Effect of optimal combination of Huangqi (Radix Astragali Mongoli-ci) and Ezhu (Rhizoma Curcumae Phaeocaulis) on proliferation and apoptosis of A549 lung cancer cells

Xu Chengyong, Wang Yuguo, Feng Jian, Qin Li, Xu Ran, Dou Yongqi

Xu Chengyong, Medical School of Chinese People’s Liberation Army, Chinese People’s Liberation Army General Hospital, Beijing 100853, China; Department of Traditional Chinese Medicine, Hainan Branch of Chinese People’s Liberation Army General Hospital, Sanya 572013, China

Wang Yuguo, Feng Jian, Qin Li, Xu Ran, Medical School of Chinese People’s Liberation Army, Chinese People’s Liberation Army General Hospital, Beijing 100853, China

Dou Yongqi, Department of Traditional Chinese Medicine, Chinese People’s Liberation Army General Hospital, Beijing 100853, China

Supported by the National Natural Science Foundation of China: the Study on Molecular Mechanism of Anti-lung Cancer Angiogenesis of Astragalus-zedoariae in Experienced Prescriptions Based on TGF-β1/MAPK / HIF-1α Signaling Pathway (No. 81673810)

Correspondence to: Dou Yongqi, Department of Traditional Chinese Medicine, Chinese People’s Liberation Army General Hospital Beijing (100853) China.dyqi_301@yeah.net

Telephone: +86-10-66939456

Accepted: February 22, 2018

Abstract

OBJECTIVE: To investigate the effect of optimal combination (E) of Huangqi (Radix Astragali Mongolici) and Ezhu (Rhizoma Curcumae Phaeocaulis) on proliferation and apoptosis of A549 lung cancer cells and the possible mechanism underpinning the action.

METHODS: A uniform design method was used to optimize the E of Huangqi (Radix Astragali Mongolici) and Ezhu (Rhizoma Curcumae Phaeocaulis) in A549 lung cancer cells. MTS assay was applied to analyze the effect of the component formula of Huangqi (Radix Astragali Mongolici) and Ezhu (Rhizoma Curcumae Phaeocaulis) on A549 cells viability in various uniform design groups. A549 cells with exponential growth in routine culture were exposed to CoCl₂ (200 μmol/L) to mimic hypoxic conditions. Group 0 was treated with RPMI-1640, the group CoCl₂ was treated with CoCl₂ (200 μmol/L), the group DDP + CoCl₂ was treated with 4 mg/L Cisplatin injection (DDP) + CoCl₂ (200 μmol/L), and the drug group was treated with various dose of E (0.5E, 1E, 2E) + CoCl₂ (200 μmol/L). All groups were cultured for 24 h. Cell apoptosis was measured by Annexin V-FITC/propidium iodide double staining and flow cytometry. Western blot assay and quantitative real-time polymerase chain reaction (qRT-PCR) were employed to detect the protein and mRNA expression of B-celllymphoma-2 (Bcl-2), Bcl-2-associated X protein (Bax) and cysteiny1 aspartate specific proteinase-3 (caspase-3).

RESULTS: The E obtained by the uniform design was comprise of 200 mg/L Astragalus polysaccharide (X1) and 32 mg/L Curcumin (X3). Group DDP + CoCl₂ group 1E + CoCl₂ and group 2E + CoCl₂ promoted the apoptosis of A549 cells (P < 0.05). Group 1E + CoCl₂ and group 2E + CoCl₂, had no statistically significant differences compared with the group DDP + CoCl₂ (P > 0.05). Compared with group 0, various doses of E + CoCl₂, could up-regulate the expression of Bax and caspase-3 and down-regulate the expression of Bcl-2 at protein and mRNA levels (P < 0.05).

CONCLUSION: Astragalus polysaccharide and Curcumin was the optimal combination of Huangqi (Radix Astragali Mongolici) and Ezhu (Rhizoma Cur-
cumae Phaeocaulis). E promoted the apoptosis of A549 cells. Combination of Astragalus polysaccharide and Curcumin increased the expression of Bax and caspase-3, and decreased the expression of Bcl-2 to initiate apoptosis in A549 cells under chemical-induced hypoxia.

© 2018 JTCM. All rights reserved.

Keywords: Lung neoplasms; A549 cells; Apoptosis; Astragalus; Huangqi (Radix Astragali Mongolici); Ezhu (Rhizoma Curcumae Phaeocaulis); Curcumin; Hypoxia

INTRODUCTION

Lung cancer has the highest death rate among all types of cancers. In 2017 the most common cause of cancer death was still lung cancer in the United States, which contributes to more than a quarter of cancer mortality among all tumor types. In China, lung cancer is also the leading cause of cancer death for both males and females. Non-small cell lung cancer (NSCLC) accounts for 80%-85% of all lung cancers. Cisplatin-based chemotherapy has been widely used for patients with NSCLC in recent years. Although chemotherapy and targeted therapy have been improved in recent decades, the efficacy of chemotherapy for NSCLC is modest at present, and the 5-year survival rate of NSCLC is still unsatisfactory.

Traditional Chinese Medicine (TCM) has been used in treatment of lung cancer for many years in China. The method of promoting Qi and activating blood is commonly used in the treatment. The combination of Huangqi (Radix Astragali Mongolici) and Ezhu (Rhizoma Curcumae Phaeocaulis) is one of the most common combinations in the method. The effective active ingredients of Huangqi (Radix Astragali Mongolici) and Ezhu (Rhizoma Curcumae Phaeocaulis), such as Astragalus saponins, Astragalus polysaccharide, β-elemene and Curcumin, have been reported to have anti-cancer effects. The research into the compatibility of Chinese herbs has risen from the herbal pieces level to the component formula level and the uniform experimental design has become a new valuable method in the compatibility research of Chinese medicine drugs.

In this study, we used the uniform design method with a 4-factor and 8-level table to determine the optimal combination (E) of four components in Huangqi (Radix Astragali Mongolici) and Ezhu (Rhizoma Curcumae Phaeocaulis), namely Astragalus polysaccharide, Astragalus saponins, Curcumin and β-Elemene. Changes in the inhibition of A549 cell proliferation were observed as screening indices, and regression analysis was used to determine E. Using the chemical approach (CoCl2) to simulate hypoxia, we analyzed the expression of Bcl-2, Bax and caspase-3 in A549 lung cancer cells treated with various doses of E.

MATERIALS AND METHODS

Drugs and reagents

Astragalus saponins and polysaccharide were purchased from Elebio Co., Ltd., (Shanghai, China). β-Elemene and Curcumin were purchased from the National Institutes for Food and Drug Control (Beijing, China). Antibody against Bax and antibody against β-actin were obtained from Abcam (Cambridge, UK). Antibody against caspase-3 was obtained from Cell Signaling Technology Inc. (Danvers, MA, USA). Antibody against Bcl-2, goat anti-rabbit IgG-horseradish peroxidase (HRP) and goat anti-mouse IgG-HRP were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Trizol reagent was obtained from Invitrogen (Carlsbad, CA, USA). Other reagents included a HiFiMMLvDNA First Strand Synthesis kit, Ultra-pure RNA extraction kit. An Annexin V-FITC/PI Apoptosis Detection kit and UltraSYBR mixture were obtained from CWbio Co., Ltd., (Beijing, China). A Cell Titer 96® AQueous One Solution Cell Proliferation Assay (MTS) was purchased from Promega (Madison, WI, USA). Cisplatin injection was obtained from Hospira (Mulgrave, Australia). CoCl2 was obtained from Sigma-Aldrich (St Louis, MO, USA).

Cell culture and treatments

The A549 human lung adenocarcinoma cell line was purchased from the Cell Center of the Chinese Academy of Medical Sciences (Beijing, China). The cells were cultured in Roswell Park Memorial Institute 1640 medium (RPMI-1640) (Gibco, NY, USA) containing 10% fetal bovine serum (Sijiqing, Hangzhou, China) and an antibiotic mixture of Penicillin-Streptomycin Solution (Pasching, Austria). The cells were seeded on culture plates for each experiment and grown at 37 °C with 5% CO2. Astragalus saponins, Astragalus polysaccharide, Curcumin and β-Elemene were dissolved in dimethyl sulfoxide (DMSO) and diluted with RPMI-1640. The final concentration of DMSO never exceeded 5% (v/v). CoCl2 was dissolved in sterile water for injection and diluted with RPMI-1640. Cisplatin was diluted with RPMI-1640.

Uniform design of the experiments and cell viability assay

In this study, the uniform design method was used to optimize the most effective component formula of Huangqi (Radix Astragali Mongolici) and Ezhu (Rhizoma Curcumae Phaeocaulis) in on effect of the proliferation of A549 lung cancer cells. A uniform design method with a 4-factor and 8-level table U8 (84) was used to optimize the proportions of four component in Huangqi (Radix Astragali Mongolici) and Ezhu (Rhizoma Curcumae Phaeocaulis), namely, Astragalus poly-
saccharide (X1), Astragalus saponins (X2), Curcumin (X3), and β-elemene (X4) (Table 1). Moreover, changes in the cellular proliferation inhibition rate (CPIR) were observed as evaluation indicator, and regression analysis was used to determine E.

For cell proliferation assays, A549 cells were harvested and transferred to 96-well plates at a density of 1 × 10^4 cells per well. After incubation for 24 h, according to the uniform design for dosing (Table 1), each combination of drugs was added into the 96-well plates. CPIR was analyzed by the Cell Titer 96® AQueous One Solution Cell Proliferation assay (MTS) according to the manufacturer’s protocol. After 24 h of treatment, the supernatant was removed, and then 100 µL RPMI-1640 and 10 µL MTS solution were added to each well, followed by incubation for 1 h at 37 °C. Cell viability was measured with PE Victor X microplate reader (PerkinElmer, USA) at 490 nm. The average absorbance value from three wells per group was calculated. CPIR (%) = [1-OD (Experimental group)/OD (control group)] × 100 (%).

**Apoptosis assay**

Apoptosis analysis was carried out by flow cytometry (FACS Calibur; BD Biosciences). We screened 200 µmol/L CoCl₂ by pre-experiment to simulate the chemical hypoxic micro-environment. Group 0 was treated with RPMI-1640, group CoCl₂ was treated with 200 µmol/L CoCl₂; group DDP + CoCl₂ was treated with 4 mg/L DDP + 200 µmol/L CoCl₂, and drug group was treated with various doses of E (0.5E, 1E, 2E) + 200 µmol/L CoCl₂. All these groups were cultured for 24 h. Next, according to the manufacturer’s protocol for the Annexin V-FITC/PI apoptosis detection kit, cells were washed with ice-cold Phosphate Buffer saline (PBS), harvested by 0.25% trypsin. Then, 5 µL Annexin V-fluorescein isothiocyanate (FITC) and 5 µL propidium iodide (PI) were added to the cells, and the mixture was kept in the dark at room temperature for 15 min. Subsequently, 400 µL binding buffer was added to each treatment group, followed by immediate flow cytometric analysis. The apoptosis rate (%) = (number of apoptotic cells / number of total cells) × 100 (%).

**RNA extraction and quantitative real-time PCR**

Total RNA from A549 cells was isolated using Trizol, according to the manufacturer’s instructions. The total RNA was reverse transcribed using Trizol, according to the manufacturer’s instructions. The total RNA was reverse transcribed using HiFi-MMLV cDNA first strand synthesis kit with random primers. RT-PCR were conducted using Ultra SYBR mixture in the ABI Prism 7500 real-time PCR instrument (Bio systems, MA, USA). The PCR amplification conditions were as follows: 95 °C for 10 min, and then 45 cycles of 95 °C for 15 s and 60 °C for 60 s. Each target gene was quantified with housekeeping gene GAPDH serving as an internal control. The primer sequences are listed in Table 2.

### Table 1 Uniform design U, (8) of the active components of Huangqi (Radix Astragali Mongolici) and Ezhu (Rhizoma Curcumae Phaeocaulis) and the CPIR in A549 cells (x ± s, n = 3)

<table>
<thead>
<tr>
<th>Test number</th>
<th>X1 (mg/L)</th>
<th>X2 (mg/L)</th>
<th>X3 (mg/L)</th>
<th>X4 (mg/L)</th>
<th>CPIR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60.00</td>
<td>140.00</td>
<td>24.00</td>
<td>20.00</td>
<td>25.1±1.1</td>
</tr>
<tr>
<td>2</td>
<td>80.00</td>
<td>100.00</td>
<td>4.00</td>
<td>50.00</td>
<td>3.3±8.6</td>
</tr>
<tr>
<td>3</td>
<td>100.00</td>
<td>180.00</td>
<td>20.00</td>
<td>80.00</td>
<td>12.0±9.9</td>
</tr>
<tr>
<td>4</td>
<td>120.00</td>
<td>60.00</td>
<td>28.00</td>
<td>60.00</td>
<td>73.9±2.1</td>
</tr>
<tr>
<td>5</td>
<td>140.00</td>
<td>200.00</td>
<td>8.00</td>
<td>30.00</td>
<td>0.3±5.0</td>
</tr>
<tr>
<td>6</td>
<td>160.00</td>
<td>80.00</td>
<td>16.00</td>
<td>10.00</td>
<td>21.0±5.9</td>
</tr>
<tr>
<td>7</td>
<td>180.00</td>
<td>160.00</td>
<td>32.00</td>
<td>40.00</td>
<td>92.2±1.6</td>
</tr>
<tr>
<td>8</td>
<td>200.00</td>
<td>120.00</td>
<td>12.00</td>
<td>70.00</td>
<td>45.7±1.0</td>
</tr>
</tbody>
</table>

Notes: X₁: Astragalus polysaccharide; X₂: Astragalus saponins; X₃: Curcumin; X₄: β-elemene. CPIR: cellular proliferation inhibition rate.

### Table 2 Polymerase chain reaction primers for PCR

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’-3’)</th>
<th>Amplified fragment length</th>
<th>Annealing temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bax</td>
<td>upstream primer</td>
<td>GACGAACCTGGACAGTAACATGGAGCT</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>downstream primer</td>
<td>GGCAAAGTAGAAAAGGGCAACAAC</td>
<td>-</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>upstream primer</td>
<td>TGGGATGCTTTTGTGAACTG</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>downstream primer</td>
<td>TCTTCAAGACAGCAGGAGG</td>
<td>-</td>
</tr>
<tr>
<td>Caspase-3</td>
<td>upstream primer</td>
<td>TACAGATGTCTGAGCAGAAAAAACC</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>downstream primer</td>
<td>GCCCTGAGCAAGCAAAACAAAAACT</td>
<td>-</td>
</tr>
<tr>
<td>GAPDH</td>
<td>upstream primer</td>
<td>CTGGGCTACACTGAGGACC</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>downstream primer</td>
<td>AAGTGCGTCGTTGAGGGCAATG</td>
<td>-</td>
</tr>
</tbody>
</table>
Proliferation inhibition of A549 cells

A uniform design method with a 4-factor and 8-level table was used to optimize the proportions of four components in Huangqi (Radix Astragali Mongolici) and Ezhu (Rhizoma Curcumae Phaeocaulis) (Table 1). All CPIR observations of the eight uniform design groups were analyzed by stepwise regression analysis using SPSS 17.0 statistical software. Screening α into = α out = 0.15, two factors entered the model (X1 and X3), R2 = 0.805, it indicated that two influencing factors resulted in 80.5% CPIR variation and the model was relatively satisfactory. The optimal regression equation is = 0.003X1 + 0.26X3-0.503. Based on the partial regression coefficient, higher levels of X1 and X3 led to a higher CPIR. X2 and X4 were removed during the regression process, it indicated that changes of X2 and X4 did not affect the changes of CPIR. Therefore, the optimal test scheme obtained by the uniform design (E) was 200 mg/L Astragalus polysaccharide (X1) and 32 mg/L curcumin (X3).

Western blot analysis

A549 cells collected at the indicated times were lysed in lysis buffer (RIPA Lysis Buffer and protease inhibitor mixture) and sonicated five times using an Ultrasonic cell disrupter. After the protein concentration was quantified by a BCA assay (CWbio, Beijing, China), the lysate was mixed with sample loading buffer and heated at 100℃ for 10 min. The protein samples were separated by SDS-polyacrylamide gel electrophoresis and electrotransferred onto polyvinylidene difluoride membranes (0.45 μm, Merck KGaA, Darmstadt, Germany). Then, the membranes were blocked with 5% bovine serum albumin (BSA) in TBST (10mmol/L NaCl, and 0.1% Tween-20) for 2 h at room temperature before incubation with the corresponding antibodies overnight at 4℃. The next day, after five washes with 1×TBST (5 min/wash), the membranes were incubated with HRP-conjugated secondary antibodies (goat anti-mouse IgG or goat anti-rabbit IgG) for 1 h at room temperature. After five washes with TBST, antibody binding was detected using High sensitivity chemiluminescence detection kit (CWbio, Beijing, China) and visualized using an Image Quant LAS 500 (Healthcare Bio-Sciences, AB, Sweden). Western blots membranes were analyzed by Image Processing and Analysis in Java software (Image J) from the National Institutes of Health (NIH).

Statistical analysis

All data were processed using SPSS 17.0 (SPSS Inc., Released 2008, SPSS for Windows, Version 17.0, Chicago, IL, USA). Data are expressed as the mean±standard deviation ( x ± s). One-way analysis of variance was used for comparisons among all groups, followed by the Student-Newman-Keuls test for comparisons between two groups. P < 0.05 was considered as statistically significant.

RESULTS

Proliferation inhibition of A549 cells

Apoptotic induction of A549 cells under chemical-induced hypoxia

Apoptosis was analysed by flow cytometry in A549 cells. Results showed no significant difference between group 0 and group CoCl2 (P > 0.05). Compared with group 0, group DDP + CoCl2, 1E + CoCl2 and 2E + CoCl2 promoted the apoptosis rates of A549 cells (P < 0.05), and the apoptosis rates of group 0.5E + CoCl2, 1E + CoCl2 and 2E + CoCl2 were dose dependent. The apoptosis rates of the group 1E + CoCl2 and the group 2E + CoCl2 had no statistically significant difference compared with the group DDP + CoCl2 (P > 0.05).

Expressions of Bcl-2, Bax and caspase-3 protein in A549 cells under chemical-induced hypoxia

Compared with group 0, Bax and caspase-3 protein expressions increased and Bcl-2 protein expression decreased in group CoCl2 (P < 0.05). Compared with group 0, Bax and caspase-3 protein expressions in each drug intervention groups increased significantly (P < 0.05) and Bcl-2 protein expression in group DDP + CoCl2, 1E + CoCl2 and 2E + CoCl2 decreased significantly (P < 0.05). Bax and caspase-3 protein expressions in each dose of E + CoCl2 groups were significantly lower than group DDP + CoCl2, (P < 0.05), but Bcl-2 protein expression of group 1E + CoCl2 and 2E + CoCl2 showed no significant differences compared with the group DDP + CoCl2 (P > 0.05) (Figure 2).

Expressions of Bcl-2, Bax, and caspase-3 mRNAs in A549 cells under chemical-induced hypoxia

Compared with group 0, the mRNA expressions of Bax, caspase-3 and Bcl-2 in group CoCl2 were consistent with the results of western blot assay. Compared with group 0, the mRNA expressions of Bax and caspase-3 in group DDP + CoCl2, 1E + CoCl2 and 2E + CoCl2 significantly increased, Bcl-2 mRNA in each drug intervention groups significantly decreased (P < 0.05). Bax mRNA expression in group 0.5E + CoCl2 and 1E + CoCl2 were significantly lower than group DDP + CoCl2 (P < 0.05). However, Bax mRNA expression showed no significant difference between the...
As the main components of Huangqi (Rhi-Radix Astragali Mongolici) and Ezhu (Rhizoma Curcumae Phaeocaulis), Astragalus saponins, Astragalus polysaccharide, β-Elemene and Curcumin have been reported to show anti-tumor effects. Astragalus polysaccharide can reduce the expression of Bcl-2 and HSP70 in tumor tissue. β-Elemene inhibits the growth of solid tumors. Curcumin induces apoptosis through the mitochondrial pathway involving caspase-8, cytochrome C release, and caspase-3 activation. β-Elemene can promote the apoptosis of tumor cells and inhibit the tumor micro-vessel formation. According to the clinical medication rule of TCM, we selected Huangqi (Radix Astragali Mongolici) and Ezhu (Rhizoma Curcumae Phaeocaulis) as a representative combination of in the method of promoting Qi and activating blood. E was found through a uniform design with a 4-factor and 8-level table from four constituents of Huangqi (Radix Astragali Mongolici) and Ezhu (Rhizoma Curcumae Phaeocaulis). The regression analysis

DISCUSSION

Uniform design has the advantage for multi-factor and multi-level experimental research. Under the same situation, compared with orthogonal design, through the application of uniform design analysis to establish the optimal composition of the recipe selection model, it is easy to obtain a great deal of information from simple experiments, and the experimental results had shown good reproducibility. As the main components of Huangqi (Radix Astragali Mongolici) and Ezhu (Rhizoma Curcumae Phaeocaulis), Astragalus saponins, Astragalus polysaccharide, β-Elemene and Curcumin have been reported to show anti-tumor effects. Astragalus polysaccharide can reduce the expression of Bcl-2 and HSP70 in tumor tissue. β-Elemene inhibits the growth of solid tumors. Curcumin induces apoptosis through the mitochondrial pathway involving caspase-8, cytochrome C release, and caspase-3 activation. β-Elemene can promote the apoptosis of tumor cells and inhibit the tumor micro-vessel formation. According to the clinical medication rule of TCM, we selected Huangqi (Radix Astragali Mongolici) and Ezhu (Rhizoma Curcumae Phaeocaulis) as a representative combination of in the method of promoting Qi and activating blood. E was found through a uniform design with a 4-factor and 8-level table from four constituents of Huangqi (Radix Astragali Mongolici) and Ezhu (Rhizoma Curcumae Phaeocaulis). The regression analysis
showed that E obtained by the uniform design was 200 mg/L Astragalus polysaccharide and 32 mg/L Curcumin.

**Hypoxia** is one of the common pathological phenome-
na in tumor tissues. In fact, most tumor cells are subjected to hypoxia because of their rapid growth. Therefore, it is important to study the effect of drugs on the apoptosis of tumor cells in hypoxic environment. Besides, clinical and basic studies have shown that hypoxia regulates changes in apoptosis-related molecules and enhances the anti-apoptotic ability of tumor cells, which make the tumor cells achieve stronger viability.

We used human A549 cells with exponential growth in routine culture, which were exposed to 200 μmol/L CoCl₂ to mimic hypoxic conditions. The results showed that 200 μmol/L CoCl₂ had little effect on the apoptotic rate of A549 cells (P > 0.05). However, 200 μmol/L CoCl₂ can regulate the expression of Bax, Bcl-2 and caspase-3 to a certain degree, which was similar to the literature report. Therefore, we should pay attention to the effect of E combined with CoCl₂ on the expression of apoptotic factor in the discussion. In this study, various doses of E (0.5, 1, 2) + CoCl₂ promoted the apoptosis rate of A549 cells in a dose-dependent manner. The group 2E + CoCl₂ was better than the group DDP + CoCl₂ (P < 0.05) for promotion of the apoptosis rate in A549 cells. Mitochondrion-dependent and mitochondrion-independent apoptosis are important pathways that mediate cell death. Caspase-3 is a central executor of the apoptotic process. In this study, various doses of E + CoCl₂ could up-regulate the expression of caspase-3 at protein and mRNA levels. The Bcl-2 family is involved in the process of tumor cell apoptosis through complex mechanisms. As an important endogenous anti-apoptotic gene, Bcl-2 plays an anti-apoptotic role in preserving the mitochondrial structure and function. As the dominant negative inhibitor of Bcl-2, Bax is the most important pro-apoptotic gene in the Bcl-2 family, which promotes apoptosis by inducing mitochondrial permeability transition. Induction of apoptosis is an important effect of many anti-tumor drugs. In this study, various doses of E + CoCl₂ could up-regulate the expression of Bax and caspase-3 and down-regulate the expression of Bcl-2 at protein and mRNA levels.

In conclusion, the present study indicated that Astragalus polysaccharide plus Curcumin was the optimal combination for active ingredients of Huangqi (Radix Astragali Mongolici) and Ezhu (Rhizoma Cucurbitae Phaenocaulis). The combination of Astragalus polysaccharide and Curcumin (E) promoted the apoptosis of A549 cells. Various doses of E initiated apoptosis in A549 cells by increasing pro-apoptotic proteins Bax and caspase-3, and decreased anti-apoptotic protein Bcl-2 to activate the mitochondrial pathway.

REFERENCES

gullapsapins induce growth inhibition and apoptosis in hu
man colon cancer cells and tumor xenograft. Carcinogene


