Original Research Article

Glutinol inhibits the proliferation of human ovarian cancer cells via PI3K/AKT signaling pathway

Yuanyuan Chen[1], Juan Li[2]*
[1]*Department of Radiation Oncology, Women's Hospital,* [2]*Department of Radiation Oncology, Affiliated Hangzhou Cancer Hospital, School of Medicine, Zhejiang University, HangZhou, ZheJiang 310002, China*

*For correspondence: **Email:** jlu.edu.1@gmail.com; **Tel/Fax:** 0086-0571-56006302*

Abstract

Purpose: To investigate the anticancer effect of glutinol on OVACAR3 human ovarian cancer cells, and to elucidate the underlying molecular mechanisms.
Methods: Cell viability was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Cell cycle distribution, reactive oxygen species (ROS) and mitochondrial membrane potential were measured via flow cytometry, while protein expression levels were determined with western blotting assay.
Results: Glutinol exerted significant growth-inhibitory effects on human OVACAR3 cells, but interestingly, it exhibited comparatively lower cytotoxic effects against normal SV40 cells. The IC_{50} of glutinol against human OVACAR3 cells was 6 µM, while the IC_{50} against normal SV40 cells was 60 µM. Flow cytometric analysis showed an increase in population of OVACAR3 cells in G2/M phase from 4.02 % in control to 29.05 % on treatment with 12 µM glutinol, suggestive of G2/M phase arrest. The G2/M arrest of OVACAR3 cells was also accompanied by suppression of cyclin B1. It was also found that increases in ROS levels and decreases in MMP activities contributed to the glutinol-induced antiproliferative effects on human OVACAR3 cells. Moreover, glutinol deactivated the PI3K/AKT signaling pathway in OVACAR-3 ovarian cancer cells.
Conclusion: Glutinol exerted potent anticancer effects against human ovarian cancer. Thus, it might be of potential benefit in the treatment of ovarian cancer.

Keywords: Ovarian cancer, Glutinol, Cell cycle arrest, Chemotherapy, Triterpenes

INTRODUCTION

Human beings have been using plant parts or their extracts for the treatment of various ailments since time immemorial [1]. The use of pure forms of plant compounds started just recently with the advent of the natural product chemistry [2]. Chemical analysis of plant extracts has now revealed that plants contain different categories of natural chemical scaffolds such as flavonoids, terpenoids and alkaloids [3]. Cancer is one of the most dreaded and devastating diseases responsible for huge mortalities and morbidities across the globe [4]. In developing countries, cancer is currently ranked as the 2nd most prevalent cause of mortality [5]. Currently, there is pressing need to evolve newer

anticancer drugs that are both effective and efficient. Plants are unparalleled sources of compounds with structural diversities and varied biological activities [6]. Triterpenes have shown remarkable potential to halt the growth of human cancer cells [7]. The PI3K/AKT signaling pathway has been shown to be dysregulated in cancer cells, and it has been implicated in the etiology of human cancers [8,9]. It is believed that drugs which block these pathways may prove highly efficient in cancer treatment [9]. The present study was designed to investigate the anticancer effects of a plant-derived compound, glutinol on human ovarian cancer cells, and to elucidate the molecular mechanisms involved in the process. Moreover, the effect of glutinol on the PI3K/AKT signaling pathway was investigated.

EXPERIMENTAL

Cell viability assay

The viability of OVACAR3 ovarian cancer cells and viability normal SV40 cells were determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The OVACAR3 cells were seeded in 96-well plates at a density of 1.5×10^4 cells per 0.2 mL for 24 h. Then, the cells were treated with different doses of glutinol ranging from 0 to 320 μM, after which 5 μL of MTT (10 mg/L) was added, and the plates were again incubated at 37 °C for 4 h. The formazan crystals formed were solubilised in 10 % dimethyl sulfoxide (DMSO), and the absorbance of each formazan solution was read at 570 nm. The absorbance readings served as index of cell viability.

Cell cycle analysis

The OVACAR3 cells were treated with glutinol at doses of 3, 6 and 12 μM for 24 h. Then, the cells were harvested, suspended in ice-cold 75 % ethanol for fixation at 24 °C. Subsequently, the OVACAR3 cells were rinsed with phosphate buffered saline (PBS) and treated with 1 mL of propidium iodide (PI). The samples were then placed in the dark for 30 min and analysed using FACScan flow cytometry.

Reactive oxygen species (ROS) and mitochondrial membrane potential (MMP) levels

The OVACAR3 cells were cultured at a density 2 × 10^4 cells/mL and exposed to glutinol at doses of 3, 6 and 12 μL for 24 h. The glutinol-treated cells were washed using phosphate buffered saline, followed by treatment with DCF-DA (10 μM) for ROS determination, and DiOC6 (1 μM) for MMP assay. Finally, the ROS and MMP levels were determined flow cytometrically as described previously [10].

Western blot assay

Glutinol-treated OVACAR3 cells were harvested and lysed with RIPA lysis buffer. After determining the lysate protein concentration with BCA method, the protein samples were subjected to separation on 10 % SDS-PAGE and subsequently transferred to nitrocellulose membranes. Following blocking with skim milk, the membranes were incubated with primary antibodies for 50 min at 25 °C, and then for 12 h at 4 °C. Then, the membranes were incubated with secondary antibody at room temperature for 2 h. Actin was used as internal control. Enhanced chemiluminescence reagent was utilised for the visualisation of the protein bands of interest.

Statistical analysis

Data are presented as mean ± SD of three replicates. Student's *t*-test was used for statistical analysis with GraphPad prism 7 software. Values of $p < 0.05$ were assumed indicative of significant differences between samples.

RESULTS

Glutinol exhibited anti-proliferative effects on ovarian cancer cells

Glutinol (Figure 1 A) exerted anti-proliferative effects on human ovarian cancer cells, as revealed from MTT assay. The viabilities of the ovarian cancer OVACAR3 and normal SV40 cells were determined after treatment with different doses of glutinol. It was found that glutinol dose-dependently suppressed the growth of human OVACAR3 cells, with an IC_{50} of 6 μM (Figure 1 B). However, with an IC_{50} of 60 μM for SV40 cells (10 times higher than LD_{50} against OVACAR-3), the anti-proliferative effect of glutinol against SV40 cells was relatively lower (Figure 1 C).

Glutinol triggered G_2/M arrest of ovarian cancer cells

The effect of glutinol on the distribution of OVACAR3 cells in different cell cycle phases was studied. At doses of 3, 6 and 12 μM, glutinol caused dose-dependent increases in the percentage of ovarian cancer cells in the G_2/M phase. The percentages of OVACAR 3 cells in

G_2/M phase were 4.022, 18.70, 20.09 and 29.05 at glutinol doses of 0, 3, 6 and 12 µM, respectively (Figure 2). The glutinol-induced G_2/M phase cell cycle arrest was associated with dose-dependent suppression of cyclin B1 expression (Figure 3).
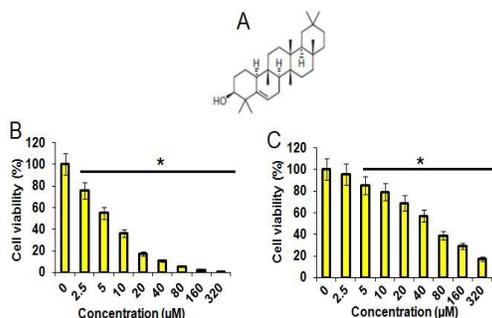

Figure 1: Effect of glutinol on the viability of ovarian cancer cells (A) Structure of glutinol; (B) viability OVACAR-3 cells, and (C) viability of hTRET-OME cells, as determined with MTT assay. The experiments were performed in triplicate, and the data are expressed as mean ± SD. *$P < 0.05$)
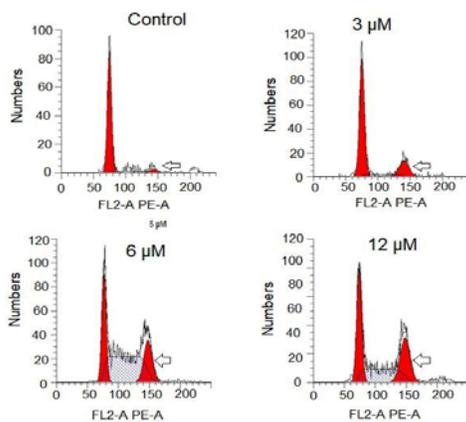

Figure 2: Effect of glutinol on cell cycle of OVACAR-3 cells. Flow cytometric analysis showed that glutinol induced G2/M arrest in OVACAR-3 cells in a dose-dependent manner. The experiments were performed in triplicate
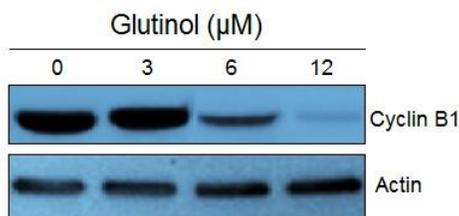

Figure 3: Glutinol inhibited the expression of cyclin B1 in a dose dependent manner, as revealed using Western blot analysis. The experiments were performed in triplicate

Glutinol increased ROS and decreased MMP levels in ovarian cancer cells

Glutinol dose-dependently increased ROS levels in OVACAR cells. The ROS levels in OVACAR cells treated with glutinol at doses of 0, 3, 6 and 12 µM were 100, 132, 165 and 225 %, respectively (Figure 4). Moreover, glutinol caused significant and dose-dependent decreases in MMP levels. The MMP levels in OVACAR cells treated with glutinol at doses of 0. 3, 6 and 12 µM were 100, 68, 42 and 22 %, respectively (Figure 5).
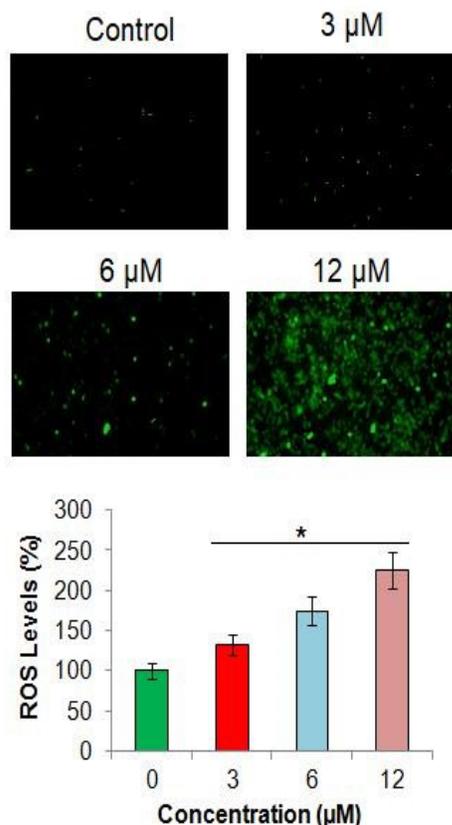

Figure 4: Effect of glutinol on ROS levels in SCC-4 cells, as determined with flow cytometry. Glutinol increased ROS levels of OVACAR-3 cells in a dose-dependent manner. The experiments were performed in triplicate, and the results are expressed as mean ± SD. *$P < 0.05$

Glutinol blocked the mTOR/AKT and β-catenin signaling pathways

Results from Western blotting assay showed that glutinol dose-dependently blocked the phosphorylations of PI3K and AKT, but it had no noticeable effects on total PI3K and AKT levels (Figure 6).
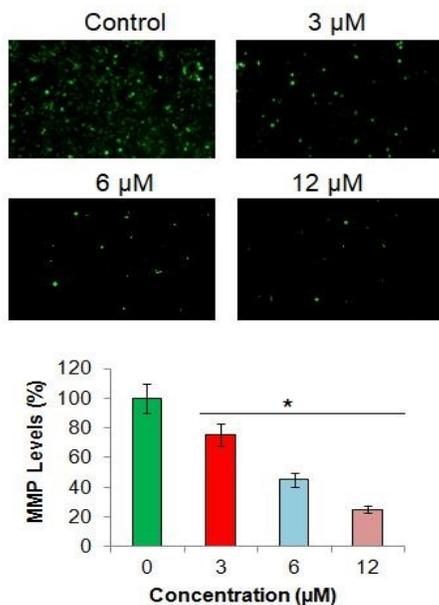
Figure 5: MMP levels at indicated concentrations of glutinol in SCC-4 cells, as determined using flow cytometry. Glutinol inhibited MMP levels in OVACAR-3 cells in a dose-dependent manner. The experiments were performed in triplicate, and the results are expressed as mean ± SD. *P < 0.05
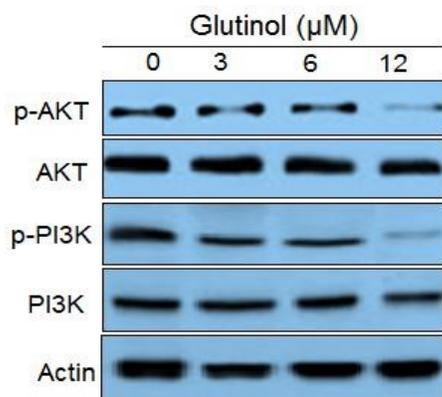

Figure 6: Effect of glutinol on expressions of PI3K/AKT signaling pathway-associated proteins. Glutinol blocked the mTOR/AKT pathway in a dose-dependent manner. The experiments were performed in triplicate

DISCUSSION

Owing to the dearth of reliable and efficient diagnostic assays for its early detection, and lack of potent chemotherapeutic drugs, ovarian cancer is currently one of the leading causes of mortality in women across the globe. Most of the patients are diagnosed at advanced stages, thereby resulting in very poor 5-year survival (just 30 %) [11].

Cytoreductive surgery and subsequent chemotherapy are currently employed as standard treatments for advanced-stage ovarian cancer. However, the severe adverse effects of chemotherapy and frequent relapses constitute impediments in the management of ovarian cancer [12]. Against this backdrop, the present study was carried out to investigate the anticancer effect of an important triterpene, glutinol against human ovarian cancer cells. It was found that glutinol selectively suppressed the growth of human ovarian cancer cells, while it exerted relatively negligible anti-proliferative effects on normal ovarian cells. These observations are in agreement with results from previous studies. For example, the triterpene lupane has been shown to suppress the growth of cancer cells [13]. Anticancer agents suppress the growth of cancer cells via multiple mechanisms such as apoptosis, autophagy, and cell cycle arrest [14,15]. In this study, it was found that the anti-proliferative effect of glutinol was mainly due to its potential to promote the arrest of ovarian cancer cells at the G2/M check point of the cell cycle by suppressing the expression of cyclin B1. The generation of ROS and disruption of MMP have been implicated in the suppression of cancer cell proliferation [16]. This study also revealed that the glutinol-induced anti-proliferative effects were accompanied by enhancement of ROS and decreases in MMP levels in the ovarian cancer cells. Studies have shown that the mTOR/AKT and β-catenin signaling pathways are dysregulated in cancer cells, and are markedly implicated in the pathogenesis of the human cancers [8,9]. Glutinol suppressed these pathways in OVACAR-3 ovarian cancer cells, indicating that it may be utilized to target these pathways in the management of ovarian cancer.

CONCLUSION

The findings of this study reveal that glutinol, a plant-derived triterpene, suppressed the growth of human ovarian cancer cells via G$_2$/M cell cycle arrest. It also exhibited potential to block mTOR/AKT and β-catenin signaling pathways. Therefore, glutinol has potential anticancer effects.

DECLARATIONS

Acknowledgement

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

Open Access

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/read), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

REFERENCES

1. Newman DJ, Cragg GM. Natural products as sources of new drugs from 1981 to 2014. J Nat Prod 2016; 79: 629-661.

2. Harvey AL, Edrada-Ebel R, Quinn RJ. The re-emergence of natural products for drug discovery in the genomics era. Nat Rev Drug Discov 2015; 14(2): 111.

3. Liu J, Liu M, Wang S, He Y, Huo Y, Yang Z, Cao X. Alantolactone induces apoptosis and suppresses migration in MCF 7 human breast cancer cells via the p38 MAPK, NF κB and Nrf2 signaling pathways. Int J Mol Med 2018; 42: 1847-1856.

4. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D, Global cancer statistics. CA-cancer J Clin 2011; 61: 69e90.

5. S Huang, L Ma, MH Tong, Y Yu, D O'Hagan, H Deng. Fluoroacetate biosynthesis from the marine-derived bacterium Streptomyces xinghaiensis NRRL B-24674. Org Biomol Chem 2014; 12: 4828e4831

6. Ma L, Bartholome A, Tong MH, Qin Z, Yu Y, Shepherd T, Kyeremeh K, Deng H, O'Hagan D. Identification of a fluorometabolite from Streptomyces sp. MA37:(2R3S4S)-5-fluoro-2, 3, 4-trihydroxypentanoic acid. Chem. Sci 2015; 61414e1419.

7. Ma L, Li Y, Meng L, Deng H, Li Y, Zhang Q, Diao A. Biological fluorination from the sea: discovery of a SAM-dependent nucleophilic fluorinating enzyme from the marine-derived bacterium Streptomyces xinghaiensis NRRL B24674. RSC Adv2016; 6: 27047e27051.

8. Bahrami A, Amerizadeh F, ShahidSales S, Khazaei M, Ghayour-Mobarhan M, Sadeghnia HR, Maftouh M, Hassanian SM, Avan A. Therapeutic potential of targeting Wnt/β-catenin pathway in treatment of colorectal cancer: Rational and progress. J Cell Biochem 2017; 118: 1979-1983.

9. Chao X, Zao J, Xiao-Yi G, Li-Jun M, Tao S. Blocking of PI3K/AKT induces apoptosis by its effect on NF-κB activity in gastric carcinoma cell line SGC7901. BiomedPharmacother 2010; 64(9): 600-604

10. Chiang JH, Yang JS, Ma CY, Yang MD, Huang HY, Hsia TC. Danthron, an Anthraquinone Derivative, Induces DNA Damage and Caspase Cascades-Mediated Apoptosis in SNU-1 Human Gastric Cancer Cells through Mitochondrial Permeability Transition Pores and Bax-Triggered Pathways. Chem Res Toxicol 2011; 24: 20-29

11. Konstantinopoulos PA, Norquist B, Lacchetti C, Armstrong D, Grisham RN, Goodfellow PJ, Kohn EC, Levine DA, Liu JF, Lu KH, Sparacio D. Germline and somatic tumor testing in epithelial ovarian cancer: ASCO guideline. J Clin Oncol 2020; 38(11): 1222-1245.

12. Lukanova A, Kaaks R. Endogenous hormones and ovarian cancer: epidemiology and current hypotheses. Cancer Epidemiol Prev Biomark2005 14: 98-107

13. Kommera H, Kaluđerović GN, Kalbitz J, Dräger B, Paschke R. Small structural changes of pentacyclic lupane type triterpenoid derivatives lead to significant differences in their anticancer properties. Eur J Med Chem 2010; 45: 3346-3353.

14. Youn CK, Kim J, Park JH, Do NY, Cho SI. Role of autophagy in cisplatin-induced ototoxicity. Int J Pediatr otorhinolaryngol 2015; 79(11): 1814-1819.Lowe SW, Lin AW. Apoptosis in cancer. Carcinogenesis. 2000;21(3): 485-895.

15. Li AX, Sun M, Li X. Withaferin-A induces apoptosis in osteosarcoma U2OS cell line via generation of ROS and disruption of mitochondrial membrane potential. Euro Rev Med Pharma Sci 2017; 21: 1368-1374.