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SIJUNZI DECOCTION DEMOLITION PARTIES INHIBIT PROLIFERATION AND INDUCE APOPTOSIS OF HUMAN GASTRIC CANCER BGC823 SIDE POPULATION

Jun Qian[1,†], Hui Xie[2,†], Chenxu Guo[1,†], Rui Sun[3, #], Lin Su[2, #], Jianguang Jia[1], Xin Jin[1], Dajun Yu[1], Jing Li[1]

[1] Third Department of Tumor Surgery, First Affiliated Hospital of Bengbu Medical College, Bengbu 233000, PR China.[2] Department of Nursing, Bengbu Medical College, Bengbu 233030, PR China.[3] Department of Gynecologic Oncology, Affiliated Hospital of Bengbu Medical College, Bengbu 233000, PR China.
[#] Correspondence to: Rui Sun: ruisunbbmc@163.com, Lin Su: sulin6303@sina.com. †These authors contributed equally to this work.

Abstract

Background: Comprehensive treatment combining with Chinese medicine has become the main therapeutic regimen of gastric cancer. Previous evidence demonstrated SD can enhance the effect of chemotherapy in advanced cancer, especially in gastric cancer. In order to investigate the anticancer mechanism of SD in gastric cancer, we observed the effects of SD parties (Ginseng, Atractylodes, Poria, Licorice) on proliferation and apoptosis of SP of human gastric cancer BGC-823.

Materials and Methods: 1. BGC-823 side population cells were sorted through flow cytometry. 2. To detect the changes of proliferation of SP and NSP before and after the intervention of serum containing SD parties using cck-8 method. 3. To detect the changes of cell cycle and apoptosis of SP and NSP before and after the intervention of serum containing SD parties through flow cytometry. 4. To detect the effects of serum containing SD parties on apoptosis-related proteins Bax and Bcl-2 of SP and NSP before and after the intervention by western-blot.

Results: It was found that four demolition parties serum treatments inhibited cell proliferation in a time-dependent manner. Compared with the control group (normal saline treatment), there were increase in G1/G0 phase population of SP and NSP, and decrease in G2/M and S phase population (P<0.05). Meanwhile, we found G1/G0 arrest induced by four demolition parties serum which was followed by apoptosis in a time-dependent manner. The apoptosis rate of drug serum treatment group was higher than the control group (P<0.05), and the apoptosis rate of 48 h treatment was higher than 24 h treatment (P<0.05). The expression of Bax protein of SP and NSP was higher than the control group in a time-dependent manner. The expression of Bcl-2 protein of SP and NSP was lower than the control group in a time-dependent manner.

Conclusion: The serum of four demolition parties of SD can inhibit the proliferation of SP of BGC-823 cell lines through G1/G0 phase arrest and followed by apoptosis which involves the up-regulation of Bax and the down-regulation of Bcl-2.

Key Words: BGC823 Side Population, Sijunzi Decoction, Ginseng, Atractylodes, Poria, Licorice

Abbreviations list: (SD) Sijunzi Decoction Demolition, (SP) side population, (NSP) non-side population, (Bcl-2) B-cell lymphoma 2, (BAX) Bcl-2 Associated X Protein, (FBS) Fetal bovine serum, (PBS) Phosphate buffer solution, (CCK-8) Cell Counting Kit-8 reagent, (AV) Annexin V-FITC, (PI) Propidium iodide, (EDTA) Ethylene Diamine Tetraacetic Acid, (PMSF) Phenylmethanesulfonyl fluoride, (RIPA) Radio Immunoprecipitation Assay, (PVDF)Poly(vinylidene fluoride), (TBST) Tris-buffered saline containing Tween-20

Introduction

Gastric cancer is the most commonly diagnosed gastrointestinal cancer with high recurrence rate, high metastatic rate and resistance to chemotherapeutic drugs (Saricanbaz et al., 2014). At present, comprehensive treatment combining with Chinese medicine has become the main therapeutic regimen of gastric cancer. Currently, the biological characteristics of gastric cancer cells are investigated mainly through studying proliferation (Kato et al., 2012), apoptosis (Akagi et al., 2013), nude mice tumorigenic (Zheng et al., 2010) and protein molecular level of gastric cancer cells (Wang et al., 2014). At this stage, cancer prevention and therapy are based on cancer stem cell theory (Kim et al., 2012; Sabet et al.,

2014; Zhao et al., 2014). Some evidence shows that cancer stem cell plays a key role in cancer occurrence and development, and is closely related with recurrence, metastasis and resistance to chemotherapeutic drugs (Singh and Settleman, 2010).

SP was proved to be a cell subset with low fluorescence as a result of the Hoechst 33342 staining, which is the founding belongs to Goodel et al. (Goodell et al., 1996), who used Hoechst 33342 and flow cytometry to stain and sort bone marrow cells in mice. SP can be sorted through ABCG-2 (adenosine triphosphate binding cassette super family G member-2 of transport protein, ABCG-2) because ABCG-2 has the feature of Hoechst 33342 excretion and expresses in high level in SP (Shaharuddin et al., 2014). Since SP has been demonstrated with stem cell properties of proliferation-added and *in vivo* tumorigenicity in nude mice (Kong et al., 2014), studying the biological characteristics of malignancies from the SP cells is of great significance.

Currently, there are an increasing number of experiments on cancer therapy concerning Chinese medicine and many of which have confirmed that traditional Chinese medicine could inhibit cancer cells and induce apoptosis of tumor cells (Zhang et al., 2012; Li-Weber, 2013). However, the study regarding cancer stem cells could be inhibited by Chinese medicine is uncommon. Classic SD is composed of Ginseng, Atractylodes, Poria and Licorice, which can nourish spleen and enrich qi. Previous evidence demonstrated that SD could enhance not only the effect of chemotherapy in advanced cancer but also the immune cell function of patients, which consequently reduces the side effects of chemotherapy and improves the life quality of patients (Huang et al., 2010). It is also implied that SD plays an important role in inhibiting tumor cell proliferation, invasion and metastasis (Deng et al., 2013), and can effectively improve the clinical efficacy of gastrointestinal cancer patients with positive resistance genes (Gan et al., 2010).

In order to collect the evidence for developing the theory of traditional Chinese medicine treating malignant tumors, we investigated the effects of SD parties on proliferation and apoptosis of Human Gastric Cancer SP.

Materials and Methods

Cell Line and Experimental Animal

Human gastric cancer cell line BGC-823 was purchased from Wuhan Cell Bank of Chinese Academy of Sciences (Wuhan, China). New Zealand white female rabbits (approx 2 kg each rabbit) were obtained from Animal Lab of Bengbu Medical College (Bengbu, China).

Reagents and Instruments

RPMI 1640 Medium and Penicillin-Streptomycin Solution were from Hyclone (USA). FBS was from Tianhang Biological Technology (Hangzhou, China). PBS was from CORNING (NY, USA). 0.25% trypsin and CCK-8 were from Beyotime (Nantong, China). SD parties herb (Ginseng, Atractylodes, Poria, Licorice) was from Department of Traditional Chinese medicine of the First Affiliated Hospital of Bengbu Medical College (Bengbu, China). 0.22um syringe filter was from Millpore (Boston, USA). Hochest 33342 was from Sigma (Saint Louis, USA). Verapamil was from Shanghai Pharmaceutical Group Co., Ltd. (Shanghai, China). AV and PI were from BD (Franklin Lakes, USA). Antibodies against Bax and Bcl-2 were from Abcam (Cambridge, England). Substrate Reagent was from Millpore (Boston, USA). β-actin antibody was from SANTA CRUZ (Dallas, USA). Horseradish peroxidase-labeled goat anti-rabbit IgG was from Biosharp (Hefei, China). Flow cytometers BD FACS ARIA II SORP and BD FACSCalibur: BD (Franklin Lakes, USA). Electrophoresis apparatus and gel imaging system: BioTeK Synergy 2 (BioTeK, USA). Power supply and gel imaging system ChemiDox XRS: Bio-Rad (Hercules, USA).

Preparation of Medicated Serum

15 pure-bred New Zealand adult female rabbits (about 2.0 kg per rabbit) were randomly divided into 5 groups (3 rabbits per group): normal saline control group (NS/control), and Ginseng group (G), Atractylodes group (A), Poria group (P), and Licorice group (L). The normal dose was calculated according to “Equivalent dose rate table of human and animal body surface area convert” (China Pharmacopoeia) as: rabbit dose (ml/kg•d) = human daily dose × 0.007/1.5 (ml/kg•d) = 14 ml/kg•d. Before each time of gavage, the rabbits were fasted for 12 h but fed water, the gavage was performed two times a day for two consecutive weeks. 1 hour after the last gavage, anesthetized the rabbits with 10% chloral hydrate, and collected the sterile cardiac blood. Serum was separated by centrifugation at 4,000 rpm for 60 min at 4 °C. 40 ml of serum was collected

from each rabbit. Serum was inactivated by water bath at 56 °C for 30 min, sterilized by filtration (0.22 μm filter), and stored in 1.5-ml eppendorf tubes at -20 °C for use.

Cell Culture

BGC-823 human gastric cells were maintained in RPMI-1640 medium containing 100 u/ml of penicillin, 100 ug/ml of streptomycin and 10% FBS, at 37 °C in a humidified and 5% CO_2 atmosphere.

Cell Sorting

The cells were harvested by detachment with 0.25% trypsin-EDTA and resuspended in PBS with 2% FBS, added hoechst 33342 (final concentration is 5 ug/ml) into the cell suspension, and control group was supplemented with 10 ul/ml verapamil additionally. Incubated all the cells at 37 °C in 5% CO_2 atmosphere for 90 min. Cells were collected and then resuspended in ice-cold PBS with 2% FBS at 1×10^6 cells/ml. Then SP and NSP cells were identified and sorted by a flow cytometry, and the signal was collected as follows: blue light, 402–446 nm; red light, 650–670 nm.

Cell Growth Standard Curve

Cells were adjusted to 1×10^4 cells/ml and 0, 20, 40, 60, 80, and 100 μl of cell suspension were added to 96-well plate (3 wells per group) and supplemented with 100 μl per well of RPMI-1640 containing 10% FBS. Then, 10 μl of CCK-8 reagent was added to each well for 3 h, and the absorbance was measured at 490 nm using a microplate reader to construct the standard curve.

Cell Proliferation Assay

Cells were cultured at 37 °C in a humidified and 5% CO_2 atmosphere. Cell proliferation was analyzed as detailed in CCK-8 kit instructions. All tests were done in triplicate and the experiments were repeated three times independently. The values were converted to cell numbers using the standard curve. The data were presented as $\overline{themean}$ ± standard error ($\bar{x}$ ±s).

Cell Cycle Analysis (Huang et al., 2014)

Cells were collected and washed with PBS twice, and resuspended in 1 ml of cold 70% ethanol, incubated at -20 °C overnight. The cells were pelleted by centrifugation and washed by PBS, resuspended in 0.5 ml PBS with 50 ug/ml of PI, and incubated in dark at 37 °C for 30 min, then cell cycle progression was analyzed by flow cytometry.

Apoptosis Assay

The cells were collected and washed with PBS twice, then the cells were resuspended in 250 ul binding buffer, 3 ul AV and 3 ul PI, incubated in dark for 15 min, then cell apoptosis rate was analyzed through flow cytometry.

Protein Analysis

Cell total protein was extracted by lysates (PMSF: RIPA=1:100). Protein concentration was adjusted to 60 ug/ul by RIPA. Protein was boiled at 99 °C for 5 min, stored at -80 °C for use. Proteins were separated by electrophoresis in 12% of separating Gel and 5% of stacking gel.

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Then the protein was transferred onto PVDF membrane. PVDF membrane was blocked with 5% non-fat milk at room temperature for 1-3 h. The PVDF membrane was incubated in primary antibody solution at 4 °C overnight. The PVDF membrane was washed by TBST, then the PVDF membrane was incubated in secondary antibody solution at 4 °C for 1 h. Protein bands exposure were performed using chromogenic substrate. The protein bands were analyzed by densitometry using Quantity One software (Bio-Rad). β-actin was used as internal control.

Statistical Analysis

All experiments were repeated 3 times independently, and the data were presented as $\overline{themean}$ ± standard error ($\bar{x}$ ±s). Statistical significance ($p < 0.05$) was assessed by the analysis of variance (ANOVA) followed by Student's *t*-test.

Results

Ratio of SP in BGC-823 Cell Line

The ratio of SP in BGC-823 cell line was (2.62±1.63)%, Meanwhile, the ratio of SP in control group (Verapamil group) was 0 (Figure 1).

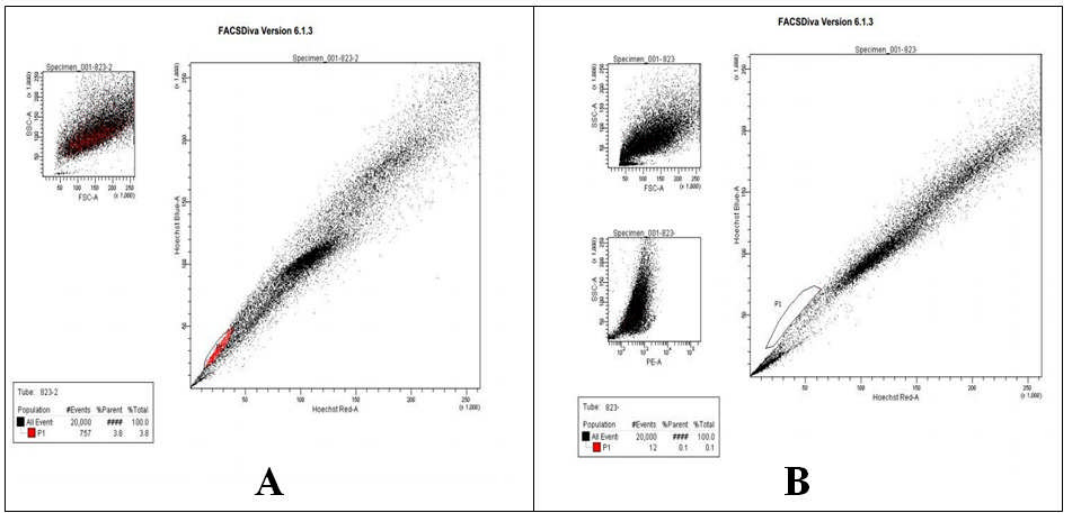

Figure 1: SP detection graph of BGC-823 cell line by flow cytometry. The SP cells in BGC 823 were sorted as detailed in "Cell sorting". The figures shown are representative of three independent experiments. A. SP detection graph without treatment of Verapammy; B. SP detection graph of Verapammy group.

Cell Proliferation Standard Curve of BGC-823 Cell Line

CCK-8 assay was used to determine cell proliferation. Cell growth standard curve was made through the regression equation y = 17640x + 314.69, r^2=0.994 ($P < 0.01$), which meet the linear relationship (Figure 2).

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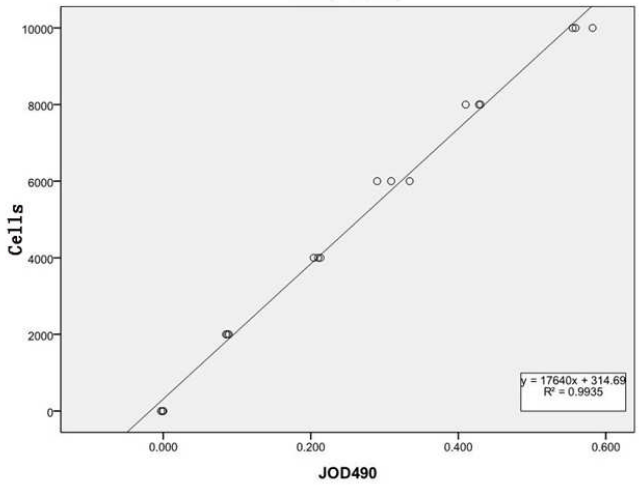

Figure 2: Cell proliferation standard curve of BGC-823 cells. The Cell proliferation standard curve was made as detailed in "Cell growth standard curve".

Changes in Cell Proliferation of SP and NSP Before and after Drug Serum (Ginseng, Atractylodes, Poria or Licorice) Intervention

After treated by the four demolition party serum or normal saline respectively, cell proliferation of SP and NSP were determined. As can be see in Figure 3, compared with the normal saline group (control group) at corresponding time point, four demolition party serum treatments inhibited cell proliferation in a time-dependent manner. Meanwhile, the inhibition rates of 168 h of SP were lower than that of NSP (Figure 4) ($P < 0.01$).

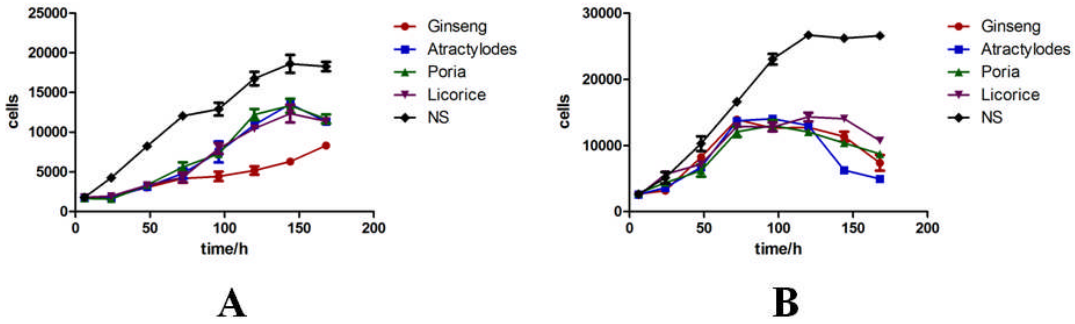

Figure 3: The effects of the drug serum (Ginseng, Atractylodes, Poria or Licorice) intervention on cell proliferation of BGC-823 SP and NSP. SP and NSP cells were seeded into 96-well plates at 1×10^3 cells per well and treated with 100 ul RPMI1640 supplemented with 10% drug serum obtained from rabbits that received Ginseng, Atractylodes, Poria, Licorice, or normal saline (control: NS) respectively. Cell proliferation was analyzed after treatments of 6 h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, and 168 h as detailed in "Cell proliferation assay". A. BGC-823 SP; B. BGC-823 NSP

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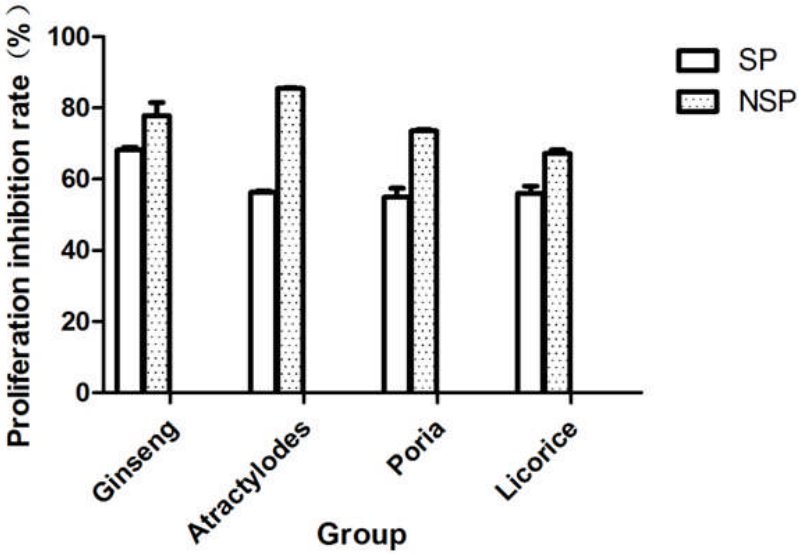

Figure 4: The comparison of proliferation inhibition rates of the four drug serum (Ginseng, Atractylodes, Poria or Licorice) in SP and NSP. SP and NSP cells were seeded into 96-well plates at 1×10^3 cells per well and treated with 100 ul RPMI1640 supplemented with 10% drug serum obtained from rabbits that received Ginseng, Atractylodes, Poria, Licorice or normal saline (control) respectively. Cell proliferation was analyzed after treated for 168 h as detailed in "Cell proliferation assay". Proliferation inhibition rates were determined using following formula.

$$\text{Inhibition rate}\,(\%) = (1 - \frac{\text{drug serum group}}{\text{control group}}) \times 100\%$$

The Changes of Cell Cycle after Treatment with Drug Serum

As we can see from Figure 5, drug serum intervention (Ginseng, Atractylodes, Poria or Licorice) induced G1/G0 arrest in cell cycle progression of SP and NSP of BGC-823 cell line in time-dependent manner. Compared with control group (normal saline group), there are a generally increase in G1/G0 phrase ($p < 0.05$) and hypodiploid population, a generally decrease in S or G2/M ($p < 0.05$) with prolonged treatment time (Figure 5). Meanwhile, these changes in NSP are generally more obvious than corresponding that of SP ($p < 0.05$) (Table 1).

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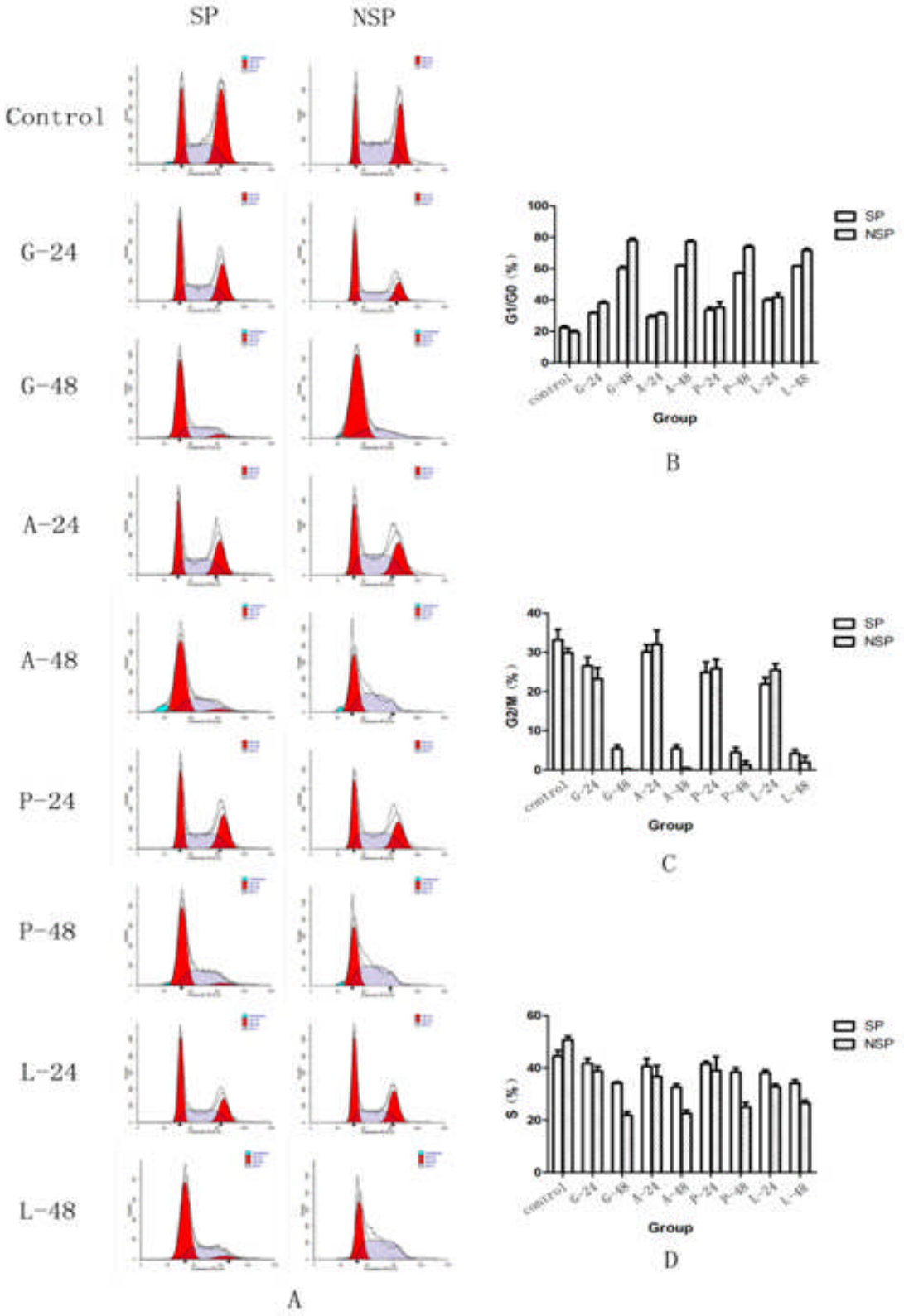

Figure 5: The changes in the cell cycle after treated with drug serum (containing Ginseng serum, Atractylodes serum, Poria serum, licorice). SP and NSP cells were all randomly divided into 5 groups, then 2 ml of SP and NSP cell suspension (5×10^5 cells/ml) were seeded into 60-mm Petri dish respectively, and were treated by cell culture medium containing Ginseng serum, Atractylodes serum, Poria serum, licorice serum or normal saline (control) serum respectively. After 24 h and 48 h of treatment, cell cycle progression was analyzed by flow cytometry as detailed in "Cell cycle analysis". A. Histogram of flow cytometry for cell cycle analysis after treated with drug serum; B. The comparison of G1/G0 phase in SP

and NSP after treated with drug serum; C. The comparison of S phase in SP and NSP after treated with drug serum; D. The comparison of G2/M phase in SP and NSP after treated with drug serum.

Table 1: The Changes in the cell cycle phases of BGC-823 SP and NSP after treated with drug serum (n=3, $\bar{X}$ ±s, %)

Groups	Cell cycle phases	Cell subsets	
		SP	NSP
Control	G1/G0	22.27±1.12	19.41±1.24
	S	44.47±2.16	50.71±1.47
	G2/M	33.26±2.61	29.88±1.13
Ginseng 24 h	G1/G0	31.68±0.59*	38.13±1.05*
	S	41.77±1.79	38.70±1.85*
	G2/M	26.56±2.18	23.17±2.90
Ginseng 48 h	G1/G0	60.16±1.32*#	78.00±1.43*#
	S	34.23±0.44*#	21.83±1.40*#
	G2/M	5.42±0.96*#	0.17±0.17*#
Atractylodes 24 h	G1/G0	29.24±1.24*	31.30±0.72*
	S	40.63±2.96	36.64±4.23*
	G2/M	30.13±1.82	32.06±3.63
Atractylodes 48 h	G1/G0	62.00±0.34*#	77.18±1.0*#
	S	32.57±1.26*#	22.52±1.28*#
	G2/M	5.43±0.92*#	0.30±0.29*#
Poria 24 h	G1/G0	33.53±1.90*	35.19±3.63*
	S	41.57±0.92	38.91±5.34
	G2/M	24.90±2.64*	25.90±2.34
Poria 48 h	G1/G0	57.14±0.40*#	73.77±1.14*#
	S	38.41±1.61*#	25.02±1.65*#
	G2/M	4.46±1.28*#	1.20±1.04*#
Licorice 24 h	G1/G0	39.99±0.80*	41.77±2.65*
	S	38.10±1.14	32.79±1.05*
	G2/M	21.91±1.67*	25.44±1.69*
Licorice 48 h	G1/G0	61.71±0.31*#	71.54±1.29*#
	S	34.12±1.30*#	26.52±1.06*#
	G2/M	4.17±1.06*#	1.95±1.50*#

Note: * $p < 0.05$ compared with corresponding cell cycle phase in control group; # $p < 0.05$ in same drug serum group, cell cycle phases in 48 h group show significant difference than corresponding cell cycle phases in 24 h group.

Drug Serum Treatment Induced Apoptosis in SP and NSP of BGC-823 Cell Line

In above results, we find that drug serum intervention result in increase in G1/G0 phrase and hypodiploid population which suggests that drug serum treatments induced G1/G0 phrase arrest followed by apoptosis. This is confirmed by next apoptosis test. As can be seen in Figure 6 and Table 2, drug serum (Ginseng, Atractylodes, Poria or Licorice) treatment induced apoptosis in SP and NSP of BGC-823 cell line in a time-dependent manner. Meanwhile, the apoptosis rate of SP was generally lower than that of NSP.

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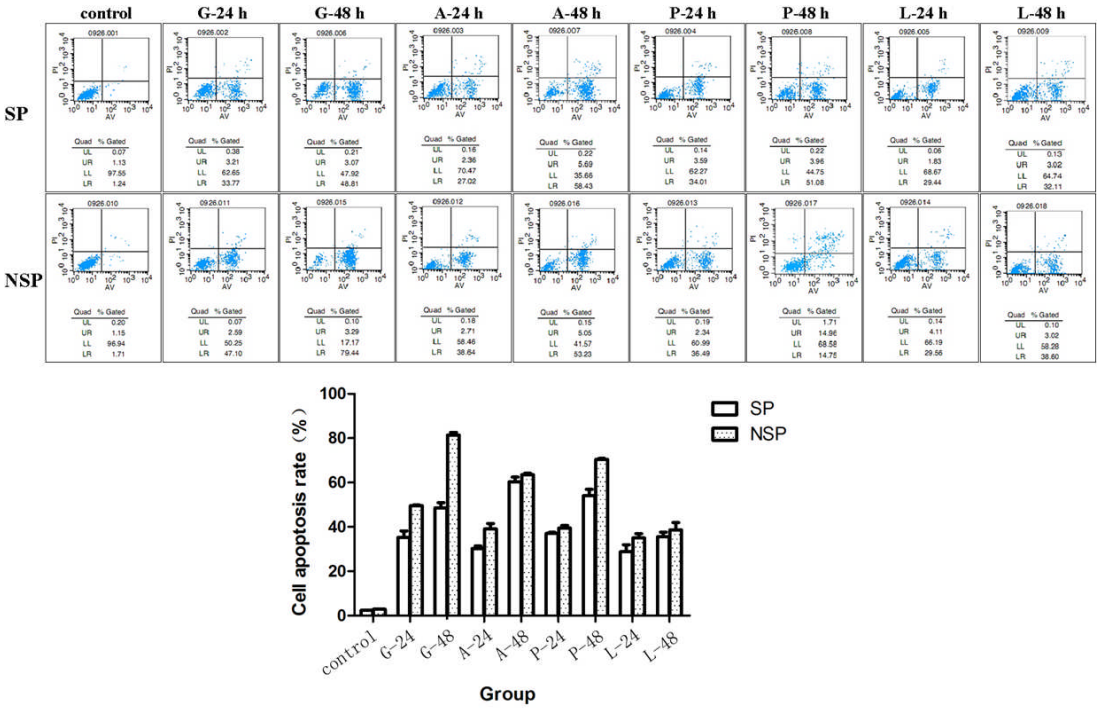

Figure 6: Drug serum (Ginseng, Atractylodes, Poria or Licorice) induced apoptosis in SP and NSP of BGC823 cells. SP and NSP cells were all randomly divided into 5 groups, then 2 ml of SP and NSP cell suspension (5×10^5 cells/ml) were seeded into 60-mm Petri dish respectively, and were treated by culture medium containing Ginseng serum, Atractylodes serum, Poria serum, licorice serum or normal saline serum (control) respectively. After 24 h and 48 h of treatment, cell apoptosis rates were analyzed through flow cytometry as detailed in "Apoptosis assay".

Table 2: Apoptosis rates of SP and NSP after treated with drug serum (n =3, $\bar{X}$ ±s, %)

Groups	Cell subsets	
	SP	NSP
Control	2.40±0.03	2.87±0.04
Ginseng 24 h	35.25±2.92*	49.59±0.28*
Ginseng 48 h	48.66±2.33*	81.44±1.20*
Atractylodes 24 h	30.31±1.09*	39.15±2.35*
Atractylodes 48 h	60.35±2.14*	63.53±0.71*
Poria 24 h	37.02±0.52*	39.46±1.19*
Poria 48 h	54.06±2.90*	70.45±0.40*
Licorice 24 h	28.83±3.14*	35.05±1.88*
Licorice 48 h	35.58±2.05*	38.59±3.39*

Note: * $p < 0.05$ compared with corresponding control group.

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Effects of Drug Serum (Ginseng, Atractylodes, Poria or Licorice) on Bax and Bcl-2 Protein Expression in SP and NSP

Based on above results, we investigated the effects of drug serum (Ginseng, Atractylodes, Poria or Licorice) on regulators of apoptosis (main inhibitor or promoter) as molecular targets for the apoptotic death of SP or NSP of BGC-823 cell line. Accordingly, we first focus our attention on the effects of drug serum (Ginseng, Atractylodes, Poria or Licorice) on pro-apoptotic protein Bax of SP and NSP of BGC-823 cell line because the protein play a key role in cell apoptosis. As shown in Figure 7 and Table 3, treatment of SP or NSP of BGC-823 cell line with drug serum (Ginseng, Atractylodes, Poria or Licorice) resulted in increased expression of Bax in a time-dependent manner.

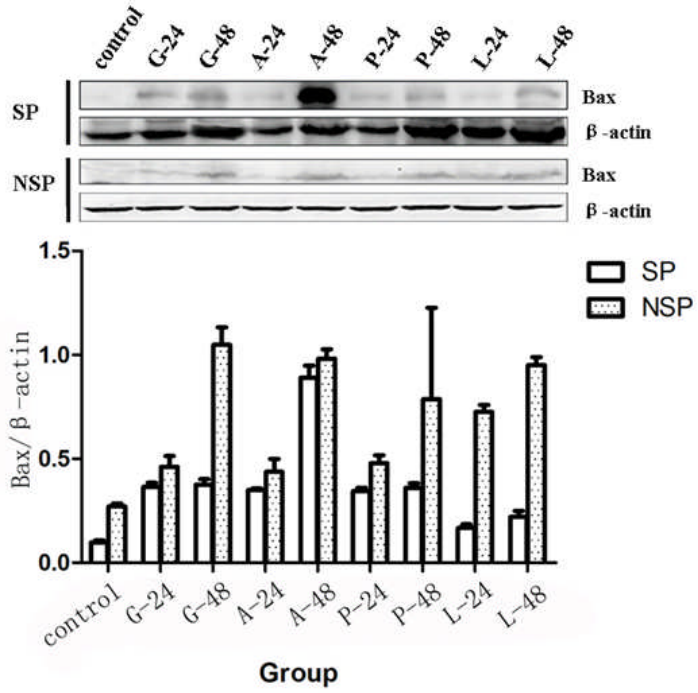

Figure 7: Bax expression in SP and NSP after treated with drug serum (containing Ginseng, Atractylodes, Poria, licorice or normal saline). SP and NSP cells were all randomly divided into 5 groups, then SP and NSP cells were seeded into cell culture flasks respectively, and were treated by culture medium containing Ginseng serum, Atractylodes serum, Poria serum, licorice serum or normal saline serum (control) respectively. After 24 h and 48 h of treatment, Bax was analyzed as detailed in “Protein analysis ”.

Table 3: Bax protein expression in SP and NSP treated with drug serum (n=3, $\bar{X}$ ±s)

Groups	Cell subsets	
	SP	NSP
Control	0.098±0.009	0.270±0.015
Ginseng 24 h	0.366±0.019*	0.461±0.052*
Ginseng 48 h	0.376±0.026*	1.048±0.084*
Atractylodes 24 h	0.351±0.006*	0.437±0.062*
Atractylodes 48 h	0.891±0.057*	0.982±0.045*
Poria 24 h	0.344±0.017*	0.479±0.038*
Poria 48 h	0.360±0.022*	0.787±0.440*
Licorice 24 h	0.167±0.017*	0.725±0.034*
Licorice 48 h	0.221±0.028*	0.951±0.038*

Note: * $p < 0.05$ compared with corresponding control group.

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In order to further investigate the apoptosis inducing effects of drug serum (Ginseng, Atractylodes, Poria or Licorice) on SP and NSP of BGC-823 cell lines, next we assessed the effects of drug serum on the expression of anti-apoptotic protein Bcl-2 of SP and NSP of BGC-823 cells. Compared with the control group, treatments of SP and NSP of BGC-823 cells with drug serum (Ginseng, Atractylodes, Poria or Licorice respectively) inhibited the expression of Bcl-2 ($p < 0.05$) in a time-dependent manner (Table 4, Figure 8).

Table 4: Bcl-2 protein expression in SP and NSP treated with drug serum（n=3, $\bar{X}$ ±s）

Group	Cell subsets	
	SP	NSP
Control	1.267±0.032	0.789±0.043
Ginseng 24h	0.743±0.005*	0.461±0.010*
Ginseng 48h	0.551±0.044*	0.328±0.012*
Atractylodes 24h	1.166±0.180*	0.576±0.086*
Atractylodes 48h	0.636±0.033*	0.328±0.023*
Poria 24h	0.903±0.039*	0.532±0.006*
Poria 48h	0.748±0.036*	0.294±0.017*
Licorice 24h	0.649±0.060*	0.602±0.053*
Licorice 48h	0.552±0.029*	0.392±0.021*

Note: * $p < 0.05$ compared with corresponding control group.

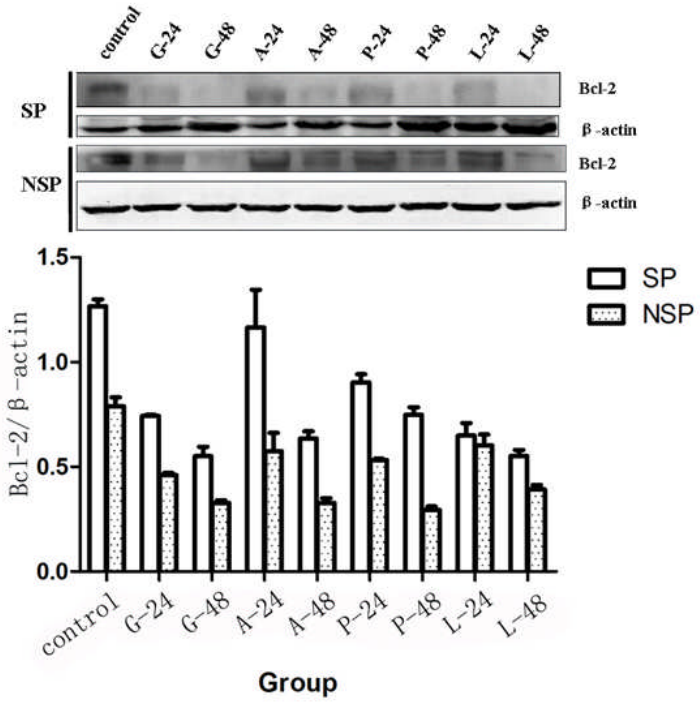

Figure 8: Bcl-2 expression in SP and NSP after treated with drug serum (containing Ginseng, Atractylodes, Poria, licorice or normal saline). SP and NSP cells were all randomly divided into 5 groups, then SP and NSP cells were seeded into cell culture flasks respectively, and were treated by culture medium containing Ginseng serum, Atractylodes serum, Poria serum, licorice serum or normal saline serum (control) respectively. After 24 h and 48 h of treatment, Bax was analyzed as detailed in “Protein analysis ”.

Discussion

Nowadays, the effects of using traditional Chinese medicine to against cancer are increasingly discussed in the reports. Cancer stem cell plays a crucial role in tumor progression. In the present work, SP cells of BGC823 cell line are employed as research object, which are special subpopulation that have the characteristics of cancer stem cells. According to our observation, the effects of drug serum on NSP cells of BGC823 were determined. In doing so, the activity of SD parties against BGC823 was preliminarily investigated by our research.

Firstly, the effects of SD parties on proliferation of SP and NSP of BGC823 cells were assessed. As a consequence, we found that the proliferation of SP and NSP was inhibited in a time-dependent manner. Moreover, the intervention activity of drug serum is higher in NSP than in SP. Based on the results, we next detected the effects of SD parties on cell cycle of SP and NSP of BGC823. It is found that treatment of SP or NSP of BGC823 with SD parties result in G1/G0 arrest in cell cycle progression of SP or NSP of BGC823 cells followed by apoptosis in a time-dependent manner. It was confirmed by subsequent apoptosis rate test. The experiment did indicate that treatment of SP or NSP of BGC823 with SD parties induced apoptosis and the apoptosis rates increased with prolonged treatment.

In order to further investigate apoptosis induction effect of drug serum (Ginseng, Atractylodes, Poria or Licorice) on SP or NSP of BGC823, Bax and Bcl-2 were selected as the apoptotic cell death studies in the research because they are the major, possibly causative apoptotic and antiapoptotic indicators of the multifactorial mechanisms of uncontrolled gastric cancer growth respectively. Apparently, the results showed that drug serum (Ginseng, Atractylodes, Poria or Licorice) induced the activity of pro-apoptotic protein Bax and inhibited anti-apoptotic protein Bcl-2 in a time-dependent manner in SP or NSP of BGC823.

Based on the findings, it is easy to conclude that: SD can inhibit the proliferation of SP and NSP of BGC-823 cell line followed by apoptosis which is partly mediated via promoting Bax but inhibiting Bcl-2.

Conflict of Interest: The authors declare that there are no conflicts of interest in this article content.

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