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Anticancer effects of Chinese red yeast rice versus monacolin K alone on colon cancer cells

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Abstract

Chinese red yeast rice (RYR) is a food herb made by fermenting *Monascus purpureus* Went yeast with white rice. RYR contains a mixture of monacolins, one of which — monacolin K (MK) — is identical to lovastatin (LV). Epidemiological studies show that individuals taking statins have a reduced risk of colon cancer. In the present study, LV decreased cellular proliferation ($P<.001$) and induced apoptosis ($P<.05$) in HCT-116 and HT-29 human colon cancer cells. RYR inhibited both tumor cell growths ($P<.001$) and enhanced apoptosis ($P<.05$) in HCT-116 cells. Inhibition of proliferation was reversed by mevalonate (MV) in LV-treated cells, since LV is a 3-hydroxy-3-methyl-glutaryl CoA reductase (HMGCR) inhibitor. However, RYR with MV did not reverse the observed inhibition of growth. MK-free RYR did not reverse the observed LV-mediated inhibition of cancer cell growth. These observations suggest that other components in RYR, including other monacolins, pigments or the combined matrix effects of multiple constituents, may affect intracellular signaling pathways differently from purified crystallized LV in colon cancer cells. RYR was purified into two fractions: pigment-rich fraction of Chinese red yeast rice (PF-RYR) and monacolin-rich fraction of Chinese red yeast rice (MF-RYR). The effect of MF-RYR was similar to that of LV, while the effect of PF-RYR was similar to the effect of the whole RYR extract on the proliferation, apoptosis and mRNA level of HMGCR and sterol response element binding protein-2. These results suggest that the matrix effects of RYR beyond MK alone may be active in inhibiting colon cancer growth. RYR with or without MK may be a botanical approach to colon cancer chemoprevention worthy of further investigation.

Keywords: Chinese red yeast rice; Colon cancer; Lovastatin; Monacolins; Pigment; Cholesterogenesis

1. Introduction

Red yeast fermented with rice is a traditional food spice consumed throughout Asia [1,2]. It is also known as "red koji," "angkak" or "red yeast rice," and its food and medicinal values date back to more than a thousand years, with the first recorded documentation of use being 800 AD [1,3,4]. Chinese red yeast rice (RYR) is derived from rice that has been allowed to ferment with the yeast *Monascus purpureus*. The fungus *Monascus* was studied by Dutch scientists in 1884 after the discovery of its use by villagers in Java [5]. A species isolated from red koji or honqu (as red rice yeast is known in East Asia) was named *M. purpureus* Went in 1895, recognizing its purple coloration [6]. RYR contains predominantly rice starches and sugars, and also yeast polyketides, fatty acids, pigments and condensed tannins [7,8]. The classes of polyketide structures that arise from the fermentation process are called monacolins, and the major monacolin found in RYR is monacolin K (MK), which is identical in structure to lovastatin (LV). Other polyketides in RYR are structural analogs of MK [7].

LV is a reversible competitive inhibitor of the key enzyme that controls cholesterol biosynthesis, 3-hydroxy-3-methyl-glutaryl CoA reductase (HMGCR), and it has been used for the management of hypercholesterolemia [9,10]. LV at a dose of 20–40 mg significantly reduces levels of cholesterol and

Abbreviations: DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; HMGCR, 3-hydroxy-3-methyl-glutaryl CoA reductase; HPLC, high-performance liquid chromatography; LDL, low-density lipoprotein; LV, lovastatin; MF-RYR, monacolin-rich fraction of Chinese red yeast rice; MK, monacolin K; MV, mevalonate; PCR, polymerase chain reaction; PF-RYR, pigment-rich fraction of Chinese red yeast rice; RT, reverse transcription; RYR, Chinese red yeast rice; SREBP-2, sterol response element binding protein-2.

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low-density lipoprotein (LDL) cholesterol [11,12]. RYR has also been shown to reduce blood cholesterol levels in rabbits on an atherogenic diet [13,14], and RYR can reduce lipid accumulation in 3T3 L1 preadipocytes [15]. In a randomized prospective controlled trial, RYR, in comparison to a placebo, decreased total cholesterol, triglycerides and apolipoprotein B in hypercholesterolemic individuals [16].

De novo cholesterogenesis is required for tumor growth, and HMGCR activities are up-regulated in colon tumors [17–20]. A growing body of evidence supports the notion that statins, including LV, may inhibit colon cancer cell growth and thereby possess preventive potential for reducing the incidence of colon cancer [21–23]. In a population-based study, statin consumption was associated with a 47% reduced risk of colon cancer [24]. The present study was designed to determine the effect of RYR containing multiple monacolins and LV versus LV alone on colon cancer cell growth and apoptosis.

Our group has previously shown that RYR administration to hypercholesterolemic individuals at a dose of 2400 mg/day resulted in an 18% decrease in total cholesterol, a 23% decrease in LDL cholesterol and a 15% decrease in triglycerol concentrations [25]. In that study, a daily dose of 2400 mg of RYR powder containing 0.4% monacolins or 5–7.5 mg of MK reduced cholesterol levels in hypercholesterolemic subjects to a degree equivalent to what is typically observed with 20 mg of LV. Therefore, we hypothesized that other constituents in the RYR matrix were bioactive beyond MK alone. In the current study, we examined the effects of LV, RYR, MK-free RYR, pigment-rich fraction of Chinese red yeast rice (PF-RYR) and monacolin-rich fraction of Chinese red yeast rice (MF-RYR) on human colon cancer cell growth, apoptosis and the transcription levels of HMGCR and sterol response element binding protein-2 (SREBP-2).

2. Materials and methods

2.1. Extract and standard preparation

RYR powder (Botanica BioScience, Ojai, CA) was extracted with methylene chloride and evaporated under vacuum at 40°C. The MK concentration of RYR extract was determined by high-performance liquid chromatography (HPLC) mass spectrometry analysis (LCQ Classic Finnigan LC-MS/MS Systems; ThermoFinnigan, San Jose, CA) using an authentic standard (AG Scientific, San Diego, CA) [26] (Fig. 1A and B). For MK-free RYR, endogenous MK in RYR was removed by injecting a sample of RYR extract into a Prep-LC 4000 system coupled with a 490E Programmable Multiwavelength UV detector (Waters Corp., Milford, MA), with conditions as follows: column, Phenomenex Spher-eclone (250×21.2 mm×10 mm); isocratic solvent system, methanol:water (8:2); flow, 5 ml/min; detection, λ=237 nm. The fraction collected between 18 and 19 min of elution time was eliminated, and the remaining fraction corresponding to MK-free RYR was collected, which was confirmed by analytical HPLC using an authentic standard of MK. For the monacolin-rich fraction (MF-RYR) and the pigment-rich fraction (PF-RYR) of RYR, RYR was dissolved in a mixture of dichloromethane and acetone (1:1, vol/vol) solution, mixed with silica gel and dried under vacuum at 40°C. Flash column chromatography was used, eluting with hexane and acetone (8:2, vol/vol) and then with pure acetone. The

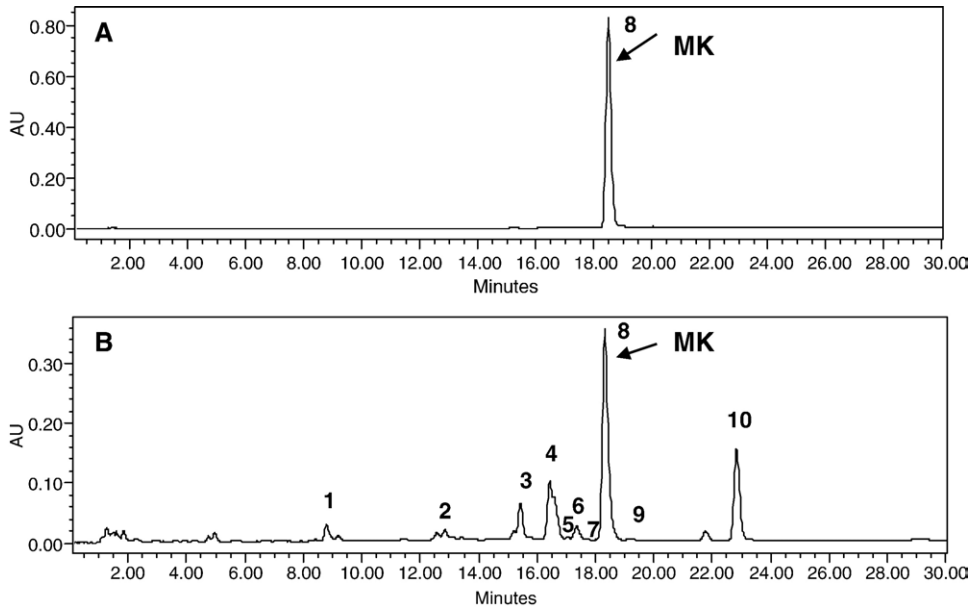

Fig. 1. HPLC. (A) MK identification using a standard LV. (B) Identification of monacolins in RYR. (1) MK analogue; (2) MK dehydro analogue; (3) hydroxy acid form of monacolin L; (4) hydroxy acid form of MK; (5) dihydromonacolin K; (6) monacolin L; (7) hydroxy acid form of dehydromonacolin K; (8) MK; (9) methyl ester of hydroxyl acid form of MK; (10) dehydromonacolin K, as previously reported [26]. AU=absorbance.

proportions of PF-RYR and MF-RYR were 10% and 90% of RYR by weight, respectively.

2.2. Cell culture

The human colon cancer cell lines HCT-116 and HT-29 were obtained from the American Type Culture Collection (ATCC; Manassas, VA) and maintained by seeding 500,000 to 1 million cells weekly into 100-mm dishes using McCoy's 5A medium (ATCC) containing 10% fetal bovine serum (Life Technologies, Grand Island, NY), 100 U/ml penicillin (Life Technologies) and 100 μg/ml streptomycin (Life Technologies). All experiments were done within <20

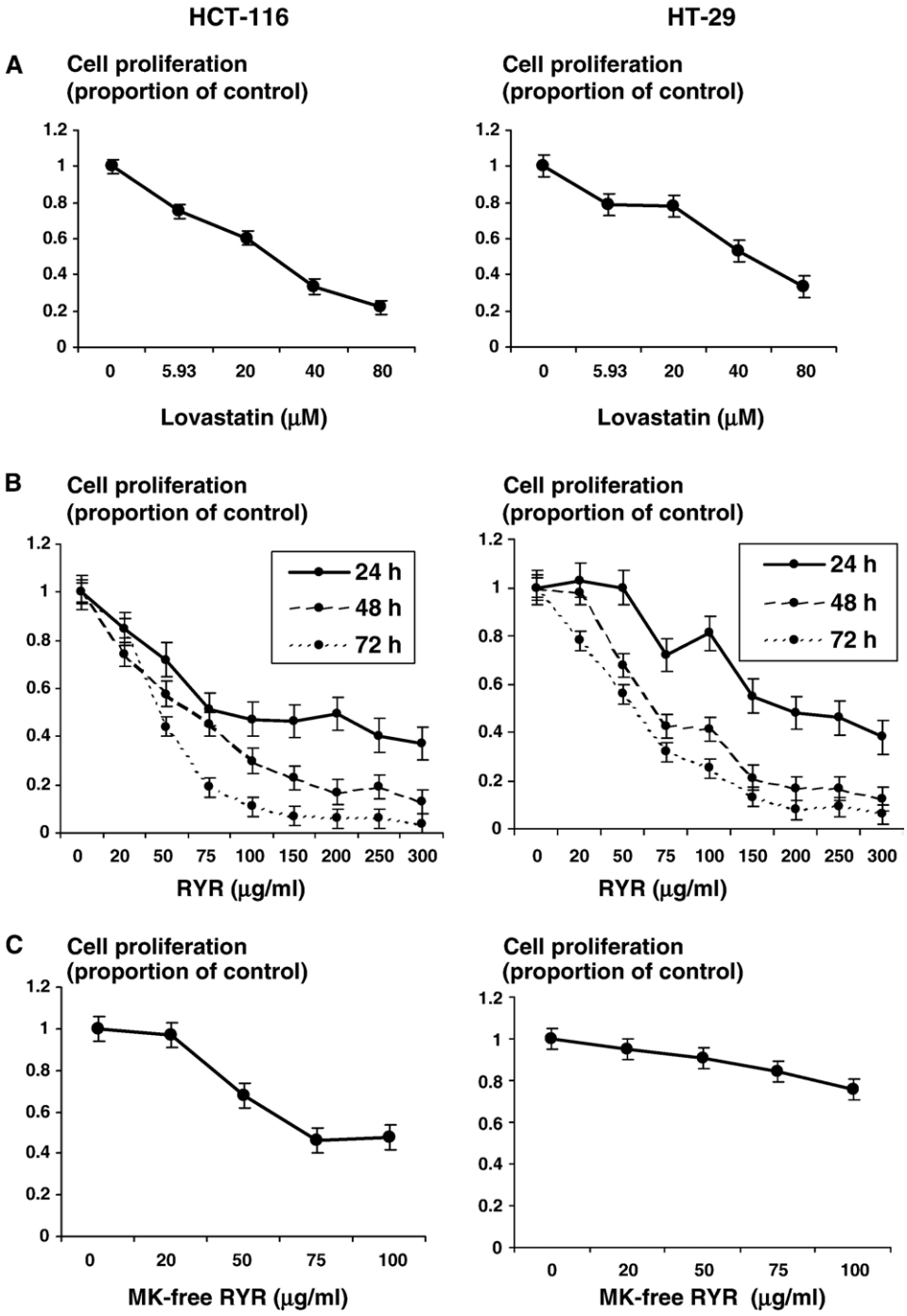

Fig. 2. LV, RYR and MK-free RYR effects on human colon cancer cell growth. (A) LV treatment for 48 h decreased cell proliferation in a dose-dependent manner in HCT-116 ($P<.01$) and HT-29 ($P<.01$) cells. (B) RYR decreased the cell proliferation of both HCT-116 and HT-29 colon cancer cells in a dose-dependent manner at 24, 48 and 72 h of treatment ($P<.001$). (C) MK-free RYR decreased cell proliferation in both cells ($P<.01$). Values are presented as mean±S.E.M. (n=3–6).

passages from the passage number they were in upon receipt from ATCC. Cells were kept in a 37°C incubator with 95% air and 5% CO_2.

2.3. MTT cell proliferation assay

Cells (5×10^3 per well) were seeded in 0.1 ml of the medium in sterile 96-well plates. After 24 h, the medium was removed and replaced with treatment media. For the LV dose curve, cells were treated with LV (5. 93, 20, 40 or 80 μM) for 48 h. A concentration of 5.93 μM LV is equivalent to an MK amount of 50 μg/ml RYR. For RYR dose experiment, cells were treated with RYR (0–300 μg/ml) for 24, 48 or 72 h. To test MK function in RYR during colon cancer cell growth, cells were treated with MK-free RYR (0–100 μg/ml) for 48 h. To compare the effects of whole RYR, MF-RYR and PF-RYR on cell growth, cells were treated with RYR, MF-RYR (90% of RYR concentration) or PF-RYR (10% of RYR concentration) for 48 h. Mevalonate (MV; Sigma-Aldrich, St. Louis, MO) in 25 or 50 μM was used to test the effect of RYR and its fraction on de novo cholesterogenesis. All stock solutions of LV, RYR, MK-free RYR, MF-RYR, PF-RYR and MV were dissolved in dimethyl sulfoxide (DMSO), and the final concentration of DMSO in the media was <0.2%. Cell proliferation was estimated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich) assay. MTT assay measures the cellular conversion of a tetrazolium salt into a formazan product, which can be detected by spectrophotometry and provides a relative estimate of cell growth. Absorbance at 570 nm was measured with SoftMax (Molecular Devices, Sunnyvale, CA). Three replicates per condition were assayed, and data

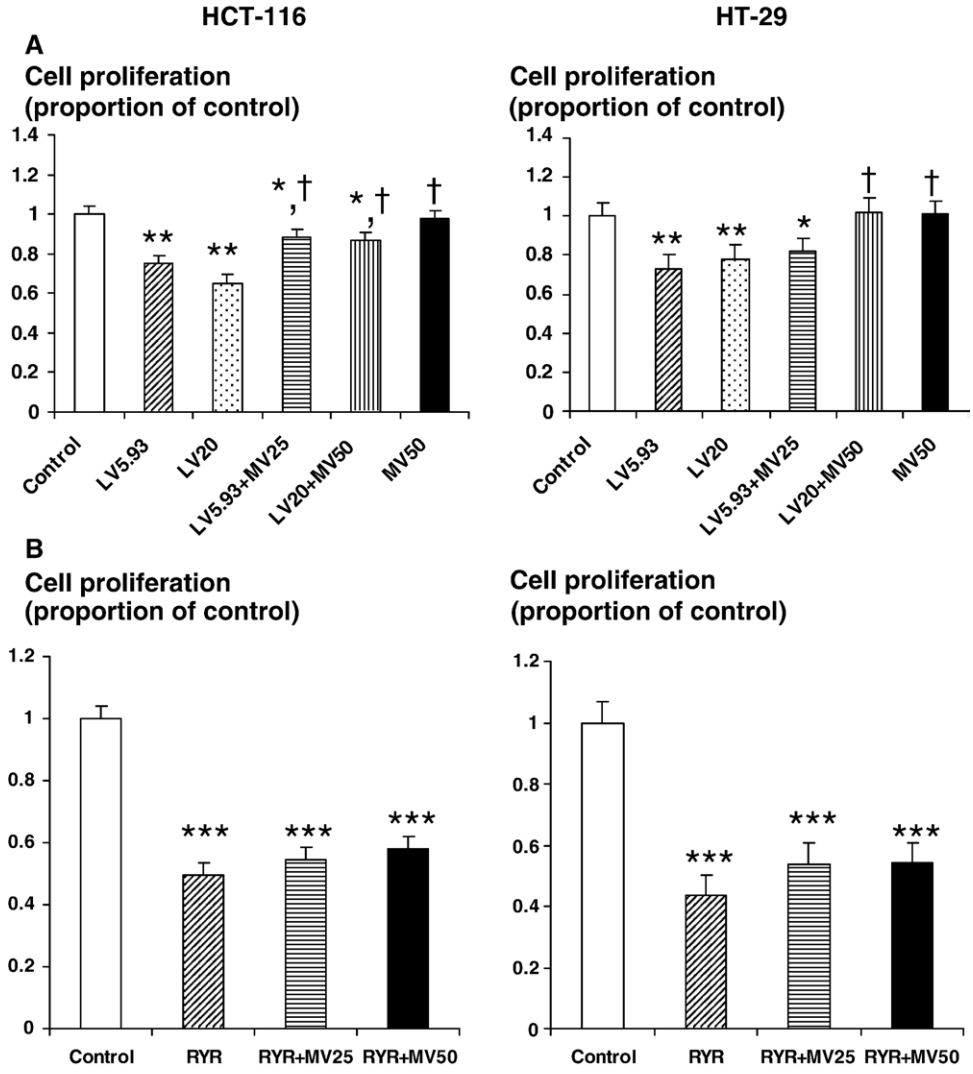

Fig. 3. MV effect on LV-treated or RYR-treated colon cancer cell growth. (A) Addition of MV (25 or 50 μM) partially or fully abolished the antiproliferative activity of LV in HCT-116 and HT-29 cells. (B) Incubation with MV for 48 h did not reverse the antiproliferative effect of RYR (50 μg/ml) in HCT-116 and HT-29 cells. Control: 0.2% DMSO. Values are presented as mean±S.E.M. (n=3–6). *Significantly different from control at $P<.05$. **Significantly different from control at $P<.01$. ***Significantly different from control at $P<.001$. †Significantly different from $LV_{5.93}$ or LV_{20} at $P<.05$.

averaged from three to six separate experiments are presented. Data are expressed as the percentage of control (0.2% DMSO).

2.4. Apoptosis assay

Apoptosis was assessed by measuring DNA fragmentation using Cell Death Detection ELISA^{PLUS} Assay (Roche, Indianapolis, IN). This assay is a photometric enzyme-linked immunosorbent assay (ELISA) that quantitatively measures the internucleosomal degradation of DNA, which occurs during apoptosis. Cells (10^5 per dish) were plated in 60-mm dishes for 24 h then treated with control (0.2% DMSO), LV (5. 93 μM), RYR (50 μg/ml), MF-RYR (45 μg/ml) or PF-RYR (5 μg/ml) for 48 h. Following treatments, nonadherent cells were collected and pelleted at 200×*g*. Adherent cells were washed with phosphate-buffered saline (Invitrogen, Carlsbad, CA), trypsinized, collected and combined with nonadherent cells into a 1 ml of the medium. Both live and dead cells were than counted via trypan blue exclusion (Pierce, Rockford, IL). An equal number of cells were added to microtiter plates for all treatment groups, and apoptosis assay was performed according to the manufacturer's instructions. Data are expressed as the percentage of control at an absorbance at 405 nm. Two replicates per condition were assayed, and data averaged from three to four separate experiments are presented.

2.5. RNA extraction and reverse transcription (RT)

Total RNA was extracted using RNeasy Mini Kit (Qiagen, Valencia, CA). Sample RNA content was quantified by measuring absorbance at 260 nm with a Gene Quant Spectrophotometer (Amersham-Pharmacia Biotech, Piscataway, NJ). RT was performed with 3 μg of RNA by using oligo(dT)$_{12–18}$ primers (Invitrogen) with SuperScript III Reverse Transcriptase (Invitrogen) according to the manufacturer's instructions.

2.6. Quantitative real-time polymerase chain reaction (PCR)

Gene expressions of HMGCR and SREBP-2 were determined with *TaqMan* Universal PCR master mix and primers (Applied Biosystems, Foster City, CA) by quantitative real-time PCR using the ABI 7900 HT Sequence Detector (Applied Biosystems). The transcription level of target genes was normalized to r18*S* expression. Every other sample had RT reactions repeated on a separate occasion, followed by PCR and quantitation to confirm the reproducibility of the assay. In addition, every set of RT reactions contains a −RT negative control to confirm that no contamination or anomaly has occurred.

2.7. Statistical analysis

Data for proliferation and apoptosis assays were analyzed by Student's *t* test or one-way analysis of variance, followed by Student–Newman–Keuls test, with GraphPad PRISM 3.0 (GraphPad Software, San Diego, CA).

3. Results

3.1. Cell proliferation

The proliferation of both human colon cancer cell lines HCT-116 ($P<.01$) and HT-29 ($P<.01$) was inhibited by the presence of LV in a dose-dependent manner (Fig. 2A). LV at a concentration of 5.93 μM decreased colon tumor cell growth by 25% and 21% in HCT-116 and HT-29 cells,

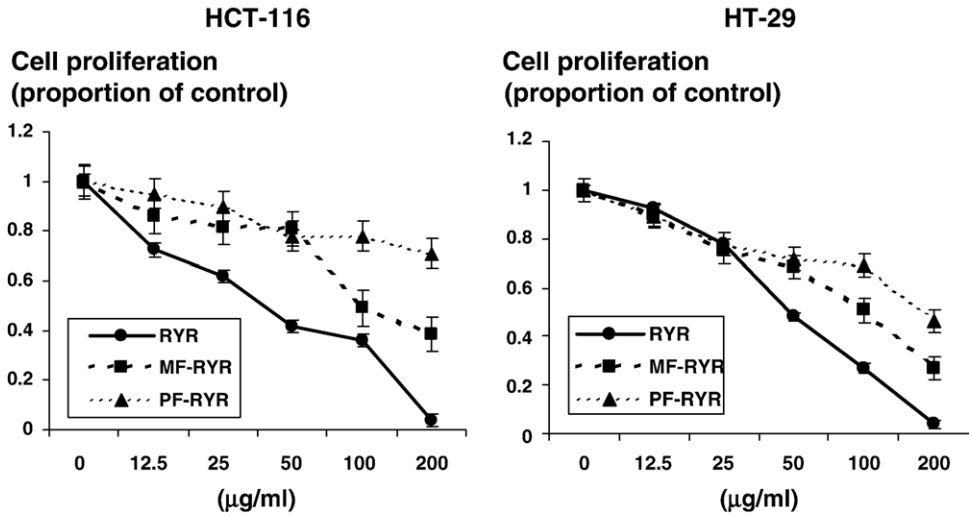

Fig. 4. PF-RYR, MF-RYR or RYR effect on colon cancer growth. PF-RYR or MF-RYR treatment for 48 h decreased cell proliferation in both HCT-116 ($P<.001$) and HT-29 cells ($P<.001$). However, the degree of antiproliferation was lower than that of RYR. Values are presented as mean±S.E.M. (n=3–6). The proportions of PF-RYR and MF-RYR were 10% and 90% of RYR by weight, respectively. For example, 50 μg/ml refers to 50 μg/ml RYR, 5 μg/ml PF-RYR or 45 μg/ml MF-RYR.

respectively ($P<.01$) (Fig. 2A). MK at a concentration of 5.93 μM is equivalent to that of 50 μg/ml RYR. Treatment with 50 μg of RYR for 48 h reduced tumor cell growth by 41% and 32% in HCT-116 and HT-29 cells, respectively ($P<.01$; Fig. 2B). RYR decreased colon cancer cell growth in a dose-dependent manner with 24, 48 and 72 h of treatment ($P<.001$; Fig. 2B). MK-free RYR treatment still decreased cell proliferation in HCT-116 cells at 50 μg/ml ($P<.001$), and in HT-29 cells at 100 μg/ml ($P<.01$; Fig. 2C). Addition of MV abolished the inhibitory activity of LV seen at 48 h at concentrations of 25 μM in HCT-116 cells ($P<.05$) and 50 μM in HT-29 cells ($P<.05$; Fig. 3A). However, the same concentration of MV had no effect on the antiproliferative activity of RYR at 48 h in both cancer cells (Fig. 3B).

In order to determine which fraction of RYR exhibited the greatest antiproliferative potential, the effects of MF-RYR or PF-RYR, as well as RYR, on tumor cell growth were compared. Both MF-RYR and PF-RYR inhibited cell growth in a dose-dependent manner in both HCT-116 and HT-29 colon cancer cells ($P<.001$; Fig. 4). However, the degree of antiproliferation was lower than that of RYR. Treatment of MV (25 μM) with MF-RYR (45 μg/ml) fully reversed its antiproliferation effect ($P<.01$) on HCT-116 cells and partially reversed its antiproliferation effect on HT-29 cells ($P<.05$; Fig. 5A). However, PF-RYR still suppressed tumor cell growth regardless of MV treatment ($P<.05$; Fig. 5B).

3.2. Apoptosis

An ELISA-based apoptosis assay, which quantitatively detects fragmented DNA, was used to measure the relative amount of apoptosis induction at 48 h. LV (5.93 μM) enhanced apoptosis in both HCT-116 and HT-29 cells by 3.8-fold and 1.6-fold, respectively ($P<.001$), and incubation with MV nullifies the proapoptotic action of LV (Fig. 6A). Visual inspection of the cells showed that RYR increased

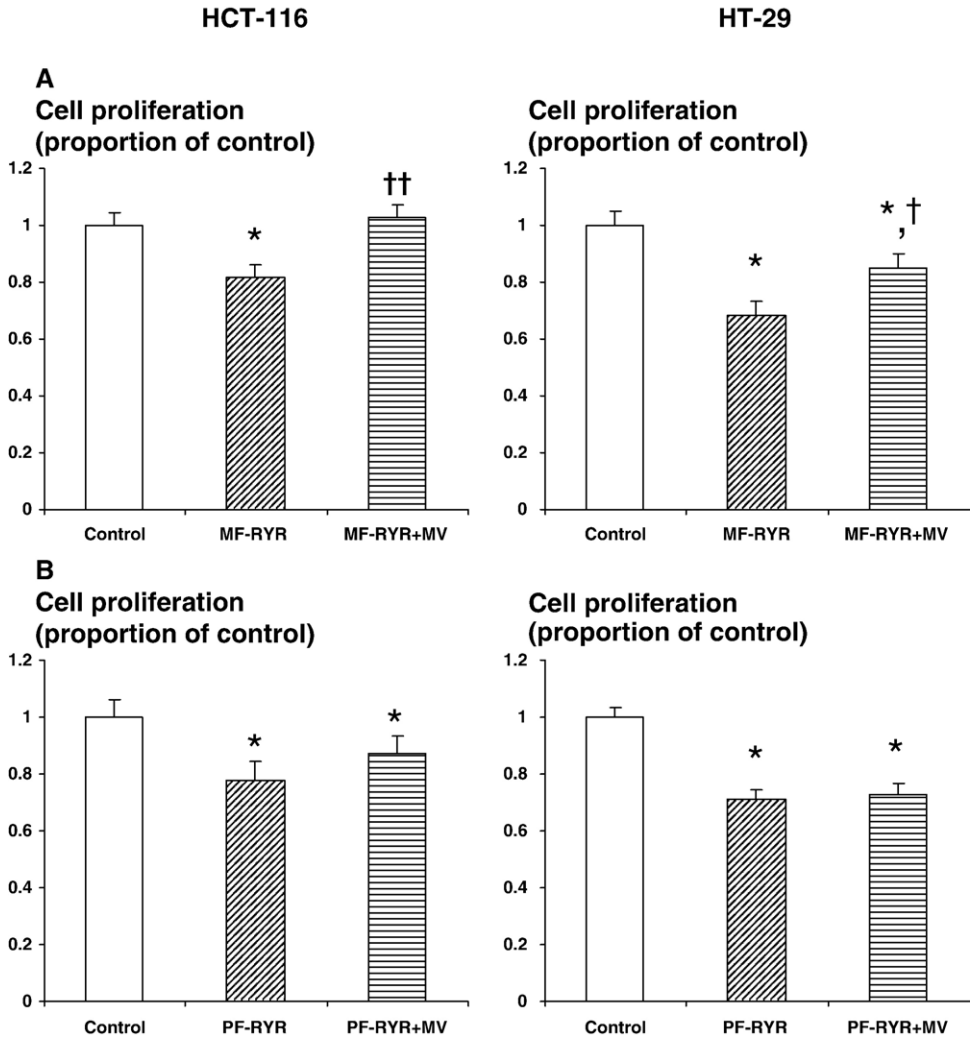

Fig. 5. MV effect on PF-RYR-treated or MF-RYR-treated colon cancer cell growth. (A) Treatment of MV (25 μM) with MF-RYR (45 μg/ml) fully reversed its antiproliferative effects ($P<.01$) on HCT-116 cells and partially reversed its antiproliferative effects on HT-29 cells ($P<.05$). (B) PF-RYR (5 μg/ml) still suppressed tumor cell growth regardless of MV treatment ($P<.05$). Control: 0.2% DMSO. Values are presented as mean±S.E.M. (n=3–6). *Significantly different from control at $P<.05$. †Significantly different from MF-RYR at $P<.05$. ††Significantly different from MF-RYR at $P<.01$.

dead (floating) cells, and the amount of rounding of the cells increased (data not shown). Apoptosis increased by 2.9-fold with RYR treatment at 50 μg/ml in HCT-116 cells ($P<.01$; Fig. 6B). Incubation with RYR and MV still increased apoptosis compared to controls ($P<.001$) in HCT-116 cells. MF-RYR fraction showed results similar to those of LV on apoptosis. MF-RYR increased apoptosis in both colon cancer cells by 3-fold and 1.7-fold, respectively ($P<.001$), and it was reversed by incubation with MV (Fig. 6C). PF-RYR enhanced apoptosis by 2.1-fold in HCT-116 cells ($P<.01$), and increase in apoptosis was also found with MV administration ($P<.05$; Fig. 6D).

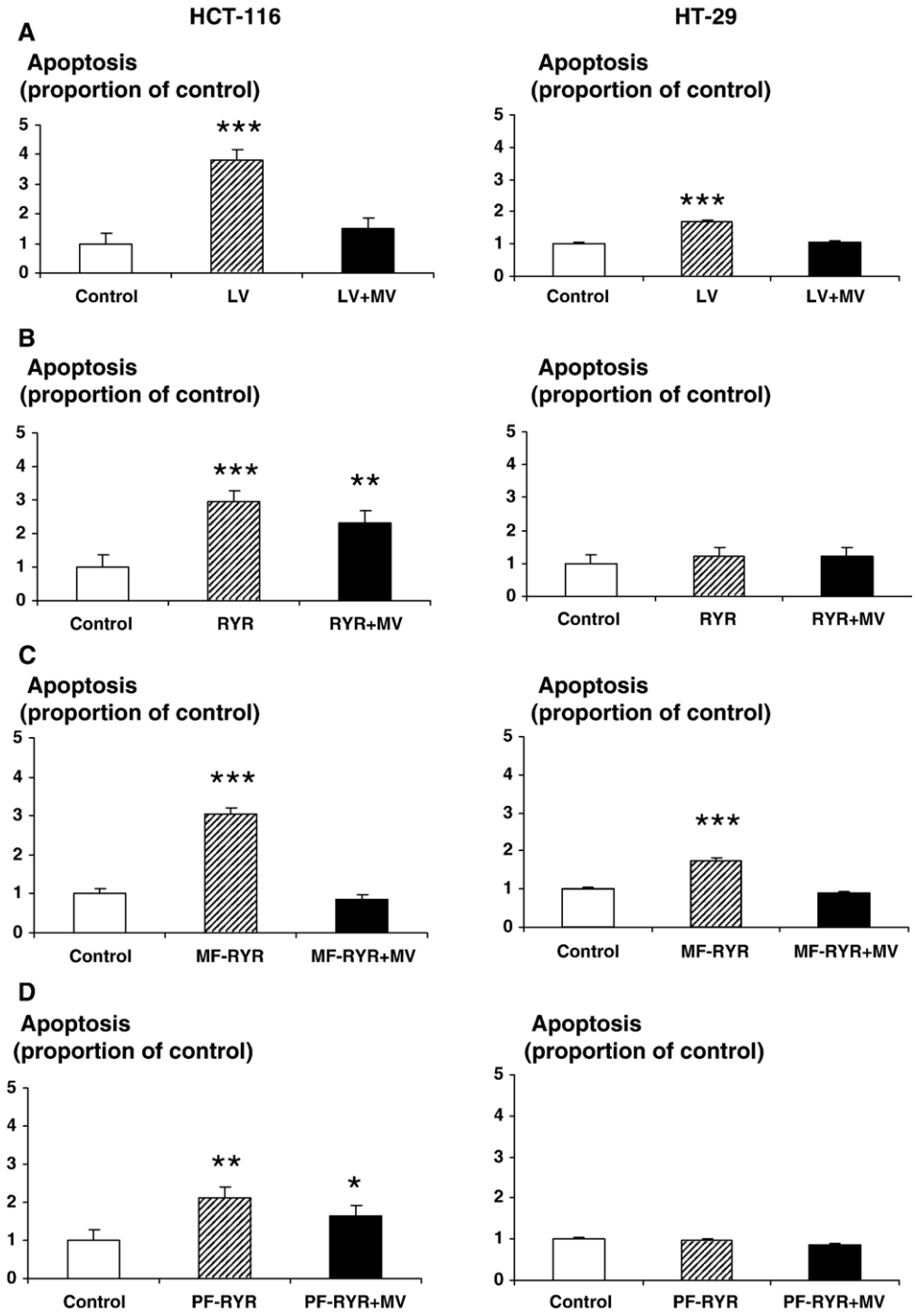

Fig. 6. LV, RYR, MF-RYR or PF-RYR effects on apoptosis. (A) LV (5.93 μM) enhanced apoptosis, and incubation with MV nullified the proapoptotic action of MK on HCT-116 and HT-29 cells ($P<.001$). (B) RYR increased apoptosis regardless of MV presence in HCT-116 cells ($P<.001$). In HT-29 cells, there was no effect of RYR with and without MV treatment. (C and D) The effect of MF-RYR on apoptosis was similar to that of LV, while the effect of PF-RYR on apoptosis was similar to that of RYR. Values are presented as mean±S.E.M. (n=3–4). *Significantly different from control at $P<.05$. **Significantly different from control at $P<.01$. ***Significantly different from control at $P<.001$.

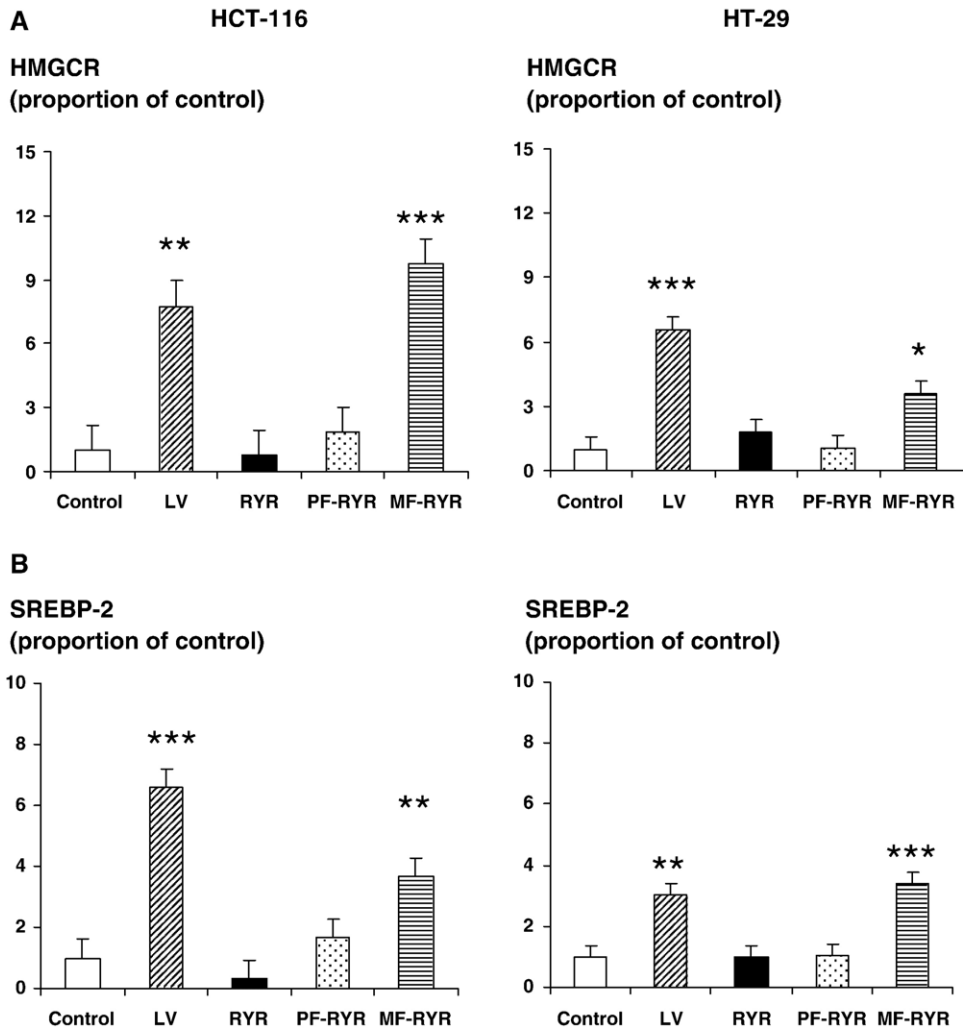

Fig. 7. LV, RYR, MF-RYR or PF-RYR effect on the transcription of HMGCR (A) and SREBP-2 (B). LV (5.93 μM) and MF-RYR (45 μg/ml) up-regulated HMGCR and SREBP-2 gene expressions in both HCT-116 and HT-29 cells. There were no significant effects of RYR (50 μg/ml) and PF-RYR (5 μg/ml) versus controls on mRNR levels of HMGCR and SREBP-2. Values are presented as mean±S.E.M. (n=3–6). *Significantly different from control at P<.05. **Significantly different from control at P<.01. ***Significantly different from control at P<.001.

3.3. HMGCR and SREBP-2 expression

LV treatment increased the mRNR level of HMGCR and SREBP-2 by more than sixfold in HCT-116 cells (P<.01) and by threefold in HT-29 colon cancer cells (P<.01; Fig. 7A and B). RYR and PF-RYR did not affect the expression of HGMCR and SREBP-2, compared to controls. MF-RYR increased the transcriptional level of HMGCR and SREBP-2 by more than threefold in HCT-116 and HT-29 colon cancer cells (P<.05; Fig. 7A and B).

4. Discussion

There is accumulating evidence that statins may reduce the risk of colon cancer based on in vitro [21–23] and in vivo [27–29] observations and population studies [24,30,31]. Our studies focused on the contributions of MK within RYR and elements in RYR other than MK. For this purpose, we prepared fractions of RYR without MK, a fraction rich in pigments and a fraction rich in monacolins but lacking pigments. In the two human colon cancer cell lines, the addition of 25 or 50 μM MV partly or fully reversed the antiproliferative and proapoptotic activities of LV. The selective reversal of the LV-mediated inhibition of proliferation and of the increase in apoptosis as a result of MV supplementation is simply due to the restoration of the de novo cholesterogenesis metabolic pathway. On the other hand, RYR's effect on cell proliferation and apoptosis was not affected by the addition of MV, even though RYR contained the same range of MK concentrations as the media containing MK alone. Furthermore, MK-free RYR still inhibited cell proliferation. These data suggest that RYR has effects on proliferation and apoptosis that are independent of MK in RYR. A matrix with other structural analogs and substances included pigments that were able to inhibit colon cancer cell proliferation and stimulate apoptosis.

RYR contains *Monascus* pigments as well as MK [7,8]. *Monascus* pigments comprise more than 10 compounds, six of which are well known: monascin, ankaflavin, monascorubrin, rubropunctatin, monascorubramin and rubropunctamin [32–36]. Recently, it has been reported that derivatives of *Monascus* pigments have antimicrobial activity [37,38] and that monascorubrin pigment inhibits skin cancer promotion in mice when applied topically or taken orally [39,40]. The anticancer effect of the pigments was also supported by our current experiment suggesting that the pigment-rich fraction of RYR showed antiproliferation and proapoptotic activities. While our studies clearly demonstrate that there are other factors besides MK that mediate some of the effects of RYR, further studies are needed to determine the effects of other active ingredients in RYR, including sterols, isoflavones and tannins, on colon cancer cell growth and apoptosis.

It has been reported that LV reduced DNA synthesis by a significant induction of p21WAF1/Cip1 protein expression in vascular smooth muscle cells [41], which may, in part, explain the potential mechanism of RYR in the inhibition of cancer cell growth. Simvastatin potentiates the apoptosis induced by tumor necrosis factor-α through the down-regulation of the nuclear factor κB signaling pathway in squamous cell carcinoma SCC4 cells [42], intestinal epithelial cells and colon cancer cells (COLO 205) [43]. It was also shown that LV decreased AKT protein expression in SCC6 cells [44], which suggests the involvement of PI-3 kinase signaling in apoptosis induction. Therefore, RYR, which naturally contains LV, may enhance apoptosis via the down-regulation of nuclear factor κB and PI-3 kinase/AKT signaling.

LV increased the transcription levels of HMGCR (the rate-limiting enzyme for cholesterogenesis) and SREBP-2 (the response element that binds to the promoter region of HMGCR). When cellular cholesterol levels are reduced following statin treatment, SREBPs are released from the endoplasmic reticulum membrane and translocate to the nucleus, where they activate SREBP target genes [45]. SREBP-2 primarily regulates the transcription of HMGCR [45,46]. The increase in HMGCR and SREBP-2 gene expressions is a compensatory response designed to restore reduced levels of cholesterol resulting from statin inhibition of HMGCR. In the present study, LV increased the expression of HMGCR and SREBP-2, while RYR did not increase the gene expression of HMGCR and SREBP-2. One of the merits in using RYR instead of LV is that it decreases cholesterol levels without elevating the gene expressions of HMGCR and SREBP-2.

Although the beneficial effects of statins are mediated by their lipid-lowering properties, experimental and clinical studies have suggested that statins also exhibit anti-inflammation activity [47–49]. Cyclooxygenase (COX-2) expression is closely related to the inflammation process and has an important role in colorectal tumorigenesis [50]. A synergistic interaction between COX-2 inhibitors and statins has been shown in colon cancer cells (colon-26 and CMT-93) [51] and mouse intestines [52]. The rationale for choosing the HCT-116 (no COX-2 expression) and HT-29 (COX-2 expression) [53] human colon adenocarcinoma cell lines was to determine whether the activity of RYR in colon cancer was associated with the inhibition of the COX-2-related inflammation process. In the present study, RYR inhibited colon cancer cell growth regardless of COX-2 expression. However, some differential responses to treatments were also observed. For example, RYR induced apoptosis in HCT-116 cells, but not in HT-29 cells. MK-free RYR still decreased cell proliferation in both cells, but the degree was much weaker in HT-29 cells. These suggest that, with or without COX-2, expression of the cell lines may partially cause different responses to LV and RYR treatments in the two cell lines; this should be further studied.

The results of the current study are significant enough to warrant further study. The mechanisms of RYR effect on apoptosis [such as via caspases 3, 8 and 9, and poly(ADP-ribose) polymerase cleavage] and RYR effect on the protein level of HMGCR and SREBP are under investigation. In vivo animal studies are needed to confirm whether RYR inhibits colon cancer risk primarily via the inhibition of de novo cholesterogenesis.

RYR, a traditional Chinese food herb and modern dietary supplement, has demonstrated in vitro effects, including inhibition of proliferation and stimulation of apoptosis, in human colon cancer cells by mechanisms involving MK and the red yeast pigment fraction. The multiple effects of RYR in vitro suggest that further investigation in animal models and, ultimately, in humans may be warranted given the unique profile of the actions of herbal supplements with multiple components versus purified crystallized drugs containing only a single component.

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