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

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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 IC₅₀ of glutinol against human OVACAR3 cells was 6 µM, while the IC₅₀ 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 x 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 x 10^4 cells/mL and exposed to glutinol at doses of 3, 6 and 12 µM 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 IC50 of 6 µM (Figure 1 B). However, with an IC50 of 60 µM for SV40 cells (10 times higher than LD50 against OVACAR-3), the anti-proliferative effect of glutinol against SV40 cells was relatively lower (Figure 1 C).

**Glutinol triggered G2/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 G2/M phase. The percentages of OVACAR 3 cells in
G2/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 G2/M phase cell cycle arrest was associated with dose-dependent suppression of cyclin B1 expression (Figure 3).

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).

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).
Chen & Li


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

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 G2/M cell cycle arrest. It also exhibited potential to block mTOR/AKT and β-catenin signaling pathways. Therefore, glutinol has potential anticancer effects.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

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