# Synergistic effects of thalidomide and cisplatin are mediated via the PI3K/AKT and JAK1/STAT3 signaling pathways in cervical cancer

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**Abstract.** Thalidomide (THD) has been found to synergize with cisplatin (DDP) in certain types of cancers; however, their combined use in the treatment of cervical cancer has not been reported to date, at least to the best of our knowledge. Thus, the present study aimed to explore the synergistic effects of THD and DDP and determine their regulatory effects on the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) and Janus kinase 1 (JAK1)/signal transducer and activator of transcription 3 (STAT3) pathways in cervical cancer. For this purpose, 0-160 $\mu$M THD and 0-64 $\mu$M DDP monotherapy or in combination were used to treat the HeLa and SiHa cervical cancer cell lines. This was followed by the calculation of the combination index (CI) and 160 $\mu$M THD and 16 $\mu$M DDP were then used to treat the cells. Relative cell viability and apoptosis, as well as the mRNA and protein levels of PI3K, AKT, JAK1 and STAT3 were evaluated. The results revealed that THD and DDP monotherapy suppressed the viability of the HeLa and SiHa cells in a concentration-dependent manner. Moreover, THD and DDP treatment exerted a more prominent suppressive effect on the relative viability of HeLa and SiHa cells compared with DDP monotherapy at several concentration settings; further CI calculation revealed that the optimal synergistic concentrations were 160 $\mu$M for THD and 16 $\mu$M for DDP. Subsequently, combined treatment with THD and DDP suppressed relative cell viability, whereas it promoted cell apoptosis compared with THD or DPP monotherapy; it also inhibited the PI3K/AKT and JAK1/STAT3 signaling pathways compared with DPP or THD monotherapy in both HeLa and SiHa cells. On the whole, the present study demonstrated that THD synergizes with DDP to exert suppressive effects on cervical cancer cell lines. This synergistic action also inactivated the PI3K/AKT and JAK1/STAT3 pathways. Thus, these findings suggest that the combined use of THD and DPP may have potential for use in the treatment of cervical cancer.

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*Abbreviations:* THD, thalidomide; CI, combination index; DDP, cisplatin

*Key words:* cisplatin, thalidomide, cervical cancer, PI3K/AKT, JAK1/STAT3

## Introduction

Cervical cancer is the global leading cause of morbidity and mortality in women and has been listed as one of the most critical issues affecting women's health (1,2). Over the past few decades, the advancements made in screening programs, vaccinations to avoid human papillomavirus (HPV) infection, targeted therapies and immunotherapies for cervical cancer have markedly reduced the disease burden (3-6). However, it should be noted that the current overall prognosis of patients with cervical cancer remains far from satisfactory (1,3). Cisplatin (DDP) is widely used in the treatment of cervical cancer; it is not only used as a chemotherapeutic agent for patients with advanced cervical cancer, but is also applied for neoadjuvant/adjuvant treatment (7,8). However, DDP monotherapy has been indicated as insufficient for the treatment of cervical cancer (8).

Thalidomide (THD), an immunomodulatory and anti-angiogenic agent, also potentially induces the apoptosis of cancer cells and has been applied for the treatment of several types of cancer over the past few decades (9-12). Notably, the combination of THD with other chemotherapeutic reagents (including DDP) has been shown to exert a synergistic anticancer effect. For instance, a previous study demonstrated that THD plus DDP exerted a synergistic inhibitory effect on tumor growth and angiogenesis in head and neck squamous cell carcinoma model mice (13). Furthermore, another study found that THD plus DDP exerted a more prominent suppressive effect on tumor volume than DDP monotherapy in glioma model rats (14). Similar results were also found in breast tumor model mice and colorectal tumor model mice (15). More importantly, a previous randomized controlled trial revealed that THD plus DDP improved the 3-year overall survival and progression-free survival rate of patients with advanced esophageal cancer (16). Based on these findings, it was hypothesized that THD plus DDP may also exert a synergistic effect on cervical cancer. However, relative information is lacking.

The phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) and Janus kinase 1 (JAK1)/signal transducer and activator of transcription 3 (STAT3) pathways regulate various biological processes, including cell survival, metabolism and protein synthesis (17,18). In cervical cancer, it has been suggested that the PI3K/AKT and JAK1/STAT3 pathways critically participate in cancer pathogenesis and progression (19,20). In addition, previous studies have indicated that both THD and DDP exert antitumor effects by modulating the PI3K/AKT and JAK1/STAT3 pathways (21-24). Therefore, the present study aimed to evaluate the effects of THD plus DDP on cell viability, apoptosis, as well as the activation of the PI3K/AKT and JAK1/STAT3 pathways in HeLa and SiHa cervical cancer cell lines.

## Materials and methods

*Cells and cell culture*. The human cervical carcinoma cell lines, HeLa (TCHu187) and SiHa (SCSP-5058), were purchased from The Cell Bank of Type Culture Collection of the Chinese Academy of Sciences. Both HeLa and SiHa cells were cultured in 90% Eagle's minimum essential medium (Nissui Pharmaceutical Co., Ltd.) and 10% fetal bovine serum (FBS) (Gibco; Thermo Fisher Scientific, Inc.). Normal human cervical epithelial (HCerEpiC) (product no. FC-0080) cells were purchased from Lifeline Cell Technology, LLC. HCerEpiC cells were cultured with 90% cervical epithelial medium (Lifeline Cell Technology, LLC) and 10% FBS. The culture was conducted in a cell incubator with a humidified atmosphere at 37˚C and 5% $CO_2$. Cells in the exponential growth phase were selected for use in the following experiments.

*Treatments and detections*. DDP and THD were purchased from MedChemExpress and were prepared into gradient solutions with dimethyl sulfoxide (MedChemExpress) for use in the following experiments. After the preparation of DDP and THD, the following treatments were carried out:

i) Single-drug treatment: Both HeLa and SiHa cells were respectively treated with a single-drug solution at various concentrations in medium containing 10% FBS for 24 h, and the concentration gradient was set as follows: DDP: 0, 2, 4, 8, 16, 32 and 64 $\mu$M; and THD: 0, 5, 10, 20, 40, 80 and 160 $\mu$M. Following 24 h of treatment with the single-drug solution, cell viability was analyzed using a Cell Counting Kit-8 (CCK-8) (Beyotime Institute of Biotechnology).

ii) Two-drug treatment: Both HeLa and SiHa cells were respectively treated with the two drugs in 12 combinations at various concentrations in medium containing 10% FBS for 24 h, and the concentration gradient was set as follows: DDP: 0, 4, 16 and 64 $\mu$M; THD: 0, 20 and 160 $\mu$M. Following 24 h of treatment with the two-drug solution, cell viability was analyzed using CCK-8 (Beyotime Institute of Biotechnology). Furthermore, the combination index (CI) was estimated to determine the optimal combination concentration, which was calculated as follows: The relative cell viability of combination treatment divided by the product of the relative cell viability of two single-drug treatments.

iii) Synergistic treatment: Both HeLa and SiHa cells were respectively categorized into four groups: Group A, cells were treated with 0 $\mu$M DDP and 0 $\mu$M THD dissolved in medium containing 10% FBS for 24 h; group B, cells were treated with 16 $\mu$M DDP and 0 $\mu$M THD dissolved in medium containing 10% FBS for 24 h; group C, cells were treated with 0 $\mu$M DDP and 160 $\mu$M THD dissolved in medium containing 10% FBS for 24 h; group D, cells were treated with 16 $\mu$M DDP and 160 $\mu$M THD dissolved in medium containing 10% FBS for 24 h. Following 24 h of treatment, in each cell group, cell viability was analyzed using CCK-8 (Beyotime Institute of Biotechnology); cell apoptosis was assessed using the Annexin V-fluorescein Isothiocyanate (FITC) Apoptosis Detection kit (Beyotime Institute of Biotechnology); the expression levels of the PI3K/AKT pathway and the JAK/STAT pathway in each group were determined using reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and western blot analysis.

iv) PI3K and JAK activation: The HeLa and SiHa cells were divided into three groups as follows: Group A, cells were treated 16 $\mu$M DDP and 160 $\mu$M THD for 24 h; group B, cells were treated with 16 $\mu$M DDP, 160 $\mu$M THD and 20 $\mu$M 740Y-P (MedChemExpress) for 24 h; group C, cells were treated with 16 $\mu$M DDP, 160 $\mu$M THD and 20 $\mu$M RO8191 (MedChemExpress) for 24 h. Cell viability and apoptosis were measured using CCK-8 assay and the Annexin V-FITC Apoptosis Detection kit, respectively.

*Cell viability determination*. The cells were seeded at $1x10^4$ per well in a 96-well plate. Following treatment, the old culture solution in the experimental wells was discarded and 90 $\mu$l medium and 10 $\mu$l CCK-8 solution were added to each well of the 96-well plate, followed by incubation for 2 h at 37˚C. Finally, a microplate reader (BioTek Instruments, Inc.) was applied to measure the absorbance of each experimental well at 450 nm and the relative cell viability was calculated based on the optical density value.

*Cell apoptosis determination*. The cells were seeded at $4x10^5$ per well in a 6-well plate. Following the treatment, the cells were digested by trypsin at 37˚C and the supernatant was removed by centrifugation (1,500 x g for 3 min at room temperature). The cells were stained with trypan blue solution (Beyotime Institute of Biotechnology) at room temperature for 2 min, followed by cell counting under an inverted microscope (Motic China Group Co., Ltd.). Following the adjustment of the cell density, 5 $\mu$l Annexin V and 5 $\mu$l propidium iodide were added to a 100-$\mu$l cell suspension, which was then maintained at room temperature for 15 min in the dark. After the cells were passed through 400-mesh sieves, a FACSCalibur flow cytometer (BD Biosciences) was applied to analyze cell apoptosis. The data were analyzed using Flowjo 7.6 (BD Biosciences).

*RT-qPCR*. The expression levels of JAK1, STAT3 and PI3K in each group were assayed using RT-qPCR. The cells were seeded at $4x10^5$ per well in a six-well plate. Following treatment, the isolation of total RNA was completed using TRIzol reagent (Beyotime Institute of Biotechnology). qPCR was performed on an AFD9600 real-time fluorescence quantitative PCR instrument (AGS) using ReverTra Ace® qPCR RT kit (Toyobo Co., Ltd.) (at 37˚C for 15 min, followed by 98˚C for

Table I. Primers sequences used for RT-qPCR.

| Gene | Forward (5'->3') | Reverse (5'->3') |
|---|---|---|
| JAK1 | TGGATTACAAGGATGACGAAGGAA | CGGACACAGACGCCATAGAG |
| STAT3 | GAGAAGGACATCAGCGGTAAGAC | GGATAGAGATAGACCAGTGGAGACA |
| PI3K | TTCTCAACTGCCAATGGACTGT | AGCACGAGGAAGATCAGGAATG |
| GAPDH | GAGTCCACTGGCGTCTTCAC | ATCTTGAGGCTGTTGTCATACTTCT |

RT-qPCR, reverse transcription-quantitative PCR; JAK1, Janus kinase 1; STAT3, signal transducer and activator of transcription 3; PI3K, phosphoinositide 3-kinase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

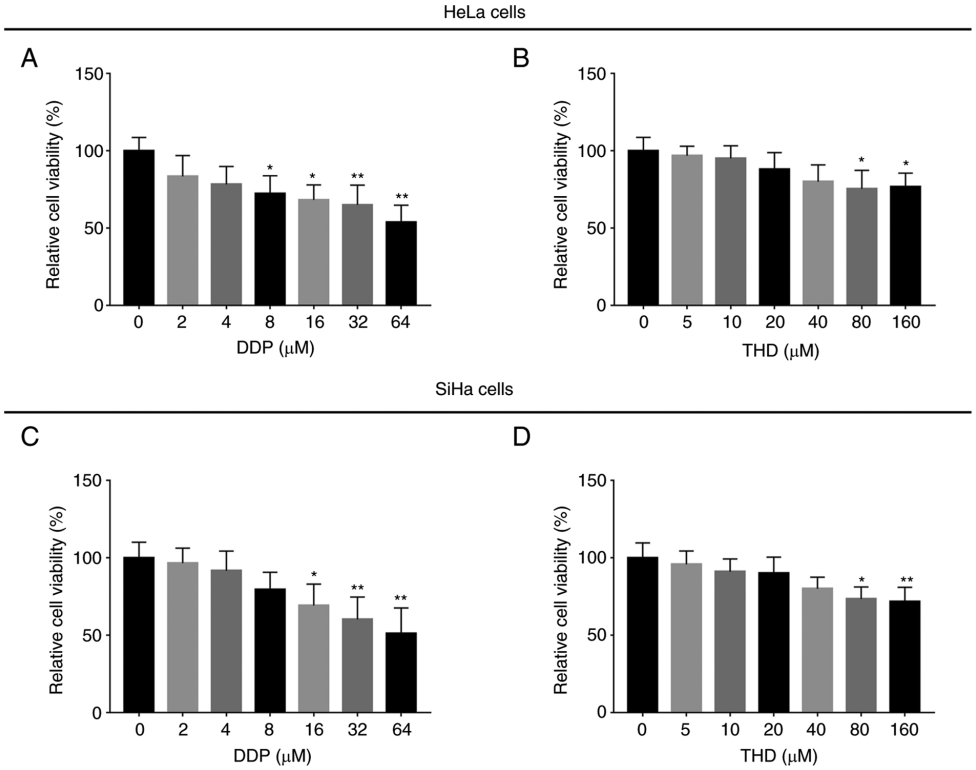
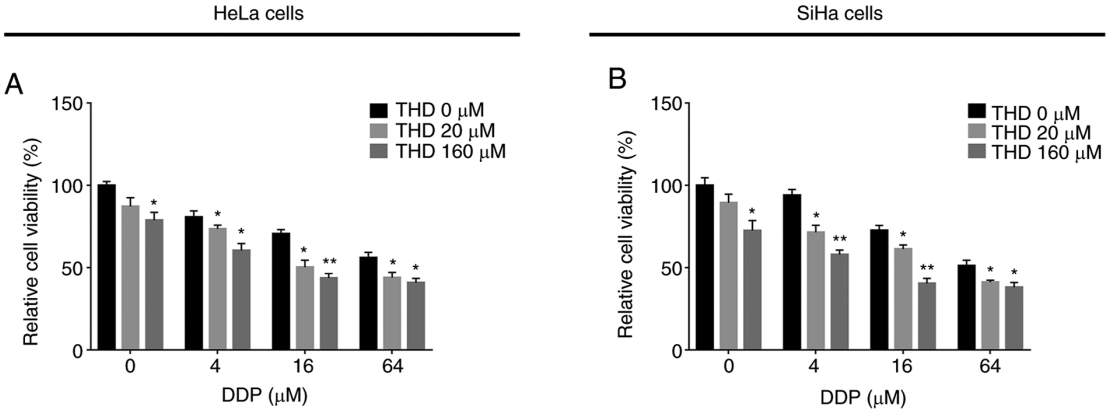



Figure 1. Comparison of relative cell viability following treatment with various concentrations of DDP or THD. Comparison of relative cell viability following treatment with various concentrations of (A) DDP or (B) THD treatment in HeLa cells; comparison of relative cell viability following treatment with various concentrations of (C) DDP or (D) THD treatment in SiHa cells. *P<0.05 and **P<0.01 compared with cells treated with 0 $\mu$M of DDP or THD. DDP, cisplatin; THD, thalidomide.

5 min) and THUNDERBIRD® SYBR® qPCR Mix (Toyobo Co., Ltd.) (95˚C for 1 min, 1 cycle; followed by 95˚C for 15 sec, 61˚C for 30 sec, 40 cycles) as per the manufacturer's protocol. The internal reference was glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The $2^{-\Delta\Delta Cq}$ method was used to calculate the relative expression of each gene (25). The primer sequences used for RT-qPCR are presented in Table I.

*Western blot analysis*. The procedures of western blot analysis were conducted according to those described in a previous study with certain modifications (26). Briefly, the cells were seeded at $4x10^5$ per well in a six-well plate. Following treatment, the cells were collected and protein was extracted using RIPA Lysis Buffer (Beyotime Institute of Biotechnology) on ice for 30 min and quantified using a Pierce™ BCA Protein Assay kit (Thermo Fisher Scientific, Inc.). Subsequently, 20 $\mu$g protein was separated by 4-20% sodium dodecyl sulfate polyacrylamide gel electrophoresis, transferred to a polyvinylidene fluoride membrane and incubated with 5% non-fat milk powder at room temperature for 1 h. Subsequently, the membrane was incubated with primary antibodies at 4˚C overnight, followed by incubation with HRP-conjugated goat anti-rabbit secondary antibodies (1:20,000; product code ab6721; Abcam) at room temperature for 1 h and visualization using Pierce™ ECL Plus Western Blotting Substrate (Thermo Fisher Scientific, Inc.). The primary and secondary antibodies for JAK1 (monoclonal antibody; 1:5,000; product code ab133666), phosphorylated (p)-JAK1 (monoclonal antibody; 1:5,000; product code ab138005), STAT3 (monoclonal antibody; 1:2,000; product code ab68153), p-STAT3 (monoclonal antibody; 1:1,000; product code ab267373), PI3K (monoclonal antibody; 1:1,500; product code ab40755), AKT




| Combination index | | DDP (μM) | | |
|---|---|---|---|---|
| | | 4 | 16 | 64 |
| THD (μM) | 20 | 1.0429 | 0.8157 | 0.9012 |
| | 160 | 0.9498 | 0.7858 | 0.9254 |

| Combination index | | DDP (μM) | | |
|---|---|---|---|---|
| | | 4 | 16 | 64 |
| THD (μM) | 20 | 0.9299 | 1.0314 | 0.9581 |
| | 160 | 0.8496 | 0.7665 | 1.0264 |

Figure 2. Comparison of relative cell viability by combination treatment involving various concentrations of DDP and THD. Comparison of relative cell viability after treatment with various concentrations of DDP and THD combination treatment in (A) HeLa cells and (B) SiHa cells. *P<0.05 and **P<0.01 compared with cells treated with 0 $\mu$M of DDP and THD. DDP, cisplatin; THD, thalidomide.

(polyclonal antibody; 1:500; product code ab8805), p-AKT (polyclonal antibody; 1:1,000; product code ab38449) and GAPDH (polyclonal antibody; 1:2,500; product code ab9485) were all purchased from Abcam. The quantification of the western blots was carried out with ImageJ 1.8 (National Institutes of Health).

*Statistical analysis*. All data processing and analysis were completed using GraphPad Prism 7.02 (GraphPad Software Inc.) and are presented in bar plots, representing the mean value and standard deviation (error bar). Multiple comparisons were performed using one-way analysis of variance (ANOVA) followed by Tukey's or Dunnett's test. A P-value <0.05 was considered to indicate a statistically significant difference. The Shapiro-Wilk normality test was used for normality test of data. The data were parametric distribution.

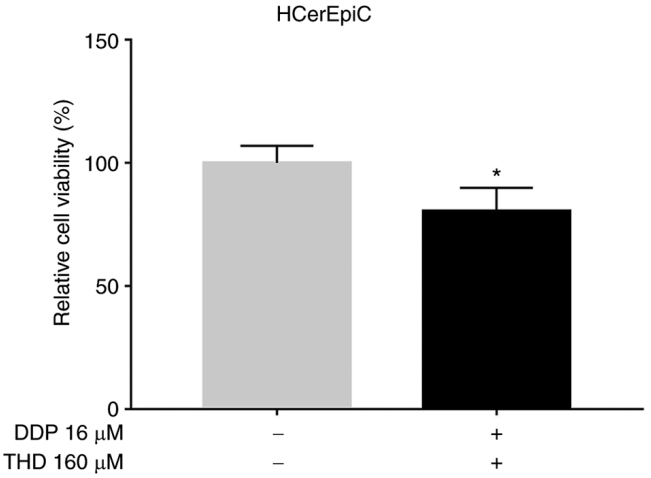



Figure 3. Effect of THD plus DDP on HCerEpiC cell viability. *P<0.05 compared with cells without treatment of DDP and THD. DDP, cisplatin; THD, thalidomide.

## Results

*Sensitivity of cervical cancer cell lines to DDP monotherapy and THD monotherapy*. DDP treatment suppressed the relative viability of HeLa cells (Fig. 1A) (P<0.05 and P<0.01 when DDP ≥8 $\mu$M) and SiHa cells (Fig. 1C) (P<0.05 and P<0.01 when DDP ≥16 $\mu$M) in a concentration-dependent manner. Moreover, THD treatment suppressed the relative viability of HeLa cells (Fig. 1B) (both P<0.05 when THD ≥80 $\mu$M) and SiHa cells (Fig. 1D) (P<0.05 and P<0.01 when THD ≥80 $\mu$M) in a concentration-dependent manner.

*Synergized effect of THD and DDP on cervical cancer cell lines*. THD plus DDP treatment exerted a more prominent suppressive effect on the relative viability of HeLa cells (Fig. 2A) and SiHa cells (Fig. 2B) compared to DDP monotherapy at several concentration settings (P<0.05 and P<0.01). Furthermore, after calculating the CI (the lower value, the more prominent the synergistic effect), it was determined that the optimal combination concentration was 16 $\mu$M for DDP and 160 $\mu$M for THD (synergistic treatment) in both cell lines (Fig. 2A and B); thus, these settings were applied in the following experiments. In addition, THD plus DDP suppressed the relative viability of HCerEpiC cells, although the inhibitory effect was weaker than that observed in the cervical cancer cells (P<0.05; Fig. 3).

*Synergistic effects of THD and DDP on the relative viability and apoptosis of cervical cancer cell lines*. In HeLa cells, treatment with both THD and DDP suppressed the relative cell viability (P<0.05 and P<0.01; Fig. 4A), while it promoted cell apoptosis (both P<0.01) (Fig. 4B and C) compared with DPP or THD monotherapy. In addition, THD and DDP exerted a similar synergistic effect on the relative viability (both P<0.05; Fig. 4D) and apoptosis (both P<0.01; Fig. 4E and F) of SiHa cells.

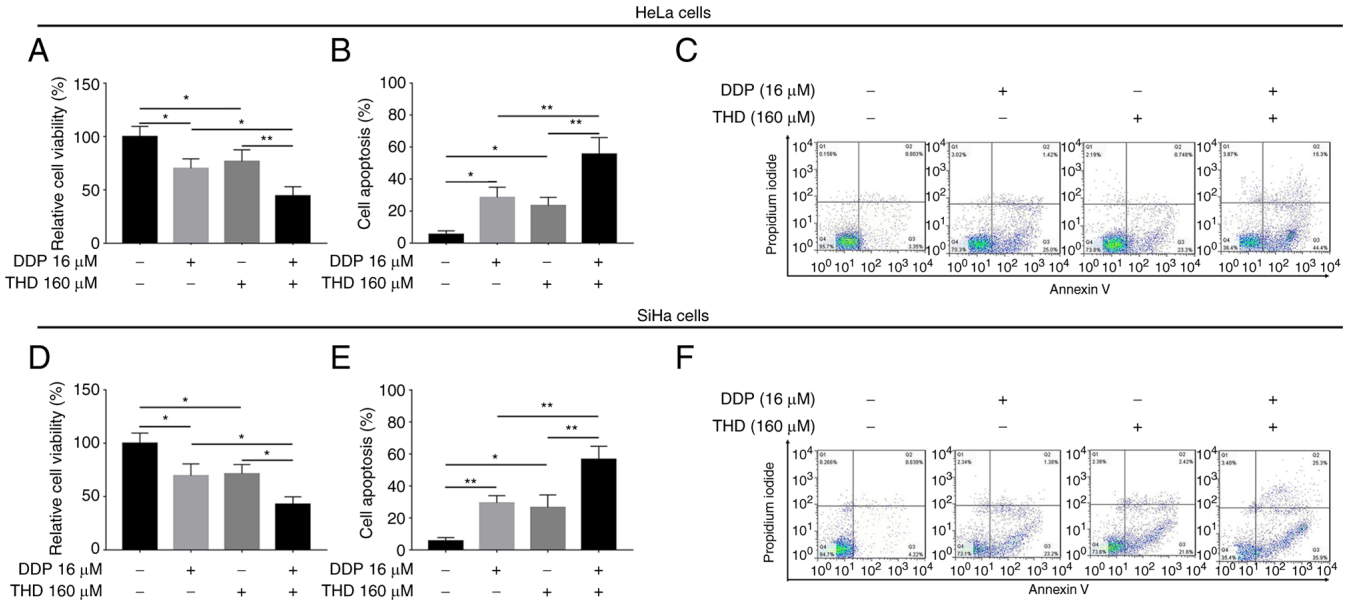



Figure 4. Comparison of relative cell viability and apoptosis by THD/DDP monotherapy and their synergistic treatment. Comparison of (A) relative cell viability and (B) apoptosis by THD/DDP monotherapy and their synergistic treatment in HeLa cells; (C) representative images of flow cytometric analysis of apoptosis in HeLa cells. Comparison of (D) relative cell viability and (E) apoptosis by THD/DDP monotherapy and their synergistic treatment in SiHa cells; (F) representative images of flow cytometric analysis of apoptosis in SiHa cells. $^{*}$P<0.05 and $^{**}$P<0.01. DDP, cisplatin; THD, thalidomide.

*Synergistic effects of THD and DDP on the PI3K/AKT pathway in cervical cancer cell lines*. In both HeLa cells and SiHa cells, THD or DDP monotherapy reduced the mRNA and protein levels of PI3K, as well as the phosphorylation levels of AKT (P<0.05 and P<0.01; Fig. 5A-H). Moreover, combined treatment with THD and DDP exerted a more prominent suppressive effect on the mRNA and protein levels of PI3K, as well as the on the phosphorylation levels of AKT, compared with DDP or THD monotherapy (P<0.05, P<0.01 and P<0.001; Fig. 5A-H).

*Synergistic effects of THD and DDP on the JAK1/STAT3 pathway in cervical cancer cell lines*. In HeLa cells, the mRNA (P<0.05, P<0.01 and P<0.001; Fig. 6A and B) and protein levels (P<0.05 and P<0.01; Fig. 6C-E) of JAK1 and STAT3 were decreased by THD or DDP monotherapy. Furthermore, the mRNA and protein levels of JAK1 and STAT3 were further reduced by THD and DDP combined treatment compared with DDP or THD monotherapy (P<0.05, P<0.01 and P<0.001; Fig. 6A-E). Moreover, the levels of phosphorylated JAK1 and STAT3 were also decreased by THD and DDP combined treatment (P<0.05 and P<0.01; Fig. 6F and G). In SiHa cells, mRNA (P<0.05 and P<0.01; Fig. 6H and I) and protein levels (all P<0.05; Fig. 6J-L) of JAK1 and STAT3 were decreased by THD or DDP monotherapy and further reduced by THD and DDP combined treatment (P<0.05 and P<0.01; Fig. 6H-L). In addition, the levels of phosphorylated JAK1 and STAT3 were also decreased by THD and DDP combined treatment (P<0.05 and P<0.01; Fig. 6M and N). Furthermore, when the PI3K/AKT or JAK1/STAT3 pathway was activated, the effects of THD plus DDP on reducing cell viability and increasing apoptosis were attenuated (P<0.05 and P<0.01; Fig. 7A-F), indicating that the PI3K/AKT and JAK1/STAT3 pathways are required for the killing effects of THD plus DDP on cervical cancer cells.

## Discussion

DDP has long been included in the treatment regimen of cervical cancer; however, its use as a monotherapy has not proven to be effective (5,7). Previous studies have demonstrated that the combination of cisplatin with other therapeutic agents exhibits an adequate treatment efficacy in several types of cancer, including non-small cell lung, bladder and cervical cancer (27-29). On the other hand, THD exerts a synergistic effect when combined with other therapeutic agents in the treatment of cancer patients. For instance, a previous study demonstrated that THD plus chemo-radiotherapy improved the 3-year overall survival rate, progression-free survival rate and median progression-free survival time compared with chemo-radiotherapy alone in patients with esophageal cancer (16). Moreover, another systematic review indicated that THD plus docetaxel improved patient prognosis and exerted a more prominent suppressive effect on prostate-specific antigen levels than docetaxel in patients with androgen-independent prostate cancer (30). In addition, it has been reported that THD plus chemotherapy exerts an improved treatment effect compared with chemotherapy alone in patients with advanced non-small cell lung or small cell lung cancer (31).

Although the aforementioned studies have indicated that the combination of THD or DDP with other treatment strategies enhances their effects on several types of cancer, including cervical cancer (13,27,29), it remains unclear whether THD plus DDP can exert a synergistic therapeutic effect on cervical cancer. Therefore, the present study found that DDP or THD monotherapy both suppressed the relative viability of cervical cancer cell lines in a concentration-dependent manner. These data were partially in line with those of previous studies (32,33). In addition, the present study demonstrated that DDP plus THD exerted a more prominent suppressive effect on the relative cell viability of cervical cancer cell lines

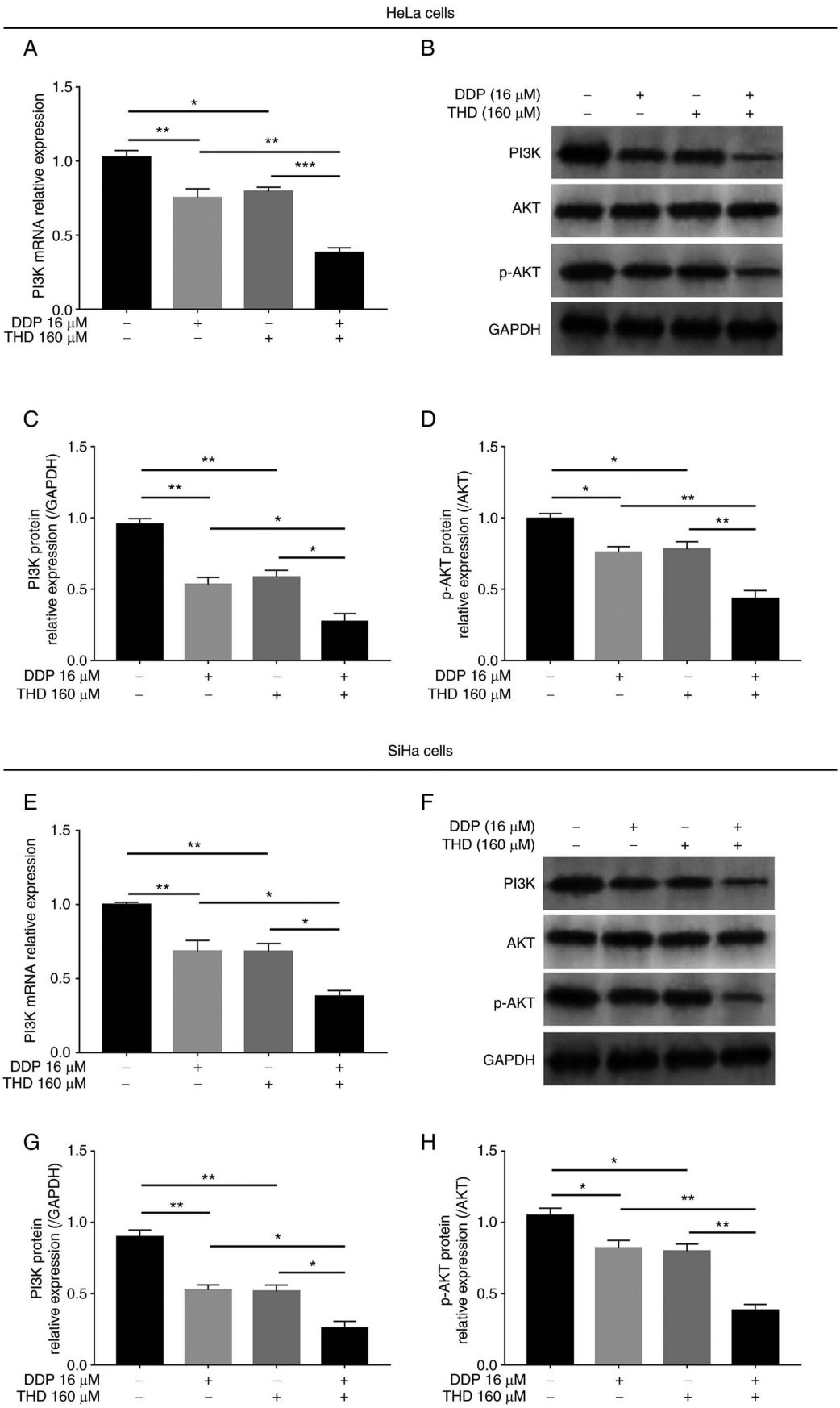



Figure 5. Comparison of PI3K and AKT expression levels. (A) Comparison of PI3K mRNA expression by THD/DDP monotherapy and their synergistic treatment in HeLa cells. (B) Representative images of PI3K, AKT and p-AKT protein expression detection by western blotting in HeLa cells. Comparison of (C) PI3K and (D) p-AKT protein expression by THD/DDP monotherapy and their synergistic treatment in HeLa cells. (E) Comparison of PI3K mRNA expression by THD/DDP monotherapy and their synergistic treatment in SiHa cells. (F) Representative images of PI3K, AKT and p-AKT protein expression detection by western blotting in SiHa cells. (G) Comparison of PI3K and (H) p-AKT protein expression by THD/DDP monotherapy and their synergistic treatment in SiHa cells. $^{*}$P<0.05, $^{**}$P<0.01 and $^{***}$P<0.001. PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; THD, thalidomide; DDP, cisplatin; p-, phosphorylated.

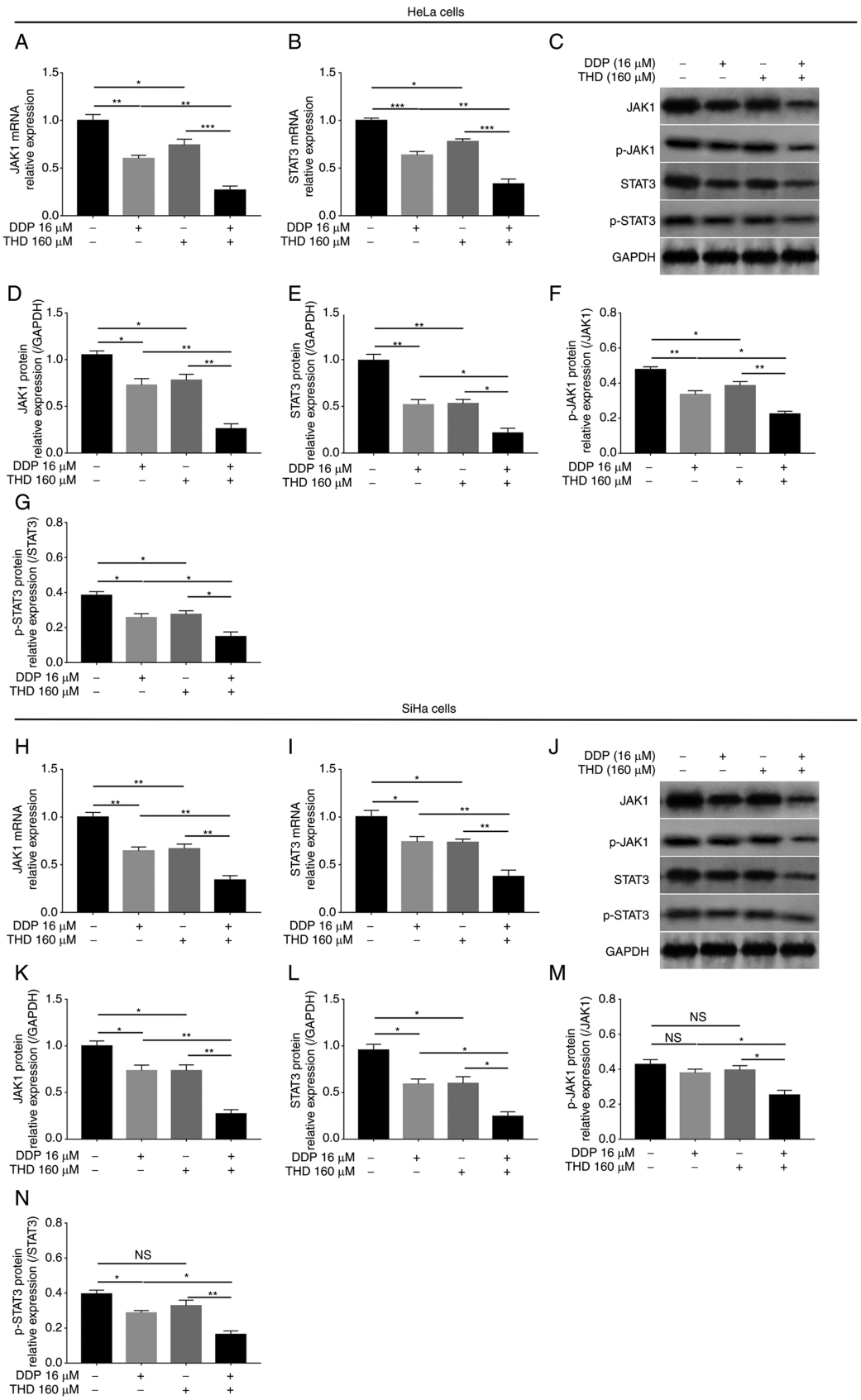



Figure 6. Comparison of JAK1 and STAT3 expression levels. Comparison of (A) JAK1 and (B) STAT3 mRNA expression levels by THD/DDP monotherapy and their synergistic treatment in HeLa cells. (C) Representative images of JAK1 and STAT3 protein expression detection by western blotting in HeLa cells. Comparison of (D) JAK1, (E) STAT3, (F) p-JAK1 and (G) p-STAT3 protein expression levels by THD/DDP monotherapy and their synergistic treatment in HeLa cells. Comparison of (H) JAK1 and (I) STAT3 mRNA expression levels by THD/DDP monotherapy and their synergistic treatment in SiHa cells. (J) Representative images of JAK1 and STAT3 protein expression detection by western blotting in SiHa cells. Comparison of (K) JAK1, (L) STAT3, (M) p-JAK1 and (N) p-STAT3 protein expression levels by THD/DDP monotherapy and their synergistic treatment in SiHa cells. $^{*}$P<0.05, $^{**}$P<0.01 and $^{***}$P<0.001. JAK1, Janus kinase 1; STAT3, signal transducer and activator of transcription 3; THD, thalidomide; DDP, cisplatin; p-, phosphorylated; NS, not significant.

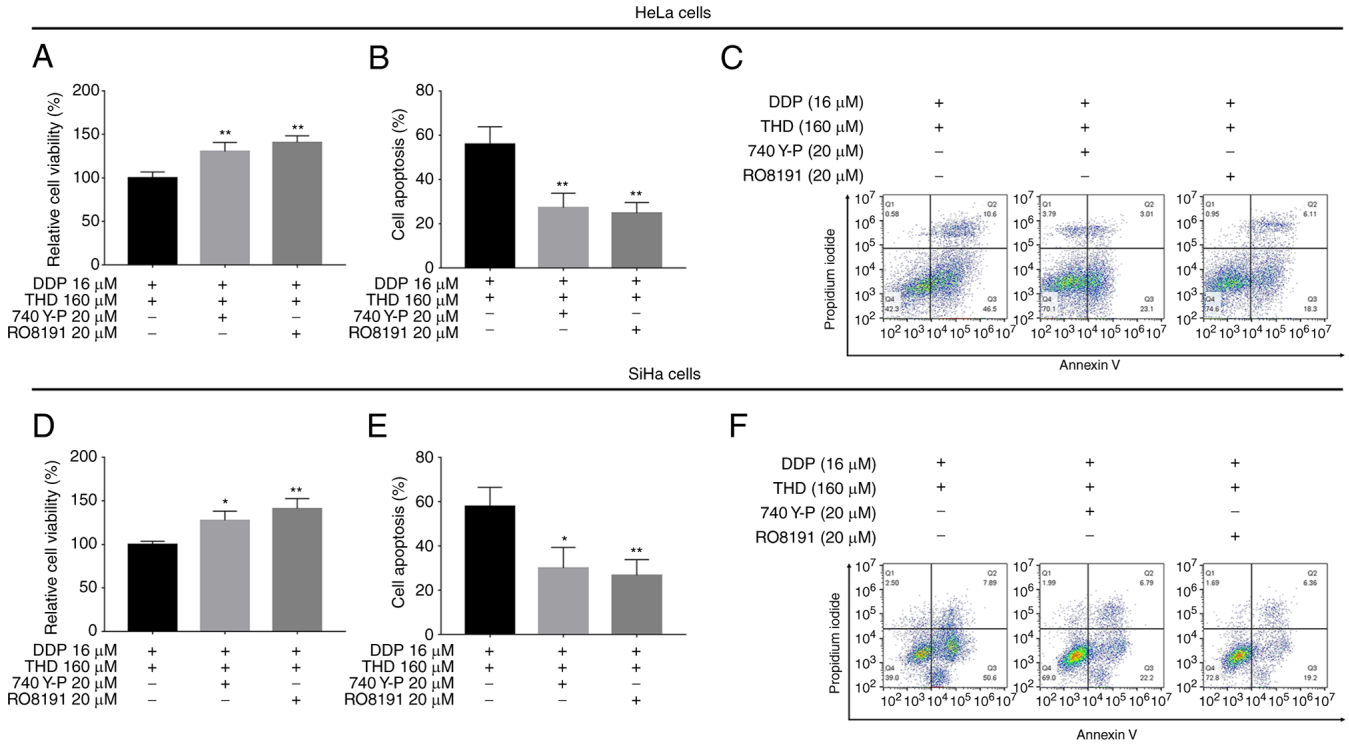



Figure 7. Role of PI3K/AKT and JAK1/STAT3 pathways on the regulation of cervical cancer cell viability and apoptosis by THD and DDP synergistic treatment. Comparison of (A) cell viability and (B) apoptosis among groups in HeLa cells. (C) Representative images of apoptosis evaluation among groups in HeLa cells. Comparison of (D) cell viability and (E) apoptosis among groups in SiHa cells. (F) Representative images of apoptosis evaluation among groups in SiHa cells. $^{*}$P<0.05 and $^{**}$P<0.01 compared with cells treated with 16 $\mu$M of DDP and 160 $\mu$M of THD but without treatment of 740 Y-P or RO8191. PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; JAK1, Janus kinase 1; STAT3, signal transducer and activator of transcription 3; THD, thalidomide; DDP, cisplatin.

than DDP or THD monotherapy, and the optimal combination concentrations were 16 $\mu$M for DDP and 160 $\mu$M for THD. It was hypothesized that THD may regulate several pathways, such as the PI3K/AKT and JAK1/STAT3 pathways (as demonstrated using western blot analysis in the present study) (34) to enhance the suppressive effects of cisplatin on the viability of cervical cancer cell lines. Moreover, it was found that combined treatment with THD and DDP enhanced apoptosis compared with DDP or THD monotherapy in cervical cancer cell lines. These data also suggest that THD may regulate several pathways to enhance the cytotoxic effects of DDP (as aforementioned).

The PI3K/AKT and JAK1/STAT3 pathways are two classic pathways that regulate cell survival in cervical cancer. Previous studies have indicated that activating the PI3K/AKT or JAK1/STAT3 pathways promotes the proliferation, whereas it suppresses the apoptosis of cervical cancer cell lines (35,36). In addition, the PI3K/AKT and JAK1/STAT3 pathways are associated with HPV infection, which is a critical risk factor of cervical cancer (37-39). In the present study, it was found that combined treatment suppressed PI3K, AKT, JAK1 and STAT3 gene expression. Concurrently, combined treatment also inhibited PI3K, p-AKT, JAK1 and STAT3 protein expression, suggesting that it suppressed the PI3K/AKT and JAK1/STAT3 pathways in cervical cancer cell lines. However, these assays did not include the human normal cervical epithelial cell line, which was a limitation of the present study.

Previous studies have demonstrated that the combination of THD and DDP exerts a suppressive synergistic effect on tumor progression in breast tumor model mice, colorectal tumor model mice and head and neck squamous cell carcinoma model mice (13,15). However, to the best of our knowledge, only one *in vitro* study found that the combination between THD and DDP exerted a synergistic suppressive effect on the proliferation of head and neck squamous cell carcinoma cells (13). Currently, studies investigating the treatment efficacy of THD plus DDP in patients with cancer are relatively limited; to the best of our knowledge, to date, only one randomized controlled trial revealed that DDP plus THD improved the prognosis of patients with advanced esophageal cancer (16). Furthermore, the use of THD plus DDP in patients with cancer also lacks pre-clinical research and theoretical support. The findings of the present study potentially contribute to this issue. However, further pilot clinical trials are required to explore the efficacy of THD plus DDP in cervical cancer patients.

In conclusion, the present study demonstrated that THD synergized with DDP in killing cervical cancer cell lines, which also inactivated the PI3K/AKT and JAK1/STAT3 pathways; this suggests their potential for use in cervical cancer treatment. However, further studies are warranted to determine whether THD and DDP also exert synergistic suppressive effects on tumor progression in cervical cancer model mice.

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## Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

## Authors' contributions

CL and LY contributed to the conception of the study. HF, LS, SL and YW contributed to data acquisition and data analysis. CL, HF, LS and YW drafted the manuscript. SL and LY revised the manuscript. CL and LY confirm the authenticity of all the raw data. All authors have approved the final version to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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