Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Original Research

miR-196a targeting LRIG3 promotes the proliferation and migration of cervical cancer cells

Yunfen Qiu, Qing Han, Hong Lu, Chunming Shi*

Department of Gynecology, Nantong Tumor Hospital,Nantong 226001,Jiangsu,China

*Correspondence to: 1980580863@qq.com

Received May 27, 2020; **Accepted** October 20, 2020; **Published** October 31, 2020

Doi: http://dx.doi.org/10.14715/cmb/2020.66.7.27

Abstract: In recent years, studies have found that miR-RNA plays a role in cell proliferation, differentiation, apoptosis and metabolism. Among them, miR-196a is closely related to cervical cancer. Therefore, this experiment investigates the effect of mir-196a expression on cervical cancer cells and related mechanisms. The expression level of miR-196a in the cervical cancer cell line was assayed with the RT-PCR method, and liposome transfection was used to investigate its up-regulation or down-regulation in cervical cancer cells. The CCK-8 method and flow cytometry were used to measure cervical cancer cell proliferation and apoptosis, while the Transwell assay was used to determine cell migration and invasion of each transfection group. Bioinformatics was used to predict the target gene of miR-196a, which was verified using dual luciferase report experiment and Western blot, and miR-196a was further transfected with si-LRIG3 to detect its reversal effect on miR-196a regulation. Inhibition of the expression of miR-196a significantly reduced the proliferation, migration and invasion of cervical cancer cells, and promoted their apoptosis. Results from dual luciferase assay showed that miR-196a and LRIG3 had direct targeting effects. Cell proliferation, migration and invasion were enhanced by a reduction in the expression level of LRIG3 protein after miR-196a inhibitor cells were transfected with si-LRIG3. The expression of miR-196a is up-regulated in cervical cancer, and it promotes the growth of cervical cancer by its targeting effect on LRIG3 expression, resulting in enhancement of the proliferation, migration and invasion ability of cervical cancer cells, and inhibition of apoptosis.

Key words: miR-196a; Cervical cancer; Cell proliferation; LRIG3.

Introduction

Cervical cancer is one of the most common malignant tumors in women. The global new incidence has exceeded 500,000 annually, with an estimate of 300,000 deaths. Moreover, the age of onset of the disease is now reduced. The risk factors for cervical cancer are human papillomavirus (HPV) infection, smoking and multiple pregnancies (1). At present, the main treatment for patients with early cervical cancer is radical hysterectomy, and for patients with local progression or advanced stage, it is mainly radiotherapy or chemotherapy. Due to tumor metastasis or drug resistance of tumor cells, the 5-year survival of cervical cancer patients is not high. Therefore, it is very important to identify new molecular markers that can be used for early diagnosis and treatment of cervical cancer targets, to improve the survival of cervical cancer patients. The occurrence of cervical cancer is a complex process involving the dysregulation of multiple gene expressions. With advancements in molecular biology, some progress has been made on the molecular mechanism of cervical cancer, but there are still many unclear issues.

MicroRNAs (miRNAs) are a class of single-stranded non-coding RNAs of 18 to 25 nucleotides in length, and they are highly conserved. They bind to the 3-URT region of the messenger RNA (mRNA) of their target genes, leading to mRNA degradation or inhibition of protein synthesis, thereby regulating the expression levels of specific proteins (2). A large number of studies have confirmed that miRNAs play important regulatory roles in biological processes such as differentiation, development, and homeostasis of various cells (3). The relationship between miRNA and tumor diagnosis, treatment and prognosis has attracted a lot of research interest. The expression of miRNA is tissue-specific, that is, the expression level of the same miRNA in different tumor tissues differs, and the expression levels of different miRNAs in the same tumor tissue are different. In addition, miRNAs play different functions in different stages of the tumor. Thus, miRNAs have great potential for use in monitoring tumor progression. In recent years, more and more studies have shown that miRNAs perform important regulatory roles in cervical cancer.

MiR-196a is differentially expressed in various malignant tumors. Previous studies have found that miR-196a is up-regulated in gastric cancer, colon cancer, breast cancer and pancreatic cancer (4-8). It targets and reduces the expressions of the tumor suppressor genes p27, FOXO1 and ING5, thereby promoting tumor cell proliferation, migration and invasion. Thus, it functions as a cancer-promoting gene. There is currently no systematic report on the regulatory mechanism and clinical value of miR-196a in cervical cancer. Therefore, this study was aimed at investigating the effect of miR-196a on various biological functions of cervical cancer cells, and the possible mechanism (s). This was done by regulating the expression of miR-196a in cervical cancer

cells through liposome transfection, determination of changes in the proliferation, apoptosis, migration and invasion ability of cervical cancer cells at different miR-196a expression levels, and preliminary identification of the regulatory mechanism (s) involved.

The leucine-rich repeats and immunoglobulin-like domains 3 (LRIG3) belongs to the LRIGx family. Studies have shown that LRIGx genes are under-expressed in a variety of malignant tumors. The chromosome of LRIG3 is located at 12q13.2. This region is also the most common site for gene deletion in many human tumors. Therefore, it can be preliminarily inferred that LRIG3 may be closely related to the occurrence of some human tumors. At present, LRIG3 has been studied in glioma, pituitary adenoma, etc., but it is rarely studied in cervical cancer. Both LRIG3 gene and miRNA are closely related to tumors. The relationship between LRIG3 and miRNA is currently unclear.

Materials and Methods

Cell lines and cell culture

Human cervical cancer cells Hela, CaSki, HCC94, C33A and normal cervical cells Ect1/E6E7 were all purchased from American ATCC. The cells were cultured in DMEM containing 10% fetal bovine serum and placed in a constant temperature incubator at 37°C and 5% CO_2. The culture medium was changed every 2-3 days.

qRT-PCR

Total cell RNA was extracted using the Trizol method, and the PrimeScript RT kit was used for reverse transcription of the RNA to cDNA. The components of the PCR reaction system were added to a 96-well plate, and the RT-PCR reaction was carried out using the SYBR Green method. The reaction conditions were: 95°C for 10 min, followed by 40 cycles; 95°C for 30 sec, and 60°C for 1 min. The PCR primer was designed by Shanghai Shenggong Biological Engineering Co. Ltd. The sequences of the primer used were: miR-196a forward: 5'-TCC AGT TCG GGA AGT GAA GTG A-3',reverse: 5'-GTC CAA AGT GGT ATT GCC ACT A-3';GAPDH (internal reference) forward: 5'-CTC CAG TAC GTA AGT GCA GAG CT-3'; reverse, 5'-CAG TGA CAC ACG TTG CGT TGT C-3'.

Each sample was analyzed in triplicate.

Cell transfection

Cells in the logarithmic growth phase were trypsinized and made into a cell suspension which was seeded into a 6-well plate at a density of 5×10^5 cells/well. The cells were transfected when the degree of cell fusion was about 80%. The transfection process was carried out strictly following the instructions of the Lipofectamine 2000 transfection kit. The cells were divided into 4 groups and were transfected with miR-196a mimics and its negative control miR-196a-con, miR-125b inhibitor and its negative control miR-196a inhibitor-con. The cells were cultured in an incubator containing 5% CO_2 at 37°C.

Cell proliferation experiment

After transfection, each group of cells was made into a single-cell suspension and seeded in a 96-well plate at a density of 1×10^5 cells/well. Three duplicate wells were set for each group. The cells were cultured in a carbon dioxide incubator at 37°C and 5% CO_2. At 0, 24, 48, and 72h, 10μl of CCK-8 solution (0.5mg/ml) was added to each well, and culturing was continued for 4 h. Thereafter, the absorbance of each well was read at 450nm (A450nm) and the results were used to construct cell growth curves.

Determination of apoptosis

Cells in each group were cultured with 3% low-concentration serum culture solution for 48 hours, digested with trypsin, washed twice with PBS buffer, and resuspended in 100μl of 1 × binding buffer. Then, the cells were stained with Annexin V/PI double stain. Annexin V and PI were added to the cell suspension, mixed well, and incubated at room temperature in the dark for 15 minutes. The cells were then subjected to flow cytometric analysis within 1h at extinction and emission wavelengths of 488nm and 530nm, respectively.

Cell cycle detection

Cells in each group were centrifuged at 2000 rpm for 5 min, and the supernatants were discarded. The cell pellets were washed 3 times with PBS buffer and fixed with 75% ice ethanol for 2h in line with the instructions in the PI single-stain kit. The cells were centrifuged again at 2000 rpm for 5min, washed thrice with PBS and stained with 150μl of PI dye, followed by incubation at room temperature in the dark for 30 min. Thereafter, flow cytometry was performed to detect the percentage of cells in each stage of the cell cycle.

Transwell assay

Matrigel was diluted to a concentration of 1 mg/ml with a serum-free medium on ice. Then, 40μl of Matrigel was added to the upper chamber of the Transwell and spread evenly. The gel was allowed to stand at room temperature for 2 hours for it to be fully solidified. Then, each group of cells in a good growth state was made into a single-cell suspension, and the cell suspension was added to the glue along the sidewall of the cell at a density of 4×10^5 cells/mL. Medium containing 20% FBS as a chemokine (600μl) was put in the lower chamber, followed by incubation in a 5% CO_2 incubator at 37°C for 24 hours. The culture medium in the lower chamber was aspirated, and the cells were washed thrice with PBS, fixed with 4% paraformaldehyde for 30 min, stained with crystal violet for 30 min, mounted over neutral resin, and examined under an inverted microscope at a magnification of ×200. The number of dye-penetrating cells in 6 randomly selected visual fields were calculated. Each experiment was repeated 3 times, and the mean value was taken.

Dual-luciferase verification of target genes

The LRIG3 wild type and mutant 3'URT were cloned into the psiCHECK-2 vector to construct wild type and mutant LRIG3-3'URT reporter gene plasmids, in line with the luciferase reporter gene detection kit. MiR-196a NC was co-transfected with cervical cancer cells, and enzyme activity was tested after 48 hours of culture. Three replicate wells were set for each sample.

Western blotting

Total protein was extracted from cervical cancer cells using RIPA cell lysis. The protein concentration of the lysate was determined with the BCA method, and the protein (50μg) was subjected to SDS-PAGE and transferred to PVDF membrane. The membrane was incubated with the LRIG3 primary antibody (1: 1000) overnight at 4°C. After washing twice with PBS and TBST, the membrane was incubated with horseradish peroxidase (HRP)-labeled goat anti-rabbit secondary antibody (1: 10000) at room temperature on a horizontal shaker for 2h, and washed with ECL chemiluminescence reagent for color development. Quantity One software was used for grayscale analysis. The internal standard was GADPH.

Statistical analyses

Using SPSS 19.0 statistical software analysis, all data are expressed as mean ± standard deviation (x ± SD). A comparison between the two groups was done with a *t*-test. A value of $p < 0.05$ was considered statistically significant.

Results

miR-196a is up-regulated in cervical cancer cell lines

Results from RT-PCR showed that the expression level of miR-196a in the four cervical cancer cell lines Hela, CaSki, HCC94 and C33A were significantly higher than those in normal cervical cells Ect1/E6E7, and it was most upregulated in Hela cells ($p<0.05$). Thus, Hela cells were used in the follow-up experiments. After cell transfection, RT-PCR was used to assay the expression level of miR-196a in each transfection group. The results showed that after transfection with miR-196a mimics, the level of miR-196a in cervical cancer cell HeLa was significantly higher than that in the negative control group. The level of miR-196a in cells after transfection with miR-196a inhibitor was significantly lower than that in the negative control group ($p<0.05$; Figure 1 B), indicating that the transfection was successful.

miR-196a promoted Hela cell proliferation

Results from the CCK-8 test showed that in the miR-196a mimics group cells, from the 2nd day, proliferation was significantly higher than that in the negative control group. In contrast, proliferation in the miR-196a inhibitor group cells from the 2nd day was significantly lower than that in the impression control group, as shown in Figure 2 (A). The results of the plate clone

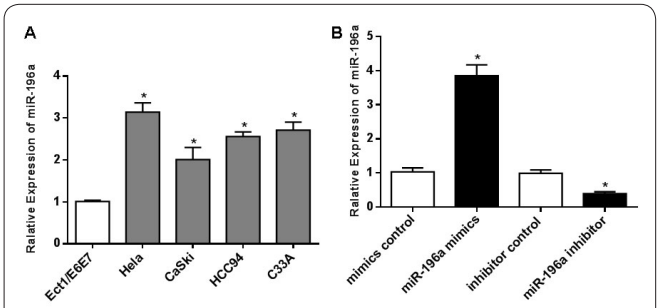

Figure 1. Expression of miR-196a in cervical cancer cell lines. (A) Expressions of MiR-196a in 4 cervical cancer cell lines. (B) Expression of miR-196a in Hela cells of different transfection groups.

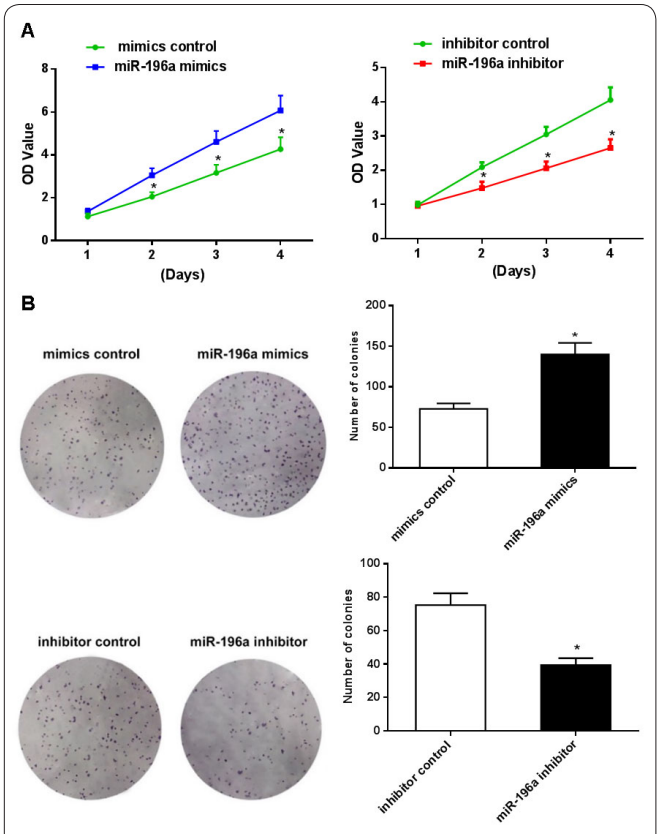

Figure 2. Effect of miR-196a expression on the proliferation of cervical cancer cells. (A) Growth curve of cells in different transfection groups. (B) Comparison of cell clone formation amongst different transfection groups.

experiment showed that the number of clones formed in the miR-196a mimics group was significantly higher than that in the mimics control group, while the number of clones formed in the miR-196a inhibitor group was significantly lower than that in the inhibitor control group ($p<0.05$). As shown in Figure 2 (B), miR-196a promoted the proliferation of cervical cancer cells.

miR-196a inhibited cervical cancer cell apoptosis

Flow cytometry was used to detect apoptosis. The results showed that the percentage of apoptosis in the miR-196a mimics group was significantly lower than that in miR-196a mimics control group ($p<0.05$). The percentage of apoptosis in the miR-196a inhibitor group was significantly higher than that in the miR-196a inhibitor control group ($p<0.05$). These results are shown in Figure 3. They suggest that miR-196a inhibited the apoptosis of cervical cancer cells.

miR-196a affected cell cycle in cervical cancer cells

Flow cytometry was used to detect changes in the cell cycle. The results showed that the proportion of cells in the S phase in the miR-196a mimics group was significantly increased, while the proportion of cells in the S phase of the miR-196 inhibitor group was significantly decreased ($p<0.05$). Cells in the miR-196 inhibitor group were mostly blocked in the G1/G0 phase, as shown in Figure 4. These results show that miR-196a promoted the proliferation of cervical cancer cells by enhancing cell progression from the G1/G0 phase to the S phase.

miR-196a promoted cervical cancer cell migration and invasion

Transwell migration experiment showed that after transfection with miR-196a mimics, the number of cervical cancer cells that migrated was significantly higher than that in the negative control group. After transfection with miR-196a inhibitor, the number of cervical cancer cells that migrated was significantly lower than that of the negative control group ($p<0.05$), as shown in Figure 5 (A). Transwell invasion experiment results showed that the number of cells crossing the membrane in the miR-196a mimics group was significantly higher than that in the negative control group. However, the number of cells crossing the membrane in the miR-196a inhibitor group was significantly lower than that in the negative control group ($p<0.05$; Figure 5 B), suggesting that miR-196a enhanced the migration and invasion of cervical cancer cells.

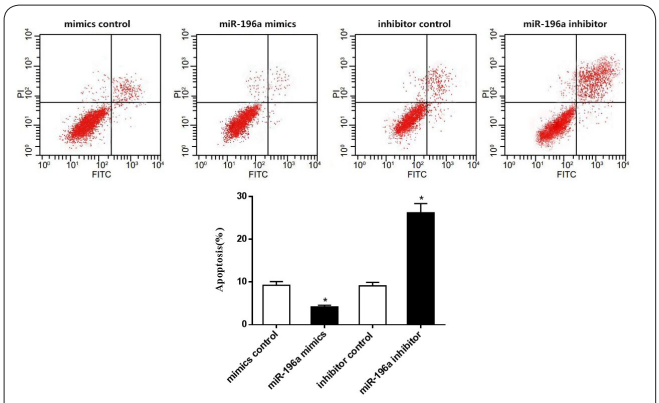

Figure 3. Comparison of cell apoptosis in different groups.

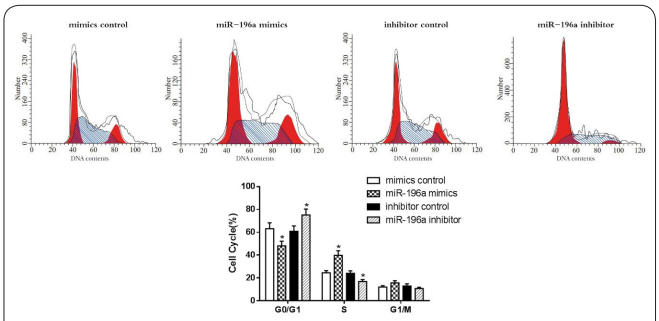

Figure 4. Comparison of cell cycle distribution of cells in different transfection groups.

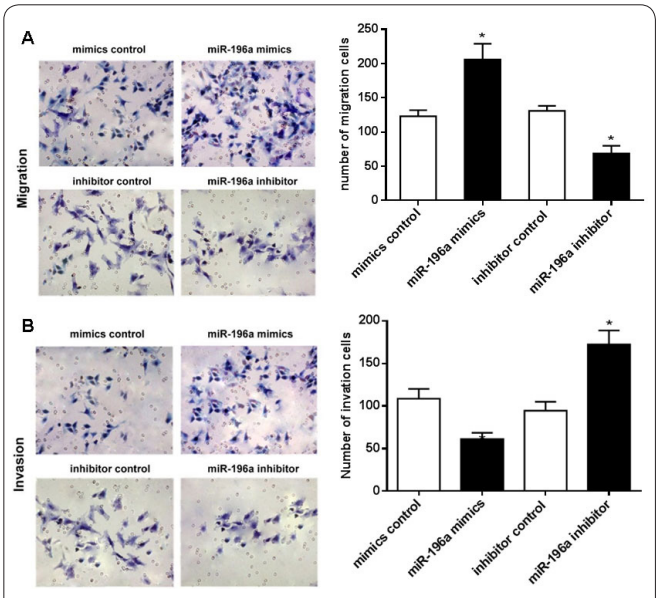

Figure 5. Number of cervical cancer cells that migrated and invaded in each group. (A): Comparison of cell migration amongst different transfection groups; (B): comparison of cell invasion amongst different transfection groups.

Direct targeting relationship between miR-196a and LRIG3

The target gene of miR-196a was analyzed using the bioinformatics software Target Scan. It was found that LRIG3 and miR-196a had binding sites which may be one of their target genes. Double luciferase experiment results showed that miR-196a significantly reduced the activity of wild-type LRIG3 plasmid luciferase ($p<0.05$). There was no significant effect on the activity of mutant luciferase ($p>0.05$). Western blot results showed that after transfection with miR-196a mimics, the expression level of LRIG3 protein in Hela cells was significantly reduced, as shown in Figure 6. Thus, miR-196a negatively regulated the expression of LRIG3.

LRIG3 promoted cervical cancer cells by blocking miR-196a

In order to further confirm that miR-196a exerted its regulatory effect on cervical cancer cells by targeting LRIG3, the expression of LRIG3 was inhibited with cell transfection in the miR-196a inhibitor group cells, and the effects of transfection on the proliferation, migration and invasion ability of each group of cells were determined. The results showed that inhibition of the expression of LRIG3 led to marked enhancements of the proliferation, migration and invasion capabilities of cervical cancer cells when compared with the miR-196a inhibitor group ($p<0.05$; Figure 7). Thus, miR-196a enhanced the viability of cervical cancer by targeting LRIG3.

Discussion

Factors such as persistent infection with high-risk HPV viruses and changes in host and environment stimulate tumor growth, invasion and metastasis of cervical cancer cells. In addition, epigenetic regulation mechanisms affect cervical cancer progression through oncogene activation and tumor suppressor gene inactivation (9). Studies have found that miRNAs play an important role in cervical cancer cell apoptosis, cell cycle, tumor metastasis, radiotherapy, chemotherapy, and drug

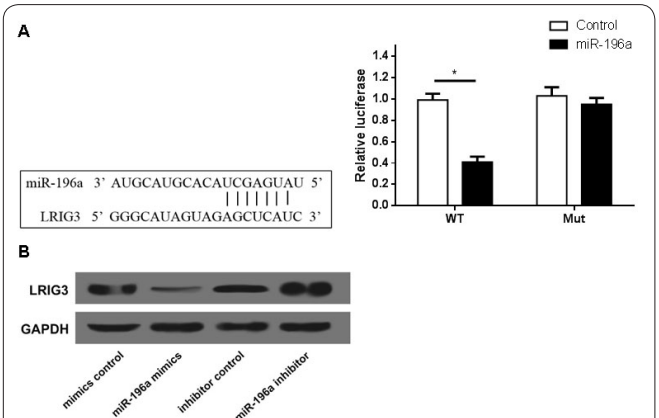

Figure 6. miR-196a regulated LRIG3. (A) Direct targeting of miR-196a to LRIG3, as verified using dual luciferase; (B) effect of miR-196a on the expression of LRIG3 protein.

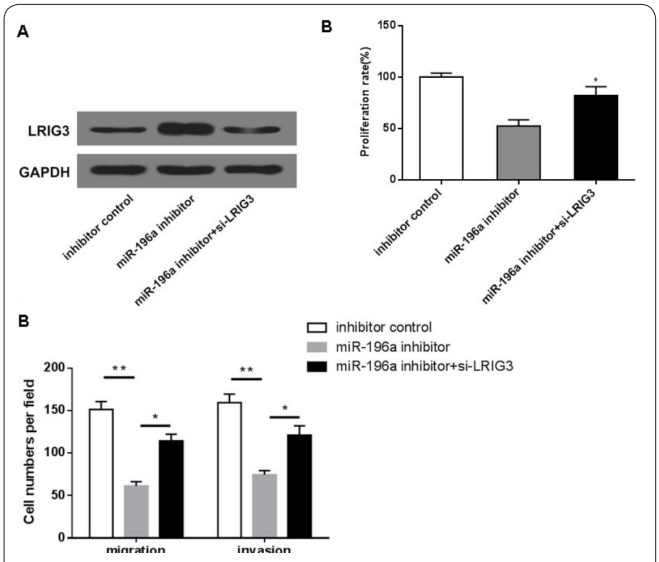

Figure 7. LRIG3 blocks miR-196a to promote cervical cancer cells. (A) effect of miR-196a on the expression of LRIG3 protein;(B) effects of LRIG3 Expression on Cervical Cancer Cells.

resistance. Different miRNAs play different regulatory roles. Studies have found that miR-31 expression is significantly increased in cervical cancer cell lines. It inhibits the expression of the pro-apoptotic protein Bax, thereby promoting cell proliferation and reducing cell apoptosis (10). Gao *et al*. found that the expression of miR-34 was down-regulated in cervical cancer. After over-expression of miR-34 by cell transfection, the invasiveness of tumor cells was reduced. The possible mechanism was identified as the targeted regulation of the expression of Notch and Jagged1 proteins (11). Wang *et al.* found that miR-214 induced cervical cancer cell apoptosis by inhibiting Bcl2 expression and increasing the sensitivity of cervical cancer cells to cisplatin (12). These studies have shown that miRNAs play an important regulatory role in the pathogenesis of cervical cancer. The miRNAs can be used for the auxiliary diagnosis and prognosis of cervical cancer. They have wide clinical application prospects and can be used as new therapeutic targets.

Recent studies have shown that miR-196a is highly expressed in various malignant tumors. It promotes tumor growth and metastasis and enhances the resistance of certain tumor cells to chemotherapy drugs. Measurement of levels of miR-196a in tumor tissues helps in assessing patients' disease state and prognosis (13-14). Sun *et al.* found that miR-196a was highly expressed in gastric cancer tissues. The miR-196a expression has a positive correlation with tumor size and clinical stage of patients. Kaplan-Meier survival curve analysis revealed that miR-196a was negatively correlated with patient survival (15). Bu *et al.* found that miR-196a was highly expressed in esophageal cancer cell lines. Studies have shown that miR-196a promoted the proliferation, migration and invasion of esophageal cancer cells by targeting the expression of Ras-related protein (Rap1A) (16). Wang *et al*. used miRNA chips and RT-PCR to show that miR-196a was highly expressed in pancreatic cancer cell PANC-1. After reducing the expression of miR-196a by cell transfection, the proliferation and migration ability of pancreatic cancer cells were reduced, and the EMT process was inhibited (17). Another study found that miR-196a was up-regulated in non-small cell lung cancer. Through targeted down-regulation of HOXA5 gene expression, miR-196a promoted cell proliferation and invasion and inhibited lung cancer cell apoptosis (18).

This study found that miR-196a was up-regulated in cervical cancer cell lines, indicating that it may function as an oncogene. The expression of miR-196a in cervical cancer cells was up-regulated and down-regulated by liposome transfection. It was observed that when miR-196a was overexpressed, the proliferation, migration and invasion of cervical cancer cells were significantly enhanced, while apoptosis was inhibited. However, when miR-196a expression was silenced, these results were reversed i.e. when miR-196a expression was suppressed in cervical cancer cells, cell proliferation, migration and invasion were reduced, and apoptosis was accentuated. Cell cycle results showed that after overexpression of miR-196a, the proportion of cervical cancer cells in the S phase increased significantly, and the proportion of cells in the G1/G0 phase was significantly reduced, indicating that miR-196a promoted cell progression from G1/G0 phase to the S phase, thereby enhancing the proliferation of cervical cancer cells.

Bioinformatics predicted that LRIG3 may be the target gene of miR-196a. The dual-luciferase experiment revealed that miR-196a significantly reduced the activity of wild-type LRIG3 plasmid luciferase, and had a direct targeting effect. After overexpression of miR-196a, the expression of LRIG3 protein in cervical cancer cells was significantly reduced. Furthermore, when the cells of the miR-196a inhibitor group were transfected with si-LRIG3 again, it was found that LRIG3 blocked the promoting effect of miR-196a on cervical cancer cells. This is further evidence that miR-196a regulated cervical cancer by controlling the expression level of LRIG3.

In conclusion, this study has shown that miR-196a is up-regulated in cervical cancer cells and that it enhances proliferation, migration and invasion of cervical cancer cells. Reduction of miR-196a expression inhibits the proliferation, migration and invasion of cervical cancer cells, and promotes their apoptosis through a mechanism related to the targeting effect LRIG3. These findings have potential applications in the effective treatment of cervical cancer.

Acknowledgments
None.

Conflicts of interest
There are no conflicts of interest in this study.

Author's contribution
All work was done by the author named in this article and the authors accept all liability resulting from claims which relate to this article and its contents. The study was conceived and designed by Chunming Shi; Yunfen Qiu, Qing Han, Hong Lu, Chunming Shi collected and analysed the data; Yunfen Qiu wrote the text and all authors have read and approved the text prior to publication.

References

1. Cancer Genome Atlas Research Network. Integrated genomic

and molecular characterization of cervical cancer. Nature 2017; 543: 378.
2. Ludwig N, Leidinger P, Becker K, Backes C, Fehlmann T, Pallasch C, et al. Distribution of miRNA expression across human tissues. Nucleic Acids Res 2016; 44: 3865-3877.
3. Reddy KB. MicroRNA (miRNA) in cancer. Cancer Cell Int 2015; 15: 38.
4. Pan Y, Shu X, Sun L, Yu L, Sun L, Yang Z, et al. miR-196a-5p modulates gastric cancer stem cell characteristics by targeting Smad4. Int J Oncol 2017; 50: 1965-1976.
5. Chen X, Du P, She J, Cao L, Li Y, Xia H. Loss of ZG16 is regulated by miR-196a and contributes to stemness and progression of colorectal cancer. Oncotarget 2016; 7: 86695.
6. Jiang CF, Shi ZM, Li DM, Qian YC, Ren Y, Bai XM, et al. Estrogen-induced miR-196a elevation promotes tumor growth and metastasis via targeting SPRED1 in breast cancer. Mol Cancer 2018; 17: 83.
7. Belvedere R, Saggese P, Pessolano E, Memoli D, Bizzarro V, Rizzo F, et al. miR-196a is able to restore the aggressive phenotype of annexin A1 knock-out in pancreatic cancer cells by CRISPR/Cas9 genome editing. Int J Mol Sci 2018; 19: 1967.
8. Bordbar M, Darvishzadeh R, Pazhouhandeh M, Kahrizi D. An overview of genome editing methods based on endonucleases. Mod Genet J 2020; 15(2): 75-92.
9. Wentzensen N, Schiffman M, Palmer T, Arbyn M. Triage of HPV positive women in cervical cancer screening. J Clin Virol 2016; 76: 49-55.
10. Zheng W, Liu Z, Zhang W, Hu X. miR-31 functions as an oncogene in cervical cancer. Arch Gynecol Obstet 2015; 292: 1083-1089.
11. Córdova-Rivas S, Fraire-Soto I, Mercado-Casas Torres A, Servín-González LS, Granados-López AJ, López-Hernández Y, et al. 5p and 3p Strands of miR-34 Family Members Have Differential Effects in Cell Proliferation, Migration, and Invasion in Cervical Cancer Cells. Int J Mol Sci 2019; 20: 545.
12. Peng R, Men J, Ma R, Wang Q, Wang Y, Sun Y, et al. miR-214 down-regulates ARL2 and suppresses growth and invasion of cervical cancer cells. Biochem Biophys Res Commun 2017; 484: 623-630.
13. Liu P, Xin F, Ma CF. Clinical significance of serum miR-196a in cervical intraepithelial neoplasia and cervical cancer. Genet Mol Res 2015; 14: 17995-18002.
14. Yang L, Peng F, Qin J, Zhou H, Wang B. Downregulation of microRNA-196a inhibits human liver cancer cell proliferation and invasion by targeting FOXO1. Oncol Rep 2017; 38: 2148-2154.
15. Tsai MM, Wang CS, Tsai CY, Huang CG, Lee KF, Huang HW, et al. Circulating microRNA-196a/b are novel biomarkers associated with metastatic gastric cancer. Eur J Cancer 2016, 64: 137-148.
16. Ma Y, Wang B, Guo Y, Zhang Y, Huang S, Bao X, et al. Inhibition of miR-196a affects esophageal cancer cell growth in vitro. Biomed Pharmacother 2016, 84: 22-27.
17. Yu Q, Xu C, Yuan W, Wang C, Zhao P, Chen L, et al. Evaluation of plasma microRNAs as diagnostic and prognostic biomarkers in pancreatic adenocarcinoma: miR-196a and miR-210 could be negative and positive prognostic markers, respectively. Biomed Res Int 2017; 2017: 6495867.
18. Li JH, Luo N, Zhong MZ, Xiao ZQ, Wang JX, Yao XY, et al. Inhibition of microRNA-196a might reverse cisplatin resistance of A549/DDP non-small-cell lung cancer cell line. Tumor Biol 2016; 37: 2387-2394.