

Original Research Article

Ginsenoside induces apoptosis, autophagy and cell cycle arrest in gastric cancer cells by regulation of reactive oxygen species and activation of MAPK pathway

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Abstract

Purpose: To study the influence of ginsenoside on apoptosis, cell cycle and autophagy in gastric carcinoma (GC), and its effect on reactive oxygen species (ROS) levels and the mitogen-activated protein kinase (MAPK) pathway.

Methods: Human gastric cancer cell line BGC-823 was randomly divided into the following groups: control, 100 μ M ginsenoside (Rg5), 150 μ M Rg5, and 200 μ M Rg5 groups. Western blot assay was used to determine the expressions of autophagy-associated protein 12 (Atg12), Beclin-1, Lc3b II, cycle-related protein, phosphorylated mitotic cyclin 25 homologous protein C (p-cdc25c), cyclin B1, and MAPK signaling pathway-related proteins.

Results: There was significantly higher apoptosis in Rg5-treated BGC-823 cells than in untreated cells. Relative protein levels of Beclin-1, Atg5, Atg12, and Lc3b II in BGC-823 cells in Rg5 groups were significantly and concentration-dependently up-regulated, relative to the corresponding expression levels in untreated cells. There were markedly up-regulated proteins of p-cdc25c, cyclin B1 and p-cdc2 in Rg5-exposed BGC-823 cells than in untreated cells, while CDC2 protein expression was significantly and concentration-dependently lower than that of control group ($p < 0.05$). Rg5 treatment resulted in marked and concentration-dependent increases in ROS levels in BGC-823 cells, relative to control cells ($p < 0.05$), whereas the expression levels of p-p38, p-JNK and p-ERK were significantly higher in Rg5-exposed cells than in unexposed cells ($p < 0.05$).

Conclusion: Ginsenoside induces apoptosis, autophagy and cycle interruption in GC cells by regulating ROS production and activating MAPK pathway. Therefore, ginsenoside may be a promising agent for the management of gastric cancer. However, there is a need to conduct in vivo studies on the compound.

Keywords: Ginsenoside, Reactive active species, MAPK, Autophagy, Protein expressions

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INTRODUCTION

Gastric cancer is associated with very high mortality and incidence, with higher incidence in

males than in females, and it poses a serious threat to human life and health [1]. Surgical resection is an important treatment strategy for gastric cancer, but it is only suitable at the early

stage of gastric cancer. If the disease progresses to the advanced stage, chemotherapy and targeted therapy are usually used in place of surgical resection [2]. Although the use of chemotherapeutic drugs in clinical practice relieves gastric cancer to a certain extent, these drugs lead to many toxic side effects and insensitivity to medication. Thus, it is crucial to identify novel therapeutic principles to replace existing chemotherapy drugs in the treatment of gastric cancer [3].

Ginsenoside is a triterpenoid compound which has been shown to exert anti-inflammatory, hypoglycemic, anti-aging, anti-tumor and immunity-enhancing properties [4]. Clinical studies have demonstrated that ginsenoside, which has been widely used in the treatment of various types of tumors, blocks the proliferation of cancer cells and promotes cancer cell apoptosis [5].

Reactive oxygen species (ROS) play crucial roles in the regulation of the growth of many tumor types [6]. Previous reports have shown that excessive release of ROS leads to suppression of tumor cell growth. Anti-tumor drugs enhanced apoptosis and autophagy of human GC cells through increases in ROS contents [7]. Clinical studies have shown that ROS accumulation activates the MAPK pathway which plays a key role in the initiation and evolution of cancer [8]. Currently, not much is known about how ginsenoside affects GC, and involvement of ROS and MAPK pathway in the process. Therefore, this study was aimed at investigating the effect of ginsenoside on apoptosis, autophagy and cell-division sequence in GC cells, and the mechanism involved.

EXPERIMENTAL

Cell lines

Human BGC-823 GC cells were product of Wuxi Xinrun Biotechnology Company Ltd., and were incubated in 1640 cell culture medium.

Main reagents and equipment

The major reagents and instruments used in this study, and their suppliers (in brackets) were: ginsenoside Rg5 (Nanjing Beiyu Biotechnology Co. Ltd.), 1640 medium (Jiangsu Qishi Biotechnology Co. Ltd), RIPA lysing buffer (Harbin Xinhai Gene Testing Co. Ltd), antibodies for Atg5, Beclin-1 and LC3B II (Beijing Baiolibio Technology Co. Ltd), and antibodies for P-ERK, P-p38, P21, Cyclin B1, P-cdc2 and P-JNK (Xiamen Huijia Biotechnology Co. Ltd).

The instruments used were biological safety cabinet (Beijing Dingguo Changsheng Biotechnology Co. Ltd), fluorescent microscope (Guangzhou Kesite Scientific Instrument Co. Ltd.), ultra-low temperature refrigerator (Hangzhou Noding Scientific Equipment Co. Ltd) and flow cytometer (Changzhou Bidako Biotechnology Co. Ltd).

Study design

The BGC-823 cells in good growth state and logarithmic growth phase were digested with 0.25 % trypsin and centrifuged. After cell counting, they were plated in six-well plates (4×10^5 cells/well). After cell adherence, Rg5 treatment was done at three doses i.e., 100, 150 and 200 μ M for 24 h. Untreated cells served as control. After 24 h, cells from each group were used for follow-up experiments. Flow cytometry was used to determine % apoptosis and ROS production in BGC-823 cells in each group. Immunoblot assay was used to determine the expression levels of autophagy-related proteins (Atg5 and Atg12); Beclin-1, LC3B-II, cycle-related proteins [phosphated cell division cyclin 25 homologous protein C (p-cdc25c), cyclin B1, cell cycle division gene 2 (Cdc2) and p-Cdc2], and MAPK signaling-related proteins.

Statistical analysis

Apoptosis and expression levels of cell cycle-related proteins in each group are expressed as mean \pm SD, and comparison between two groups was done with *t*-test, while one-way ANOVA was used for comparison amongst many groups. Statistical analyses were done with SPSS version 23.0 app. Significance was considered at $p < 0.05$.

RESULTS

Apoptosis in BGC-823 cells

The Rg5 treatment resulted in significant and dose-dependent increase in percentage apoptosis of BGC-823 cells, relative to untreated cells (Table 1 and Figure 1).

Table 1: Apoptosis in various groups

Group	Apoptosis (%)
Control	8.23 \pm 1.01
100 μ M Rg5	14.01 \pm 2.28 ^a
150 μ M Rg5	69.11 \pm 4.73 ^b
200 μ M Rg5	85.79 \pm 4.09 ^c

^{a,b,c} $P < 0.05$: ^avs control; ^bvs 100 μ M Rg5 exposure, ^cvs 150 μ M Rg5 group

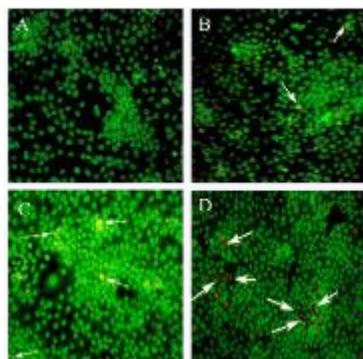


Figure 1: Photomicrographs of apoptosis. A: Untreated cells, B: 100 μ M Rg5-exposed; C: 150 μ M Rg5 group, D: 200 μ M Rg5 group

Autophagy of BGC-823 cells

Protein amounts of Atg5, Atg12, Beclin-1 and LC3B II were markedly and concentration-dependently raised in Rg5-treated cells, relative to control (Table 2 and Figure 2).

Table 2: Relative levels of autophagy-related proteins in BGC-823 cells in each group

Group	Atg5	Atg23	Beclin-1	LC3B II
Control	0.19±0.01	0.12±0.02	0.13±0.01	0.19±0.02
100 μ M Rg5	0.35±0.03 ^a	0.21±0.03 ^a	0.29±0.05 ^a	0.39±0.01 ^a
150 μ M Rg5	0.49±0.04 ^b	0.42±0.02 ^b	0.59±0.04 ^b	0.48±0.03 ^b
200 μ M Rg5	0.59±0.03 ^c	0.56±0.03 ^c	0.68±0.04 ^c	0.65±0.04 ^c

^{a,b,c} $P < 0.05$: ^avs control; ^bvs 100 μ M Rg5 group, ^cvs 150 μ M Rg5 group

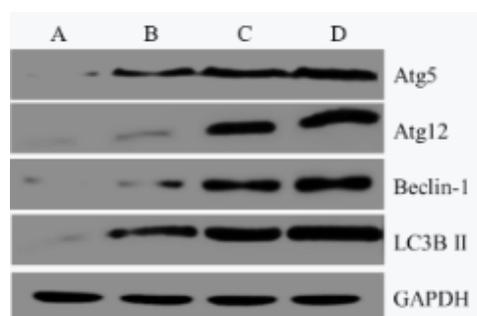


Figure 2: Autophagy-related proteins in BGC-823 cells. A: Untreated, B: 100 μ M Rg5-treated, C: 150 μ M Rg5-treated, and D: 200 μ M Rg5 groups

Expression levels of BCC-823 cycle-related proteins

Levels of p-CDC25C, Cyclin B1 and p-CDC2 in BGC-823 cells were markedly up-regulated in Rg5 group, relative to those in untreated cells, while Cdc2 expression was significantly and

concentration-dependently lower than that in control group ($p < 0.05$). These data are presented in Table 3 and Figure 3.

Table 3: Levels of BCC-823 cell cycle-related proteins in each group

Group	p-Cdc25c	Cyclin B1	Cdc2	p-Cdc2
Control	0.11±0.01	0.31±0.02	0.61±0.05	0.50±0.01
150 μ M Rg5	0.25±0.02 ^a	0.41±0.03 ^a	0.51±0.03 ^a	0.68±0.01 ^a
200 μ M Rg5	0.44±0.02 ^b	0.49±0.04 ^b	0.43±0.03 ^b	0.52±0.05 ^b
250 μ M Rg5	0.71±0.05 ^c	0.58±0.03 ^c	0.32±0.01 ^c	1.14±0.05 ^c

^{a,b,c} $P < 0.05$: ^avs control; ^bvs 100 μ M Rg5-treated cells; ^cvs 150 μ M Rg5 group

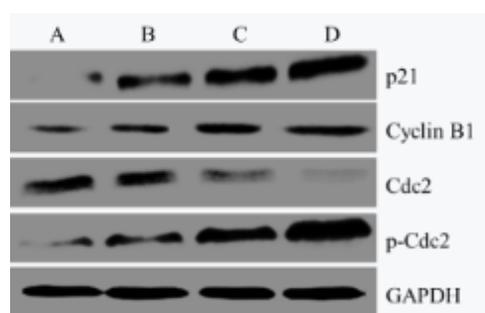


Figure 3: Comparison of expression levels of BCC-823 cycle-related protein amongst all groups. A: Untreated, B: 100 μ M Rg5-treated, C: 150 μ M Rg5-treated, D: 200 μ M Rg5 group

ROS levels in BGC-823 cells

The Rg5 treatment led to significant and dose-based elevations in ROS levels, when compared with control ($p < 0.05$; Table 4).

Table 4: ROS levels in BGC-823 cells among all groups

Group	ROS
Control	401.02±111.33
100 μ M Rg5	895.48±249.31 ^a
150 μ M Rg5	987.18±298.14 ^b
200 μ M Rg5	1189.89±307.99 ^c

^{a,b,c} $P < 0.05$: ^avs control; ^bvs 100 μ M Rg5-treated cells; ^cvs 150 μ M Rg5 group

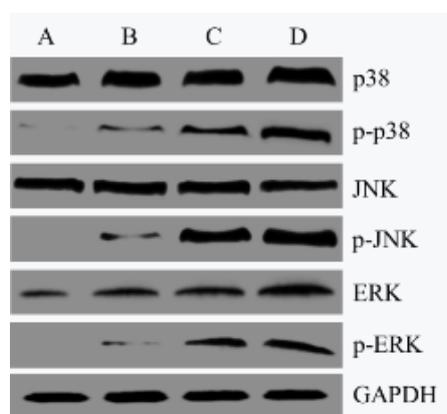
MAPK signaling pathway-related protein expression levels

As presented in Figure 4 and Table 5, protein concentrations of P-ERK, P-P38 and P-JNK in BGC-823 cells were significantly and dose-dependently increased by Rg5, when compared to the corresponding control expressions.

Table 5: Expression levels of MAPK signal route-related proteins in each group

Group	p-p38/p38	p-JNK/JNK	p-ERK/ERK
Control	0.19±0.01	0.21±0.03	0.25±0.02
150 µM Rg5	0.45±0.05 ^a	0.36±0.04 ^a	0.74±0.02 ^a
200 µM Rg5	0.85±0.04 ^b	0.89±0.04 ^b	1.10±0.03 ^b
250 µM Rg5	1.16±0.03 ^c	1.19±0.05 ^c	1.28±0.04 ^c

^{a,b,c}*P* < 0.05: ^avs control; ^bvs 100 µM Rg5-treated cells; ^cvs 150 µM Rg5 group

**Figure 4:** Expression levels of MAPK signal route-related factors. A: Untreated, B: 100 µM Rg5-treated cells, C: 150 µM Rg5-treated cells, D: 200 µM Rg5 group

DISCUSSION

Ginsenoside Rg5 exerts a wide range of pharmacological effects, and studies have shown that it relieved cisplatin-induced renal toxicity in mice by blocking inflammatory and oxidative stress responses [9]. Other reports have shown that ginsenoside Rg5 mitigated insulin resistance and decreased blood glucose by reducing the accumulation of diacylglycerol and ceramide, and blocking the translocation of protein kinase C [10]. Some researchers have reported that Rg5 enhanced cell death and DNA lesions in human cervix carcinoma, and significantly blocked the proliferation of cervical cancer cells. However, there are limited studies on the anti-tumor effect of ginsenoside Rg5 in gastric cancer, and the underlying mechanism. The present study was carried out to address this gap in knowledge.

Apoptosis has been widely used in the study of gastric cancer. It is triggered by mitochondrion- and death receptor-mediated pathways. Clinical reports have shown that some chemotherapeutic drugs induced both endogenous and exogenous apoptosis in gastric cancer cells. In the present research, the percentage apoptosis of BGC-823

cells in Rg5 group was markedly and dose-dependently higher than that in the control group. Ginsenoside Rg5 enhanced apoptosis of gastric cancer cells. Autophagy is relatively conserved in evolution. Clinical studies have shown that autophagic cell death and gastrointestinal cancer increased protein expression levels of Atg5 and Atg12 [11].

Other reports have shown that Beclin-1 and LC3B II are autophagy-related genes which play important roles in autophagy [12]. Beclin-1 is involved in autophagy regulation, and it blocks tumor growth by enhancing autophagy. Thus, it may serve as a candidate tumor suppressor gene. The number of autophagosomes generated is proportional to the extent of transformation of LC3 I protein into LC3 II protein [13]. In the present investigation, protein expressions of Atg5, Atg12, Beclin-1 and LC3B II in Rg5-treated cells were markedly and concentration-dependently raised, relative to those in untreated group. These results suggest that ginsenoside Rg5 significantly promoted autophagy in gastric cancer cells. The increased expression of P-CDC25C enhanced initiation of mitosis and regulated the expression of cdc2/Cyclin B1 complex which is an important marker of G2/M stage arrest. Protein expressions of p-cdc25c, cyclin B1 and p-CDC2 in Rg5-treated BGC-823 cells were markedly raised, relative to those in control, while protein expression of cdc2 was significantly and concentration-dependently down-regulated. Thus, ginsenoside Rg5 induced peripheral block in gastric cancer cells.

Reactive oxygen species (ROS) participate in the regulation of cancer initiation. Indeed, it has been shown in gastric carcinoma that excessive ROS caused cell damage which led to cell cycle arrest at G2-phase, as well as apoptotic and autophagic lesions, leading ultimately to cell death [14]. In this study, ROS levels in BGC-823 cells treated with Rg5 were significantly and concentration-dependently higher than those in control group. This suggests that ginsenoside accentuated ROS levels, and induced apoptotic lesions, autophagy, and arrest of cell sequence.

The MAPK signaling pathway is divided into three parts: P38 pathway, JNK pathway and ERK pathway. The P38 pathway exists as cancer suppressor in gastric carcinoma, and many drugs promote apoptotic lesions by activating this pathway. The JNK pathway is closely associated with apoptosis and autophagy, and it has been suggested that suppression of JNK by chemotherapeutic agents is traditionally associated with phenotypic resistance to many

types of genotoxic stimuli. Research has revealed that many drugs promote apoptotic and autophagic lesions in gastric carcinoma cancer cells by regulating ERK route. The results of this study showed marked and concentration-dependent increases in protein concentrations of p-ERK, p-P38 and p-JNK in Rg5-treated cells, relative to those in untreated group.

CONCLUSION

Ginsenoside induces apoptosis, autophagy and cell cycle stagnation in GC cells by regulating ROS generation and activating MAPK pathway. Therefore, ginsenoside is a potential anticancer drug that can effectively target gastric cancer. However, there is need for *in vivo* studies on this compound.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest 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. Qicong Zhu designed the study, supervised the data collection, and analyzed the data. Jingfen Lu interpreted the data and prepared the manuscript for publication. Jingfen Lu, LinYang, Qicong Zhu, Peng Gao, +Zhengyi Zhang, Wei Wang and Yongsheng Fu supervised the data collection, analyzed the data and reviewed the final draft of the manuscript.

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