

Original Article

Volasertib suppresses tumor growth and potentiates the activity of cisplatin in cervical cancer

Feng-Feng Xie^{1*}, Shi-Shi Pan^{1*}, Rong-Ying Ou^{1*}, Zhen-Zhen Zheng¹, Xiao-Xiu Huang¹, Meng-Ting Jian¹, Jian-Ge Qiu², Wen-Ji Zhang², Qi-Wei Jiang², Yang Yang², Wen-Feng Li³, Zhi Shi², Xiao-Jian Yan¹

¹Department of Gynecology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, Zhejiang, China; ²Department of Cell Biology & Institute of Biomedicine, National Engineering Research Center of Genetic Medicine, Guangdong Provincial Key Laboratory of Bioengineering Medicine, College of Life Science and Technology, Jinan University, Guangzhou 510632, Guangdong, China; ³Department of Chemoradiotherapy, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, Zhejiang, China. *Equal contributors.

Received October 20, 2015; Accepted October 27, 2015; Epub November 15, 2015; Published December 1, 2015

Abstract: Volasertib (BI 6727), a highly selective and potent inhibitor of PLK1, has shown broad antitumor activities in the preclinical and clinical studies for the treatment of several types of cancers. However, the anticancer effect of volasertib on cervical cancer cells is still unknown. In the present study, we show that volasertib can markedly induce cell growth inhibition, cell cycle arrest at G2/M phase and apoptosis with the decreased protein expressions of PLK1 substrates survivin and wee1 in human cervical cancer cells. Furthermore, volasertib also enhances the intracellular reactive oxidative species (ROS) levels, and pretreated with ROS scavenger N-acety-L-cysteine totally blocks ROS generation but partly reverses volasertib-induced apoptosis. In addition, volasertib significantly potentiates the activity of cisplatin to inhibit the growth of cervical cancer *in vitro* and *in vivo*. In brief, volasertib suppresses tumor growth and potentiates the activity of cisplatin in cervical cancer, suggesting the combination of volasertib and cisplatin may be a promising strategy for the treatment of patients with cervical cancer.

Keywords: Volasertib, cisplatin, cervical cancer, combination therapy

Introduction

Cervical cancer is the third most common and fourth lethal gynecologic cancer with more than 500,000 new case in the worldwide [1]. Human papillomavirus infection is involved in the development of over 90% of cases [2]. Treatment of cervical cancer includes the combination of surgery, chemotherapy or radiotherapy. However, patients with metastatic or recurrent cancers after platinum-based chemotherapy are still lack of effective therapeutic agents [3]. Therefore, development of new therapeutic drugs against cervical cancer is an urgent necessity.

Volasertib (BI 6727) is a highly selective and potent small molecule inhibitor of the polo-like kinase 1 (PLK1) with IC₅₀ of 0.87 nM. It shows 6- and 65-fold greater selectivity against two closely related kinases PLK2 and PLK3 [4]. PLK1 proteins are located in the nuclei of cells

and play key role in controlling multiple stages of cell cycle, including mitotic entry, centrosome maturation, maintenance of the bipolar spindle and mitotic exit [5, 6]. Overexpression of PLK1 is found in up to 80% of malignancies, where it has been associated with poorer prognosis [6-8]. Therefore, PLK1 may be an important target for cancer treatment. Preclinical data have demonstrated that volasertib shows broad antitumor activity in multiple cancer models by inducing cell cycle arrest and apoptosis [4, 9-11]. In a phase I clinical study including 65 patients with progressive advanced or metastatic solid tumors received a single 1-h infusion of volasertib at doses of 12-450 mg every 3 weeks, volasertib shows a favorable pharmacokinetic profile and encouraging preliminary antitumor activity with manageable toxicities such as anaemia, neutropenia, fatigue and thrombocytopenia [12]. The results of another phase I clinical trial enrolled 59 Asian patients with advanced solid tumors has shown that

Sildenafil suppresses cervical cancer growth

volasertib is generally well tolerated with a promising pharmacokinetic properties with dose-limiting toxicities including thrombocytopenia, neutropenia and febrile neutropenia [13]. The phase II trial in 50 patients with locally advanced or metastatic urothelial cancer has concluded that volasertib has an acceptable safety profile but insufficient antitumor activity as a monotherapy [14]. Furthermore, evaluation of volasertib alone or combined with other chemotherapeutic drugs for the treatment of multiple cancers currently is ongoing. In this study, we investigate the antitumor effects of volasertib alone or in combination with cisplatin in cervical cancer.

Material and methods

Cell lines, cell culture, and reagents

The human cervical cell lines Caski and HeLa were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/ml) and streptomycin (100 ng/ml) in a humidified incubator at 37°C with 5% CO₂. Volasertib and cisplatin were ordered from ApexBio and Qilu Pharmaceutical, respectively. N-acety-L-cysteine (NAC) and dihydroethidium (DHE) were purchased from Sigma-Aldrich. Anti-PARP (9542) and Anti-Survivin (2808) antibodies were from Cell Signaling Technologies. Anti-Wee1 (SC-5285) antibodies were from Santa Cruz Biotechnology. Anti-GAPDH (LK9002T) antibodies were from Tianjin Sungene Biotech.

Cell viability assay

Cells were firstly seeded into a 96-well plate at a density of 5000 cells per well, and incubated with drugs in three parallel wells for 72 hr. Then 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well at a final concentration of 0.5 mg/ml. After incubation for 4 hr, formazan crystals were dissolved in 100 µl of DMSO, and absorbance at 570 nm was measured by plate reader. The concentrations required to inhibit growth by 50% (IC₅₀) were calculated from survival curves using the Bliss method [15-17]. For drug combinational experiments, cells were treated with the indicated concentrations of volasertib combined with cisplatin for 72 h.

Cell cycle analysis

Cells were harvested and washed twice with cold phosphate-buffered saline (PBS), then

fixed with ice-cold 70% ethanol for 30 min at 4°C. After centrifugation at 200 × g for 10 min, cells were washed twice with PBS and resuspended with 0.5 ml PBS containing PI (50 µg/ml), 0.1% Triton X-100, 0.1% sodium citrate, and DNase-free RNase (100 µg/ml), and detected by FCM after 15 min incubation at room temperature in the dark. Fluorescence was measured at an excitation wavelength of 480 nm through a FL-2 filter (585 nm). Data were analyzed using ModFit LT 3.0 software (Becton Dickinson) [18, 19].

Apoptosis assay

Cell apoptosis was evaluated with flow cytometry (FCM) assay. Briefly, cells were harvested and washed twice with PBS, stained with Annexin V-FITC and propidium iodide (PI) in the binding buffer, and detected by FACS Calibur FCM (BD, CA, USA) after 15 min incubation at room temperature in the dark. Fluorescence was measured at an excitation wavelength of 480 nm through FL-1 (530 nm) and FL-2 filters (585 nm). The early apoptotic cells (Annexin V positive only) and late apoptotic cells (Annexin V and PI positive) were quantified [20, 21].

Reactive oxygen species (ROS) assay

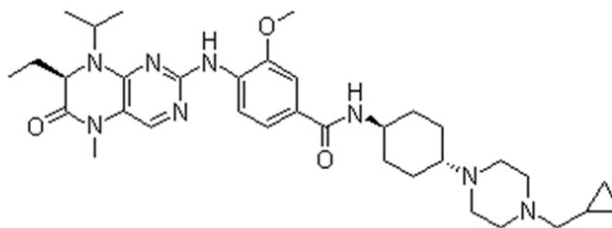
Cells were incubated with 10 µM of DHE for 30 min at 37°C, washed twice with PBS and immediately photographed under fluorescent microscope (Olympus, Japan). For each well, 5 fields were taken randomly [22, 23].

Western blot analysis

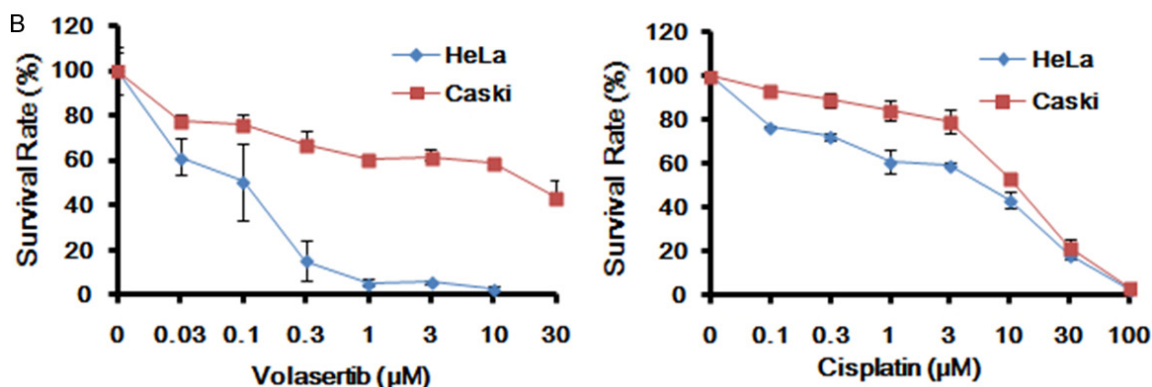
Cells were harvested and washed twice with cold PBS, then resuspended and lysed in RIPA buffer (1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 10 ng/ml PMSF, 0.03% aprotinin, 1 µM sodium orthovanadate) at 4°C for 30 min. Lysates were centrifuged for 10 min at 14,000 × g and supernatants were stored at -80°C as whole cell extracts. Total protein concentrations were determined with Bradford assay. Proteins were separated on 12% SDS-PAGE gels and transferred to polyvinylidene difluoride membranes. Membranes were blocked with 5% BSA and incubated with the indicated primary antibodies. Corresponding horseradish peroxidase-conjugated secondary antibodies were used against each primary antibody. Proteins were detected using the chemiluminescent detection reagents and films [24, 25].

Sildenafil suppresses cervical cancer growth

A



Volasertib



C

Summary of IC₅₀ of volasertib and cisplatin in cervical cancer cells.

IC ₅₀	Volasertib(µM±SD)	Cisplatin (µM±SD)
Caski	2.02±0.16	10.44±2.75
HeLa	0.02±0.01	8.18±1.21

Figure 1. Volasertib inhibits the growth of cervical cancer cells *in vitro*. A. Chemical structure of volasertib. B. Summary of IC₅₀ of volasertib and cisplatin in the indicated cervical cancer cells is shown. Cells were grown in 96-well plates for 24 hr and treated with the indicated concentrations of volasertib or cisplatin for 72 hr, and cell survival was determined by MTT assay. C. The representative growth curves of cells treated with dinaciclib and cisplatin are shown. Data are mean ±SD of three independent experiments.

Nude mice xenograft tumor assay

Balb/c nude mice were obtained from the Guangdong Medical Laboratory Animal Center and maintained with sterilized food and water. Six female nude mice with 5 weeks old and 20 g weight were used for each group. Each mouse was injected subcutaneously with Caski cells (2×10^6 in 200 µl of medium) under the shoulder. When the subcutaneous tumors were approximately 0.3×0.3 cm² (two perpendicular diameters) in size, mice were randomized into four groups, and were injected intraperitoneally with vehicle alone

(20% hydroxypropyl-β-cyclodextrin), volasertib alone (15 mg/kg), cisplatin alone (2 mg/kg), and a combination of volasertib with cisplatin every two days. The body weights of mice and the two perpendicular diameters (A and B) of tumors were recorded. The tumor volume (V) was calculated according to the formula.

$$V = \frac{\pi}{6} \left(\frac{A+B}{2} \right)^3$$

The mice were anaesthetized after experiment, and tumor tissue was excised from the mice

Sildenafil suppresses cervical cancer growth

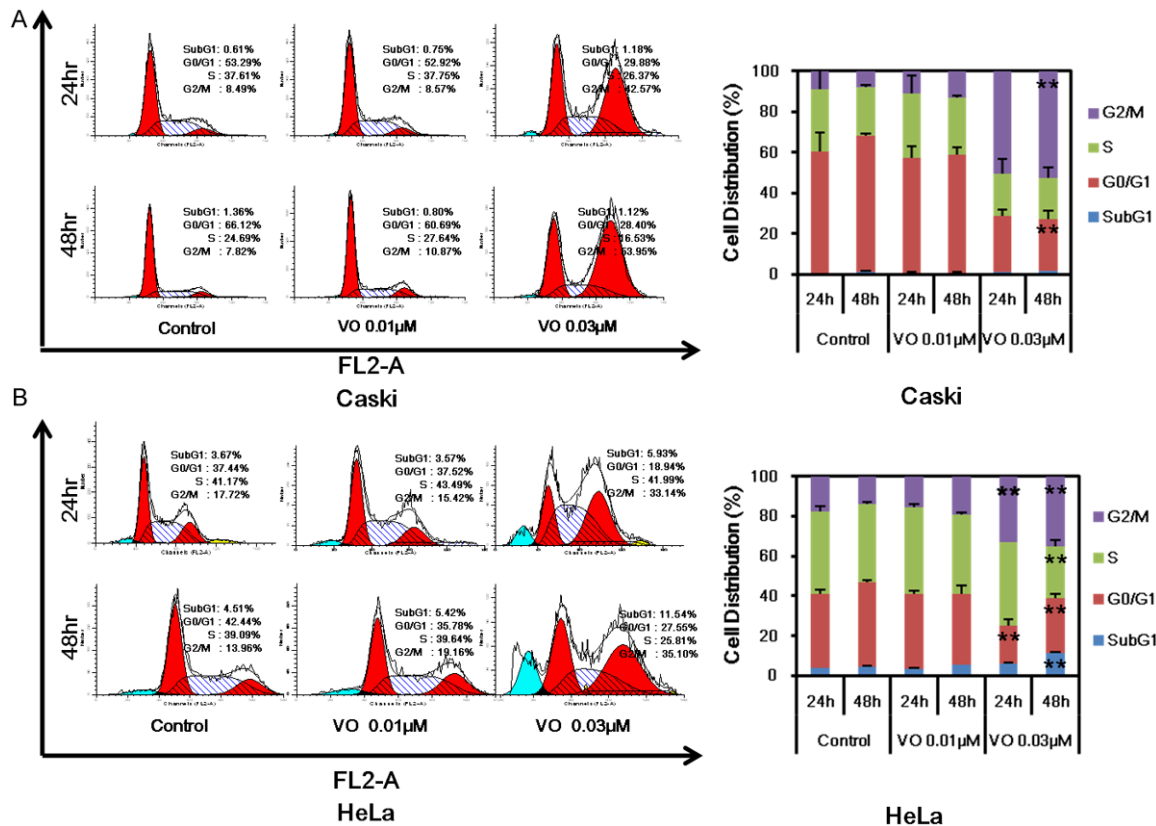


Figure 2. Volasertib induces cell cycle arrest at G2/M Phase in cervical cancer cells. Caski (A) and HeLa (B) were treated with volasertib at the indicated concentrations and times. The distribution of cell cycle was detected by FCM with PI staining. The percentages of subG1, G1/G0 and G2/M phases were calculated by ModFit LT 3.0 software. The representative charts and quantified results of three independent experiments are shown. VO: volasertib. * $P < 0.05$ and ** $P < 0.01$ vs. corresponding control.

and weighted. The rate of inhibition (IR) was calculated according to the formula [26, 27].

$$IR = 1 - \frac{\text{Mean tumor weight of experimental group}}{\text{Mean tumor weight of control group}} \times 100\%$$

Statistical analysis

All results are expressed as mean \pm standard deviation (SD). Statistical analysis of the differences between two groups is performed with Student's t-test. Values of $P < 0.05$ are considered as significant differences.

Result

Volasertib inhibits the growth of cervical cancer cells in vitro

To evaluate the single agent effect of volasertib on cervical cancer cells, two cervical cancer cell lines Caski and HeLa were treated with

either vehicle control or increasing concentrations of volasertib range from 0.03 μM to 30 μM for 72 hr. As shown in **Figure 1B**, the survivals of two cervical cancer cell lines are decreased in a dose-dependent manner after volasertib treatment. The IC_{50} values of volasertib in HeLa and Caski cells are 0.02 μM and 2.02 μM , respectively. In addition, cisplatin also dose-dependently inhibits the growth of HeLa and Caski cells with IC_{50} values of 8.18 μM and 10.44 μM , respectively.

Volasertib induces cell cycle arrest at G2/M phase in cervical cancer cells

To assess whether the growth inhibition of volasertib in cervical cancer cells is due to induction of cell cycle arrest, both Caski and HeLa cells were treated with volasertib at 0.01 μM and 0.03 μM for 24 hr and 48 hr, stained with PI and detected by FCM. The cell cycle distribution was analyzed with ModFit LT 3.0 software.

Sildenafil suppresses cervical cancer growth

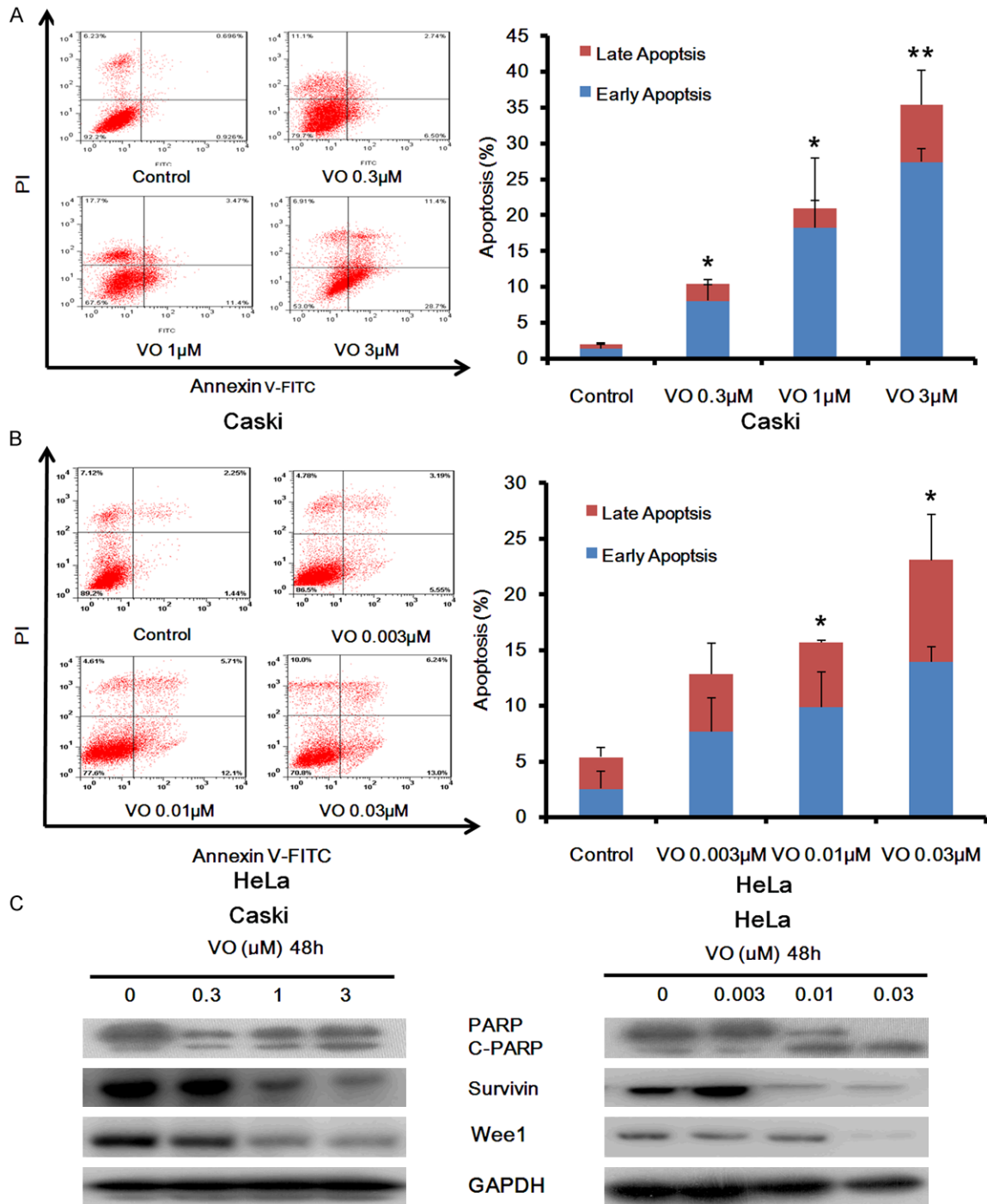


Figure 3. Volasertib induces apoptosis in cervical cancer cells. Caski (A) and HeLa (B) cells were treated with volasertib at the indicated concentrations. The apoptosis was calculated by FCM Annexin V/PI staining. The proportion of Annexin V⁺/PI⁻ and Annexin V⁺/PI⁺ cells indicated the early and late stage of apoptosis. The protein expression was examined by western blot, and GAPDH was used as loading control. The representative charts, quantified results and Western blot results (C) of three independent experiments are shown. VO: volasertib. Statistical analysis of the difference between two groups is performed with Student's t-test. * $P < 0.05$ and ** $P < 0.01$ vs. corresponding control.

As shown in **Figure 2A** and **2B**, treatment with volasertib at 0.03 µM for 24 hr and 48 hr increases the distribution of cells at G2/M

phase in both Caski and HeLa cells, suggesting that volasertib can induce cell cycle arrest at G2/M phase in cervical cancer cells.

Sildenafil suppresses cervical cancer growth

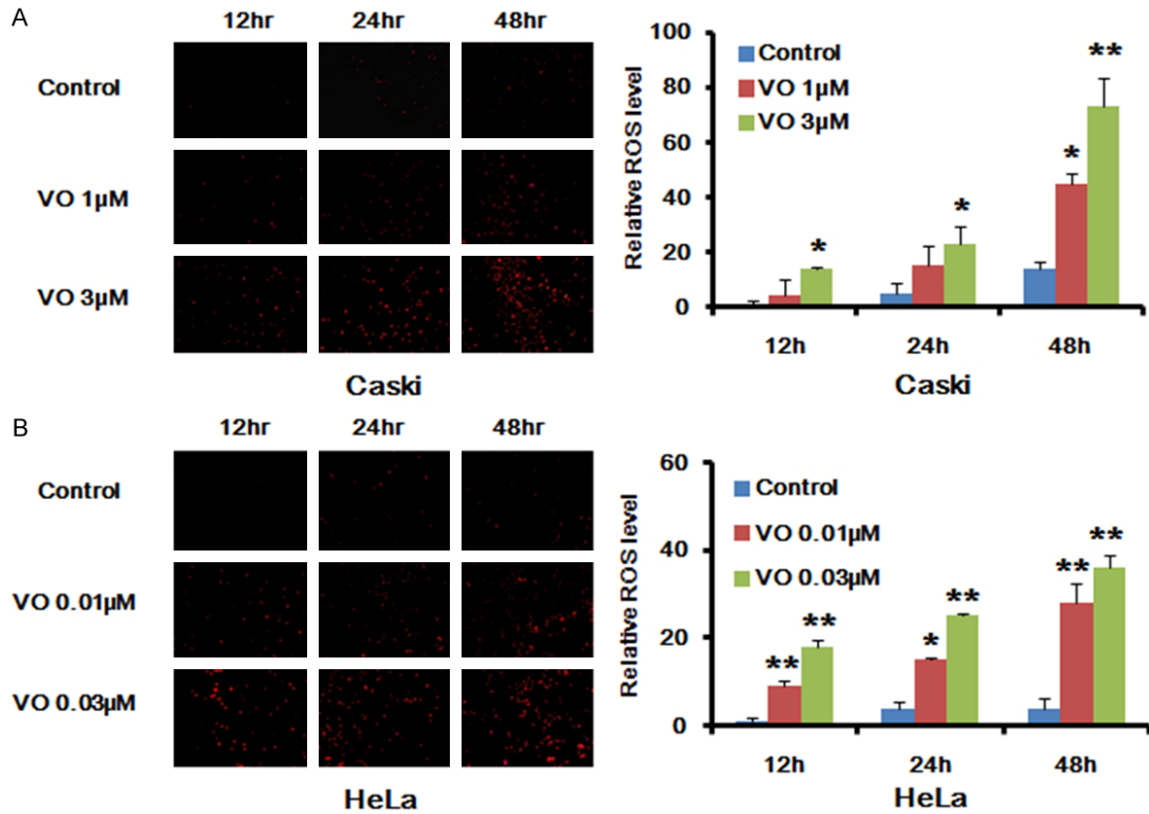


Figure 4. Volasertib induces ROS accumulation in cervical cancer cells. Caski (A) and HeLa (B) cells were treated with volasertib at the indicated times and concentrations, stained with DHE and photographed under fluorescent microscope. The representative micrographs and quantified results of three independent experiments are shown. VO: volasertib. Statistical analysis of the difference between two groups is performed with Student's t-test. * $P < 0.05$ and ** $P < 0.01$ vs. corresponding control.

Volasertib induces apoptosis in cervical cancer cells.

To further investigate whether volasertib is able to induce apoptosis in cervical cancer cells, both Caski and HeLa cells were treated with volasertib at different concentrations for 48 hr, stained with AnnexinV/PI and analyzed by FCM. As shown in **Figure 3A** and **3B**, volasertib dose-dependently induces apoptosis in both Caski and HeLa cells. Additionally, the protein levels of cleaved PARP which is a molecular marker of apoptosis are increased in a dose-dependent manner after volasertib treatment (**Figure 3C**). PLK1 phosphorylates survivin and wee1 proteins to enhance their stabilities, and inhibition of PLK1 results in the decrease of survivin and wee1 proteins levels [28, 29]. Indeed, volasertib dose-dependently suppresses the expression of survivin and wee1 proteins in both Caski and HeLa cells (**Figure 3C**).

Volasertib induces ROS accumulation in cervical cancer cells

It has been demonstrated that the intracellular level of ROS plays key role in cancer treatment with anticancer drugs, which usually induce cancer cell death with the elevated intracellular ROS [30, 31]. We used the common ROS fluorescent probe DHE to stain cervical cancer cells treated with volasertib. As shown in **Figure 4A** and **4B**, volasertib augmented the fluorescent intensity of DHE in both Caski and HeLa cells in a dose- and time-dependent manner, suggesting volasertib can increase the intracellular ROS level in cervical cancer cells.

Inhibition of ROS partially rescues volasertib-induced apoptosis in cervical cancer cells

To further certify the relationship between cell apoptosis and ROS generation elicited by volasertib, both cells were treated with volasertib

Sildenafil suppresses cervical cancer growth

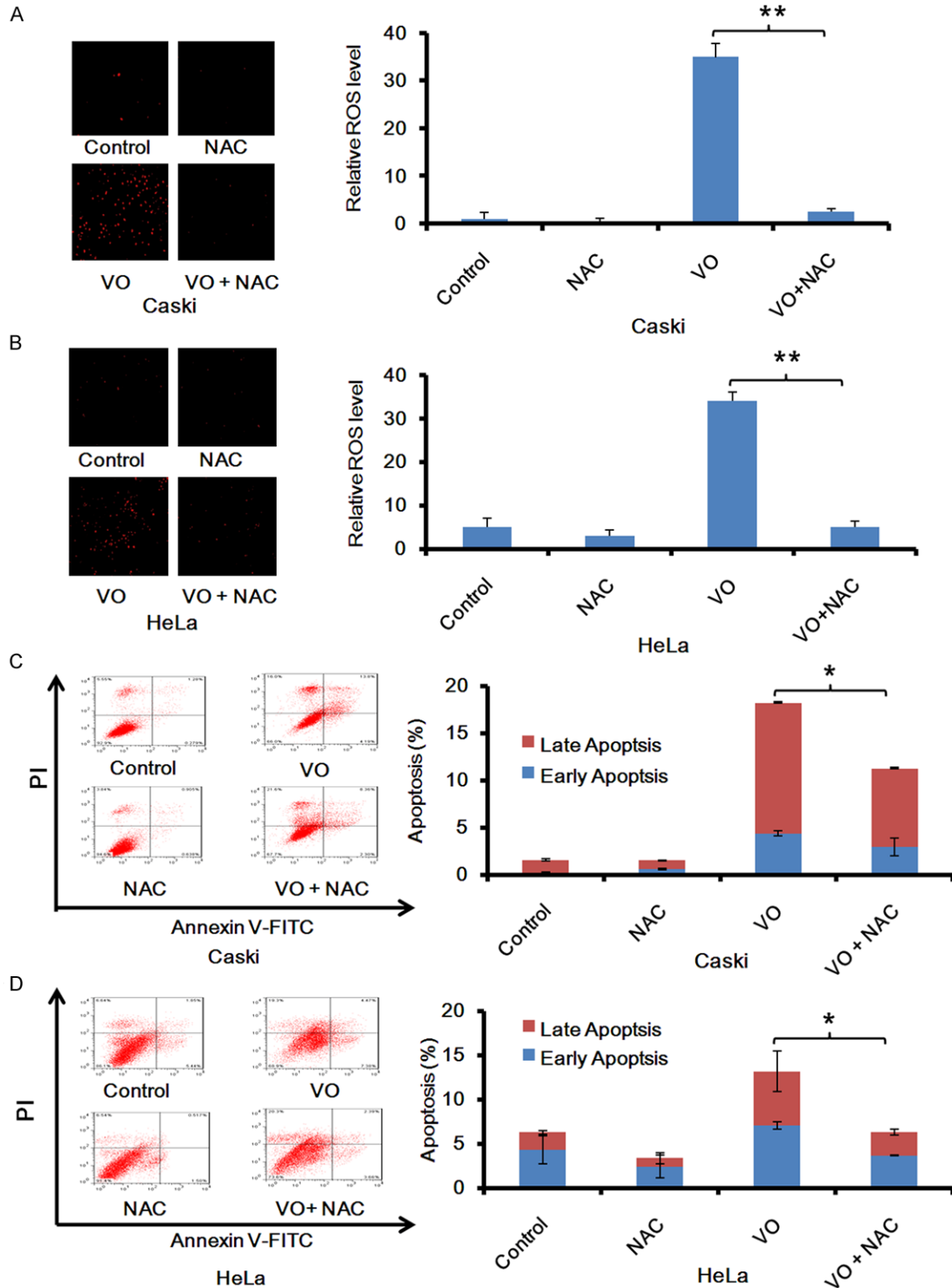


Figure 5. Inhibition of ROS partially rescues volasertib-induced apoptosis in cervical cancer cells. Caski (A, C) and HeLa (B, D) cells were treated with volasertib at 1 μ M and 0.01 μ M respectively for 48 hr in the presence or absence of 5 mM NAC pretreatment for 1 hr, stained with DHE and photographed under fluorescent microscope. The apoptosis was detected by FCM with Annexin V/PI staining. The proportions of Annexin V+/PI- and Annexin V+/PI+ cells indicated the early and late stage of apoptosis. The representative micrographs, charts and quantified results of three independent experiments are shown. VO: volasertib. Statistical analysis of the difference between two groups is performed with Student's t-test. * P <0.05 and ** P <0.01 vs. corresponding group.

Sildenafil suppresses cervical cancer growth

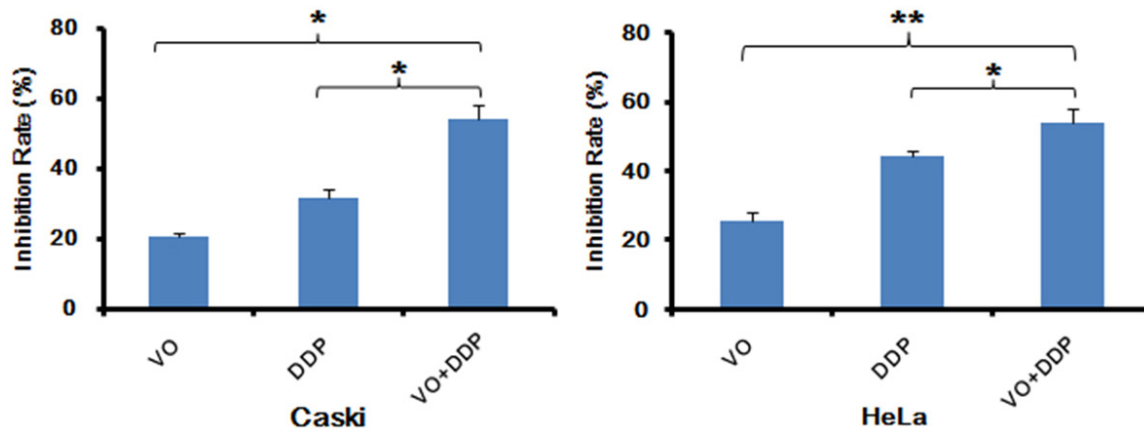


Figure 6. Volasertib potentiates the activity of cisplatin to inhibit the growth of cervical cancer cells *in vitro*. Caski and HeLa cells were treated with volasertib at 1 μ M and 0.01 μ M respectively and cisplatin at 3 μ M for 72 hr, and cell survival was detected by MTT assay. The inhibition rates of three independent experiments are shown. VO: volasertib; DDP: cisplatin. Statistical analysis of the difference between two groups is performed with Student's t-test. * P <0.05 and ** P <0.01 vs. corresponding group.

for 48 h with or without the antioxidative agent NAC pretreated for 1 hr and stained with DHE. As shown in **Figure 5A** and **5B**, the volasertib-induced DHE fluorescent signals were totally reversed by NAC in both cells. Furthermore, cell apoptosis was analyzed by FCM with Annexin V/PI staining. The volasertib-induced cell apoptosis were partly reversed by NAC (**Figure 5C** and **5D**). These results suggest volasertib can trigger both ROS dependent and independent apoptosis in cervical cancer cells.

Volasertib potentiates the activity of cisplatin to inhibit the growth of cervical cancer cells in vitro

Cisplatin currently is one of the main chemotherapeutic drugs for cervical cancer in clinic. To examine the combined effects of volasertib and cisplatin on cervical cancer cells, the inhibition of cell growth was detected by MTT assay. As shown in **Figure 6**, the inhibition of cell growth in the combined treatment with volasertib and cisplatin was significantly enhanced in comparison with volasertib or cisplatin alone treatment in both Caski and HeLa cells, suggesting the combination of volasertib and cisplatin may synergistically inhibit the growth of cervical cancer cells.

Volasertib potentiates the activity of cisplatin to inhibit xenograft tumor growth of cervical cancer cells in nude mice

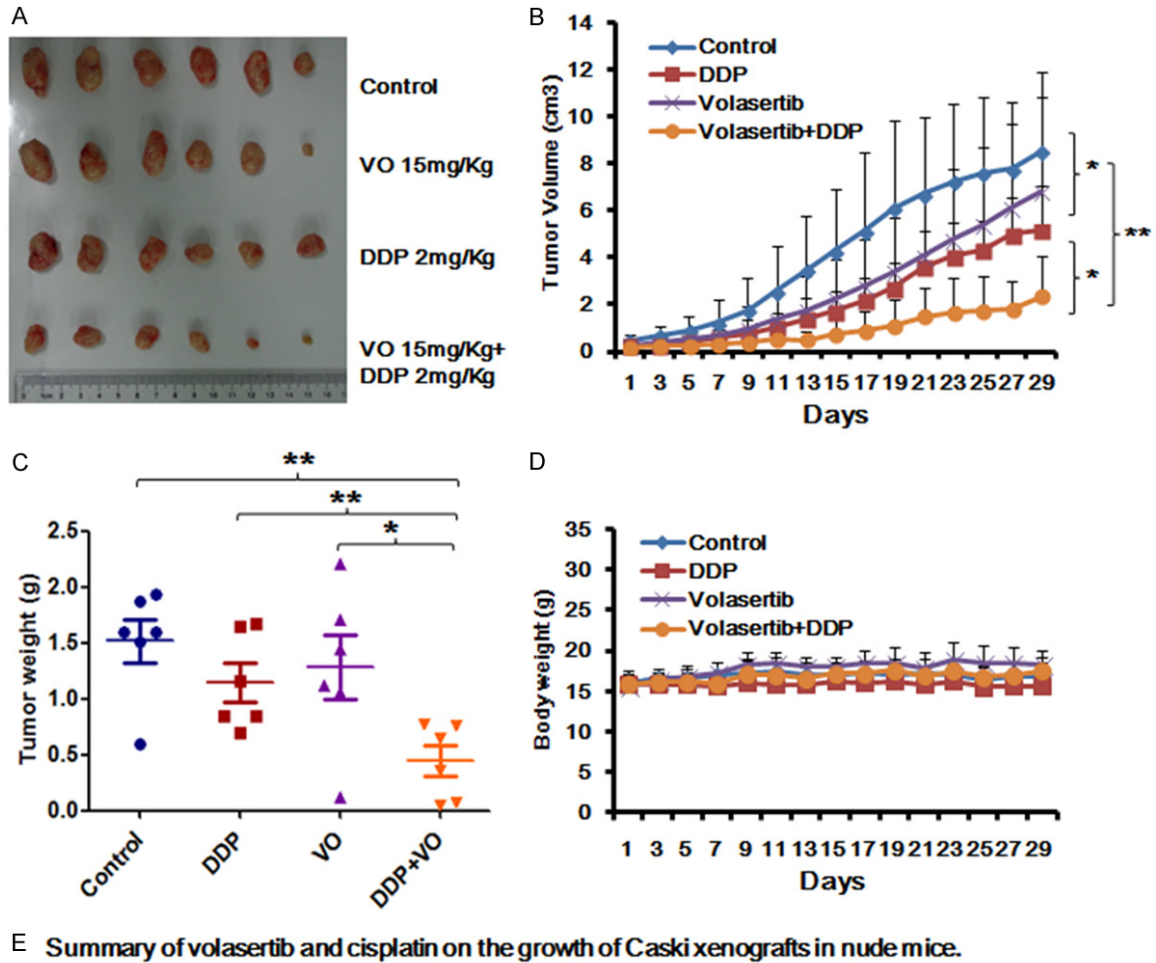
To further estimate the combined antitumor effects of volasertib and cisplatin *in vivo*, we

generated the subcutaneous xenograft tumor models by transplanting Caski cells into nude mice. The original tumors were showed in the **Figure 7A**, combination of volasertib and cisplatin apparently inhibited the tumors growth by reducing the volume and weight of Caski tumors **Figure 7B** and **7C**. The inhibition rates of tumor growth in the combined group was 78%, which was significantly higher than those in volasertib (15.6%) and cisplatin (24.5%) alone (**Figure 7E**). In addition, the weight of nude mice did not exhibit the statistical difference during experiments, which suggests volasertib or cisplatin alone and combination at the indicated dose did not cause toxicities in mice (**Figure 7D**).

Discussion

In the present study, our data show that volasertib can markedly induce cell growth inhibition, cell cycle arrest at G2/M phase and apoptosis with the decreased protein expressions of PLK1 substrates survivin and wee1 in human cervical cancer cells, which are consistent with previous reports that have demonstrated volasertib can induce mitotic arrest and promote apoptosis by targeting PLK in multiple cancer models [4, 9]. In addition, volasertib also enhances the intracellular ROS levels, and pretreated with ROS scavenger NAC totally blocks ROS generation but partly reverses volasertib-induced apoptosis. The intracellular ROS plays a critical role in multiple cell physiological processes, and cancer cells usually are suscepti-

Sildenafil suppresses cervical cancer growth



E Summary of volasertib and cisplatin on the growth of Caski xenografts in nude mice.

Groups	Before Experiment		After Experiment		Weight of Tumor(g)	Inhibition (%)
	Animals	Weight (g)	Animals	Weight (g)		
Control	6	16.1±0.97	6	16.9±1.56	1.51±0.48	
DDP	6	15.9±1.72	6	15.6±1.75	1.14±0.43	24.5*
VO	6	15.6±1.39	6	18.2±1.83	1.28±0.71	15.6
DDP+VO	6	15.8±1.21	6	17.5±1.67	0.44±0.33	70.8**

Figure 7. Volasertib potentiates the activity of cisplatin to inhibit xenograft tumor growth of cervical cancer cells in nude mice. Each mouse was injected subcutaneously with Caski cells (2×10^6 in 100 μ l of medium) under the shoulder. When the tumors developed into 0.3×0.3 cm² (two perpendicular diameters) in size, mice were randomized into four groups, and were injected intraperitoneally with vehicle alone (20% hydroxypropyl- β -cyclodextrin), volasertib alone (15 mg/kg), cisplatin alone (2 mg/kg), and the combination of volasertib with cisplatin every two days. The body weights of mice and the two perpendicular diameters of the tumor were recorded. The mice were anaesthetized after experiment, and tumor tissue was excised from the mice and weighed. The original tumors (A), tumor volume (B), tumor weight (C), body weight (D) and summary data (E) are shown. The values presented are the means \pm SD for each group. VO: volasertib; DDP: cisplatin. Statistical analysis of the difference between two groups is performed with Student's t-test. * $P < 0.05$ and ** $P < 0.01$ vs. corresponding group.

ble to anticancer drugs that enhancing ROS generation due to their increased basal levels

of intrinsic oxidative stress [32-34]. Therefore, our results suggest volasertib can prompt both

Sildenafil suppresses cervical cancer growth

ROS dependent and independent apoptosis in cervical cancer cells.

Combination therapy currently is the main mode of cancer chemotherapy due to its remarkable advantages such as less drug resistance and more treatment success [35]. Several groups have investigated the combined anticancer effects of volasertib and other agents in the preclinical and clinical studies. Combination of volasertib with sepantronium potently inhibits the growth of various non-small cell lung cancer cell lines than either drug alone *in vitro* [31]. Moreover, volasertib is highly efficacious as a single agent and in combination with cytarabine, decitabine, azacitidine and quizartinib in multiple preclinical models of acute myeloid leukemia [36]. In a phase I study of 57 patients with advanced solid tumors, volasertib combined with afatinib has manageable adverse effects and limited antitumor activity without clearly pharmacokinetic interference [37]. The results of another phase I study of 30 patients with advanced solid tumors has demonstrated that volasertib in combination with nintedanib also has manageable safety profile and antitumor activity without obviously pharmacokinetic disturbance [38]. In a phase II trial of 87 patients with acute myeloid leukemia, the combination of volasertib and cytarabine significantly prolongs median event-free survival and median overall survival with an increased frequency of adverse events compared with cytarabine alone [39]. Another phase II trial in patients with advanced non-small-cell lung cancer has showed that the combination of volasertib with pemetrexed does not improve efficacy without increasing toxicity and drug-drug interactions compared with pemetrexed alone [40]. In our study, volasertib significantly potentiates the activity of cisplatin to inhibit the growth of cervical cancer *in vitro* and *in vivo*. This is consistent with a previous report which has shown volasertib alone significantly inhibits the growth and invasion of bladder cancer cells, and combinations with cisplatin or methotrexate shows auspicious results but combinations with doxorubicin shows mostly antagonistic effects [41]. Furthermore, a recent phase I study in solid tumors has concluded that volasertib plus cisplatin or carboplatin has manageable safety and antitumor activity without pharmacokinetics affection of each drug [42]. Accordingly, the combination of volasertib with cisplatin for the

treatment of cervical cancer and other cancers may be worth to further evaluate in the future clinical trials.

In summary, our study provided strong evidence to demonstrate that volasertib not only significantly suppresses the growth of cervical cancer cells by induction of cell cycle arrest and apoptosis as single agent, but also potentiates the activity of cisplatin *in vitro* and *in vivo*. The combination of volasertib and cisplatin may be a promising strategy for the treatment of patients with cervical cancer.

Acknowledgements

This work was supported by funds from the Chinese National Natural Science Foundation No. 31271444 and No. 81201726 (Z. S.), No. 81503293 (X. J. Y.) and No. 81373075 (R. Y. O), the Specialized Research Fund for the Doctoral Program of Higher Education No. 20124-401120007 (Z. S.), the Guangdong Natural Science Funds for Distinguished Young Scholar No. 2014A030306001 (Z. S), and the Science and Technology Program of Guangzhou No. 2014J4100009 (Z. S), and the Natural Science Foundation of Zhejiang Province, China No. LQ12H16004 (X. J. Y.).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Zhi Shi, Department of Cell Biology & Institute of Biomedicine, National Engineering Research Center of Genetic Medicine, Guangdong Provincial Key Laboratory of Bioengineering Medicine, College of Life Science and Technology, Jinan University, Room 708, The 2nd Engineer and Scientific Building, 601 Huangpu Road West, Guangzhou 510632, China. Tel: +86-20-852-245-25; Fax: +86-20-852-259-77; E-mail: tshizhi@jnu.edu.cn; Dr. Xiao-Jian Yan, Department of Gynecology, The First Affiliated Hospital of Wenzhou Medical University, Shangcai Village South, Ouhai District, Wenzhou 325000, Zhejiang China. Tel: +86-577-555-791-63; Fax: +86-577-555-780-33; E-mail: yxjbetter@126.com

References

- [1] Siegel R, Ma J, Zou Z and Jemal A. Cancer statistics, 2014. *CA Cancer J Clin* 2014; 64: 9-29.
- [2] Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, Snijders PJ and Meijer CJ. Epidemiologic classification of human papillomavirus types associated with cer-

Sildenafil suppresses cervical cancer growth

- vical cancer. *N Engl J Med* 2003; 348: 518-527.
- [3] Waggoner SE. Cervical cancer. *Lancet* 2003; 361: 2217-2225.
- [4] Rudolph D, Steegmaier M, Hoffmann M, Grauert M, Baum A, Quant J, Haslinger C, Garin-Chesa P and Adolf GR. BI 6727, a Polo-like kinase inhibitor with improved pharmacokinetic profile and broad antitumor activity. *Clin Cancer Res* 2009; 15: 3094-3102.
- [5] Liu X and Erikson RL. Polo-like kinase 1 in the life and death of cancer cells. *Cell Cycle* 2003; 2: 424-425.
- [6] Strebhardt K and Ullrich A. Targeting polo-like kinase 1 for cancer therapy. *Nat Rev Cancer* 2006; 6: 321-330.
- [7] Gjertsen BT and Schoffski P. Discovery and development of the Polo-like kinase inhibitor volasertib in cancer therapy. *Leukemia* 2015; 29: 11-19.
- [8] Degenhardt Y and Lampkin T. Targeting Polo-like kinase in cancer therapy. *Clin Cancer Res* 2010; 16: 384-389.
- [9] Gorlick R, Kolb EA, Keir ST, Maris JM, Reynolds CP, Kang MH, Carol H, Lock R, Billups CA, Kurmasheva RT, Houghton PJ and Smith MA. Initial testing (stage 1) of the Polo-like kinase inhibitor volasertib (BI 6727), by the Pediatric Preclinical Testing Program. *Pediatr Blood Cancer* 2014; 61: 158-164.
- [10] Liu X. Targeting Polo-Like Kinases: A Promising Therapeutic Approach for Cancer Treatment. *Transl Oncol* 2015; 8: 185-195.
- [11] Brandwein JM. Targeting polo-like kinase 1 in acute myeloid leukemia. *Ther Adv Hematol* 2015; 6: 80-87.
- [12] Schoffski P, Awada A, Dumez H, Gil T, Bartholomeus S, Wolter P, Taton M, Fritsch H, Glomb P and Munzert G. A phase I, dose-escalation study of the novel Polo-like kinase inhibitor volasertib (BI 6727) in patients with advanced solid tumours. *Eur J Cancer* 2012; 48: 179-186.
- [13] Lin CC, Su WC, Yen CJ, Hsu CH, Su WP, Yeh KH, Lu YS, Cheng AL, Huang DC, Fritsch H, Voss F, Taube T and Yang JC. A phase I study of two dosing schedules of volasertib (BI 6727), an intravenous polo-like kinase inhibitor, in patients with advanced solid malignancies. *Br J Cancer* 2014; 110: 2434-2440.
- [14] Stadler WM, Vaughn DJ, Sonpavde G, Vogelzang NJ, Tagawa ST, Petrylak DP, Rosen P, Lin CC, Mahoney J, Modi S, Lee P, Ernstoff MS, Su WC, Spira A, Pilz K, Vinisko R, Schloss C, Fritsch H, Zhao C and Carducci MA. An open-label, single-arm, phase 2 trial of the Polo-like kinase inhibitor volasertib (BI 6727) in patients with locally advanced or metastatic urothelial cancer. *Cancer* 2014; 120: 976-982.
- [15] Shi Z, Jain S, Kim IW, Peng XX, Abraham I, Youssef DT, Fu LW, El Sayed K, Ambudkar SV and Chen ZS. Sipholenol A, a marine-derived sipholane triterpene, potently reverses P-glycoprotein (ABCB1)-mediated multidrug resistance in cancer cells. *Cancer Sci* 2007; 98: 1373-1380.
- [16] Shi Z, Tiwari AK, Shukla S, Robey RW, Kim IW, Parmar S, Bates SE, Si QS, Goldblatt CS, Abraham I, Fu LW, Ambudkar SV and Chen ZS. Inhibiting the function of ABCB1 and ABCG2 by the EGFR tyrosine kinase inhibitor AG1478. *Biochem Pharmacol* 2009; 77: 781-793.
- [17] Shi Z, Tiwari AK, Shukla S, Robey RW, Singh S, Kim IW, Bates SE, Peng X, Abraham I, Ambudkar SV, Talele TT, Fu LW and Chen ZS. Sildenafil reverses ABCB1- and ABCG2-mediated chemotherapeutic drug resistance. *Cancer Res* 2011; 71: 3029-3041.
- [18] Li J, Ouyang Y, Zhang X, Zhou W, Wang F, Huang Z, Wang X, Chen Y, Zhang H and Fu L. Effect of HM910, a novel camptothecin derivative, on the inhibition of multiple myeloma cell growth in vitro and in vivo. *Am J Cancer Res* 2015; 5: 1000-1016.
- [19] Qiao D, Tang S, Aslam S, Ahmad M, To KK, Wang F, Huang Z, Cai J and Fu L. UMMS-4 enhanced sensitivity of chemotherapeutic agents to ABCB1-overexpressing cells via inhibiting function of ABCB1 transporter. *Am J Cancer Res* 2014; 4: 148-160.
- [20] Shi Z, Park HR, Du Y, Li Z, Cheng K, Sun SY, Fu H and Khuri FR. Cables1 complex couples survival signaling to the cell death machinery. *Cancer Res* 2015; 75: 147-158.
- [21] Shi Z, Li Z, Li ZJ, Cheng K, Du Y, Fu H and Khuri FR. Cables1 controls p21/Cip1 protein stability by antagonizing proteasome subunit alpha type 3. *Oncogene* 2015; 34: 2538-2545.
- [22] Chen XX, Xie FF, Zhu XJ, Lin F, Pan SS, Gong LH, Qiu JG, Zhang WJ, Jiang QW, Mei XL, Xue YQ, Qin WM, Shi Z and Yan XJ. Cyclin-dependent kinase inhibitor dinaciclib potently synergizes with cisplatin in preclinical models of ovarian cancer. *Oncotarget* 2015; 6: 14926-39.
- [23] Gong LH, Chen XX, Wang H, Jiang QW, Pan SS, Qiu JG, Mei XL, Xue YQ, Qin WM, Zheng FY, Shi Z and Yan XJ. Piperlongumine induces apoptosis and synergizes with cisplatin or paclitaxel in human ovarian cancer cells. *Oxid Med Cell Longev* 2014; 2014: 906804.
- [24] Shi Z, Liang YJ, Chen ZS, Wang XH, Ding Y, Chen LM and Fu LW. Overexpression of Survivin and XIAP in MDR cancer cells unrelated to P-glycoprotein. *Oncol Rep* 2007; 17: 969-976.
- [25] Shi Z, Parmar S, Peng XX, Shen T, Robey RW, Bates SE, Fu LW, Shao Y, Chen YM, Zang F and Chen ZS. The epidermal growth factor tyrosine kinase inhibitor AG1478 and erlotinib reverse

Sildenafil suppresses cervical cancer growth

- ABCG2-mediated drug resistance. *Oncol Rep* 2009; 21: 483-489.
- [26] Qiu JG, Zhang YJ, Li Y, Zhao JM, Zhang WJ, Jiang QW, Mei XL, Xue YQ, Qin WM, Yang Y, Zheng DW, Chen Y, Wei MN and Shi Z. Trametinib modulates cancer multidrug resistance by targeting ABCB1 transporter. *Oncotarget* 2015; 6: 15494-509.
- [27] Shi Z, Liang YJ, Chen ZS, Wang XW, Wang XH, Ding Y, Chen LM, Yang XP and Fu LW. Reversal of MDR1/P-glycoprotein-mediated multidrug resistance by vector-based RNA interference in vitro and in vivo. *Cancer Biol Ther* 2006; 5: 39-47.
- [28] Colnaghi R and Wheatley SP. Liaisons between survivin and Plk1 during cell division and cell death. *J Biol Chem* 2010; 285: 22592-22604.
- [29] Watanabe N, Arai H, Nishihara Y, Taniguchi M, Hunter T and Osada H. M-phase kinases induce phospho-dependent ubiquitination of somatic Wee1 by SCFbeta-TrCP. *Proc Natl Acad Sci U S A* 2004; 101: 4419-4424.
- [30] Jiang QW, Cheng KJ, Mei XL, Qiu JG, Zhang WJ, Xue YQ, Qin WM, Yang Y, Zheng DW, Chen Y, Wei MN, Zhang X, Lv M, Chen MW, Wei X and Shi Z. Synergistic anticancer effects of trip-tolide and celastrol, two main compounds from thunder god vine. *Oncotarget* 2015; 6: 32790-804.
- [31] Mei XL, Yang Y, Zhang YJ, Li Y, Zhao JM, Qiu JG, Zhang WJ, Xue YQ, Zheng DW, Chen Y, Qin WM, Wei MN and Shi Z. Sildenafil inhibits the growth of human colorectal cancer *in vitro* and *in vivo*. *Am J Cancer Res* 2015; 6: 15494-509.
- [32] Trachootham D, Alexandre J and Huang P. Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? *Nat Rev Drug Discov* 2009; 8: 579-591.
- [33] Schumacker PT. Reactive oxygen species in cancer cells: live by the sword, die by the sword. *Cancer Cell* 2006; 10: 175-176.
- [34] Sabharwal SS and Schumacker PT. Mitochondrial ROS in cancer: initiators, amplifiers or an Achilles' heel? *Nat Rev Cancer* 2014; 14: 709-721.
- [35] Bozic I, Reiter JG, Allen B, Antal T, Chatterjee K, Shah P, Moon YS, Yaqubie A, Kelly N, Le DT, Lipson EJ, Chapman PB, Diaz LA Jr, Vogelstein B and Nowak MA. Evolutionary dynamics of cancer in response to targeted combination therapy. *Elife* 2013; 2: e00747.
- [36] Rudolph D, Impagnatiello MA, Blaukopf C, Sommer C, Gerlich DW, Roth M, Tontsch-Grunt U, Wernitznig A, Savarese F, Hofmann MH, Albrecht C, Geiselman L, Reschke M, Garin-Chesa P, Zuber J, Moll J, Adolf GR and Kraut N. Efficacy and mechanism of action of volasertib, a potent and selective inhibitor of Polo-like kinases, in preclinical models of acute myeloid leukemia. *J Pharmacol Exp Ther* 2015; 352: 579-589.
- [37] Machiels JP, Peeters M, Herremans C, Surmont V, Specenier P, De Smet M, Pilz K, Strelkowa N, Liu D and Rottey S. A phase I study of volasertib combined with afatinib, in advanced solid tumors. *Cancer Chemother Pharmacol* 2015; 76: 843-851.
- [38] de Braud F, Cascinu S, Spitaleri G, Pilz K, Clementi L, Liu D, Sikken P and De Pas T. A phase I, dose-escalation study of volasertib combined with nintedanib in advanced solid tumors. *Ann Oncol* 2015; 26: 2341-6.
- [39] Dohner H, Lubbert M, Fiedler W, Fouillard L, Haaland A, Brandwein JM, Lepretre S, Reman O, Turlure P, Ottmann OG, Muller-Tidow C, Kramer A, Raffoux E, Dohner K, Schlenk RF, Voss F, Taube T, Fritsch H and Maertens J. Randomized, phase 2 trial of low-dose cytarabine with or without volasertib in AML patients not suitable for induction therapy. *Blood* 2014; 124: 1426-1433.
- [40] Ellis PM, Leigh NB, Hirsh V, Reaume MN, Blais N, Wierzbicki R, Sadrolhefazi B, Gu Y, Liu D, Pilz K and Chu Q. A Randomized, Open-Label Phase II Trial of Volasertib as Monotherapy and in Combination With Standard-Dose Pemetrexed Compared With Pemetrexed Monotherapy in Second-Line Treatment for Non-Small-Cell Lung Cancer. *Clin Lung Cancer* 2015; 16: 457-465.
- [41] Brassesco MS, Pezuk JA, Morales AG, de Oliveira JC, Roberto GM, da Silva GN, Francisco de Oliveira H, Scrideli CA and Tone LG. In vitro targeting of Polo-like kinase 1 in bladder carcinoma: comparative effects of four potent inhibitors. *Cancer Biol Ther* 2013; 14: 648-657.
- [42] Awada A, Dumez H, Aftimos PG, Costermans J, Bartholomeus S, Forceville K, Berghmans T, Meeus MA, Cescutti J, Munzert G, Pilz K, Liu D and Schoffski P. Phase I trial of volasertib, a Polo-like kinase inhibitor, plus platinum agents in solid tumors: safety, pharmacokinetics and activity. *Invest New Drugs* 2015; 33: 611-620.