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RESEARCH ARTICLE

P22077 enhances the antitumor efficacy of Cisplatin and its mechanism

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Abstract: Activation of DNA damage repair pathways in tumor cells may reduce the treatment efficacy of platinum-based chemotherapeutic agents. Ubiquitin-specific protease 7 (USP7) is one of the deubiquitinating enzymes that can remove the ubiquitin from target proteins and protect substrate proteins from degradation. Although ubiquitin-specific protease 7(USP7) is highly expressed in cervical cancer tissues and plays an important role in DNA damage repair, the role of USP7 inhibition in the antitumor efficacy of cisplatin remains unknown. This study explored the effects and mechanisms of a USP7 inhibitor P22077 on the anti-cervical cancer efficacy of cisplatin. In *in vitro* studies, P22077 and cisplatin both significantly reduced HeLa cell proliferation and colony formation, and the combination produced preferable effects. In *in vivo* xenograft tumor model, P22077 and cisplatin both demonstrated significant antitumor efficacy. The drug combination produced greater antitumor activity than the individual drug alone. Cisplatin evoked DNA damage repair-related molecules and P22077 tended to prevent this change. The drug combination produced higher cell death rate than the individual drug alone. Collectively, These results suggest that the USP7 inhibitor P22077 alone has significant antitumor efficacy and also can enhance the antitumor effects of cisplatin. The USP7 inhibitor P22077 combined with cisplatin may be an effective treatment strategy for cervical cancer.

Keywords: cervical cancer, DNA damage repair, combination therapy strategy

1 Introduction

Cervical cancer remains one of the leading causes of cancer-related death in women. The prognosis of patients with advanced/recurrent cervical cancer is not optimistic, with an estimated 604,000 new cases and 342,000 deaths worldwide in 2020 [1–4]. Cisplatin is the most effective chemotherapeutic agent for advanced/recurrent cervical cancer [5,6]. However, tumor cells may undergo DNA damage repair when receiving chemotherapy, which will lead to unsatisfactory tumor treatment effects [7–10].

Cisplatin is generally considered as a cytotoxic drug which kills cancer cells by damaging DNA and inhibiting DNA synthesis. One of the cisplatin-induced DNA damages is the generation of DNA double strand break (DSB) [11]. The Mre11-Rad50-Nbs1 (MRN) complex plays a crucial role in the detection and repair of DNA damage. In response to DNA damage [12, 13], the MRN complex first detects DSB and then recruits the major DNA damage response (DDR) signal kinase ataxia telangiectasia mutation [14] to phosphorylate histone H2AX. Subsequently, DNA damage checkpoint protein 1 (MDC1), another important core component of the initial DDR signaling, binds to phosphorylated H2AX (γH2AX), and the γH2AX-MDC1 binding module further promotes the recruitment of the MRN complex and thus ATM to amplify and sustain the damage-sensing and repair signal [15–17]. This continuous activation of DNA damage repair signal can reduce the antitumor efficacy of cisplatin and even lead to drug resistance [6].

Ubiquitin-specific protease 7 (USP7) is one of the deubiquitinating enzymes that erases ubiquitin and protects substrate protein from degradation [18, 19]. Many proteins that play crucial roles in cell cycle, DNA repair, chromatin remodeling and epigenetic regulation have been identified as substrates of USP7, which makes USP7 an emerging therapeutic target for the treatment of cancer [20–24]. USP7 is overexpressed in cervical cancer and the level of its expression positively correlates with worse survival rates in patients with cervical cancer [25]. Thus, USP7 inhibition could be a useful approach against cervical cancer but this hypothesis has not been tested. In this study, we examined the effects of a USP7 inhibitor, P22077, on the anti-cervical cancer efficacy of cisplatin and also explored the underlying mechanisms. The

goal of the study was to evaluate a novel combination therapy by combining a USP7 inhibitor and cisplatin for the treatment of cervical cancer. (see in Figure 1)

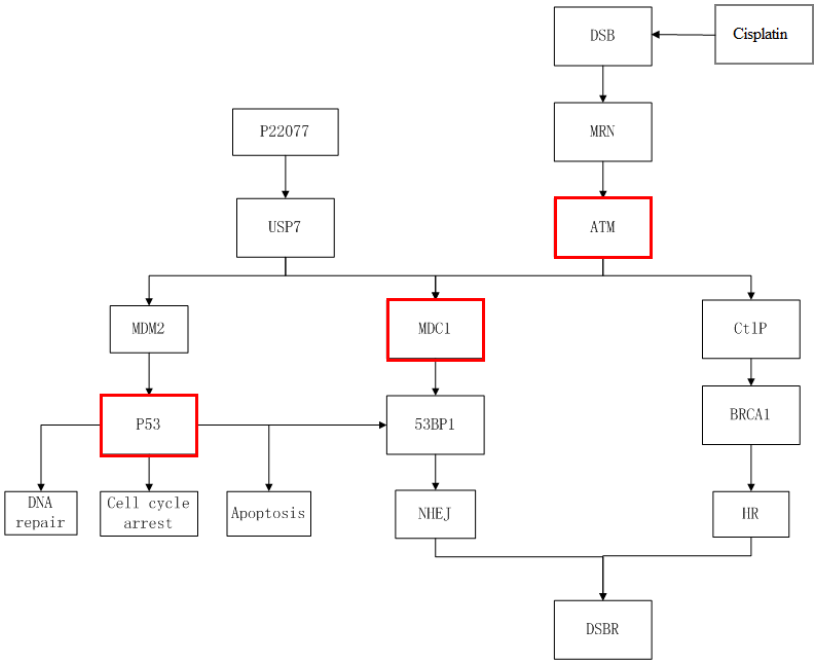

Figure 1 Mechanism of Cisplatin action

2 Materials and methods

2.1 Antibodies and chemicals

The following antibodies were used in the study: USP7 (abcam, UK, 1:1000) MDC1 (abcam, UK, 1:5000) MDM2 (CST, USA, 1:1000) Cleaved Caspase-3 (CST, USA, 1:1000)γ-H2AX (CST, USA, 1:1000) ATM (CST, USA, 1:1000) ATR (CST, USA, 1:1000) P-ATM (CST, USA, 1:1000) P-ATR (CST, USA, 1:1000) P53 (Santa Cruz Biotechnology, USA, 1:1000) β-actin (Beyotime, China, 1:1000). P22077 and Cisplatinwas purchased from MedChemExpress (MCE, USA, HY-13865, HY-17394)

2.2 Cell culture and treatments

HeLa, SiHa, and CaSki cancer cell lines were purchased from ATCC (Manassas, VA, USA).Cells were regularly screened for mycoplasma contamination using Mycoplasma Detection Kits MycoFluor™ (M7006, Thermo Fisher Scientific, USA). The cells were cultured in DMEM media supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin, and incubated at 37 °C in a humidified atmosphere containing 5% CO_2. All cells were harvested during the exponential growth phase.

2.3 Cell proliferation assay

The MTT detection assay was based on our previous protocol [26]. Briefly, cells were seeded into 96-well plates and the test compounds were added the next day. For drug treatments, cisplatin was given first followed by the addition of the USP7 inhibitor P22077 4 hours later. Cells were then incubated for 72 h. An MTT solution was added to the cells, which were incubated for 2 h, followed by the addition of 150 mL DMSO and gently shaking of the 96-well plates for 10 min. The optical density of the cultures was measured at 570 nm using a plate reading spectrophotometer (BioTek, USA) to calculate the cell survival rate.

2.4 Colony formation assay

The colony formation experiment was performed following our previously published protocol [27]. Briefly, cells were treated with the drugs for 72 h after which they were trypsinized and seeded in a 6-well plate at a count of 1000 cells per plate. After 14 days of incubation, the colonies were fixed with methanol and stained with crystal violet and then the colony numbers

were counted. The colonies with $\geq$ 50 cell count as observed under a stereomicroscope were included for analysis. The number of colonies derived from the untreated control cells was set as 100% (reference) for comparison. The surviving fraction was calculated by dividing the average number of visible colonies in treated versus untreated dishes. All experiments were performed at least three times, and representative results are shown in Fig. 1D.

2.5 Tumor xenograft experiments

Nude mice (6–8 weeks old, BALB/c, female) were used to develop the ectopic xenograft tumor model following our published protocol [28]. Briefly, HeLa cells (5×10^6) were implanted into the back of the mice by subcutaneous injection. When the tumor mass reached 100–200 mm^3, the mice were randomly divided into five groups (six animals per group): control group; P22077 (100 mg/kg); Cisplatin (5 mg/kg); Cisplatin combined with P22077 group (simultaneous administration), and Cisplatin combined with P22077 group (administered sequentially). Cisplatin was administered on the first day of treatment via intraperitoneal injection, and P22077 was administered via gavage daily. The tumor growth was measured every 3^{rd} day during the treatment. At the end of the treatment, mice were sacrificed and the tumors were removed and weighed. All animals were examined prior to the initiation of studies to ensure that they were healthy and had acclimated to the laboratory environment. The animals were housed in standard laboratory conditions (temperature 25 °C, 12h light/dark cycle, food and water were provided ad libitum).

2.6 siRNA transfection

siRNAs were provided by Gene Pharma (Shanghai, China) and used to transfect cells according to our previous protocol [29]. Briefly, cells seeded in 6-well plates were transfected with siRNA (80 pmol) using Lipofectamine 3000 (L3000015, Thermo Fisher Scientific, USA), and the silencing efficiency was examined using western blot assay 48 h after transfection. siRNA sequences targeting USP7-1, USP7-2, and USP7-3 were 5′-GACGUUUCGAAUAGAGGAA-3′, 5′-GCACUAAUGCUUACAUGUU-3′ and 5′-GACUUUGAGAACAGGCGAA-3′. respectively. The Negative control sequence was 5′-UUCUCCGAACGUGUCACGUTT-3′.

2.7 Western blot

Cells were collected after incubation and lysates were prepared to detect the target proteins as previously described [29]. Briefly, cells were exposed to the test compounds at the indicated concentrations and then collected and lysed in RIPA (Radio Immuno-precipitation Assay) buffer. The total cellular protein extract was electrophoresed on 8% SDS-polyacrylamide gels and then transferred to a PVDF membrane (Millipore, USA). The membranes were incubated overnight with primary antibodies, followed by incubation with secondary antibodies. The membrane was developed and then visualized by Image Quant LAS4000 (GE, USA).

2.8 Flow cytometry assay

Cell apoptosis analysis was performed using flow cytometry following our previously published protocol [30]. Briefly, the HeLa cells were seeded into 6-well plates with a density of 2×10^5 cells/well to incubate overnight, and then treated with respective drugs for 24, 48, or 72 hours according to the dosing regimen. The effects of the drugs on apoptosis were determined using PI/FITC-labeled annexin stained cells according to the manufacturer's recommended procedures and analyzed by a flow cytometer (BD Biosciences, SanJose, CA, USA).

2.9 Statistical analysis

For statistical comparisons, GraphPad Prism 8 Software was used with one-way ANOVA and two-way ANOVA. Data were expressed as mean $\pm$ SD and $P < 0.05$ was considered statistically significant.

3 Results

3.1 Differential sensitivity of the three cervical cancer cell lines to P22077

As shown in Fig. 1, the expression level of USP7 protein in three cervical cancer cell lines (HeLa, SiHa, CaSki) was detected by western blot (Figure 2(A)). Meanwhile, the cytotoxicity of the USP7 inhibitor P22077 on the three cell lines was detected by the MTT (Figure 2(B)).

The results show that USP7 is highly expressed in HeLa cells, which was also the most sensitive cell line to the cytotoxic effect of P22077 (IC_{50} = 15.92). SiHa cells and CaSki cells were ranked lower than HeLa cells both in the USP7 expression level and the sensitivity to P22077 cytotoxicity, demonstrating a clear positive correlation between the antitumor activity of P22077 and USP7 expression level. Thus, all subsequent *in vitro* cell studies only used HeLa cells.

3.2 Cytotoxic effects of P22077 and cisplatin used alone or in combination

The MTT method was used to examine the survival rate of HeLa cells after treatment with P22077 or cisplatin alone, and in combination (Figure 2(C)). P22077 concentration-dependently reduced the survival rate between 5 μM and 40 μM across all the three timepoints (24 hr, 48 hr, 72 hr) ($P < 0.001$). Cisplatin (1-5 μM) also significantly reduced the HeLa cell survival rate at all time points ($P < 0.001$). When treated as a combination, 1 μM cisplatin and 10 μM P22077 produced significantly greater cytotoxic effect in HeLa cells as compared to the individual drug alone ($P < 0.001$), suggesting the combination produced cytotoxic potentiation (Figure 2(C)). Importantly, this potentiation was seen regardless of the treatment strategy, as both combinatorial and sequential treatments produced similar results. Similar results were also observed in the colony formation experiment as shown in Figure 2(D). While both P22077 at concentrations of 1 μM and 2 μM and cisplatin at the concentration of 0.1 μM significantly reduced the colony formation (39% for 1 μM P22077 and 13% for 0.1 μM cisplatin), Compared with the single treatment the colony formation rate was significantly lower under combined treatment condition when 1 μM P22077 and 0.1 μM cisplatin were used in combination (5%) or sequentially (4%) ($P < 0.05$). These data strongly suggest that the combination of P22077 and cisplatin produced cytotoxic potentiation against HeLa cells.

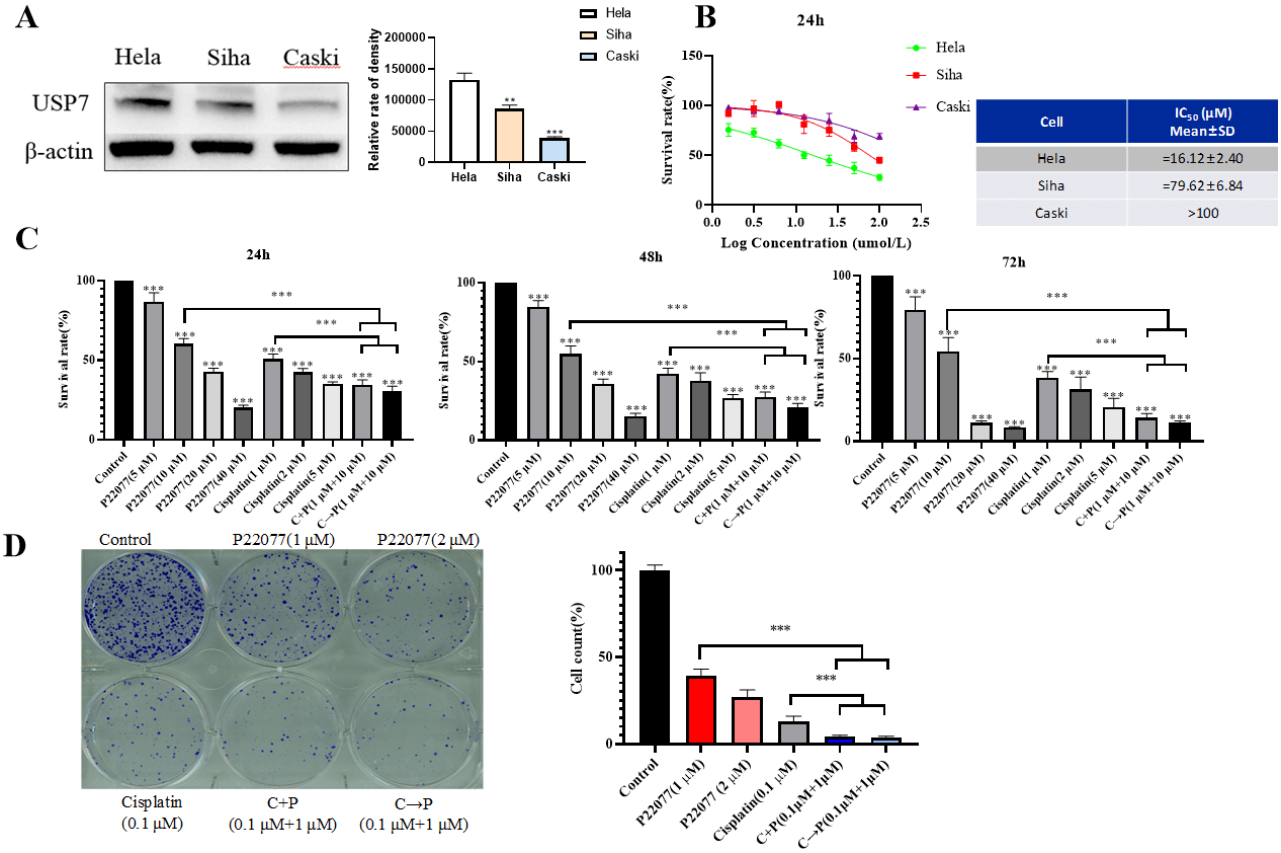

Figure 2 Effects of P22077 and Cisplatin on HeLa Cells. (A) The expression of USP7 in different cervical cancer cell lines; (B) The effect of P22077 on the survival rate of different cervical cancer cells. Administration time 24 h; (C) The effect of cisplatin and P22077, alone or in combination, on the survival rate of HeLa cells after 24 h, 48 h, 72 h. C+P: Simultaneous administration of cisplatin P22077; C→ P: P22077 given 4h after cisplatin administration. (D) The effect of different treatments on the colony formation in HeLa cells. *P < 0.05 vs control, ** P < 0.01 vs control, *** P < 0.001 vs control. One-way ANOVA with a post-hoc Bonferroni test was used for all the statistical analyses.

3.3 *In vivo* antitumor activity of P22077 and cisplatin, alone or in combination, in mice

Given the clear potentiated cytotoxic effects of the P22077-cisplatin combination *in vitro*, *in vivo* antitumor activity of the combination was evaluated in a mouse HeLa cell xenograft tumor model. As shown in Figure 3, although both 100 mg/kg P22077 and 5 mg/kg cisplatin tended to reduce the tumor weight (Figure 3(B)) and tumor volume (Figure 3(C)) without affecting the body weight (Figure 3(D)) of the mice, but the drug combination produced significantly greater antitumor activity ($P < 0.05$). These results confirmed the greater antitumor efficacy of the combined P22077 and cisplatin treatment in the *in vivo* tumor model, suggesting the potential therapeutic value of this novel combination therapy against cervical cancer.

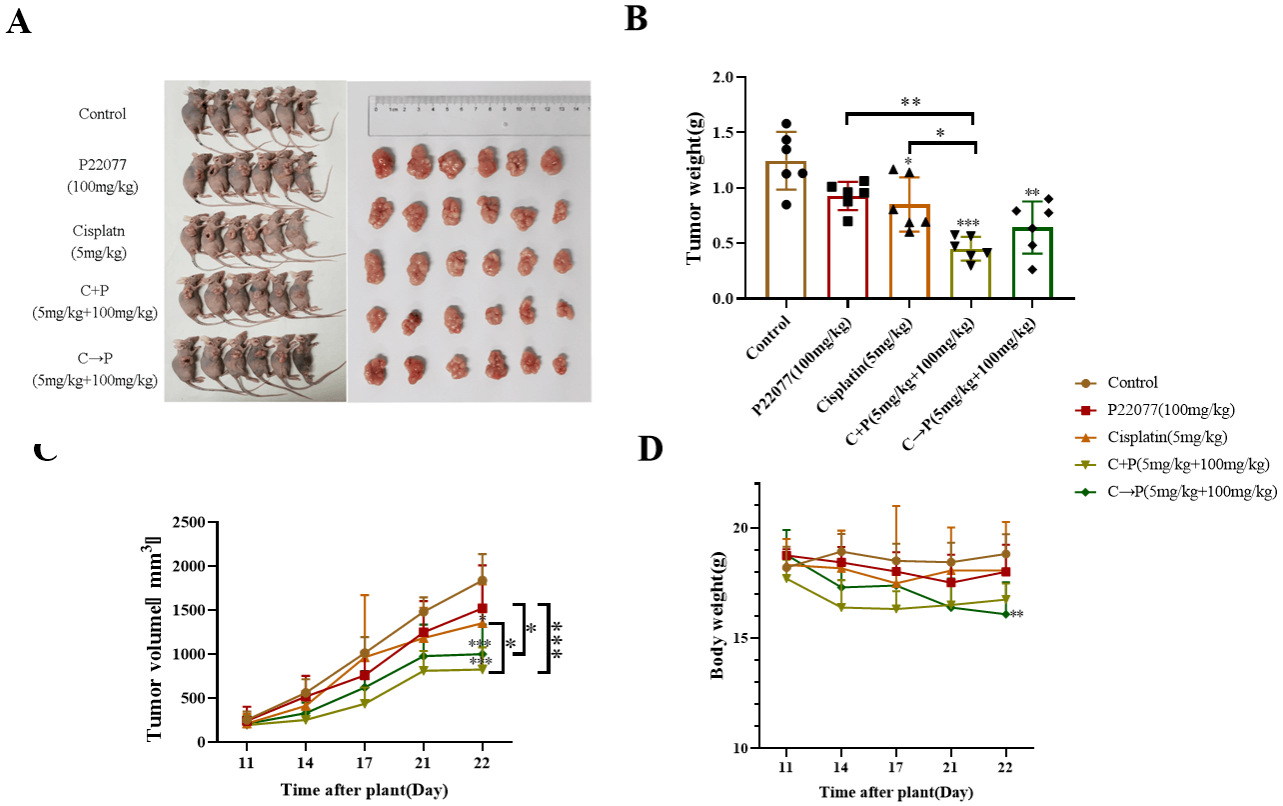

Figure 3 Enhanced anti-tumor activity of combined P22077 and Cisplatin in the xenograft mouse model. Hela cells were implanted in the BALB/c mice and mice were treated with test compounds after tumors grew to require ed size. (A) Representative photographs of mice and tumors after last treatment; (B) Tumor weight; (C) Tumor volume; (D) Bodyweight were measured. *$P < 0.05$ *vs* control, ** $P < 0.01$ *vs* control, *** $P < 0.001$ *vs* control. One-way ANOVA with a post-hoc Bonferroni test was used for Figure 3(B); Two-way ANOVA was used for Figure 3(C, D).

3.4 Effects of P22077 and cisplatin, alone or in combination, on DNA damage and cell apoptosis

In order to verify the role of USP7 in the DNA damage response, we compared the effect of adding cisplatin to HeLa cells and knockdown of USP7 on the expression of downstream molecules. It can be seen from Figure 4(A) that the siRNA used is very effective in knocking down USP7. With the knockdown of USP7, the expression of MDC1 decreased significantly, and the expression of P53 increased significantly. Importantly, MDC1, ATM, and ATR, which are vital molecules in the process of DNA damage repair, will be increased significantly under the stimulation of cisplatin, but this increase is significantly suppressed in cells with USP7 knockdown. In addition, we found that knocking down USP7 has no significant effect on ATM and ATR. Similarly, we used P22077 to inhibit USP7 and observed similar results. suggesting the potential that USP7 inhibition via P22077 may be able to reduce cisplatin-induced DNA damage repair, thus increasing the antitumor activity of cisplatin. In addition, the combined use of P22077 and cisplatin also increased the expression of apoptotic proteins γ-H2AX and C-Caspase3, suggesting the drug combination also can promote tumor cell apoptosis.

At the whole cell level, flow cytometry detection showed that the combination of P22077 and cisplatin significantly increased the death rate as compared to cisplatin treatment alone (Figure 5, $P < 0.05$), suggesting that P22077 could enhance cisplatin-induced cell death, which may contribute to the observed enhanced *in vivo* antitumor efficacy by the P22077 and cisplatin combination.

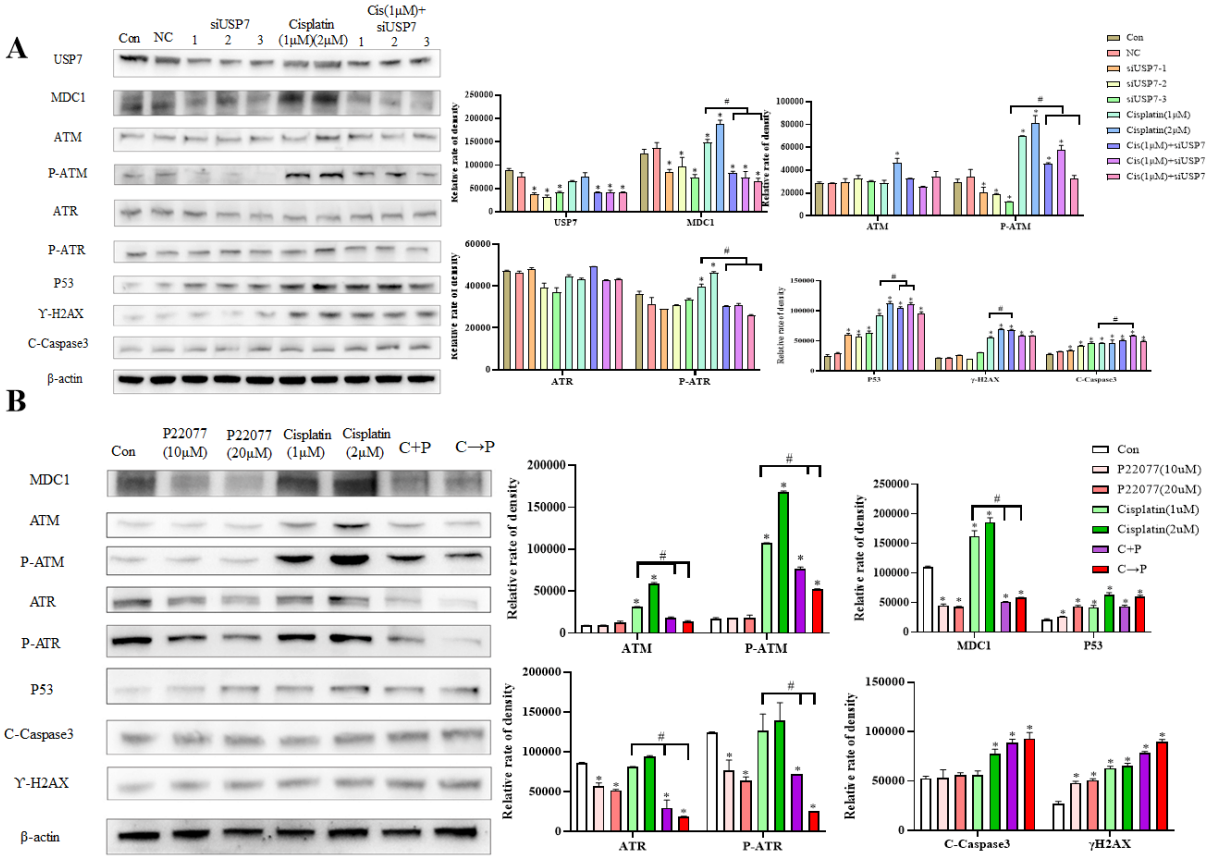

Figure 4 The effect of P22077 on cisplatin-induced DNA damage and repair. (A) Effects of silencing USP7 on the expression of USP7 downstream pathway-related proteins; (B) Effects of different treatments on proteins related to DNA damage and apoptosis. *P < 0.05 *vs* control #P < 0.05 *vs* Cisplatin (1μM) Two-way ANOVA with a post-hoc Bonferroni test was used for all the statistical analyses.

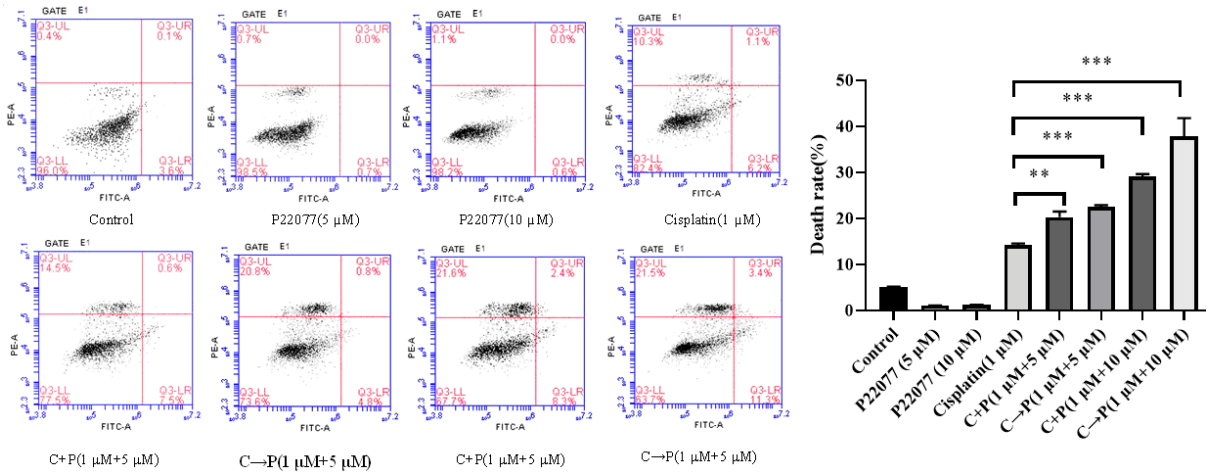

Figure 5 Effects of different treatments on HeLa cell death as measured by flow cytometry. *P < 0.05 *vs* control, ** P < 0.01 *vs* control, *** P < 0.001 *vs* control. One-way ANOVA with a post-hoc Bonferroni test was used for all the statistical analyses.

4 Discussion

The primary findings of the present study were that the USP7 inhibitor P22077 demonstrated significant cytotoxic activity against HeLa cells, a cervical cancer-derived cell line, and enhanced the cytotoxic activity of cisplatin. Furthermore, using a xenograft tumor model in mice, we demonstrated that P22077 produced significant antitumor efficacy and enhanced the antitumor activity of cisplatin. Mechanistically, P22077 reduces cisplatin-activated molecular changes associated with DNA damage repair and promotes cisplatin-induced apoptosis. Together, these results demonstrate for the first time that pharmacological inhibition of USP7 exerts significant anti-cervical cancer activity and that the USP7 inhibitor-cisplatin combination could be a beneficial combination therapy against cervical cancer.

Deubiquitinase is involved in the regulation of cell cycle, progression, signal transduction pathway regulation, DNA damage repair, and other biological functions [31–33]. USP7 is a member of the deubiquitinating enzyme family and is involved in host-virus interaction, DNA damage and repair, gene expression and protein function regulation and other cellular processes [14, 34, 35]. USP7 reportedly is involved in the development of several tumors, including esophagus cancer, myeloma, ovarian cancer and hepatocellular carcinoma. It is also highly expressed in cervical cancer [25, 36]. In cancer, USP7 can stabilize MDM2 by de-ubiquitination and subsequently promote the degradation of p53 and has been suggested as an emerging new drug target for chemotherapy against cancer. Therefore, it is reasonable to hypothesize that USP7 inhibitor could have antitumor efficacy against cervical cancer. Indeed, in consistent with the literature [25, 37, 38], we found that the USP7 protein was expressed in all the three cervical cancer-derived cell lines, HeLa, SiHa and CasKi, with highest expression level detected in HeLa cells. Using the HeLa cell line as the cell model, we examined the cytotoxic effects of a USP7 inhibitor, P22077. Indeed, P22077 demonstrated dose-dependent cytotoxic efficacy against HeLa cells with highest drug concentration (40 μM) showing violent efficacy. More importantly, although it is not surprising that cisplatin also showed cytotoxic effect, the combination of a marginally effective dose of P22077 with a dose of cisplatin further potentiated the cytotoxic efficacy and colony formation suppressing effect, revealing a clear enhancement on the cytotoxic effect of the drug combination. To confirm the observed *in vitro* cytotoxic effects of the P22077-cisplatin combination, we examined the antitumor efficacy of the drug combination in a mouse xenograft tumor model. Indeed, in this *in vivo* tumor model, both P22077 and cisplatin demonstrated significant antitumor activity and the combination showed greater effect. Again, the *in vivo* research support the *in vitro* results and suggests that the USP7 inhibitor/cisplatin could be a very useful combination therapy strategy against cervical cancer.

Given that USP7 is physically associated with the MRN-MDC1 complex and that MRN-MDC1 acts as a platform for USP7 to efficiently deubiquitinate and stabilize MDC1 and thereby sustains DDR [25] and that USP7 promotes cervical carcinogenesis, USP7 inhibition may produce cytotoxic and antitumor effects via disrupting DDR [39, 40]. Indeed, our mechanistic study shows that USP7 knockdown significantly reduced MDM2 and MDC1 expression and increased p53 expression level, which likely contribute to the antitumor activity of the USP7 inhibitor P22077. Interestingly, because one of the mechanism underlying cisplatin-induced chemoresistance is cisplatin-evoked DNA damage repair process [41], which involves the MRN-MDC1 complex, the findings that P22077 in combination with cisplatin canceled or reduced cisplatin-evoked increases of MRN-MDC1 and related molecules suggest that P22077 may also reduce cisplatin-induced chemoresistance. In addition, because it is known that USP7 inhibitor can induce cell death in ovarian cancer [42], the current finding that P22077 and cisplatin combination increased the cell apoptosis related molecules such as γH2AX and C-caspase 3 suggests that the USP7 inhibitor-cisplatin combination also can promote cell apoptosis.

In summary, this research proves for the first time demonstrated that a USP7 inhibitor P22077 alone has anti-cervical cancer activity and facilitates the antitumor efficacy of the traditional solid tumor chemotherapeutic drug cisplatin and that these effects may be due to the inhibition of cisplatin-induced DDR and promotion of cell apoptosis. In summary, this research has proved that P22077 has anti-tumor activity in cervical cancer cells, and P22077 can enhance the therapeutic effect of cisplatin by inhibiting cisplatin-induced DNA damage repair. These results suggest that the combination of USP7 inhibitor and cisplatin could be a useful chemotherapeutic approach against cervical cancer.

Acknowledgements

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Availability of data and materials

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

Authors' contributions

Qiu, Ren, Wang, Xie, Liu performed the experiments and interpretated the results. Hongbo Wang Yu and Tian conceived and designed the current study. Qiu wrote the manuscript. All authors read and approved the final manuscript. Qiu Yu and Tian confirm the authenticity of all raw data.

Conflict of interest

The authors declare that there are no conflicts of interest.

Ethical statement

The use of animals was approved by the Animal Experimentation Ethics Committee of Yantai University in accordance with the guidelines for ethical conduct in the care and use of animals.

References

[1] Roden RBS and Stern PL. Opportunities and challenges for human papillomavirus vaccination in cancer. Nature Reviews Cancer, 2018, **18**(4): 240-254.
https://doi.org/10.1038/nrc.2018.13

[2] Tsu V and Jeronimo J. Saving the World's Women from Cervical Cancer. New England Journal of Medicine, 2016, **374**(26): 2509-2511.
https://doi.org/10.1056/NEJMp1604113

[3] Olawaiye AB, Baker TP, Washington MK, *et al*. The new (Version 9) American Joint Committee on Cancer tumor, node, metastasis staging for cervical cancer. CA: A Cancer Journal for Clinicians, 2021, **71**(4): 287-298.
https://doi.org/10.3322/caac.21663

[4] Sung H, Ferlay J, Siegel RL, *et al*. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians, 2021, **71**(3): 209-249.
https://doi.org/10.3322/caac.21660

[5] Wieringa HW, van der Zee AG, de Vries EG, *et al*. Breaking the DNA damage response to improve cervical cancer treatment. Cancer Treatment Review, 2016, **42**: 30-40.
https://doi.org/10.1016/j.ctrv.2015.11.008

[6] Zhu H, Luo H, Zhang W, *et al*. Molecular mechanisms of cisplatin resistance in cervical cancer. Drug Design, Development and Therapy, 2016, **10**: 1885-1895.
https://doi.org/10.2147/DDDT.S106412

[7] Gottesman MM, Fojo T and Bates SE. Multidrug resistance in cancer: role of ATP-dependent transporters. Nature Reviews Cancer, 2002, **2**(1): 48-58.
https://doi.org/10.1038/nrc706

[8] Zisowsky J, Koegel S, Leyers S, *et al*. Relevance of drug uptake and efflux for cisplatin sensitivity of tumor cells. Biochemical Pharmacology, 2007, **73**(2): 298-307.
https://doi.org/10.1016/j.bcp.2006.10.003

[9] Siddik ZH. Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene, 2003, **22**(47): 7265-7279.
https://doi.org/10.1038/sj.onc.1206933

[10] Dasari S and Tchounwou PB. Cisplatin in cancer therapy: molecular mechanisms of action. European Journal of Pharmacology, 2014, **740**: 364-378.
https://doi.org/10.1016/j.ejphar.2014.07.025

[11] Khanna KK and Jackson SP. DNA double-strand breaks: signaling, repair and the cancer connection. Nature Genetics, 2001, **27**(3): 247-254.
https://doi.org/10.1038/85798

[12] Stracker TH and Petrini JH. The MRE11 complex: starting from the ends. Nature Reviews Molecular Cell Biology, 2011, **12**(2): 90-103.
https://doi.org/10.1038/nrm3047

[13] Cherry SM, Adelman CA, Theunissen JW, *et al*. The Mre11 complex influences DNA repair, synapsis, and crossing over in murine meiosis. Current Biology, 2007, **17**(4): 373-378.
https://doi.org/10.1016/j.cub.2006.12.048

[14] Reverdy C, Conrath S, Lopez R, *et al*. Discovery of Specific Inhibitors of Human USP7/HAUSP Deubiquitinating Enzyme. Chemistry & Biology, 2012, **19**(4): 467-477. https://doi.org/10.1016/j.chembiol.2012.02.007

[15] Uziel T, Lerenthal Y, Moyal L, *et al*. Requirement of the MRN complex for ATM activation by DNA damage. EMBO Journal, 2003, **22**(20): 5612-5621. https://doi.org/10.1093/emboj/cdg541

[16] Jungmichel S, Clapperton JA, Lloyd J, *et al*. The molecular basis of ATM-dependent dimerization of the Mdc1 DNA damage checkpoint mediator. Nucleic Acids Research, 2012, **40**(9): 3913-3928. https://doi.org/10.1093/nar/gkr1300

[17] Stewart GS, Wang B, Bignell CR, *et al*. MDC1 is a mediator of the mammalian DNA damage checkpoint. Nature, 2003, **421**(6926): 961-966. https://doi.org/10.1038/nature01446

[18] Dikic I, Wakatsuki S and Walters KJ. Ubiquitin-binding domains - from structures to functions. Nature Reviews Molecular Cell Biology, 2009, **10**(10): 659-6571. https://doi.org/10.1038/nrm2767

[19] Vucic D, Dixit VM and Wertz IE. Ubiquitylation in apoptosis: a post-translational modification at the edge of life and death. Nature Reviews Molecular Cell Biology, 2011, **12**(7): 439-452. https://doi.org/10.1038/nrm3143

[20] Wang Z, Kang W, You Y, *et al*. USP7: Novel Drug Target in Cancer Therapy. Frontiers in Pharmacology, 2019, **10**: 427. https://doi.org/10.3389/fphar.2019.00427

[21] Zhu Q, Sharma N, He J, *et al*. USP7 deubiquitinase promotes ubiquitin-dependent DNA damage signaling by stabilizing RNF168. Cell Cycle, 2015, **14**(9): 1413-1425. https://doi.org/10.1080/15384101.2015.1007785

[22] Zhang P, Wei Y, Wang L, *et al*. ATM-mediated stabilization of ZEB1 promotes DNA damage response and radioresistance through CHK1. Nature Cell Biology, 2014, **16**(9): 864-875. https://doi.org/10.1038/ncb3013

[23] Alonso-de Vega I, Martin Y and Smits VA. USP7 controls Chk1 protein stability by direct deubiquitination. Cell Cycle, 2014, **13**(24): 3921-3926. https://doi.org/10.4161/15384101.2014.973324

[24] Faustrup H, Bekker-Jensen S, Bartek J, *et al*. USP7 counteracts SCFbetaTrCP- but not APCCdh1-mediated proteolysis of Claspin. Journal of Cell Biology, 2009, **184**(1): 13-19. https://doi.org/10.1083/jcb.200807137

[25] Su D, Ma S, Shan L, *et al*. Ubiquitin-specific protease 7 sustains DNA damage response and promotes cervical carcinogenesis. Journal Of Clinical Investigation, 2018, **128**(10): 4280-4296. https://doi.org/10.1172/JCI120518

[26] Lv G, Sun D, Zhang J, *et al*. Lx2-32c, a novel semi-synthetic taxane, exerts antitumor activity against prostate cancer cells in vitro and in vivo. Acta Pharmaceutica Sinica B, 2017, **7**(1): 52-58. https://doi.org/10.1016/j.apsb.2016.06.005

[27] Li F, Liu Z, Sun H, *et al*. PCC0208017, a novel small-molecule inhibitor of MARK3/MARK4, suppresses glioma progression in vitro and in vivo. Acta Pharmaceutica Sinica B, 2020, **10**(2): 289-300. https://doi.org/10.1016/j.apsb.2019.09.004

[28] Ma YT, Yang Y, Cai P, *et al*. A Series of Enthalpically Optimized Docetaxel Analogues Exhibiting Enhanced Antitumor Activity and Water Solubility. Journal Of Natural Products, 2018, **81**(3): 524-533. https://doi.org/10.1021/acs.jnatprod.7b00857

[29] Yang Y, Guan D, Lei L, *et al*. H6, a novel hederagenin derivative, reverses multidrug resistance in vitro and in vivo. Toxicology And Applied Pharmacology, 2018, **341**: 98-105. https://doi.org/10.1016/j.taap.2018.01.015

[30] Wang H, Ma X, Ren S, *et al*. A small-molecule inhibitor of MDMX activates p53 and induces apoptosis. Molecular Cancer Therapeutics, 2011, **10**(1): 69-79. https://doi.org/10.1158/1535-7163.MCT-10-0581

[31] Clague MJ, Urbe S and Komander D. Breaking the chains: deubiquitylating enzyme specificity begets function. Nature Reviews Molecular Cell Biology, 2019, **20**(6): 338-352. https://doi.org/10.1038/s41580-019-0099-1

[32] D'Arcy P, Wang X and Linder S. Deubiquitinase inhibition as a cancer therapeutic strategy. Pharmacology & Therapeutics, 2015, **147**: 32-54. https://doi.org/10.1016/j.pharmthera.2014.11.002

[33] Mevissen TET and Komander D. Mechanisms of Deubiquitinase Specificity and Regulation. Annual Review of Biochemistry, 2017, **86**: 159-192. https://doi.org/10.1146/annurev-biochem-061516-044916

[34] Nicholson B and Kumar KGS. The Multifaceted Roles of USP7: New Therapeutic Opportunities. Cell Biochemistry and Biophysics, 2011, **60**(1-2): 61-68. https://doi.org/10.1007/s12013-011-9185-5

[35] Turnbull AP, Ioannidis S, Krajewski WW, *et al*. Molecular basis of USP7 inhibition by selective small-molecule inhibitors. Nature, 2017, **550**(7677): 481-486. https://doi.org/10.1038/nature24451

[36] Xu L, Wang J, Yuan XN, *et al*. IU1 suppresses proliferation of cervical cancer cells through MDM2 degradation. International Journal of Biological Sciences, 2020, **16**(15): 2951-2963. https://doi.org/10.7150/ijbs.47999

[37] Tavana O and Gu W. Modulation of the p53/MDM2 interplay by HAUSP inhibitors. Journal of Molecular Cell Biology, 2017, **9**(1): 45-52. https://doi.org/10.1093/jmcb/mjw049

[38] Li M, Brooks CL, Kon N, *et al*. A dynamic role of HAUSP in the p53-Mdm2 pathway. Molecular Cell, 2004, **13**(6): 879-886. https://doi.org/10.1016/s1097-2765(04)00157-1

[39] Zlatanou A, Sabbioneda S, Miller ES, *et al*. USP7 is essential for maintaining Rad18 stability and DNA damage tolerance. Oncogene, 2016, **35**(8): 965-976. https://doi.org/10.1038/onc.2015.149

[40] Agathanggelou A, Smith E, Davies NJ, *et al*. USP7 inhibition alters homologous recombination repair and targets CLL cells independently of ATM/p53 functional status. Blood, 2017, **130**(2): 156-166. https://doi.org/10.1182/blood-2016-12-758219

[41] Singh N, Bhakuni R, Chhabria D, *et al*. MDC1 depletion promotes cisplatin induced cell death in cervical cancer cells. BMC Research Notes, 2020, **13**(1): 146. https://doi.org/10.1186/s13104-020-04996-5

[42] Wang M, Zhang Y, Wang T, *et al*. The USP7 Inhibitor P5091 Induces Cell Death in Ovarian Cancers with Different P53 Status. Cell Biochemistry And Biophysics, 2017, **43**(5): 1755-1766. https://doi.org/10.1159/000484062