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Original Research

USP22 promotes the expression of GLUT1 and HK2 to facilitate growth and glycolysis in cervical cancer cells

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Summary

Background: Cervical cancer is one of the most aggressive cancers in women, of which the pathogenesis should be further investigated to improve the prognosis. *Objectives:* The goal of this study is to investigate the role of ubiquitin-specific peptidase 22 (USP22) in cervical cancer and reveal its involvement in the signaling pathway. *Results:* Upregulation of USP22 was observed in cervical cancer tissues and cell lines. Cell proliferation, lactate production, glucose uptake and lactate dehydrogenase (LDH) activity was increased by overexpression of USP22 and decreased by suppression of USP22. The protein expression levels of Forkhead Box M1 (FoxM1), glucose transporter 1 (GLUT1) and hexokinase-2 (HK2) were upregulated by overexpression of USP22 and downregulated by suppression of USP22. Co-transfection of shUSP22 and FoxM1 attenuated the inhibitory effects of shUSP22 on the growth and glycolysis of cervical cancer cells. *Conclusion:* upregulation of USP22 in cervical cancer could promote cell proliferation and glycolysis through mediating FoxM1, indicating that USP22 and FoxM1 could be therapeutic targets in cervical cancer.

Key words: USP22; GLUT1; HK2; Glycolysis; Cervical cancer cells.

Introduction

Cervical cancer ranks as the fourth most frequently diagnosed tumor and the fourth leading cause of death in women, with approximately 570,000 new cases and 311,000 deaths worldwide in 2018 [1]. With the development of vaccine and screening technology, the morbidity of cervical cancer has decreased in developed countries [2, 3]. Despite the development in surgery, chemotherapy and radiotherapy, the recurrence rate is still 35 % in patients with cervical cancer [4-6]. Therefore, Exploring new biomarkers and therapeutic regimens are needed to improve the patient outcomes in patients with cervical cancer.

The pathogenesis of cervical cancer is miscellaneous, including DNA methylation, histone modification, dysregulation of RNAs [7]. The Forkhead Box M1 (FoxM1) is a transcription factor that belongs to the Forkhead box protein superfamily [8]. FoxM1 is a proliferation-associated transcription factor, which can directly or indirectly initiate target gene expression and transcription [9]. FoxM1 was upregulated in many human cancers, including cervical cancer, hepatoma, breast cancer, ovarian cancer, melanoma, lung cancer, and could regulate cancer cell proliferation, differentiation and transformation [10, 11]. Moreover, FoxM1 could also promote epithelial–mesenchymal transition through activation of ROCKs in colorectal cancer [12]. Therefore, FoxM1 plays an essential role in the pathogenesis and progression of various cancers.

Ubiquitin-specific peptidase 22 (USP22) is a family member of the ubiquitin-specific processing proteases and is highly conserved among species [13]. USP22 acts as a transcriptional activator and responsible for target gene transcription in in various cancers, such as breast cancer, lung cancer, as well as prostate cancer [14]. Furthermore, overexpression of USP22 promoted cell cycle initiation from G1 phase to S phase in pancreatic cancer cell lines [15]. However, the mechanism underlying the effects of USP on regulating the progression of cervical cancer is still unknown. Thus, the goal is to investigate the role of USP22 in cervical cancer and reveal the signaling pathway.

Materials and Methods

Human tissue samples

60 pairs of cervical cancer tissues and adjacent normal tissues were collected during operation at Changzhou Maternal and Child Health Hospital Hospital and written informed consent was obtained from all patients. The protocol of this research project has been approved by the Ethics Committee of Changzhou Maternal and Child Health Hospital (Approval no.2020122) and was in accordance with the Declaration of Helsinki

Cell culture and transfection

Three human cervical cancer cell lines (ME180, Siha and Caski cells) and ectocervical cell line Et1/E6E7 cells were purchased from the China Center for Type Culture

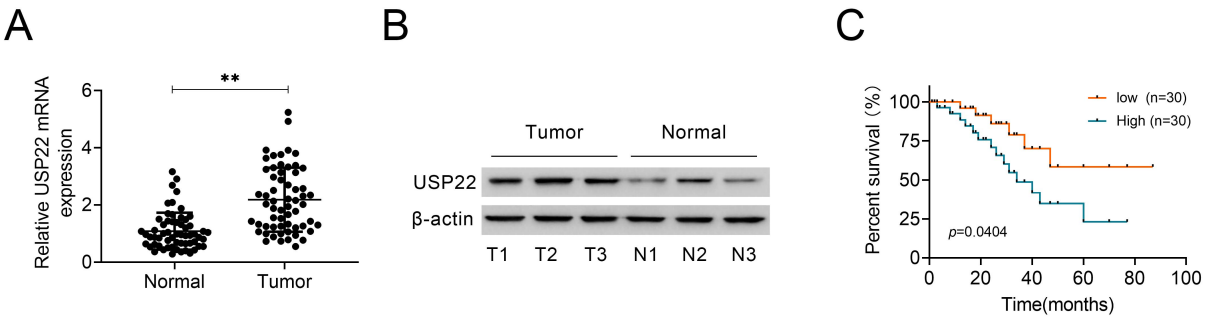

Figure 1. — USP22 was overexpressed in cervical cancer tissues. (A) the mRNA expression of USP22 was upregulated in cervical cancer tissues; (B) the protein expression of USP22 was upregulated in cervical cancer tissues; (C) the expression of USP22 was negatively correlated with the survival rate of patients with cervical cancer. $^{**}p < 0.01$ versus (*vs.*) normal.

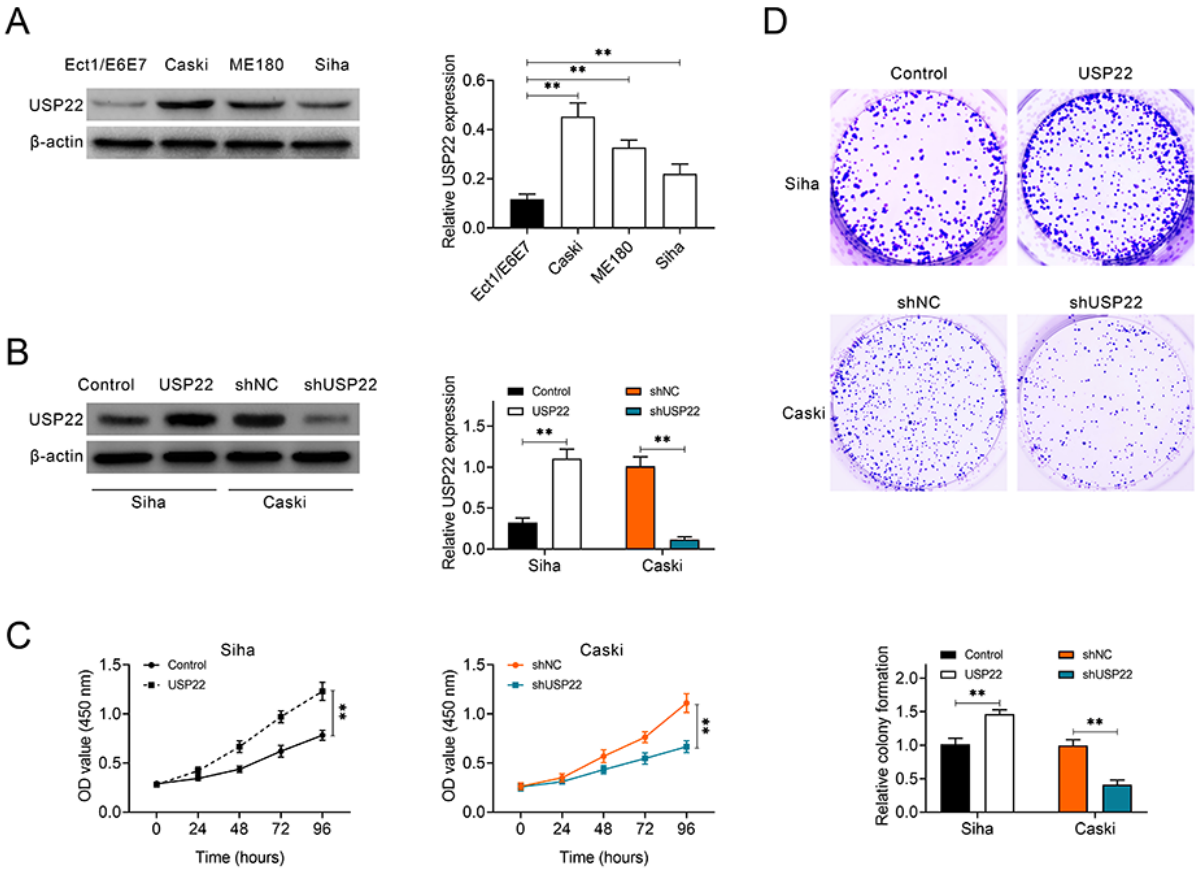

Figure 2. — Upregulation of USP22 promoted the proliferation of cervical cancer cell lines. (A) USP22 was overexpressed in cervical cancer cell lines; (B) Expression of USP22 was increased by USP22 plasmid and repressed by shUSP22; (C) Cell viability was increased by overexpression of USP22 and decreased by suppression of USP22; (D) The number of clone was increased by overexpression of USP22 and decreased by suppression of USP22. $^{**}p < 0.01$ *vs.* control or shNC. Control: negative control of USP22 plasmid; shNC: negative control of shNC.

Collection (China). Cells were cultured in RPMI 1640 media containing 1% penicillin and streptomycin (Invitrogen, USA) and 10 % fetal bovine serum (FBS, Invitrogen, USA) under 37 ºC with 5 % CO_2.

Human-specific shUSP22 and its negative control (shNC) were purchased from Shanghai Genechem Co., Ltd (Shanghai, China) and used to knock down the expression of USP22 in Caski cells. The coding sequence of USP22

(Ribobio, China) and its negative control were cloned into pcDNA3.1 and then transfected into Siha cells to overexpress USP22. Siha cells and Caski cells were cultured in a sterile 12-well plated. The transfection was conducted using Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's instruction.

CCK-8 assay and clone formation assay

Siha and Caski cells were seeded in 12-well plates. After transfection, cell activity was measured at 24, 48, 72, and 96 hours (h) using a commercial CCK-8 kit (Beyotime, China) according to the manufacturer's instruction. The absorbance value at 450 nm was recorded using a spectrophotometer.

For clone formation assay, after transfection for 24 h, Siha and Caski cells were mixed with 0.4 % of agarose and then cultured with 2 ml of culture medium in a 12-well plate. The cells were fixed and stained with 0.1 % crystal violet at Day 7. The number of colonies were recorded and counted under a microscope (Olympus, Japan).

Measurement of lactate production, glucose uptake and lactate dehydrogenase (LDH) activity

The lactate production, glucose uptake and LDH activity was measured using commercial kits, Lactate Assay Kit (BioLabs, USA), Glucose Uptake Assay Kit (Abcam, UK) and LDH Assay kit (Abcam, UK), respectively, according to the manufacturer's instruction.

Reverse transcription-quantitative PCR (RT-qPCR)

Siha and Caski cells were collected after transfection and total RNA was extracted from transfected cells using TRIzol reagent (Thermo Fisher, USA). cDNA was synthesized and RT-qPCT was performed. After RT-qPCR, the relative RNA expression was calculated the $2^{-\Delta\Delta Ct}$ method. The primer sequences used were: USP22, forward 5'- AGCAGCGGATTCACCATCTC-3' and reverse 5'- TGATGTATGCGATCACCAGTGT-3'; β-actin, forward 5'- GAATCAATGCAAGTTCGGTTCC -3' and reverse 5'- TCATCTCCGCTATTAGCTCCG -3'.

Western blotting

Cell lysates was extracted by RIPA cell lysis buffer (Beyotime, China). Total proteins (5 μg) were separated via electrophoresis in SDS-PAGE and then transferred to PVDF membrane followed by blockade of membranes with 5 % of milk. The blocked membranes were probed with the proper primary and secondary antibodies. The signal of protein bands was detected using ECL Detection reagents (Sigma-Aldrich, USA). The primary antibodies used were as follows: USP22 (ab227523, 1 : 1000 dilution), glucose transporter 1 (GLUT1) (ab15309, 1 : 1000 dilution), hexokinase-2 (HK2) (MBS200075, 1 : 1500 dilution), FoxM1 (ab180710, 1 : 1000 dilution) and β-actin (CST#4967, 1 : 5000 dilution).

Statistical analysis

All results were analyzed using SPSS 19.0 (IBM, USA) and shown as mean $\pm$ SEM. Differences between two groups were analyzed using by *t*-test. Differences among multiple groups were analyzed using by one-way ANOVA. The OS were analyzed using Kaplan-Meier's method (log-rank test). $p < 0.05$ was considered statistically significant difference.

Results

Expression of USP22 was upregulated in cervical cancer

Samples were collected from 60 pairs of cervical cancer and adjacent normal tissues, and the mRNA expression of USP22 was examined using RT-qPCR assay. The results demonstrated that the mRNA expression of USP22 was higher in cervical cancer tissues than that in normal tissues (Figure 1A). Western blotting results showed that the protein expression of USP22 was upregulated in cervical cancer tissues compared with normal tissues (Figure 1B). USP22 expression was also negatively correlated with survival rate, which means high expression of USP22 was associated with poor outcomes in patients with cervical cancer (Figure 1C).

Upregulation of USP22 promoted cell proliferation in cervical cancer cells

In cervical cancer cell lines, the protein expression of USP22 was higher in ME180, Siha and Caski cells than that in normal cell line Et1/E6E7 (Figure 2A). Expression of USP22 was upregulated in Siha and Caski cells that transfected with USP22 plasmid while the expression of USP22 was downregulated in Siha and Caski cells that transfected with shUSP22 compared with the corresponding control groups (Figure 2B and S1A). 24, 48, 72 and 96 h after transfection, the cell proliferative activity was increased by overexpression of USP22 and decreased by suppression of USP22 in both Siha and Caski cells (Figure 2C and S1B). Similarly, the number of clone spots was increased by overexpression of USP22 and decreased by suppression of USP22 in cervical cancer cells (Figure 2D).

Overexpression of USP22 accelerated aerobic glycolysis in cervical cancer cells.

The lactate production, glucose uptake and LDH activity was measured using commercial kits. Upregulation of USP22 increased the production of lactate while downregulation of USP22 caused the contrary results in Siha and Caski cells (Figure 3A and S1C). Glucose uptake was increased transfected with USP22 plasmid while was decreased transfected with shUSP22 compared with the corresponding control groups (Figure 3B and S1D). LDH activity was significantly increased by overexpression of USP22 and decreased by suppression of USP22 in cervical cancer cells (Figure 3C and S1E).

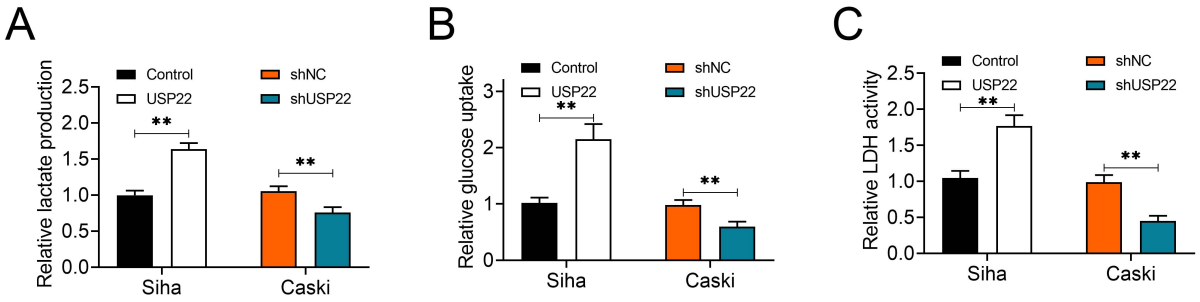

Figure 3. — Overexpression of USP22 accelerated aerobic glycolysis in cervical cancer cells. (A) Lactate production was increased by overexpression of USP22 and decreased by suppression of USP22; (B) Glucose uptake was increased by overexpression of USP22 and decreased by suppression of USP22; (C) LDH activity was increased by overexpression of USP22 and decreased by suppression of USP22. $^{**}p < 0.01$ *vs.* control or shNC.

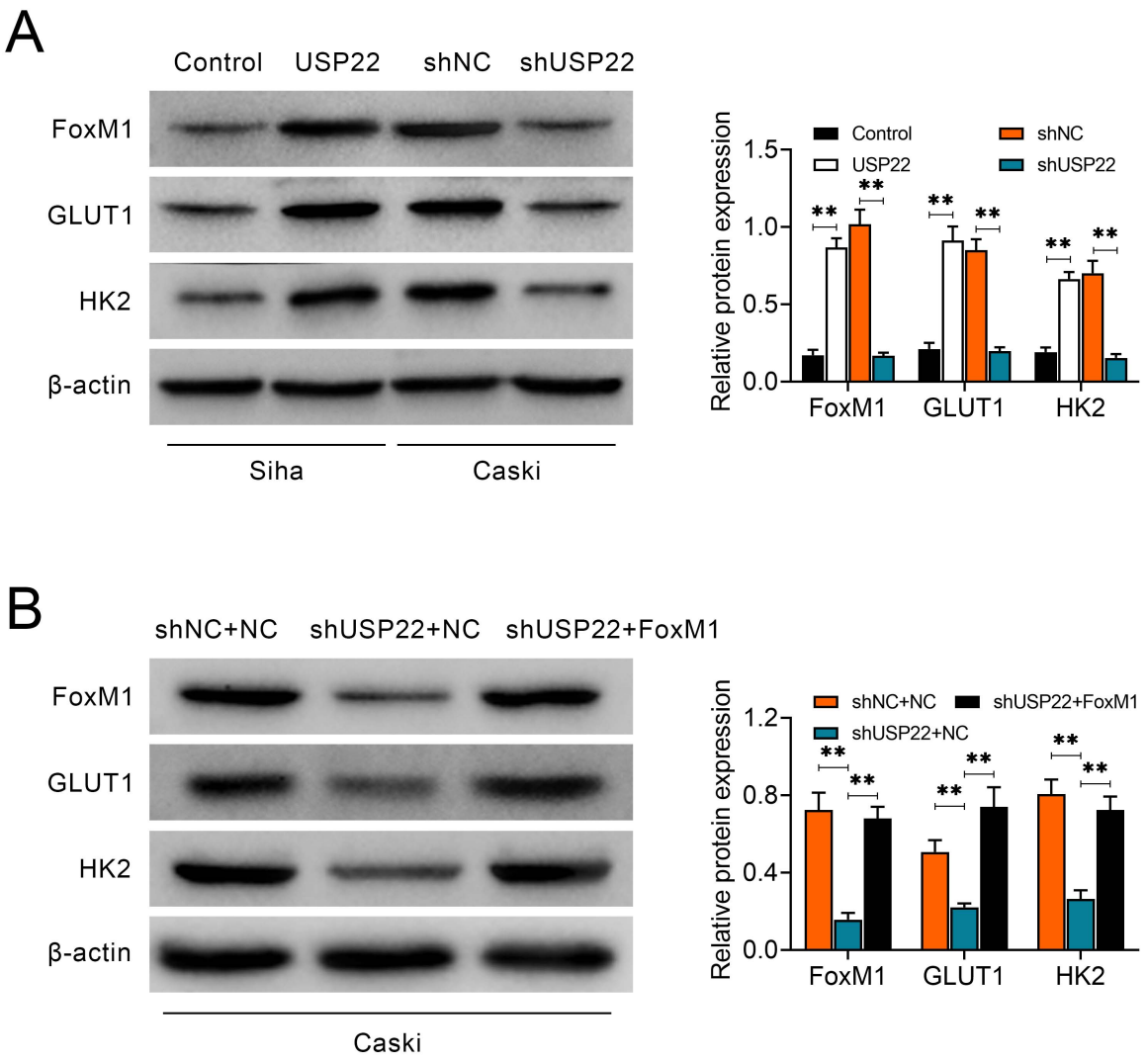

Figure 4. — USP22 upregulated the expression of GLUT1 and HK2 through FoxM1 in cervical cancer cells. (A) The protein expression levels of FoxM1, GLUT1 and HK2 were upregulated by overexpression of USP22 and downregulated by suppression of USP22; (B) The protein expression levels of FoxM1, GLUT1 and HK2 were downregulated by shUSP22 and upregulated by co-transfection of shUSP22 and FoxM1. $^{**}p < 0.01$ *vs.* shNC + NC or shUSP22 + NC. NC: negative control of FoxM2 plasmid.

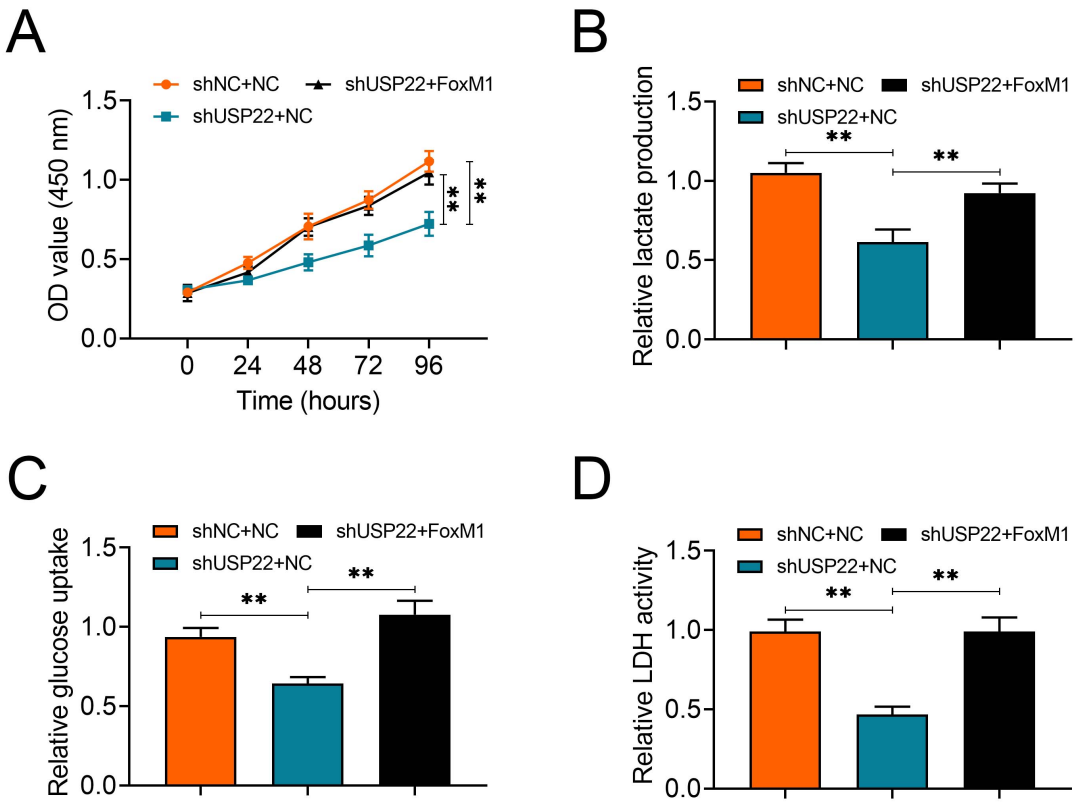

Figure 5. — FoxM1 mediated USP22-induced cell proliferation and glycolysis in cervical cancer cells. (A) Cell proliferation was inhibited by shUSP22 and promoted by co-transfection of shUSP22 and FoxM1; (B) Lactate production was decreased by shUSP22 and increased by co-transfection of shUSP22 and FoxM1; (C) Glucose uptake was decreased by shUSP22 and increased by co-transfection of shUSP22 and FoxM1; (D) LDH activity was decreased by shUSP22 and increased by co-transfection of shUSP22 and FoxM1. $^{**}p < 0.01$ *vs.* shNC + NC or shUSP22 + NC.

USP22 upregulated the expression of GLUT1 and HK2 through FoxM1 in cervical cancer cells.

The protein expression of FoxM1 was upregulated by overexpression of USP22 in Siha cells and downregulated by suppression of USP22 in Caski cells (Figure 4A). Besides, the protein expression levels of both GLUT1 and HK2 were increased in Siha cell transfected with USP22 plasmid and decreased in Caski cell transfected with shUSP22 compared with the corresponding control groups (Figure 4A). In Caski cells that co-transfected with shUSP22 and FoxM1 plasmid, FoxM1 was overexpressed compared with that transfected with shUSP22 alone (Figure 4B). The protein expression levels of both GLUT1 and HK2 were upregulated by co-transfection of shUSP22 and FoxM1 plasmid compared with transfection of shUSP22 alone (Figure 4B).

Effects of USP22 were mediated by FoxM1 in cervical cancer cells.

CCK-8 assay indicated that the cell activity was increased in Caski cells that co-transfected with shUSP22 and FoxM1 plasmid compared with that transfected with shUSP22 alone (Figure 5A). Lactate production was significantly increasedin cells that co-transfected with shUSP22 and FoxM1 plasmids compared with that transfected with shUSP22 alone (Figure 5B). Similarly, both glucose uptake and LDH activity were reduced by shUSP22 while was attenuated by co-transfection of shUSP22 and FoxM1 in Caski cells (Figure 5C and 5D).

Discussion

Upregulation of USP22 has been proved in various cancers and it is reported that upregulation of USP22 is correlated with poor prognosis [14]. The results from this study demonstrated that USP22 was upregulated in cervical cancer and high expression of USP was associated with poor outcomes, which was consistent with previous studies [16]. It has been reported that overexpression of USP22 could promote cell cycle initiation from G1 phase to S phase and facilitate cell proliferation [15]. In this study, the effects of USP22 on cell proliferation were confirmed by CCK-8 assay and clone formation assay, demonstrating that upregulation of USP22 was involved in the tumorigenesis of cervical cancer.

Aerobic glycolysis, frequently occurred in cancers, is the process of oxidation of glucose into pyruvate [17]. Under anaerobic condition, LDH could catalyze pyruvate to lactate and cause increased production of lactate [18]. In the present study, both LDH activity and lactate production was increased by overexpression of USP22 and decreased by shUSP22 in cervical cancer, indicating that USP22 could accelerate aerobic glycolysis. In addition, aerobic glycolysis could also facilitate the glucose uptake of cancer cell [17]. This study also demonstrated that glucose uptake was upregulated by overexpression of USP22 and decreased by suppression of USP22, further confirming that USP22 promoted aerobic glycolysis in cervical cancer. GLUT1 is the most common glucose transporter and high expression of GLUT1 indicates the enhancement of glucose uptake and results in the aggressiveness of cancers [19]. Overexpression of GLUT1 was also positively correlated with poor prognosis in cancer [20]. This study showed that expression of GLUT1was upregulated by USP22, implying that UPS22 might promote the progression of cervical cancer by regulating GLUT1. Besides, glycolysis was also tightly controlled by hexokinases (HKs), and inhibition of HK2 repressed the cell proliferation and decreased glycolysis activity [21]. Results from this study demonstrated that expression of HK2 was regulated by USP22, suggesting that regulation of glycolysis might be closely associated with USP22 and HK2. Taken together, these results manifested that overexpression of USP22 facilitated aerobic glycolysis through overexpression of GLUT1 and HK2.

FoxM1 has been reported to be involved in USP22-induced cancer progression, including pancreatic cancer and lung cancer. [15, 22]. However, no published studied considering the relationship between USP22 and FoxM1 in cervical cancer. The present study revealed that FoxM1 was upregulated by overexpression of USP22 in cervical cancer cells, which was consistent with previous studies, showing that overexpression of FoxM1 was associated with the development and progression of cervical cancer [23]. Moreover, this study also found that overexpression of FoxM1 attenuated shUSP22-induced repression of cervical cancer cell proliferation, proving that the progression of cervical cancer induced by overexpression of USP22 was mediated by FoxM1.

In conclusion, this study showed that expression of USP22 was upregulated in cervical cancer tissues and cell lines, which was associated with poor outcomes in patients with cervical cancer. In addition, this study showed that the promoter effects of USP22 on regulating cell proliferation and glycolysis in cervical cancer was mediated by FoxM1, suggesting that USP22 and FoxM1 might be potential biomarkers and therapeutic targets for patients with cervical cancer.

Authors' contributions

JX designed the study, supervised the data collection, analyzed the data, QT interpreted the data and prepare the manuscript for publication, and TL supervised the data collection, analyzed the data and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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Conflict of Interest

The authors state that there are no conflicts of interest to disclose.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.31083/j.ejgo.2020.05.2158.

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(Forkhead box M1): Proliferation-Specific Expression, Transcription Factor Function, Target Genes, Mouse Models, and Normal Biological Roles. Advances in Cancer Research, 2013, volume 118, pages 97–398". In: Tew K.D., Fisher P.B., editors. Advances in Cancer Research: Academic Press; 2013. p. 191.

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