JBUON 2021; 26(5): 1782-1788
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
Email: editorial_office@jbuon.com

ORIGINAL ARTICLE

miR-99a inhibits proliferation and migration of cervical cancer cells by targeting IGF1R

Li Han

Department of Clinical Laboratory, Huai'an Hospital Affiliated of Xuzhou Medical University, Huai'an 223002, China.

Summary

Purpose: *To explore the effects of miR-99a on the proliferation and migration of cervical cancer cells (CCCs) by targeting IGF1R.*

Methods: *miR-99a and IGF1R expression in C-33 A and C-4 II cells was interfered. Their effects on the proliferation, apoptosis, and migration of CCCs were analyzed by MTT assay, flow cytometry, and Transwell assay. The mechanism of action of miR-99a was analyzed by a rescue experiment and a dual luciferase reporter gene assay (DLRGA). Differences in miR-99a and IGF1R expression were detected in cervical cancer and adjacent tissues (n=30 each), and the correlation of the expression with clinicopathological characteristics of patients with cervical cancer was analyzed*

Results: *miR-99a expression was lower but IGF1R expression was higher in C-33 A and C-4 II cells than that in normal cervical epithelial cells. The results showed that both the promotion and the inhibition significantly decreased the proliferation and migration of the two CCCs, but increased their apoptosis. To further verify the correlation of miR-99a with IGF1R in cervical cancer, we co-transfected miR-99a and IGF1R overexpression vectors into the cells and found that compared with CCCs transfected with miR-99a overexpression vectors alone, the expression of IGF1R in the co-transfection group increased, while the expression of miR-99a did not change significantly. Additionally, the proliferation and migration of the cells in the co-transfection group increased, while their apoptotic rate decreased. DLRGA showed the targeted inhibition of miR-99a on IGF1R expression.*

Conclusions: *miR-99a can specifically inhibit IGF1R expression and thus inhibit the proliferation and migration of CCCs.*

Key words: *miR-99a, IGF1R, cervical cancer, proliferation, migration*

Introduction

As a major cause of cancer-related deaths all over the world, cervical cancer has the highest incidence and mortality rates among all gynecologic tumors, with 528,000 new cases and 266,000 deaths each year [1,2]. Currently, surgery, radiotherapy, and chemotherapy are commonly used to treat this disease, but conventional chemotherapy has a limited therapeutic effect and causes serious systemic toxicity. Moreover, the tolerance of tumor cells to radiotherapy and chemotherapy gradually enhances with the extension of treatment time [3,4]. Therefore, it is necessary to find new targets for the treatment of cervical cancer.

We have a new understanding of tumor pathogenesis due to the discovery of microRNAs (miRNAs/miRs), which has also provided an important research direction for finding new therapeutic targets. miRNAs are generally about 22 nucleotides in length and regulate target gene expression through the targeted complementary pairing with the 3'-untranslated region (UTR) of mRNAs [5,6]. Located at human chromosome 21q21.1, miR-99a

Corresponding author: Dr.Li Han Department of Clinical Laboratory, Huai'an Hospital Affiliated of Xuzhou Medical University, 62 Huaihai South Rd, Qingjiangpu District, 223002, Huai'an, China.
Email: zhx3dx@163.com
Received: 20/02/2021; Accepted: 19/03/2021

inhibits cervical cancer cells (CCCs) to proliferate and invade via targeting mTOR signaling pathway [7]. Later research has found that the target of miR-99a, insulin-like growth factor 1 receptor (IGF1R) inhibits breast cancer cells to proliferate, migrate and invade [8] and also esophageal cancer cells [9] through inhibiting IGF1R. This receptor promotes glycolysis, and glycolysis disorders are an important feature of cancer cells, so its activation significantly enhances the growth, survival, and movement of tumor cells [10,11]. IGF1R is also reported to regulate the biological behavior of CCCs such as proliferation and invasion [12,13]. However, the role of miR-99a regulating IGF1R on CCCs has been rarely studied.

In this study, the influence of miR-99a targeting IGF1R on CCC proliferation and migration were explored to provide more experimental basis for clinical search for therapeutic targets of cervical cancer.

Methods

Cell sources

Human primary cervical epithelial cells (PC-480-011), C-33 A cells (HTB-31), C-4 II (CRL-1595), and their culture media were purchased from ATCC. The medium for the cervical epithelial cells was a Cervical Epithelial Cell Basal Medium, and the culture medium for C-33 A cells was Eagle's Minimum Essential Medium + 10% fetal bovine serum (FBS), and the culture medium for C-4 II cells was Walmouth's MB 752/1 medium + 10% FBS. The cells were cultured at 37°C and with 95% air + 5% carbon dioxide, and the culture medium was changed 2-3 times weekly.

Cell passage

After recovery, the cells were adherently cultured, and then rinsed with 0.25% trypsin + 0.03% ethylenediaminetetraacetic acid (EDTA) solution after the old culture medium was sucked out. After the liquid was removed, the cells were added with trypsin-EDTA solution (1-2 mL), which was removed after the cells were separated. After the fresh culture medium was added, the cells were suspended, and then separately cultured for 2-3 generations.

Construction and transfection of expression vectors

All primers and expression vectors were designed and synthesized by TaKaRa (Tokyo, Japan), including a miR-99a interference vector (inhibitor), a miR-99a overexpression vector (mimic), a blank vector miR-NC, an IGF1R interference vector (si-IGF1R), an IGF1R overexpression vector (sh-IGF1R), and blank vectors (sh-NC, PGL3-wt-IGF1R, and PGL3-mt-IGF1R). Trypsin was used to digest the cells 24 h before transfection, and the expression vectors were transfected into the cells when they were fused to about 80%, with specific operating steps referring to the kit instruction. After that, the cells were cultured in an incubator for 48 h (37°C, 5% CO_2), with the culture medium changed every 6 h. qRT-PCR and Western blotting were used to measure the transfection results. LipofectamineTM2000 (11668019) was purchased from ThermoScientific.

Cell grouping and intervention

The cells were divided into the control, miR-NC, miR-99a mimic, si-NC, si-IGF1R, and co-transfection groups. Those in the first group were not treated, while those in other groups were transfected with miR-NC, miR-99a mimic, si-NC, si-IGF1R, and miR-99a mimic+sh-IGF1R, respectively.

Tissue sources

The cervical cancer tissues and their corresponding adjacent tissues (n=30 each) were selected, preserved from August 2013 to May 2014 and confirmed by pathology. The patients had complete clinical medical records and follow-up data. This study was approved by the Medical Ethics Committee of our hospital. The patients consented through telephone or text message and signed the informed consent form.

qRT-PCR

qRT-PCR was performed to detect miR-99a expression in cells/tissues, from which total RNA was drawn using the TRIzol reagent. An UV spectrophotometer and agarose gel electrophoresis were applied to detect its purity, concentration, and integrity. Experimental requirements were met with A260/A280 value between 1.8 and 2.1. Based on the kit instruction, the first strand cDNA was synthesized, and then PCR amplification was performed. The system was upstream and downstream primers (0.4 μL each), 2×TransTaq® Tip Green qPCR SuperMix (10 μL), Passive Reference Dye (50X) (0.4 μL), and ddH_2O finally added to make up to 20 μL. The conditions were pre-degeneration (94°C, 30 s), degeneration (94°C, 5 s), annealing and extension (60°C, 30 s), for a total of 40 cycles. With U6 used as a reaction internal reference, its upstream and downstream sequences were 5'-GCGCGTCGTGAAGCGTTC-3' and 5'-GTGCAGGGTCCGAGGT-3'. miR-99a sequences were 5'-AGTTACTTAAGCTCGGGCCC-3' and 5'-TTGTTCTCGGTGGGCTTGGC-3'. The primer sequences were designed by Thermo, and the TransStart® Tip Green qPCR SuperMix (AQ142-21) was purchased from TransGen Biotech Co., Ltd, Beijing.

Western blotting

The repeated freeze-thaw method was used to extract protein from the cells/tissues, and its concentration was detected using the BCA method. The protein was separated with 12% polyacrylamide gel electrophoresis (PAGE) after the concentration was adjusted to 4 μg/μL, with the voltage initially being 90 V and then increasing to 120V to move the samples to the appropriate position of the separation gel. Then, the protein was transferred to the membrane (100V constant voltage, 100 min) and sealed (37°C, 60 min). Next, the transferred membrane

was sealed in 5% skimmed milk for immune reaction. It was incubated with primary antibody (1: 1000) (all night, 4°C), warmly cleaned with PBS (5 min) for 3 times on the next day, and then incubated with secondary antibody (1: 1000) (room temperature, 1 hour). Finally, it was developed and fixed with an ECL reagent. Protein bands scanned by the film were statistically analyzed by Quantity One. The relative expression of the protein = gray values of protein bands / gray values of internal reference. The BCA protein kit (23250), ECL kit (35055), and trypsin (90058) were all purchased from Thermo Scientific™. Rabbit anti-IGF1R (monoclonal antibody, ab182408) and goat anti-rabbit IgG (secondary antibody, ab6721) were all purchased from Abcam, USA.

MTT assay for cell proliferation

The cells transfected for 24 h were collected, and conventionally digested. After their density was adjusted to 3×10^4 cells/well, they were inoculated on 96-well plates, incubated (37°C, 48 h), then added with 20 μL of MTT solution (5 mg/mL), and finally cultured (37°C, 4 hours). 150 μL of dimethyl sulfoxide was added to each well. Subsequently, optical density (OD) values in each group were measured at 490 nm using a microplate reader (Shanghai Flash Spectrum Biological Technology Co., Ltd.). The MTT assay kit (V13154) was purchased from Thermo Fisher (China).

Flow cytometry for cell apoptosis

The cells were digested with 0.25% trypsin, cleaned twice with PBS, then added with binding buffer (100 μL), and finally prepared into a suspension (1×10^6 cells/mL). After sequentially added with Annexin V-FITC and PI, the cells were incubated in the dark (room temperature, 5 min). A FACSCalibur flow cytometer (BD Biosciences, CA, USA) was used for the detection, and the experiment was repeatedly conducted for 3 times to obtain the average value. The Annexin V-FITC/PI apoptosis detection kit (V35113) was purchased from Invitrogen, USA.

Detection of cell migration and invasion

The upper chamber of Transwell was inoculated with 5×10^4 cells, and the L-15 medium (10% FBS) was added into the lower one. After the Transwell was cultured for 24 h, the cells under the membrane were fixed with 75% methanol, and stained with crystal violet, so as to count the number of transmembrane cells in 5 visual fields under 40*10 field of view. Three parallel experiments were conducted. The Transwell chamber and its related reagents were purchased from Corning, New York, USA.

Dual luciferase reporter gene assay (DLRGA)

Human embryonic kidney cells 293T (BNCC100530) were purchased from BeNa Culture Collection. After cultured to logarithmic growth phase, the cells were transfected with PGL3-wt-IGF1R or PGL3-mt-IGF1R, miR-99 mimic, miR-99 inhibitor, and miR-NC for 48 h. After that, the fluorescence intensity was detected by the dual luciferase reporter assay system (CytoFLEX S flow cytometer, Beckman, USA).

Statistics

SPSS 19.0 (Asia Analytics Formerly SPSS China) was used for statistical analyses. Measurement data were expressed as mean±SD, and their comparison between two groups was analyzed by independent samples t-test, while their comparison between multiple groups was analyzed by one-way analysis of variance (ANOVA). *Post hoc* test was used for LSD test, and Pearson test was used to analyze the correlation. P values were bilateral, and the difference was statistically significant when $p<0.05$.

Results

miR-99a expression and IGF1R in cervical cancer cell lines

Compared with normal cervical epithelial cells, C-33 A and C-4 II cells had remarkably lower miR-99a expression ($p<0.05$), but remarkably higher IGF1R expression ($p<0.05$) (Figure 1).

Effects of miR-99a on proliferation and migration of CCCs

miR-99a was lowly expressed in CCCs, so we transfected miR-99a mimic vectors into the cells. After the transfection, miR-99a expression in the CCCs remarkably rose ($p<0.05$); the cells' proliferation and migration in the mimic group were remarkably lower than those of the un-transfected cells ($p<0.05$), but their apoptosis was remarkably higher in the mimic group ($p<0.05$) (Figure 2).

Effects of IGF1R on proliferation and migration of CCCs

Similarly, we transfected si-IGF1R vectors. After the transfection, IGF1R expression in CCCs remarkably rose ($p<0.05$); the cells' proliferation and migration in the si-IGF1R group were remarkably lower than those of the un-transfected cells ($p<0.05$), but their apoptosis was remarkably higher in the si-IGF1R group ($p<0.05$) (Figure 3).

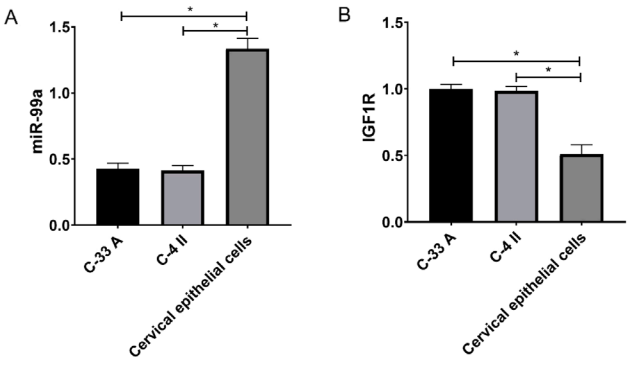

Figure 1. Expression of miR-99a and IGF1R in cervical cancer cell lines. **A:** miR-99a. **B:** IGF1R. *$p<0.05$.

Effects of co-transfection with miR-99a mimic+sh-IGF1R on proliferation and migration of CCCs

In order to verify the effects of IGF1R on tumor inhibition by miR-99a, we co-transfected miR-99amamic+sh-IGF1R vectors. According to the results, IGF1R expression in the co-transfection group was remarkably higher than that of the cells transfected with miR-99a mimic vectors alone ($p<0.05$), but miR-99a expression was not different between the two cells ($p>0.05$). According to the comparison of the biological behavior, the proliferation and migration of the cells in the co-transfection group were restored to a certain extent ($p<0.05$), and their apoptotic rate also reduced ($p<0.05$). (Figure 4).

DLRGA

The binding site between miR-99a and IGF1R was predicted through targetscan7.2, so as to design the wild and mutant types of IGF1R. According to the DLRGA, the up-regulation of miR-99a expression could significantly inhibit the fluorescence intensity of PGL3-wt-IGF1R ($p<0.05$), and the down-regulation of miR-99a could increase the fluorescence intensity ($p<0.05$), but the two interference vectors had no effect on the fluorescence intensity of PGL3-mt-IGF1R (Figure 5).

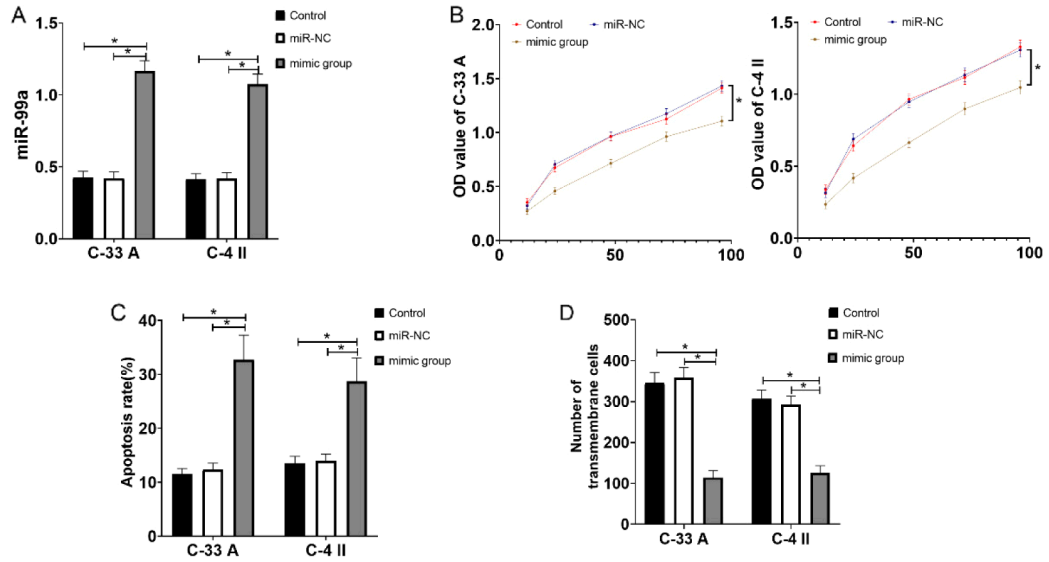

Figure 2. Effects of miR-99a on proliferation and migration of CCCs. **A:** Results of transfection with miR-99a mimic. **B:** Changes in the proliferation of CCCs. C: Changes in the apoptosis of CCCs. D: Changes in the migration of CCCs. *p<0.05.

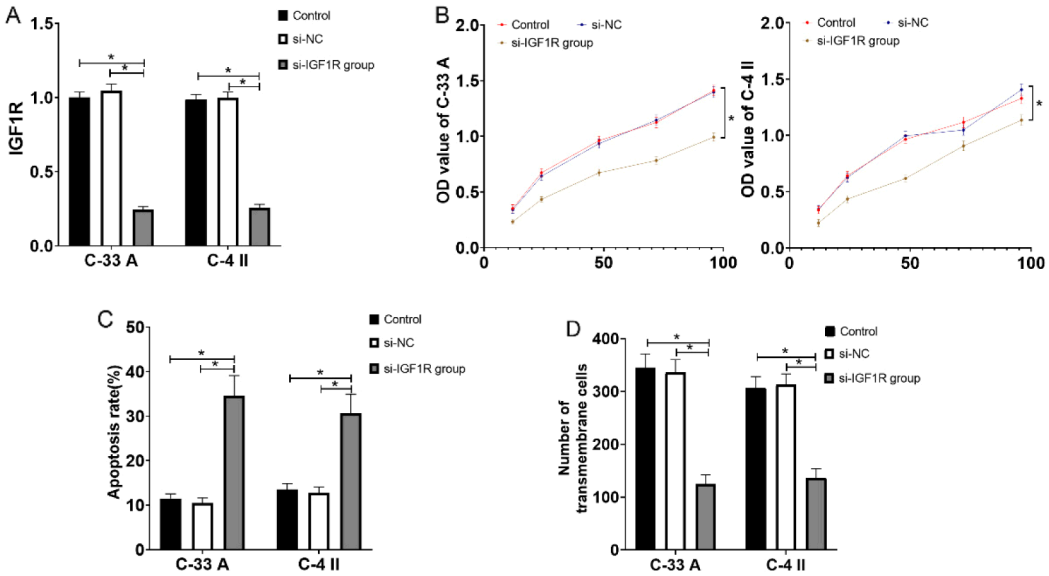

Figure 3. Effects of IGF1R on proliferation and migration of CCCs. **A:** Results of transfection with si-IGF1R. **B:** Changes in the proliferation of CCCs. **C:** Changes in the apoptosis of CCCs. **D:** Changes in the migration of CCCs. *p<0.05.

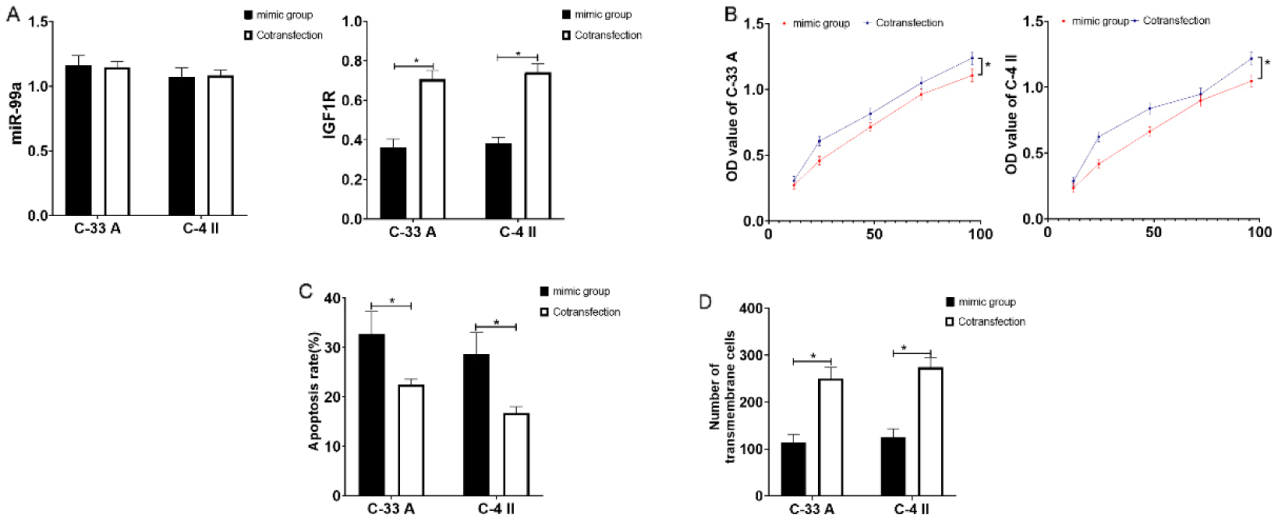

Figure 4. Effects of co-transfection with miR-99a mimic+sh-IGF1R on proliferation and migration of CCCs. **A:** Results of transfection with miR-99amimic+sh-IGF1R. **B:** Changes in the proliferation of CCCs. **C:** Changes in the apoptosis of CCCs. **D:** Changes in the migration of CCCs. *p<0.05.

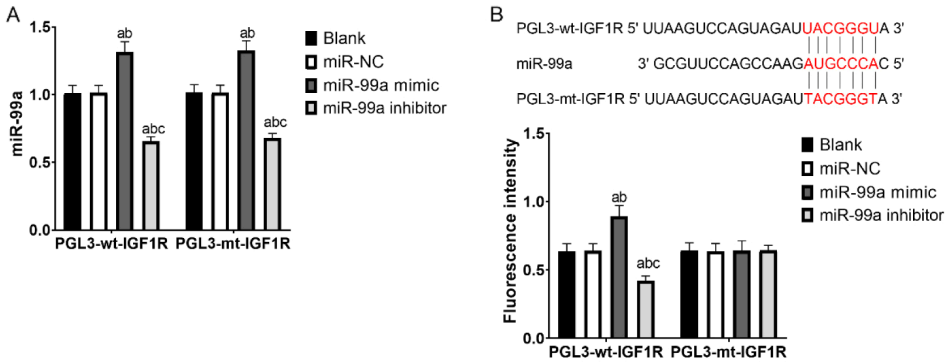

Figure 5. DLRGA. **A:** Results of miR-99a intervention. **B:** Effects of miR-99a on fluorescence intensity. **a** indicates p<0.05 compared with that in the blank group. **b** indicates p<0.05 compared with that in the miR-NC group. **c** indicates p<0.05 compared with that in the miR-99a mimic group.

miR-99a and IGF1R expression in cervical cancer tissues

miR-99a expression reduced (p<0.05), while IGF1R expression rose in cervical cancer tissues. Their expression was associated with the grade of differentiation, FIGO staging, and 5-year survival rate (p<0.05). (Table 1).

Discussion

As one of the most destructive gynecologic tumors, cervical cancer affects women's health and quality of life. Advanced cervical cancer has a 5-year survival rate of only 40%, so new and effective therapeutic methods for treating this disease are urgently needed [14,15]. In this study, the mechanism of action of miR-99a on affecting CCC proliferation and migration was analyzed, so as to find potential targets for its treatment.

Previous studies have reported the anti-cancer effect of miR-99a. In a study by Wang et al, miR-99a has a negative correlation with lymph node metastasis, so its overexpression inhibits CCC growth and invasion through the targeted inhibition of mTOR signaling pathway [7]. According to Li et al, the targeted inhibition of miR-99a on this pathway also inhibits endometrial cancer cells to proliferate and invade, and the regulatory effect of miR-99a on cell cycles blocks the cells' G1/S phase transition and induce their apoptosis [16]. At present, no research has reported the regulatory relationship between miR-99a and IGF1R, but the promotion of IGF1R in tumor occurrence and development (including cervical cancer) can be confirmed. Wu et al have found that miRNA-375 may inhibit the growth of CCCs by inhibiting IGF1R signaling pathway [17]. According to an earlier report, knocking out IGF1R can inhibit the

Table 1. Correlations of miR-99a and IGF1R with pathological characteristics

	n	*miR-99a*	x^2/t	*p*	*IGF1R*	x^2/t	*p*
Cancer tissues	30	1.235±0.106	23.244	<0.001	1.121±0.121	14.984	<0.001
Adjacent tissues	30	1.845±0.137			0.742±0.067		
Age, years			1.865	0.073		0.698	0.491
<50	10	1.927±0.117			0.754±0.077		
≥50	30	1.839±0.124			0.736±.062		
Tumor diameter			0.652	0.520		0.594	0.598
<4cm	13	1.851±0.117			0.733±0.058		
≥4cm	17	1.882±0.136			0.748±0.073		
Degree of differentiation			3.590	0.001		3.674	0.001
Medium-high	17	1.930±0.108			0.709±0.533		
Low	13	1.788±0.105			0.784±0.058		
FIGO staging			4.211	<0.001		4.722	<0.001
I and II	16	1.941±0.097			0.701±0.046		
III and IV	14	1.785±0.106			0.788±0.056		
5-year survival rate			3.108	0.004		2.841	0.008
Surviving	23	1.983±0.057			0.685±0.040		
Dead	7	1.834±0.122			0.759±0.064		

cells' growth and colony formation [18]. Many studies have also found that this receptor can be used as a predictor of efficacy on cervical cancer, and its high expression is associated with the formation of chemoradiotherapy resistance in patients with the disease, resulting in the reduction of long-term local control of the patients [19-21]. Additionally, patients with its high expression have significantly reducing 5-year recurrence-free survival rate and overall survival rate [22]. It is clear that miR-99a has a targeted inhibitory and regulatory effect on IGF1R, which is similar to reports on bladder cancer and gastric cancer [23]. Accordingly, miR-99a regulates the growth of tumor cells through the targeted inhibition of IGF1R. This study supplements the role of miR-99a on the growth of CCCs through the targeted inhibition.

We purchased PC-480-011, C-33 A, and C-4 II cells from ATCC. The detection results showed that miR-99a expression was lower but IGF1R expression was higher in the two CCCs than that in the normal cervical epithelial cells. Therefore, we promoted miR-99a expression and inhibited IGF1R expression, and then analyzed the cells' biological behavior after the interference. The results showed that both the promotion and the inhibition significantly decreased the proliferation and migration of the two CCCs, but increased their apoptosis. To further verify the correlation of miR-99a with IGF1R in cervical cancer, we co-transfected miR-99a and IGF1R overexpression vectors into the cells. During this process, we found that compared with CCCs transfected with miR-99a overexpression vectors alone, the expression of IGF1R in the co-transfection group increased, while the expression of miR-99a did not change significantly. Additionally, the cell proliferation and migration in the co-transfection group increased, while their apoptotic rate decreased. The DLRGA showed targeted inhibition of miR-99a on IGF1R expression. Therefore, from the cell experiment level, we have preliminarily verified that miR-99a can specifically inhibit IGF1R expression and thus inhibit the proliferation and migration of CCCs. We also analyzed the correlation of miR-99a and IGF1R expression in cervical cancer tissues with clinicopathological characteristics of the patients. miR-99a and IGF1R were correlated with the grade of differentiation, FIGO staging, and 5-year survival rate, which supports the conclusions of our cell experiments to some extent.

However, there are still deficiencies in this study. Animal experiments were not conducted for further verification. *In vitro* experiments cannot simulate the microenvironment *in vivo* that has a great influence on gene action, so *in vivo* experiments are needed to further verify our conclusions. Moreover, miR-99a and IGF1R expression in normal people are unknown, and there are few

clinical reports on miR-99a and IGF1R in cervical cancer, so the clinical significance of their expression in the disease requires more research.

In summary, miR-99a is lowly expressed but IGF1R is highly expressed in CCCs, both of which are associated with the grade of differentiation, FIGO staging, and 5-year survival rate of patients with the disease. miR-99a can specifically inhibit IGF1R expression and thus inhibit the proliferation and migration of CCCs.

Conflict of interests

The authors declare no conflict of interests.

References

1. Cancer Genome Atlas Research Network. Integrated genomic and molecular characterization of cervical cancer. Nature 2017; 543:378.
2. Berman TA, Schiller JT. Human papillomavirus in cervical cancer and oropharyngeal cancer: one cause, two diseases. Cancer 2017; 123:2219-29.
3. Zaman MS, Chauhan N, Yallapu MM et al. Curcumin nanoformulation for cervical cancer treatment. Sci Rep 2016; 6:20051.
4. Ramirez PT, Frumovitz M, Pareja R, et al. Minimally invasive versus abdominal radical hysterectomy for cervical cancer. N Engl J Med 2018; 379:1895-904.
5. Rupaimoole R, Calin GA, Lopez-Berestein G, Sood AK. miRNA deregulation in cancer cells and the tumor microenvironment. Cancer Discov 2016; 6:235-46.
6. Xie H, Ren X, Xin S et al. Emerging roles of circRNA_001569 targeting miR-145 in the proliferation and invasion of colorectal cancer. Oncotarget 2016; 7:26680.
7. Wang L, Chang L, Li Z et al. miR-99a and-99b inhibit cervical cancer cell proliferation and invasion by targeting mTOR signaling pathway. Med Oncol 2014; 31:934.
8. Xia M, Li H, Wang JJ, Zeng HJ, Wang SH. MiR-99a suppresses proliferation, migration and invasion through regulating insulin-like growth factor 1 receptor in breast cancer. Eur Rev Med Pharmacol Sci 2016; 20:1755-63.
9. Mei LL, Qiu YT, Huang MB, Wang WJ, Bai J, Shi ZZ. MiR-99a suppresses proliferation, migration and invasion of esophageal squamous cell carcinoma cells through inhibiting the IGF1R signaling pathway. Cancer Biomark 2017; 20:527-37.
10. Lenz G, Hamilton A, Geng S et al. t-Darpp activates IGF-1R signaling to regulate glucose metabolism in trastuzumab-resistant breast cancer cells. Clin Cancer Res 2018; 24:1216-26.
11. Ekyalongo RC, Yee D. Revisiting the IGF-1R as a breast cancer target. NPJ Precis Oncol 2017; 1:1-7.
12. Yu X, Zhao W, Yang X, Wang Z, Hao M. miR-375 affects the proliferation, invasion, and apoptosis of HPV16-positive human cervical cancer cells by targeting IGF-1R. Int J Gynecol Cancer 2016; 26:851-8.
13. Sin STK, Li Y, Liu M, Ma S, Guan XY. TROP-2 exhibits tumor suppressive functions in cervical cancer by dual inhibition of IGF-1R and ALK signaling. Gynecol Oncol 2019; 152:185-93.
14. Zhang J, Yao T, Wang Y, Yu J, Liu Y, Lin Z. Long noncoding RNA MEG3 is downregulated in cervical cancer and affects cell proliferation and apoptosis by regulating miR-21. Cancer Biol Ther 2016; 17:104-13.
15. Xin M, Qiao Z, Li J et al. miR-22 inhibits tumor growth and metastasis by targeting ATP citrate lyase: evidence in osteosarcoma, prostate cancer, cervical cancer and lung cancer. Oncotarget 2016; 7:44252.
16. Li Y, Zhang Z, Zhang X et al. A dual PI3K/AKT/mTOR signaling inhibitor miR-99a suppresses endometrial carcinoma. Am J Transl Res 2016; 8:719.
17. Wu S, Chen H. Anti Condyloma acuminata mechanism of microRNAs 375 modulates HPV in cervical cancer cells via the UBE3A and IGF 1R pathway. Oncol Lett 2018; 16:3241-7.
18. Luo M, Shen D, Zhou X, Chen X, Wang W. MicroRNA-497 is a potential prognostic marker in human cervical cancer and functions as a tumor suppressor by targeting the insulin-like growth factor 1 receptor. Surgery 2013; 153:836-47.
19. Lloret M, Lara PC, Bordón E et al. IGF-1R expression in localized cervical carcinoma patients treated by radiochemotherapy. Gynecol Oncol 2007; 106:8-11.
20. Zhang R, Hao S, Yang L, Xie J, Chen S, Gu G. LINC00339 promotes cell proliferation and metastasis in pancreatic cancer via miR-497-5p/IGF1R axis. J BUON 2019; 24: 729-38.
21. Velinovic M, Jankovic R, Jovanovic D, et al. Tumor characteristics, expressions of ERCC1, Bax, p53, IGF1R, Bcl2, Bcl2/Bax and prognostic factors for overall survival in patients with lung carcinoid. J BUON 2019; 24:256-66.
22. Huang YF, Shen MR, Hsu KF, Cheng YM, Chou CY. Clinical implications of insulin-like growth factor 1 system in early-stage cervical cancer. Br J Cancer 2008; 99:1096.
23. Xu XL, Guo AX, Pan QY, Chang AL, Zhao CR. MiR-99a suppresses cell migration and invasion by regulating IGF1R in gastric cancer. Eur Rev Med Pharmacol Sci 2019; 23:7375-82.
24. Lin JF, Tsai TF, Lin YC, Chen HE, Chou KY, Hwang TIS. Benzyl isothiocyanate suppresses IGF1R, FGFR3 and mTOR expression by upregulation of miR-99a-5p in human bladder cancer cells. Int J Oncol 2019; 54:2106-16.