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ORIGINAL ARTICLE

Effects of resveratrol on the protein expression of survivin and cell apoptosis in human gastric cancer cells

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Summary

Purpose: *This study aimed to investigate the effects of resveratrol on cell proliferation, apoptosis and protein expression of survivin in human gastric cancer (SGC7901) cell line.*

Methods: *SGC7901 cell proliferation and G0/G1 phase of the cell cycle induced by resveratrol (treatment group) and phosphate buffer solution (PBS) (control group) were assessed by flow cytometry. In addition, protein expression of survivin was assessed by immunohistochemistry after treatment with resveratrol.*

Results: *SGC7901 cell apoptosis rates were 0.00% and 3.45% in the control and treatment groups, respectively. Furthermore, cell cycles were significantly changed in the resveratrol group; for example, the proportion of the G0/G1 phase increased, whereas the proportion of the S and G2/M phases decreased. Survivin protein expression was significantly reduced ($p<0.01$) in the treatment group compared with that in the control group.*

Conclusion: *Resveratrol inhibited the proliferation of SGC7901 cancer cells by inducing cell apoptosis and down-regulating survivin expression.*

Key words: *apoptosis, proliferation, resveratrol, survivin*

Introduction

Resveratrol (3, 5, 4'-trihydroxy-trans-stilbene) is a non-flavonoid polyphenol produced by plants and is richly found in the skin of red grapes, peanuts and blueberries [1]. However, natural resveratrol has a cis-isomer and is almost insoluble in water but is easily soluble in ethanol, ethyl acetate and acetone. Resveratrol has multiple bioactivities, such as regulation of lipid metabolism, inhibition of platelet aggregation and protection of the cardiovascular system through suppression of lipid peroxidation and eicosanoid synthesis; moreover, resveratrol has antiinflammatory properties and forms a lauric acid-like substance that can prevent atherosclerosis [2]. Recent research found that resveratrol has anticancer effects [3,4]. Resveratrol can inhibit the proliferation of many types of tumor cells, including liver cancer cells, pancreatic cancer cells, gastric cancer cells, breast cancer cells and prostatic cancer cells [5-8]. However, the exact mechanisms underlying the anticancer effects remain largerly unclear. Previously published research showed that resveratrol is a natural prophylactic agent that can interfere with three different stages of carcinogenesis -initiation, promotion and progression. Resveratrol can induce cell apoptosis and possesses antimutational and antioxidant properties. However, the specific regulation pathway and the mechanism of antitumor activities are unknown.

In our previous studies [9,10] we demonstrated that resveratrol can induce apoptosis of colorectal and gastric cancer cells. In the present study we focused on the possible mechanisms by which resveratrol inhibits SGC7901 cell proliferation to provide a theoretical foundation for gastric cancer treatment.

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Methods

Cell lines and grouping

Human gastric cancer cell line SGC7901 was provided by the Department of Pathophysiology, Chongqi Medical University (Chongqi, China). The experimental cells were divided into two groups: the control and the treatment groups. The SGC7901 cells in the treatment group were treated with resveratrol (Sigma, USA), the concentrations of which were determined in previous trials [9,10]. However, the concentration of resveratrol was one-third that of a previous concentration [11] determined by flow cytometry and immunocytochemistry.

Cell culture

SGC7901 cells were cultured for 72 hrs in RPMI-1640 medium with 10% fetal calf serum (FCS) in a humidified incubator at 37 °C and 5% CO_2 atmosphere, and the viable cells were counted using the trypan blue exclusion assay.

SGC7901 cells were collected and seeded into a culture bottle (5.0×10^4/mL) after being digested with 0.1% trypsin. The cells were then divided into the control and treatments groups, which were respectively treated with PBS and resveratrol for 72 hrs. The cells were then centrifuged at 1000 rpm for 10 min, and the supernatant fluid was washed off. After repeating the previous operation, cells were fixed with 70% alcohol for 1 h. The cell suspension was then washed twice with PBS and mixed with propidium iodide (PI) at 4 °C for 30 min, and the tubes (with 1×10^5/mL cells) were examined by flow cytometry (FCM). The exciting wavelength was 488 nm, and red fluorescence represented dead cells. All data were put into a Macintosh 650 computer, and the cell cycle and apoptosis rate were analysed by Modifit 1.0 software.

Sample preparation

SGC7901 cells were seeded into 24 well plates (1×10^5/well), on which coverslips (9×9 mm) were placed. The cells were treated with or without resveratrol for 72 hrs at 37 °C in 5% CO_2 atmosphere. Then, the cells were washed thrice with PBS for 5 min each, after which they were fixed with 95% alcohol for 30 min. All samples were stored at 4 °C.

Immunocytochemistry assay

The coverslips with cells were washed thrice with PBS for 5 min each and then placed into 0.3% H_2O_2 for 10 min at room temperature. The coverslips were again washed with PBS thrice after nonspecific antigens were blocked with serum for 5 min. The coverslips were again washed thrice with PBS, and survivin antibody (Jianglai Biotech Company, Shanghai, China) was added at 4 °C overnight. The secondary antibody (rabbit anti-human, Jianglai Biotech Company, Shanghai, China) was then added at 37 °C for 30 min. The coverslips were again washed thrice with PBS, after which DAB staining was implemented. The coverslips were then observed under optical microscope.

Ranking of cells

Ten cells from a single field were randomly chosen under a 10×10 low-power magnification, and 50 cells for every field were counted under a 10×20 middle-power magnification. According to Deborah methods for classifying four different ranks [12], cells without color scored 0, cells with light yellow color scored 1, cells with brownish-yellow color scored 2 and cells with chocolate brown color scored 3.

HSCORE = $\sum Pi(I + 1)$ (i = 0, 1, 2, 3; pi is the proportion of i. HSCORE values were presented as mean ± standard error of the mean (SEM). This method reflected the protein expression in cells.

Statistics

Statistical analysis was performed by SPSS 10.0 software (SPSS Inc., Chicago, Ill), and all results were presented as mean±standard deviation (SD). One-way analysis of variance (ANOVA) was used to compare the results of different groups. T-test and chi square test were also used. A p-value <0.05 denoted a statistically significant difference.

Results

FCM showed that resveratrol promoted SGC7901 cell apoptosis

The cells at the S and M phases were significantly decreased in the treatment group compared with those in the control group ($p<0.05$; Figures 1 and 2; Table 1), indicating that resveratrol inhibited cell proliferation. Furthermore, cell apoptosis rate in the treatment group was higher than that in the control group ($p<0.01$), demonstrating that resveratrol promoted cell apoptosis effectively.

Survivin protein assessment showed that resveratrol inhibited survivin expression in SGC7901 cells

Survivin protein expression was located in the cytoplasm and exhibited brownish-yellow staining after usage of immunocytochemistry. Compared with the control group, the treatment group exhibited increased survivin protein expression after resveratrol treatment. Under optical microscopy, the stained SGC7901 cells displayed brownish-yellow and chocolate-brown colors (Figure 3). In the treatment group, positive cells exhibited a light-yellow stain; however, the survivin

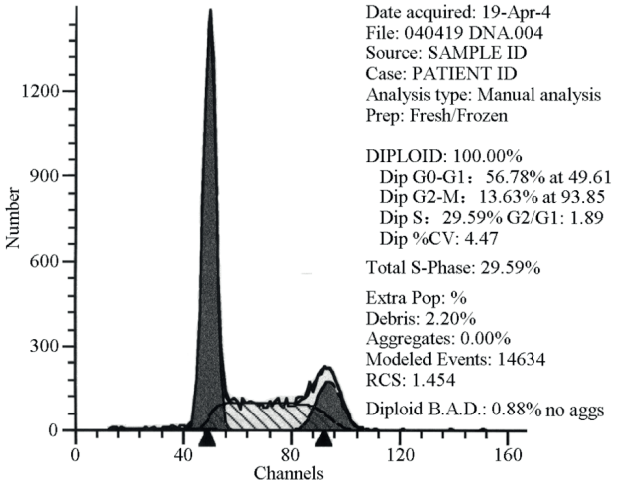

Figure 1. Cell cycle distribution of the SGC7901 control group.

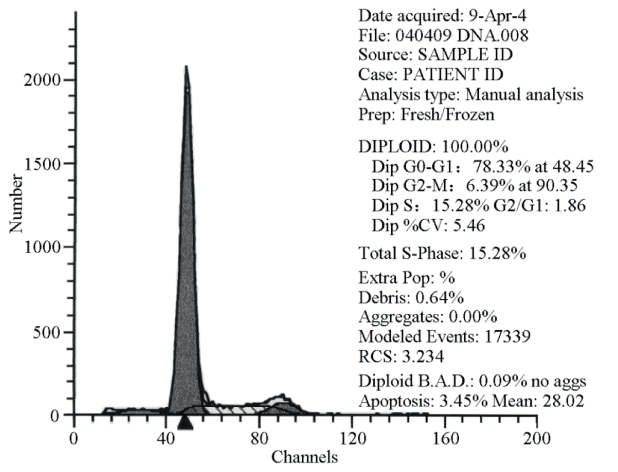

Figure 2. Cell cycle distribution of the SGC7901 group exposed to 35.69 μM resveratrol for 72 hrs.

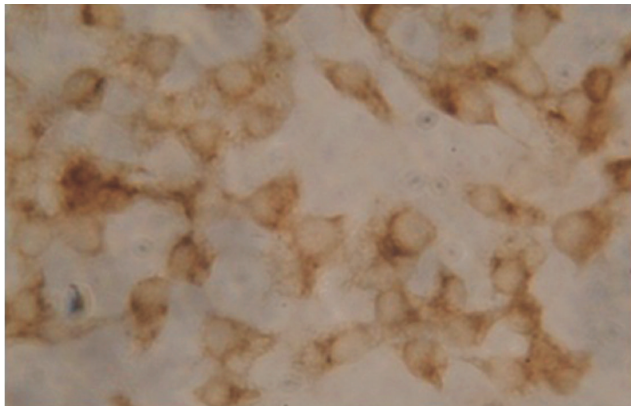

Figure 3. Expression of survivin protein in SGC7901 cancer cells of the control group (×400).

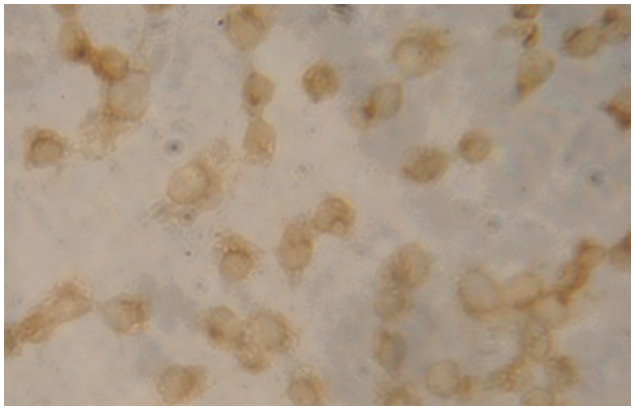

Figure 4. Expression of survivin protein in SGC7901 cancer cells of the resveratrol group (×400).

Table 1. Effect of resveratrol on SGC7901 cell cycle (%)

Group	*SGC7901*			
	G_0/G_1	*S*	G_2/M	*Apoptosis rate*
Control	56.78	29.59	13.63	0.00
Resveratrol	78.33*	15.28*	6.39**	3.45**

*p < 0.05, **p < 0.01 vs the control group (x2 test)

Table 2. HSCORE in SGC7901 cells

Group	*Mean*±SD
Control	2.9380±0.0280
Resveratrol	2.2500±0.0213*

*p<0.01 vs the control group (t-test)

protein expression was weaker in the treatment group compared with the control group (p<0.05) (Figure 4).

Table 2 shows that survivin expression decreased significantly after resveratrol treatment compared with the control group (p<0.01).

Discussion

Although the absolute mortality rates of gastric cancer have slightly decreased, this disease still ranks fourth in global cancer-related deaths. Despite improvements in the treatment of gastric cancer its prognosis has not significantly improved [13,14].

Resveratrol was originally isolated from the roots of *Veratrum grandiflorum* in 1940 and was later found in the skin of red grapes, peanuts, and red wines and blueberries. In 1998, Jang et al. reported that topical resveratrol application showed anticancer effects and reduced the skin cancer development in mice [15]. Since then, numerous studies have found that resveratrol prevented tumorigenesis by inhibiting three stages

of cancer: initiation, promotion and progression [16-18]. Resveratrol induced tumor cell apoptosis through different signalling pathways in different tumor cells [19,20]. In our previous studies [9,10], we found that resveratrol could promote the apoptosis of colorectal cancer cells. Based on this observation, we decided to explore whether resveratrol could promote the apoptosis of gastric cancer cells, as well as to determine the pathway through which apoptosis is promoted.

In this study, resveratrol was used as treatment to interfere with the growth of SGC7901 cells, and FCM assay showed that the apoptosis rate of SGC7901 cells was 4.5% after 72 hrs of treatment with resveratrol. By contrast, no apoptotic cancer cells were found in the control group. Moreover, the difference in apoptosis rate was significant. This result indicates that resveratrol promoted SGC7901 apoptosis. In addition, the G0/G1 phase of SGC7901 cells was increased after resveratrol treatment.

Survivin is a newly discovered apoptosis-inhibiting protein, and its gene is located on chromosome 17q25 [21]; this study showed that survivin can inhibit the apoptosis of tumor cells [22-24].

Thus, further exploration of the molecular mechanisms of resveratrol-induced apoptosis is important. Apoptosis pathways are usually associated with the gene expressions of caspase, inhibitor of apoptosis (IAP) and Bcl-2 families. Survivin, which is also known as baculoviral inhibitor of apoptosis repeat-containing 5 or BIRC5, is a member of the IAP family that was found in recent years. In 1997, Ambrosini from Yale University [21] isolated survivin protein from human genomic library for the first time through the effect of protein receptor-1. The survivin protein elicited negative regulation of apoptosis by binding with caspase-3 and -7 to block cell cycle arrest and inhibit cell apoptosis [25,26]. Furthermore, survivin promoted the growth of tumors by increasing cell proliferation [20,27]. Thus, the decrease in survivin, which is a factor contributing to chemotherapy resistance and apoptosis induction therapies, will render cancer cells more prone to cancer treatments. Moreover, several studies indicated that survivin can activate Cdk-2/cyclinE complexes to cause Rb phosphorylation to promote cell mitosis further [28]. Survivin is known to be highly expressed in most tumor cells but absent in normal cells. Therefore, survivin inhibition is an ideal target for cancer therapy.

In this study, immunohistochemistry showed that resveratrol downregulated the protein expression of survivin in SGC7901 cells. Statistical analysis showed a significant difference between the treatment and control groups ($p<0.01$), which confirmed that resveratrol can induce SGC7901 cell apoptosis by inhibiting the survivin expression. Thus, we conclude that resveratrol inhibited cell proliferation not only by inducing SGC7901 cell apoptosis but also by causing cell cycle arrest.

Riles et al. [29] reported that resveratrol caused nuclear DNA double-strands break in gastric cancer cells without damaging the integrity of mitochondria. Moreover, the expression of survivin mediated by resveratrol was relevant to the type of cell lines. Different cells might have different apoptotic pathways [21]. Yan et al. [26] also demonstrated that silencing the gene expression of survivin effectively reduces the mRNA and protein expression in oral squamous carcinoma cell lines KB and KBv200. This study also demonstrated that through the silencing of survivin gene expression, the apoptosis of KBv200 can be mediated.

In conclusion, this study fully confirmed that resveratrol application can decrease SGC7901 cell proliferation and inhibit the expression of survivin *in vitro*. Moreover, the overexpression of survivin was related to the resistance of gastric tumor cells [25]. These results revealed that resveratrol suppressed SGC7901 cell proliferation by mediating apoptosis, changing the cell cycle and inhibiting survivin expression. Such evidence provides a new perspective for the clinical application of resveratrol for cancer prevention.

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