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Original Article

Patrinia scabiosaefolia Inhibits Growth of 5-FU-Resistant Colorectal Carcinoma Cells via Induction of Apoptosis and Suppression of AKT Pathway*

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ABSTRACT **Objective:** To investigate the effects of ethanol extract of *Patrinia scabiosaefolia* (EEPS) on chemo-resistance of colorectal cancer cells (CRC) and explore the possible molecular mechanisms. **Methods:** 5-fluorouracil (5-FU)-resistant human colorectal carcinoma cell line (HCT-8/5-FU) and its parental cells HCT-8 were treated with EEPS (0, 0.25, 0.50, 1 or 2 mg/mL), or 5-FU (0, 100, 200, 400, 800 or 1600 μmol/L). The 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay was performed to evaluate the cell viability. Cell density was observed by phase-contrast microscope, cell counting and colony formation assay were used to determine the cell proliferation of HCT-8/5-FU cells treated with 0, 0.5, 1 or 2 mg/mL EEPS. Cell apoptosis was determined by Hoechst staining. Western-blot was performed to detect the phosphorylation of AKT as well as the protein expression level of B-cell CLL/lymphoma 2 (Bcl-2) and Bcl-2-associated X protein (Bax). **Results:** Compared with HCT-8 cells, MTT assay results indicated that HCT-8/5-FU cells were resistant to 5-FU treatment ($P<0.05$), and sensitive to EEPS treatment ($P>0.05$). Moreover, compared with untreated HCT-8/5-FU cells, 1 and 2 mg/mL of EEPS treatment significantly reduced cell density, cell number, inhibited cell survival ($P<0.05$), and induced apoptosis in HCT-8/5-FU cells. Furthermore, 1 and 2 mg/mL of EEPS significantly decreased the phosphorylation level of p-AKT and Bcl-2 protein expression, and increased the expression of Bax protein ($P<0.05$). **Conclusion:** EEPS is a promising therapeutic agent that may overcome chemo-resistance in cancer cells, likely through suppression of the AKT pathway and promotion of cancer cell apoptosis.

KEYWORDS AKT pathway, colorectal cancer, 5-fluorouracil resistance, *Patrinia scabiosaefolia*, Chinese medicine

Colorectal cancer (CRC) is the third most common cancer worldwide and causes over 600,000 deaths every year.[1] Despite recent advances in chemotherapy, 5-fluorouracil (5-FU)-based regimens remain to be the key therapeutic modality for advanced CRC patients.[2,3] However, long-term use of 5-FU usually results in the initiation and development of drug resistance, which is a major cause of cancer chemotherapy failure. Thus, there is an urgent need for development of novel therapeutic strategies and agents.[4-8]

Multiple mechanisms have been reported to be involved in the development of 5-FU resistance, including acquired anti-apoptotic capacity of cancer cells to protect tumors from chemotherapeutics-induced apoptosis.[9-13] Phosphatidylinositol 3-kinase (PI3K)-dependent AKT pathway is critical for cell growth and survival. The anti-apoptotic protein B-cell CLL/lymphoma (Bcl-2) and the pro-apoptotic protein Bcl-2 associated X (Bax) are important down-stream factors of PI3K/AKT signaling pathway. It has been reported that aberrant activation of AKT signaling and dysregulation of Bcl-2/Bax expression was commonly found in 5-FU-resistant cancer cells, which lead to 5-FU treatment resistance.[14-20] Therefore, modulation of AKT pathway and its downstream target gene expression may provide a promising strategy to overcome drug resistance.

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Recently, Chinese medicines (CMs) have gained great interest, due to their therapeutic efficacy and lower side effects compared with modern chemotherapeutic agents.[21,22] *Patrinia scabiosaefolia* (PS), a perennial plant native to Eastern Asia, has been clinically used to treat edema, appendicitis, endometritis, and other inflammatory diseases in China.[23-25] PS has also been used as a major component in several CM formulae for the treatment of breast carcinoma and myeloma.[26,27] Recent studies have also indicated that PS could inhibit CRC growth through induction of apoptosis, tumor angiogenesis and proliferation both *in vivo* and *in vitro*.[28-30] However, the mechanisms mediating its antitumor activities remain poorly understood. In the present study, we evaluated the effects of PS on chemo-resistance in a 5-FU-resistant human colorectal carcinoma cell line, and investigated the underlying mechanisms.

METHODS

Materials and Reagents

Roswell Park Memorial Institute (RPMI) 1640 medium, fetal bovine serum (FBS), penicillin-streptomycin, and trypsin-ethylenediaminetetraacetic acid (EDTA) were purchased from Thermo Fisher Scientific (Carlsbad, CA, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and crystal violet were obtained from Amresco (Solon, OH, USA). Hoechst 33258 staining was obtained from the Beyotime Institute of Biotechnology (Nanjing, China). p-AKT, AKT, Bcl-2, Bax, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primary antibodies, as well as horseradish peroxidase (HRP)-conjugated secondary antibodies were obtained from Cell Signaling Technology, Inc. (Beverly, MA, USA).

Preparation of Ethanol Extract from PS

An ethanol extract from PS (EEPS) was prepared as described previously.[30] Stock solutions of EEPS were prepared before use by dissolving EEPS powder in 50% dimethyl sulfoxide (DMSO) to a concentration of 250 mg/mL. Working concentrations of EEPS were made by diluting the stock solution in culture medium. The final concentration of DMSO in the medium for all cell experiments was < 0.5%.[28-30]

Cell Culture

The human colorectal carcinoma HCT-8 cell line and the 5-FU resistant HCT-8/5-FU cell line were obtained from Nanjing KeyGen Biotech. Co. Ltd. (No. KG333, Nanjing, China). Cells were grown in RPMI 1640 medium containing 10% (v/v) FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin in a 37 ℃ humidified incubator with 5% CO_2. The cells were sub-cultured at 80%–90% confluency. HCT-8/5-FU cells were cultured in RPMI-1640 containing 15 μg/mL of 5-FU.

Evaluation of Cell Viability by MTT Assay

The cell viability of HCT-8 and HCT-8/5-FU cells was determined by MTT colorimetric assay. Cells were seeded into 96-well plates at a density of 6×10^3 cells/well in 100 μL medium for 24 h, and then treated with various concentrations of 5-FU (100, 200, 400, 800 or 1600 μmol/L) or EEPS (0.25, 0.50, 1 or 2 mg/mL)[28-30] for 24 h, un-treated cells were set as control. MTT [100 μL; 0.5 mg/mL in phosphate-buffered saline (PBS)] was added to each well, and the samples were incubated for an additional 4 h at 37 ℃. The purple-blue MTT formazan precipitate was dissolved in 100 μL DMSO. The absorbance was measured at 570 nm using an ELISA reader (BioTek, Model ELX800, USA).

Observation of Cell Density

HCT-8/5-FU cells were seeded into 6-well plates at a density of 2.0×10^5 cells/ well in 2 mL medium. Cells were treated with 0.5, 1 or 2 mg/mL of EEPS for 24 h, untreated cells were set as control (0 mg/mL). Cell density was observed using a phase-contrast microscope (Leica, Mannheim, Germany). Images were captured at a magnification of ×200.

Cell Number Counting

HCT-8/5-FU cells were seeded into 6-well plates at a density of 2.0×10^5 cells /well in 2 mL culture medium. Cells were then treated with 0.5, 1 or 2 mg/mL of EEPS for 24 h, untreated cells were set as control (0 mg/mL). Cells were trypsinized, mixed with 0.4% Trypan blue solution (Sigma Aldrich, USA), and counted using a Countstar Automated Cell Counter (Inno-Alliance Biotech, USA) using the Trypan blue exclusion principle.

Colony Formation Assay

HCT-8/5-FU cells were seeded in 12-well plates at a density of 1×10^5 cells/well in 1 mL culture medium. Cells were treated with 0.5, 1 or 2 mg/mL of EEPS for 24 h, untreated cells were set as control

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(0 mg/mL). After treatment, the cells were collected and diluted in fresh medium in the absence of EEPS, and then reseeded in 12-well plates at a density of 500 cells/well. Following incubation for 10–12 days in a 37 ℃ humidified incubator with 5% CO_2, the formed colonies were fixed with 4% paraformaldehyde for 15 min, stained with 0.01% crystal violet solution for 10–15 min, washed with water, and air-dried.

Hoechst Staining Analysis

HCT-8/5-FU cells were seeded into 12-well plates at a density of 1×10^5 cells/ well in 1 mL culture medium. Cells were treated with 0.5, 1 or 2 mg/mL of EEPS for 24 h, untreated cells were set as control (0 mg/mL). After treatment, cell apoptosis was determined by Hoechst staining according to the manufacturer's instructions. Briefly, at the end of the experiment, cells were fixed with 4% polyoxymethylene and then incubated in Hoechst 33258 solution for 10–15 min in the dark. The stained images were recorded using a fluorescence microscope (Leica, Mannheim, Germany). Images were captured at a magnification of ×100.

Western-Blot Analysis

HCT-8 and HCT-8/5-FU cells were seeded into 25 cm^2 flasks at a density of 1.5×10^6 cells/flask in 5 mL culture medium. HCT-8/5-FU cells were treated with 0.5, 1 or 2 mg/mL of EEPS for 24 h, while untreated cells were set as control. After treatment, cells were lysed with RIPA buffer containing protease and phosphatase inhibitor cocktails. Total protein concentrations were determined by the bicinchoninic acid (BCA) assay. A total of 50 μg of proteins were resolved in 12% SDS-PAGE gels using 80 V for 2 h. The proteins were then electrophoretically transferred onto PVDF membranes. Membranes were blocked for 1 h with blocking solution at room temperature, washed in Tris buffered saline (TBS) with 0.25% Tween-20 (TBS-T), and exposed to primary antibodies against p-AKT, AKT, Bcl-2, and Bax (1:1,000) overnight at 4 ℃. GAPDH (1:2,000) was measured as an internal control for protein loading. After the membranes were washed in TBS-T, secondary HRP-conjugated antibodies (anti-rabbit or anti-mouse) were added at 1:2,000 dilutions for 1 h at room temperature. Membranes were then washed again in TBS-T followed by enhanced chemiluminescence detection. Band intensities were quantified relative to intensity of GAPDH using ImageLab software. The relatively reduced or increased level of target proteins were compared with the un-treated HCT-8/5-FU cells, which was set as 1.00.

Statistical Analysis

Data are presented as means ± standard deviation ($\bar{x} \pm s$) for the indicated number of independently performed experiments, and analyzed using the SPSS package for Windows (version 20.0). Statistical analyses of the data were performed with the one-way ANOVA and Student's *t*-test. Values of $P<0.05$ were considered as statistically significant.

RESULTS

EEPS Inhibits Growth of HCT-8/5-FU Cells

As shown in Figure 1A, the viability of parental HCT-8 cells was significantly decreased after treatment with 5-FU ($P<0.05$), whereas HCT-8/5-FU cell viability did not remarkably change after 5-FU treatment ($P>0.05$). Compared with un-treated HCT-8 cells or HCT-8/5-FU cells, administration of EEPS significantly reduced the viability of both HCT-8 and HCT-8/5-FU cells ($P<0.05$, Figure 1B).

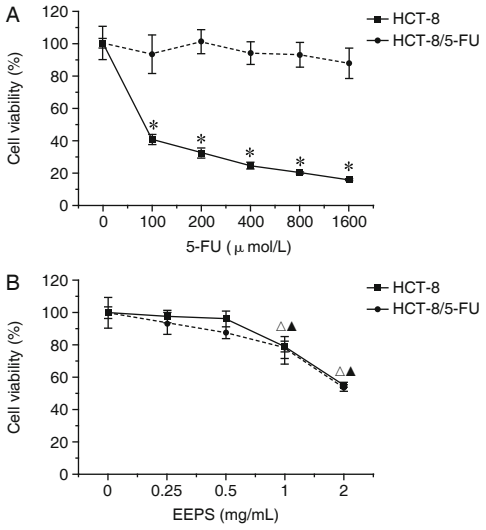

Figure 1. Effect of EEPS and 5-FU on Viability of HCT-8/5-FU and HCT-8 Cells

Notes: HCT-8/5-FU and HCT-8 cells were treated with 5-FU (A; 100, 200, 400, 800 or 1,600 μmol/L) or EEPS (B; 0.25, 0.5, 1 or 2 mg/mL) for 24 h. *$P<0.05$, *vs*. HCT-8 cells after treatment with the indicated concentrations of 5-FU; △$P<0.05$, *vs*. untreated HCT-8 cells; ▲$P<0.05$, *vs*. untreated HCT-8/5-FU cells

As shown in Figure 2, compared with untreated HCT-8/5-FU cells, EEPS treatment significantly decreased cell confluence and cell number ($P<0.05$). Furthermore, evaluation of the long term cellular proliferation capacity by colony formation assay indicated that EEPS treatment significantly reduced the number of colonies in HCT-8/ 5-FU cells compared with the untreated group ($P<0.05$, Figure 3).

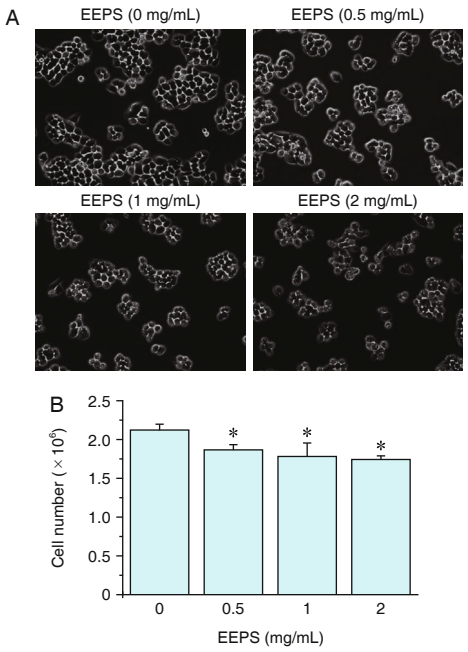

Figure 2. Effect of EEPS on Cell Growth of HCT-8/5-FU Cells

Notes: A: Cell density was observed using phase-contrast microscopy. The images were captured at a magnification of ×200. B: The cell number was counted by Trypan blue staining with a Countstar Automated Cell Counter. *P<0.05, *vs.* untreated HCT-8/5-FU cells

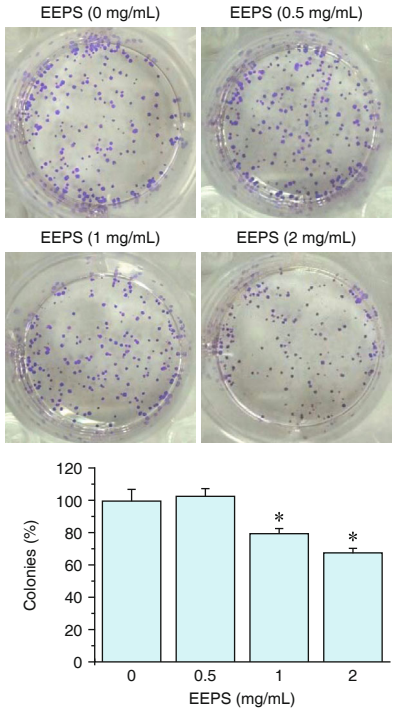

Figure 3. Effect of EEPS on Colony Formation Capacity of HCT-8/5-FU Cells

Notes: The number of colonies were counted and the data were normalized to the colonies of untreated cells. *P<0.05, *vs.* untreated HCT-8/5-FU cells

EEPS Induces Apoptosis of HCT-8/5-FU Cells

As shown in Figure 4, EEPS-treated cells showed condensed chromatin and fragmented nuclear morphology, typical apoptotic morphological features, whereas the nuclei of untreated cells were homogeneously stained and less intense.

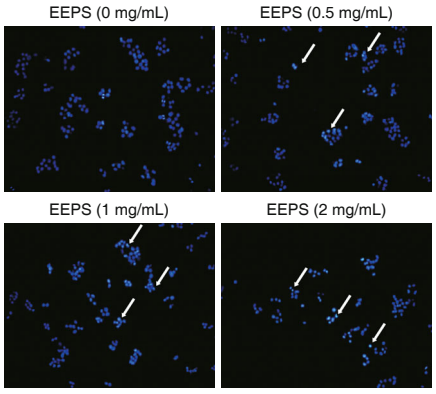

Figure 4. Effect of EEPS on Apoptosis in HCT-8/5-FU Cells (Hoechst staining, ×100)

Note: White arrows indicate the morphology of condensed chromatin or fragmented nuclear

EEPS Suppresses AKT Pathway Activation and Modulated Bcl-2/Bax Expression

As shown in Figure 5, EEPS treatment significantly decreased the phosphorylation level of AKT and Bcl-2 protein expression, whereas the protein expression of Bax significantly increased after EEPS treatment (P<0.05). The level of non-phosphorylated AKT remained unchanged (P>0.05).

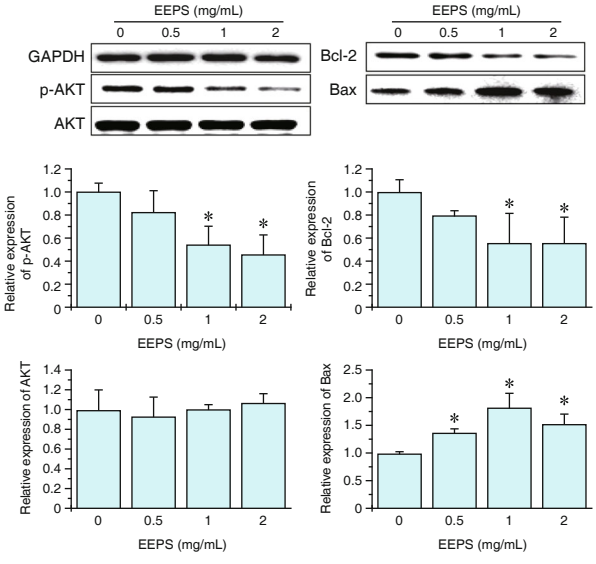

Figure 5. Effect of EEPS on Expression of p-AKT, AKT Bax and Bcl-2 in HCT-8/5-FU Cells by Western Blot

Note: *P<0.05, *vs.* untreated HCT-8/5-FU cells

DISCUSSION

As an important Chinese herb for heat-clearing and detoxifying, PS has been widely used as an

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anti-cancer agent in clinical cancer treatment.[27,31] It has been shown that PS could inhibit tumor growth both *in vitro* and *in vivo* through suppressing cancer cell proliferation and tumor angiogenesis, as well as promoting cell apoptosis.[27-30]

In the present study, we evaluated the effect of EEPS on drug resistance using a 5-FU-resistant human colorectal carcinoma cell line HCT-8/5-FU. Our findings indicated that HCT-8/5-FU cells were resistant to 5-FU treatment and sensitive to EEPS treatment, suggesting the potential effect of EEPS in overcoming 5-FU resistance in CRC. Moreover, since insensitivity to drug-induced apoptosis is one of the major mechanisms of 5-FU resistance,[8] we further assessed the effect of EEPS on cellular proliferation and apoptosis in HCT-8/5-FU cells. The findings indicated that EEPS treatment significantly suppressed cell growth by inhibiting cellular proliferation and inducing apoptosis. These studies confirmed that PS may possess the capacity to surmount chemo-resistance.

Increasing evidence has demonstrated that abnormal activation of AKT signaling pathway and the dysregulation of its down-stream mediators, including Bcl-2 and Bax, may contribute to the resistance of cancer cells to 5-FU treatment,[14-20] implicating that suppressing AKT pathway activation and modulating the expression of its associated down-stream genes could be an essential strategy for overcoming drug resistance.[16,17] Therefore, to further explore the underlying mechanisms of anti-cancer activity of EEPS, we investigated the effect of EEPS treatment on the activation of AKT signaling pathway and the expression of its down-stream genes. Our results indicated that EEPS treatment significantly decreased the phosphorylation of AKT and the expression of anti-apoptotic Bcl-2, but increased the expression of pro-apoptotic Bax, suggesting that suppression of AKT signaling pathway might be one of the mechanisms of EEPS in terms of its ability to overcome 5-FU resistance in CRC. Based on the present and our previous studies,[27-30] we proposed that PS results may exert its anti-tumor activities via multiple mechanisms, such as promoting apoptosis of cancer cells, inhibiting cell proliferation and tumor angiogenesis, as well as overcoming chemo-resistance.

The pathogenic mechanisms mediating CRC progression are complicated, involving many intracellular signaling transduction cascades, such as signal transducer and activator of transcription 3 (STAT3), extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), P38 mitogen-activated protein kinase (p38MAPK) and AKT pathways. Moreover, PS consists of numerous chemical compounds including triterpenoids, iridoids, flavonoids, alkaloids, polysaccharides and steroides. It is unclear which of these compounds possess anti-cancer activity. In addition, the effect of PS on the activation of other signaling remains unknown. These intriguing questions should be further addressed to better understand the molecular mechanisms underlying tumoricidal activities of PS, eventually helping to develop better multi-target drugs for cancer therapy.

Conflict of Interest

The authors declare no financial or commercial conflict of interest.

Author Contributions

Peng J designed the research and revised paper; Huang SZ, Liu WY, Huang Y and Liu LY performed the research; Shen AL analyzed the data; Huang SZ and Shen AL wrote the paper.

REFERENCES

1. Bonnet M, Buc E, Sauvanet P, Darcha C, Dubois D, Pereira B, et al. Colonization of the human gut by *E. coli* and colorectal cancer risk. Clin Cancer Res 2014;20:859-867.
2. Alvarez P, Marchal JA, Boulaiz H, Carrillo E, Velez C, Rodriguez-Serrano F, et al. 5-Fluorouracil derivatives: a patent review (2012–2014). Expert Opin Ther Pat 2015;25:107-123.
3. Gustin DM, Brenner DE. Chemoprevention of colon cancer: current status and future prospects. Cancer Metast Rev 2002;421:323-348.
4. Longley DB, Allen WL, Johnston PG. Drug resistance, predictive markers and pharmacogenomics in colorectal cancer. Biochim Biophys Acta 2006;1766:184-196.
5. De Rosa M, Pace U, Rega D, Costabile V, Duraturo F, Izzo P, et al. Genetics, diagnosis and management of colorectal cancer (Review). Oncol Rep 2015;34:1087-1096.
6. Gieseler F, Rudolph P, Kloeppel G, Foelsch UR. Resistance mechanisms of gastrointestinal cancers: why does conventional chemotherapy fail? Int J Colorectal Dis 2003;18:470-480.
7. Tentes IK, Schmidt WM, Krupitza G, Steger GG, Mikulits W, Kortsaris A, et al. Long term persistence of acquired resistance to 5 fluorouracil in the colon cancer cell line SW620. Exp Cell Res 2010;316:3172-3181.

8. Van Cutsem E, Costa F. Progress in the adjuvant treatment of colon cancer: has it influenced clinical practice? JAMA 2005;294:2758-2760.
9. Zhang N, Yin Y, Xu SJ, Chen WS. 5-fluorouracil: mechanisms of resistance and reversal strategies. Molecules 2008;13:1551-1569.
10. Mader RM, Müller M, Steger GG. Resistance to 5-fluorouracil. Gen Pharmacol 1998;31:661-666.
11. Li B, Li J, Xu WW, Guan XY, Qin YR, Zhang LY, et al. Suppression of esophageal tumor growth and chemo-resistance by directly targeting the PI3K/AKT pathway. Oncotarget 2014;5:11576-11587.
12. Yang SY, Miah A, Sales KM, Fuller B, Seifalian AM, Winslet M. Inhibition of the p38 MAPK pathway sensitives human colon cancer cells to 5-fluorouracil treatment. Int J Oncol 2011;38:1695-1702.
13. Urick ME, Chung EJ, Shield WP, Gerber N, White A, Sowers A, et al. Enhancement of 5-fluorouracil-induced *in vitro* and *in vivo* radiosensitization with MEK inhibition. Clin Cancer Res 2011;17:5038-5047.
14. You F, Aoki K, Ito Y, Nakashima S. AKT plays a pivotal role in the acquisition of resistance to 5-fluorouracil in human squamous carcinoma cells. Mol Med Rep 2009;2:609-613.
15. Xiao ZM, Wang XY, Wang AM. Periostin induces chemoresistance in colon cancer cells through activation of the PI3K/Akt/survivin pathway. Biotechnol Appl Biochem 2015;62:401-406.
16. Zhang B, Zhang B, Chen X, Bae S, Singh K, Washington MK, et al. Loss of Smad4 in colorectal cancer induces resistance to 5-fluorouracil through activating Akt pathway. Br J Cancer 2014;110:946-957.
17. Shin JY, Kim JO, Lee SK, Chae HS, Kang JH. LY294002 may overcome 5-FU resistance via down-regulation of activated p-AKT in *Epstein-Barr* virus-positive gastric cancer cells. BMC Cancer 2010;10:425.
18. Violette S, Poulain L, Dussaulx E, Pepin D, Faussat AM, Chambaz J, et al. Resistance of colon cancer cells to long-term 5-fluorouracil exposure is correlated to the relative level of Bcl-2 and Bcl-X(L) in addition to Bax and p53 status. Int J Cancer 2002;98:498-504.
19. Zhang L, Yu J, Park BH, Kinzler KW, Vogelstein B. Role of BAX in the apoptotic response to anticancer agents. Science 2000;290:989-992.
20. Hung JH, Teng YN, Wang LH, Su IJ, Wang CC, Huang W, et al. Induction of Bcl-2 expression by hepatitis B virus pre-S2 mutant large surface protein resistance to 5-fluorouracil treatment in Huh-7 cells. PLoS One 2011;6:e28977.
21. Qi F, Zhao L, Zhou A, Zhang B, Li A, Wang Z, et al. The advantages of using traditional Chinese medicine as an adjunctive therapy in the whole course of cancer treatment instead of only terminal stage of cancer. BioSci Trends 2015;9:16-34.
22. Yu C, Liu SL, Qi MH, Zou X, Wu J, Zhang J. Herbal medicine Guan Chang Fu Fang enhances 5-fluorouracil cytotoxicity and affects drug-associated genes in human colorectal carcinoma cells. Oncol Lett 2015;9:701-708.
23. Cho EJ, Shin JS, Noh YS, Cho YW, Hong SJ, Park JH, et al. Anti-inflammatory effects of methanol extract of *Patrinia scabiosaefolia* in mice with ulcerative colitis. J Ethnopharmacol 2011;136:428-435.
24. Gao L, Zhang L, Li N, Liu JY, Cai PL, Yang SL. New triterpenoid saponins from *Patrinia scabiosaefolia*. Carbohydr Res 2011;346:2881-2885.
25. Ju HK, Baek SH, An RB, Bae K, Son KH, Kim HP, et al. Inhibitory effects of nardostachin on nitric oxide, prostaglandin E2, and tumor necrosis factor-alpha production in lipopolysaccharide activated macrophages. Biol Pharm Bull 2003;26:1375-1378.
26. Chiu LC, Ho TS, Wong EY, Ooi VE. Ethyl acetate extract of *Patrinia scabiosaefolia* downregulates anti-apoptotic Bcl-2/Bcl-XL expression, and induces apoptosis in human breast carcinoma MCF-7 cells independent of caspase-9 activation. J Ethnopharmacol 2006;105:263-268.
27. Peng J, Chen YQ, Lin JM, Zhuang QC, Xu W, Hong ZF, et al. *Patrinia scabiosaefolia* extract suppresses proliferation and promotes apoptosis by inhibiting the STAT3 pathway in human multiple myeloma cells. Mol Med Rep 2011;4:313-318.
28. Zhang M, Sun G, Shen A, Liu L, Ding J, Peng J. *Patrinia scabiosaefolia* inhibits the proliferation of colorectal cancer *in vitro* and *in vivo* via G1/S cell cycle arrest. Oncol Rep 2015;33:856-860.
29. Chen L, Liu L, Ye L, Shen A, Chen Y, Sferra TJ, et al. *Patrinia scabiosaefolia* inhibits colorectal cancer growth through suppression of tumor angiogenesis. Oncol Rep 2013;30:1439-1443.
30. Liu L, Shen A, Chen Y, Wei L, Lin J, Sferra TJ, et al. *Patrinia scabiosaefolia* induces mitochondrial-dependent apoptosis in a mouse model of colorectal cancer. Oncol Rep 2013;30:897-903.
31. Liu ZH, Ma RJ, Yang L, Li JY, Hou B, Hu JM, et al. Triterpenoids and iridoids from *Patrinia scabiosaefolia*. Fitoterapia 2017;119:130-135.

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