JMJD2A facilitates growth and inhibits apoptosis of cervical cancer cells by downregulating tumor suppressor miR-491-5p

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Received March 27, 2017; Accepted August 10, 2018

DOI: 10.3892/mmr.2019.9916

Abstract. Cervical cancer remains the second most common malignancy for women worldwide. Jumonji domain containing 2A (JMJD2A), a member of the JmjC domain-containing family of JMJD2 proteins, is capable of regulating cancer-associated genes, including genes involved in the cell cycle, proliferation, apoptosis, invasion and metastasis. However, its role in human cervical cancer has yet to be elucidated. microRNA (miR)-491-5p, a mature form of miR-491, has been shown to function as a tumor suppressor gene *in vitro* by inducing apoptosis and inhibiting proliferation and invasion in various types of cancer. However, the underlying mechanism remains to be elucidated. In the present study it was observed that JMJD2A expression was significantly upregulated in human cervical cancer cell lines and cervical epithelial carcinoma tissues. A high JMJD2A level predicted poor overall and disease-free survival rate and may serve as an independent prognostic factor for adverse outcome. JMJD2A increased cervical cancer cell and colony numbers *in vitro*, increased the tumor weight in a mouse xenograft model, and decreased the apoptotic rate by downregulating the pro-apoptotic proteins Bax, p21 and active caspase-3, and upregulating the anti-apoptotic protein Bcl-2. Transfection experiments indicated that the role of JMJD2A in cervical cancer was mediated, at least in part, by the repression of miR-491-5p. In summary, JMJD2A was identified as an oncogenic protein in human cervical cancer that significantly affected cell and colony numbers, tumor weight and apoptosis via the downregulation of miR-491-5p, which acts as a tumor suppressor in cervical cancer. Therefore, JMJD2A may serve as a prognostic factor and potential target for intervention in cervical cancer.

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Key words: jumonji domain containing 2A, microRNA-491-5p, cervical cancer

Introduction

Cervical cancer is a preventable disease, and reductions in its incidence and mortality have been achieved (1); however, it remains the second common malignancy in women worldwide (2). Research into the molecular mechanisms associated with the pathogenesis of cervical cancer is important for its treatment and prevention (3).

microRNAs (miRNAs) are a class of short, non-coding RNAs ~22 nucleotides long that regulate gene expression by binding to the 3' untranslated regions (3'-UTRs) of target mRNAs in a sequence-specific manner, resulting in translational repression and/or gene silencing (4,5). A number of studies have demonstrated that miRNAs, functioning as either onco-miRNAs or tumor suppressors, perform important roles during cancer progression (6-8). At present, >2,500 miRNAs have been identified in humans (miRBase database version 20.0) (9). However, few studies have investigated the association between miRNA and the tumorigenesis of cervical cancer (10). miRNA (miR)-491-5p, which is a mature form of miR-491, functions as a tumor suppressor gene *in vitro,* as it induces the apoptosis and inhibits the proliferation of ovarian (11), colorectal (12), pancreatic (13) and breast (14) cancer cells. In addition, miR-491-5p inhibits the invasion of glioma (15), breast (16) and oral squamous (17) cancer cells. However, the role of miR-491 in cervical cancer cells remains unknown.

Jumonji domain containing 2A (JMJD2A), a member of the JmjC domain-containing family of JMJD2 proteins (JMJD2A-JMJD2D), recognizes di- and tri-methylated histone H3 lysine 9 (H3K9) and H3K36, and trimethylated H1.4K26 as substrates (18). This leads to the promotion of an open chromatin state and contributes to transcriptional activation and the regulation of cancer-associated genes, including those involved in the cell cycle, cell proliferation, apoptosis, invasion and metastasis (19). JMJD2A is involved in several types of cancer, including ductal carcinoma, lung, breast, ovarian, bladder and colon cancer, renal adenocarcinoma,

and head and neck squamous cell carcinoma (20-24). It has been demonstrated that miR-491-5p exerts inhibitory effects on breast cancer cell growth by directly targeting the 3'UTR of JMJD2B mRNA and blocking the estrogen receptor (ER) α-mediated signaling pathway (25). Additionally, a previous study indicated that JMJD2A may contribute to breast tumor formation by stimulating ERα activity (26). These results suggest that JMJD2A may exhibit its oncogenic function by regulating the expression of miR-491-5p.

Thus, the aim of the present study was to investigate the function of JMJD2A in human cervical cancer and determine whether its role is dependent on miR-491-5p. The expression of JMJD2A in human cervical cancer cell lines was evaluated to determine whether it is an oncogenic protein. Additionally, the association of JMJD2A levels with overall and disease-free survival rates and the potential of JMJD2A as an independent prognostic factor for adverse outcomes were investigated. Furthermore, the effects of JMJD2A on cervical cancer cell growth and apoptosis were evaluated.

Materials and methods

Human specimens. A total of 38 primary cervical epithelial carcinoma tissues were collected from patients (n=38; mean age of 50±0.7 years old). Normal cervical tissues were collected from patients who underwent hysterectomy as a result of benign gynecological diseases (n=20; mean age of 46±0.9 years old). Specimens were obtained from patients admitted to the Banan People's Hospital of Chongqing (Chongqing, China) between November 2014 and July 2016. Informed consent was obtained, and the present study was approved by the Ethics Committee of the Banan People's Hospital of Chongqing. Tissue samples were immediately snap-frozen in liquid nitrogen and stored at -80˚C prior to use. The mean JMJD2A level of the cervical cancer tissues was evaluated using western blotting as later detailed. Tissues exhibiting lower JMJD2A expression compared with the mean were classified as the JMJD2A low group, while tissues exhibiting higher JMJD2A expression compared with the mean were classified as the JMJD2A high group. The mean miR-491-5p level of the cervical cancer tissues was evaluated using reverse transcription-quantitative polymerase chain reaction (RT-qPCR) as later detailed. Tissues in which miR-491-5p levels were lower than the mean level were classified as the miR-491-5p low group, while tissues with higher miR-491-5p levels compared with the mean level were classified as the miR-491-5p high group.

Cell culture and oligonucleotide transfection. The human cervical cancer cell lines HeLa, CaSki, C-4-I, SiHa and C-33 A were obtained from the Cell Bank of Shanghai, Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). Human cervical cancer cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) under an atmosphere of 5% CO_2 at 37˚C. Normal human cervical cells were grown in a 100-mm plastic dish and cultured under the aforementioned conditions. All procedures involving clinical specimens were approved by the Banan People's Hospital of Chongqing.

The locked-nucleic-acid-modified oligonucleotide (LNA) of miR-491-5p (LNA-miR-491-5p) and negative control (LNA-NC) were synthesized and transfected into cervical cancer cells, as previously described (27).

Cell viability assay. The viability of cervical cancer cells was determined using an MTS kit (CellTiter 96 AQ; Promega Corporation, Madison, WI, USA). Briefly, cells ($5x10^3$ cells/well) were seeded in 96-well plates. After 12 h, a fresh mixture of MTS and phenazine methosulfate was added and the cells were incubated for 2-4 h at 37˚C. An MR7000 microplate reader (Dynatech International, LLC, Melville, NY, USA) was used to measure the absorbance at 490 nm.

Colony formation assay. Cells were trypsinized and plated on 6-well plates and cultured for 2 weeks under an atmosphere of 5% CO_2 at 37˚C. Following fixation with 4% paraformaldehyde for 30 min at room temperature, the colonies were stained with 1% crystal violet for 30 sec at room temperature. The number of colonies, defined as >50 cells/colony, were counted from nine random visual fields using an inverted microscope (magnification, x200; IX83; Olympus Corporation, Tokyo, Japan).

Apoptosis analysis. Apoptosis was detected using an Annexin V-FITC/PI double staining kit (Beyotime Institute of Biotechnology, Jiangsu, China) according to the manufacturer's protocol. Cells were harvested and stained with Annexin V-FITC and PI. Cell samples were analyzed using a flow cytometer (FACScan; BD Biosciences, Franklin Lakes, NJ, USA). Data were analysed using FlowJo software version 10.0.5 for Microsoft (Tree Star, Inc., Ashland, OR, USA).

Vector construction and transfection. Adenovirus-based JMJD2A overexpression (Ad-JMJD2A) was performed as described previously (28). Briefly, cells ($2x10^4$) were infected with Ad-JMJD2A ($1x10^{10}$ PFU/ml; 1 μl) and Ad-negative control (Ad-NC; 1 μl) using LipoFiter™ reagent (Hanbio, Shanghai, China). HEK293 cells were used to generate the third-generation adenovirus. For deletion of JMJD2A, short hairpin (sh) RNA (sh-JMJD2A) sequences were adopted (Ambion; Thermo Fisher Scientific, Inc.). In addition, cells ($2x10^5$) were transfected with sh-JMJD2A (6 μl) or sh-negative control (sh-NC; 6 μl) using HiPerFect Transfection Reagent (6 μl; Qiagen GmbH, Hilden, Germany). The cells were harvested 48 h post-transfection and the RNA was then extracted for RT-qPCR analysis to determine the overexpression or knockdown efficiency.

RNA isolation and RT-qPCR. Post-transfection, total RNA (tRNA) was extracted from human cervical cancer cells using TRIzol® (Thermo Fisher Scientific, Inc.), following the manufacturer's protocol. Following spectrophotometric measurement (260 nm) of tRNA concentration using an UV spectrophotometer and subsequent analysis of the quality of the tRNA using 2% agarose gels stained with ethidium bromide at room temperature for 30 min, 3 mg tRNA was subjected to reverse transcription (RT) using M-MLV reverse transcriptase (Invitrogen; Thermo Fisher Scientific, Inc.) at 25˚C for 10 min, 37˚C for 100 min and 90˚C for 5 sec and 4˚C for 5 min.

RT-qPCR was performed using the miScript SYBR-Green PCR kit (Qiagen GmbH) on an ABI 7900 Real-time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The sequences of PCR primers used were as follows: JMJD2A forward, 5'-ATCCCAGTGCTAGGATAATGACC-3' and reverse, 5'-ACTCTTTTGGAGGAACCCTTG-3'; miR-491-5p forward, 5'-ACACTCCAGCTGGGAGTGGGGAACCCT TC-3' and reverse, 5'-TGGTGTCGTGGAGTCG-3'; GAPDH forward, 5'-TGACGCTGGGGCTGGCATTG-3' and reverse, 5'-GCTCTTGCTGGGGCTGGTGG-3'; and U6 forward, 5'-CTCGCTTCGGCAGCACA-3' and reverse, 5'-AACGCT TCACGAATTTGCGT-3'. The thermocycling conditions used were as follows: 95˚C for 15 min; followed by 40 cycles of 94˚C for 15 sec, 55˚C for 30 sec and 70˚C for 30 sec. All assays were performed in triplicate. Quantitative analysis of the relative expression of RNA was calculated using the $2^{-\Delta\Delta Cq}$ method (29). The mRNA expression values of JMJD2A and miR-491-5p were normalized to the internal controls GAPDH and U6, respectively.

Western blot analysis. Following transfection, protein was isolated from human cervical cancer cells and cervical cancer tissues using a radioimmunoprecipitation assay lysis buffer kit (Santa Cruz Biotechnology, Inc., Dallas, TX, USA) at 4˚C for 30 min. Protein concentrations were quantified using a Bio-Rad protein assay (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Proteins (20 μg/lane) were separated by 10-12% SDS-PAGE. The separated proteins were then transferred onto a PVDF membrane (EMD Millipore, Billerica, MA, USA) at 250 mA for 2 h. The membranes were blocked with 5% bovine serum albumin (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) for 1 h at room temperature and then incubated with primary antibody at 4˚C overnight: Anti-JMJD2A (cat. no. ab105953; 1:1,000), anti-Bcl-2 (cat. no. ab32124; 1:1,000), anti-Bax (cat. no. ab32503; 1:1,000), anti-p21 (cat. no. ab109520; 1:1,000), anti-caspase-3 (cat. no. ab4051; 1:500), active-caspase-3 (cat. no. ab13847; 1:500), and anti-GAPDH (cat. no. ab9483; 1:1,000; all purchased from Abcam, Cambridge, MA, USA). Following 5 washes with TBST, the membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (cat. no. A50-106P; 1:1,000; Origene Technologies, Inc, Beijing, China) for 1 h at room temperature. Each band was visualized using an enhanced chemiluminescence kit (EMD Millipore). Quantitiative analysis was performed using Alpha View Analysis Tools (AlphaViewSA software version 3.2.2; ProteinSimple, San Jose, CA, USA).

Mouse xenograft experiment. Mouse xenograft experiments were performed as described previously (30). A total of 40 female athymic nude (nu/nu) mice (3-4 weeks old; weighing 14-16 g) were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. (Shanghai, China). The mice were bred and maintained under specific pathogen-free conditions. Animals were housed under a controlled temperature (25±1˚C) and humidity (50%), with a 12/12 h light/dark cycle and free access to food and water. HeLa, c-4-1 or SiHa cells ($5x10^6$) transfected with Ad-JMJD2A, Sh-JMJD2A or the corresponding controls (Ad-NC and Sh-NC) were inoculated subcutaneously into the left and right flanks of the mice (n=10/group). The mice were sacrificed and the tumors were weighed 3 weeks after inoculation. The weight of the animals at the time of sacrifice was 12-18 g and there was no significant change in the weight of each animal over the experimental period. Tumor volumes were calculated using the following formula: $\pi/6 \times a^2 \times b$, where a is the short axis and b is the long axis. The largest subcutaneous tumor detected in the present study had a diameter of 1.9 cm. No mouse exhibited multiple subcutaneous tumors. Notably, discomfort was considered a humane endpoints and euthanasia by isoflurane inhalation was applied to immediately sacrifice those animals exhibiting such symptoms. The protocol was approved by the Ethics Committee of Banan People's Hospital of Chongqing.

Statistical analysis. Statistical analysis was performed using SPSS 13.0 (SPSS, Inc., Chicago, IL, USA). Data are presented as the mean ± standard error of the mean. One-way analysis of variance followed by Dunnett's post hoc test was used to analyze differences among multiple groups. The association between JMJD2A levels and miR-491-5p was tested using Spearman rank correlation. Survival rate analysis was performed using the Kaplan-Meier method, and differences between the groups were tested using the log-rank test.

Results

JMJD2A is highly expressed in cervical cancer cells and strongly associated with survival rate. To investigate the role of JMJD2A in cervical cancer, the expression of JMJD2A in five cervical cancer cell lines (HeLa, CaSki, C-4-I, SiHa and C-33 A) was examined. The reverse transcription-quantitative polymerase chain reaction (RT-qPCR) results (Fig. 1A) and western blot analysis (Fig. 1B) revealed that JMJD2A mRNA and protein levels were clearly higher in cervical cancer cell lines compared with normal cervical cells, suggesting that JMJD2A is highly expressed in human cervical cancer.

To further investigate the association of JMJD2A with cervical cancer, the 60-month overall survival rates and disease-free survival rates of patients with low (lower than the mean level) and high (higher than the mean level) JMJD2A expression levels were analyzed. The results demonstrated that a low JMJD2A level was associated with high overall and disease-free survival rates, while a high JMJD2A level was associated with poor overall and disease-free survival rates (Fig. 1C and D). These observations indicate that JMJD2A has an association with survival rate and may potentially serve as an independent prognostic factor.

JMJD2A overexpression induces the growth of cervical cancer cells. To explore whether JMJD2A participates in the growth and apoptosis of cervical cancer cells, JMJD2A was knocked down or overexpressed in the human cervical cancer cell lines HeLa, C-4-I, and SiHa. The knockdown and overexpression efficiencies were confirmed by RT-qPCR (Fig. 2A). Next, the cell viability at different time-points after transfection (0, 24, 48 and 72 h) was determined. The results demonstrated that JMJD2A knockdown (sh-JMJD2A) significantly reduced the number of surviving cells, and the inhibition of cell growth appeared to be time-dependent. In addition, JMJD2A knockdown significantly decreased the number of colonies *in vitro*. In the mouse xenograft model, tumor weights for mice implanted

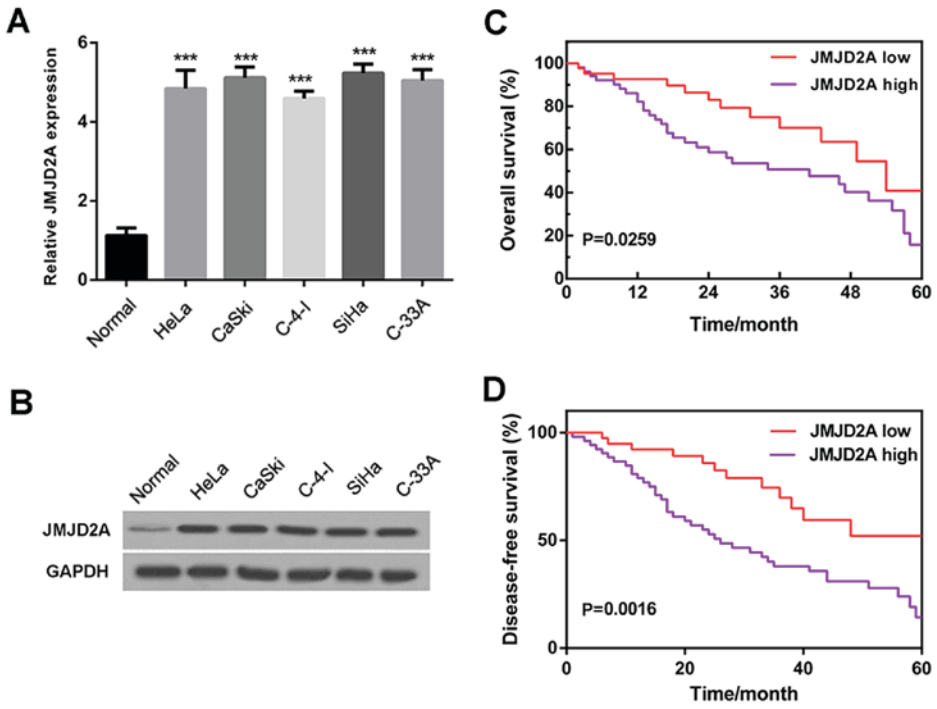

Figure 1. JMJD2A is highly expressed in cervical cancer cells and strongly associated with survival rate. (A) JMJD2A mRNA levels are upregulated in HeLa, CaSki, C-4-I, SiHa and C-33 A human cervical cancer cell lines. Normal and cancerous cervical cells were subjected to reverse transcription-quantitative polymerase chain reaction to detect the mRNA levels of JMJD2A. ***P<0.001 vs. the normal control. (B) JMJD2A is upregulated in HeLa, CaSki, C-4-I, SiHa and C-33 A human cervical cancer cell lines. Representative western blotting results are shown. Kaplan-Meier survival rate analysis demonstrates that (C) overall survival rate and (D) disease-free survival rates were lower in cervical cancer patients with higher JMJD2A expression. The mean JMJD2A level of the cervical cancer tissues was evaluated using western blotting. Cases with a JMJD2A level lower than the mean were enrolled in the JMJD2A low group, while the others were enrolled in the JMJD2A high group. Data are presented as the mean ± standard error of the mean. JMJD2A, jumonji domain containing 2A.

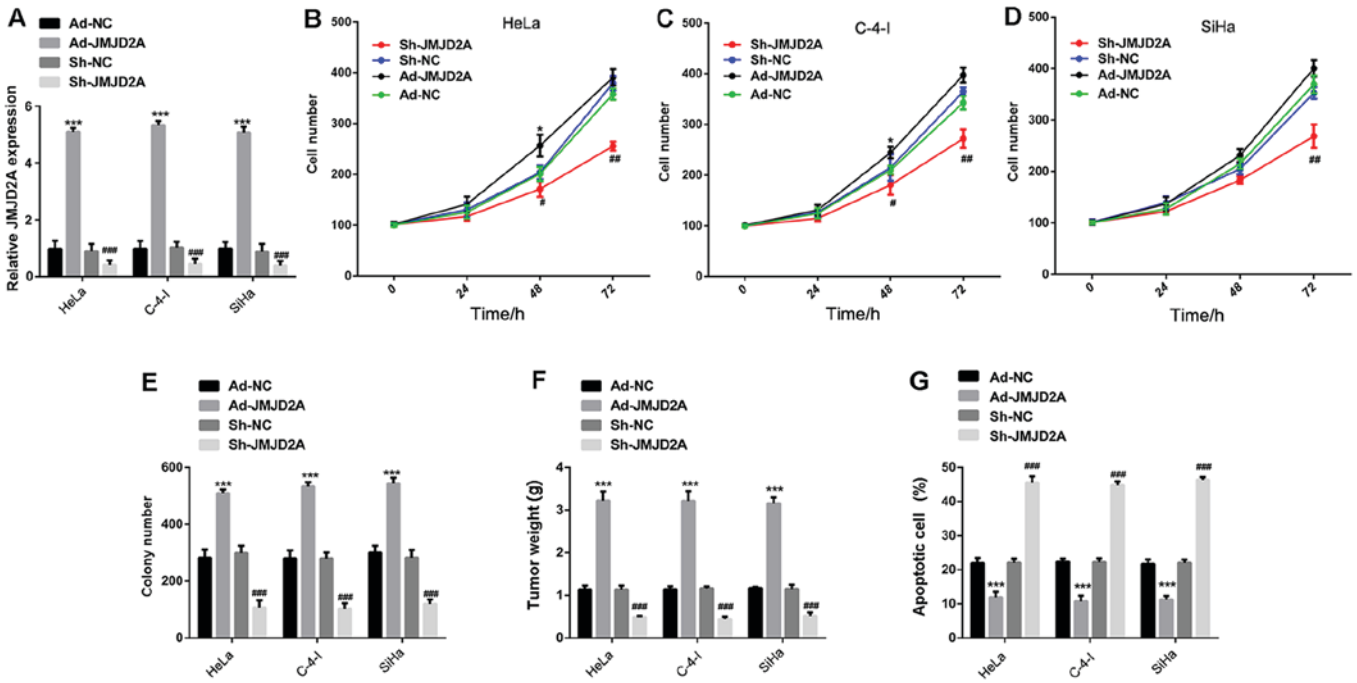

Figure 2. JMJD2A overexpression induces growth and inhibits apoptosis of cervical cancer cells. (A) The knockdown and overexpression efficiencies of Sh-JMJD2A and Ad-JMJD2A were confirmed by reverse transcription-quantitative polymerase chain reaction. JMJD2A overexpression increased the number of (B) HeLa, (C) C-4-I and (D) SiHa cells, and MJD2A knockdown demonstrated the opposite effect. The cell numbers were evaluated at 0, 24, 48 and 72 h post-transfection. (E) JMJD2A overexpression increased the colony number of HeLa, C-4-I and SiHa cells, and JMJD2A knockdown demonstrated the opposite effect. The number of colonies was counted at 2 weeks after transfection. (F) JMJD2A overexpression increased the weights of HeLa, C-4-I and SiHa cell-derived tumors, and JMJD2A overexpression demonstrated the opposite effect. Tumor weight was evaluated 3 weeks after the inoculation of the cervical cancer cells into female athymic nude (nu/nu) mice. (G) JMJD2A overexpression inhibited cellular apoptosis in HeLa, C-4-I, and SiHa cells, and JMJD2A overexpression demonstrated the opposite effect. Data are presented as the mean ± standard error of the mean. *P<0.05 and ***P<0.001 vs. Ad-NC; #P<0.05, ##P<0.01 and ###P<0.001 vs. sh-NC. JMJD2A, jumonji domain containing 2A; sh, short hairpin; Ad, adenovirus; NC, negative control.

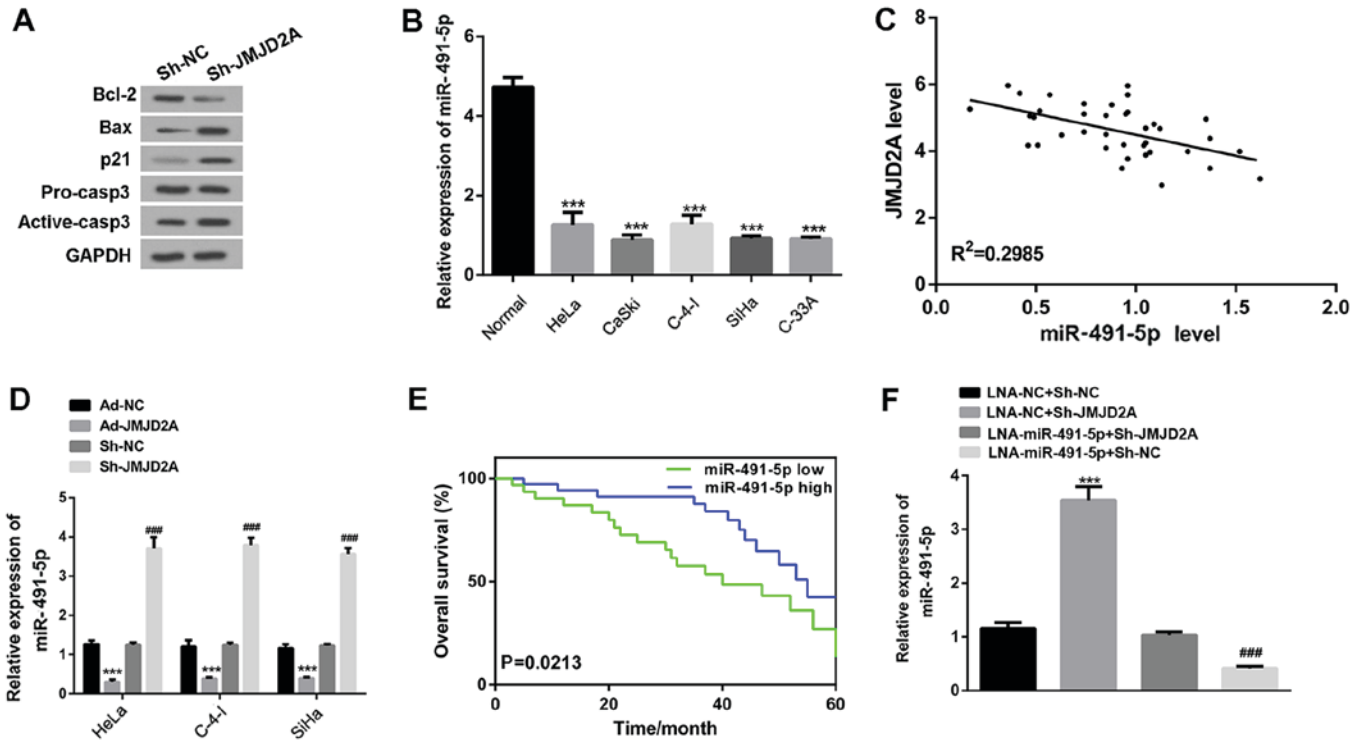

Figure 3. JMJD2A knockdown induces miR-491-5p expression in cervical cancer cells. (A) Western blotting demonstrates that JMJD2A knockdown induced the expression of pro-apoptotic proteins (active caspase-3, Bax and p21) and inhibited the expression of the anti-apoptotic protein Bcl-2 in HeLa cells. (B) miR-491-5p mRNA levels are downregulated in HeLa, CaSki, C-4-I, SiHa and C-33 A human cervical cancer cell lines. ***P<0.001 vs. normal. (C) The miR-491-5p and JMJD2A levels were negatively correlated in the human cervical cancer specimens (n=38). (D) JMJD2A knockdown significantly increased the level of miR-491-5p, and JMJD2A overexpression significantly decreased the level of miR-491-5p in cervical cancer cell lines. ***P<0.001 vs. Ad-NC; ###P<0.001 vs. sh-NC. (E) Kaplan-Meier survival rate analysis demonstrates that the overall survival rate is poor for cervical cancer patients with low miR-491-5p expression. The mean miR-491-5p level of the cervical cancer tissues was evaluated using reverse transcription-quantitative polymerase chain reaction. The cases whose miR-491-5p level was lower than the mean miR-491-5p level were enrolled to the miR-491-5p low group, while the others were enrolled in the miR-491-5p high group. (F) miR-491-5p levels following the transfection of LNA-miR-491-5p and LNA-NC into sh-JMJD2A and sh-NC cells. ***P<0.001 vs. LNA-NC + Sh-NC; ###P<0.001 vs. LNA-miR-491-5p + Sh-JMJD2A. JMJD2A, jumonji domain containing 2A; sh, short hairpin; Ad, adenovirus; NC, negative control; Bax, Bcl-2-like protein 4; Bcl-2, B-cell lymphoma.

with the sh-JMJD2A HeLa, C-4-I and SiHa cells were significantly reduced compared with those in the respective sh-NC group (Fig. 2B-F). Conversely, JMJD2A overexpression (Ad-JMJD2A) increased the proliferation and colony formation of cervical cancer cells *in vitro*, and increased the weight of the xenograft tumors compared with those in the respective control group (Ad-NC). These findings indicate that JMJD2A regulates cervical cancer growth *in vitro*.

JMJD2A overexpression inhibits apoptosis of cervical cancer cells. Flow cytometric analysis (Fig. 2G) revealed that JMJD2A knockdown induced cellular apoptosis in HeLa, C-4-I and SiHa cells, whereas JMJD2A overexpression significantly attenuated cellular apoptosis in these cell lines, resulting in a markedly low apoptotic rate. The results of western blot analysis (Fig. 3A) demonstrated that JMJD2A knockdown upregulated the expression of pro-apoptotic proteins (Bax, p21 and active caspase-3) and downregulated the level of the anti-apoptotic protein Bcl-2. However, it exhibited no clear effect on the expression of the apoptosis-related protein pro caspase-3.

JMJD2A knockdown induces miR-491-5p expression in cervical cancer cells. miR-491-5p has been reported to act as a tumor suppressor and is downregulated in a number of cancer cell lines (31-33). The results of the present study demonstrated that miR-491-5p was downregulated in the human cervical cancer lines HeLa, CaSki, C-4-I, SiHa and C-33 A (Fig. 3B). To explore the correlation between JMJD2A and miR-491-5p levels in the cervical cancer tissues, linear regression analysis was performed. The data demonstrated that the miR-491-5p and JMJD2A levels were significantly and negatively correlated (Fig. 3C). In addition, when JMJD2A was knocked down or overexpressed in cervical cancer cells, significant inductive and suppressive effects on miR-491-5p expression were observed, respectively (Fig. 3D). The miR-491-5p mRNA levels of the cervical cancer tissues were used to divide the patients into miR-491-5p low and miR-491-5p high expression groups. Kaplan-Meier curves were plotted for analysis of the overall survival rates (Fig. 3E) and indicated that a low miR-491-5p level predicted a poor overall survival rate. Together, these results suggest that miR-491-5p expression is downregulated in cervical cancer and a low miR-491-5p expression level is predictive of a poor survival rate.

miR-491-5p knockdown reverses the effects of sh-JMJD2A on cervical cancer growth. In order to examine the potential regulative mechanism, LNA-miR-491-5p and LNA-NC were respectively transfected into sh-JMJD2A and sh-NC cells. The miR-491-5p levels of the transfected were validated using RT-qPCR (Fig. 3F).

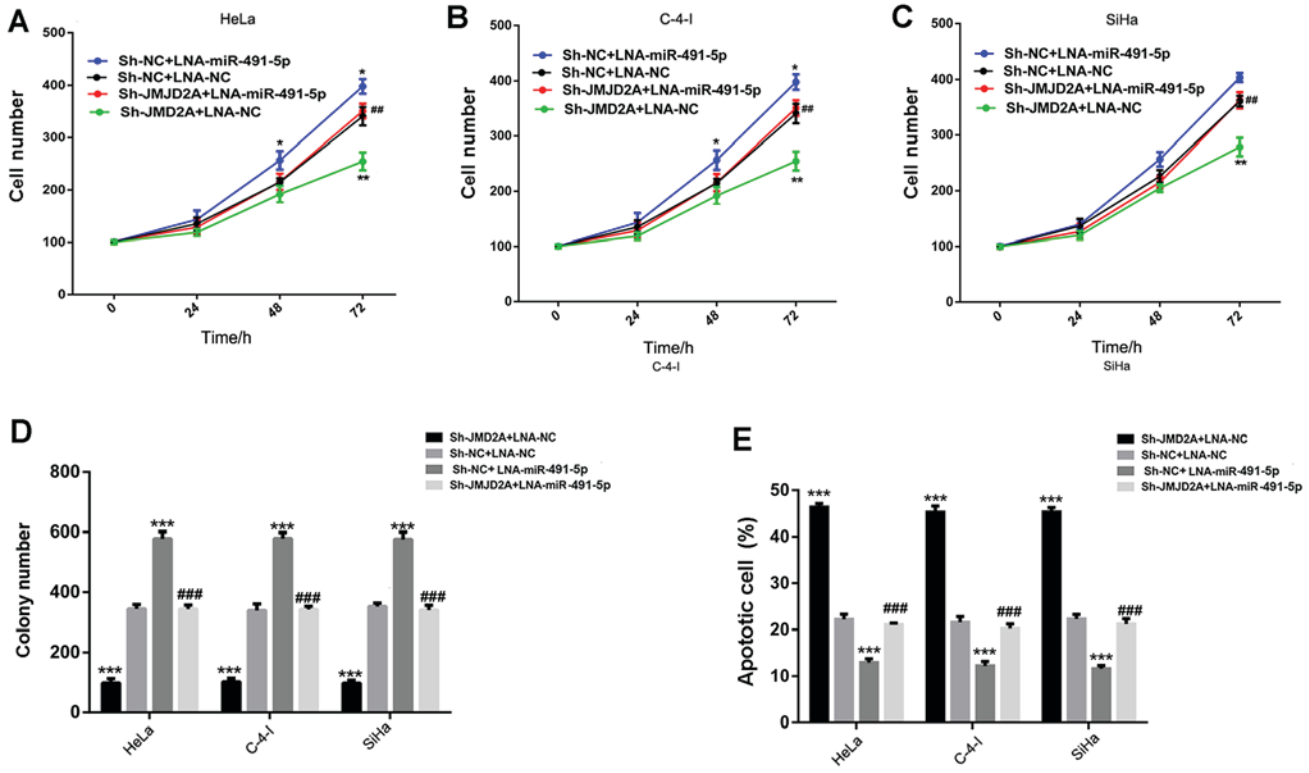

Figure 4. miR-491-5p knockdown reverses the effects of sh-JMJD2A on cervical cancer cells. miR-491-5p knockdown reversed the effects of sh-JMJD2A on cell number in the cervical cancer cell lines (A) HeLa, (B) C-4-I and (C) SiHa. The cell numbers were evaluated at 0, 24, 48 and 72 h post-transfection. (D) miR-491-5p knockdown reversed the effects of sh-JMJD2A on cervical cancer colony number. The number of colonies was analyzed at 2 weeks after transfection. (E) miR-491-5p knockdown reversed the effects of sh-JMJD2A on cervical cancer cell apoptosis. Apoptotic rates were evaluated 2 weeks after transfection. Data are presented as the mean ± standard error of the mean. *P<0.05, **P<0.01 and ***P<0.001 vs. sh-NC + LNA-NC; $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001 vs. sh-JMJD2A + LNA-NC. JMJD2A, jumonji domain containing 2A; sh, short hairpin; Ad, adenovirus; NC, negative control.

The cell number at the 0, 24, 48 and 72 h time-points, the colony number and apoptotic cell rates were detected in the transfected HeLa, C-4-I and SiHa cell lines. The results (Fig. 4) demonstrated that following the knockdown of miR-491-5p (sh-NC + LNA-miR-491-5p), the cell and colony numbers were significantly higher, and the apoptotic rate was significantly lower than those in the control (sh-NC + LNA-NC). In addition, JMJD2A knockdown (sh-JMJD2A + LNA-NC) significantly decreased the cell and colony numbers, and induced cell apoptosis in comparison with the control (sh-NC + LNA-NC). In the cells with knockdown of miR-491-5p and JMJD2A (sh-JMJD2A + LNA-miR-491-5p), the effects of JMJD2A knockdown were significantly attenuated, as evidenced by the increase of cell and colony numbers and the reduction of cell apoptotic rate compared with those in the sh-JMJD2A + LNA-NC group, and the results were comparable to those in the control group (sh-NC + LNA-NC).

Discussion

Although the functions of JMJD2A in various types of cancer have been demonstrated previously, its role in human cervical cancer remains unclear. The present study identified that JMJD2A is overexpressed in human cervical cancer, which is consistent with a previous study reporting that JMJD2A is overexpressed in human breast, lung, head and neck, uterine, endometrial and ovarian cancer, and stomach and renal adenocarcinoma (34). In addition, log-rank analysis in the present study indicated that high JMJD2A expression predicts poor overall and disease-free survival rates. These results suggest that JMJD2A is overexpressed in human cervical cancer and may be an independent prognostic factor.

The potential mechanisms of JMJD2A in various types of cancer have been investigated in previous studies. In one study, the overexpression of JMJD2A was detected in human breast cancer cells, and it was observed that JMJD2A forms a complex with estrogen receptor (ER)α *in vivo*, and the down-regulation of JMJD2A decreased the expression of cyclin D1, a prominent ERα target gene and cell cycle regulator (26). Another study demonstrated that JMJD2A promotes cellular transformation by blocking cellular senescence through transcriptional repression of the tumor suppressor CHD5 (23). Furthermore, JMJD2A has been reported to be involved in human carcinogenesis through regulation of the G1/S transition in human bladder and lung cancers (22). Another study suggested that the JMJD2A level correlates with the level of the pro-apoptotic microRNA miR-34a in gastric cancer tissues and JMJD2A represses the expression of miR-34a by decreasing its promoter activity (35).

The potential mechanism underlying the roles of JMJD2A in human cervical cancer was investigated in the present study. JMJD2A was found to regulate cervical cancer cell growth and apoptosis; more specifically, JMJD2A deficiency induced cervical cancer cell apoptosis by upregulating pro-apoptotic proteins (Bax, p21 and active caspase-3) and downregulating the anti-apoptotic protein Bcl-2. In addition, miR-491-5p,

which is reported to act as tumor suppressor in a number of different cancers, was demonstrated to be negatively correlated with the expression of JMJD2A in cervical cancer. The present study also revealed that JMJD2A knockdown significantly upregulated the expression of miR-491-5p. The significant inhibition of cell growth and increased apoptosis of cervical cancer cells induced by sh-JMJD2A were markedly reversed by the suppression of miR-491-5p. Together, these results indicate that JMJD2A participates in the proliferation and apoptosis of human cervical cancer cells and its role is partly mediated via the regulation of miR-491-5p. However, the mechanism underlying the effects on the regulation of miR-491-5p expression requires further exploration.

The mechanism by which miR-491-5p acts in tumor suppression was not fully investigated in the present study. Previous studies found that miR-491-5p suppressed the growth of oral squamous cell carcinoma, pancreatic cancer and glioblastoma by targeting GIT1, EGFR and CDK6 genes (14,17,36). Another study indicated that miR-491-5p targeting TP53 and Bcl-X_L induced cell apoptosis in SW1990 pancreatic cancer cells through a mitochondria-mediated pathway (13). However, the genes that miR-491-5p targets in human cervical cancer have not yet been identified.

In summary, JMJD2A was identified as an oncogenic protein in human cervical cancer. Its effects on cell and colony numbers, tumor weight and apoptosis may be mediated by the downregulation of miR-491-5p, which serves a role as an anti-onco-miRNA in cervical cancer. Therefore, JMJD2A could serve as an independent prognostic factor and potential target for intervention in cervical cancer.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YL performed the experiments; YW designed the study and reviewed the manuscript; ZX analysed and interpreted the data; HH acquired the data, and drafted and edited the manuscript.

Ethics approval and consent to participate

Patient specimens were obtained with informed consent and the present study was approved by the Ethics Committee of the Banan People's Hospital of Chongqing.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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