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Original Article

USP5 promotes glycolysis in cervical cancer by stabilizing FOXM1

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Abstract: Cervical cancer treatment faces challenges like limited efficacy, side effects, and high costs. Hence, unearthing feasible objectives targeting pathogeny is compulsory for the management of cervical cancer. Ubiquitin-specific peptidase 5 (USP5) acts as an oncogene in various cancers, but its role in cervical cancer continues vague. USP5 expression was assessed bioinformatically and experimentally. Studies involving gain and loss of function were performed in Hela and CaSki cells as well as in a xenograft mouse model. Assays included reverse transcription-quantitative polymerase chain reaction (RT-qPCR), cell counting kit-8 (CCK-8), clonogenesis, flow cytometry, western blot, co-immunoprecipitation (Co-IP), and immunohistochemistry. High USP5 expression predicted poor prognosis in cervical cancer patients. USP5 knockdown inhibited cell proliferation, glycolysis (reducing GLUT1, PGC-1α, HK, LDHA expression, glucose uptake, and lactate production), and induced apoptosis, while overexpression had opposite effects. USP5 stabilized FOXM1 via deubiquitination, which was crucial for USP5-mediated oncogenic effects. *In vivo*, USP5 silencing suppressed tumor growth and glycolytic gene expression. USP5 promoted cervical cancer progression by enhancing proliferation and glycolysis and suppressing apoptosis through deubiquitination and stabilization of FOXM1, identifying it as a potential therapeutic target.

Keywords: Cervical cancer, USP5, FOXM1, glycolysis, deubiquitination

Introduction

Cervical cancer is one of the most prevalent gynecologic malignancies in females. The year 2022 saw reports of 662,301 new cases and 348,874 deaths, with a 14.8% increase in new cases and a 17.8% increase in deaths by 2030 worldwide [1]. Constant infection with carcinogenic human papillomavirus (HPV) HPV strains is the most important cause of cervical cancer [2]. Thus, screening and HPV vaccination have effectively contributed to the elimination of cervical cancer [3]. However, females in low- and middle-income countries remain unevenly impacted by cervical cancer, due to the lack of these interventions [4]. Effectively therapeutical approaches, such as surgery, radiation and chemotherapy, are used alone or in combination, depending on the stage of the disease [5]. Also, medications, including paclitaxel and cisplatin consistently show efficacy [6]. In addition, emerging interventions, such as immunotherapy, nanotechnology, photodynamic therapy and gene therapy, have improved the outcomes of cervical cancer [7]. Unfortunately, finite availability, side effects and high cost bring intensively physical and psychosocial challenges, seriously influencing the quality of life of survivors. Therefore, elucidating the pathogeny, and exploiting feasible targets are momentous for the management for cervical cancer.

One of the delineating features of cancers is the metabolic rearrangement, acclimatized to favor ceaseless proliferation, mobility and assault [8]. In 1956, Warburg effect was noted as a metabolic transformation through elevated aerobic glycolysis, in which more lactate is produced and more glucose is consumed by cancer cells than by healthy cells [9]. During glycolysis, the completion of glucose uptake is required for glucose transporters (GLUTs), among which, GLUT1 is essential for tissues that are

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primarily dependent on glucose for energy, due to a greater affinity for glucose than other GLUTs [10]. Subsequently, several crucial enzymes are involved in glycolytic process. In the glycolytic pathway, the first enzyme, hexokinase (HK), is responsible for catalyzing the conversion of glucose into glucose-6-phosphate (G6P) through phosphorylation [11]. G6P further affects the efficiency of extracellular glucose transport through the cell membrane [12]. Lactate dehydrogenase A (LDHA) that reversibly catalyzes pyruvate to lactate, are also the rate-limiting enzyme of glycolytic process [13]. Both HK and LDHA have been elaborated to largely expressed in diverse cancers, including cervical cancer [14]. Additionally, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) is becoming a crucial modulator of several metabolic processes, especially in mitochondrial activity and biogenesis [15]. Besides, PGC-1α is reported to be involved in glycometabolism, and serve as a marker of Warburg effect [16, 17].

Ubiquitin-specific peptidase 5 (USP5), a key deubiquitinating enzyme (DUB) within the ubiquitin-proteasome system, cleaves unanchored polyubiquitin chains to facilitate ubiquitin recycling and maintain cellular ubiquitin homeostasis [18, 19]. It is ubiquitously expressed in multiple tissues, such as the testis, brain, skin, and lung, and localizes to both the cytosol and nucleoplasm, implicating it in diverse processes including development, DNA repair, and immune regulation [20, 21]. Beyond these physiological roles, USP5 has garnered attention in oncology for its ability to modulate signaling pathways associated with tumor progression and therapy resistance [22]. Aberrant expression of USP5 is linked to pathogenesis in several malignancies, such as lung cancer [23, 24], melanoma [25], clear cell renal cell carcinoma [26], head and neck squamous cell carcinoma [27], hepatocellular carcinoma [28], bladder cancer [29], nasopharyngeal carcinoma [30] and breast cancer [31]. Furthermore, studies using chalcone-derivatives have shown that USP5 inhibition can induce cell cycle arrest and apoptosis in breast cancer, ovarian cancer, and cervical cancer [32]. In spite of these advances, the specific role and mechanism of USP5 in cervical cancer remain largely unexplored.

Therefore, this study aimed to explore the role and underlying mechanism of USP5 in the progress of cervical cancer. Based on existing literature and bioinformatic predictions from the Ubibrowser database, we hypothesized that USP5 promoted cervical cancer progression by stabilizing FOXM1 via deubiquitination, thereby enhancing proliferation and glycolytic activity. Using a combination of cellular and animal models, along with molecular and biochemical assays, we sought to clarify USP5's function in cervical cancer, with the intention of finding new therapeutic targets.

Materials and methods

Bioinformatic analysis of USP5 expression

USP5 expression in cervical squamous cell carcinoma (CESC) and adjacent normal tissues was analyzed using the GEPIA2 database (http://gepia2.cancer-pku.cn). The prognostic value of USP5 expression in CESC patients was evaluated via the KM-plot online tool (http://kmplot.com/).

Clinical specimens

From patients diagnosed and surgically treated at the Affiliated Hospital of YouJiang Medical University for Nationalities, we acquired paired cervical cancer and nearby normal tissue samples (n=20). None of the patients had received preoperative radiotherapy, chemotherapy, or immunotherapy, or had other malignancies. Normal tissues were collected ≥ 5 cm from the tumor margin. Each sample was promptly frozen using liquid nitrogen. The study was approved by the Institutional Review Board of YouJiang Medical University for Nationalities, which adhered to the Declaration of Helsinki, and written informed consent was secured from every participant.

Reverse transcription quantitative PCR (RT-qPCR)

TRIzol reagent (15596026, Thermo Fisher Scientific) was employed to extract total RNA, and the Bio-Rad ScripTM cDNA Synthesis Kit (1708890) was used for cDNA synthesis. The procedure for RT-qPCR involved using SYBR Master mix (RR820A, Takara) on a Bio-Rad CFX system, with the following settings: 94°C for 5 minutes; 40 cycles of 94°C for 10 seconds,

Table 1. The primer sequences used in RT-qPCR

Name	Forward (5'-3')	Reverse (5'-3')
Homo sapiens		
USP5	TTCTGGGCTTTGGGAAACAGT	TCCGCCTTCAACACCAATAGC
GLUT1	CGGGCCAAGAGTGTGCTAAA	TGACGATACCGGAGCCAATG
PGC-1α	ACACTTTGCGCAGGTCAAAC	AGCAGGGTCAAAGTCATCTGAG
HK2	GAGCCACCACTCACCCTACT	CCAGGCATTCGGCAATGTG
LDHA	CGTCAGCAAGAGGGAGAAAG	GCCACGTAGGTCAAGATATCC
β-actin	TCACCATGGATGATGATATCGC	ATAGGAATCCTTCTGACCCATGC
Mus musculus		
GLUT1	CCATCCACCACACTCACCAC	GCCCAGGATCAGCATCTCAA
PGC-1α	AGCCGTGACCACTGACAACGAG	GCTGCATGGTTCTGAGTGCTAAG
HK2	GTGTGCTCCGAGTAAGGGTG	CAGGCATTCGGCAATGTGG
LDHA	GGAGATTCCAGTGTGCCTGT	GTCCAATAGCCCAGGATGTG
β-actin	GCAGGAGTACGATGAGTCCG	ACGCAGCTCAGTAACAGTCC

58°C for 45 seconds, and 72°C for 1 minute. The qPCR reaction mixture (20 μL total volume) contained 10 μL of 2× SYBR Green Master Mix, 1 μL of forward primer (10 μM), 1 μL of reverse primer (10 μM), 2 μL of cDNA template, and 6 μL of RNase-free water. Gene expressions (USP5, GLUT1, PGC-1α, HK2, LDHA) were adjusted relative to β-actin and determined by the $2^{-\Delta\Delta CT}$ approach. Primer sequences were detailed in **Table 1**.

Cell culture

Human cervical cancer cells Hela (CL-0101) and CaSki (CL-0048), and immortalized human cervical epithelial cells H8 (BFN607200572), were acquired from Procell and BLUEFBIO, respectively. Hela cells were maintained in MEM with NEAA (PM150410, Procell), while CaSki and H8 cells were cultured in RPMI-1640 medium (PM150110, Procell), both supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C with 5% CO_2.

Cell transfection

Lentiviral vectors encoding shRNA targeting USP5 (shUSP5, 5'-CTTTGCCTTCATTAGTCACAT-3') and a non-targeting control (shCtrl, 5'-CCTAAGGTTAAGTCGCCCTCG-3') were constructed by GenePharma. Full-length USP5 and FOXM1 cDNA were cloned into pcDNA3.1 vectors for overexpression. Cells were transfected using Lipofectamine 3000 (L3000001, Invitrogen), and transfection efficiency was confirmed by western blot.

Cell viability

To assess cell viability, a CCK-8 kit (CA1210, Solarbio, Beijing, China) was employed as per the manufacturer's directions. Each well was treated with 10 μl of CCK-8 reagents and incubated at 37°C for 2 hours. Thermo Fisher Scientific's microplate reader from Waltham (MA, USA), was used to read the absorbance at 450 nm.

Clonogenic assay

For colony formation, 500 cells per well were placed in 6-well plates and grown for 14 days. The colonies were then fixed using 4% paraformaldehyde (P1110, Solarbio), dyed by crystal violet (G1063, Solarbio), and counted by hand.

Apoptosis analysis

Apoptosis was evaluated using a cell death ELISA kit (11544675001, Roche, Basel, Switzerland) in keeping with the operating manual. The optical density value was measured at 405 nm using a Thermo Fisher Scientific microplate reader.

Metabolic assays

The measurement of glucose consumption and lactate production was carried out using Sigma's (CBA086) and Biovision's (K607) commercial kits.

Co-immunoprecipitation (Co-IP) assay

The supernatant was harvested by lysing Hela cells with lysis buffer (R0100, Solarbio) and protease inhibitors (A8260, Solarbio) on ice for 40 minutes. Antibodies targeting USP5 (ab24-1311, Abcam), FOXM1 (ab245309, Abcam), or IgG (ab172730, Abcam) were used to detect the binding between USP5 and FOXM1, with an overnight incubation at 4°C. Following this, protein A/G-agarose beads (78609, Thermo Fisher Scientific) were introduced and mixed at 4°C. The cells were pelleted and cleansed with lysis

buffer. The precipitates underwent a 5-minute boiling process with loading buffer (P1040, Solarbio) before being detected via western blot.

Ubiquitination assay

Hela cells transfected with shCtrl or shUSP5 underwent immunoprecipitation using an anti-FOXM1 antibody, followed by immunoblotting with an anti-ubiquitin antibody (ab19247, Abcam). Re-extraction of the immunoprecipitates was performed in a lysis buffer containing 1% sodium dodecyl sulfate (SDS, S8010, Solarbio), followed by denaturation through 10 minutes of heating and centrifugation at 12,000 g for 1 minute to separate the supernatants. Afterward, the supernatants were processed for immunoblotting with the anti-FOXM1 antibody.

Cycloheximide assay

Hela cells with OE-USP5 and NC-OE transfections were incubated with cycloheximide (100 μg/mL, 239763-M, Sigma-Aldrich) to halt new protein synthesis at intervals of 0, 1, 3, and 6 hours. Hela cells were subsequently broken down using RIPA buffer containing protease inhibitors for western blot analysis.

Animal studies

Four-week-old BALB/c nude mice were sourced from Cyagen in Jiangsu, China, and maintained under specified pathogen-free (SPF) conditions with a 12-hour light-dark cycle and regulated temperature. Random allocation placed the mice into either the shCtrl group or the shUSP5 group, with each group containing six mice. Mice were subcutaneously injected with shCtrl- or shUSP5-transfected Hela cells (1×10^6) [33]. Tumor volume was measured every two days. Following a 20-day period, mice were sacrificed through an intraperitoneal dose of 100 mg/kg sodium pentobarbital. Tumors were excised, weighed, and processed for further analysis. Approved by the Animal Ethics Committee of YouJiang Medical University for Nationalities, all procedures complied with the Guide for the Care and Use of Laboratory Animals [34].

Immunohistochemistry

The tumor tissues were preserved in 4% paraformaldehyde and dehydrated using a gradient ethanol process. Afterward, the tissues were embedded in paraffin (YA0011, Solarbio) for sectioning into 5 μm thick slices. Restoration was performed with a sodium citrate buffer (pH 6.0, P0081, Beyotime, Shanghai, China) at a temperature of 94°C for 15 minutes. Following this, slices were sealed with 1% bovine serum albumin (BSA, ST2249, Beyotime) for an hour and exposed to primary antibodies against Ki67 (1:200, ab15580, Abcam, Cambridge, UK) overnight at 4°C. An HRP-tagged anti-rabbit IgG secondary antibody (ab288151, Abcam) was applied to the slices and incubated at 37°C for 30 minutes. Hematoxylin (G1080, Solarbio) was used to re-stain the slices, which were then imaged under a light microscope (Olympus).

Western blot

Using RIPA lysis buffer (R0010, Solarbio), tumor cells and tissues were lysed to gather total proteins, and the BCA Protein Assay Kit (PC0020, Solarbio) was employed to measure the protein concentrations. The proteins (20 μg) were electrophoresed using 10% SDS-PAGE and transferred onto PVDF membranes (IPVH00010, EMD Millipore, Billerica, MA, USA). The membranes were blocked in 5% BSA Blocking Buffer (SW3015, Solarbio) for 1 h at room temperature, and incubated with primary antibodies at 4°C overnight. The primary antibodies included anti-USP5 (1:2000, ab241311, Abcam), anti-FOXM1 (1:2000, 13147-1-AP, Proteintech, Wuhan, China) and anti-β-actin (1:1000, ab8226, Abcam). For an hour at room temperature, the membranes were exposed to secondary antibodies, Rabbit Anti-Mouse IgG H&L (HRP) (1:10000, ab6728, Abcam). A BeyoECL Plus kit (P0018S, Beyotime) was used to develop the bands, and Image-ProPlus software (Media Cybernetics, Inc., Rockville, MD, USA) was employed to determine the gray value.

Statistical analysis

The data are shown as mean ± SD and analyzed by SPSS 20.0 software (IBM, Armonk, New York, USA). Differences were assessed using Student's t-test or one-way ANOVA followed by Bonferroni post-hoc test. Specially, repeated-measures ANOVA was employed for all datasets involving multiple groups measured across sequential time points, including CCK-8 proliferation assays and CHX chase ex-

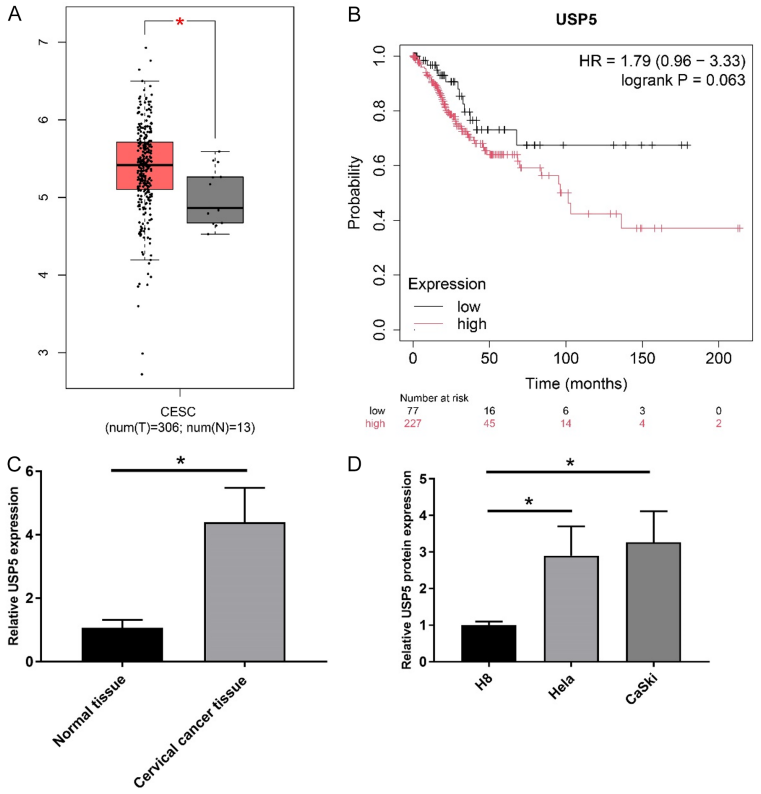

Figure 1. USP5 was upregulated in cervical cancer and correlates with poor prognosis. A. USP5 expression in cervical squamous cell carcinoma (CESC) and normal tissues from the GEPIA database. B. Kaplan-Meier overall survival analysis of cervical cancer patients stratified by USP5 expression. C. Relative USP5 mRNA levels in 20 paired clinical cervical cancer and adjacent normal tissues measured by RT-qPCR. D. USP5 transcript levels in normal cervical epithelial (H8) and cervical cancer (Hela, CaSki) cell lines. Data normalized to *β-actin*. *P < 0.05.

periments. Survival analysis utilized the Kaplan-Meier method with a log-rank test, and a *p*-value of less than 0.05 was deemed significant.

Results

USP5 is upregulated in cervical cancer tissues and cell lines

Analysis of the GEPIA database revealed significantly higher USP5 expression in cervical squamous cell carcinoma (CESC) samples compared to normal controls (P < 0.05, **Figure 1A**). Patients with high USP5 expression showed markedly shorter overall survival (**Figure 1B**). Consistent with bioinformatic findings, RT-qPCR analysis of 20 paired clinical samples confirmed pronounced USP5 upregulation in tumor tissues (P < 0.05, **Figure 1C**). USP5 transcript levels were similarly elevated in cervical cancer cell lines (Hela and CaSki) when compared to normal cervical epithelial cells (P < 0.05, **Figure 1D**). Moreover, USP5 protein levels were markedly elevated in both Hela and CaSki cells compared to H8 cells (Figure S1), consistent with the RT-qPCR findings (**Figure 1D**). This confirmed that USP5 overexpression in cervical cancer cells was evident at both the transcriptional and translational levels. Hence, USP5 was overexpressed in cervical cancer and correlates with poor prognosis.

USP5 knockdown suppresses proliferation and induces apoptosis

Experiments involving both gain and loss of function were conducted to evaluate the role of USP5. Western blot confirmed successful USP5 overexpression and knockdown (P < 0.05, **Figure 2A**). USP5 overexpression enhanced cell viability and clonogenic ability while reducing apoptosis in Hela and CaSki cells (P < 0.01, **Figure 2B-D**). Conversely, USP5 knockdown produced opposite effects, suppressing growth and promoting apoptosis (P < 0.01, **Figure 2B-D**). Thus, USP5 promoted proliferation and repressed apoptosis in cervical cancer cells.

USP5 knockdown attenuates the glycolysis

To further resolve the role of USP5 in the progression of cervical cancer, the function of USP5 on glycolysis was addressed in Hela and CaSki cells. USP5 overexpression significantly increased the mRNA levels of glycolytic genes (GLUT1, PGC-1α, HK, LDHA), glucose consumption, and lactate production (P < 0.05, **Figure 3A-F**). USP5 knockdown suppressed these indicators (P < 0.05, **Figure 3A-F**), indicating that USP5 promotes aerobic glycolysis in cervical cancer cells.

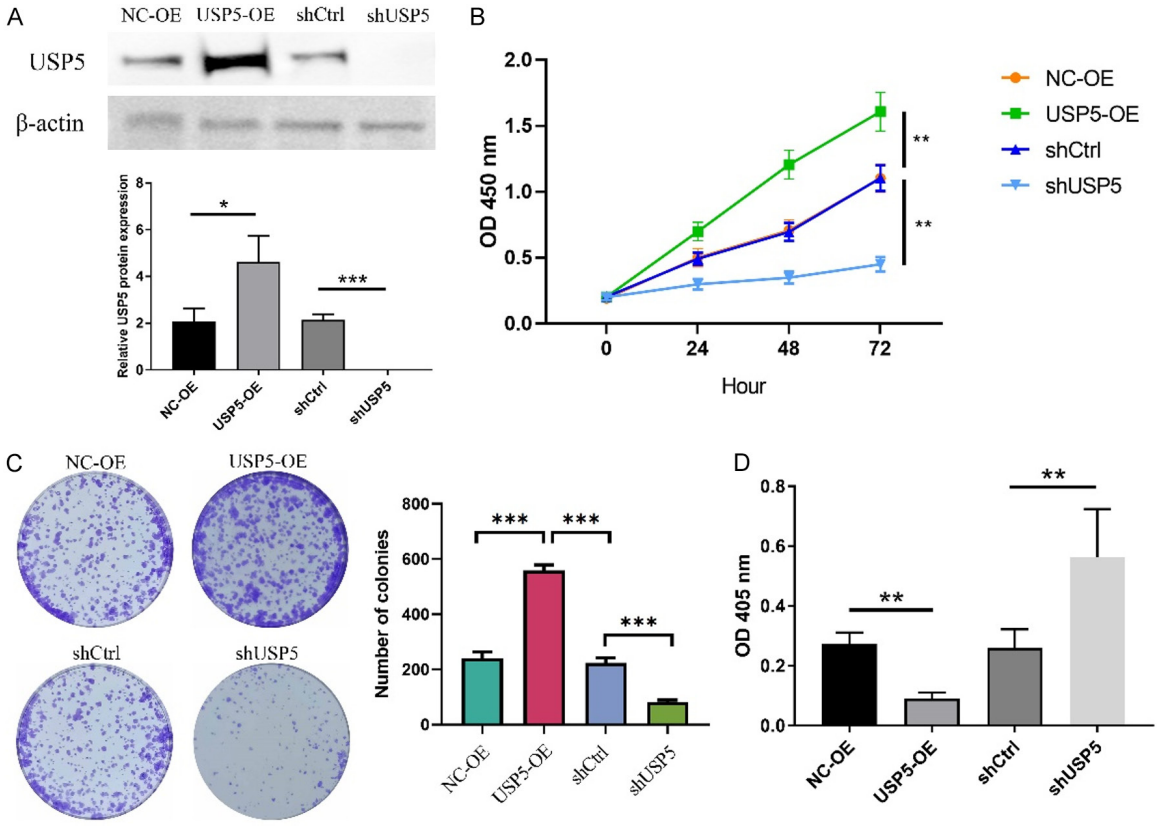

Figure 2. USP5 knockdown suppressed proliferation and promotes apoptosis in cervical cancer cells. Hela and CaSki cells were transfected with USP5 overexpression plasmid (USP5-OE), USP5-targeting shRNA (shUSP5), or corresponding controls. A. Western blot verification of USP5 protein levels. B. Cell viability assessed by CCK-8 assay. C. Colony formation ability. D. Apoptosis rate measured by flow cytometry. *P < 0.05, **P < 0.01 and ***P < 0.001.

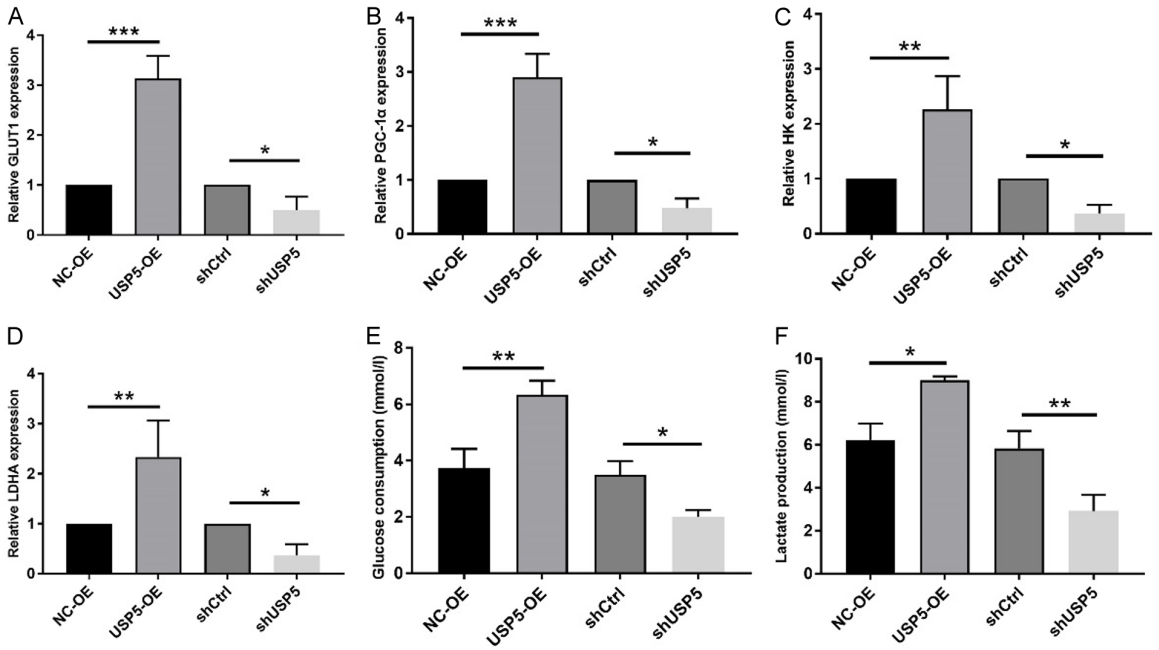

Figure 3. USP5 knockdown attenuates aerobic glycolysis (Warburg effect) in cervical cancer cells. A-D. mRNA expression of glycolytic genes (GLUT1, PGC-1α, HK, LDHA) by RT-qPCR. Data normalized to *β-actin*. E. Cellular glucose consumption. F. Lactate production levels. *P < 0.05, **P < 0.01 and ***P < 0.001.

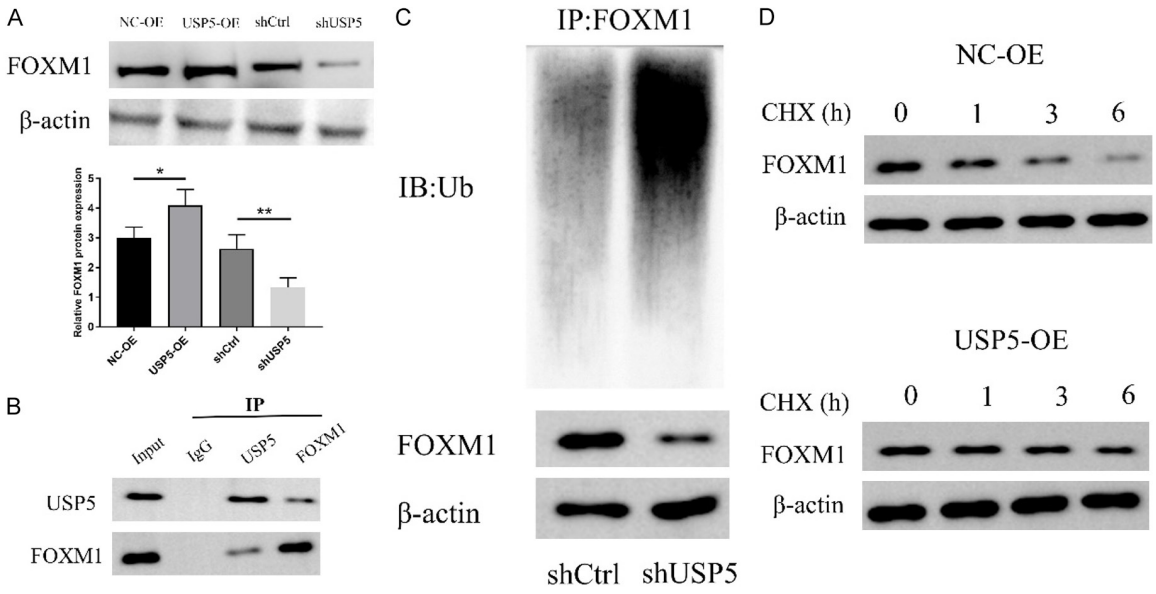

Figure 4. USP5 stabilized FOXM1 through deubiquitination. A. FOXM1 protein levels after USP5 modulation. Data normalized to β-actin. B. USP5-FOXM1 interaction detected by co-immunoprecipitation in Hela cells. C. Ubiquitination levels of FOXM1 following USP5 knockdown. D. FOXM1 protein stability assay using CHX. *P < 0.05 and **P < 0.01.

USP5 stabilizes FOXM1 through deubiquitination

A bioinformatic prediction was performed using the UbiBrowser database (http://ubibrowser.bio-it.cn), which identified FOXM1 as a high-confidence candidate substrate of USP5. In addition, extensive literature has established that FOXM1 was overexpressed in cervical cancer and drove tumor progression by regulating proliferation, apoptosis, and radioresistance, with its protein stability governed by the ubiquitin-proteasome pathway [35, 36]. Based on these converging lines of evidence, we hypothesized that USP5 may stabilize FOXM1 through deubiquitination to promote cervical cancer malignancy. USP5 overexpression elevated FOXM1 protein levels, while USP5 knockdown reduced them (P < 0.05, **Figure 4A**). Co-immunoprecipitation confirmed a physical interaction between USP5 and FOXM1 (**Figure 4B**). USP5 knockdown increased FOXM1 ubiquitination (**Figure 4C**), and USP5 overexpression delayed FOXM1 degradation in cycloheximide-treated cells (P < 0.05, **Figure 4D**), indicating that USP5 enhances FOXM1 stability via deubiquitination.

FOXM1 mediates the oncogenic effects of USP5

To confirm the direct role of FOXM1 in the malignant progression of cervical cancer mediated by USP5, FOXM1 was overexpressed in Hela cells with the shUSP5 transfection (P < 0.05, **Figure 5A**). Rescue experiments showed that FOXM1 overexpression reversed the suppressive effects of USP5 knockdown on viability, colony formation, glycolytic gene expression, glucose uptake, and lactate production (P < 0.05, **Figure 5B-I**), confirming that FOXM1 acted downstream of USP5 in promoting malignant phenotypes.

USP5 silencing inhibits tumor growth and glycolysis in vivo

In a xenograft model, USP5 knockdown reduced tumor size, weight, and levels of Ki67 and FOXM1 (P < 0.001, **Figure 6A-D**), and downregulated glycolytic gene expression (P < 0.05, **Figure 6E-H**), confirming that USP5 promotes tumor growth and glycolysis *in vivo*.

Discussion

Cervical cancer remains a major health burden among women worldwide. While early-stage patients often benefit from surgical or radiotherapeutic interventions, overall survival rates in China continue to show an unfavorable trend, largely due to late diagnosis and high rates of metastasis and recurrence [37]. There is an urgent necessity to identify robust molecular targets to enhance therapeutic strategies. In this study, we found that USP5 was upregulated

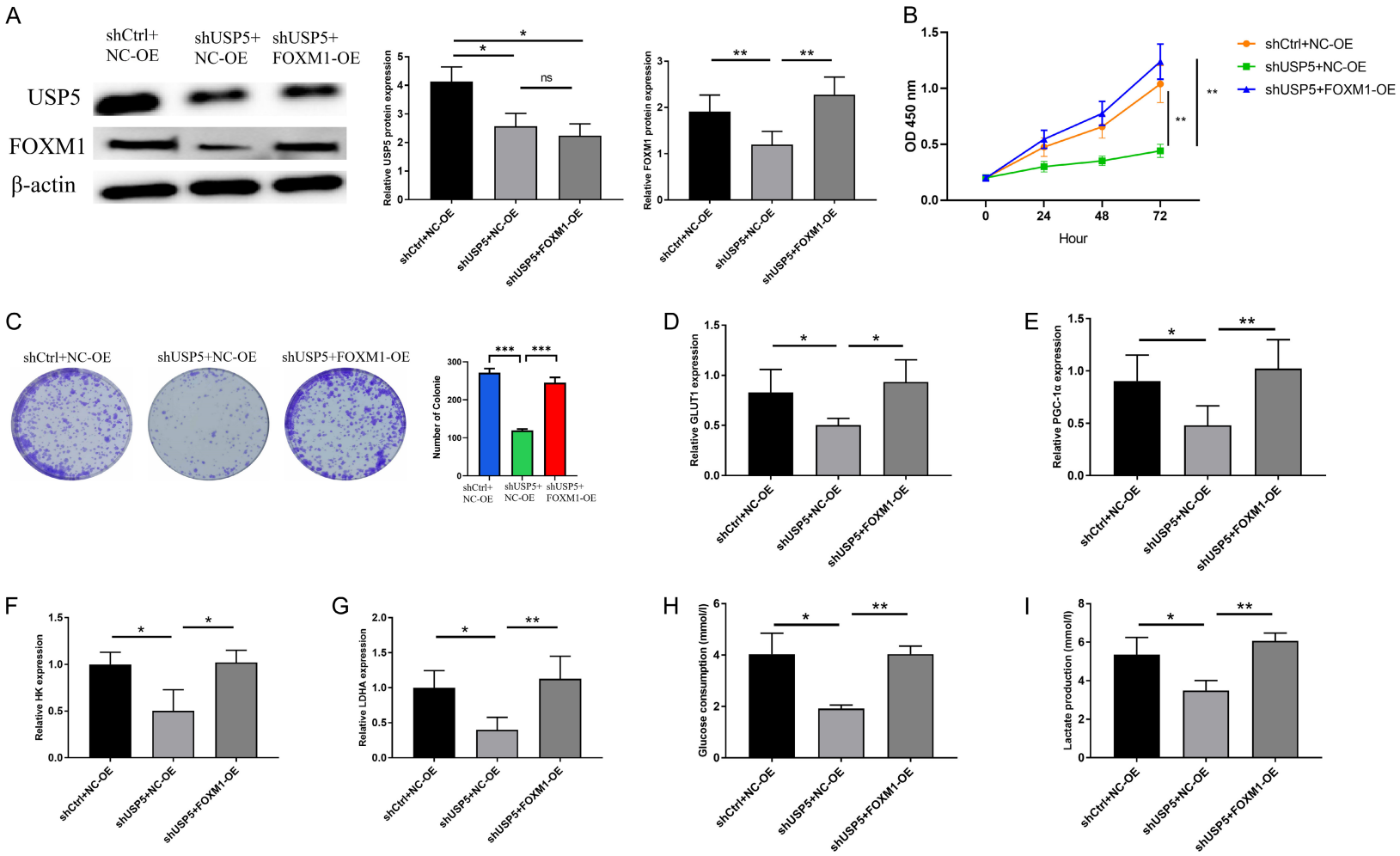

Figure 5. FOXM1 mediated USP5-induced proliferation and glycolysis. Hela cells were transfected with shUSP5 and/or FOXM1 overexpression plasmid. A. USP5 and FOXM1 protein expression. Data normalized to β-actin. B. Cell viability. C. Colony formation. D-G. Glycolytic gene expression. Data normalized to *β-actin*. H. Glucose consumption. I. Lactate production. *P < 0.05, **P < 0.01 and ***P < 0.001.

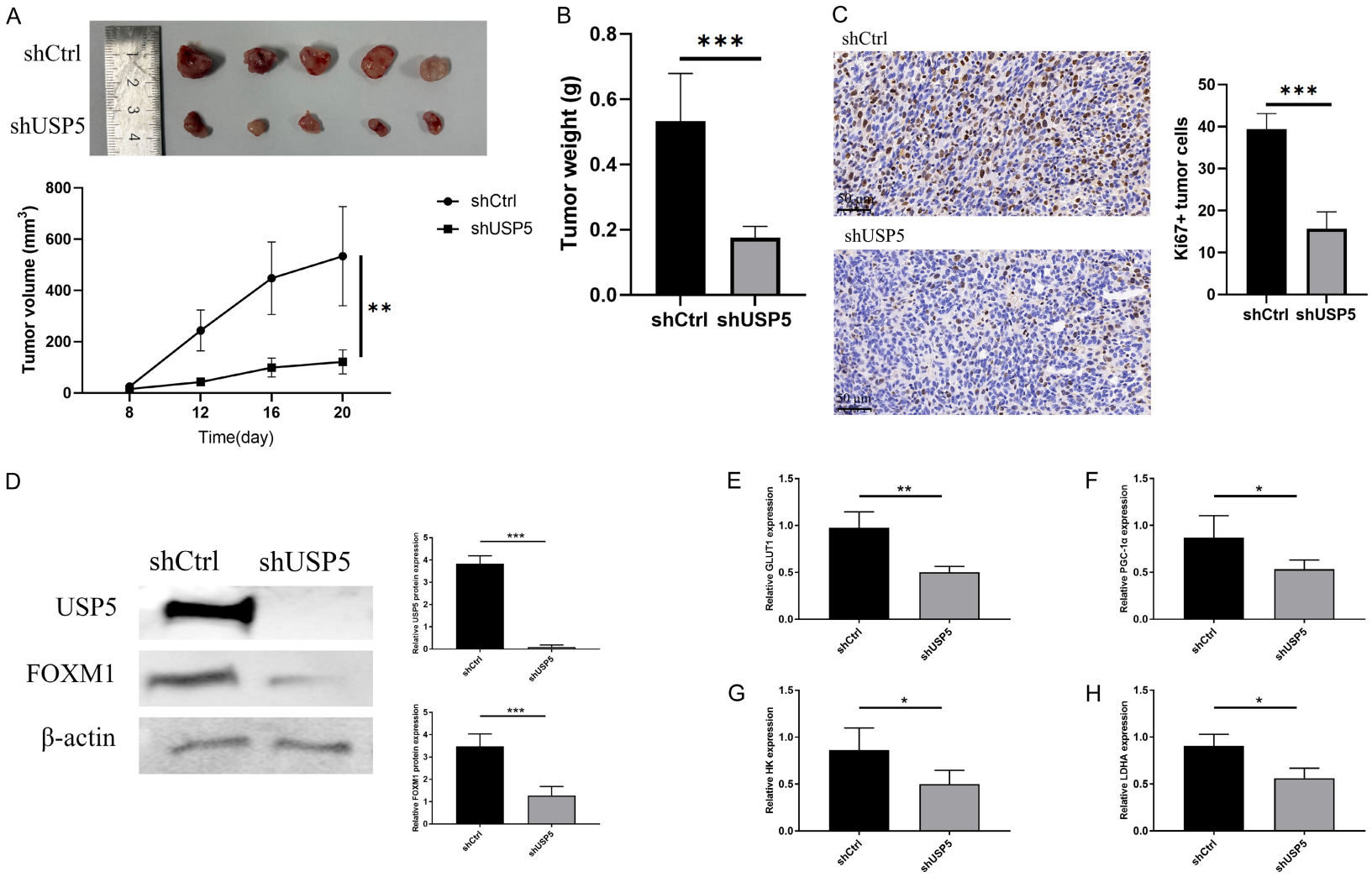

Figure 6. USP5 knockdown suppressed tumor growth and glycolysis *in vivo*. Nude mice were subcutaneously injected with shUSP5- or shCtrl-transfected Hela cells (1×10^6) into the right flank of nude mice. Tumor volume was monitored two days for consecutive 12 days and quantified by the following formula: volume = 0.5 × length × width^2. After 20 days, the tumors samples were excised and weighed. A. Tumor images (up) and growth curves (down). B. Tumor weight. C. Ki67 immunohistochemistry. Scale bar=50 μm. D. USP5 and FOXM1 protein levels in xenografts. Data normalized to β-actin. E-H. Glycolytic gene expression in tumor tissues. Data normalized to *β-actin*. *P < 0.05, **P < 0.01 and ***P < 0.001.

in cervical cancer and associated with poor prognosis. Functionally, USP5 promoted tumor cell proliferation and glycolytic metabolism, but inhibited apoptosis. Mechanistically, we found that USP5 stabilized FOXM1 by reducing its ubiquitin-mediated degradation. These findings were further corroborated in a xenograft model, in which the knockdown of USP5 suppressed tumor growth and glycolysis. Taken together, these results indicated that USP5 exerted an oncogenic effect on cervical cancer through deubiquitinating and stabilizing FOXM1.

USP5 is revealed as an oncogene in several malignancies, such as acute myeloid leukemia [38], triple-negative breast cancer [31], multiple myeloma [39], head and neck squamous cell carcinoma [27], hepatocellular carcinoma [28], bladder cancer [40] and non-small cell lung cancer [41]. Consistent with these findings, an elevated expression of USP5 was found in cervical cancer tissues and cell lines based on *in silicon*, *in vitro* and *in vivo* analysis. Furthermore, high levels of USP5 was correlated with shorter overall survival among patients with triple-negative breast cancer [31], head and neck squamous cell carcinoma [27], bladder cancer [40] and lung cancer [42], supporting its clinical relevance as a prognostic marker.

Dysregulation of proliferation and apoptosis is an established hallmark of cancer [43]. Several ubiquitin-specific peptidases (USPs) have been revealed to regulate the progression of cervical cancer. For instance, downregulation of USP18 enhances proliferation, migration and aggressiveness of HeLa cells [44]. Overexpression of USP26 suppresses proliferation and migration of HeLa cells [45]. USP53 reduces the radiosensitivity of cervical cancer [46]. In this study, we found that USP5 increased cell viability and colony formation, but inhibited apoptosis in cervical cancer cells. These results were line with the previous reports of USP5-driven growth in lung cancer [24], head and neck squamous cell carcinoma [27], cholangiocarcinoma [47], bladder cancer [29], triple-negative breast cancer [31], non-small cell lung cancer [41] and acute myeloid leukemia [48]. Together, these findings indicated that USP5 enhanced growth but repressed apoptosis of cervical cancer.

Metabolic reprogramming, particularly the Warburg effect, is increasingly recognized as important for sustaining rapid tumor growth [49]. Glycolysis is markedly upregulated in cervical cancer, and its inhibition represents a promising therapeutic strategy [50]. Our study employed a multi-dimensional approach to systematically evaluate glycolytic activity: (1) RT-qPCR analysis of key glycolysis-related genes (GLUT1, PGC-1α, HK, LDHA) at the transcriptional level; (2) functional quantification of glucose consumption and lactate production; and (3) *in vivo* validation of suppressed glycolytic gene expression upon USP5 silencing. These complementary lines of evidence, spanning from transcriptional regulation to metabolic end-product accumulation, collectively formed a coherent evidence chain supporting the conclusion that USP5 promoted glycolysis in cervical cancer through FOXM1 stabilization. This pro-glycolytic role has also been observed in lung cancer [23] and multiple myeloma [39], underscoring the conserved function of USP5 in cancer metabolism. However, seahorse XF-based ECAR/OCR profiling as an important future direction for further elucidating the impact of USP5 on the metabolic switch between mitochondrial oxidative phosphorylation and glycolysis.

Mechanistically, USP5 can stabilize numerous oncoproteins through deubiquitination, such as c-Myc [51], FcεRI [52], HOXA10 [24], PD-L1 [25], P4HB [53], IMPDH2 [28], METTL3 [54], SLUG [40] and IRF3 [55]. FOXM1 is a known driver of cervical cancer progression [56-59]. In this study, FOXM1 was identified as a novel substrate of USP5. We confirmed their physical interaction and showed that USP5 decreased the FOXM1 ubiquitination, thereby extending its protein half-life. Rescue assays further established that overexpression of FOXM1 reversed the anti-tumor effects of USP5 knockdown, confirming FOXM1 as a key downstream effector.

In brief, our study established USP5 as a clinically significant promoter of cervical cancer progression via FOXM1 stabilization. Several limitations remain to be explored in the future. The correlation between USP5 expression and specific clinicopathological features warrants further clinical investigation, as do its potential roles in migration, invasion, and EMT. Future studies should also explore the USP5/FOXM1 axis using more complex *in vivo* models and identify the exact ubiquitination sites on FOXM1

targeted by USP5. Besides, the limited clinical sample size was a study limitation in this study and a multi-center cohort expansion should be a future direction. In addition, the log-rank *P* value of 0.063 in **Figure 1B** did not reach conventional significance. This marginal result was likely attributable to limited statistical power due to imbalanced group sizes (227 *vs.* 77). However, the HR of 1.79 (95% CI: 0.96-3.33) indicated a clear trend toward worse prognosis in the high-expression group, with the confidence interval narrowly crossing unity. Moreover, the central focus of this study was to delineate the mechanistic role of USP5 in promoting glycolysis via FOXM1 stabilization, and the survival analysis served as supportive clinical evidence. The observed trend was directionally consistent with our mechanistic findings. Thus, a bigger and balanced group size is needed to be included in the future. These findings nonetheless provide preclinical support for targeting USP5 as a potential therapeutic strategy in cervical cancer.

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Written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article.

Disclosure of conflict of interest

None.

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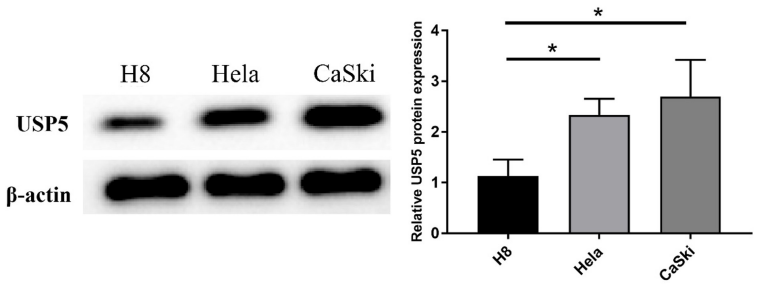

Figure S1. USP5 protein levels were markedly elevated in both Hela and CaSki cells compared to H8 cells. Western blot verification of USP5 protein levels in H8, Hela and CaSki cells. *P < 0.05.