



The reversal effect of Ginsenoside Rh2 on drug resistance in human colorectal carcinoma cells and its mechanism

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Abstract

Recent studies hint that Ginsenoside is involved in cancer prevention and treatment. In this study, we investigated the effect of Ginsenoside Rh2 on drug resistance in human colorectal carcinoma (CRC) cells and its mechanism. The resistance reversion effect of Ginsenoside Rh2 in CRC cells was analyzed using CCK-8 assay. After treating with Ginsenoside Rh2, the cell cycle distribution and cellular apoptosis were analyzed by flow cytometry, cell migration was determined by transwell migration assay, the expression of drug-resistance genes and proteins were evaluated using quantitative real-time polymerase chain reaction (qRT-PCR) and Western blot, respectively. Ginsenoside Rh2 could enhance the cytotoxicity of 5-FU in drug-resistant CRC cells (LoVo/5-FU and HCT-8/5-FU). Treatment with Ginsenoside Rh2 could result in an increase of cell numbers in G0/G1 phase accompanied with a decrease in S-phase, and induced cellular apoptosis in drug-resistant CRC cells. In addition, the migration process and EMT process of drug-resistant CRC cells were suppressed by treatment of Ginsenoside Rh2. Compared to control group, expression of drug-resistance genes, such as MRP1, MDR1, LRP and GST, were negatively correlated to Ginsenoside Rh2. All these results indicated that Ginsenoside Rh2 could effectively reverse drug resistance in human colorectal carcinoma cell and its mechanism involved the prevention of cellular proliferation and migration, the promotion of cellular apoptosis and the alteration of drug-resistance genes, which suggested that Ginsenoside Rh2 may act as a promising candidate for drug resistance in human colorectal carcinoma chemotherapy.

Keywords Ginsenoside Rh2 · Drug resistance · Human colorectal carcinoma

Introduction

Colorectal carcinoma (CRC) is a common malignant tumor and the third leading cancer-related mortality cause around the world [1]. Owing to considerable improvements in systemic therapy, the mortality rate of colorectal cancer has been reduced, still surgery and chemotherapeutic strategies are CRC patients' two major options. As for chemotherapeutic strategies, 5-fluorouracil (5-FU) is one of the most widely used chemotherapeutic agents for patients [2]. However, frequently occurring drug resistance resulted in treatment failure in CRC [3]. The acquisition of drug resistance could be associated with many mechanisms, including increased

drug efflux, alterations in the drug target and drug inactivation, modification of cell cycle checkpoints and defective apoptotic pathways [4–8]. Therefore, novel agents of high efficiency and low toxicity are needed for overcoming 5-FU resistance and increasing patients' survival rate.

Ginseng is a commonly used Chinese herbal medicine which is considered to be linked with significantly decreased risk of a variety of cancers [9]. Researches proved that ginsenosides is one of the main ingredients with anti-tumor activity in Ginseng [10–12]. Ginsenoside Rh2 has been found with a strong ability to inhibit glioma cell proliferation [13] and induced cellular apoptosis in both hepatoma [14] and leukemia Reh cells [15]. In addition, Han's study suggested that Rh2 has an anti-cancer activity in CRC cells [16]. However, studies related to the resistance reversion effect and mechanism of Ginsenoside Rh2 in human colorectal carcinoma cells were limited.

In the present study, we systematically investigated the reversal effect of Ginsenoside Rh2 on drug resistance in human colorectal carcinoma (CRC) cell line

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HCT-8/5-FU and LoVo/5-FU. Moreover, the mechanisms that may be involved, such as cellular proliferation, apoptosis, migration and some drug-resistance genes were also studied.

Materials and methods

Reagents and antibodies

Rh2 was purchased from Sigma-Aldrich and the powder was dissolved in dimethylsulfoxide(DMSO). The chemical structure of Rh2 is shown in Fig. 1A. Antibodies of Bcl-2, caspase-3, I κ B- α , phospho-I κ B- α (S32), E-cadherin, N-cadherin, Vimentin, MMP9, GAPDH were purchased from Cell

Signaling Technology (Beverly, MA). Antibodies of MRP1, MDR1, LRP and GST were purchased from Santa Cruz Biotech (Santa Cruz, CA). Antibodies of CyclinD1, CDK2, Rb and phospho-Rb (S807/811) were purchased from Signalway Antibody (SAB, USA). Cell Counting Assay Kit-8 (CCK-8) was purchased from Guangzhou Ladder Biotech (Guangzhou, China). Fetal bovine serum (FBS) and RPMI-1640 medium was purchased from Gibco (Gibco, USA).

Cell culture

Human CRC cells (HCT-8 and LoVo) were cultured at 37 °C in RPMI-1640 medium containing 10% fetal bovine serum (FBS), in a humidified atmosphere of 5% CO₂. Human 5-FU-resistant CRC cells (HCT-8/5-FU and LoVo/5-FU)

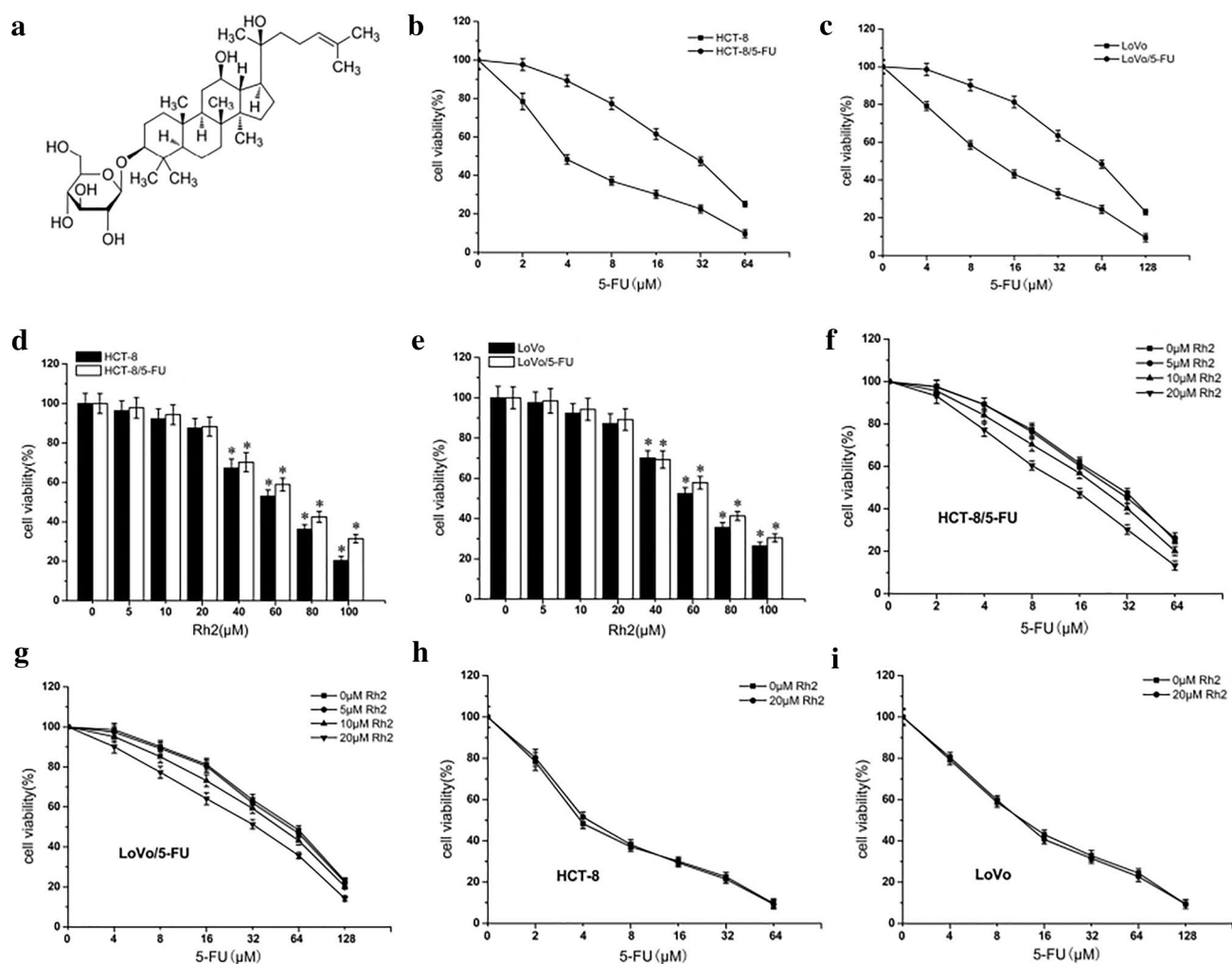


Fig. 1 The reversal effect of Rh2 on 5-Fu resistance in HCT/5-FU and LoVo/5-FU cells. **a** The chemical structure of Rh2. **b** Cell viability of HCT-8/5-FU cells and its parental HCT-8 cells. **c** Cell viability of LoVo/5-FU cells and its parental LoVo cells. **d** Toxic effect of RH2 on HCT-8/5-FU cells and its parental HCT-8 cells. Bars represent mean \pm SD, $n = 6$. * p < 0.05 vs. 0 μ M. **e** Toxic effect of

RH2 on LoVo/5-FU cells and its parental LoVo cells. Bars represent mean \pm SD, $n = 6$. * p < 0.05 vs. 0 μ M. **f** Effect of Rh2 on HCT-8/5-FU cell viability. **g** Effect of Rh2 on LoVo/5-FU cell viability. **h** Effect of Rh2 on HCT-8 cell viability. **i** Effect of Rh2 on LoVo cell viability

were established and cultured in RMPI-1640 medium supplemented with 6.5 or 15.0 $\mu\text{g/ml}$ 5-FU. The establishment of human 5-FU-resistant CRC cells was briefly described as follows: parent human CRC cells HCT-8 were exposed stepwise to escalating concentrations of 5-FU, ranging from 0.5 to 2 $\mu\text{g/ml}$ for about 7 months; and parent human CRC cells Lovo were exposed stepwise to escalating concentrations of 5-FU, ranging from 1 to 2.5 $\mu\text{g/ml}$ for about 7 months. Then the 5-FU resistance characteristics of HCT-8/5-FU and LoVo/5-FU cells were confirmed by CCK-8 assay.

Cell viability assay

The effect of Rh2 on the viability of CRC cells was measured by CCK-8 assay. Single-cell suspensions were seeded in 96-well plates. After incubation with Rh2 for 24 h, 10 μL CCK-8 reagents were added and incubated for another 2 h; the absorbance at 450 nm was measured using a microplate reader (AMR-100, ThermoRui-OS, Jiangsu).

Multidrug resistance reversal assay

The effect of Rh2 on human 5-FU-resistant CRC cells (HCT-8/5-FU and LoVo/5-FU) was detected with CCK-8 assay. Single-cell suspensions were seeded in 96-well plates. Cells were pretreated with different concentrations of 5-FU for 30 min, then the cells were incubated with varying concentrations of Rh2 for 24 h; 10 μL CCK-8 reagents were added and incubated for another 2 h; the absorbance at 450 nm was measured using a microplate reader (AMR-100, ThermoRui-OS, Jiangsu).

Cell cycle analysis

Cell cycle distribution was detected by propidium iodide (PI) staining using flow cytometry (CytoFLEX, Beckman, USA), according to the manufacturer's instructions. Briefly, HCT-8/5-FU cells or LoVo/5-FU cells were pretreated with 4 or 8 μM 5-FU for 30 min, respectively, then the cells were incubated with varying concentrations of Rh2 for 24 h. All the treated cells were collected and fixed in ice-cold 70% methanol. Following centrifugation, cells were resuspended in ice-cold PBS, and incubated with RNase at 37 °C for 30 min, and then were incubated with propidium iodide (PI) at room temperature for 30 min. Finally, cell cycle distribution was analyzed using flow cytometer (CytoFLEX, Beckman, USA). The percentage of cells containing different multiple DNA was quantified.

Cell apoptotic analysis

Cellular apoptosis was measured by Annexin-V/PI double staining using flow cytometry (CytoFLEX, Beckman, USA),

according to the manufacturer's instructions. Briefly, HCT-8/5-FU cells or LoVo/5-FU cells were pretreated with 4 or 8 μM 5-FU for 30 min, respectively, then the cells were incubated with varying concentrations of Rh2 for 24 h. All the treated cells were harvested and resuspended in binding buffer; then incubated with Annexin-V-FITC for 15 min and PI for another 5 min in the dark at room temperature. Finally, each sample was quantitatively analyzed using flow cytometer (CytoFLEX, Beckman, USA).

Transwell assay

Cellular migration was analyzed by transwell assay according to manufacturer's instructions (Corning, USA). Briefly, HCT-8/5-FU cells or LoVo/5-FU cells were pretreated with 4 or 8 μM 5-FU for 30 min, respectively, then the cells were incubated with varying concentrations of Rh2 for 24 h. All the treated cells were harvested and 1×10^5 cells cultured in 100 μL RMPI-1640 medium containing 1% FBS were seeded on the upper chamber insert with 8- μm -pore membrane (Corning, Lowell, USA), and 600 μL RMPI-1640 medium containing 5% FBS was added to the lower chamber. After migration for 12 h in a 37 °C, 5% CO_2 atmosphere, cells that migrated into the lower chamber were fixed in methanol, and stained with 0.5% crystal violet solution. Cell migration was quantized using microscopy (IX73, Olympus, Japan).

Western blot analysis

Protein expressions and phosphorylation levels were measured by Western blot assay. HCT-8/5-FU cells or LoVo/5-FU cells were pretreated with 4 or 8 μM 5-FU for 30 min, respectively, then the cells were incubated with varying concentrations of Rh2 for 24 h. All the treated cells were lysed in RIPA lysis buffer with protease and phosphatase inhibitor cocktail. Protein concentrations were quantized by BCA protein assay kit. 60 μg proteins were separated by SDS-PAGE on 8% gels and transferred to PVDF membranes followed by blockage with 5% non-fat milk diluted with TBST (Tris-HCl 20 mmol/l, NaCl 150 mmol/l, pH 7.5, 0.1% Tween 20) at room temperature for 60 min. Then PVDF membranes were probed with primary antibody overnight at 4 °C. After washing with TBST for 3×5 min, the membranes were incubated with HRP-conjugated secondary antibody at room temperature for 90 min, followed by ECL detection. The density of the bands was measured using the Image J software (1.48 μ , USA), and GAPDH was used as internal control.

qRT-PCR

Total RNA was extracted with Trizol reagent (Invitrogen) and 2 μg RNA was applied for gene-specific RT-PCR using

a Onestep RT-PCR Kit (Qiagen) according to manufacturer's instructions. The PCR primer sequences were as follows:

MRP1 forward 5'-TTGCCGTCTACGTGACCATT-3' and reverse: 5'-AGGCGTTTGAGGGAGACACT-3';

MDR1 forward 5'-CTTGGCAGCAATTAGAAC-3' and reverse: 5'-TCAGCAGGA AAGCAGCAC-3';

LRP forward 5'-TATGTGCCATCTGCCAAAGT-3' and reverse: 5'-CATGTAGGTGCTTCCAATCA-3';

GST forward 5'-TTCCTGTGGCATAATGTGAT-3' and reverse: 5'-CTGATTCAAAGGCAAATCTC-3';

GAPDH forward 5'-CCACCCATGGCAAATTCCATG GCA-3' and reverse: 5'-TCTAGACGGCAGGTCAGGTCC ACC-3'.

PCR reactions were performed on Applied Biosystems 7500 real-time PCR system with the following conditions: 94 °C, 12 min for 1 cycle; then 94 °C, 40 s; 75 °C, 1 min, 59 °C, 40 s and 72 °C, 1 min for 32 cycles. GAPDH mRNA was used as an internal control. And the levels for each gene were counted by standardizing the quantified mRNA amount to the GAPDH mRNA.

Statistical analysis

All data were expressed as the mean \pm SD. Comparisons between 2 groups were analyzed using Student's *t* test and among three groups by ANOVA test using the least significant difference test (SPSS 17.0). Differences were considered statistically significant at a *p* value of < 0.05 .

Results

Rh2 could reverse drug resistance in CRC cells

To investigate the effect of Rh2 on drug resistance in CRC cells, we first established drug-resistant CRC cells (HCT-8/5-FU and LoVo/5-FU cells). As shown in Fig. 1b, c, compared to the half-maximal inhibitory concentration of 5-FU in the parental HCT-8 (IC₅₀ 2.82 \pm 0.12 μ M) and LoVo (IC₅₀ 12.3 \pm 1.14 μ M) cells, HCT-8/5-FU (IC₅₀ 28.8 \pm 3.22 μ M) and LoVo/5-FU (IC₅₀ 62.7 \pm 4.24 μ M) cells were more resistant against 5-FU. And cytotoxicity test of RH2 (Fig. 1d, e) showed that 0-20 μ M RH2 does not have toxic effect on CRC cell growth. Then treatment with Rh2 (20 μ M) could remarkably reverse 5-FU resistance in HCT-8/5-FU and LoVo/5-FU cells (Fig. 1f, g), while it does not have significant impact on the growth of HCT-8 and LoVo cells (Fig. 1h, i). Furthermore, the resistance reversion effect of Rh2 on the IC₅₀ of 5-FU in HCT-8/5-FU and LoVo/5-FU cells was calculated, as shown in Table 1; the IC₅₀ of 5-FU in HCT-8/5-FU and LoVo/5-FU cells remarkably decreased

Table 1 The resistance reversion effect of Rh2 on the IC₅₀ of 5-FU in HCT/5-FU and LoVo/5-FU cells

Rh2(μ M)	IC ₅₀ of 5-FU (μ M)	
	HCT-8/5-FU	LoVo/5-FU
0	28.8 \pm 0.22	62.7 \pm 0.24
5	27.1 \pm 0.2*	57.3 \pm 0.21*
10	22.7 \pm 0.23*#	49.6 \pm 0.22*#
20	13.7 \pm 0.19*#&	34.3 \pm 0.26*#&

Mean \pm SD, *n* = 6

**p* < 0.05 vs. 0 μ M; #*p* < 0.05 vs. 5 μ M; &*p* < 0.05 vs. 10 μ M

to 13.7 \pm 0.19 and 34.3 \pm 0.26 μ M by pretreatment with 20 μ M Rh2.

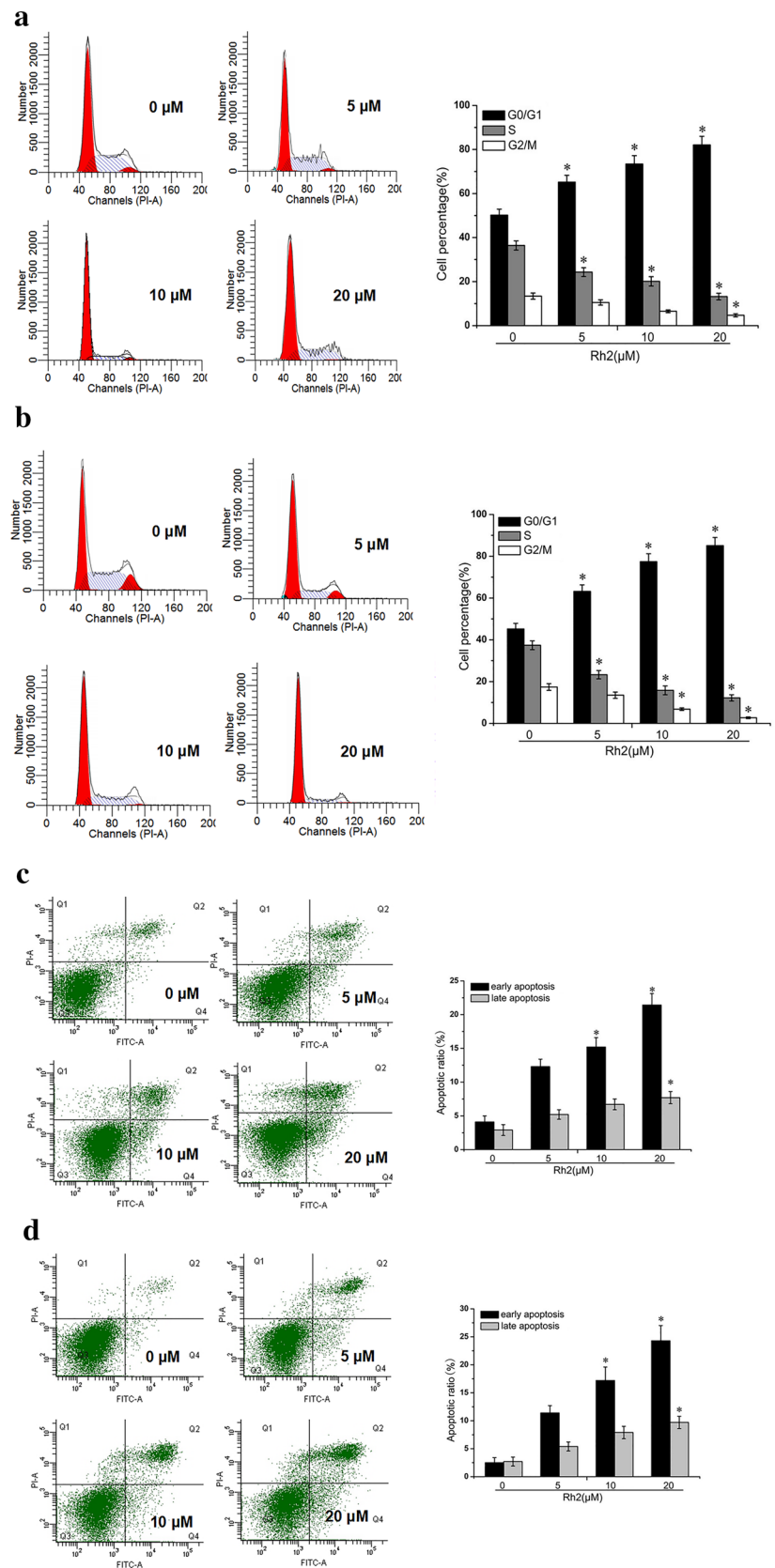
Rh2 induces cell cycle arrest and cell apoptosis in drug-resistant CRC cells

Imbalance between cell proliferation and apoptosis was thought to be related to the progression of cancer and its drug resistance, so we set out to investigate whether RH2 could inhibit the growth of drug-resistant CRC cells. Cell cycle analysis showed that treatment with 5, 10, 20 μ M Rh2 could dramatically increase the percentage of cells in the G1-phase and decrease the percentage of cells in the S-phase in both HCT-8/5-FU and LoVo/5-FU cells (Fig. 2a, b). Furthermore, we explored whether pro-apoptosis participated in Rh2-induced anti-proliferative effect. In Fig. 2c, d, Annexin-V-FITC/PI flow cytometry analysis revealed that the number of early apoptotic HCT-8/5-FU and LoVo/5-FU cells induced by 5-FU was evidently higher after treatment with Rh2 at the concentrations of 10 and 20 μ M. The data hint that Rh2 sensitized CRC cells to 5-FU by inhibiting proliferation and promoting 5-FU-induced apoptosis.

The effects of Rh2 on the expression of cell cycle and apoptosis-related molecules in drug-resistant CRC cells

To investigate the possible mechanism of Rh2 on proliferation, cell cycle and apoptosis, we tested the effect of Rh2 on several cell cycle and apoptosis-related proteins. As shown in Fig. 3, treatment with Rh2 at the concentrations of 10 μ M and 20 μ M markedly decreased the protein levels of cyclin D1, CDK2, p-Rb and Bcl-2, while increasing the protein levels of cleaved-caspase 3 in both HCT-8/5-FU and LoVo/5-FU cells, which suggested that Rh2 induced cell cycle arrest and apoptosis by regulation of cell cycle and apoptosis-related proteins. In addition,

Fig. 2 Rh2 induces cell cycle arrest and cell apoptosis in drug-resistant CRC cells. Drug-resistant CRC cells were pre-treated with 4 μ M(HCT-8/5-FU cells) or 8 μ M(LoVo/5-FU cells) 5-FU for 30 min, then the cells were incubated with varying concentrations of Rh2 for 24 h. Cell cycle distribution of HCT-8/5-FU (**a**) and LoVo/5-FU (**b**) were detected by PI staining using flow cytometry. Cell apoptosis of HCT-8/5-FU (**c**) and LoVo/5-FU (**d**) were measured by Annexin-V/PI double staining using flow cytometry. Bars represent mean \pm SD, $n = 6$. * $p < 0.05$ vs. 0 μ M



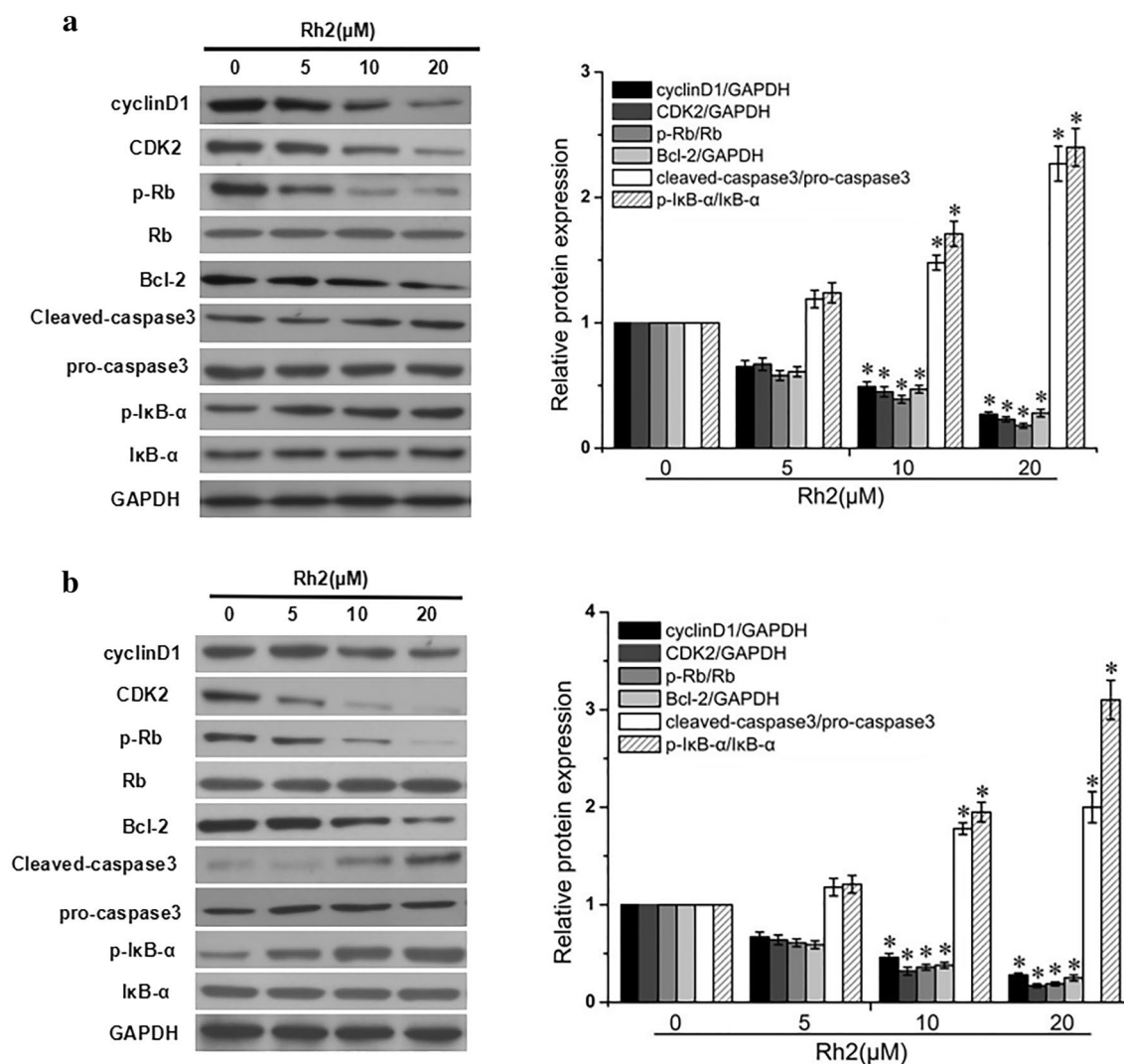


Fig. 3 The effects of Rh2 on the expression of cell cycle and apoptosis-related proteins in drug-resistant CRC cells. Drug-resistant CRC cells were pretreated with 4 μ M (HCT-8/5-FU cells) or 8 μ M (LoVo/5-FU cells) 5-FU for 30 min, then the cells were incubated with varying concentrations of Rh2 for 24 h. The protein expression

of cyclin D1, CDK2, p-Rb/Rb, Bcl-2, cleaved-caspase3 and p-IkB- α /IkB- α in HCT-8/5-FU (a) and LoVo/5-FU (b) cells, were determined by Western blot. Bars represent mean \pm SD, $n = 6$. * $p < 0.05$ vs. 0 μ M

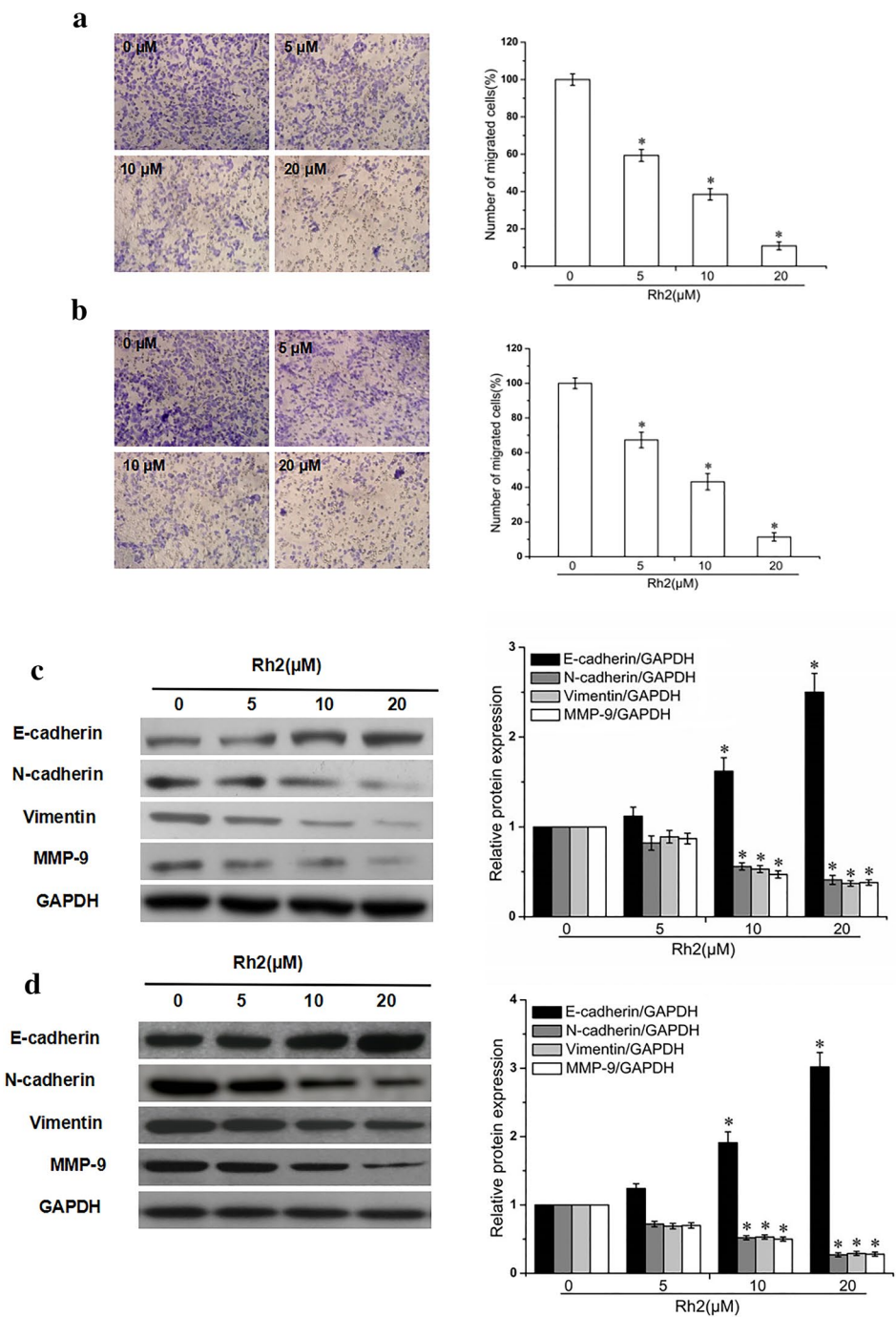
Rh2 up-regulated the expression of p-IkB, suggesting the association of Rh2 with NF- κ B activity.

Rh2 inhibits the migration and EMT process in drug-resistant CRC cells

To detect whether Rh2 affects the migration and EMT process in drug-resistant CRC cells, transwell migration assay was performed to assess the proportion of cells that migrated to the lower chamber treated with or without Rh2. The results showed that the number of migrated HCT-8/5-FU and LoVo/5-FU cells was markedly decreased after treatment with Rh2 at the concentrations of 10 and 20 μ M (Fig. 4a, b), which indicated that Rh2 might inhibit cell migration in HCT-8/5-FU

and LoVo/5-FU cells. Furthermore, we examined the effect of Rh2 on the protein expression of EMT-related molecules by Western blot assay. Rh2 at the concentrations of 10 and 20 μ M lead to up-regulation of the epithelial marker E-cadherin, and down-regulation of the mesenchymal marker N-cadherin, Vimentin and MMP9 at protein levels in HCT-8/5-FU and LoVo/5-FU cells (Fig. 4c, d). Taken together, our results indicated that Rh2 impaired the migration and EMT process in drug-resistant CRC cells.

Fig. 4 The effects of Rh2 on migration and the EMT process in drug-resistant CRC cells. Drug-resistant CRC cells were pretreated with 4 μ M (HCT-8/5-FU cells) or 8 μ M (LoVo/5-FU cells) 5-FU for 30 min, then the cells were incubated with varying concentrations of Rh2 for 24 h. The migration of HCT-8/5-FU (a) and LoVo/5-FU (b) cells were assessed by transwell assay. The protein expression of EMT-related molecules: E-cadherin, N-cadherin, Vimentin and MMP9 was determined by Western blot in HCT-8/5-FU (c) and LoVo/5-FU (d) cells. Bars represent mean \pm SD, $n = 6$. * $p < 0.05$ vs. 0 μ M



Rh2 attenuates the expression of MRP1, MDR1, LRP and GST in drug-resistant CRC cells

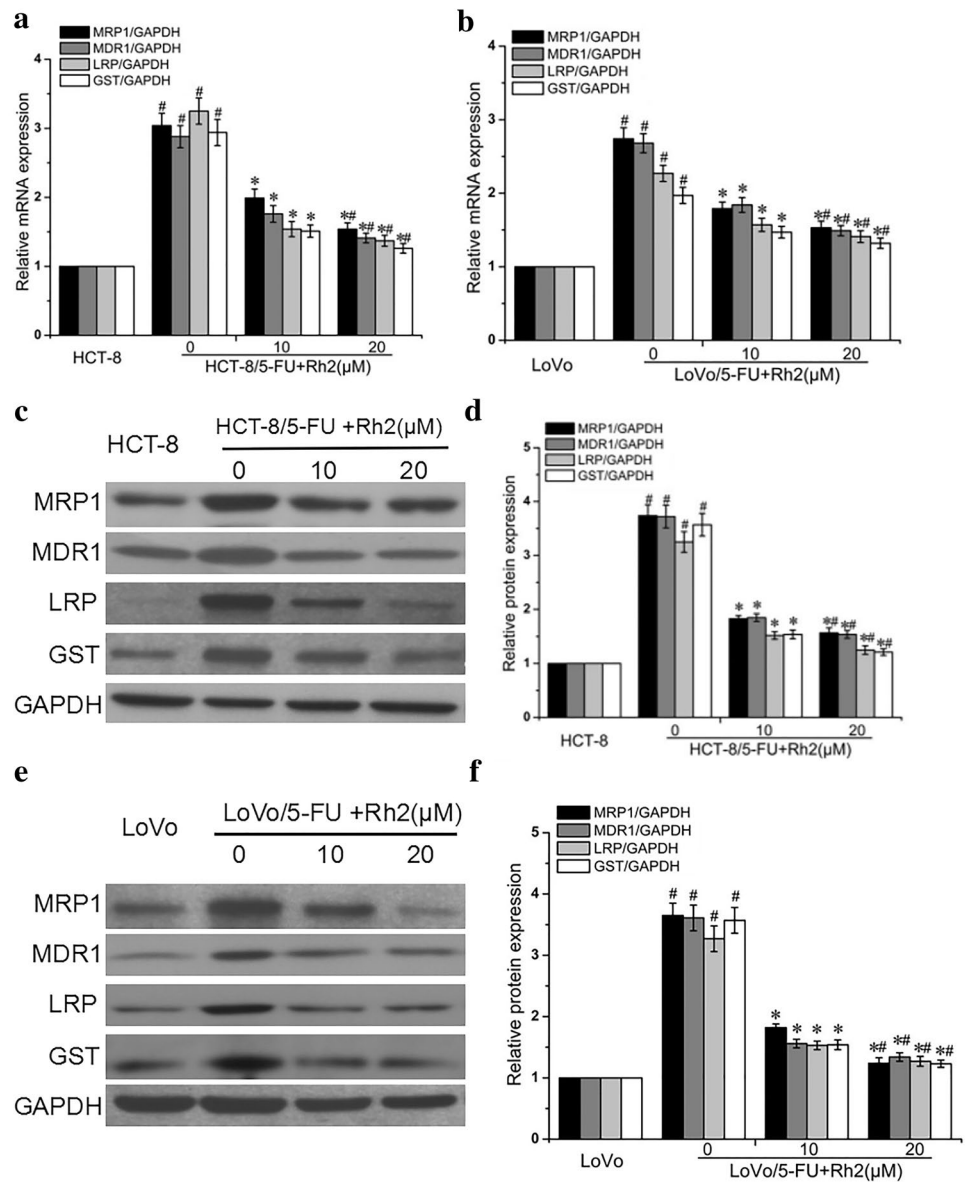
To further investigate the underlying mechanism of Rh2 on drug resistance in CRC cells, we examined the expression of drug-resistance gene MRP1, MDR1, LRP and GST. Results of qRT-PCR and Western blot (Fig. 5a, c, d) showed that HCT-8/5-FU cells had a higher expression of MRP1, MDR1, LRP and GST in both mRNA level and protein level than its parental HCT-8 cells, while treatment with

Rh2 partly reverses this high expression of MRP1, MDR1, LRP and GST in HCT-8/5-FU cells. And similar results were observed in LoVo/5-FU and LoVo cells (Fig. 5b, e, f).

Discussion

Recently, many groups have confirmed that the use of herbal medicine improved therapeutic effects and reversed drug resistance caused by the long-term chemotherapy. A

Fig. 5 The effects of Rh2 on the protein expression of drug-resistant MRP1, MDR1, LRP and GST. Drug-resistant CRC cells were pretreated with 4 μ M (HCT-8/5-FU cells) or 8 μ M (LoVo/5-FU cells) 5-FU for 30 min, then the cells were incubated with varying concentrations of Rh2 for 24 h. The mRNA expression of MRP1, MDR1, LRP and GST in HCT-8/5-FU (a) and LoVo/5-FU (b) cells, were determined by qRT-PCR. The protein expression of MRP1, MDR1, LRP and GST in HCT-8/5-FU (c, d) and LoVo/5-FU (e, f) cells, was determined by Western blot. Bars represent mean \pm SD, $n = 6$. * $p < 0.05$ vs. 0 μ M; # $p < 0.05$ vs. HCT-8 or LoVo cells



synergistic effect was observed on the prostate cancer mode treated with Rh2 and paclitaxel or mitoxantrone [17]. Also, there are evidences that suggested that Rh2 might be connected to drug resistance of cancer therapy [18]. In the present study, we confirmed that Rh2 played a protective role in CRC drug resistance. Then we designed a series of experiments to explore its involving mechanism. Consistent with previous reports, low doses of Rh2 inhibited HCT-8/5-FU and LoVo/5-FU cells proliferation and migration, promoted cells apoptosis, and adversely affected 5-FU-resistant tumor therapy.

In sorting out the molecular mechanisms of the anti-drug-resistance activity of Rh2, we need to take into account its known actions on cell abnormal proliferation and apoptosis [19, 20]. In the present study, we found that

Rh2 could inhibit proliferation and promote apoptosis of 5-FU-resistant HCT-8 and LoVo cells. As known, cell proliferation always related to cell cycle, and our data also showed that Rh2 inhibited transition of G0/G1 to G2/M, avoiding cell repair for DNA damage induced by 5-FU. To confirm the possible mechanism of Rh2 on regulation of cell cycle and apoptosis, we then investigated the effects of Rh2 on cell cycle and apoptosis-related molecules. The cyclins, CDKs and phosphorylation of Rb have been proved to be pivotal regulators of the G1/S transition of the cell cycle [21, 22]. Bcl-2, an anti-apoptotic protein, and caspase3, a downstream effective protein of apoptotic pathway, are considered to be related to poor response to conventional cancer treatment [23–25]. In this paper, we found that treatment with Rh2 at the concentrations of

10 and 20 μM markedly decreased the protein levels of cyclin D1, CDK2, p-Rb and Bcl-2, while increasing the protein levels of cleaved-caspase 3 in both HCT-8/5-FU and LoVo/5-FU cells, which indicated that Rh2 induced cell cycle arrest and apoptosis by regulation of cyclin D1, CDK2, p-Rb, Bcl-2 and caspase 3. In addition, Rh2 up-regulated the expression of p-I κ B, suggesting the association of Rh2 with NF- κ B activity.

High migration and invasion characteristic is always thought to contribute to the high mortality rate of malignant CRC, so inhibition of cell migration could be vital for controlling drug resistance [26, 27]. Transwell assay showed that Rh2 dramatically inhibited the migration of HCT-8/5-FU and LoVo/5-FU cells. Furthermore, we examined the effect of Rh2 on the protein expression of EMT-related molecules by Western blot assay. Rh2 at the concentrations of 10 and 20 μM led to up-regulation of the epithelial marker E-cadherin, and down-regulation of the mesenchymal marker N-cadherin, Vimentin and MMP9 at protein levels in HCT-8/5-FU and LoVo/5-FU cells. Taken together, our results indicated that Rh2 impaired the migration by restraining EMT process in drug-resistant CRC cells.

Exceptional drug-metabolizing could also be a reason for chemotherapeutic resistance of CRC [28]. Multi-drug resistant protein1 (MRP1) is an important ATP-binding cassette transporter protein, which affects the intracellular drug concentration through the alteration of drug influx or efflux. MRP1 was reported to mediating multidrug resistance in various cancers, such as breast and ovarian cancer [29–31]. Over-expression of MDR1 could also increase the level of drug efflux and limit the efficacy of chemotherapy [32, 33]. Lung resistance protein (LRP) was identified as a major vault protein (MVP) implicated in drug resistance of cancer cells in a cell-type-dependent manner by mediating drug efflux from the nucleus to the cytosol [34, 35]. Glutathione-S-transferases (GSTs) are a family of Phase II detoxification enzymes. It could detoxify chemotherapeutic drugs through the GSH-conjugate export pump, thus contributing to drug resistance [36, 37]. Thus, we analyzed the expression of these drug resistance-related proteins in the present study. Results of qRT-PCR and Western blot show that HCT-8/5-FU cells had a higher expression of MRP1, MDR1, LRP and GST in both mRNA level and protein level than its parental HCT-8 cells, while treatment with Rh2 partly reverses this high expression of MRP1, MDR1, LRP and GST in HCT-8/5-FU cells. And the similar results were observed in LoVo/5-FU and LoVo cells.

In conclusion, our study demonstrated that Ginsenoside Rh2 could effectively reverse drug-resistance in human colorectal carcinoma cell and its mechanism involved the prevention of cell proliferation and migration, the promotion of cell apoptosis and the alteration of drug-resistance genes, which suggested that Ginsenoside Rh2 might act as a promising

candidate for drug resistance in human colorectal carcinoma chemotherapy.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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