

CHAF1A–PCNA interaction promotes cervical cancer progression via the PI3K/AKT/FoxO1 signaling pathway

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Primary research

Keywords: Cervical cancer, CHAF1A, PCNA, Interaction

Posted Date: April 12th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-390228/v1>

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Abstract

Background

An increasing number of studies demonstrate that histone chaperones play critical roles in tumorigenesis and development. In previous research, we confirmed that CHAF1A was highly expressed in cervical cancer (CC) and was correlated with poor prognosis. However, the biological function and specific mechanism of CHAF1A in the development of CC have not been reported.

Methods

CHAF1A knockdown in SiHa and HeLa cells by lentivirus vector is verified by RT-PCR and Western blot analysis. CCK-8, flow cytometry assays, colony formation, cell migration assay and real-time cell analysis assay were performed to determine the cellular function of CHAF1A in CC. Tumor xenograft assay was conducted on nude mice to assess the effect of CHAF1A in vivo. Cell immunofluorescence and co-immunoprecipitation were applied to examine the interaction between CHAF1A and PCNA.

Results

We found that CHAF1A knockdown in SiHa and HeLa cell lines inhibited proliferation and promoted its apoptosis. Further research indicated that CHAF1A promoted proliferation and inhibited apoptosis in CC by activating the PI3K/Akt/FoxO1 signaling pathway. Moreover, CHAF1A directly interacts with PCNA and co-promotes CC progression.

Conclusions

The discovery of the mechanism of action of CHAF1A in CC may provide a new direction for the treatment of CC.

Introduction

Cervical cancer (CC) is one of the most common malignant tumors worldwide. It is the second most common malignant tumor in females⁽¹⁾. However, despite significant advancements in CC therapies, such as surgical resection, chemotherapy, and radiotherapy, the prognoses of patients with CC remain poor. CC continues to be a major cause of death from cancer worldwide⁽¹⁾. Persistent human papillomavirus infection is the main etiological factor for CC development. CC

is a multistep process involving the deregulation of multiple genes. The mechanisms of carcinogenesis are quite complex, and both genetic and epigenetic alterations can lead to carcinogenesis. In the last decade of cancer research, abnormalities in epigenetics have been consistently observed. Aberrations of

epigenetics in cancer mainly involve aberrant histone modifications and altered DNA methylation (2, 3) . Accumulating evidence suggests that cancer initiation, progression and the involvement of histone variants and their partners are closely linked (4, 5) . CAF-1 is a trimeric complex protein that includes P150, P60, and p48. CAF-1 is important for chromatin assembly during DNA synthesis, and interfering with CAF expression may affect DNA synthesis, leading to DNA damage or even programmed cell death (6-11). As the largest subunit of CAF-1 and a major component exercising biological functions, CHAF1A plays a very important role in DNA mismatch repair, epigenetic regulation of embryonic stem cells, and diverse biological behaviors such as cell division and, proliferation, among others (7, 11-13) .

An increasing number of studies have confirmed that the dysregulated expression of CHAF1A

is associated with the development and progression of several tumors, such as: glioblastoma,

hepatocellular carcinoma, colorectal cancer, and gastric cancer (14-22). Our previous study verified its high expression in CC by immunohistochemistry and association with the poor prognosis of CC (23). However, the specific biological functions and mechanisms of action of CHAF1A in cervical cancer have not been reported. In the present study, we clarified that CHAF1A could promote cell proliferation in CC, thereby inhibiting apoptosis and enhancing migration. Moreover, CHAF1A and proliferating cell nuclear antigen (PCNA) interacted to promote CC

progression. PCNA can regulate CHAF1A expression.

Materials And Methods

2.1 Cell culture

Caksi, HaCat and C33A cells were purchased from the Cell Bank of the Chinese Academy of Sciences in 2018. The CC cell lines HeLa and SiHa cells were purchased from Shanghai Jikai Gene (Shanghai, CHN). HeLa cells were maintained in DMEM, supplemented with 10% fetal bovine serum (FBS); HaCat cells were cultured in the RPMI-1640 medium; and SiHa, C33A, and Caksi were cultured in MEM with 10% FBS. All cells were cultured with 1% penicillin–streptomycin antibiotics (Beyotime, China).

2.2 Lentivirus-mediated assay

Lentivirus sh-CHAF1A was purchased from Shanghai Genechem Co. Ltd. (Shanghai, China). PCNA lentiviruses and vectors were supplied by Genecopoeia (GeneCopoeia, Rockville, MD, USA). The following were the shRNA target sequences: 5'- ACCCGGAATGCAGATATTT -3' for CHAF1A. The negative control was 5'- TTCTCCGAACGTGTCACGT -3'. The PCNA control lentiviral(sh-ctrl:5'- CGCTAGTATTTGAAGCACCTCAAGAGGGTGCTTCAAATACTAGCGCC3'); Sh-PCNA5'GCTTCGCGCCGTAGTCTTATCAAGAGTAAGACTACGGCGCGAAGC3'.

The multiplicity of infection was determined before lentiviral transfection. The cells were passaged and seeded at 2×10^4 cells / well in 6 well plates. After the cells were plated for 24 h, the original medium was

discarded and the fresh medium containing lentiviral particles and polybrene was added (the concentration of polybrene used was in accordance with the amount recommended by the manufacturer). The medium was then replaced with completely fresh medium after 24 h. After the cells were allowed to recover and grow further for 24 h, they were switched to 2.5 µg/mL of puromycin for week-long culture and selection of cells successfully transfected with the lentivirus. The culture was further maintained for a week with puromycin, and the culture medium was replaced with the complete medium.

The cells that were successfully transfected with lentiviral particles were subcultured for subsequent experiments.

2.3 RT-PCR

RT-PCR was performed using a RT-PCR kit (Takara, Shiga, Japan) in accordance with the instructions provided by the manufacturer. Total RNAs from cells were extracted using TRIzol Reagent, and then reversely transcribed into cDNA by using the Prime Script RT Reagent Kit (TaKaRa). RT-qPCR was performed with the S1000 Real-Time System (Bio-Rad, Hercules, California, USA). SYBR Green (SYBR Premix Ex Taq™ II; TaKaRa) was used for fluorescent quantification. The following cycling conditions were used for RT-PCR: pre-denaturation (95 °C, for 30 s), denaturation (95 °C, for 5 s), and 40 cycles; annealing (60 °C for 30 s). The relative mRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method. All RT-PCR results were repeated at least 3 times. The primers used are listed in Table 1.

2.4 Flow cytometry

The effects of CHAF1A knockdown on cell cycle and apoptosis were assessed by flow cytometry. When the total number of cells reached 1.0×10^6 in the culture flask, the cells were trypsinized. Cells were collected after centrifugation at 1000g for 5 min, washed three times with PBS, and resuspended in 100 µL of PBS solution. Subsequently 500 µL pre-cooled 75% alcohol in a lysis buffer containing 1 mg / mL RNase, 0.05% Triton X-100, 1 mg / mL RNase, and 50 mg / mL propidium iodide (Sigma, Aldrich, St. Louis, MO, USA) was fixed in their solution, incubated in the dark at room temperature for 30 min, and immediately analyzed using

a FACS Calibur instrument (Becton Dickinson, CA, USA). Apoptotic samples were resuspended in 500 µL of PBS without ethanol. The cell instrument was analyzed and the results were recorded.

2.5 Colony Formation Assay

Cells (500 cells/well) were seeded in a 6-well plate and then incubated for the next 14 d until cell colonies appeared. The cells were subsequently fixed with 4% paraformaldehyde for 15 min and then stained with Giemsa for 20 min. The rate of cell clone formation was ultimately calculated.

2.6 CCK8 Assay

Cell Counting Kit-8 (CCK8) (Boiss, Beijing, China) was used to determine cell viability. The cells were counted after digestion and the concentration was adjusted to 2000 cells / 100 UL, Two sets of control and experimental samples with five replicate wells per set were then

set up. The plates were seeded for 24, 48, 72, 96, and 120 h and left to stand for 1 h, in accordance with the instructions provided by the manufacturer, The 450 nm was then determined.

2.7 Real-time cell analysis (Proliferation)

Baseline values were first verified by adding 100 UL of the complete medium per well onto a dedicated 16 well electronic plate. The HeLa and SiHa cells were digested, centrifuged, resuspended, and seeded onto a baseline 16 well electronic plate containing 5000 cells / well with gold microelectrode arrays attached. The cell index was monitored using the xCELLigence system (Roche, Mannheim, Germany) for 96 h with measurements taken every 15 min. The results were analyzed using the RTCA software (version 2.0).

2.8 Cell Migration Assay

Cell migration was quantified using the in vitro scratch wound assay. HeLa and Siha cells (1×10^6 /well) were seeded in a 6-well plate. After adhesion, a linear wound was made using a 10 μ L micropipette tip. The scratched wells were washed with PBS several times to remove the detached debris caused by scratching. Images of the wounds (0, 24 h) were taken with a light microscope (20 \times magnification). After 24 h images were again recorded. The number of migrated cells was then determined.

2.9 Real-time cell analysis (Migration)

The baseline values were determined. The medium was placed in the lower chamber of a 16-well electronic plate, and a cell culture medium containing 10% FBS was added. The upper chamber was mounted, and 30 μ L of the serum-free medium in the upper chamber electronic well plate for 1 h at 37 °C and 5% CO₂ incubator. 100 UL of serum-free medium (100 UL containing 50000 cells) was seeded into

each well of the upper chamber of the cell migration plate. The cell migration index was recorded every 15 min for approximately 90 h.

2.10 Western blot analysis

Proteins were extracted from cervical cancer cells and prepared using RIPA lysis and an extraction buffer was supplemented with a protease and phosphatase inhibitor cocktail for 30 min on ice. Cell lysates were ultrasonically disrupted three times for 10 s each, followed by centrifugation at 12000 g for 15 min at 4 °C. Supernatants were collected, denatured by boiling after the addition of the Laemmli sample buffer without a reducing agent, and then assayed. Protein samples after quantification were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA). The membranes were then blocked and then incubated with a specific primary antibody diluent in a room at 4 °C overnight. Details of the primary antibodies are listed in Table 1. The membranes were then washed with TBST 3 times and then incubated with a secondary antibody (1:7500 dilution) for 1 h. The blots were washed 3 times in TBST. The immunoblots were detected with an ECL kit and visualized using an imaging system (Fusion, Germany).

2.11 Cell immunofluorescence

The CC cell lines SiHa and HeLa were seeded in 24-well plates. The well surfaces were covered with 13 mm glass coverslips. Immunofluorescence assay was performed when the cell confluence reached 90%. The cell culture medium was discarded. The cells were washed three times with PBS, fixed in 4% paraformaldehyde for 10 min at room temperature, washed again with PBS three times, permeabilized with 0.3% Triton X-100 for 10 min, and blocked with 5% goat serum for 30min. The samples were incubated with the primary antibody (mouse polyclonal PCNA antibody, 1:2400; rabbit polyclonal CHAF1A antibody, 1:200) overnight in a room at 4 °C. The samples were washed 3 times with PBS solution and then incubated in the dark at room temperature for Alexa Fluor 488 conjugated Goat anti rabbit (Abcam, ab150115) and Alexa Fluor 647 conjugated goat anti mouse antibodies (Abcam, 150077) (both 1:1000). DAPI containing fluorescence quenched coverslips were added and then observed by confocal laser scanning microscopy.

2.12 Co-immunoprecipitation

Protein immunoblotting and co-immunoprecipitation were performed to investigate the interactions of the proteins. Protein interaction was verified using the Pierce Co Immunoprecipitation Kit (Thermo Scientific, 26149). Specific experimental procedures were conducted, strictly following the instructions provided in the kit. Cells were lysed using a kit-specific cell lysate for 15 min, sonicated for 10 s three times, and centrifuged at $12000 \times g$ for 10 min. Upper protein lysate was aspirated, and the protein was incubated with 10 μg corresponding antibody overnight at 4°C . About 50 μg of a mock antibody (Rabbit Anti mouse IgG) was used as a control. The next day the resin bound to the protein was washed 4 times following the instructions provided in the kit. Specifically, 50 μL of the eluate was used. Eluted proteins were immediately boiled and denatured for Western blot analysis.

2.11 Tumor xenografts

Ten BALB/c female nude mice ((aged 5 weeks, with an average weight of 20 g).) were provided by China Chongqing Enville Biotechnology Co. Ltd. The mice were divided into two groups consisting of five mice each. All mice were given water and chow *ad libitum*. Control cells and cells with stable CHAF1A knockdown (shCHAF1A) were suspended in cold PBS. The right forelimb of each nude mouse was subcutaneously injected with 1.0×10^6 cells.

On Day 5 of modeling, the width (W), weight and length (L), of the tumor were measured for 3 d (volume $[V] = W^2 \times L \times 0.52$). The nude mice were sacrificed using 2% isoflurane anesthesia. High concentration of CO₂ asphyxia led to death by asphyxiation 21 d after injection, and the transplanted tumors were removed. Part of the tumor was sent for immunohistochemistry. The research protocol for this animal study conformed to the ethical guidelines in laboratory animal research and the Declaration of Helsinki. A approval was obtained from the ethics committee of the First Affiliated Hospital of Chongqing Medical University (accession number 2021-11).

2.12 Statistical analysis

All statistical analyses were performed using GraphPad Prism 7.0. The data are expressed as mean \pm standard deviation (SD). Between-group comparisons were conducted using a 2-tailed paired Student t-test to test for statistical significance. Statistical differences between groups were determined using one-way ANOVA. $P \leq 0.05$ was considered statistically significant in all cases. All experiments were performed in triplicate.

Results

3.1 Knockdown of CHAF1A inhibits cervical cancer cell proliferation

The expression of CHAF1A in cervical cancer cell lines was significantly higher than that in HaCat cells ($P < 0.05$) (Fig. 1A). To evaluate the functional proliferation of CHAF1A overexpression in CC, we performed CHAF1A knockdown in CC cells and evaluated the cellular outcomes. CHAF1A cells were depleted in SiHa

and HeLa cells by RNA interference, and CHAF1A knockdown efficiency after lentiviral transfection was verified by qRT PCR and Western blot analysis. Knockdown efficacy in both SiHa and HeLa cells is shown in Figs. 1.B and 1.C. The stable transfected cells were selected for the subsequent experiment. Cell proliferation was observed using the CCK8 assay. The reduced CHAF1A expression in the HeLa and SiHa cells was found to inhibit cell proliferation. Moreover, the colony formation assay and RTCA presented a trend similar to that of the CCK8 assay after CHAF1A knockdown ($P < 0.05$, Figs. 1D–1F).

3.2 Induction of cell cycle arrest and promotion of apoptosis in cervical cancer cells upon inhibition of CHAF1A expression

We further validate the biological function of CHAF1A in CC cells by conducting additional

in vitro experiments. The cell cycle was analyzed by flow cytometry. CHAF1A knockdown resulted in a significant increase in the percentage of cells in the peak G1 / G0 phase and a decrease in the percentage of cells in peak S phases. CHAF1A inhibition was found to induce G1 arrest in CC cells (Fig. 1G). The number of apoptotic cells in the cells of the CHAF1A knockdown group was significantly higher than that in the cells of the control group (Figs.2A,2B). On the basis of these findings, we speculated that CHAF1A could regulate the abnormal proliferation of CC by interfering with the cell cycle to inhibit apoptosis.

3.3 CHAF1A promotes the migration of cervical cancer

To examine the migratory properties of SiHa and HeLa, we conducted a scratch wound assay. Using a scratch assay, we found that CHAF1A silencing resulted in decreased cell migration (Figs. 2C, 2D). Meanwhile, RTCA assays were used to examine SiHa and HeLa cell migration after transfection with the sh-CHAF1A. The results indicated that the migration of SiHa and HeLa cells was inhibited by CHAF1A knockdown (Fig. 2E).

3.4 CHAF1A promotes cervical cancer progression via the PI3K / Akt signaling pathway

Activation of the PI3K / Akt signaling pathway mainly promotes cell growth and survival. We detected the proteins related to PI3K / Akt / FoxO1 by Western blot assay. The results showed that the phosphorylated PI3K (tyr458) and Akt (ser473) levels were decreased after CHAF1A knockdown, whereas PI3K and Akt levels remained unchanged in the total protein relative to the control group (Fig. 3E). This finding suggested that the high expression of CHAF1A could induce CC cell proliferation and invasion via the PI3K / Akt pathway.

3.5 CHAF1A interaction with PCNA promotes tumor progression

Localization was determined by confocal immunofluorescence microscopy, which revealed the colocalization of CHAF1A and PCNA proteins in the nucleus (Fig. 3A). These proteins were localized in the nucleus (Fig. 3A), suggesting that they could interact with each other in the nucleus. This interaction was confirmed by co-immunoprecipitation. We first co-precipitated the PCNA protein with the CHAF1A antibody (Fig. 3B), and the CHAF1A protein was precipitated with PCNA (Fig. 3B). We also found

that CHAF1A expression was decreased after PCNA knockdown, which was verified by both mRNA and protein levels (Figs. 3C, 3D).

3.6 Effects of CHAF1A on tumorigenicity *in vivo*

To further validate the biological role of CHAF1A *in vivo*, HeLa cells with control and lentiviral knockdown were injected into the right flank of nude mice (Fig.4A). The diameter of the tumor was measured every 3 d to observe tumorigenesis and the average tumor volume was calculated. The mice were sacrificed 20 d after subcutaneous injection, Subsequently, the size and weight of the tumors were determined. The tumor volumes (Fig. 4B) and tumor weights (Fig. 4C) of the nude mice were significantly higher than those of the mice in the control group. Moreover, the tumorigenicity assay of the nude mice and immunohistochemistry showed that CHAF1A knockdown inhibited cell proliferation in the tumor. Ki67 and PCNA immunostaining indicated high proliferative indexes within these control groups (Figs. 4D, 4E).

Discussion

Increasing evidence suggests that histone chaperones play an important role in tumor development. The anti-silencing function 1A(ASF1A), Aprataxin and PNK-like factor, and the FAcilitates Chromatin Transcription complex were highly expressed in tumor tissues and poor prognosis was predicted(24-26). The histone chaperone CAF-1 is similarly involved in multiple biological processes and plays an irreplaceable role (6-11). CAF-1 has been shown to cause severe loss

of cell viability, as well as delay S-phase progression and impaired DNA synthesis in human

cells if they are unable to perform poor prognosis of their normal function (12, 27, 28).CHAF1A, as the core component of CAF-1, participated in the progression of various tumor diseases, tumor proliferation, differentiation, and invasiveness. High expression of CHAF1A in a variety of human tumors is strongly associated with poor tumor outcome (14-22). In our previous research, we confirmed by immunohistochemical experiments that CHAF1A and PCNA were highly expressed in CC tissues; moreover, they were positively correlated, and their high expression was associated with poor prognosis (23) . We demonstrated through numerous experiments the biological function of CHAF1A in CC. Finally, we found that overexpressed CHAF1A is involved in tumor cell proliferation and migration, and inhibits apoptosis. We used CRISPR / Cas9 technology for CHAF1A knockdown and then inhibited the activation of the PI3K / Akt signaling pathway. Thus, we confirmed that CHAF1A overexpression promotes tumorigenesis and the progression of cervical cancer. This finding was further confirmed by our tumorigenesis experiments in nude mice. Upon lentiviral knockdown of CHAF1A, the growth of tumor cells was significantly slower than the negative control. The transplanted tumor tissue was removed from the nude mice and the immunohistochemical expression levels of the proliferation markers Ki67 and PCNA were lower in the experimental group than in the control group. To further explore the specific mechanism of CHAF1A in CC, we first confirmed that in the SiHa and HeLa cells, both CHAF1A and PCNA

were localized in the nucleus, as determined by cell immunofluorescence experiments. This result suggests, their direct interaction. We precipitated the protein extracted from HeLa cells with CHAF1A and PCNA antibodies and then detected the protein precipitated by the immunoprecipitation (IP) antibody with another antibody. The results showed that both antibodies could precipitate the other protein, confirming the interaction between CHAF1A and PCNA in HeLa cells. This conclusion is consistent with previous studies (29-32). After the PCNA gene expression was knocked out by CRISPR/Cas9 technology, CHAF1A expression was significantly reduced. This decrease suggests that PCNA acts directly on CHAF1A and regulates CHAF1A expression. Previous studies have reported that PCNA-driven DNA synthesis can also attract CAF-1 to the S site of DNA repair, such as nucleotide excision repair (33, 34). Meanwhile, specific PCNA mutation weakens the role of CAF-1 in telomeric silencing in *Saccharomyces cerevisiae*, and PCNA mutations reduce its interaction with CAF-1(32, 35) . Whether they act directly and interact with the target of the other has yet to be determined.

Overall, the present study confirmed that the oncogenic role of CHAF1A in CC. CHAF1A overexpression could promote CC cell proliferation and inhibit cell apoptosis, which predicted a

poor outcome in CC patients. The mechanism of CHAF1A in CC was thoroughly discussed,

which could provide a new direction for precisely targeted therapy for cervical cancer. Meanwhile, the interaction of CHAF1A with PCNA promotes DNA replication and cell proliferation(9). Studies have reported that CHAF1A can be used as a marker for cell quiescence and proliferation(36-38). The combined results of this study and previous studies, indicate that CHAF1A may serve as a biomarker for predicting postoperative recurrence and malignancy in cervical cancer.

Declarations

Ethics approval and consent to participate

Our study was approved by the Ethics Review Board of the First Affiliated Hospital of Chongqing University (number 2021-11).

Consent for publication

All authors agreed to publish the manuscript.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by grants from the Science and Technology Project of Chongqing City (Grant No: estc2016jcyAX0022).

Authors' contributions

YJ Corresponding author and LW conceived and designed the experiments and wrote the paper. LY, YL, XL and ZD performed the experiments and statistical analysis. Funding acquisition, YL and YJ.

All authors read and approved the final manuscript.

Acknowledgements Not applicable.

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Figures

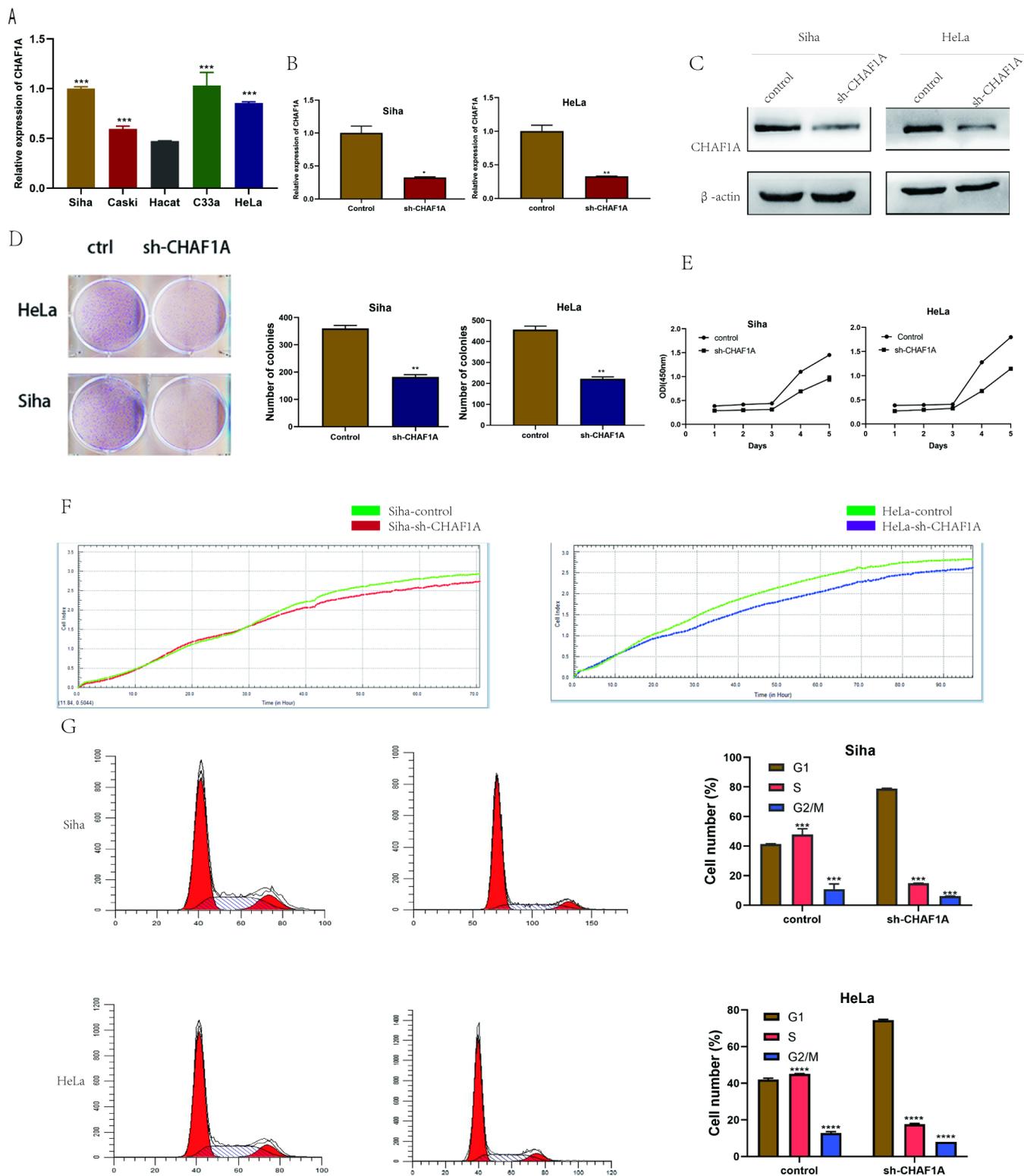


Figure 1

CHAF1A knockdown suppressed cervical cancer cell proliferation. (A) The expression of CHAF1A mRNA in cervical cancer cell was significantly higher than that in human normal immortalized epithelial cells ($P < 0.0001$). (B) Real time PCR analysis of CHAF1A mRNA in SiHa and HeLa cells infected with EGFP shRNA and CHAF1A shRNA revealed significant levels of CHAF1A downregulation ($P < 0.05$). (C) Western blot analysis of CHAF1A protein expression in SiHa and HeLa cells after lentiviral infection. (D) After

CHAF1A knockdown, cell proliferation was significantly suppressed. The number of cell clones formed was also significantly reduced in the CHAF1A shRNA group relative to that in the experimental control group ($P < 0.01$). (E) HeLa and SiHa cell proliferation was measured by CCK8 assay. (F) RTCA showed that the proliferation index of the control group was significantly higher than that of the knockdown group. (F) Using flow cytometry to analyze the effects of high CHAF1A expression on the cell cycle, we found that CHAF1A knockdown significantly induced G1 arrest in SiHa and HeLa cells. **** represents $P < 0.0001$, *** represents $P < 0.001$, and ** represents $P < 0.01$.

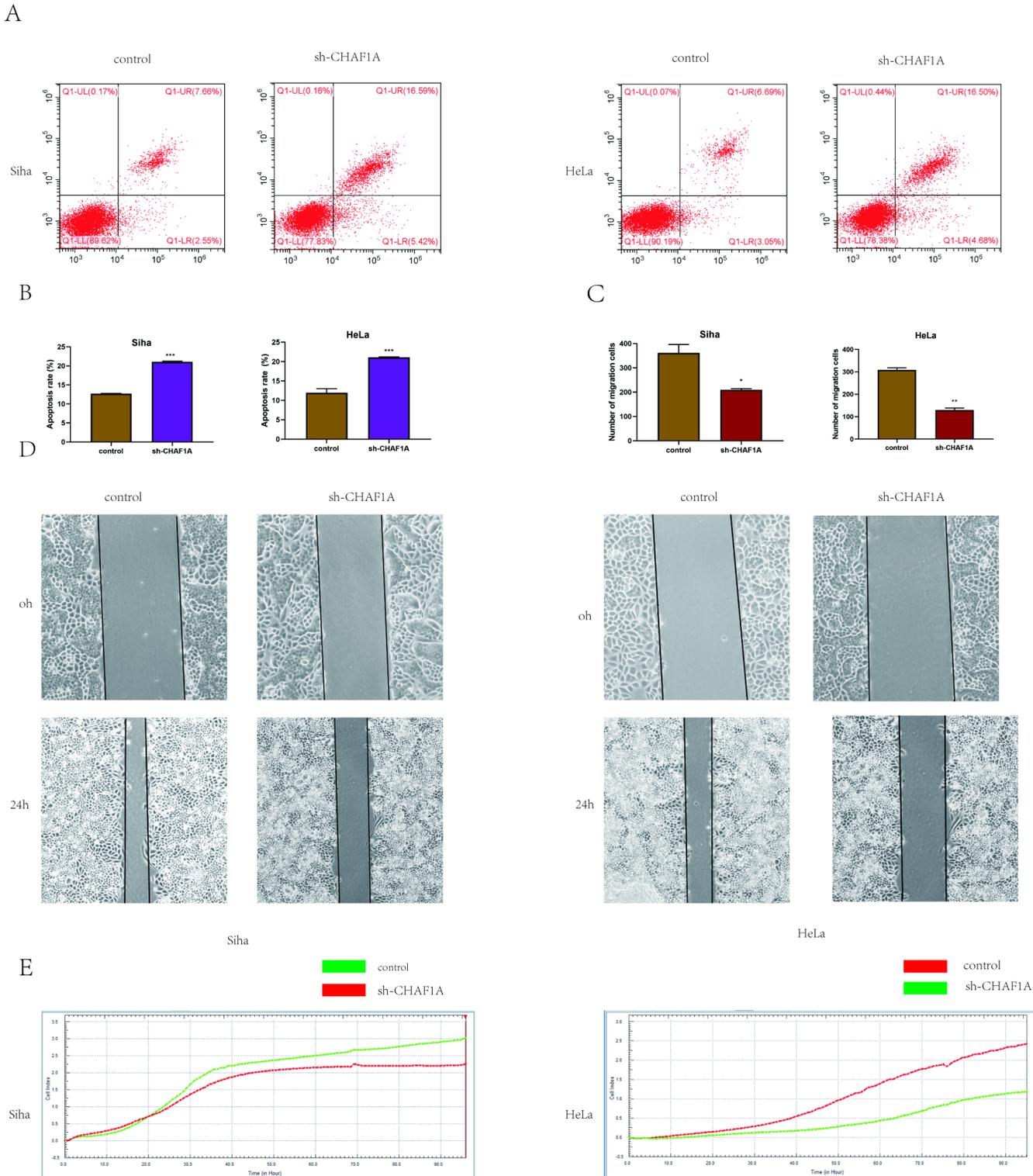


Figure 2

Knockdown of CHAF1A affects the apoptosis and invasive ability of cervical cancer cells. (A) The effect on apoptosis after CHAF1A knockdown was examined by Annexin V / PI staining and flow cytometry. This histogram shows the statistical apoptosis rate in (B). The calculation was as follows: Apoptosis rate = $Q1-LR + Q1-UR$ ($P < 0.05$). (C) An invasive wound healing assay was performed to evaluate the influence of CHAF1A on cell migration ability. This technique showed that CHAF1A silencing significantly delayed the mobility of SiHa and HeLa cells (magnification, $\times 40$). The histogram indicated a statistically significant difference ($P < 0.05$). (D) Cell migration in real time was further analyzed using the xCELLigence RTCA system.

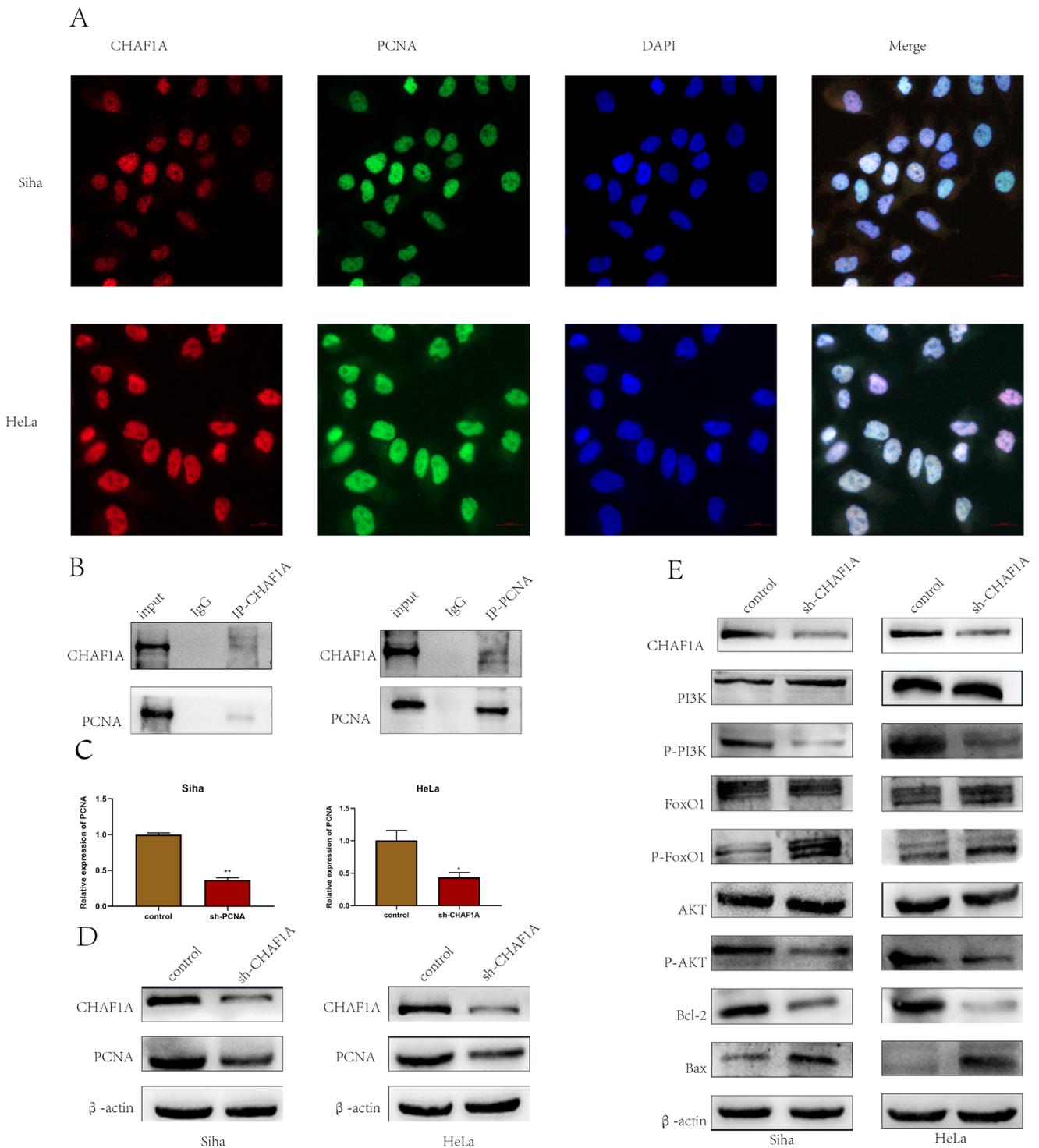


Figure 3

CHAF1A–PCNA interaction promotes cell proliferation and inhibits apoptosis. (A) CHAF1A is co-localized with PCNA in SiHa and HeLa cells. They are all localized in the nucleus. (B) PCNA is co-immunoprecipitated by CHAF1A, which is then co-immunoprecipitated by PCNA. The results are confirmed by Western blot experiments. (C) Real-time PCR analysis of PCNA mRNA expression in SiHa and HeLa cells infected with the EGFP shRNA and PCNA shRNA lentivirus. The mRNA level is significantly

reduced ($P < 0.05$). CHAF1A protein expression also decreased after PCNA knockdown. As shown in (D). (F) CHAF1A promotes cell proliferation and inhibits apoptosis via the PI3K/AKT/FoXO1 pathway in both SiHa and HeLa cells.

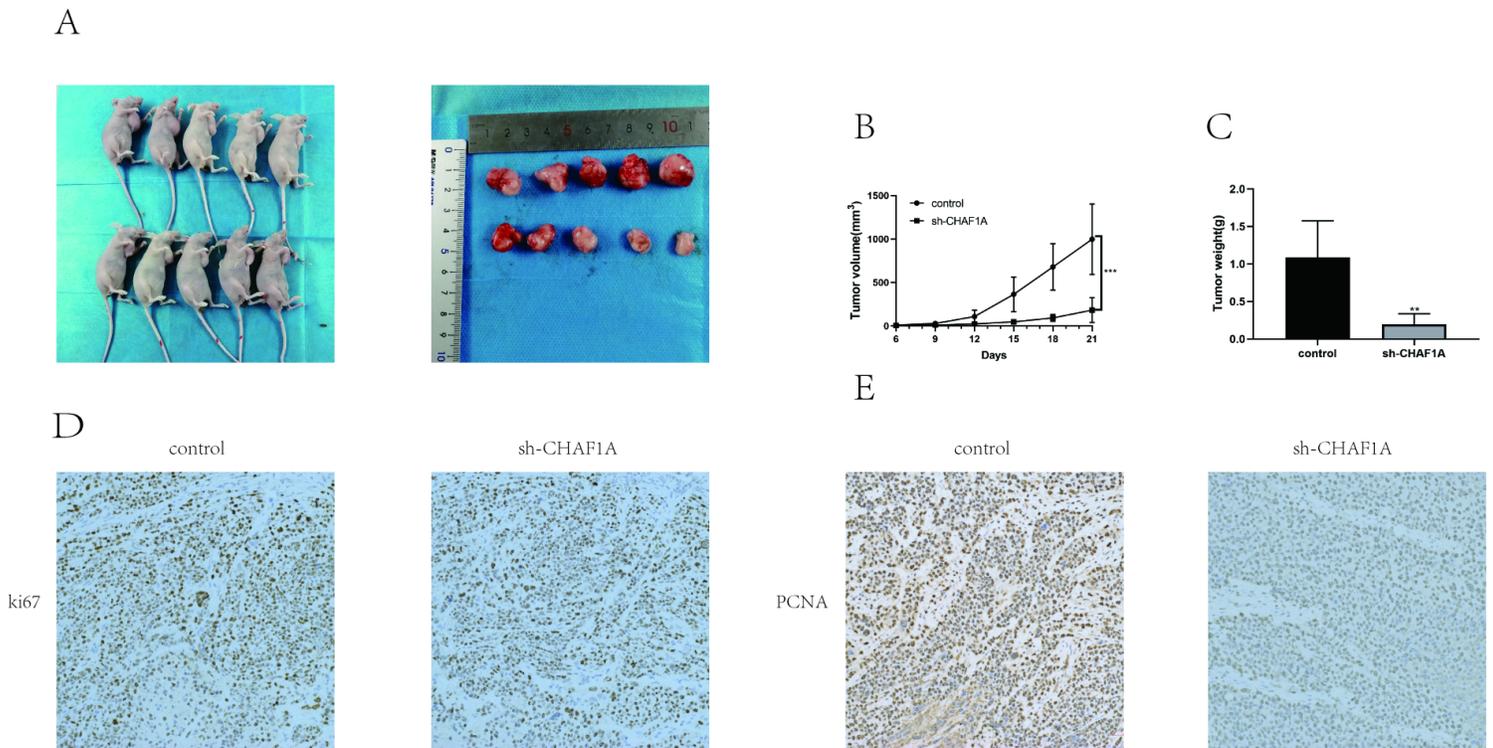


Figure 4

Tumor-promoting effect of CHAF1A on a human cervical cancer (CC) HeLa cell xenograft model. (A) Nude mice transplanted with human CC HeLa cell xenografts are randomly divided into 2 groups; the right forelimb is administered with HeLa-transfected negative control cells and sh-CHAF1 A-transfected cells via axillary injection. Representative photographs of tumor xenograft nude mice. (B) Representative photographs of the tumors 3 weeks after injection. (C) Photographs of the tumor volume, expressed as mean (+SD) ($n=5$ for each group). (C) Histopathological examination of tumor tissues; expression of Ki67 and PCNA proliferation-related markers, as determined by immunohistochemistry.