed growth and clonogenicity of cervical cancer cells. **a** HeLa cells were transfected with miR-195-overexpressing construct or the empty vector, and the levels of miR-195 were determined by real-time PCR using U6 as the internal control. **b** The transfected HeLa cells were subjected to MTT assay to assess proliferation. **c** Colony formation assay was performed to examine single-cell clonogenicity of miR-195-overexpressing HeLa cells. **d** SiHa cells were transfected with miR-195-overexpressing construct or

the empty vector, and the levels of miR-195 were determined by real-time PCR using U6 as the internal control. **e** MTT assay was performed to assess proliferation of miR-195-overexpressing SiHa cells. **f** Single-cell clonogenicity of miR-195-overexpressing SiHa cells was assessed by colony formation assay. The data are expressed as the mean \pm SD of three independent experiments. Compared with the nontransfected cells, * P <0.05, ** P <0.01, *** P <0.001

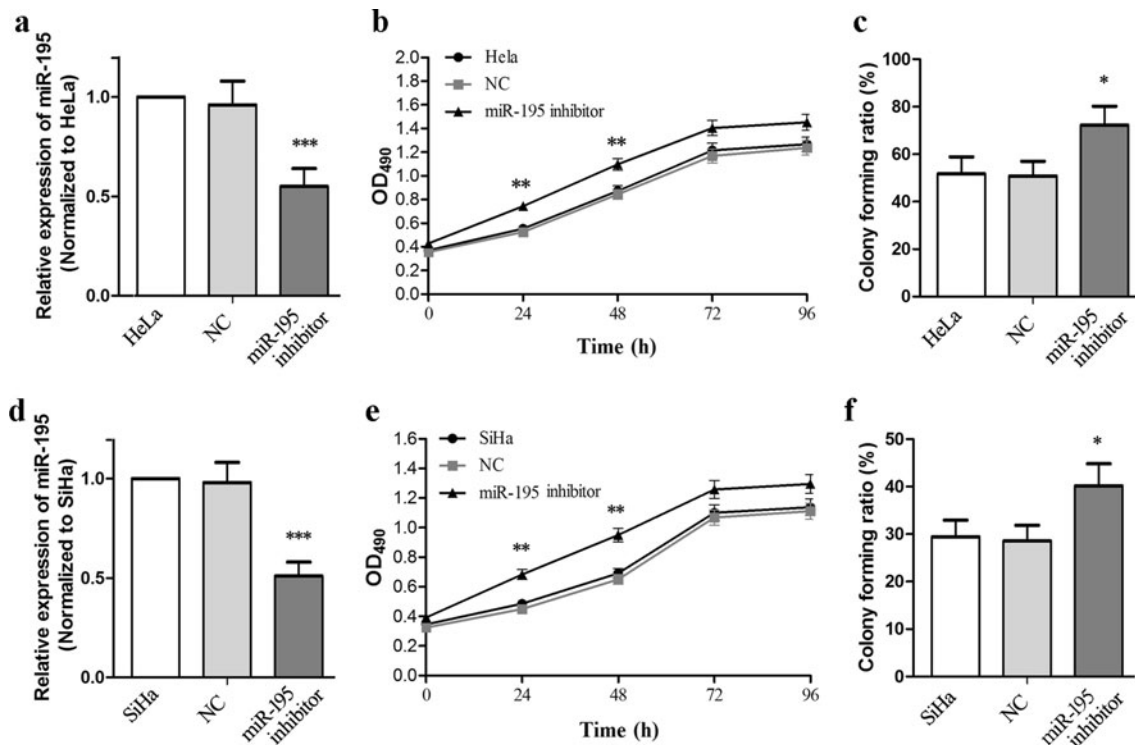


Fig. 3 Reduction of miR-195 by miR-195 inhibitor promoted growth and clonogenicity of cervical cancer cells. **a** HeLa cells were transfected with miR-195 inhibitor sequence or the negative control miRNA (NC), and the levels of miR-195 were determined by real-time PCR using U6 as the internal control. **b** The transfected HeLa cells were subjected to MTT assay to assess proliferation. **c** Colony formation assay was performed to examine single-cell clonogenicity of HeLa cells with reduced miR-195

expression. **d** SiHa cells were transfected with miR-195 inhibitor or NC, and the levels of miR-195 were determined by real-time PCR using U6 as the internal control. **e** MTT assay was performed to assess proliferation of the transfected SiHa cells. **f** Single-cell clonogenicity of SiHa cells was assessed by colony formation assay. The data are expressed as the mean \pm SD of three independent experiments. Compared with the nontransfected cells, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

accelerated proliferation rate and enhanced clonogenicity of SiHa cells (Fig. 3d–f). Therefore, these results confirmed the inhibitory role of miR-195 in the growth of cervical cancer cells.

MiR-195 acts as a cell cycle suppressor in cervical cancer cells

Cell cycle of cervical cancer cells with overexpression or underexpression of miR-195 was analyzed by flow cytometry. The results indicated that overexpression of miR-195 led to prolonged G1 phase in HeLa cells (Fig. 4a, b). At molecular level, Rb, p-Rb, and PCNA, the key cell cycle regulators, were examined by western blot analysis. Rb normally binds to E2F and inhibits the transcriptional activity of E2F. Upon phosphorylation, p-Rb dissociates from E2F, activating E2F-dependent transcription of various components that promote cell cycle progression [20]. Hence, the phosphorylation status of Rb is commonly employed as an indicator of cell cycle activity. Here, we found that the level of p-Rb was significantly decreased in miR-195-overexpressing HeLa cells, as compared to the control cells (Fig. 4c). Moreover, PCNA, a protein synthesized in early G1 and S phases and functions in DNA

replication and repair [21], was downregulated in miR-195-overexpressing HeLa cells. The altered levels of p-Rb and PCNA caused by miR-195 overexpression were consistent with the prolonged G1 phase in the cells, and similar results were also observed in SiHa cells (Fig. 4d–f).

We further analyzed the cell cycle of HeLa and SiHa cells with reduced miR-195 expression. As expected, G1 phase was shortened in miR-195 inhibitor-transfected HeLa cells as compared to the control (Fig. 5a, b). Consistently, underexpression of miR-195 resulted in an increased level of Rb phosphorylation and upregulation of PCNA (Fig. 5c). Consistently, reduced expression of miR-195 in SiHa cells resulted in accelerated cell cycle and increased level of p-Rb and PCNA (Fig. 5d–f). In summary, the above results suggest that miR-195 may act as a cell cycle suppressor in cervical cancer cells.

MiR-195 regulates cyclin D1 expression by targeting 3'-UTR of cyclin D1a mRNA

To further explore the molecular mechanism underlying the suppressive role of miR-195 in cell cycle, we predicted the putative targets of miR-195 with TargetScan. Among the predicted candidates, cyclin D1 was selected for further

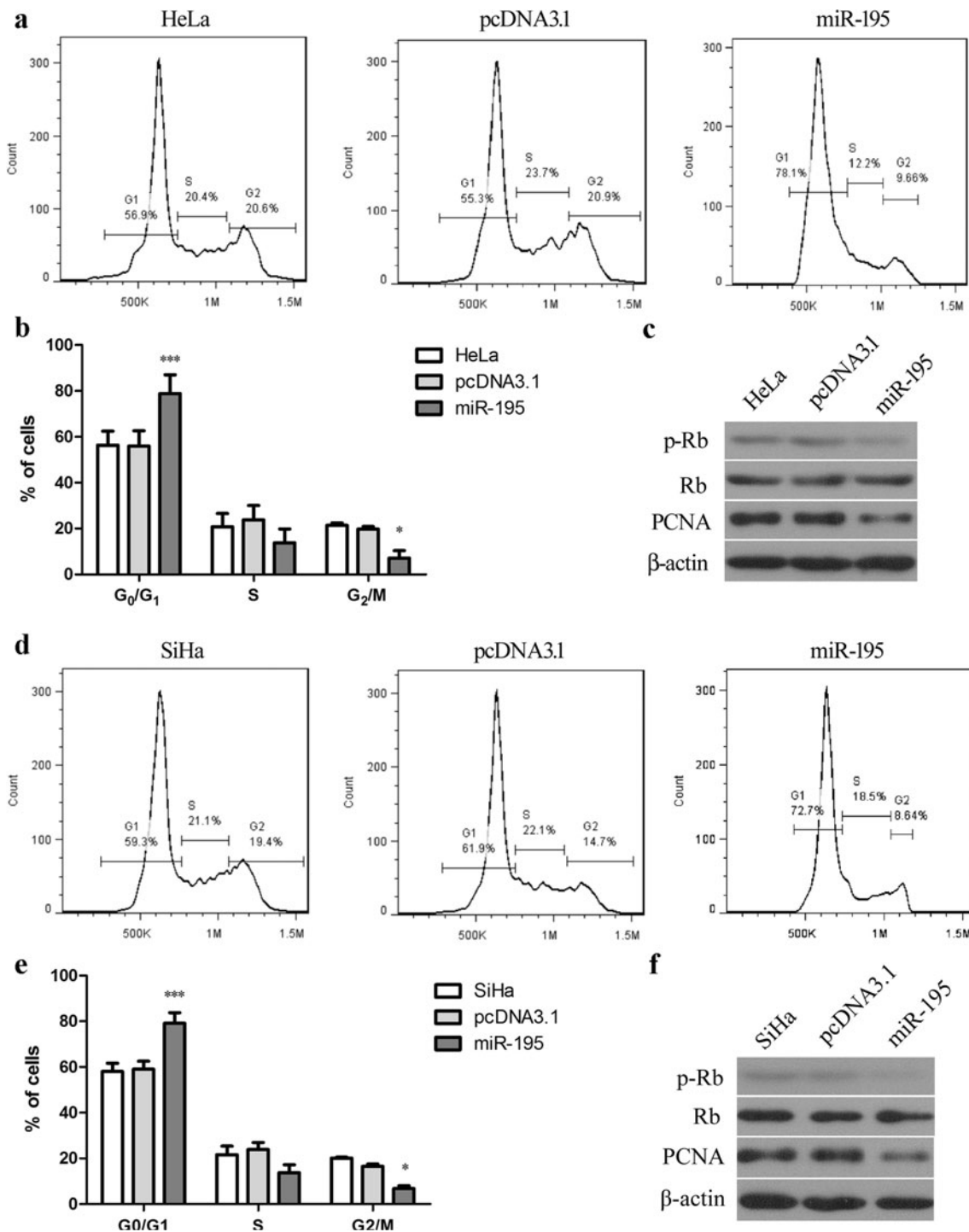


Fig. 4 Overexpression of miR-195 induced cell cycle arrest. **a** The cell cycle of HeLa cells that were transfected with miR-195 or pcDNA3.1 vector was analyzed by flow cytometry. **b** Statistical analysis of FACS results in **(a)**. **c** The levels of phosphorylated Rb (p-Rb), total Rb, and PCNA in miR-195-overexpressing HeLa cells were examined by western blotting. β -Actin served as the internal control. **d** The cell cycle of SiHa cells that were transfected with miR-195 or pcDNA3.1 vector was

analyzed by flow cytometry. **e** Statistical analysis of FACS results in **(d)**. **f** The levels of p-Rb, total Rb, and PCNA in miR-195-overexpressing SiHa cells were examined by western blotting. The figure shows the representative images of three independent experiments, and the data are expressed as the mean \pm SD. Compared with the nontransfected cells, * $P < 0.05$, *** $P < 0.001$

investigation for the good alignment of miR-195 with cyclin D1 3'-UTR (Fig. 6a) as well as their potential interaction in cell cycle and oncogenesis as previously reported [14, 16].

Western blot results revealed that overexpression of miR-195 remarkably reduced the expression of cyclin D1 in both HeLa and SiHa cells (Fig. 6b). Furthermore, the binding of

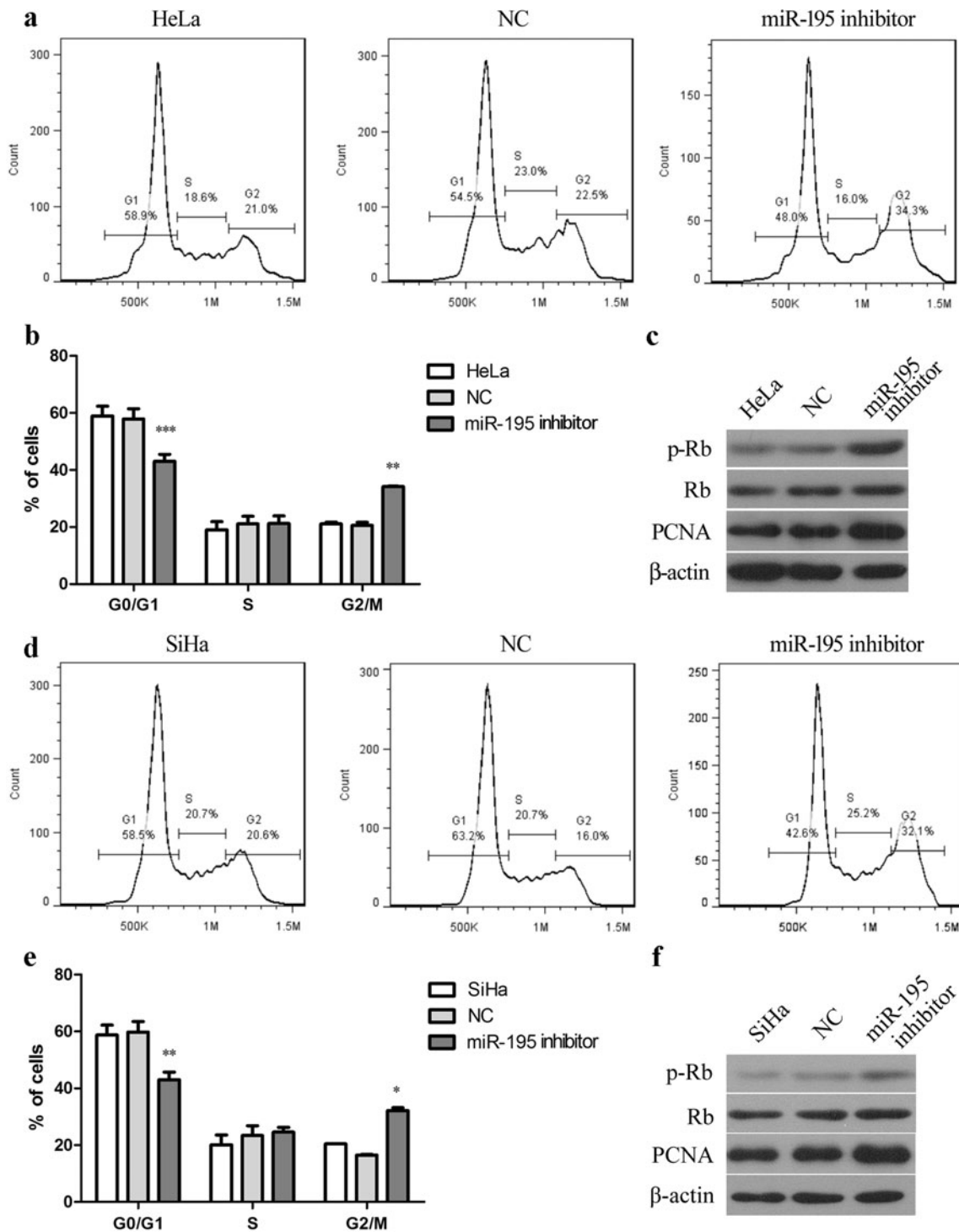


Fig. 5 Reduction of miR-195 promoted cell cycle progression. **a** The cell cycle of HeLa cells that were transfected with miR-195 inhibitor or NC was analyzed by flow cytometry. **b** Statistical analysis of FACS results in (a). **c** The levels of p-Rb, total Rb, and PCNA in HeLa cells with miR-195 underexpression were examined by western blotting using β-actin as the internal control. **d** The cell cycle of SiHa cells that were transfected with

miR-195 inhibitor or NC was analyzed by flow cytometry. **e** Statistical analysis of FACS results in (d). **f** The levels of p-Rb, total Rb, and PCNA in SiHa cells with miR-195 underexpression were examined by western blotting. The figure shows the representative images of three independent experiments, and the data are expressed as the mean±SD. Compared with the nontransfected cells, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

miR-195 on 3'-UTR of cyclin D1a transcript, the full length isoform of cyclin D1, was assessed in HeLa and SiHa cells by Dual-Luciferase Reporter Assay. As shown in Fig. 6c, d,

overexpression of miR-195 significantly inhibited the activity of firefly luciferase reporter, the coding sequence of which was linked to the wild-type 3'-UTR of cyclin D1a, whereas

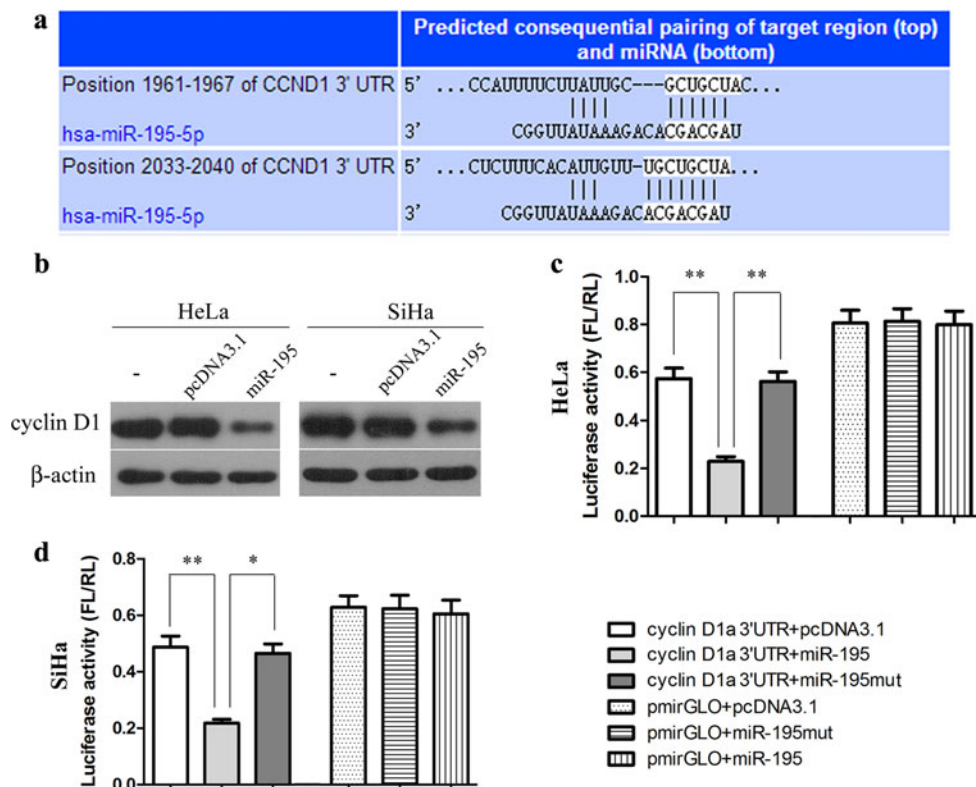


Fig. 6 MiR-195 inhibited cyclin D1 expression by directly targeting 3'-UTR of cyclin D1a. **a** The binding sites of miR-195 on cyclin D1 3'-UTR is predicted by TargetScan. **b** Western blot analysis of cyclin D1 expression in HeLa and SiHa cells after transfection with miR-195 or pcDNA3.1 vector. β -Actin served as the internal control. **c** The pmirGLO-cyclin D1a 3'-UTR or pmirGLO firefly (FL) reporter plasmid was co-transfected into HeLa cells with pcDNA3.1 vector, miR-195, and

miR-195 mutant (mut). Renilla (RL) reporter plasmid was also co-transfected as the internal reference. The luciferase activity was measured 48 h after transfection. **d** Luciferase assay was conducted in SiHa cells with the same settings as in HeLa cells. The figure shows the representative images, and the data are expressed as the mean \pm SD of three independent experiments. * P <0.05, ** P <0.01

such inhibitory effect was abrogated when miR-195 was mutated. These results indicated that miR-195 bound specifically to the 3'-UTR of cyclin D1a transcript such as to interfere with the expression of cyclin D1.

Discussion

Cervical cancer was once considered to be one of the most serious cancers in women. Cervical cancer usually undergoes a precancerous stage before developing into an actual one. At this early stage, these dysplastic cells can be removed and the condition can often be cured. Although the incidence of cervical cancer decreases in the recent years owing to the effective screening with the vaginal Pap smear, the mortality remains stably high [1]. Therefore, underlying the molecular mechanism for the tumorigenesis and progression of cervical cancer may help in the development of novel targets for anti-cancer therapies. In the present study, we first examined the level of miR-195 in primary cervical cancer tissues and found that miR-195 was downregulated in cervical cancer, which is consistent with the previous report [17]. Further, by

manipulating the levels of miR-195 in HeLa and SiHa cells, we showed that miR-195 suppressed the growth and clonogenicity of HeLa and SiHa cells in vitro and induced cell cycle arrest by reducing Rb phosphorylation and PCNA expression. In addition, Dual-Luciferase Report Assay revealed that miR-195 regulated the cell cycle of cervical cancer cells through directly targeting on the 3'-UTR of cyclin D1a mRNA.

Aberrant expression of miR-195 was reported to be associated with a variety of tumors, including hepatocellular carcinoma, glioma, gastric cancer, and breast cancer [14, 16, 22, 23], and miR-195 was implicated in the proliferation and tumorigenicity of tumor cells [14, 16]. MTT assay, a commonly used method to determine cell proliferation in vitro, is based on the formation of dark-colored formazan dye through the reduction of the tetrazolium salt MTT by metabolically active cells [24]. Colony formation assay is a well-established assay to assess clonogenicity, which is an indicator of tumorigenicity in vitro [25]. MTT and colony formation assay results demonstrated that miR-195 inhibited proliferation and tumorigenicity of cervical cancer cells, whereas reducing the endogenous miR-195 by miR-195 inhibitor promoted cervical

cancer cell proliferation and tumorigenicity. These results suggest that miR-195 plays a suppressive role in cell proliferation and that reduced expression of miR-195 in cervical cancer may contribute to the abnormal proliferation of cervical cancer cells and promote tumor growth.

Cancer is frequently considered to be a condition of disordered cell cycle [26]. From the MTT results that miR-195 affected the proliferation rate of cervical cancer cells, we postulated that the cell cycle may be altered during this process. Fluorescence-activated cell sorting (FACS) results confirmed the suppressive role of miR-195 in cell cycle progression. At molecular level, the expression levels of several key cell cycle players, such as p-Rb and PCNA, were decreased by overexpressing miR-195 and were enhanced by reducing endogenous miR-195, suggesting that miR-195 interfered with the cycle of cervical cancer cells through modulating the level of these cell cycle players.

As a member of cell cycle regulatory proteins, cyclin D1 is one of the most frequently deregulated proteins in human cancers [26]. Moreover, the expression level of cyclin D1 has been implicated to be related to the malignant degree of many cancers [27–29]. In this study, we identified cyclin D1 to be a target of miR-195 via direct binding to the 3'-UTR of cyclin D1a transcript by Dual-Luciferase Reporter Assay. Meanwhile, western blot analysis confirmed that overexpression of miR-195 downregulated the expression of cyclin D1. It was previously reported that miR-195 inhibited the proliferation of glioma cells and hepatocellular carcinoma cells through targeting cyclin D1 [14, 16]. Here, we demonstrated that targeting cyclin D1a by miR-195 was an important regulatory mechanism for cell cycle in cervical cancer cells. The findings across various tumor cell lines suggest that cyclin D1 might be a common target of miR-195 among different types of cells and that deregulated expression of miR-195 may contribute to aberrant cell proliferation and tumorigenesis by losing the control on cyclin D1.

Our study demonstrates that miR-195 plays a suppressive role in the proliferation and cell cycle of cervical cancer cell lines by direct targeting on 3'-UTR of cyclin D1a. These findings suggest that miR-195 may be a potential candidate for anti-cancer therapy.

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Compliance with ethical standards

Conflicts of interest None

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