Article

Discovery Medicine 2023; 35(176): 208–220
https://doi.org/10.24976/Discov.Med.202335176.22

FOXM1 Contributes to Chemotherapy Sensitivity in Cervical Cancer by Regulating TTK

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Published: 2 June 2023

Background: The emergence of chemotherapy resistance usually causes therapeutic failure in advanced cervical cancer. Forkhead box protein M1 (FOXM1) and threonine tyrosine kinase (TTK) are closely associated with cancer drug sensitivity, but the mechanism of FOXM1 on TTK involvement in chemo-treated cervical cancer remains unclear. Here, we aimed to observe the effects of FOXM1 on TTK and on chemotherapy sensitivity in cervical cancer.

Methods: The expressions of FOXM1 and TTK in cervical cancer tissues and para-cancerous tissues were analyzed by immunohistochemistry. SiHa and Hela cells were transfected with human lentivirus-FOXM1, small interfering RNA (siRNA) or pcDNA3.1/FOXM1 to analyze the changes in TTK protein expression. Furthermore, the cells were treated with paclitaxel (8 μM) or cisplatin (10 μM) to analyze the effects of FOXM1 on chemotherapy sensitivity. SiHa cells were used to construct a xenograft model to study the effects of FOXM1 expression in response to paclitaxel treatment. The tumor size and weight were observed. The expressions of Ki-67, FOXM1, and TTK protein in tumor tissues were measured by immunohistochemistry.

Results: High expression of FOXM1 and TTK were found in the cervical cancer tissues ($p < 0.05$). The TTK protein expressions were decreased by FOMX1-siRNA transfection in SiHa and Hela cells ($p < 0.01$). The cell viability and cell cycle were also suppressed by FOMX1-siRNA transfection ($p < 0.01$) but enhanced by pcDNA3.1/FOXM1 transfection ($p < 0.01$). For paclitaxel or cisplatin treatment, the cell viability and cell DNA damage were improved due to the FOXM1 overexpression ($p < 0.01$). TTK inhibitor significantly suppressed the effects of FOXM1 overexpression ($p < 0.01$).

Conclusions: FOXM1 regulated TTK and affected the therapeutic efficacy of cisplatin and paclitaxel in cervical cancer.

Keywords: forkhead box protein M1 (FOXM1); paclitaxel; cisplatin; cervical cancer; threonine tyrosine kinase (TTK)

Introduction

Cervical cancer is the fourth most common cancer among women [1,2]. In cervical cancer, a total of 15 high-risk strains of human papillomavirus (HPV) are identified, of which the most high-risk HPV strains are HPV 16 and 18, accounting for more than 70% of cervical cancer [1,3]. For cervical cancer treatment, patients are usually treated with radiotherapy or surgery, then performed with systemic chemoradiotherapy [4,5]. However, the emergence of chemotherapy resistance usually causes therapeutic failure, promoting researchers continuing to study the mechanism of chemotherapy resistance to cervical cancer.

Forkhead box protein M1 (FOXM1), a member of the forkhead superfamily of transcription factors, is closely involved with the processes of cell proliferation, self-renewal and tumorigenesis [6,7]. FOXM1 is overexpressed in most human cancers, including the cervix, liver, prostate, breast, etc., which indicates a poor prognosis for cancer patients [8,9]. Furthermore, many researchers have found that FOXM1 is closely associated with cancer drug sensitivity, and downregulation of FOXM1 abrogates drug resistance in different cancer cells [10–12], including cervical cancer [13,14]. However, the mechanisms of FOXM1 involvement in chemo-treated cervical cancer remain unclear.

Threonine tyrosine kinase (TTK), also known as monopolar spindle 1 (MPS1), is a mediator of the spindle assembly checkpoint, which plays an indispensable role in maintaining genomic integrity by delaying anaphase until all chromosomes are properly attached to the mitotic spindle [15,16]. For many cancer treatments, TTK suppression is a promising therapeutic strategy and many TTK inhibitors have been assessed in clinical trials [17,18]. Therefore, the mechanisms of TTK-mediated drug sensitivity needed further investigation. Several studies have shown that FOXM1 is widely spatiotemporally expressed during the cell cycle, cell proliferation and DNA damage repair [12,19], suggesting there is a potential interaction of FOXM1 and TTK in cancer chemotherapy. However, their potential interaction and effects on chemo-treated cervical cancer have not been explored.

Here, we observed the expressions of FOXM1 and TTK in cervical cancer tissues and analyzed whether FOXM1 regulates TTK to contribute to chemotherapy sensitivity in chemo-treated cervical cancer cells.

Materials and Methods

Participants

A total of 62 cervical cancer patients were recruited from January 1st 2020 to January 28th 2021 at the Department of Gynecology. The cervical tissues of all cases were histologically confirmed by pathologists and no patients had received chemotherapy or radiotherapy. All tissue specimens were immediately frozen at –80 °C. The characteristics of all patients are summarized in Table 1.

Table 1. Clinical characteristics of 62 cervical cancer patients.

Variable	Case numbers (%)
Age (year)	46.66 ± 9.42
Weight (kg)	60.73 ± 9.72
Histopathology	
Squamous cell carcinoma	57 (91.94%)
Small cell carcinoma	5 (8.06%)
FIGO stage	
IB1	7 (11.29%)
IB2	49 (79.03%)
IB3	6 (9.68%)
Disease sites	
Cervix	62 (100%)

FIGO, Federation International of Gynecology and Obstetrics; IB, Grade I and part B.

Cell Lines

Human cervical cancer cell lines, SiHa (#FH0309, HPV 16) and Hela (#FH0313, HPV 18), were obtained from Fuheng Biology (https://www.fudancell.com/, Shanghai, China), and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% streptomycin at 37 °C and 5% CO_2. The cell lines were detected by mycoplasma testing and authenticated using short tandem repeats (STR). The STR results, cell culture and preservation are shown in **Supplementary Material**.

Cells Transfection

SiHa and Hela cells (3×10^5 cells/well) were transfected with 10 μg human pcDNA3.1 plasmid or pcDNA3.1/FOXM1 (#T03025, GenePharma, Shanghai, China) for 48 h, according to a previous report [20]. Briefly, the prepared plasmids were cultured with cells for 48 h, then the cells were selected using puromycin over 2–3 weeks to obtain stably transfected cells.

SiHa and Hela cells (3.13×10^5 cells/well) were treated with 100 nM control small interfering RNA (siRNA) or specific FOXM1 siRNA (5′-CUCUUCUCCCUCAGAUAUATT-3′) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 48 h using Lipofectamine™ 2000 Transfection Reagent (#11668019, ThermoFisher Scientific, Pittsburgh, PA, USA).

Cell Treatment

In the first design, the SiHa and Hela cells were randomly divided into the control group, FOXM1-siRNA group, pcDNA3.1/FOXM1 (FOXM1) group, TTK inhibitor group and pcDNA3.1/FOXM1 plus TTK inhibitor (FOXM1+TTK inhibitor) group.

In the TTK inhibitor or FOXM1+TTK inhibitor groups, the cells were treated with 2 μM TTK selective small-molecule inhibitor MPS1-IN-3 (#HY-12401, MedChemExpress, Shanghai, China) [21] for 24 h.

In the second design (based on the first design), the cells of each group were treated with 8 μM paclitaxel (#HY-B0015, MedChemExpress, Shanghai, China) or 10 μM cisplatin (#HY-17394, MedChemExpress, Shanghai, China).

Cell Viability

The cell viability of SiHa and Hela cells (1×10^4 cells/well) was measured using a cell counting kit-8 (CCK-8) (#HY-K0301, MedChemExpress, Shanghai, China). In each well, 10 μL of CCK-8 solution was added for 4 h at 37 °C, and the absorbance (450 nm) was obtained using a microplate reader (#ELx808, BioTek, Burlington, VT, USA).

Cell Cycle Analysis

The harvested SiHa and Hela cells were fixed overnight using 70% precooled alcohol at 4 °C. After washing and suspending with phosphate-buffered saline (PBS), the cells were incubated with RNase (100 mg/mL) and propidium iodide (PI, 50 mg/mL) for 30 min at 4 °C. The cell cycle was analyzed using flow cytometry (batch number: FACS Calibur, BD, Franlin Lakes, NJ, USA) within 60 min.

Cell DNA Damage

The cell DNA damage was observed by γ-H2A histone family member X (H2AX) immunofluorescence (#C2035S, Beyotime, Shanghai, China). The fixed cells were washed and blocked with immunostaining blocking solution for 15 min, then cultured with γ-H2AX antibody at room temperature overnight. The nucleus was labeled with 4,6-diamidino-2-phenylindole (DAPI).

Western Blotting

Total protein from cells was collected using radioimmunoprecipitation assay buffer (RIPA) buffer (#R0010, Solarbio, Beijing, China), separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-

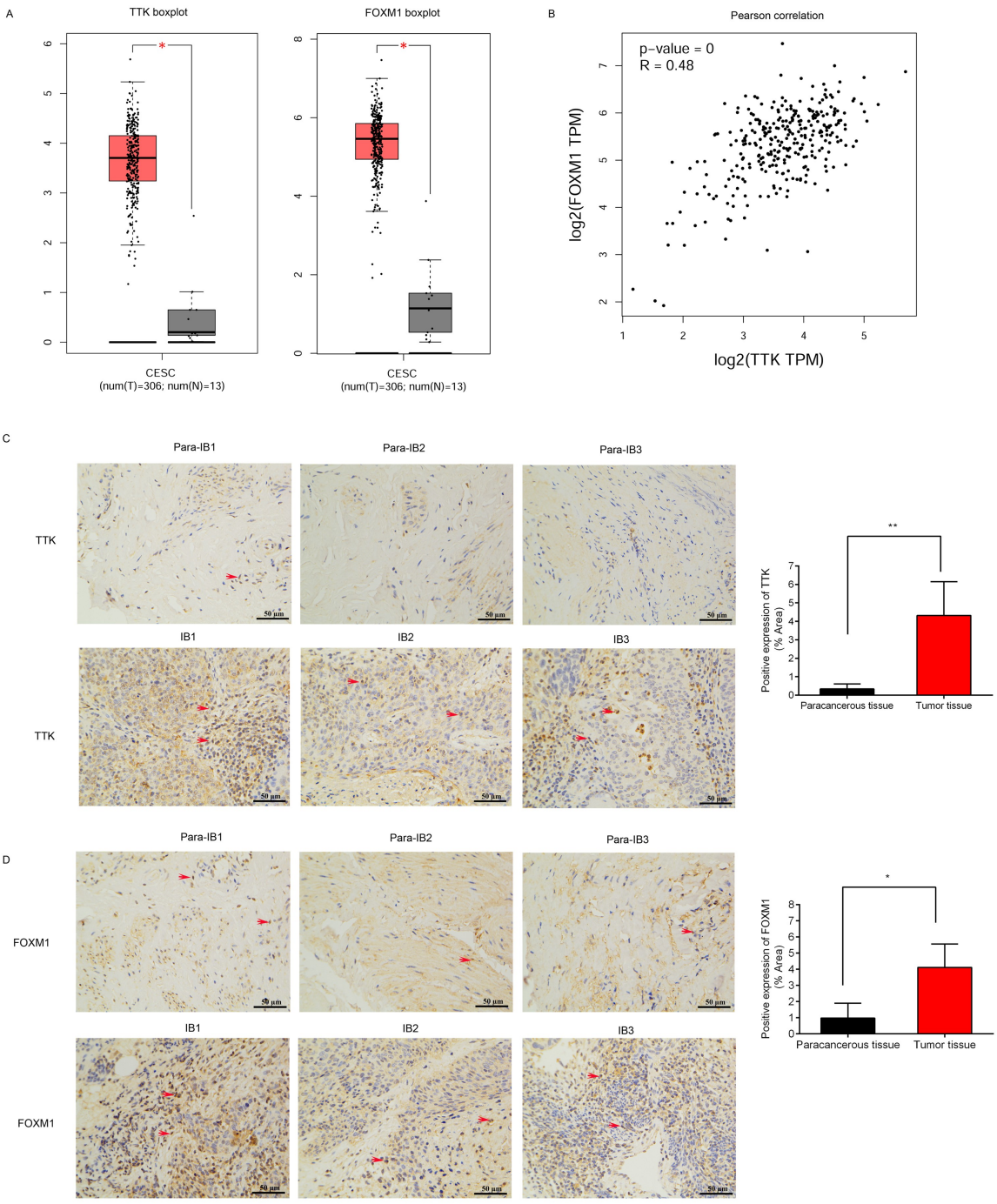

Fig. 1. TTK and FOXM1 expression levels in cervical cancer tissues. (A) The significance of TTK and FOXM1 expression between cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) tissues and normal tissues was analyzed through the GEPIA online website. (B) The correlation of TTK and FOXM1 in the CESC. The (C) TTK and (D) FOXM1 expression levels in tumor tissues and para-cancerous tissues were measured using immunohistochemistry. Red arrows: Positive expression. $^{*}p < 0.05$, $^{**}p < 0.01$.

PAGE) (#3450121, Bio-Rad, Shanghai, China), and then transferred to polyvinylidene fluoride (PVDF) membranes (#1706527, Bio-Rad, Shanghai, China). The blocked membranes were incubated with appropriate primary antibodies TTK (1:1000, #3255S), FOXM1 (1:1000, #5436), cleaved-caspase-3 (1:1000, #9661), cleaved-caspase-9 (1:1000, #7237) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:1000, #5174) overnight at 4 °C. After washing with tris-buffered saline (TBS)-0.01% Tween 20, the membranes were cultured with the secondary antibody Goat anti-Rabbit IgG (H+L) (1:1000, #14708) for 2 h at 25 °C. All the above antibodies were obtained from Cell Signaling Tech-

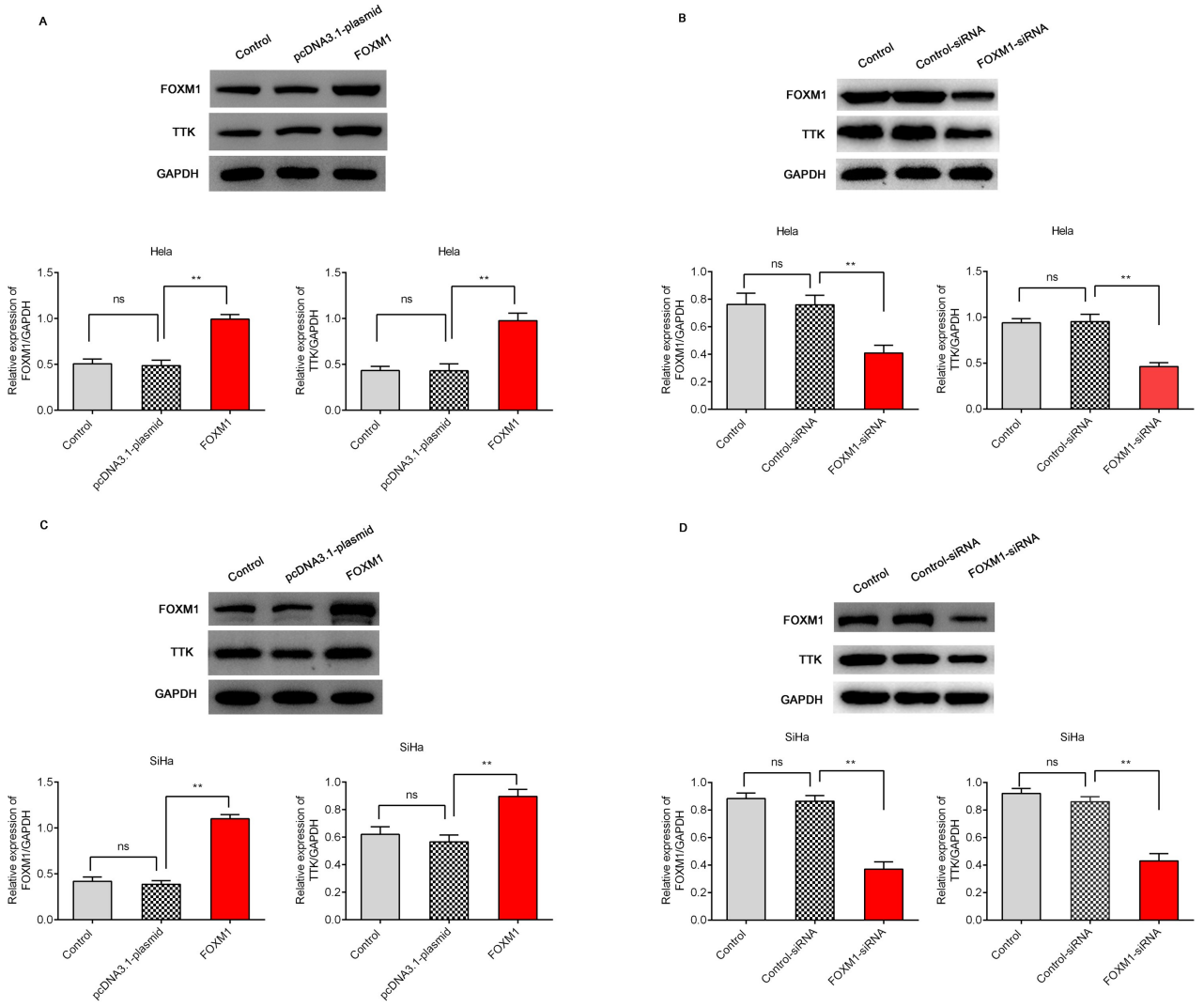

Fig. 2. The expression levels of TTK and FOXM1 protein were observed by western blot in different transfected Hela and SiHa cells (n = 3). The Hela cells were treated with pcDNA3.1 plasmid or pcDNA3.1/FOXM1 (A), and transfected with control siRNA or FOXM1 siRNA (B). The SiHa cells were treated with pcDNA3.1 plasmid or pcDNA3.1/FOXM1(C), and transfected with control siRNA or FOXM1 siRNA (D). ns, no significance. $^{**}p < 0.01$.

nology (Shanghai, China). An enhanced chemiluminescence reagent (#D085075, Bio-Rad, Shanghai, China) was used to visualize the protein bands. The quantitative analysis of protein bands was performed using ImageJ software (Version 2020, National Institutes of Health, Bethesda, MD, USA).

Animals

Twenty-four female BALB/c nude mice (5–7 weeks old) were obtained (Beijing Vital River Laboratory Animal Technology Co., Ltd., Beijing, China) and fed under a sterile environment with a 12-h/12-h light-dark cycle, providing unlimited access to water and food.

Mouse Xenograft Model

In this study, SiHa cells were used to construct a cervical cancer xenograft model to study whether FOXM1 regulates TTK in response to paclitaxel treatment. Mice were anesthetized with 1% pentobarbital sodium (50 mg/kg), and then SiHa cells (1×10^6 cells) were transfected with control plasmid or stable pcDNA3.1/FOXM1 and they were respectively injected into the subcutaneous tissue of the right hind limb of the mice.

Animal Treatment

The mice were randomly divided into the following groups: Control group, in which the mice were injected with SiHa cells transfected with control plasmid for 7 days, then treated with 5 mg/kg paclitaxel [22] by tail vein in-

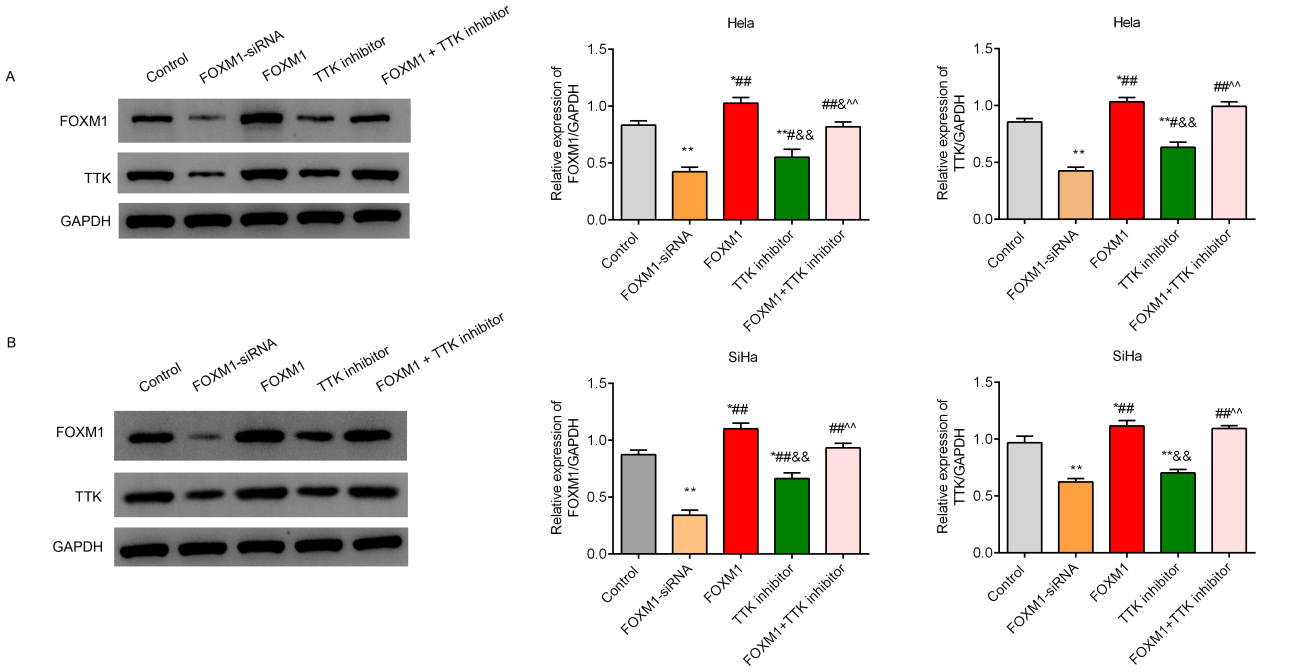

Fig. 3. The FOXM1 and TTK protein expressions in (A) Hela and (B) SiHa cells with different treatments were measured by western blot (n = 3). (A) The FOXM1 and TTK expression in Hela cells. (B) The FOXM1 and TTK expression in SiHa cells. TTK inhibitor: Cells were treated with 2 μM MPS1-IN-3 *vs* the control group, $^{*}p < 0.05$, $^{**}p < 0.01$ *vs* the FOXM1-siRNA group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$. *vs* the FOXM1 group, $^{\&}p < 0.05$, $^{\&\&}p < 0.01$ *vs* the TTK inhibitor group, $^{\wedge\wedge}p < 0.01$.

jection per week for 3 weeks; TTK inhibitor group, in which the mice were injected with SiHa cells transfected with control plasmid for 7 days, then treated with 2 mg/kg MPS1-IN-3 [21] and 5 mg/kg paclitaxel by tail vein injection per week for 3 weeks; FOXM1 group, in which the mice were injected with SiHa cells transfected with stable pcDNA3.1/FOXM1 for 7 days, then treated with 5 mg/kg paclitaxel by tail vein injection per week for 3 weeks; And FOXM1+TTK inhibitor group, in which the mice were injected with SiHa cells transfected with stable pcDNA3.1/FOXM1 for 7 days, then treated with 2 mg/kg MPS1-IN-3 and 5 mg/kg paclitaxel by tail vein injection per week for 3 weeks. There were 6 mice in each group.

Tumor volume (width^2 $\times$ depth $\times$ 0.5) was estimated every week. After 28 days, all anesthetized mice were sacrificed through decapitation. The tumor weights were obtained.

Immunohistochemistry

The tumor sections were soaked in xylene and in different volume fractions of ethanol (100%, 95%, 80%, and 70%). After washing, the sections were submerged in sodium citrate antigen repair solution. After boiling, the sections were cooled and then blocked for 30 min. All antibodies were purchased from Cell Signaling Technology (Shanghai, China). After washing, diluted primary Ki-67 rabbit-antibody (1:400, #9027), TTK (1:400, #3255S) and FOXM1 (1:400, #5436) were added and incubates at 4 °C overnight. After washing with PBS, the sections were incubated with the secondary antibody Goat anti-Rabbit IgG (H+L) (1:800, #14708) at 37 °C for 20 min. Finally, the sections were soaked in different volume fractions of ethanol (70%, 80%, 90%, 95%, 100%) and xylene. Neutral glue was used to seal the slides.

Data Analysis

All data were analyzed by SPSS Statistics, version 19.0 (IBM Corp., Armonk, NY, USA) and showed as mean $\pm$ standard deviation. For differences among groups, a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was performed. $p < 0.05$ was considered significant.

Results

FOXM1 and TTK Showed High Expression in Cervical Cancer Tissues

Through the Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/) website, we found that FOXM1 and TTK expressions were significantly increased in cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) tissues by contrast to the normal tissues ($p < 0.05$) (Fig. 1A). Furthermore, a significant association was found between the FOXM1 and TTK expressions by Pearson correlation ($p = 0.00$) (Fig. 1B). To confirm the TTK (Fig. 1C) and FOXM1

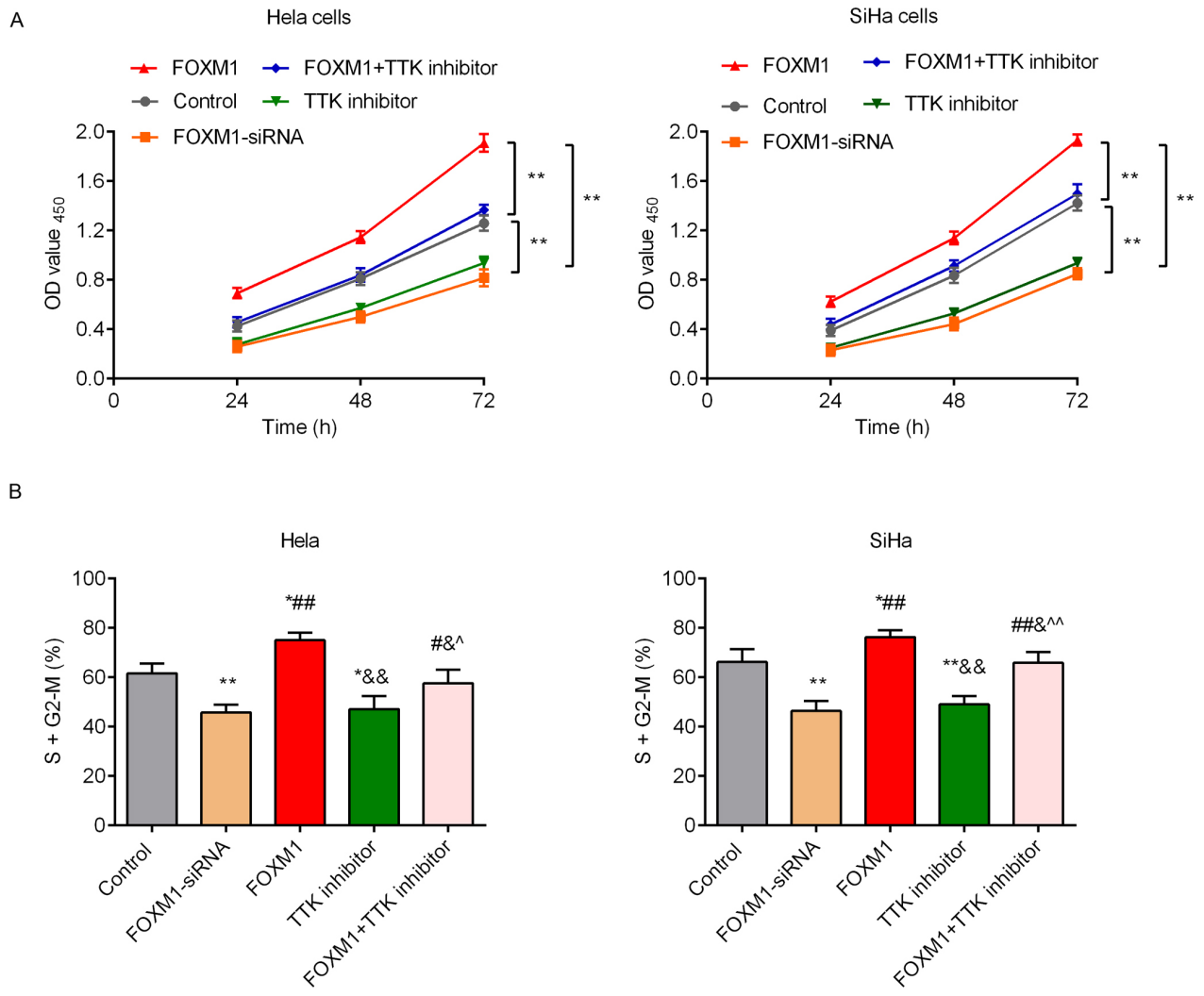

Fig. 4. The cell viability and cell cycle were observed in cervical cancer cells of each group (n = 3). (A) Cell viability was measured by CCK-8. (B) The cell cycle was measured by flow cytometry. The images of the cell cycle are shown in **Supplementary Fig. 1**. *vs* the control group, $^{*}p < 0.05$, $^{**}p < 0.01$. *vs* the FOXM1-siRNA group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$. *vs* the FOXM1 group, $^{\&}p < 0.05$, $^{\&\&}p < 0.01$. *vs* the TTK inhibitor group, $^{\wedge}p < 0.01$, $^{\wedge\wedge}p < 0.01$.

(Fig. 1D) expressions in cervical cancer, we analyzed their expression in tumor tissue and para-cancerous tissue by immunohistochemistry. The results showed that the TTK and FOXM1 expressions were higher in the tumor tissues than that in the para-cancerous tissues ($p < 0.05$).

FOXM1 Regulated TTK in Hela and SiHa Cells

After transfection with pcDNA3.1-plasmid control or pcDNA3.1/FOXM1, the FOXM1 and TTK protein expression levels were observed in Hela cells (Fig. 2A). The FOXM1 and TTK protein expression levels were significantly upregulated after being transfected with pcDNA3.1/FOXM1 compared with the pcDNA3.1-plasmid control treatment ($p < 0.01$). Meanwhile, the Hela cells were transfected with control siRNA or FOXM1 siRNA to observe the changes in FOXM1 and TTK protein expression (Fig. 2B). The FOXM1 and TTK expression levels were significantly downregulated after transfection with FOXM1 siRNA compared with the control siRNA ($p < 0.01$). Compared with the control cells, no difference was found in the two protein expression levels after transfected with pcDNA3.1-plasmid control or control siRNA in cells ($p > 0.05$).

Similar effects were observed in SiHa cells (Fig. 2C,D). Compared with the control transfection, the FOXM1 and TTK expression levels were markedly upregulated by pcDNA3.1/FOXM1 transfection, and downregulated by FOXM1 siRNA transfection ($p < 0.01$). No significant difference was found between the control cells and the cells transfected with pcDNA3.1-plasmid control or control siRNA ($p > 0.05$).

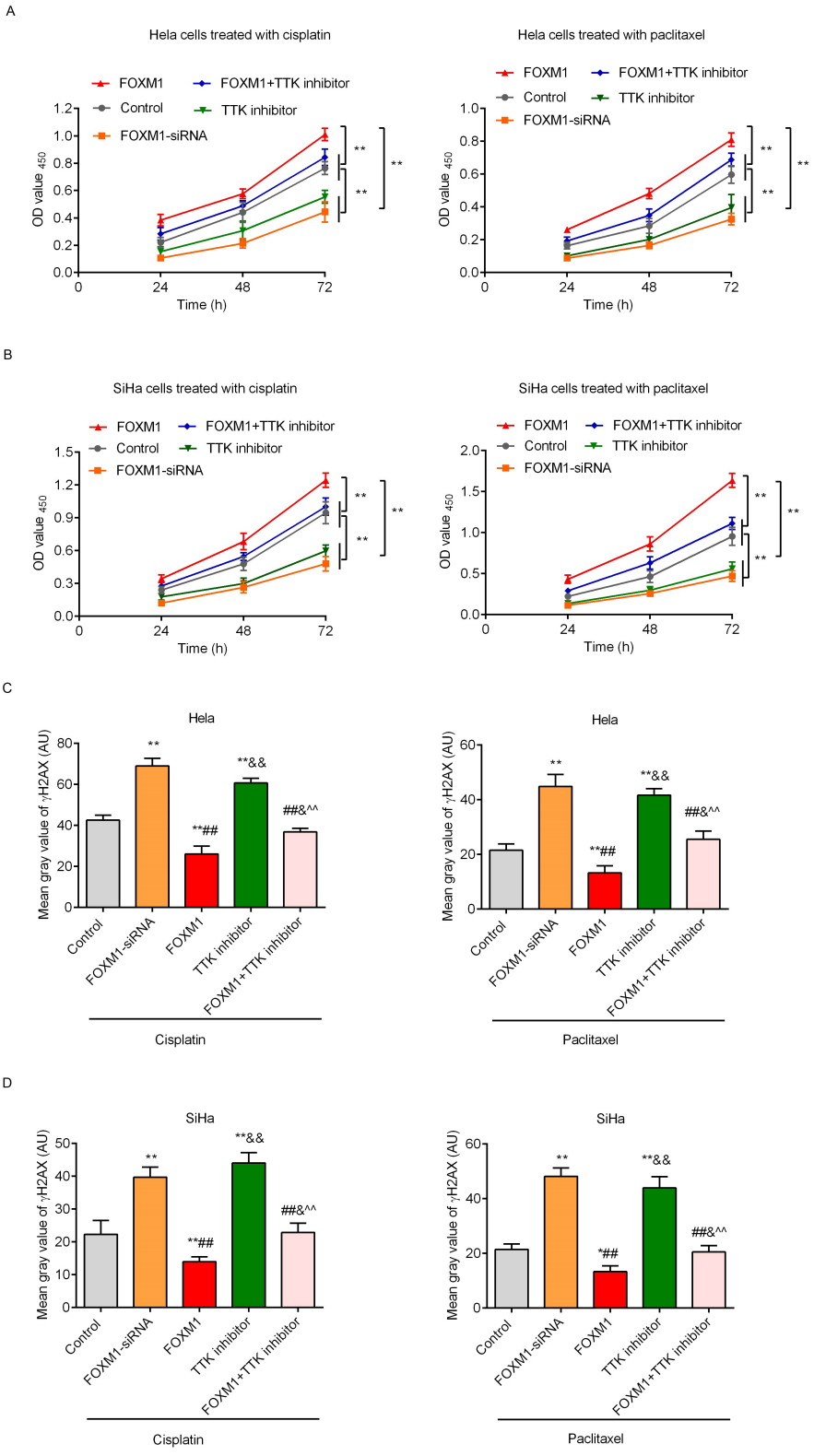

Fig. 5. The Hela and SiHa cells in each group were treated with10 μM cisplatin or 8 μM paclitaxel (n = 3). The cell viability of (A) Hela and (B) SiHa cells were analyzed by CCK-8. $^{**}p < 0.01$. The cell DNA damage of (C) Hela and (D) SiHa cells was analyzed by γ-H2AX immunofluorescence. The images of cell DNA damage are shown in **Supplementary Figs. 2 and 3**. *vs* the control group, $^{*}p < 0.05$, $^{**}p < 0.01$. *vs* the FOXM1-siRNA group, $^{\#\#}p < 0.01$. *vs* the FOXM1 group, $^{\&}p < 0.05$, $^{\&\&}p < 0.01$. *vs* the TTK inhibitor group, $^{\wedge\wedge}p < 0.01$.

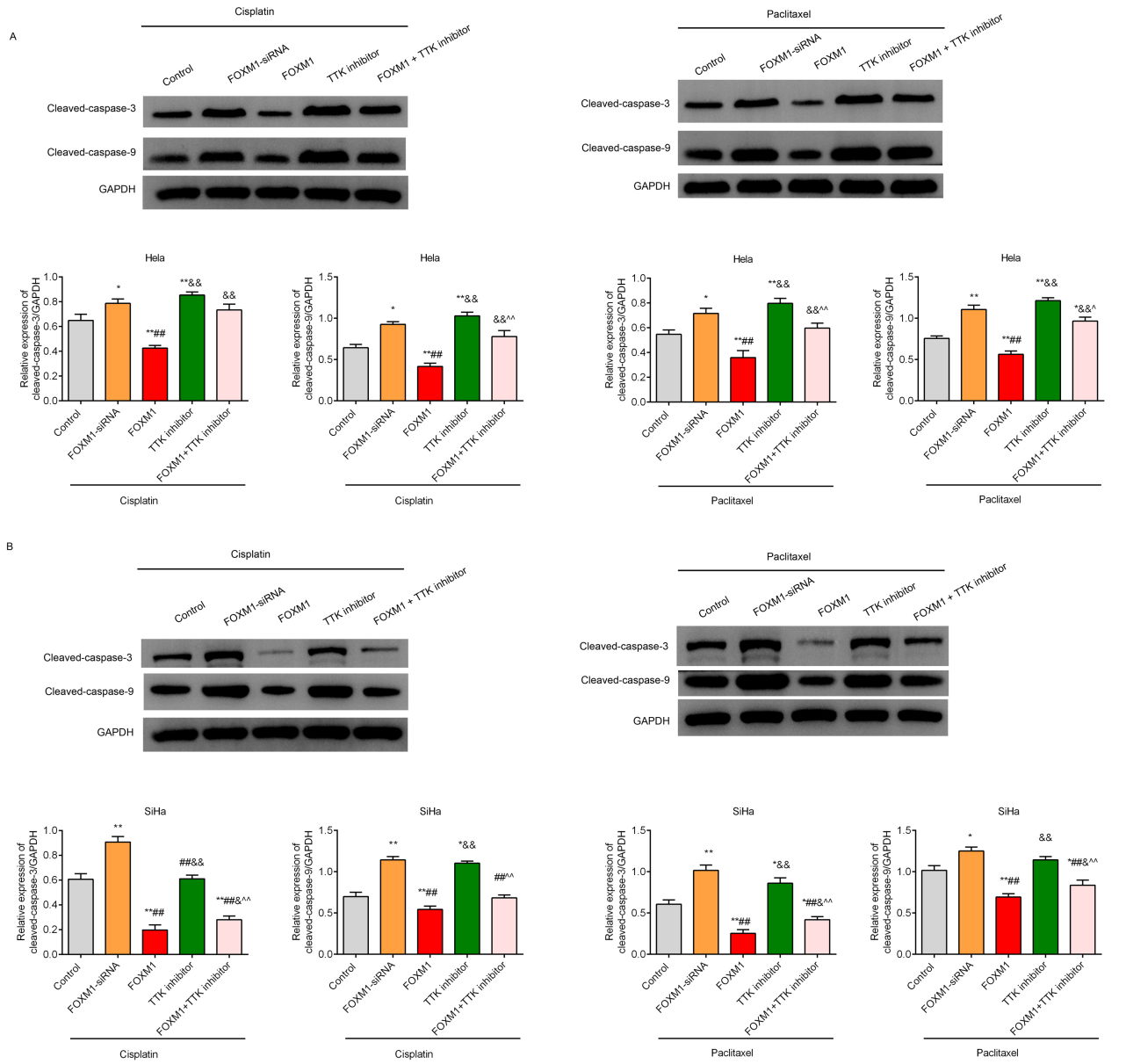

Fig. 6. The cleaved-caspase-3 and cleaved-caspase-9 protein expression levels in the cisplatin-treated or paclitaxel-treated (A) Hela and (B) SiHa cells (n = 3). (A) The cleaved-caspase-3 and cleaved-caspase-9 expression in Hela cells. (B) The cleaved-caspase-3 and cleaved-caspase-9 expression in SiHa cells. $^{*}p < 0.05$, $^{**}p < 0.01$. *vs* the control group, $^{\#\#}p < 0.01$. *vs* the FOXM1-siRNA group, $^{\&}p < 0.05$, $^{\&\&}p < 0.01$. *vs* the FOXM1 group, $^{\wedge}p < 0.05$, $^{\wedge\wedge}p < 0.01$. *vs* the TTK inhibitor group.

FOXM1 Regulated Cell Viability and Cell Cycle in Hela and SiHa Cells by Regulating TTK

Furthermore, cells with differential FOXM1 expression were used to analyze the effects of FOXM1 on TTK. Cells were divided into five groups (control, FOXM1-siRNA, FOXM1, TTK inhibitor, and FOXM1+TTK inhibitor). The FOXM1 and TTK protein levels in the Hela (Fig. 3A) and SiHa (Fig. 3B) cells of each group were measured by western blot. Compared with the control cells, the FOXM1 and TTK expression levels were clearly downregulated by FOXM1-siRNA transfection ($p < 0.01$) and were markedly upregulated by the pcDNA3.1/FOXM1 treatment ($p < 0.05$). Meanwhile, the FOXM1 and TTK protein levels were also decreased by TTK inhibitor relative to that in the control cells ($p < 0.05$).

The cell viability (Fig. 4A) and cell cycle (Fig. 4B) were observed in each group. The images of the G0–G1, S and G2/M phases of cells in each group were shown in **Supplementary Fig. 1**. The results showed that FOXM1 overexpression promoted cell viability and S+G2/M phase ratios compared with the control cells ($p < 0.01$). Silencing of FOXM1 significantly decreased cell viability and

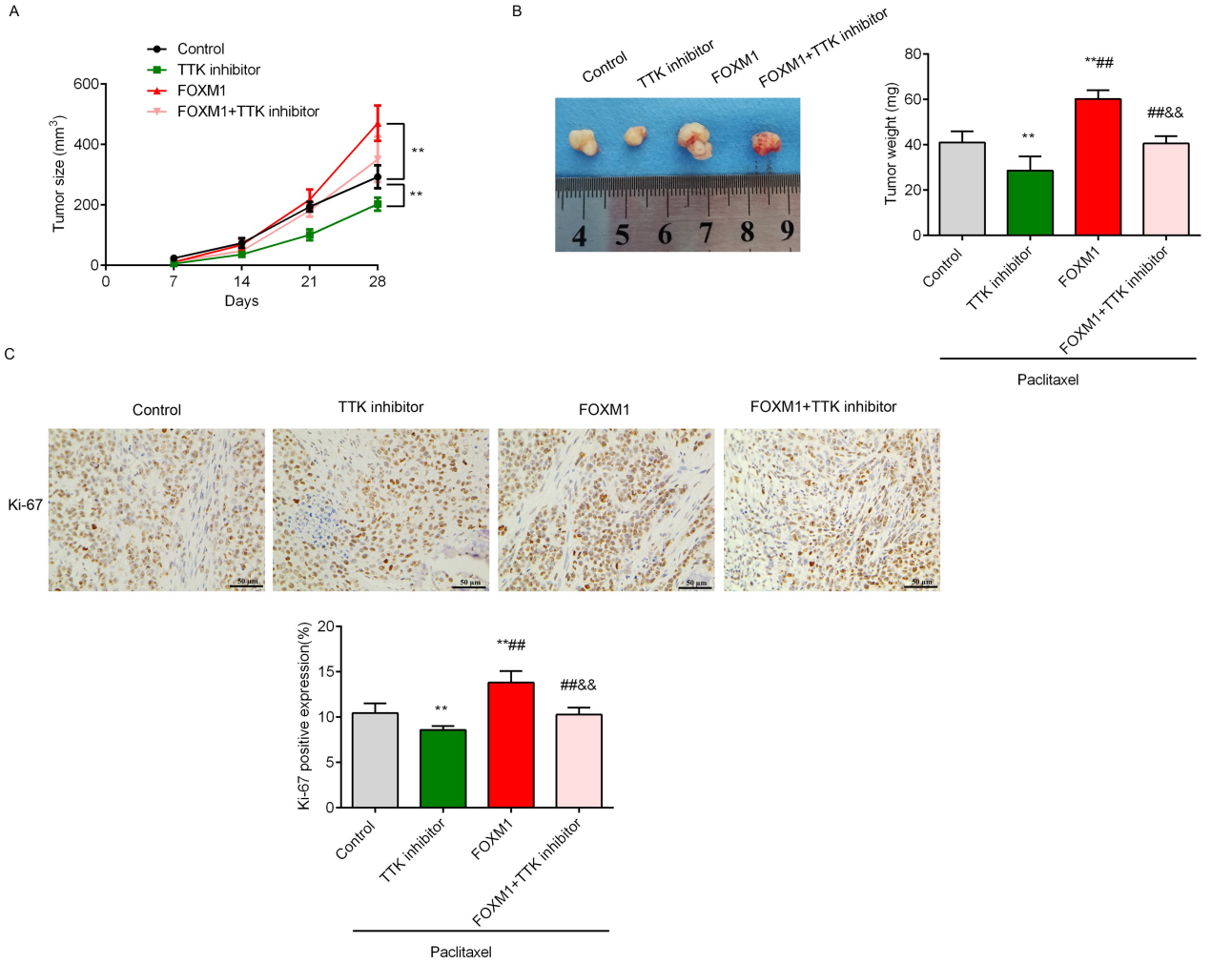

Fig. 7. The xenograft model was established by injection of SiHa cells transfected with control plasmid or stable pcDNA3.1/FOXM1. After 7 days of injection, 2 mg/kg MPS1-IN-3 and/or 5 mg/kg paclitaxel was injected into the tail vein once a week for 3 weeks (n = 6). (A) Tumor size. $^{**}p < 0.01$. (B) Tumor images and weight. (C) The Ki-67 positive expression in tumor tissues was analyzed. $^{**}p < 0.01$. *vs* the control group, $^{\#\#}p < 0.01$. *vs* the TTK inhibitor group, $^{\&\&}p < 0.01$. *vs* the FOXM1 group.

S+G2/M phase ratios compared with the control cells ($p < 0.01$). However, TTK inhibitor significantly suppressed the results of FOXM1 overexpression on cell viability and cell cycle ($p < 0.05$).

FOXM1 Regulated Cell Viability and DNA Damage by Regulating TTK in Chemo-Treated Hela and SiHa Cells

To evaluate the effects of FOXM1 on TTK in cisplatin-treated or paclitaxel-treated cervical cancer cells, the cells with differential FOXM1 expression were treated with 10 μM cisplatin or 8 μM paclitaxel. The cell viability of Hela (Fig. 5A) and SiHa cells (Fig. 5B) were observed, and the results showed that FOXM1-siRNA or TTK inhibitor significantly decreased the cell viability ($p < 0.01$). However, the cell viability was increased in the cervical cancer cells with FOXM1 overexpression ($p < 0.01$). Moreover, the effects of FOXM1 overexpression were suppressed by the TTK inhibitor ($p < 0.01$). Additionally, we investigated the DNA damage of Hela (Fig. 5C) and SiHa cells (Fig. 5D) by γ-H2AX immunofluorescence. The images of γ-H2AX immunofluorescence in each group are shown in **Supplementary Figs. 2 and 3**. The data further confirmed that FOXM1 overexpression significantly decreased the cell DNA damage induced by cisplatin or paclitaxel treatment ($p < 0.01$), and TTK inhibitor weakened the effects of FOXM1 overexpression ($p < 0.05$).

FOXM1 Regulated Cleaved-Caspase-3, -9 by Regulating TTK in Chemo-Treated Hela and SiHa Cells

The protein expression levels of cleaved-caspase-3, and -9 were measured in the cisplatin or paclitaxel-treated Hela and SiHa cells. In Fig. 6A, compared with the control cell, the FOXM1-siRNA or TTK inhibitor significantly increased the protein levels of cleaved-caspase-3, -9 in cis-

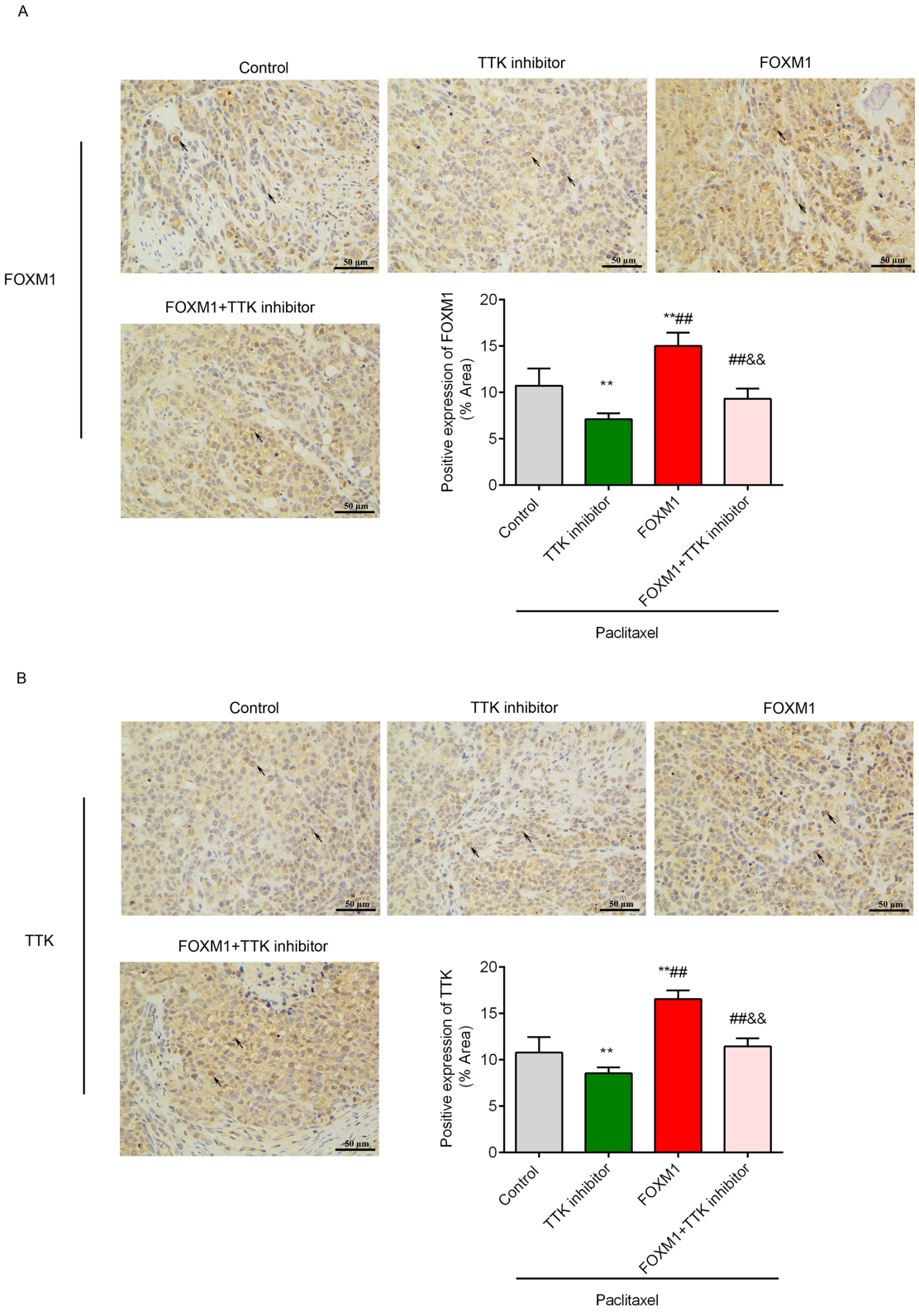

Fig. 8. The positive (A) FOXM1 and (B) TTK expression levels were analyzed in tumor tissues of each group (n = 6). (A) The FOXM1 expression in tumor tissues. (B) The TTK expression in tumor tissues. Black arrows represented the positive expression. $^{**}p < 0.01$. *vs* the control group, $^{\#\#}p < 0.01$. *vs* the TTK inhibitor group, $^{\&\&}p < 0.01$. *vs* the FOXM1 group.

platin or paclitaxel-treated Hela cells ($p < 0.05$), but the FOXM1 overexpression was decreased in the two protein levels ($p < 0.01$). TTK inhibitor suppressed the effects of FOXM1 overexpression on the two expression levels ($p < 0.05$). Similarly, in the cisplatin or paclitaxel-induced SiHa cells (Fig. 6B), FOXM1 overexpression downregulated the protein expression levels of cleaved-caspase-3, -9 relative to that in the control cells ($p < 0.01$), whereas the TTK inhibitor treatment suppressed the effects of FOXM1 overexpression ($p < 0.05$).

FOXM1 Regulated Paclitaxel Chemotherapy by Regulating TTK in the SiHa-Induced Xenograft Model

Next, we sought to confirm whether FOXM1 regulated TTK to affect the paclitaxel chemotherapy *in vivo*. We injected SiHa cells transfected with control plasmid or stable pcDNA3.1/FOXM1 into nude mice, and compared tumor size (Fig. 7A) and weight (Fig. 7B) among the different treatment groups. The results showed that the tumor growth was exacerbated due to the FOXM1 overexpression relative to the control group ($p < 0.01$). The TTK inhibitor treatment significantly suppressed the tumor growth and weakened the promoting tumorigenesis effects of FOXM1 ($p < 0.01$). Meanwhile, the Ki-67 expression in tumor tissues was also observed (Fig. 7C). The FOXM1 overexpression hampered the chemotherapeutic effect of paclitaxel in mice compared with the control group ($p < 0.01$), whereas the effects of FOXM1 overexpression were blocked by the TTK inhibitor ($p < 0.01$).

The expression of FOXM1 (Fig. 8A) and TTK (Fig. 8B) in tumor tissues of each group was measured. When contrasted to the control group, the FOXM1 and TTK expression levels were significantly decreased after treatment with TTK inhibitor ($p < 0.01$), but increased in the FOMX1overexpression group ($p < 0.01$). However, the increased FOXM1 and TTK expression levels induced by FOXM1 overexpression were significantly suppressed by TTK inhibitor treatment ($p < 0.01$).

Discussion

In this study, we confirmed that FOXM1 and TTK showed high expression levels in the cervical cancer tissues, which were consistent with many studies [23–25]. FOXM1 and TTK play important roles in cell cycle progression. Our data showed that FOXM1 might be a transcriptional regulator factor of TTK, affecting the chemosensitivity of cervical cancer cells.

The FOXM1 transcription factor is crucial for cell proliferation, cell cycle control, cell survival, senescence and DNA damage repair [6,12]. Several studies have indicated that high FOXM1 expression enhances chemotherapy resistance in many cancers, including prostate cancer [20], breast cancer [26], and cervical cancer [14]. FOXM1 regulates many cellular processes, for example, DNA repair, cell survival, drug efflux, and deregulated mitosis [12]. For instance, FOXM1 regulates cAMP-responsive element-binding protein (CREB) [27], kinesin family member 20A (KIF20A) [20], and signal transducer and activator of transcription 3 (STAT3) [28] to enhance cancer cell chemosensitization. In this study, we first found that FOXM1 is a regulator factor of TTK in Hela and SiHa cells, and further confirmed that FOXM1 regulates TTK to increase the therapeutic efficacy of cisplatin or paclitaxel in cervical cancer cells.

In cell cycle progression, high TTK expression may lead to the subsequent development of aneuploid tumors [29]. Furthermore, TTK has been shown that it could mediate multiple drug resistance in various cancers, such as lung cancer [30], ovarian cancer [29] and breast cancer [15]. Cisplatin and paclitaxel are the most widely used anticancer drug. The pharmacological effects of cisplatin are mediated by DNA binding, leading to DNA damage [29]. Paclitaxel is an antineoplastic drug with an impact on the stabilization of microtubules, which could act on different levels to inhibit tumor growth [31]. In this study, we used cisplatin and paclitaxel to treat the Hela and SiHa cells with differential FOXM1 protein expression and found that FOXM1 regulated TTK protein expression to affect the chemosensitivity of cervical cells through observing the cell DNA damage and apoptosis-related proteins, cleaved-caspase-3, -9. Cleaved-caspase-3, -9 could be activated by the apoptosome [32,33]. In the paclitaxel-induced SiHa xenograft model, we also found that the tumor growth was enhanced by FOXM1 overexpression, and TTK inhibitor suppressed the effects of FOXM1 overexpression.

However, the exact mechanism of how FOXM1 regulates TTK is unclear. Furthermore, the FOXM1 is involved in the process of cervical cancers by regulating other factors that remain unclear. Further studies are needed to further understand the underlying mechanisms.

Conclusions

In this study, we found that FOXM1 regulated TTK to affect the chemosensitivity of cervical cells, which provides a potential strategy for modulating chemosensitivity in cervical cancer.

Availability and Data and Materials

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Author Contributions

QT and ALX—designed the research; QT, ALX and YY—performed the research; YY, YMZ and JNS—analyzed the data and provide the help on research ex-

periments. All authors contributed to editorial changes in the manuscript. All authors read and approved the finial manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

In this study, human material and human data were performed in accordance with the Declaration of Helsinki, approved by the Ethics Committee of Yantai Yuhuangding Hospital (Approval No. 20022-017), and glistered in Chinse Clinical Trial Registry (No. ChiCTR2200057436) (https://www.chictr.org.cn). Written informed consents were obtained from all subjects.

Animal experimental research was in compliance with the revised Animals (Scientific Procedures) Act 1986 in the UK and Directive 2010/63/EU in Europe, and approved by the Ethics Committee of Yantai Yuhuangding Hospital (Approval No. 20022-017).

Acknowledgement

This manuscript was edited by the Besting Author Services.

Funding

This study is supported by the Yantai Science and Technology Bureau Support Grant/Science and Technology Innovation Development Project (No. 2022YD012 and 2022YD013).

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.24976/Discov.Med.202335176.22.

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