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MiR-499b-5p inhibits cervical cancer cell proliferation and induces apoptosis by targeting the Notch1 signaling pathway

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Abstract. – OBJECTIVE: We investigated the effect of miR-499b-5p on the tumorigenesis and development of cervical cancer by targeting the Notch1 signaling pathway to identify a new potential clinical target of cervical cancer.

PATIENTS AND METHODS: Quantitative real-time polymerase chain reaction (qRT-PCR) was applied to determine the mRNA expression levels of Notch1 and miR-499b-5p in cervical cancer tissues/cell lines. Cell counting kit-8 (CCK-8) assay, transwell assay, and flow cytometry were conducted to detect cell viability, cell migration, and cell apoptosis abilities. A Dual-Luciferase reporter assay was performed to test the binding site between miR-499b-5p and Notch1. An *in vivo* experiment was carried out using nude mice, and xenograft tumor models were established.

RESULTS: OD450 of the SiHa and HeLa cells of the miR-499b-5p agomir group was lower than that of the miR-499b-5p agomir-NC group. More apoptotic cells and fewer invasive cells were found in the former than in the latter. MiR-499b-5p inhibited the viability and migration of cervical cancer cells and promoted their apoptosis. Further detection of the Luciferase reporter gene confirmed the binding site of miR-499b-5p to Notch1. Western blot results showed that miR-499b-5p inhibited the expression of Notch1 and activated the expression of ChK2 and p-p38MAPK. Notch1 knockdown also inhibited the viability and migration of cervical cancer cells and promoted their apoptosis. MiR-499b-5p overexpression prevented the tumorigenesis and development of cervical cancer in xenograft tumor models.

CONCLUSIONS: MiR-499b-5p inhibits the proliferation of cervical cancer cells and induces their apoptosis by targeting the Notch1 signaling pathway.

Key Words:
MiR-499b-5p, Cervical cancer, Notch1.

Introduction

Cervical cancer is one of the most common gynecological malignancies and has become an important public health problem in the world[1]. Currently, surgery, chemotherapy, and radiotherapy are the most commonly used treatment methods for cervical cancer, but most cervical cancer cells are resistant to chemotherapy drugs; consequently, these drugs have a poor efficacy against cervical cancer[2,3]. Effective treatments are also lacking for advanced and recurrent cervical cancer with poor prognosis[4]. Therefore, the exploration of innovative treatment methods may be the key to the breakthrough of cervical cancer treatment. The molecular mechanism of cervical cancer development should also be further studied, and new molecular targets should be determined.

MicroRNAs (miRNAs), which are noncoding endogenous small-molecule single-stranded RNA with a targeted regulatory function, can cause the degradation of a target mRNA or inhibit its translation. Their mechanism of action involves binding to the 3′-UTR of the target mRNA and altering gene expression at a post-transcriptional level[6]. MiRNAs are abnormally expressed in the development and progression of various tumors[7,8]. They also play an important role in tumor development[9]. For example, various miRNAs in malignant tumors of cervical cancer are downregulated or upregulated and involved in biological processes such as the occurrence, invasion, and metastasis of cervical cancer[10].

MiR-499b-5p is a promising anticancer star miRNA. Early studies[11,12] revealed that miR-499b-5p can inhibit the occurrence of leuke-

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mia. In malignant breast tumors and malignant esophageal tumors, miR-499b-5p can inhibit tumor invasion and metastasis[13]. In addition, the miR-499b-5p expression is downregulated in the malignant transformation of liver cancer stem cells, indicating that miR-499b-5p may inhibit the occurrence and development of malignant liver cancer[14]. Unfortunately, it has been explored[15] the role of miR-499b-5p in the occurrence and development of cervical cancer.

The role of the Notch1 signaling pathway in cell development, proliferation, differentiation, and apoptosis has been widely reported[16]. It is involved in the malignant phenotypes of some cancers, such as breast cancer and T-cell leukemia[17]. Notch1 inhibits p53-regulated apoptosis and affects lung adenocarcinoma growth by regulating the stability of p53. This pathway is activated by the binding of a ligand to its corresponding receptor[18]. The ligand-receptor combination then induces a series of changes in Notch1 conformation, eventually exposing the S2 site. The released protein-active Notch form, namely, NICD, enters the nucleus and performs its functions[19]. The target genes of NICD mainly include Cyclin D1, c-Myc, p21, NF-kB, and Nanog, which promote the occurrence and development of cancer. However, the role of the Notch1 signaling pathway in the occurrence and development of cervical cancer has been rarely described[20]. Previous studies[21,22] preliminarily confirmed that the expression of the Notch1 signaling pathway in cervical cancer specimens is related to the tumor stage, tumor metastasis, and prognosis of patients with cervical cancer; therefore, Notch1 is a potentially important target for studying the malignant biological behavior of cervical cancer.

In this study, a bioinformatic database was used to predict that the intersection of the regulatory miRNAs of the Notch signaling pathway was miR-499b-5p. The correlation between the clinicopathological factors of cervical cancer and the expression level of miR-499b-5p was further analyzed. Experiments on cell biology and applied gene function were conducted to investigate the expression and influence of miR-499b-5p in the abnormal cell biological behavior of cervical cancer and to determine its target genes and biological mechanism. This study was also conducted to provide a scientific basis and propose strategies for preventing and controlling the occurrence of cervical cancer and developing new mechanisms.

Patients and Methods

Patients and Cervical Cancer Samples

Twenty pairs of matched cervical cancer tissues (n=20) and adjacent tissues (n=20) were surgically removed from patients with cervical cancer in the First Affiliated Hospital, School of Medicine, Shihezi University from January 2018 to March 2019. The tumor staging of cervical cancer was evaluated in accordance with the criteria proposed by the UICC. Clinical indicators and follow-up data were collected, and informed consent was obtained from the patients. This study was approved by the Ethics Committee of the First Affiliated Hospital, School of Medicine, Shihezi University and conducted in accordance with the Declaration of Helsinki.

Cell Culture

The human normal cervical epithelial cell line HCerEpiC and the human cervical cancer cell lines SiHa, HeLa, C33A, and CaSki were purchased from the Cell Bank of the Shanghai Institute of Cell Biology (Shanghai, China). The cells were cultured in high-glucose Dulbecco's Modified Eagle's Medium (DMEM) and Roswell Park Memorial Institute-1640 (RPMI-1640) (Gibco, Rockville, MD, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA) and 1% penicillin (100 μg/ml)-streptomycin (100 μg/ml; Hycult, Life Technologies, Rockville, MD, USA) in an incubator with a humidified 5% CO_2 atmosphere at 37°C (Thermo Fisher Scientific, Waltham, MA, USA).

Cell Transfection

Oligonucleotides (miR-499b-5p agomir-NC, miR-499b-5p agomir, si-NC, and si-Notch1) were purchased from Shanghai GenePharma Co., Ltd. (Shanghai, China). The cells were transfected with Lipofectamine 2000 (Gibco, Rockville, MD, USA) in accordance with the manufacturer's protocol. After transfection for 24-48 h, the cells were used to test the transfection efficiency.

CCK-8 Assays

The transfected cells were seeded into 96-well plates at a density of 2×10^3 cells per well. On days 1, 2, 3, and 4, a CCK-8 kit (Dojindo Laboratories, Kumamoto, Japan) was added to each well and incubated for 2 h in the dark. Absorbance was recorded at 450 nm with a microplate reader

(MultiskanEX, Lab systems, Helsinki, Finland). After the detection value was obtained, the cell proliferation rate was calculated.

Transwell

The transfected cells were seeded into 24-well plates at 2×10^5 cells per well. Then, 200 μL of the cell suspension was added to the transwell upper chamber (Millipore, Billerica, MA, USA), and 600 μL of the complete medium with 10% FBS was added to the transwell lower chamber. After 48 h of incubation, the cells were fixed in methanol for 15 min and stained with crystal violet for 10 min. Cell migration was observed under a microscope (Nikon, Japan).

RNA Extraction and Real-Time PCR Analysis

Total RNA was extracted using a TRIzol reagent (Invitrogen, Carlsbad, CA, USA). For cDNA synthesis, the total RNA was used for cDNA amplification with a HiScript®II reverse kit (Vazyme, China). For real-time qPCR analysis, an AceQ RT-qPCR kit (Vazyme, China) was utilized in accordance with the manufacturer's instructions. qPCR amplification was performed using SYBR Green PCR Mix (Boster, China) on an ABI 7500 Fast real-time PCR system (Applied Biosystems, Foster City, CA, USA). The reaction protocol was as follows: 94°C for 1 min, 94°C for 30 sec, 55°C for 30 sec, and 72°C for 30 s with 40 cycles. The following primers were used: Notch1, sense: 5′-GACATCACGGATCATATGGA-3′, antisense: 5′-CTCGCATTGACCATTCAAAC-3′; miR-499b-5p, sense: 5′-AGCAGAAAGTACACATAAACACA-3′, antisense: 5′-AGAGTTCTGCCACTATGTTTCA-3′; U6, sense: 5′-CTCGCTTCGGCAGCACA-3′, antisense: 5′-ACGCTTCACGAATTTGC-3′; and GAPDH, sense: 5′-AGAAGGCTGGGGCTCATTTG-3′, antisense: 5′-AGGGGCCATCCACAGTCTTC-3′. The relative expression levels of Notch1 and miR-499b-5p were calculated with $2^{-\Delta\Delta Ct}$. U6 was used as an internal control for the miR-499b-5p expression, and GAPDH was set as the internal control for the Notch1 expression.

Western Blot

The cells were collected and resuspended in 0.5 ml of ice-cold RIPA lysis buffer (Solarbio, Beijing, China). Then, the cell lysates were lysed by vortexing with acid-washed glass beads for 2 min and placed in an ice bath for 2 min. This cycle was repeated 10 times. After centrifugation at 12,000 rpm for 30 min, the sample protein concentration was determined using Nanotrop (Thermo Fisher Scientific, Waltham, MA, USA). Afterward, 50 μg of the lysate samples were electrophoresed on 8% or 10% polyacrylamide gels and transferred onto a polyvinylidene difluoride membrane (PVDF; Millipore, Bedford, MA, USA). The samples were blocked with 5% nonfat milk or bovine serum albumin (BSA) in a Tris-buffered saline solution containing 0.1%-0.2% Tween 20 (TBST-20) at room temperature for 2 h and then probed with Notch1, Jagged1, Hes1, c-Src, CSL, and GAPDH primary antibody (Boster, China; dilution at 1:3000) at 4°C overnight. The secondary antibodies used for detection included horseradish peroxidase (HRP)-conjugated anti-mouse IgG, anti-rat IgG, or anti-rabbit IgG. The protein expression was detected using an enhanced chemiluminescence (ECL) detection system (Millipore; Catalog: MA01821).

Xenograft Tumor Models

The female Balbc/nude mice (4-5 weeks old; Beijing Vital River Laboratory Animal Technology Co., Ltd.) were randomly divided into control, miR-499b-5p agomir, and miR-499b-5p agomir-NC groups (10 per group) and housed under standard conditions. Stably transfected cells (5×10^6 cells per mouse) were subcutaneously injected into the right flanks of the mice. Tumor volumes were measured every 4-5 days. After 4 weeks, the nude mice were anesthetized with an intraperitoneal injection of 0.5% pentobarbital sodium (50 mg/kg; Sigma-Aldrich, St. Louis, MO, USA) and sacrificed with CO_2. Subcutaneous tumor tissues were isolated, and the volume and weight of the dissected tumors were measured. Tumor volume was calculated as follows: V (mm^3) = (length×width2)/2. This study was approved by the Ethics Committee of the First Affiliated Hospital, School of Medicine, Shihezi University.

Dual-Luciferase Reporter Assays

After the cotransfection of plasmids for 36-48 h, the medium was discarded, and the cells were washed with 100 μl of 1× PBS. Deionized water was used to dilute 5× PLB (lysate) to 1× PLB (equipped with current use) and placed at normal temperature before it was used. Then, 50 μL of the diluted 1× PLB was added to each well and shaken on a shaker for 20-30 min to ensure that the cells were completely lysed by the lysis buf-

fer. A white opaque 96-well microtiter plate was added with 10 μL of the supernatant to each well, and 100 μL of premixed Luciferase assay reagent II was added to examine the data and measure the Luciferase reaction intensity. After the measurement was completed, 100 μL of premixed Stop & Glo Reagent was added to each well and allowed to stand for 2 s. The data were investigated to determine the intensity of the internal reference, i.e., *Renilla* Luciferase reaction. The ratio of the two sets of data was calculated.

TUNEL Assays

A TUNEL assay was performed to evaluate the apoptotic cells in cervical cancer tissues. Tissue slices were incubated initially with deparaffin and protease K and subsequently with TdT enzyme at 37°C for 1 h. The slides were incubated in horseradish peroxidase (HRP)-labeled streptavidin and detected with stable chromogen DAB. The slices were observed using a confocal laser scanning microscope (LSM880, Germany).

Immunohistochemistry Assay

An immunohistochemistry (IHC) assay was performed in accordance with the standard protocol[23]. An anti-mouse-Notch1 monoclonal antibody (Boster, China) was employed. After the IHC-stained slides were observed, the images were analyzed using Image J (National Institutes of Health, Bethesda, MD, USA).

Statistical Analysis

All statistical analyses were performed in SPSS 22.0 (IBM, Armonk, NY, USA). Experimental data were expressed as mean ± standard deviation. Differences between groups were analyzed with Student's *t*-test. Multiple groups were compared through one-way ANOVA and Tukey's multiple comparison test. Results were considered statistically significant at $p < 0.05$.

Results

MiR-499b-5p Is Downregulated in Cervical Cancer Tissues and Cell Lines

The miR-499b-5p expression in cervical cancer and adjacent cancer tissues was detected. The miR-499b-5p expression was significantly downregulated in cervical cancer tissues compared with that in adjacent cancer tissues (Figure 1A). The expression level of miR-499b-5p was also detected in the cervical cell lines. Similarly, the expression level of miR-499b-5p in the cervical cancer cells was significantly lower than that of the normal cervical epithelial cells (HCerEpiC; Figure 1B). Among the four cervical cancer cell lines, SiHa and HeLa cell lines showed the lowest miR-499b-5p abundance. Therefore, SiHa and HeLa were used in subsequent experiments.

Overexpression of MiR-499b-5p Inhibits the Malignant Progression in SiHa and HeLa

To our knowledge, miR-499b-5p related to cervical cancer has not been reported. MiR-499b-5p agomir-NC and miR-499b-5p agomir were successfully transfected into SiHa and

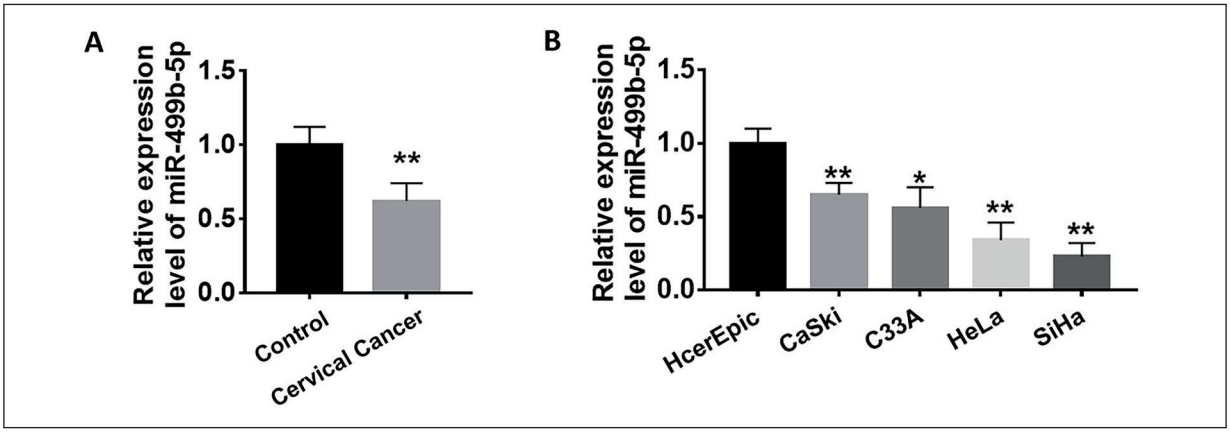

Figure 1. MiR-499b-5p is downregulated in cervical cancer tissues and cell lines**. A,** MiR-499b-5p expression levels in cervical cancer tissues and adjacent cancer tissues. **B,** MiR-499b-5p levels in cervical cancer cells and normal cervical epithelial cells. *p<0.05, **p<0.01.

HeLa and compared with the control and miR-499b-5p agomir-NC groups to investigate the function of miR-499b-5p in cervical cancer cells. qPCR results indicated that miR-499b-5p agomir transfection significantly enhanced the expression of miR-499b-5p in SiHa and HeLa (Figures 2A and 2B). Next, cell viability was determined via the CCK8 assay. The CCK-8 assay results indicated that OD450 of SiHa and HeLa of the miR-499b-5p agomir group was remarkably lower than that of the control group at 72 h after transfection (Figures 2C and 2D). The transwell assay revealed that miR-499b-5p inhibited the invasion ability of the above cells (Figures 2E and 2F). Cell apoptosis was detected via flow cytometry. As a result, more apoptotic cells were observed in the miR-499b-5p agomir group than that in the miR-499b-5p agomir-NC group (Figures 2G and 2H). Therefore, the miR-499b-5p overexpression inhibited the proliferation of cervical cancer cells and promoted the apoptosis of cervical cancer cells.

Notch1 Is the Target Gene of MiR-499b-5p

The Notch signaling pathways that bound to miR-499b-5p were analyzed to further explore the mechanism of miR-499b-5p in cervical cancer. Bioinformatics showed that miR-499b-5p could be combined with Notch1 (Figure 3A). Dual-Luciferase reporter assay was used to verify the regulatory relationship between Notch1 and miR-499b-5p. The relative Luciferase activity of the Notch1 WT reporter significantly decreased in the miR-499b-5p mimic group compared with that in the miR-499b-5p NC group. However, the relative Luciferase activity of the Notch1-Mut reporter was not significantly changed in the miR-499b-5p mimic group compared with that in the miR-499b-5p NC group (Figure 3B). MiR-499b-5p was overexpressed and knocked down to verify the regulatory effect of miR-499b-5p on Notch1 (Figures 3C and 3D). Moreover, the Notch1 expression level in the cervical cancer tissues dramatically increased compared with that in the normal tissues (Figure 3E). The co-expression test results of miR-499b-5p and Notch1 showed that they had a negative coexpression correlation (Figure 3F). Therefore, miR-499b-5p might inhibit cervical cancer development by inhibiting the Notch1 expression.

Overexpression of MiR-499b-5p Inhibits the Notch1 Signaling Pathway and Upregulates the Expression of the Signaling Pathways of the Tumor Suppressors ChK2 and p-P38 MAPK

On the basis of the results, we wondered whether the overexpression of miR-499b-5p inhibited the development of cervical cancer via multiple signaling pathways. Therefore, we detected the three classical signaling pathways via Western blot and found that the miR-499b-5p agomir group could inhibit the Notch1 signaling pathway and upregulate the two important tumor-suppressing pathways, namely, ChK2 and p-P38 MAPK signaling pathways, compared with those of the control and miR-499b-5p agomir-NC groups (Figures 4A and 4B).

Notch1 Knockdown Inhibits the Malignant Progression of Cervical Cancer Cells

We successfully transfected si-NC and si-Notch1 into SiHa and HeLa and compared them with the si-NC group. The results showed that si-Notch1 transfection significantly inhibited the expression of si-Notch1 in the SiHa and HeLa cell lines (Figures 5A and 5B). Interestingly, OD450 of the si-Notch1 group was significantly lower than that of the si-NC groups at 72 h; furthermore, the apoptotic cells and the invasive cells of SiHa and HeLa were fewer in the si-Notch1 group than in the si-NC groups (Figures 5C-5H). Therefore, Notch1 knockdown inhibited the malignant progression of cervical cancer cells.

Xenograft Tumor Models Demonstrate that the Overexpression of MiR-499b-5p Inhibits Cervical Cancer

The mouse xenograft tumor model was successfully established to further confirm whether miR-499b-5p could inhibit the proliferation of cervical cancer cells by targeting the Notch1 signaling pathway. The subcutaneous tumor tissues of the mice were obtained 28 days after subcutaneous injection. The results showed that the tumor weight and volume of the miR-499b-5p agomir group decreased significantly compared with those of the control and miR-499b-5p agomir-NC groups under almost the same bodyweight (Figures 6A-6D). In the miR-499b-5p agomir group, the Notch1 expression was significantly inhibited

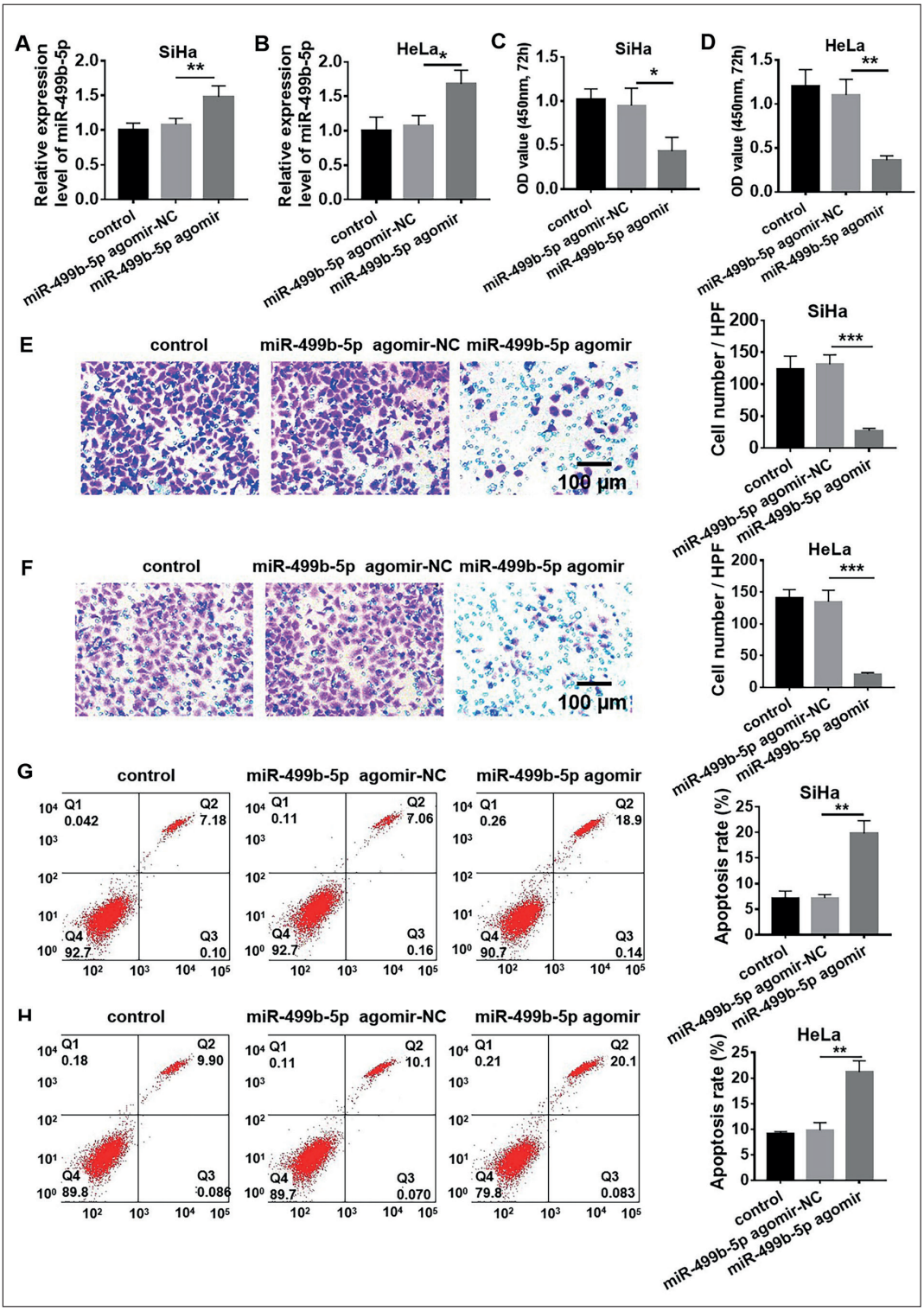

Figure 2. Overexpression of miR-499b-5p inhibits the malignant progression of SiHa and HeLa cervical cancer cell lines. **A,** Verification of miR-499b-5p overexpression in the SiHa cell line. **B,** Verification of miR-499b-5p overexpression in the HeLa cell line. **C,** MiR-499b-5p inhibits cervical cancer cell proliferation in the SiHa cell line. **D,** MiR-499b-5p inhibits cervical cancer cell proliferation in the HeLa cell line. **E,** MiR-499b-5p inhibits cervical cancer cell migration in the SiHa cell line. Scale bar = 100 μm. **F,** MiR-499b-5p inhibits cervical cancer cell migration in the HeLa cell line. Scale bar = 100 μm. **G,** MiR-499b-5p induces cervical cancer cell apoptosis in the SiHa cell line. **H,** MiR-499b-5p induces cervical cancer cell apoptosis in the HeLa cell line. *p<0.05, **p<0.01, ***p<0.001.

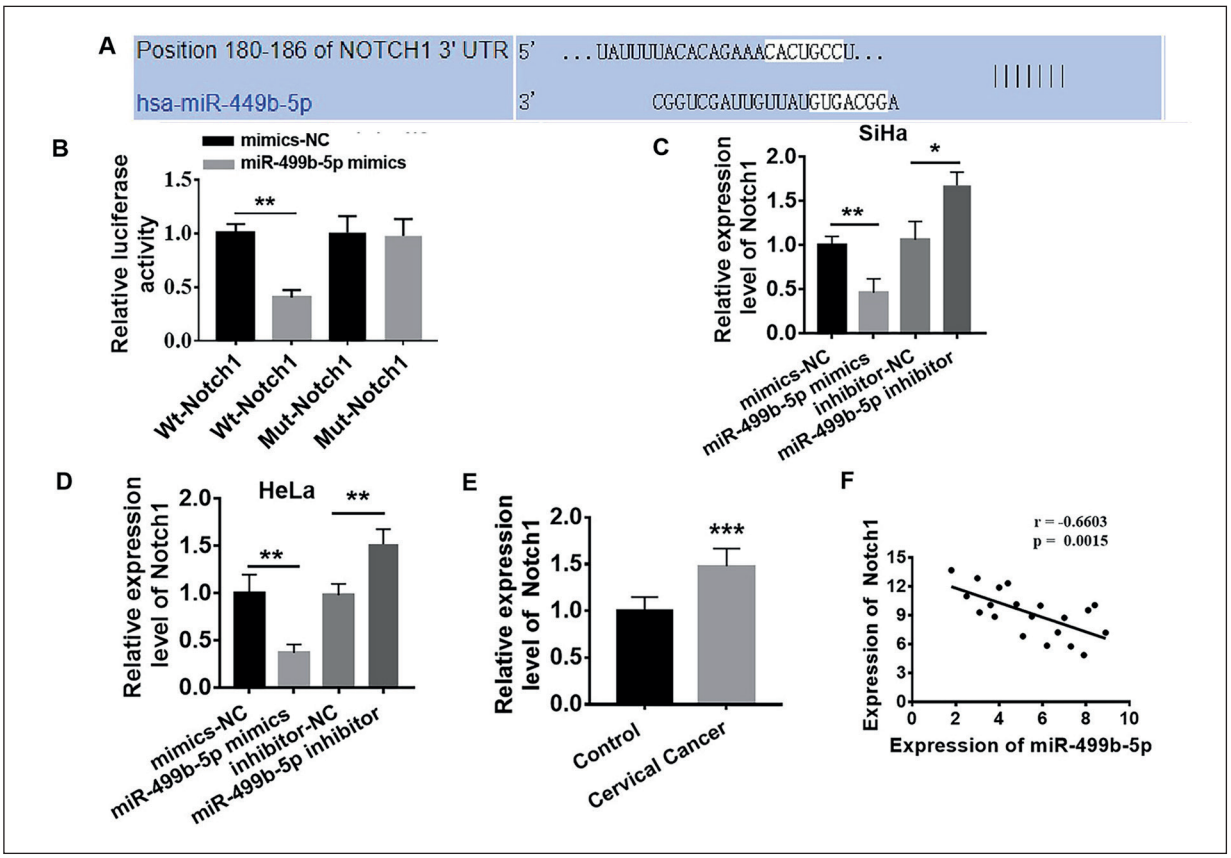

Figure 3. MiR-499b-5p regulates the detection of Notch1. **A,** MiR-499b-5p and Notch1 bind site information maps. **B,** Dual-Luciferase report detects miR-499b-5p binding to Notch1. **C,** Verification of miR-499b-5p overexpression and expression after knockdown in the SiHa cell line. **D,** Verification of miR-499b-5p overexpression and expression after knockdown in the HeLa cell line. **E,** Overexpression of Notch1 in cervical cancer tissues. **F,** Correlation analysis between miR-499b-5p and Notch1 coexpression. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

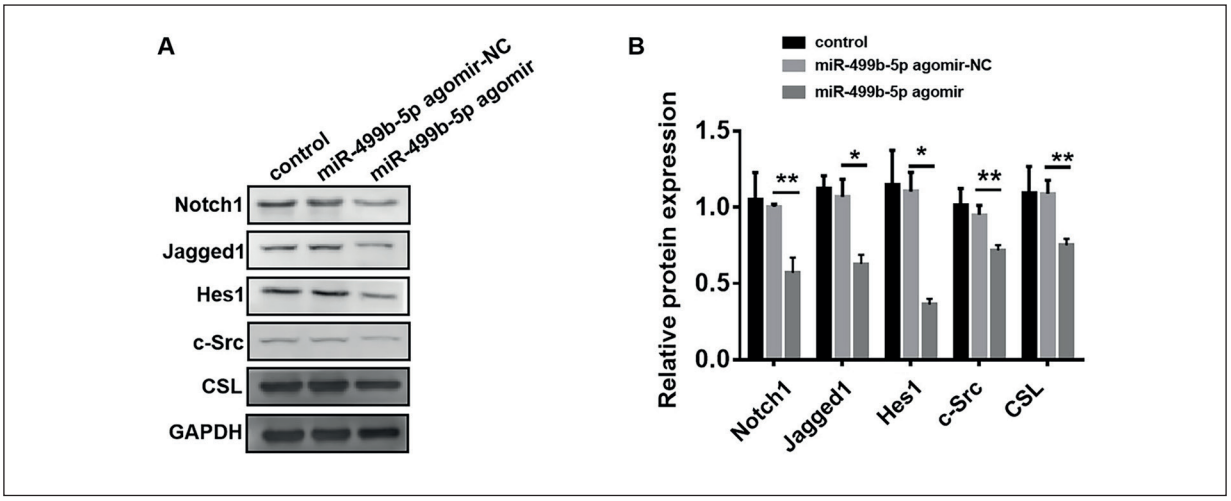

Figure 4. Overexpression of miR-499b-5p inhibits the Notch1 signaling pathway and upregulates the expression of the signaling pathways of the tumor suppressors ChK2 and p-P38 MAPK. **A-B,** MiR-499b-5p regulates the protein expression of Notch1, ChK2, and p-P38 MAPK signaling pathways. *$p<0.05$, **$p<0.01$.

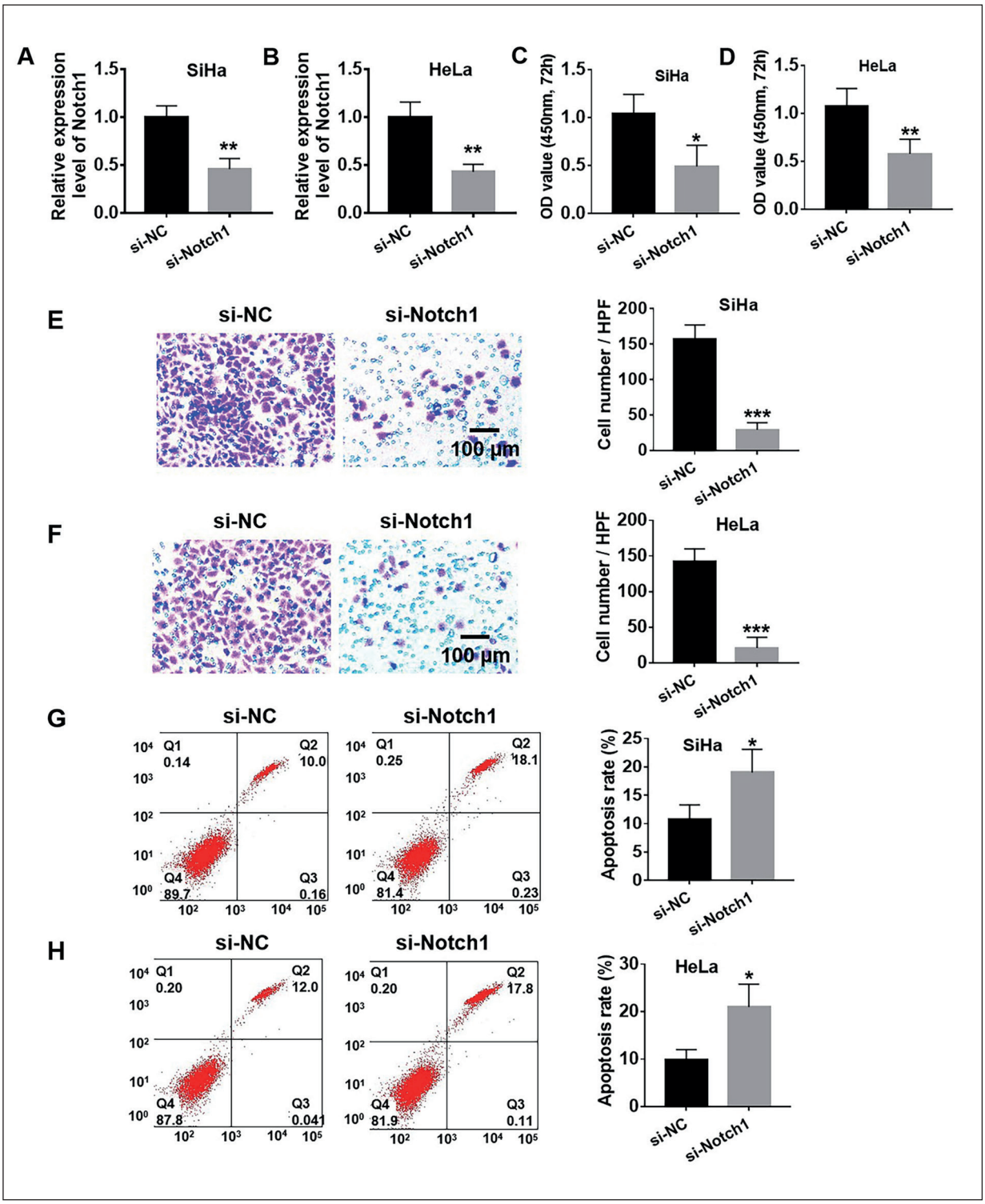

Figure 5. Notch1 knockdown inhibits the malignant progression of cervical cancer cells. **A,** Verification of Notch1 expression after knockdown in the SiHa cell line. **B,** Verification of Notch1 expression after knockdown in the HeLa cell line. **C,** Notch1 knockdown inhibits cervical cancer cell proliferation in the SiHa cell line. **D,** Notch1 knockdown inhibits cervical cancer cell proliferation in the HeLa cell line. **E,** Notch1 knockdown inhibits cervical cancer cell invasion in the SiHa cell line. Scale bar = 100 μm. **F,** Notch1 knockdown inhibits cervical cancer cell invasion in the HeLa cell line. Scale bar = 100 μm. **G,** Notch1 knockdown induces cervical cancer cell apoptosis in the SiHa cell line. H. Notch1 knockdown induces cervical cancer cell apoptosis in the HeLa cell line. *p<0.05, **p<0.01, ***p<0.001.

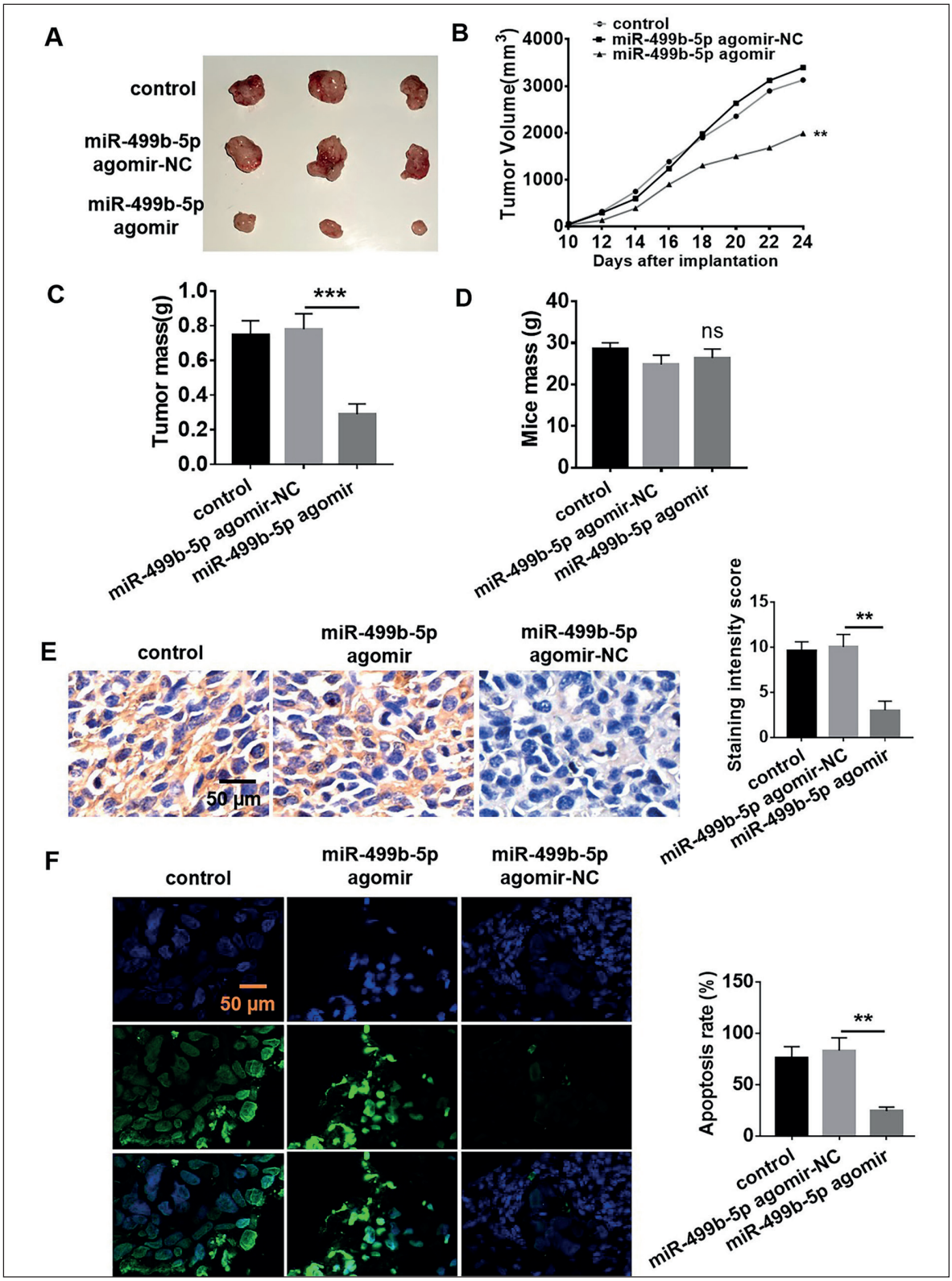

Figure 6. Xenograft tumor model demonstrates that the miR-499b-5p overexpression inhibits cervical cancer. **A,** Gross observation of tumor. **B,** MiR-499b-5p inhibits the proliferation of cervical tumors. **C,** Detection of the weight of cervical tumors. **D,** Detection of mouse weight. **E,** IHC staining intensity of Notch1 in cervical tumors. Scale bar = 50 μm. **F,** MiR-499b-5p induces cervical cell apoptosis in cervical tumors. Scale bar = 50 μm. *p<0.05, **p<0.01, ***p<0.001.

(Figure 6E), thereby intensifying the apoptosis of cervical cancer cells (Figure 6F). Therefore, the xenograft tumor model demonstrated that the miR-499b-5p overexpression inhibited cervical cancer *in vivo*.

Discussion

Different tumor cells have miRNA molecules with different characteristics and their expression profiles, and existing research results show that these cells differ. Thus, the biological functions and mechanisms of one or several miRNA molecules with the most significant changes in tumor development should be studied. Previous studies[24,25] found that miRNA molecules are closely related to the biological functions of the occurrence and development of tumors and are involved in tumor formation.

These include embryonic development, division, differentiation, apoptosis, metastasis, angiogenesis, and immune responses[26]. MiR-499b-5p is a closely related miRNA, which regulates proto-oncogenes and tumor suppressor genes in tumors and downregulates the miR-499b-5p expression in endometrial carcinoma, thyroid papillary carcinoma, pancreatic cancer, and pituitary adenocarcinoma[27]. In our study, the miR-499b-5p expression was significantly downregulated in cervical cancer tissues compared with that in adjacent tissues, which were closely related to tumor proliferation, invasion, and metastasis. The CCK-8 assay, transwell migration assay, and flow cytometry data revealed that miR-499b-5p overexpression or Notch1 knockdown inhibited the proliferation and migration of cervical cancer cells and promoted the apoptosis of cervical cancer cells in vitro. The xenograft tumor model demonstrated that the miR-499b-5p overexpression inhibited tumor weight and volume *in vivo*. The expression and prognosis analysis in cervical cancer tissues showed that the metastatic and recurrence-free survival rate and overall survival rate of those with the downregulated miR-499b-5p expression were significantly lower than those with the upregulated miR-499b-5p expression[28]. The results suggested that miR-499b-5p might act as a tumor suppressor gene in the development of cervical cancer and then inhibit the metastasis and development of cervical cancer. Moreover, the low miR-499b-5p expression is closely related to the metastasis and poor prognosis of cervical cancer. Therefore, the expression level of miR-499b-5p is likely an important reference and evaluation index for the prediction of metastasis and the prognosis of cervical cancer.

However, the Notch1 signaling pathway is a classical and highly conserved signaling network in biological evolution; implicated in regulating cell division, differentiation, proliferation, apoptosis, and tumor occurrence; and involved in all links in the metabolic process of life[29]. Notch1 is a downstream gene of miR-499b-5p. Tumor cells can initiate cervical cancer stem cells, and cervical cancer stem cells can be modified by tumor stem cells. Therefore, miR-499b-5p may be involved in the Notch1 signaling pathway to regulate liver cancer stem cells, thereby enhancing the ability of self-renewal. MiR-499b-5p can inhibit the Notch1 signaling pathway and activate tumor suppressor signaling pathways, namely, CHK2 and p-p38MAPK signaling pathways. This result demonstrated that the molecular regulatory mechanism involved in the invasion and metastasis of cervical cancer cells is a consequence of the joint regulation of multiple protein pathways.

In summary, the expression level of miR-499b-5p in cervical cancer tissues and cells is significantly downregulated and closely related to the prognosis of patients and cervical cancer cell proliferation. MiR-499b-5p could inhibit the proliferation and metastasis of cervical cancer cells by regulating the expression of the Notch1 signaling pathway. MiR-499b-5p and Notch1 genes may be potential targets for the treatment of cervical cancer. However, in the metastasis and recurrence of cervical cancer cells, their precise regulatory mechanism should be further studied.

Conclusions

MiR-499b-5p is downregulated in cervical cancer tissues and cell lines and is related to the poor prognosis of patients with cervical cancer and the proliferation and metastasis of cervical cancer cells. MiR-499b-5p inhibits cervical cancer cell proliferation and induces cervical cancer cell apoptosis by targeting the Notch1 signaling pathway.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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