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# The regulating role of miR-494 on HCCR1 in cervical cancer cells

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**ABSTRACT**

This study focused on miR-494 inhibiting the proliferation, migration and invasion of cervical cancer cells by regulating HCCR1. During the study, 30 pairs of primary cervical cancer tissues and adjacent tissues diagnosed by pathology were collected, and then placed in a cryopreservation tube, immediately placed in liquid nitrogen to freeze, and then moved to a -80 °C refrigerator for storage until use. They were dipped with crystal violet staining solution for 20 minutes, washed 3 times with PBS, and dried at room temperature. The polycarbonate film on the chamber was gently cut, placed on a glass slide, and covered with neutral resin. The number of cells in 5 random fields was counted under a microscope, and the differences between the groups were compared. The qRT-PCR results showed that compared with the normal cervical tissue cell line HCCR1, the expression levels of miR-494 in cervical cancer cell lines HCCR1 and C-33A were significantly reduced. Compared with the transfection of mimics NC, the level of HCCR1mRNA and protein decreased significantly when transfected with miRNA-494mimics ($P < 0.05$); compared with the transfection of inhibitor NC, the level of HCCR1 mRNA and protein increased significantly when transfected with miRNA-494 inhibitor ($P < 0.05$).

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## Introduction

The specific mechanism of the disease is currently unknown. MicroRNA (microRNA, miRNA) refers to a large family of endogenous small non-coding RNAs containing 18-25 nucleotides (1). About half of miRNA genes are located in fragile sites and genomic regions. Cancer is often controlled by genomic analysis (2). The miRNA does not directly encode proteins, it mainly combines with target gene mRNA through complete or incomplete pairing, induces target mRNA degradation or translation inhibition, inhibits the normal expression of related proteins in the cell, and regulates gene expression through post-transcriptional levels, and then participates The occurrence and development of diseases (3). In order to reduce the harm of cervical cancer to humans, this paper screens out the specific miRNA targeting cervical cancer HCCR1, which provides a new theoretical basis for the targeted treatment of cervical cancer?

Cervical cancer cells are common cell malignancies. Hu X believes that salidroside has effective antioxidant, anti-inflammatory and anti-tumor activity. (1) The effect of different concentrations of salidroside was considered on the viability, cell cycle and apoptosis of cervical cancer SiHa cells and its underlying mechanism (2-3). Method: Cell growth potential and colony formation were determined by Cell Counting Kit-8. The cell cycle distribution was measured by flow cytometry (4-5). The morphology of SiHa cells was examined with an optical microscope. The protein expression of the indicator gene was measured using Western blotting. Results: Different concentrations of salidroside treatment significantly reduced the viability of SiHa cells in a dose- and time-dependent manner (6-7). Salidroside treatment caused changes in the morphology of SiHa cells and induced cell cycle arrest at the G2 / M and/or S phase, which was related to a significant decrease in the expression levels of Cyclin B1, Cyclin A and cyclin-dependent kinase 2, but was related to the regulation of P21 Expression (8-10). In addition, treatment with different

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concentrations of salidroside can induce apoptosis of SiHa cells, and its effect is dose-dependent. Finally, treatment with salidroside increased the relative levels of cleaved caspase 3, Bax, and Fas, but down-regulated the relative levels of BcL-2 and FasL in SiHa cells (11-13). These results indicate that salidroside has strong cytotoxicity to SiHa cells by inducing cell cycle arrest and apoptosis in cervical cancer. Salidroside may be a promising candidate for chemotherapy for cervical cancer.

The expression of miR-494 in cells affects the activity of cervical cancer cells. Esser J S believes that MicroRNAs are small non-coding RNAs that negatively regulate the expression of post-transcriptional genes (14). Several microRNAs have been described to regulate the process of angiogenesis (15-16). It shows that bone morphogenetic protein 4 (BMP4) increases the angiogenic activity of endothelial cells (17-19). In this project, study how microRNA mediates the BMP4 effect of promoting angiogenesis (20-21). First, microRNA array analysis was performed on human umbilical vein endothelial cells (HUVEC) stimulated with BMP4 (22-23). In the up-regulated microRNA, miR-494 expression was detected to be reduced, while miR-126-5p expression was increased. Next, we analyzed the canonical Smad and alternative signaling pathways through which BMP4 regulates the expression of miR-126-5p and miR-494. In addition, the functional effects of miR-494 and miR-126-5p on endothelial cells were studied (24-25). MicroRNA-494 overexpression reduces endothelial cell proliferation, migration and sprout formation. Studies have shown that miR-494 inhibition can increase endothelial cell function.

This article mainly studies the relationship between miR-494 and HCCR1, using RT-qPCR and Western-blot technology to prove the negative correlation between miRNA-494 and HCCR1 expression, and through bioinformatics analysis found miRNA-494 and HCCR1 Targeting relationship, and further verified by the dual-luciferase experiment. On this basis, cell function experiments further confirmed that overexpression of miRNA-494 can target the expression of HCCR1, thereby inhibiting the proliferation, migration and invasion of cervical cancer cells.

## Materials and methods

### Experimental Materials

(i)Experimental cells. Human cervical cancer HeLa cell line

(ii)Cervical tissue specimens. Thirty pairs of primary cervical cancer tissues and adjacent tissues diagnosed by pathology were collected, and then placed in a cryopreservation tube, immediately placed in liquid nitrogen for freezing, and then moved to a -80 °C refrigerator for storage until use.

### Main Reagents and Instruments

(i) Main reagents

HceEpic cell lines were cultured in MEM medium (EMEM, 10% BFS, Gibco, Grand Island, NY, USA), Hela cell lines were cultured in RPMI1640 medium (10% FBS, Gibco), C-33A and HEK-293T cell lines DMEM medium (10% FBS, DMEM, Gibco) culture.

Other reagents: cell transfection reagent Effectene, TrizolReagent, pancreatin, PBS, DMSO, isopropanol, absolute ethanol, primers, mouse anti-human GAPDH antibody, 8μm transwell chamber, reverse transcription kit (MicroRNA), Real-TimePCR Kit (MicroRNA), MTT kit, transfection kit (miR-381-3pinhibitor), DACH1 antibody, DNA reverse transcription kit (AppliedBiosystems), SYBRSelectMasterMix (AppliedBiosystems) / crystal violet dye stain

(ii) Main instruments

CO2 constant temperature incubator, inverted microscope, inverted phase-contrast microscope, ultra-clean workbench, microplate reader, pipette, centrifuge, refrigerated centrifuge, vertical constant temperature oscillator, counting microscope, flow cytometer, blood cell counting plate, 24-well plate, EP tube ELISA special gun, baking machine, magnetic stirrer, laboratory pure water system, cover glass, measuring cylinder, -80 °C refrigerator, liquid nitrogen tank.

### Experimental Method

(i) Extraction of miR-494

Paraffin section dewaxing and hydration: rinse twice with xylene, once every 10 minutes; wash with 100%, 90%, 80%, 75% ethanol and DEPCH2O in turn, once every 5 minutes. Ensure that miRNA is extracted according to the instructions of the miRNA extraction kit in an environment free of RNase.

(ii) Reverse transcription

After adding the above ingredients to a 0.2mL microcentrifuge tube, mix it thoroughly and put it into the PCR instrument to complete the cycle program of 37°C 1h, 85°C 5min, 4°C to obtain cDNA, and the cDNA obtained by reverse transcription is diluted with 90μL of sterile water Until the total volume is 100μL, put it at -20°C for use.

(iii) Extraction of tissue total protein samples

1) Fresh tissues are taken out of liquid nitrogen and weighed, put into a pre-cooled homogenizer (-20°C refrigerator pre-cooling for about 30min), and cut them as much as possible;

2) Add the pre-produced protein lysate (the PMSF with a final concentration of 1mM has been added to RIPA) to the homogenizer at a rate of 2mL of protein lysate per 1g of tissue.

3) The protein lysate was fully lysed for about 30 minutes and then transferred to a 1.5 mL EP tube, centrifuged at 4°C, 12000 rpm, and 15 minutes. After the centrifugation, the supernatant was transferred to another EP tube and stored at -80°C.

(iv) Extraction of total cell protein samples

1) Prepare cell lysate on ice (RIPA: phosphorylase inhibitor = 100: 1).

2) Take the cells that have grown to the logarithmic phase and cover the bottom of the culture flask, discard the medium, wash the adherent cells twice with 2mL of pre-chilled PBS, and aspirate all the remaining liquid in the culture flask.

3) Add 100 μL of pre-produced cell lysate, scrape the cells repeatedly with a cell scraper, remove the whole

Part of the cell suspension was transferred into an EP tube and placed in an ice bath for 60 minutes to fully lyse the cells.

4) Low-temperature centrifuge Centrifuge at 4 °C, 12000rpm, 15min. After centrifugation, transfer all the supernatant into a new centrifuge tube, mark it, mark the date, and store it at -80 °C.

(v) Cell invasion experiment

Place the cell in a 24-well plate in a biological safety cabinet, add 20 μL of diluted Matrigel gel to the upper layer, gently shake to avoid air bubbles, and spread the matrigel evenly on the cell. Leave at 37 ° C overnight to ensure that Matrigel is fully set. Place the chamber in 4% POM for 20 minutes, wash with PBS 3 times, and dry at room temperature. Dip with crystal violet staining solution for 20 minutes, wash 3 times with PBS, and dry at room temperature. The polycarbonate film on the chamber was gently cut, placed on a glass slide, and covered with neutral resin. The number of cells in 5 random fields was counted under a microscope to compare the differences between the groups.

(vi) Cell migration experiment

The steps of the cell migration experiment and cell invasion experiment are roughly the same, except that the Matrigel is not needed on the microporous membrane of the cell.

**Statistical Methods**

SPSS20.0 statistical software was used to analyze all the data. The expression difference of miR-494 in cervical cancer tissues and normal cervical tissues was analyzed by t-test. The expression of miR-494 in cervical cancer tissues and clinical-pathological data were analyzed by t-test. Compared with the approximate t-test, a one-way analysis of variance (Oneway ANOVA) was used for comparison between multiple groups, with $P < 0.05$ indicating that the difference was statistically significant.

**Results and discussion**

**Pathological Features of Cervical Cancer and miRNA-494 Expression**

The miRNA expression is shown in Figure 1. The qRT-PCR results verified that miR-381-3p is lowly expressed in cervical cancer tissues and cells HCCR1. Therefore, choose miR-494 as the object of further research. The figure shows differentially expressed miRNA. B: Heat map shows differentially expressed genes. The expression of miR-494 in cervical cancer tissue is significantly lower than that of adjacent normal cervical tissue. C: qRT-PCR results verified that the expression level of miR-494 in cervical cancer tissue was significantly lower than that of adjacent normal cervical tissue, the number of adjacent tissues = 20, and the number of cancer tissues = 20. The qRT-PCR results showed that compared with the normal cervical tissue cell line HCCR1, the expression levels of miR-494 in cervical cancer cell lines HCCR1 and C-33A were significantly reduced. $P < 0.01$ indicates that there is a significant difference compared with adjacent tissues.

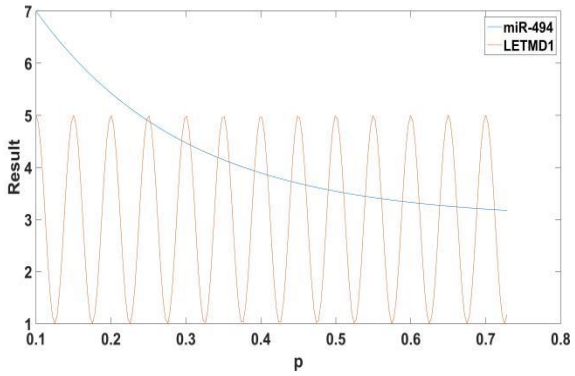


**Figure 1.** miRNA expression

When P <0.05, the expression of miRNA494 is shown in Table 1. However, there was no statistically significant difference in SCC level from the patient's age and degree of differentiation. In this experiment, endometrial cancer cell lines HEC-1A and Ishikawa were selected to study the function of normal endometrium with Ishikawa cells and HEC-1A cells to study the function of endometrial adenocarcinoma. Real-time PCR was used to detect the differential expression of miRNA494 in Ishikawa and HCCR1 cells. In the detected cell lines, the expression of miRNA494 in HEC-1A cells was significantly higher than that of HCCR1 (** p <0.01)

**Table1.** Expression of miRNA494 when P <0.05

| Age <45 years | Number of cases | X | P |
|---|---|---|---|
| FIGO installments 1 to 2 | 12 | 16 | 0.015 |
| FIGO installments 3 to 4 | 18 | 19 | 0.013 |
| The high degree of differentiation | 24 | 19 | 0.014 |
| SCC<1.5 | 6 | 18 | 0.6 |

**Expression of mir-494 in Cervical Cancer Tissues and Normal Tissues**

The study found that overexpression of miR-494 inhibited the migration and invasion of NSCLC cells, and down-regulated miR-494 expression reversed this effect. It is suggested that miR-494 as a tumor suppressor gene in NSCLC is a new potential therapeutic target and prognostic marker of NSCLC. In NSCLC cells, by increasing the expression level of miR-494, it can restore its sensitivity to tnf-related apoptosis-inducing ligand (TRAIL) treatment, thereby inhibiting the anti-apoptotic protein PED / PEA-15 in tumor therapy Drug resistance. The experimental results under different numbers of groups are shown in Figure 2. miR-494 is a promising anti-tumor gene. It has the ability to selectively induce cancer cell apoptosis without affecting the viability of healthy bypass cells. Compared with the transfection of mimics NC, the level of HCCR1mRNA and protein decreased significantly when transfected with miRNA-494mimics (P <0.05); compared with the transfection of inhibitor NC, the level of HCCR1mRNA and protein increased significantly when transfected with miRNA-494 inhibitor (P <0.05).

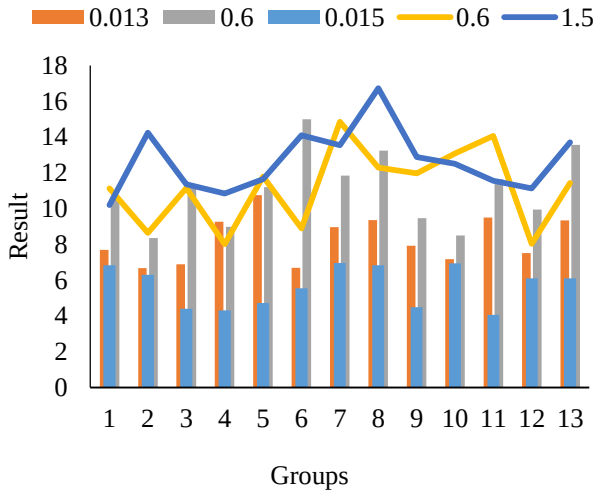


**Figure 2.** Experimental results under different groups

The expression results under different types are shown in Figure 3. At the same time, reducing the expression of FOXA1 can inhibit the proliferation and induce apoptosis of HCCR1 cells. MiR-494 can inhibit the growth and development of HCC tumors by inhibiting the expression of FOXA1, which is an effective prognostic marker for HCC. The miR-212-RBP2-CDKI pathway plays an important role in the occurrence and development of liver cancer. Overexpression of miR-494 can inhibit the expression of HCCR1, and down-regulation of RBP2 results in high expression of CDKIs. Highly expressed CDKIs inhibit cell proliferation and induce apoptosis: after inhibiting miR-494 expression, RBP2 expression is up-regulated, CDKIs are down-regulated in HCC Tumor cell proliferation and apoptosis inhibition decreased. Low expression of hsamiR-212 and RBP2 overexpression may be prognostic markers of miR-494.

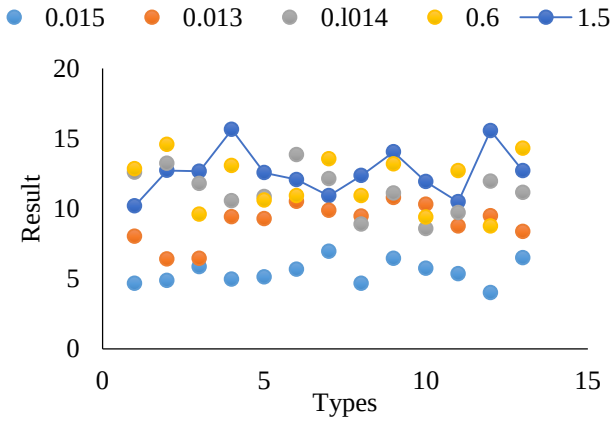


**Figure 3.** Expression results under different types

### Mir-494 Regulates the Process of HCCR1 Inhibiting Cervical Cancer Cells

The mechanism of miR-494 regulating HCCR1 is shown in Figure 4. Immunohisto chemistry, qRT-PCR and Western blot experiments found that compared with the adjacent normal mucosal tissue, the expression of HCCR1mRNA and its protein in CRC tissue was significantly increased. And through the MTT experiment and Transwell experiment found that over-expressed HCCR1 can promote the proliferation and invasion of CRC cells. In CRC, the expression of HCCR1 is directly controlled by miR-494. The down-regulated miR-494 will up-regulate the expression of HCCR1, induce the EMT process, promote tumor metastasis and recurrence, and when miR-494 is overexpressed, it can reduce SOX4. Expression, HCCR1 plays an oncogene role in CRC. Consistent with the "enhancer" of wnt signaling, the increased expression of HCCR1 in cervical cancer cells is related to poor prognosis.

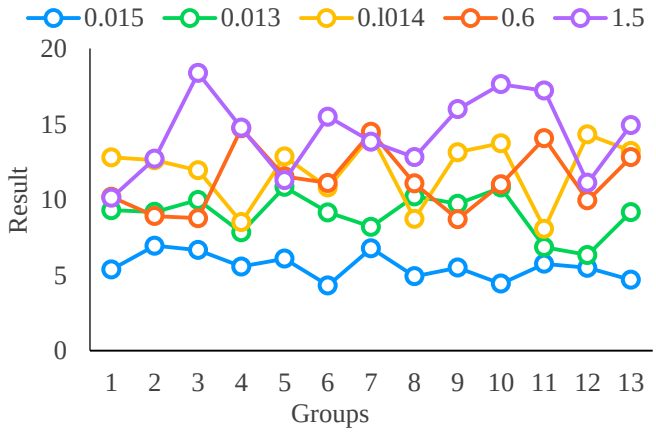


**Figure 4.** Mechanism of miR-494 regulating HCCR1

Cervical cancer is one of the most common gynecological malignancies (5). In recent years, the incidence of cervical cancer has shown a younger trend, increasing at a rate of 2% to 3% per year. At present, the treatment of cervical cancer mostly uses methods such as surgery, radiotherapy and adjuvant chemotherapy, and the 5-year survival rate has always hovered around 50% (8). Therefore, it is particularly important to further find early molecular markers and potential therapeutic targets for cervical cancer.

In this paper, RT-qPCR technology was used to detect the content of miR-494 in cervical cancer tissues and normal cervical tissues. It was found that compared with normal tissues, the expression of miR-494 in cervical cancer tissues was significantly down-regulated; The relationship between pathological features found that miR-494 was significantly related to the FIGO stage of cancer patients.

At the cellular level, clone formation experiments confirmed that miR-494 could negatively regulate HCCR1 to inhibit cervical cancer cell proliferation, while Alvarado-Ruiz *et al*. (11) confirmed that miR-494 could negatively regulate HCCR1 to inhibit cervical cancer cell migration and invasion. miR-494 plays the role of tumor suppressor gene in the occurrence and development of cervical cancer, and in the course of discussing its internal mechanism of action, it was found that HCCR1 is the target gene of miR-494, and miR-494 can target HCCR1 to bind and inhibit it Expression, thereby inhibiting the proliferation, migration and invasion of cervical cancer cells and other malignant biological behaviors, which provides a new theoretical basis and ideas for elucidating the development mechanism and precise treatment of cervical cancer in the future.

### Acknowledgements

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### Interest conflict

The authors declare no conflict of interest.

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