編號：99001

Experimental Study on Effect of Compounds in Inhibiting HCT-116 Human Colon Cancer Cells: The Preliminary Results

中文摘要

目的: 分析數種藥物對於HCT-116人類大腸癌細胞株生長抑制的影響。

材料及方法: 一些具有抗發炎或抗氧化或具有自由基清除作用之藥物被選用，包括厚朴酚(honokiol),大黃素(emodin),硫辛酸(lipoic acid),黃連素(berberine),皂甘(diosgenin),白藜蘆醇(resveratrol),呂宋揪莢粉素(rottlerin), pinolo, 薑黃素(curcumin), 退黑激素(melatonin), 以及丁酸鈉(sodium butyrate)。把HCT-116細胞培養在無血清環境之培養盤，分別加入上述藥物，分別以不同藥物濃度之條件下，於百分之五的二氧化碳濃度處理二十四或四十八小時。接著，再以3-[4,5,-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay 分析HCT-116細胞生長受影響的情形。

結果:厚朴酚，大黃素，白藜蘆醇，呂宋揪莢粉素，薑黃素，這些藥物對於HCT-116細胞生長有明顯的抑制效果。經藥物處理四十八小時後，百分之五十的生長壓制劑量分別是厚朴酚爲18.5μM，大黃素爲17.3μM，白藜蘆醇爲25.3μM，呂宋揪莢粉素爲6.9μM，薑黃素爲22.3μM。

結論: 厚朴酚，大黃素，白藜蘆醇，呂宋揪莢粉素，薑黃素，這些藥物對於HCT-116細胞生長有明顯的抑制效果。對於已篩選出的這些藥物，應當設計進一步的實驗探討牽涉在其中的機轉。

關鍵字: 增生,大腸癌, MTT 分析

類別: 原著論文

ABSTRACT

OBJECTIVE: Several compounds were studied for their growth inhibitory effects on cultured HCT-116 human colon cancer cells.

MATERIALS AND METHODS: Compounds with anti-inflammation, anti-oxidation, or free-radical scavenging ability were used, including honokiol, emodin, lipoic acid, berberine, diosgenin, resveratrol, rottlerin, pinolo, curcumin, melatonin, and sodium butyrate. Cultured cells were incubated in a serum-free medium with various concentrations of different compounds for 24 and 48 hours in a 5% CO_2 incubator, after which the proliferation of HCT-116 cells was assessed by 3-[4,5,-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay and the significance of differences was analyzed by Student's t test.

RESULTS: Honokiol, emodin, resveratrol, rottlerin, and curcumin used in this study were more effective than lipoic acid, berberine, diosgenin, pinolo, melatonin, and sodium butyrate. The 50% suppression doses after 48-hour exposure were 18.5 μM for honokiol, 17.3 μM for emodin, 25.3 μM for resveratrol, 6.9 μM for rottlerin, and 22.3 μM for curcumin respectively.

CONCLUSION: Further investigations should be conducted to elucidate the mechanisms modulating anti-tumor effects on HCT-116 cells for honokiol, emodin, resveratrol, rottlerin, and curcumin.

KEYWORDS: proliferation, colon cancer, MTT assay

CATEGORY: original article

INTRODUCTION

Colon cancer is the second leading cause of death for cancer worldwide. In Taiwan, the Bureau of Health Promotion, Department of Health has proclaimed that about 19.5 people per 100 thousand die per year of colorectal cancer. Even though surgical resection is curative for early stage diseases, currently used chemotherapeutic agents for advanced stage colon cancer are palliative. Much research has been undertaken in the battle against colon cancer over the past few decades. However, limited advances have been obtained in spite of a substantial body of new discoveries about the molecular biology of cancer cells.[1] In addition, side effects of drugs are also potential obstacles to successful chemotherapies. Compounds with anti-inflammation, anti-oxidation, or free-radical scavenging ability have also been demonstrated to possess varied degrees of anti-tumor activity in the literature. One promising approach involves the administration of dietary phytochemicals that possess cancer-preventative activity but with greater safety, better availability, and minimal toxicity. Here we selected several candidate compounds with one or more of the aforementioned properties (anti-inflammation, anti-oxidation, or free-radical scavenging ability) for investigating the *in-vitro* anti-proliferative activity against the HCT-116 cancer cell line in culture by 3-[4,5,-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay.

Berberine is found in plants such as *Berberis*, *Hydrastis canadensis,* and *Coptis chinensis*, usually in the roots, rhizomes, stems, and bark. The traditional clinical applications of berberine include anti-infection[2] and diabetic control.[3] In addition, berberine has been shown to suppress the growth of a wide variety of tumor cells including prostate cancers[4] and colon cancers.[5]

Diosgenin, a steroid sapogenin, is extracted from the tubers of Dioscorea wild yam. Diosgenin is a well-known precursor of various synthetic steroidal drugs.[6] Over the past decade, much research has been conducted to understand the role of diosgenin on human cancers and diosgenin has been found to have a role in multi-target based chemopreventive or therapeutic properties.[7]

Lipoic acid is a naturally-occurring co-factor present in many enzyme complexes regulating human metabolism. Lipoic acid has been demonstrated to have properties of anti-oxidant activity[8] and diabetic control.[9] In addition, because of its free-radical scavenging ability, lipoic acid has the potential to interfere with processes within malignant cells.[10]

Pinolo is a nonselective beta-adrenergic blocker, possessing partial beta-adrenergic receptor agonist activity. It also has membrane-stabilizing effects. Clinically, pinolo has been used in angina pectoris, hypertension, arrhythmias, and prophylasix of acute stress reactions. Several studies have also investigated its role on inhibition of tumor

growth.[11]

Melatonin is a natural human hormone, produced by the pineal gland. It is essential in the regulation of the circadian rhythms of several biological functions. Melatonin's biological effects are produced through activation of melatonin receptors or through its powerful antioxidant activities.[12] It has been demonstrated to have the properties of antioxidant activities[13] and prevention of ischemia brain damage.[14] A systematic review, involving 643 cancer patients, using melatonin found a reduced incidence of death.[15] Moreover, reduced melatonin level has been proposed as a likely carcinogenic factor in night workers.[16]

Sodium butyrate, a short-chain volatile fatty acid in a non-toxic short-chain fatty acid, is the product of large bowel microbial fermentation of dietary fiber in the colon. Numerous studies have demonstrated the anti-proliferative effect of sodium butyrate treatments in breast[17], prostate[18], and colon cancers.[19]

Curcumin is the chief ingredient in both traditional Chinese and Indian medicine and in Indian turmeric spice, which is a member of the ginger family (Zingiberaceae). Curcumin has been reported to have anti-inflammatory[20] and anti-oxidant [21] activities. It has been used for thousands of years by Asians in various clinical applications including cancer treatment.[22]

Resveratrol is a natural product highly enriched in grapes, red wine, and many

other food sources. It has been demonstrated to have anti-inflammatory [23]activities and cancer chemopreventive properties.[24]

Honokiol is a pure biphenolic compound, present in cones, barks, and leaves of Magnolia officinalis extracts, which is used in traditional Chinese medicine. Recent research demonstrated that honokiol has variable biological activities including anti-inflammatory[25] and anti-oxidant effects.[26]

Emodin (1,3,8-trihydroxy-6-methylanthaquinone) is the main component in the rhizome of *Rheum palmatum* L. (Polygonaceae). Emodin has been demonstrated to have anti-bacterial[27] and anti-tumor activities.[28]

Rottlerin, a compound from Indian tree, is a selective inhibitor of protein kinase C-delta (PKC-delta). The PKC family is a major group of intracellular phosphorylating enzymes which play a role in proliferation, differentiation, as well as tumor promotion and progression.

MATERIALS AND METHODS

Materials

The compounds (purity > 99%) were obtained from Alexis Biocompounds (San Diego, CA). Culture medium RPMI-1640, fetal bovine serum (FBS) and trypsin-ethylenediaminetetraacetic acid (EDTA) were purchased from Invitrogen (Carlsbad, CA).

Cell culture and treatment

HCT-116 cells are derived from a colon carcinoma [American Type Culture Collection (ATCC), Rockville, MD; ATCC # CCL247] and serve as a useful model for study. The culture medium used was RPMI 1640, containing 10% fetal bovine serum, 20 mmol/L HEPES buffer, and 100 μg/mL gentamicin. Cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. The candidate compounds were dissolved in dimethyl sulfoxide (DMSO) as a stock solution at 100 mmol/L and diluted with an FBS-free medium to achieve the designated concentrations. The same concentration of DMSO without any compounds added was used as a control.

MTT viability assay

Cell viability was assessed by MTT assay. HCT-116 cells cultured onto 24-well plates were treated with various compounds. After 24 hours or 48 hours incubation, the cells (10^4/well) in 24-well plates were washed twice with phosphate-buffered

saline (PBS), and MTT (100 μg/ 0.1 mL of PBS) was added to each well. The cells were incubated at 37°C for 4 hours. Culture medium was then replaced with an equal volume of DMSO to dissolve formazan crystals. The absorbance was measured at 550 nm by microplate reader (Bio-Tek, Winooski, VT). The cell proliferation inhibition rate was calculated as 1 - (average OD value of wells with administered drug/average OD value of control wells) × 100. All experiments were performed a minimum of 3 times and data was presented as the average value ± the standard error of the mean (SEM).

Statistical analysis

The values given are means ± S.E.M. The significance of difference between the experimental group and control was assessed by Student's *t* test. The difference is considered significant if the p value is < 0.05.

RESULT

To determine the optimal conditions for cytotoxicity in cultures of HCT-116 colon cancer cells, a variety of compounds were used in different concentrations for the indicated time periods. These compounds shown in Figure 1 include honokiol, emodin, lipoic acid, berberine, diosgenin, resveratrol, rottlerin, pinolo, curcumin, melatonin, and sodium butyrate. Cell viability at each time point was then assessed by MTT assay.

The HCT-116 human colon cancer cells were treated with various compounds at different concentrations in 10% FBS for 48 hours. Incubation with lipoic acid, berberine, diosgenin, pinolo, melatonin, and sodium butyrate had no significant anti-tumor effect on HCT-116 cells, while honokiol, emodin, resveratrol, rotterlin, and curcumin caused a significant ($*P < 0.05$) reduction in total cell numbers (Figure 1). Subsequently, HCT-116 cells were treated with 3, 10, and 30 μM of the five potentially cytotoxic compounds (honokiol, emodin, resveratrol, rotterlin, and curcumin) for 24 and 48 hours in serum-free medium (Figure 2 and 3). The results showed that the inhibitory effect of honokiol, emodin, resveratrol, rottlerin, and curcumin on HCT-116 cell proliferation was both time-dependent and concentration-dependent (Figure 3).

The half maximal inhibitory concentration (IC50) is a measure of the effectiveness

of a compound in inhibiting biological or biochemical function. According to the criteria of the American National Cancer Institute, values of IC50 lower than 30μg/mL suggest a compound worth further purification.[29] After 24 hour treatment, the IC50 values were 18.0 μM for honokiol, 21.5 μM for emodin, infinity for resveratrol, 13.1 μM for rottlerin, and 20.8 μM for curcumin (Table 1). However, only the IC50 of rottlerin significantly decreased after 48 hour treatment, while the IC50 values of honokiol, emodin, resveratrol, and curcumin had no significant changes (Table 1).

DISCUSSION

In Cragg's study [30], over 50% of the drugs in clinical trials for anticancer activity were isolated from natural sources or were related to them. Recently the search for natural compounds with active anti-tumor properties has been a popular research topic. In this study, the cytotoxicities of the ten candidate compounds were tested against the human colon carcinoma (HCT-116) cell line, using the thiazolyl blue test (MTT) assay. Honokiol, emodin, resveratrol, rottlerin, and curcumin significantly inhibited the growth of the HCT-116 cell line in a concentration- and time-dependent manner. However, the anti-tumor activity of lipoic acid, berberine, diosgenin, pinolo, melatonin or sodium butyrate on HCT-116 cells was not significant in the present study.

Extensive research over previous decades demonstrated that curcumin has potent cancer-killing activity *in vitro* against various types of cancers including colon, prostate, and breast.[31] Mahmoud et al proved that dietary curcumin could suppress intestinal carcinogenesis in a mouse model of familial adenomatous polyposis.[32] Ushida et al confirmed that curcumin could prevent esophageal carcinogenesis in mice.[33] Huang et al demonstrated that curcumin could reduce the incidence lymphomas and leukemias in rats.[34] Our results demonstrated that proliferation of HCT-116 cells in the presence of curcumin was inhibited in a concentration- and

time-dependent manner. On the other hand, we also demonstrated that 24 hour treatment was optimal for curcumin-induced cytotoxicity in cultures of HCT-116 colon cancer cells, because the IC50 value had no significant change after 48 hour treatment. Our results are consistent with other research that curcumin is cytotoxic for HCT-116 human colon cancer cell line.[35, 36] Moreover, curcumin is also cytotoxic against many other types of cancer cells.[37-39] Taken together, this suggests that curcumin may have broad applications in cancer chemoprevention, although we did not determine whether curcumin was selectively cytotoxic for neoplastic cells, Watson et al demonstrated that the viability of normal human dermal fibroblasts was not altered following 72 hour exposure to lower concentrations (10 and 20 μM) of curcumin.[40] This result is also consistent with Chen's research.[41]

Resveratrol has been shown to have growth-inhibitory activity in several human cancer cell lines including glioma [42], colorectal cancer [43], and epidermoid carcinoma. [44] In this study, we found that a 24 hour resveratrol exposure did not significantly alter the number of viable cells, suggesting that the anti-proliferative effect was not associated with obvious cell death at this exposure time. However, a 48 hour exposure to 30 μM resveratrol resulted in a significant (**$P < 0.01$) decrease in cell viability. This result might be indicative for delayed apoptotic death in HCT-116 cells exposure to resveratrol. No previous investigations reported any resveratrol-induced HCT-116

cancer cell death. However, in Wolter's study[45] about resveratrol-induced Caco-2 human colon cancer cell death, the growth inhibitory results were similar to ours.

Honokiol also exhibited apoptosis induction and growth inhibition in some studies including lung[46], prostate[47], and colon cancer cells.[48] In this study, we demonstrated that proliferation of HCT-116 cells in the presence of honokiol was inhibited in a concentration- and time-dependent manner with an IC50 value of 18.5 μM (48-hour treatment) and 18.0 μM (24-hour treatment) in serum-free condition. The sole research investigating the anti-tumor effect of honokiol on human colon cancer cell (HCT-116) also demonstrated similar results with an IC50 value of 23μM in 10% FBS condition.[48] The different IC50 values may be due to the presence or absence of serum in medium. Recent study [49] also elucidated that the underlying mechanism is through blocking of Nuclear Factor-kappa B.

A number of research has demonstrated the growth inhibitory effect of emodin on cancer cells such as ovarian cancers,[50] colon cancers,[51] and breast cancers.[52] Here, proliferation of HCT-116 cells in the presence of emodin was inhibited in a concentration- and time-dependent manner with an IC50 value of 17.3 μM (48 hour treatment) and 21.5 μM (24 hour treatment) in serum-free condition. No previous investigations reported any emodin-induced HCT-116 cancer cell deaths. However, several articles also demonstrated similar anti-tumor effects on human colon cancer

cells other than HCT-116.[51, 53]

Some research has already demonstrated the inhibitory effects of rottlerin on cancer cells.[54, 55] In the present study, we have shown that rottlerin inhibits HCT-116 cell proliferation in a concentration-dependent and time-dependent manner both in 24 hour and 48 hour exposures. Although there has been no research about the anti-tumor effects of rottlerin on the HCT-116 cell line, a few articles also demonstrated the significant growth inhibition of rottlerin on various human colon cancer cell lines.[56, 57]

Progression of colon cancer is associated with activation of multiple signaling pathways. Cell death is an essential event in normal life and in the pathophysiological processes which lead to diseases. A pattern of cell death has emerged where each of several distinct organelles (plasma membrane, mitochondrion, nucleus, endoplasmic reticulum, lysosome) give rise to signals which induce cell death. Endoplasmic reticulum (ER) is a central organelle engaged in protein production, folding and maturation. Various toxic insults can perturb ER function and result in ER stress.[58] There is increasing evidence that ER stress plays an important role in the regulation of cell death.[59] Until now, only one study reported about ER stress-mediated HCT-116 cell death in the literature.[60] In the future, treatment of HCT-116 human colon carcinoma cells with these five potential compounds (honokiol, emodin, resveratrol, rottlerin, and curcumin) will be conducted to elucidate the induction of signature ER

stress markers: phosphorylation of eukaryotic initiation factor-2 α (eIF-2 α), up-regulation of glucose-regulated protein (GRP)-78, and up-regulation of CCAAT / enhancer-binding protein homologous protein (CHOP).

CONCLUSION

Here, we only presented the preliminary anti-tumor results of selected candidate compounds on HCT-116 human colon cancer cells. Further investigations should be conducted to elucidate the underlying mechanisms modulating anti-tumor effects on HCT-116 cells for honokiol, emodin, resveratrol, rottlerin, and curcumin.

FIGURES AND TABLES

Figure 1

HCT-116 cells were treated with various concentrations (3, 10, 30μM) of different compounds, in complete medium with 10% fetal bovine serum (FBS), for 48 hours as described in the text. The cell viability of cells was counted by MTT assay. The results represented the mean ± S.D. of three independent experiments and the significant difference was established at *p<0.05, **p<0.01, ***p<0.001 compared with the control group. DMSO served as the solvent control. Each column showed growth inhibition after normalizing untreated cells to100%.

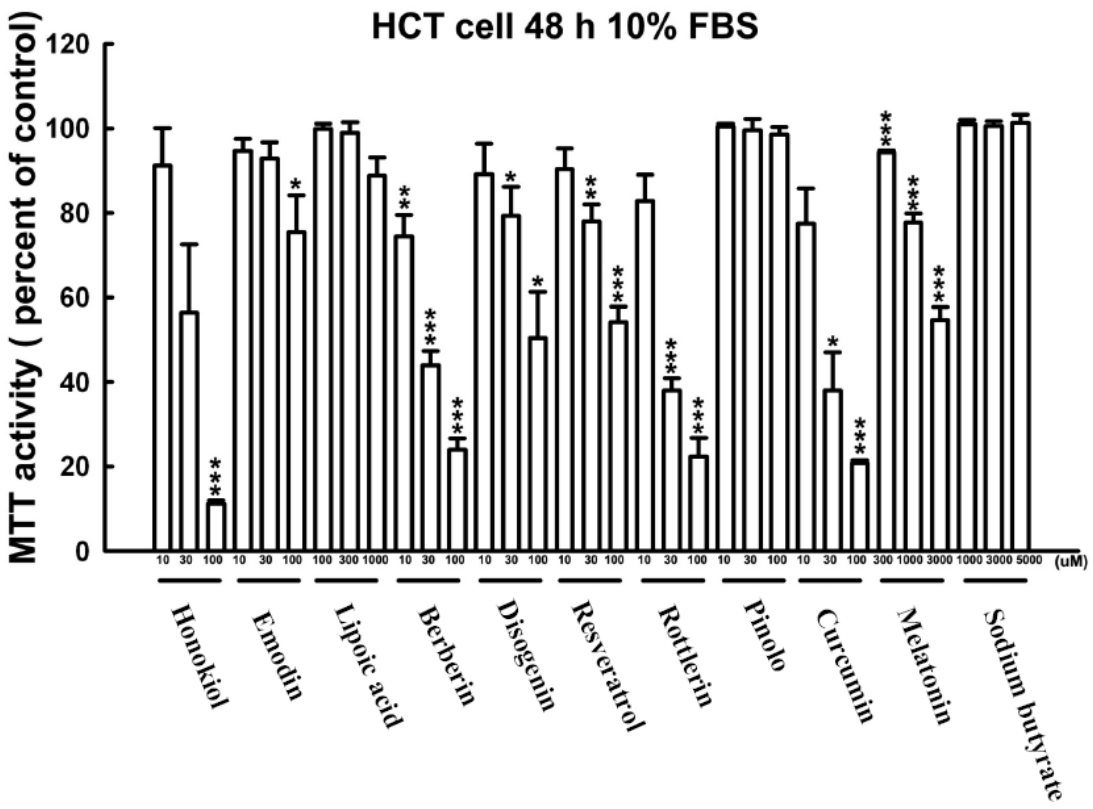
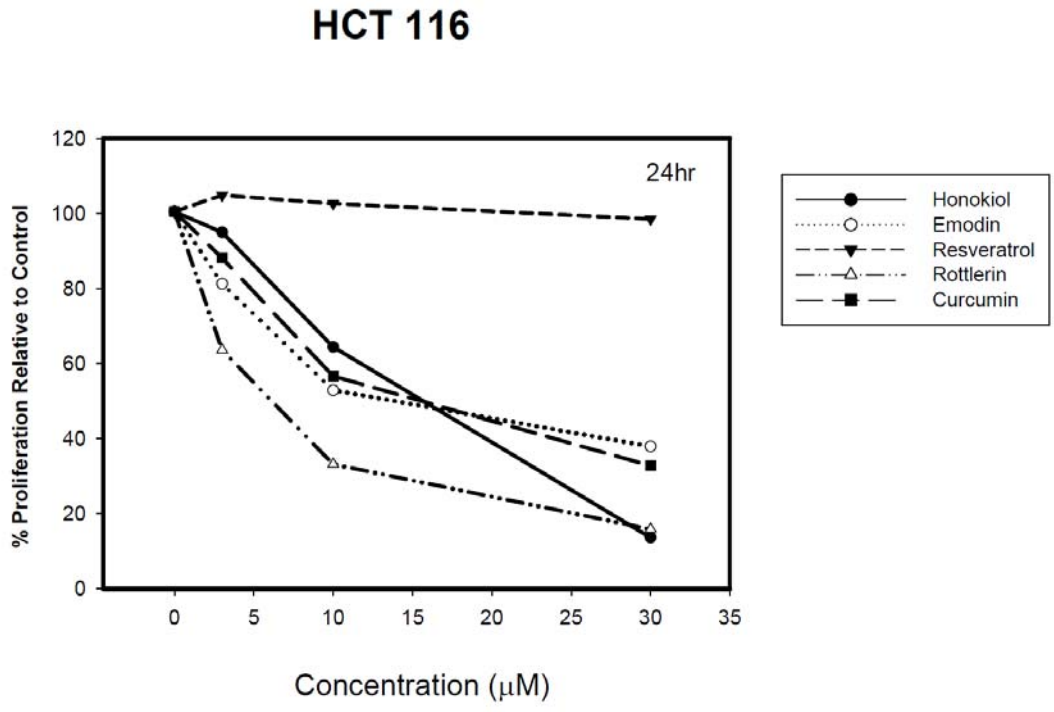


Figure 2

HCT-116 cells were treated with various concentrations (3, 10, 30μM) of different compounds (honokiol, emodin, resveratrol, rottlerin, and curcumin), in serum-free medium, for 24 and 48 hours respectively, depicted in the linear regression mode. **A**. Viable cells were detected by proliferation assay using MTT assay after 24-hour exposure. **B**. Viable cells were detected by proliferation assay using MTT assay after 48-hour exposure.

A.



B.

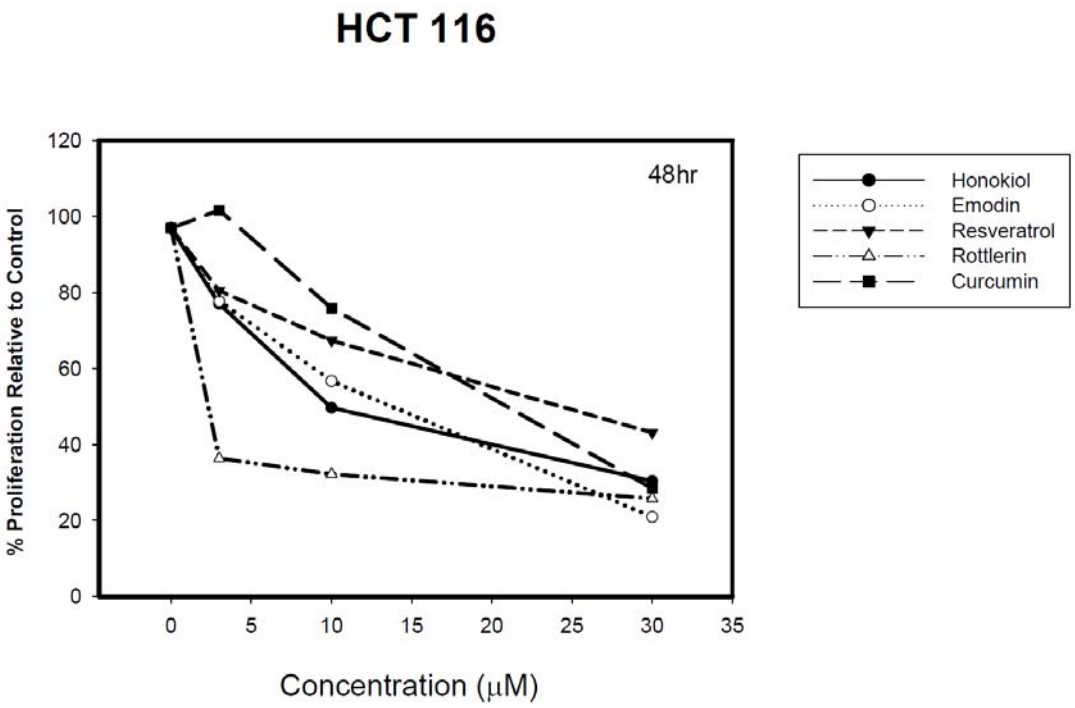

HCT 116
48hr
% Proliferation Relative to Control
120
100
80
60
40
20
0
0
5
10
15
20
25
30
35
Concentration (μM)
Honokiol
Emodin
Resveratrol
Rottlerin
Curcumin

Figure 3

Merged from Figures 2A and 2B. HCT-116 cells were treated with various concentrations (3, 10, 30μM) of different compounds (honokiol, emodin, resveratrol, rottlerin, and curcumin) in serum-free medium, for 24 (black columns) and 48 hours (white columns) respectively, measured by MTT viability assay. Each column showed growth inhibition after normalizing untreated cells to100%. The results represented the mean ± S.D. of thouree independent experiments and the significant difference was established at *p<0.05, **p<0.01, ***p<0.001 compared with the control group for the indicated time. DMSO served as the solvent control.

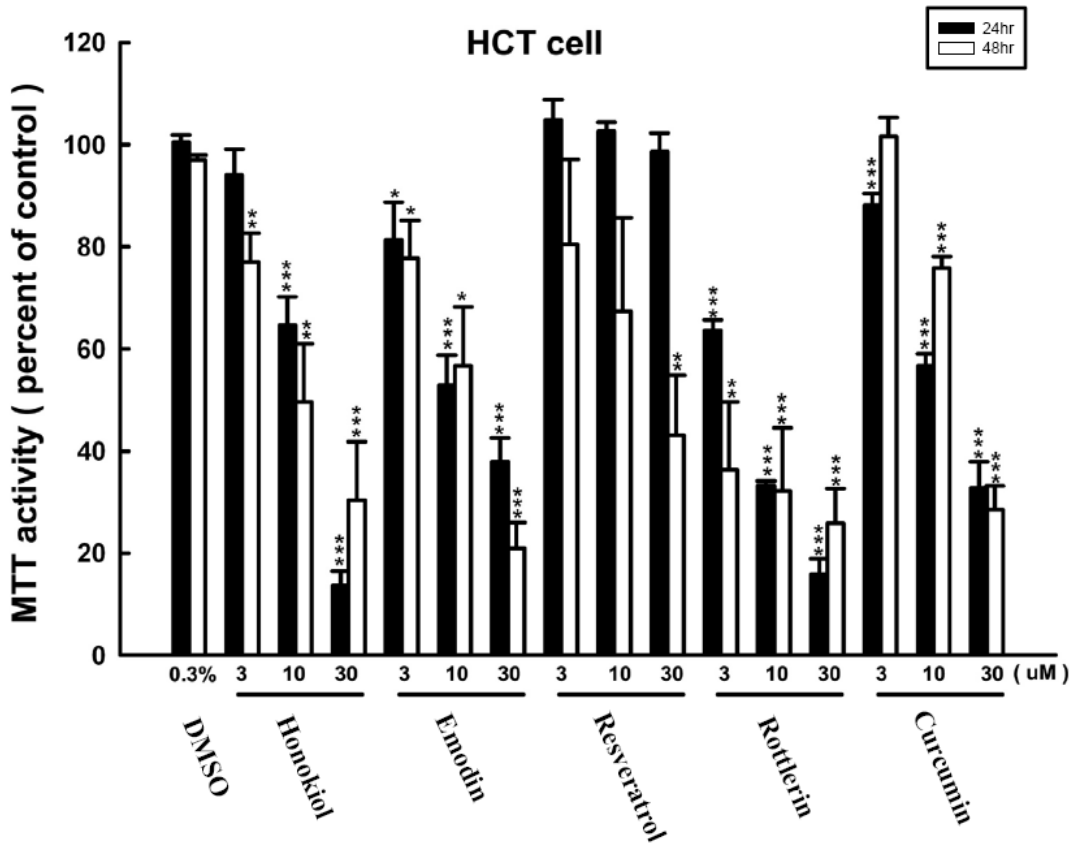


Table 1

Effective doses (μM) of various compounds (honokiol, emodin, resveratrol, rottlerin, and curcumin) that inhibited cell growth to 50% of control (IC50) in different treatment time period (24 hours and 48 hours).

	Incubation time	
Compounds	**24 hrs**	**48 hrs**
Honokiol	18.0±5 μM	18.5±11 μM
Emodin	21.5±5 μM	17.3±11 μM
Resveratrol	infinity	25.3±11 μM
Rottlerin	13.1±1 μM	6.9±14 μM
Curcumin	20.8±2 μM	22.3±2 μM

$2x10^4$/ml cells were incubated in the presence of various compounds at different concentrations (0, 3, 10, 30 μM) for 24 and 48 hours, respectively. Anti-tumor effect on the proliferation was measured with MTT test. IC50 was calculated with the Sigmaplot software.

LEGENDS

Figure 1

HCT-116 cells were treated with various concentrations (3, 10, 30μM) of different compounds, in complete medium with 10% fetal bovine serum (FBS), for 48 hours as described in the text. The cell viability of cells was counted by MTT assay. The results represented the mean ± S.D. of three independent experiments and the significant difference was established at *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared with the control group. DMSO served as the solvent control. Each column showed growth inhibition after normalizing untreated cells to100%.

Figure 2

HCT-116 cells were treated with various concentrations (3, 10, 30μM) of different compounds (honokiol, emodin, resveratrol, rottlerin, and curcumin), in serum-free medium, for 24 and 48 hours respectively, depicted in the linear regression mode. **A**. Viable cells were detected by proliferation assay using MTT assay after 24-hour exposure. **B**. Viable cells were detected by proliferation assay using MTT assay after 48-hour exposure.

Figure 3

Merged from Figures 2A and 2B. HCT-116 cells were treated with various concentrations (3, 10, 30μM) of different compounds (honokiol, emodin, resveratrol, rottlerin, and curcumin) in serum-free medium, for 24 (black columns) and 48 hours (white columns) respectively, measured by MTT viability assay. Each column showed growth inhibition after normalizing untreated cells to100%. The results represented the mean ± S.D. of thouree independent experiments and the significant difference was established at *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared with the control group for the indicated time. DMSO served as the solvent control.

Table 1

Effective doses (μM) of various compounds (honokiol, emodin, resveratrol, rottlerin, and curcumin) that inhibited cell growth to 50% of control (IC50) in different treatment time period (24 hours and 48 hours).

REFERENCES

1. Sporn MB. The war on cancer.[see comment]. *Lancet* 1996; 347: 1377-81.

2. Anonymous. Berberine. *Alternative Medicine Review* 2000; 5: 175-7.

3. Zhang Y, Li X, Zou D, Liu W, Yang J, Zhu N, Huo L, Wang M, Hong J, Wu P, Ren G, Ning G, Zhang Y, Li X, Zou D, Liu W, Yang J, Zhu N, Huo L, Wang M, Hong J, Wu P, Ren G, Ning G. Treatment of type 2 diabetes and dyslipidemia with the natural plant alkaloid berberine. *Journal of Clinical Endocrinology & Metabolism* 2008; 93: 2559-65.

4. Choi MS, Oh JH, Kim SM, Jung HY, Yoo HS, Lee YM, Moon DC, Han SB, Hong JT, Choi MS, Oh JH, Kim SM, Jung HY, Yoo HS, Lee YM, Moon DC, Han SB, Hong JT. Berberine inhibits p53-dependent cell growth through induction of apoptosis of prostate cancer cells. *International Journal of Oncology* 2009; 34: 1221-30.

5. Fukuda K, Hibiya Y, Mutoh M, Koshiji M, Akao S, Fujiwara H. Inhibition by berberine of cyclooxygenase-2 transcriptional activity in human colon cancer cells. *Journal of Ethnopharmacology* 1999; 66: 227-33.

6. Djerassi C. Steroid research at Syntex: "the pill" and cortisone.[see comment]. *Steroids* 1992; 57: 631-41.

7. Raju J, Mehta R, Raju J, Mehta R. Cancer chemopreventive and therapeutic

effects of diosgenin, a food saponin. *Nutrition & Cancer* 2009; 61: 27-35.

8. Biewenga GP, Haenen GR, Bast A. The pharmacology of the antioxidant lipoic acid. *General Pharmacology* 1997; 29: 315-31.

9. Packer L, Kraemer K, Rimbach G. Molecular aspects of lipoic acid in the prevention of diabetes complications. *Nutrition* 2001; 17: 888-95.

10. Novotny L, Rauko P, Cojocel C. alpha-Lipoic acid: the potential for use in cancer therapy. *Neoplasma* 2008; 55: 81-6.

11. Abdul M, Anezinis PE, Logothetis CJ, Hoosein NM. Growth inhibition of human prostatic carcinoma cell lines by serotonin antagonists. *Anticancer Research* 1994; 14: 1215-20.

12. Hardeland R, Hardeland R. Antioxidative protection by melatonin: multiplicity of mechanisms from radical detoxification to radical avoidance. *Endocrine* 2005; 27: 119-30.

13. Tan DX, Manchester LC, Reiter RJ, Qi WB, Karbownik M, Calvo JR. Significance of melatonin in antioxidative defense system: reactions and products. *Biological Signals & Receptors* 2000; 9: 137-59.

14. Lee MY, Kuan YH, Chen HY, Chen TY, Chen ST, Huang CC, Yang IP, Hsu YS, Wu TS, Lee EJ, Lee M-Y, Kuan Y-H, Chen H-Y, Chen T-Y, Chen S-T, Huang C-C, Yang IP, Hsu Y-S, Wu T-S, Lee EJ. Intravenous administration of melatonin reduces

the intracerebral cellular inflammatory response following transient focal cerebral ischemia in rats. *Journal of Pineal Research* 2007; 42: 297-309.

15. Mills E, Wu P, Seely D, Guyatt G, Mills E, Wu P, Seely D, Guyatt G. Melatonin in the treatment of cancer: a systematic review of randomized controlled trials and meta-analysis. *Journal of Pineal Research* 2005; 39: 360-6.

16. Schernhammer ES, Rosner B, Willett WC, Laden F, Colditz GA, Hankinson SE, Schernhammer ES, Rosner B, Willett WC, Laden F, Colditz GA, Hankinson SE. Epidemiology of urinary melatonin in women and its relation to other hormones and night work.[see comment]. *Cancer Epidemiology, Biomarkers & Prevention* 2004; 13: 936-43.

17. Chopin V, Slomianny C, Hondermarck H, Le Bourhis X. Synergistic induction of apoptosis in breast cancer cells by cotreatment with butyrate and TNF-alpha, TRAIL, or anti-Fas agonist antibody involves enhancement of death receptors' signaling and requires P21(waf1). *Experimental Cell Research* 2004; 298: 560-73.

18. Kuefer R, Genze F, Zugmaier W, Hautmann RE, Rinnab L, Gschwend JE, Angelmeier M, Estrada A, Buechele B, Kuefer R, Genze F, Zugmaier W, Hautmann RE, Rinnab L, Gschwend JE, Angelmeier M, Estrada A, Buechele B. Antagonistic effects of sodium butyrate and N-(4-hydroxyphenyl)-retinamide on prostate cancer. *Neoplasia (New York)* 2007; 9: 246-53.

19. Nguyen KA, Cao Y, Chen JR, Townsend CM, Jr., Ko TC, Nguyen KA, Cao Y, Chen JR, Townsend CM, Jr., Ko TC. Dietary fiber enhances a tumor suppressor signaling pathway in the gut. *Annals of Surgery* 2006; 243: 619-25; discussion 25-7.

20. Shishodia S, Sethi G, Aggarwal BB, Shishodia S, Sethi G, Aggarwal BB. Curcumin: getting back to the roots. *Annals of the New York Academy of Sciences* 2005; 1056: 206-17.

21. Huang TS, Lee SC, Lin JK. Suppression of c-Jun/AP-1 activation by an inhibitor of tumor promotion in mouse fibroblast cells. *Proceedings of the National Academy of Sciences of the United States of America* 1991; 88: 5292-6.

22. Balasubramanyam M, Koteswari AA, Kumar RS, Monickaraj SF, Maheswari JU, Mohan V. Curcumin-induced inhibition of cellular reactive oxygen species generation: novel therapeutic implications. *J Biosci* 2003; 28: 715-21.

23. Lu CC, Lai HC, Hsieh SC, Chen JK, Lu C-C, Lai H-C, Hsieh S-C, Chen J-K. Resveratrol ameliorates Serratia marcescens-induced acute pneumonia in rats. *Journal of Leukocyte Biology* 2008; 83: 1028-37.

24. Aggarwal BB, Bhardwaj A, Aggarwal RS, Seeram NP, Shishodia S, Takada Y, Aggarwal BB, Bhardwaj A, Aggarwal RS, Seeram NP, Shishodia S, Takada Y. Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. *Anticancer Research* 2004; 24: 2783-840.

25. Lin YR, Chen HH, Ko CH, Chan MH, Lin Y-R, Chen H-H, Ko C-H, Chan M-H. Effects of honokiol and magnolol on acute and inflammatory pain models in mice. *Life Sciences* 2007; 81: 1071-8.

26. Gu LH, Wu T, Zhang ZJ, Chou GX, Wang ZT, Gu L-H, Wu T, Zhang Z-J, Chou G-X, Wang Z-T. [Evaluation of antioxidant activity of Radix Linderae and other two Chinese drugs using TLC-bioautography]. *Yao Hsueh Hsueh Pao - Acta Pharmaceutica Sinica* 2006; 41: 956-62.

27. Kim JR, Oh DR, Cha MH, Pyo BS, Rhee JH, Choy HE, Oh WK, Kim YR, Kim JR, Oh D-R, Cha MH, Pyo BS, Rhee JH, Choy HE, Oh WK, Kim YR. Protective effect of polygoni cuspidati radix and emodin on Vibrio vulnificus cytotoxicity and infection. *Journal of Microbiology* 2008; 46: 737-43.

28. Koyama M, Kelly TR, Watanabe KA. Novel type of potential anticancer agents derived from chrysophanol and emodin. Some structure-activity relationship studies. *Journal of Medicinal Chemistry* 1988; 31: 283-4.

29. Suffness, Pezzuto. Assays related to cancer drug discovery. *Methods in plant biochemistry: assays for bioactivity* 1990; 6 Academic press: 71-133.

30. Cragg GM, Newman DJ. Antineoplastic agents from natural sources: achievements and future directions. *Expert Opinion on Investigational Drugs* 2000; 9: 2783-97.

31. Sharma RA, Gescher AJ, Steward WP. Curcumin: the story so far. *Eur J Cancer* 2005; 41: 1955-68.

32. Mahmoud NN, Carothers AM, Grunberger D, Bilinski RT, Churchill MR, Martucci C, Newmark HL, Bertagnolli MM. Plant phenolics decrease intestinal tumors in an animal model of familial adenomatous polyposis. *Carcinogenesis* 2000; 21: 921-7.

33. Ushida J, Sugie S, Kawabata K, Pham QV, Tanaka T, Fujii K, Takeuchi H, Ito Y, Mori H. Chemopreventive effect of curcumin on N-nitrosomethylbenzylamine-induced esophageal carcinogenesis in rats. *Jpn J Cancer Res* 2000; 91: 893-8.

34. Huang MT, Lou YR, Xie JG, Ma W, Lu YP, Yen P, Zhu BT, Newmark H, Ho CT. Effect of dietary curcumin and dibenzoylmethane on formation of 7,12-dimethylbenz[a]anthracene-induced mammary tumors and lymphomas/leukemias in Sencar mice. *Carcinogenesis* 1998; 19: 1697-700.

35. Subramaniam D, May R, Sureban SM, Lee KB, George R, Kuppusamy P, Ramanujam RP, Hideg K, Dieckgraefe BK, Houchen CW, Anant S. Diphenyl difluoroketone: a curcumin derivative with potent in vivo anticancer activity. *Cancer Res* 2008; 68: 1962-9.

36. Watson JL, Hill R, Lee PW, Giacomantonio CA, Hoskin DW. Curcumin induces

apoptosis in HCT-116 human colon cancer cells in a p21-independent manner. *Exp Mol Pathol* 2008; 84: 230-3.

37. O'Sullivan-Coyne G, O'Sullivan GC, O'Donovan TR, Piwocka K, McKenna SL. Curcumin induces apoptosis-independent death in oesophageal cancer cells. *Br J Cancer* 2009; 101: 1585-95.

38. Rowe DL, Ozbay T, O'Regan RM, Nahta R. Modulation of the BRCA1 protein and induction of apoptosis in triple negative breast cancer cell lines by the polyphenolic compound curcumin. *Breast Cancer* 2009; 3: 61-75.

39. Sahu RP, Batra S, Srivastava SK. Activation of ATM/Chk1 by curcumin causes cell cycle arrest and apoptosis in human pancreatic cancer cells. *Br J Cancer* 2009; 100: 1425-33.

40. Watson JL, Hill R, Lee PW, Giacomantonio CA, Hoskin DW, Watson JL, Hill R, Lee PW, Giacomantonio CA, Hoskin DW. Curcumin induces apoptosis in HCT-116 human colon cancer cells in a p21-independent manner. *Experimental & Molecular Pathology* 2008; 84: 230-3.

41. Chen HW, Huang HC. Effect of curcumin on cell cycle progression and apoptosis in vascular smooth muscle cells. *Br J Pharmacol* 1998; 124: 1029-40.

42. Jiang H, Zhang L, Kuo J, Kuo K, Gautam SC, Groc L, Rodriguez AI, Koubi D, Hunter TJ, Corcoran GB, Seidman MD, Levine RA, Jiang H, Zhang L, Kuo J, Kuo K,

Gautam SC, Groc L, Rodriguez AI, Koubi D, Hunter TJ, Corcoran GB, Seidman MD, Levine RA. Resveratrol-induced apoptotic death in human U251 glioma cells. *Molecular Cancer Therapeutics* 2005; 4: 554-61.

43. Schneider Y, Vincent F, Duranton B, Badolo L, Gosse F, Bergmann C, Seiler N, Raul F. Anti-proliferative effect of resveratrol, a natural component of grapes and wine, on human colonic cancer cells. *Cancer Letters* 2000; 158: 85-91.

44. Ahmad N, Adhami VM, Afaq F, Feyes DK, Mukhtar H. Resveratrol causes WAF-1/p21-mediated G(1)-phase arrest of cell cycle and induction of apoptosis in human epidermoid carcinoma A431 cells. *Clinical Cancer Research* 2001; 7: 1466-73.

45. Wolter F, Akoglu B, Clausnitzer A, Stein J. Downregulation of the cyclin D1/Cdk4 complex occurs during resveratrol-induced cell cycle arrest in colon cancer cell lines. *J Nutr* 2001; 131: 2197-203.

46. Yang SE, Hsieh MT, Tsai TH, Hsu SL, Yang SE, Hsieh MT, Tsai TH, Hsu SL. Down-modulation of Bcl-XL, release of cytochrome c and sequential activation of caspases during honokiol-induced apoptosis in human squamous lung cancer CH27 cells. *Biochemical Pharmacology* 2002; 63: 1641-51.

47. Shigemura K, Arbiser JL, Sun SY, Zayzafoon M, Johnstone PA, Fujisawa M, Gotoh A, Weksler B, Zhau HE, Chung LW, Shigemura K, Arbiser JL, Sun S-Y,

Zayzafoon M, Johnstone PAS, Fujisawa M, Gotoh A, Weksler B, Zhau HE, Chung LWK. Honokiol, a natural plant product, inhibits the bone metastatic growth of human prostate cancer cells. *Cancer* 2007; 109: 1279-89.

48. Lee SY, Yuk DY, Song HS, Yoon DY, Jung JK, Moon DC, Lee BS, Hong JT. Growth inhibitory effects of obovatol through induction of apoptotic cell death in prostate and colon cancer by blocking of NF-kappa B. *European Journal of Pharmacology* 2008; 582: 17-25.

49. Lee SY, Yuk DY, Song HS, Yoon do Y, Jung JK, Moon DC, Lee BS, Hong JT, Lee SY, Yuk DY, Song HS, Yoon DY, Jung JK, Moon DC, Lee BS, Hong JT. Growth inhibitory effects of obovatol through induction of apoptotic cell death in prostate and colon cancer by blocking of NF-kappaB. *European Journal of Pharmacology* 2008; 582: 17-25.

50. Li J, Liu P, Mao H, Wanga A, Zhang X, Li J, Liu P, Mao H, Wanga A, Zhang X. Emodin sensitizes paclitaxel-resistant human ovarian cancer cells to paclitaxel-induced apoptosis in vitro. *Oncology Reports* 2009; 21: 1605-10.

51. Lu Y, Zhang J, Qian J, Lu Y, Zhang J, Qian J. The effect of emodin on VEGF receptors in human colon cancer cells. *Cancer Biotherapy & Radiopharmaceuticals* 2008; 23: 222-8.

52. Zhang L, Chang CJ, Bacus SS, Hung MC. Suppressed transformation and

induced differentiation of HER-2/neu-overexpressing breast cancer cells by emodin. *Cancer Research* 1995; 55: 3890-6.

53. Kamei H, Koide T, Kojima T, Hashimoto Y, Hasegawa M. Inhibition of cell growth in culture by quinones. *Cancer Biotherapy & Radiopharmaceuticals* 1998; 13: 185-8.

54. Won YK, Ong CN, Shen HM, Won Y-K, Ong C-N, Shen H-M. Parthenolide sensitizes ultraviolet (UV)-B-induced apoptosis via protein kinase C-dependent pathways. *Carcinogenesis* 2005; 26: 2149-56.

55. Williams SR, Son DS, Terranova PF, Williams SR, Son D-S, Terranova PF. Protein kinase C delta is activated in mouse ovarian surface epithelial cancer cells by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). *Toxicology* 2004; 195: 1-17.

56. Lim JH, Park JW, Kim SH, Choi YH, Choi KS, Kwon TK, Lim JH, Park J-W, Kim SH, Choi YH, Choi KS, Kwon TK. Rottlerin induces pro-apoptotic endoplasmic reticulum stress through the protein kinase C-delta-independent pathway in human colon cancer cells. *Apoptosis* 2008; 13: 1378-85.

57. Chen ZF, Fang JY, Weng YR, Sun DF, Wang X, Lu R, Chen Z-f, Fang J-y, Weng Y-r, Sun D-f, Wang X, Lu R. [The effect of PKC-delta inhibitor Rottlerin on human colon cancer cell line SW1116 and its mechanism]. *Chung-Hua Chung Liu Tsa Chih [Chinese Journal of Oncology]* 2006; 28: 564-7.

58. Yung HW, Korolchuk S, Tolkovsky AM, Charnock-Jones DS, Burton GJ. Endoplasmic reticulum stress exacerbates ischemia-reperfusion-induced apoptosis through attenuation of Akt protein synthesis in human choriocarcinoma cells. *FASEB J* 2007; 21: 872-84.

59. Moenner M, Pluquet O, Bouchecareilh M, Chevet E. Integrated endoplasmic reticulum stress responses in cancer. *Cancer Res* 2007; 67: 10631-4.

60. Payne CM, Crowley-Weber CL, Dvorak K, Bernstein C, Bernstein H, Holubec H, Crowley C, Garewal H. Mitochondrial perturbation attenuates bile acid-induced cytotoxicity. *Cell Biol Toxicol* 2005; 21: 215-31.