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Effects of alisol B 23-acetate on ovarian cancer cells: G1 phase cell cycle arrest, apoptosis, migration and invasion inhibition

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ABSTRACT

Background: Ovarian cancer is the first leading cause of death among gynecologic malignancies worldwide. Discovery of new chemotherapeutic drugs is still imperative for the improvement of the survival rate.

Purpose: This study aims to investigate the anti-cancer potential of alisol B 23-acetate (AB23), a protostane-type triterpene isolated from the *Alismatis Rhizoma*, in the parental and paclitaxel-resistant ovarian cancer cells.

Methods: MTT assay was performed to evaluate cell viability after treatment with AB23, along with flow cytometry for apoptosis and cell cycle analysis. Western blotting was conducted to determine the relative protein level. Wound healing and transwell assays were performed to investigate the effect of AB23 on cell migration and invasion.

Results: AB23 obviously inhibited proliferation of the three ovarian cancer cell lines, down-regulated the protein levels of CDK4, CDK6, and cyclin D1, and blocked the cell cycle progressions in G1 phase. Meanwhile, AB23 induced accumulation of the sub-G1 phase in the three cell lines in a concentration dependent manner. The protein levels of cleaved poly ADP-ribose polymerase (PARP) and the ratio of Bax/Bcl-2 were up-regulated after treatment with AB23. Further study showed that AB23 induced endoplasmic reticulum stress through IRE1 signaling pathway and silencing of IRE1α partially enhanced AB23-induced apoptosis. Wound healing and transwell assays showed that AB23 could also suppress the migration and invasion of HEY cells. Moreover, it down-regulated the protein levels of matrix metalloproteinases MMP-2 and MMP-9.

Conclusion: AB23 possessed anti-proliferation, anti-migration and anti-invasion activities as a single agent on ovarian cancer cells.

Introduction

Ovarian cancer is the first leading cause of death among gynecologic malignancies worldwide. According to the 2014 World Cancer Report, approximately 239,000 women were diagnosed with ovarian cancer and 152,000 women died of this disease in 2012 (Stewart and Wild 2014). Approximately 22,280 women will receive a new diagnosis of ovarian cancer in the United States and 14,240 women will die from this disease in 2016 (Siegel et al. 2016). It remains extremely difficult to treat, and the 5 year survival rate of ovarian cancer remains below 40%, unchanged for the past decades (Colombo et al. 2014, Vaughan et al. 2011). Surgery is still the first choice for the treatment of ovarian

Abbreviations: AB23, alisol B 23-acetate; DMSO, dimethyl sulfoxide; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; PBS, phosphate-buffered saline; PI, propidium iodide; PARP, poly ADP-ribose polymerase; MMP, matrix metalloproteinases; CDK, cyclin-dependent kinase; A2780/Taxol, paclitaxel-resistant A2780; IC_{50}, half inhibitory concentrations; ER, endoplasmic reticulum; UPR, unfolded protein response.

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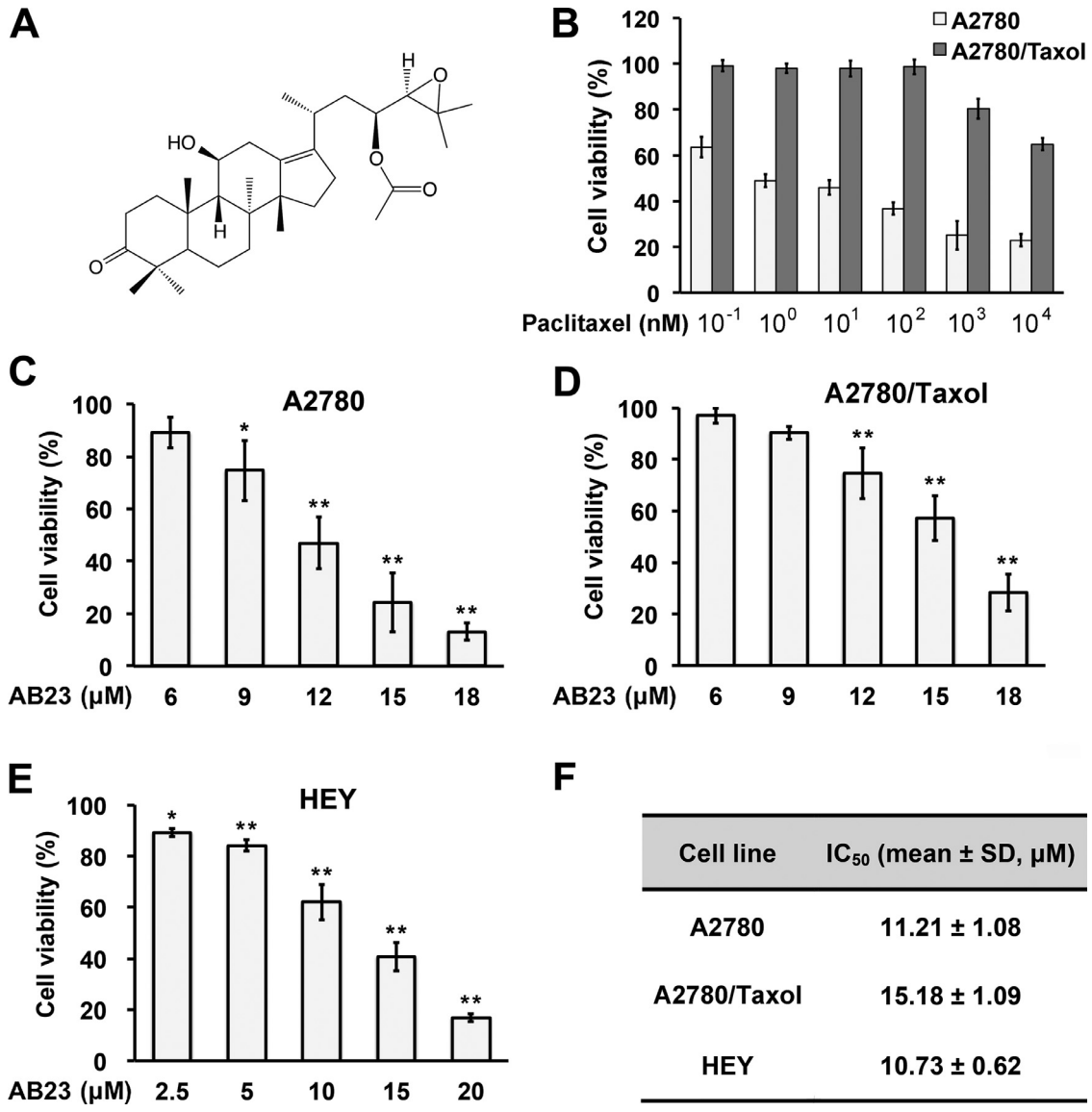

Cell line	IC_{50} (mean ± SD, μM)
A2780	11.21 ± 1.08
A2780/Taxol	15.18 ± 1.09
HEY	10.73 ± 0.62

Fig. 1. AB23 decreased the cell viability of ovarian cancer cells. (A) Chemical structure of AB23. (B) A2780 and A2780/Taxol cells were treated with different concentrations of paclitaxel for 48 h and the cell viability was tested by MTT assay. (C-E) A2780, A2780/Taxol and HEY cells were treated with series concentrations of AB23 for 24 h and the cell viability was tested by MTT assay. $^{*}P < 0.05$ and $^{**}P < 0.01$, compared with the 0 μM AB23 treatment (control). (F) IC_{50} values of AB23 in A2780, A2780/Taxol and HEY cells.

cancer. However, owing to the difficulty in diagnosing the disease at an early stage, high rates of metastasis and recurrence, surgery alone is not sufficient to control these tumors. Adjuvant radiotherapy, chemotherapy and biological therapy after resection have been clinically adopted to improve the survival status of patients, among which chemotherapy has been recommended as the relatively effective strategy. According to the 2015 National Comprehensive Cancer Network ovarian cancer treatment guidelines, for most patients with stage III-IV ovarian cancer, the standard platinum-taxane doublet is the first-line chemotherapy, most commonly carboplatin/cisplatin and paclitaxel (Sayal et al. 2015). Although initial chemotherapy is effective in many patients, the relapse rate remains high and tumors become increasingly resistant. Relapse occurs in up to 70% of patients faced with chemoresistance within 5 years. Chemoresistance has become a major cause of treatment failure in human ovarian cancer. Therefore, it is imperative to develop new chemotherapeutic drugs to overcome the intractable chemoresistance.

Discovery of novel anti-cancer compounds from natural products have received more and more attention owing to their rich source and enormous structural diversity (Li et al. 2013, Wu et al. 2013, Xu et al. 2014). Alisol B 23-acetate (AB23) is a protostane-type triterpene isolated from *Alismatis Rhizoma* which is a medicinal plant widely used in traditional Chinese medicine for urological disease for a long time. Its chemical structure is shown in Fig. 1A. In recently years, the biological characterization of AB23 has been identified and several pharmacological activities have been defined, including hepatoprotective (Meng et al. 2015a, Meng et al. 2015b), anti-hepatitis virus (Jiang et al. 2006), anti-bacterial (Jin et al. 2012), etc. Furthermore, AB23 has been demonstrated to possess anti-proliferative activity in our recent study on the screening of anti-proliferative activities of terpenoids isolated from *Alismatis Rhizoma* (Xu et al. 2015). It also induced Bax nuclear translocation and apoptotic cell death in human hormone-resistant prostate cancer PC-3 cells (Huang et al. 2006). However, there are still few systematical studies about the effects of AB23 on multiple processes of tumor progression in ovarian cancer cells.