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Quercetin Inhibit Human SW480 Colon Cancer Growth in Association with Inhibition of Cyclin D_1 and Survivin Expression through Wnt/β-Catenin Signaling Pathway

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ABSTRACT

Aim: **The Wnt signaling pathway plays a pivotal role in cellular developmental processes and human carcinogenesis. The aim of this study was to investigate the effects of quercetin on the growth of the colon carcinoma cell line and the regulation effect of quercetin on the Wnt/β-catenin signaling pathway.** ***Methods:*** **MTT assay was used to determine the reduction of cell viability of quercetin on SW480 cells and clone 26 cells. The apoptotic rate and cell-cycle analysis after treatment with quercetin was determnined by flow cytometry. Effects of quercetin on mRNA expression of cyclin D_1 and survivin were detected by semiquantitative RT-PCR. After treatment with quercetin, the protein expression of cyclin D_1 and survivin in SW480 cells was analyzed by Western blot analysis. We built a Wnt/β-catenin signaling pathway reporter gene model. The regulation effect of quercetin on the Wnt/β-catenin signaling transcription was investigated by using this reporter gene model.** ***Results:*** **Quercetin reduced cell viability in a dose- and time-dependent manner in SW480 and clone 26 cells. The percentages of SW480 cells and clone 26 cells at G_2/M phase were increased significantly after treatment with 40∼80 μmol/Lquercetin for 48 hours. Quercetin induced the apoptosis of SW480 cells in a dose-dependent manner at the concentration of 20, 40, 60, anf 80 μmol/L. However, quercetin only induced the apoptosis of clone 26 cells at the concentration of 80 μmol/L. Quercetin downregulated transcriptional activity of β-catenin/Tcf in SW480 cells transiently transfected with the TCF-4 reporter gene. Within 24 hours of treatment, a 160-μmol/L concentration of quercetin reduced β-catenin/Tcf transcriptional activity by about 18-fold. Cyclin D_1 and the survivin gene were downregulated markedly by quercetin in a dose-dependent manner at both the transcription and protein expression levels.** ***Conclusion:*** **The results indicate that the molecular mechanism underlying the antitumor effect of quercetin in SW480 colon cancer cells is related to the inhibition of expression of cyclin D_1 and survivin as well as the Wnt/β-catenin signaling pathway. Therefore, the Wnt/β-catenin signaling pathway could be qualified as one of the promising targets for innovative treatment strategies of colorectal cancer.**

Keywords: Quercetin, colon cancer, Wnt/β-catenin signaling pathway, cyclin D_1, survivin
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INTRODUCTION

The Wnt signaling pathway, also called the APC/β-catenin/Tcf pathway, plays a pivotal role in cellular developmental processes and human carcinogenesis (1–3). Wnts activated transmembrane frizzled receptors and the disheveled protein, leading to inhibition of glycogen synthase kinase 3β (GSK-3β) activity. β-catenin plays a dual role in cells. It associates with the cytoplasmic domain of E-cadherin and α-catenin in order to link these proteins to the actin cytoskeleton (4) and is involved in the Wnt signaling pathway to transactivate

T-cell factor (Tcf)/lymphocyte enhancing factor (Lef) transcription factors in the nucleus (5). Some of the genes activated by β-catenin/Tcf signaling are c-jun, c-myc, fibronectin, cyclin D_1, and fra-1. etc. (6–8). In normal cells, most β-catenin protein is present in cell-cell junctions with very little in the cytoplasm or nucleus. Cytosolic β-catenin is phosphorylated by Axin–adenomatous polyposis coli (APC)-GSK-3β complex and recognized by β-TrCP, an F-box component of the E3 ubiquitin ligase complex that promotes ubiquitination of β-catenin and increases its susceptibility to degradation by a ubiquitin-proteasome system (9). If Wnt signaling is activated, β-catenin degradation is inhibited due to the decreased ability of GSK-3β to phosphorylate β-catenin and β-catenin translocates into the nucleus to transactivate the Tcf/Lef transcription factor, leading to the upregulation of many genes responsible for cell proliferation.

Activated β-catenin/Tcf signaling by the accumulation of β-catenin in the cells has been implicated in human carcinogenesis. This accumulation may result from the mutation of either the β-catenin gene itself or the tumor suppressor gene, APC. Actually, the APC gene or serine–threonine phosphorylation sites for the GSK3β within exon 3 of the β-catenin gene are mutated in many cancer cells, including colorectal cancer (CRC), melanoma, hepatocellular carcinoma, and gastric carcinoma, and the transcriptional activity of β-catenin is upregulated in these cancer cells (10–12). More recently, approximately half of the largest group of sporadic colorectal cancers and CRC cell lines lacking APC mutations were shown to possess somatic mutations in the β-catenin gene (13, 14). This means that the dysregulation of β-catenin plays a crucial role in colon cancer cells. Therefore, we hypothesized that reduced β-catenin/Tcf transcriptional activity may lead to suppressed tumor growth.

Flavonoids are a group of more than 4000 polyphenolic compounds that occur naturally in foods of plant origin. They have been shown to possess a variety of biological activities at nontoxic concentrations in organisms. The role of dietary flavonoids in cancer prevention is widely discussed. Quercetin, the major representative of the flavonol subclass of flavonoids, is an integral part of the human diet and the average human intake has been estimated to be 25 mg/day. In animals developing aberrant crypt foci by the application of a chemical carcinogen, a diet supplemented with 2% quercetin markedly reduced the development of the initial stages of colorectal cancer development (15). In addition, it exerts an antiproliferative effect *in vitro* on colonic cancer cell lines of diverse lineages (16, 17). Despite these promising actions of quercetin as significant antitumor activities, its molecular mechanisms underlying these effects are generally unknown. To elucidate why intake of quercetin is disadvantageous to colonic cancer cells, we investigated the effect of quercetin on β-catenin/Tcf signaling, which is particularly important in colon cancer. In this paper, we describe the evidence that quercetin could downregulate expression of cyclin D_1 and surviving, and its inhibitory mechanism is related to the decreased β-catenin/Tcf signaling.

MATERIALS AND METHODS

Cell lines and reagents

Quercetin (Q0125,MW:338.27) was purchased from Sigma. Stock solutions (50 mmol/L) were made in dimethyl sulfoxide (DMSO). TOPFLASH, FOPFLASH, and pRL-null plasmid were provided by Dr. Hong Ma (American Chemical Genomics Institute). 3-(4,5-dimethyiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and propidium iodide were obtained from Sigma (St. Louis, MO, USA). RNase, sodium dodecyl sulfate (SDS), proteinase K, trypsin, and agarose were purchased from Sino-American Biotec Co. (Beijing, Beijing, China) RPMI 1640 medium was purchased from GIBCO. Fetal bovine serum (FBS) was obtained from Hangzhou Sijiqing Biotec Co. (Hangzhou, Zhejiang, China).

Cell culture

Human colon cancer SW480 cells and mouse colorectal cancer clone 26 cells, obtained from the Shanghai Cell Database of Chinese Academy of Sciences, were cultured in RPMI 1640 medium supplemented with 10% (v/v) FBS, 100 units/mL of penicillin, and 100 μg/mL streptomycin at 37°C in a humidified, CO_2-controlled (5%) incubator.

MTT assays

The cell viability was determined by MTT assay. The SW480 cells or clone 26 cells in the exponential phase of growth were harvested and seeded in 96-well plates (Costar, USA) at a density of 50,000 cells per well and cultured for 24 hours. Quercetin (10, 20, 40, 80, and 160 μmol/L) and control (phosphate-buffered saline; PBS) was then added into the wells and incubated continuously for 24, 48, and 72 hours at 37°C with 5% CO_2. A 10-μL sample of MTT solution (5 g/Ldissolved in PBS) was added to each well and the plates were incubated at 37°C for 4 hours. The supernatant was discarded and 150 μL of dimethylsulfoxide was added to dissolve the blue insoluble MTT formazan produced by mitochondrial succinate dehydrogenase. The absorbance was measured at 570 nm in a spectrophotometer (Zhengzhou Bosai Biotech Co., Zhengzhou, Henan, China), the negative control wells contained medium only. All determinations were performed in quadruplicate, and each experiment was repeated at least three times.

Determination of cell cycle and apoptosis by flow cytometric analysis

After coculturation with different concentration of quercetin (10, 20, 40, 60, and 80 μmol/L) for 48 hours, SW480 or clone 26 cells were harvested by centrifugation, washed with ice-cold PBS once, and fixed in 70% ethanol at 4°C overnight. The cells were then washed once with ice-cold PBS and resuspended in PBS (pH 7.4), 20 mg/L RNase, and 50 mg/L of propidium iodide at room temperature for 1 hour and 10 minutes, respectively. Finally, cells were analyzed by flow cytometry on a Epics-XL

(Becton Coulter Corporation, Miami, FL, USA), using the Single Histogram Statistic software to determine the percentage of the apoptotic cells and the proportion of cells in G_0/G_1, S, and G_2/M phases of the cell cycle.

Western blot analysis

Expression of cyclin D_1 and survivin on the whole-extract proteins in SW480 cells were performed by Western blot analysis. The whole-cell protein was obtained by lysing the cells on ice for 20 minutes in 700 μL of lysis buffer (0.05 mol/L Tris-HCl, pH 7.4, 0.15 mol/L NaCl, 1% Nonidet P-40, 0.5 mol/L PMSF, 50 μg/mL aprotinin, 10 μg/mL leupeptin, 50 μg/mL pepstatin, 0.4 mmol/L sodium orthovanadate, 10 mmol/L sodium fluoride, and 10 mmol/L sodium pyrophosphate) (Sigma, Sino-American Co., Shanghai, China). The lysates were then sonicated for 20 seconds and spun at 15,000*g* for 10 minutes, and the supernatant was saved. Protein determinations were made by using the method of Bradford. The supernatants were analyzed by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under the following conditions. Separated proteins (50 μg) were mixed 1:1 with 2× sample buffer (20% glycerol, 4% SDS, 10% 2-ME, 0.05% bromophenol blue, and 1.25 mol/L Tris–HCl; pH 6.8), loaded onto a 10% SDS-PAGE gel and run at 120 V for 2 hours. Cell proteins were transferred to a polyvinylidene fluoride (PVDF) membrane (Promega Co., Sino-American Co., Beijing, China) for 3 hours at 250 mA. The PVDF membrane was then blocked with 5% milk in TBST overnight, washed four times, and then incubated with the primary antibody (Ab) (anticyclin D_1 and survivin antibody, Santa Cruz Biotechnology, Santa Cruz, California, USA) for 2 hours at room temperature. The blots were washed four times with TBST and incubated for 1 hour with horseradish peroxidase (HRP)-conjugated anti-IgG Ab (Santa Cruz Biotechnology). Immunoreactive bands were developed by using the ECL system (Santa Cruz). An autoradiograph was obtained with exposure times of 1–10 minutes. The images were analyzed by using the gel-pro Analyzer 3.1 software (Bethesda, MD, USA).

RT-PCR analysis

Total RNA was isolated from cancer cells by using TRIZOL isolation reagent (Gibco BRL, Catlsbad, CA, USA), according to the manufacturer's instructions. RNA concentration was measured spectrophotometrically at 260 nm and was seperated on 1% agarose gel to check integrity by estimating the ratio of 18S/28S rRNA. The polymerase chain reaction (PCR) was carried out by use of a set of forward and reverse primers (Table 1). cDNA was synthesized by the reverse transcription of 2 μg of total RNA at 37°C for 45 minutes. First-strand cDNA was then performed by using a reverse transcriptase (RT)-PCR kit (Sino-American Co., China) in a 30-μL reaction volume, following the manufacturer's instructions. After initional denaturation for 5 minutes at 94°C the amplification were carried out for 30 cycles as follows: denaturation at 94°C for 30 seconds, annealing at 60°C for 45 seconds, and extension at 72°C for 1 minute, followed by 10 minutes at 72°C. All PCR products were analyzed by electrophoresis on a 1.5% agarose gel and photographed. The sequences of primers (Sino-American Co., China) used in the RT-PCR are shown in Table 1.

Table 1. Cyclin D_1, surviving, and β-actin primer sequence

Gene	Primer sequence	Product size (bp)
Cyclin D_1		
Sense	5′-ATGCCAACCTCCTCAACGACC-3′	512
Anti-sense	5′-TGGCACAGAGGGCAACGAAGG-3′	
Survivin		
Sense	5′-AGCCCTTTCTCAAGGACCAC-3′	432
Anti-sense	5′-GCACTTTCTCCGCAGTTTCC-3′	
β-actin		
Sense	5′-ATCTGGCACCACACCTTCTACAA TGAGCTGCG-3′	838
Anti-sense	5′-CGTCATACTCCTGCTTGCTG ATCCACATCTGC-3′	

Potential contamination by genomic DNA was checked for by running the reactions without RT and using β-actin primers in subsequent PCR amplifications. Only RNA samples that showed no bands after that procedure were used for further investigation.

Transfection and luciferase assay

Transient transfection was performed by using lipofectamine and plus reagent (Invitrogen, Carlsbad, CA, USA). Briefly, 1.5 × 10^6 SW480 cells were seeded in the medium onto a 60-mm dish. After 24 hours, cells were transfected with 1.0 μg of the luciferase reporter constructs (0.8 μg TOPFLASH or FOPFLASH plus 0.2 pRL-null plasmid; Figure 1). After 4 hours of post-transfection, quercetin (40, 80, and 160 μmol/L) was added with a medium containing FBS. Cells were incubated for 24 hours, lysed in Passive Lysis Buffer (Promega, Madison, WI, USA), and collected for assays of luciferase activity, using the Dual-Luciferase Reporter Assay System (Promega).

RESULTS

Quercetin inhibited the proliferation of SW480 cells and clone 26 cells

To investigate putative growth modulating effects of quercetin in colonic cancer cells, cell proliferation assays were performed with SW480 cells and clone 26 cells. The proliferation of SW480 cells was significantly inhibited in dose- and time-dependent manner by 40, 80, and 160 μmol/L of quercetin for 24, 48, and 72 hours of incubation. The inhibitory fraction of SW480 cells exposed to 40, 80, and 160 μmol/L of quercetin for 72 hours was 38.08, 70.14, and 87.96%, respectively (Figure 2). The proliferation of clone 26 cells was significantly inhibited in a dose- and time-dependent manner by 20, 40, 80, and 160 μmol/L of quercetin for 48 and 72 hours of incubation, and 80 and 160 μmol/L of quercetin for 24 hours incubation, respectively.

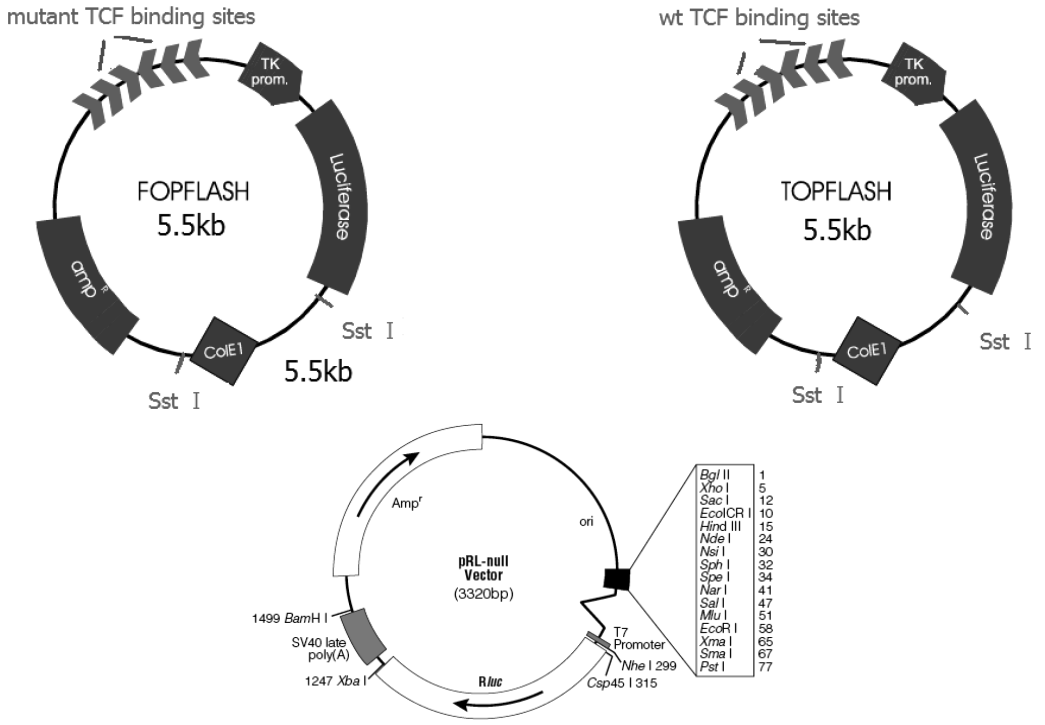

Figure 1. Plasmid circle map and sequence reference points of TOPFLASH, FOPFLASH, and pRL-null (vehicle).

Quercetin blocked the cell cycle of SW480 cells and clone 26 cells at G_2/M–phase

To investigate if the observed growth-inhibiting effects of quercetin on colon cancer cells were caused by cell-cycle arrest, cell-cycle analysis was performed. The proportion of cells in the G_2/M-phase of the cell cycle was significantly increased, and the G_0/G_1- and S-phases of cells were significantly decreased after sustained incubation of SW480 cells with quercetin (20, 40, 60, and 80 μmol/L) for 48 hours and clone 26 cells with quercetin (60 and 80 μmol/L) for 48 hours, respectively. These data suggested that quercetin inhibited the cell proliferation via G_2/M-phase arrest (Figure 3).

Quercetin induced the apoptosis of SW480 cells and clone 26 cells

To check whether the antiproliferative effects of quercetin were caused not only by cell-cycle arrest, but also by an induction of programmed cell death, the apoptotic rate was investigated in SW480 cells and clone 26 cells by flow cytometry. Quercetin induced the apoptosis of SW480 cells in a dose-dependent manner at the concentration between 40 and 80 μmol/Lfor 48 hours. The apoptotic rate of SW480 cells treated with quercetin are markedly higher than the control ($P < 0.01$). However, quercetin only induced the apoptosis of clone26 cells at the concentration of 80 μmol/L (Table 2).

Quercetin downregulated the β-catenin/Tcf signaling

To investigate whether quercetin modulates β-catenin/Tcf signaling, we used SW480 human colon cancer cells transiently transfected with wild and mutant TCF-4 genes (Figure 1). The transcriptional activity of β-catenin/Tcf is constitutively active in the SW480 cell line. We used reporter gene constructs containing two copies of either an optimized wild (TOPflash) or mutant (FOPflash) Tcf-binding element. SW480 cells were cotransfected with TOPflash or FOPflash and pRL-null to normalize for transfection effciency and sequentially treated with quercetin. Figure 4 shows that quercetin suppresses the Tcf transcriptional activity in the SW480 cell line in a concentration-dependent manner. Within 24 hours

Table 2. Effects of quercetin on apoptotic rate of SW480 cells and clone 26 cells ($\bar{x} \pm s$, $n = 3$)

Groups (μmol/L)		Apoptotic rate (%) SW480 cells	Clone 26 cells
Control		2.68 ± 1.04	1.61 ± 1.02
Quercetin	10	2.74 ± 1.82	2.02 ± 1.32
	20	3.91 ± 2.65	2.73 ± 1.59
	40	9.23 ± 2.74^c	4.10 ± 2.62
	60	12.59 ± 4.65^c	3.16 ± 2.82
	80	13.32 ± 4.62^c	5.37 ± 1.42^b

$^bP < 0.05$, $^cP < 0.01$, vs. control group.

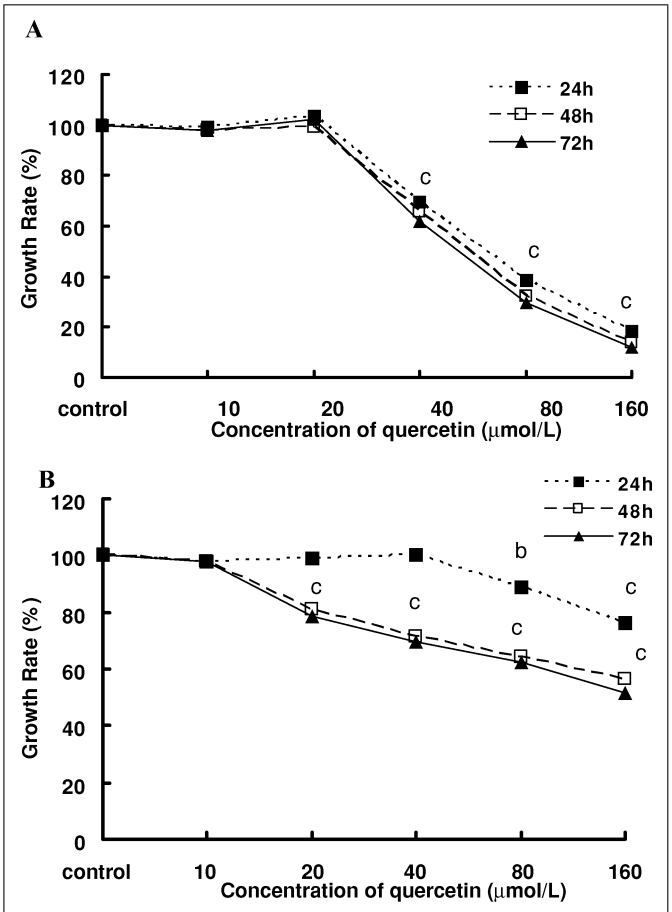

Figure 2. Growth curve of SW480 cells (**A**) and clone 26 cells (**B**) treated with quercetin ($n = 3$). $^bP < 0.05$, $^cP < 0.01$ vs. control group.

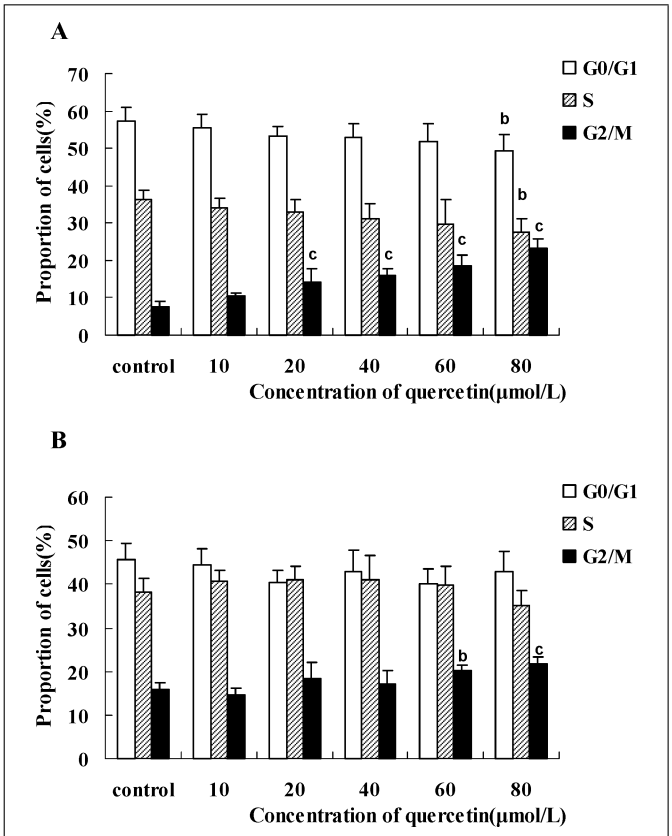

Figure 3. Effects of quercetin on cell cycle of SW480 cells (**A**) and clone 26 cells (**B**) ($\bar{x} \pm s$, $n = 3$). $^bP < 0.05$, $^cP < 0.01$ vs. control group.

of treatment, the 160-μmol/L concentration of quercetin reduced β-catenin/Tcf transcriptional activity from 195.65 $\pm$ 12.25 of the control to 11.54 $\pm$ 5.60, whereas the FOPflash activity, a mutant for β-catenin/Tcf binding, remained unchanged after quercetin treatment. Quercetin treatment decreased Tcf signaling by about 18-fold. This proposes that the functional binding of β-catenin/Tcf may be important for TOPflash. It is of note that the results presented in Figure 4 show that quercetin is also a good inhibitor of β-catenin/Tcf signaling.

Quercetin downregulated the expression of cyclin D_1 and survivin gene and protein

We investigated whether quercetin affects the expression of cyclin D_1 and survivin in SW480 cells. It is well known that cyclin D_1 and survivin are β-catenin/Tcf target genes (18, 19). Therefore, we hypothesized that quercetin would downregulate cyclin D_1 and survivin gene expression by inhibiting β-catenin/Tcf signaling. In SW480 cells, as we expected, cyclin D_1 and survivin gene transcription and proteins expression (Figure 5–10) were downregulated markedly by quercetin in a dose-dependent manner. These results indicate that the function of β-catenin, as a transcription activator, is rendered inoperative by the inhibitor.

DISCUSSION

Flavonoids constitute one of the largest groups of naturally occurring phenols and are commonly present in plants

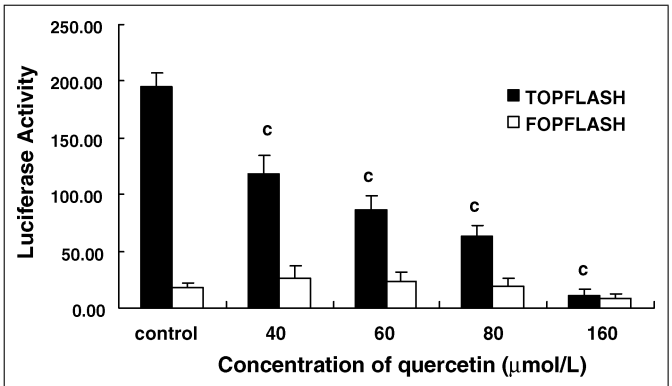

Figure 4. Effect of quercetin on transcriptional activity of β-catenin/Tcf in SW480 cells ($\bar{x} \pm s$, $n = 3$). SW480 cells were cotransfected with reporter genes harboring Tcf-binding sites (TOPflash) or mutant Tcf-binding sites (FOPflash), repectively. $^bP < 0.05$, $^cP < 0.01$ vs. control group.

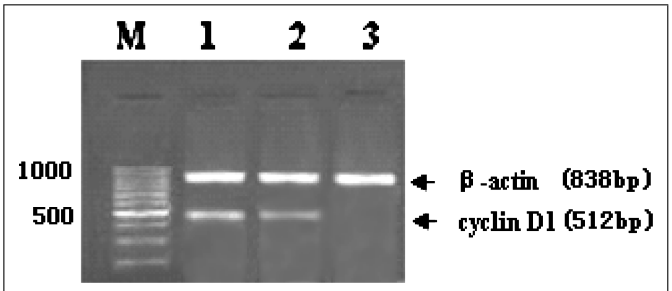

Figure 5. β-catenin/Tcf target gene, cyclin D_1 mRNA expression in SW480 cells treated with quercetin, was detected by RT-PCR analysis. M, marker; 1, control; 2, 60 μmol/L quercetin; 3, 120 μmol/L quercetin. The picture is representative of three independently repeated experiments.

as glycosides. These compounds possess a common phenylbenzopyrone structure (C6–C3–C6), and they are categorized into flavones, flavanols, isoflavones, flavonols, flavanones, and flavanonols (20). These dietary anti oxidants are well known to exert significant antitumor effects and have been extensively reviewed (21, 22).

Here, we tested the effect of quercetin, which is the most famous one of various flavonoids, on the growth inhibition and against the transcriptional activity of β-catenin/Tcf. Quercetin has demonstrated chemopreventive activity in a variety of laboratory animal models, including azoxymethane (AOM)-induced colonic tumorigenesis in mice and rats, dimethylbenz[α]anthracene (DMBA), and N-nitrosomethylurea (NMU)-treated mammary glands of rats (23). In this paper, our data show that an increase of the G_2/M phase in colonic cells was noted in our experimental cells treated with quercetin. Yang et al. (24) also found that quercetin inhibitied lung cancer cell growth via G_2/M arrest. In contrast, Yoshida et al. (25) and Hosokawa et al. (26) reported that quercetin arrested leukemic and colonic cell growth in cancer cell lines at the late G_1 phase. It was found by Wang et al. (27) that the percentage of SW480 cells in the G_2/M phase decreased at doses of greater than 40 μmol/L of quercetin.

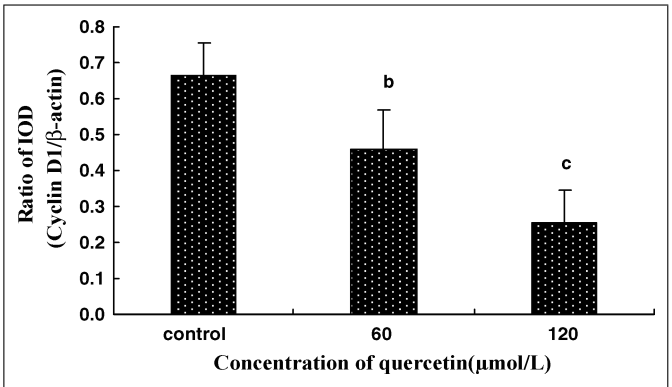

Figure 6. Results of cyclin D_1 mRNA expression in SW480 cells treated with quercetin was analyzed with semiquantitative analysis of RT-PCR ($\bar{x} \pm s$, $n = 3$). $^bP < 0.05$, $^cP < 0.01$ vs. control group.

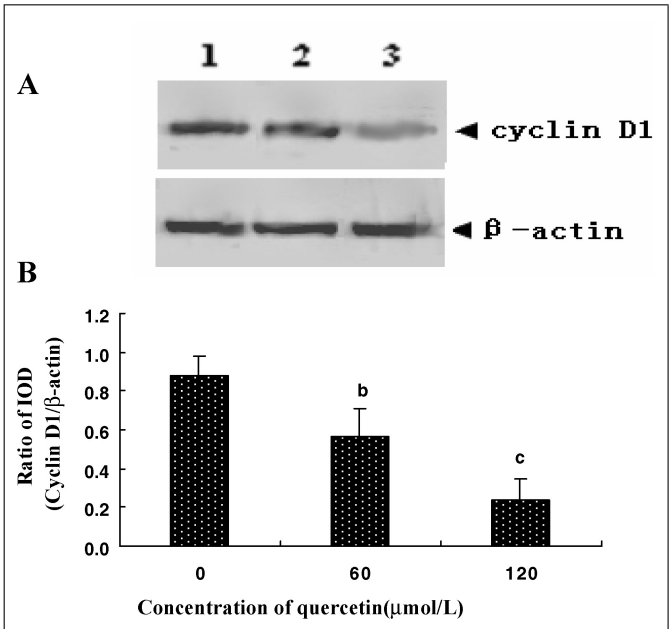

Figure 7. β-catenin/Tcf downstream protein, cyclin D_1 expression in SW480 cells treated with quercetin, was analyzed by Western blot analysis. (**A**) lane 1. Control, lanes 2~3. quercetin at 60 and 120 μmol/L, respectively. (**B**) Relative expression level of cyclin D_1 protein analyzed with Gel-Pro Analyzer Software. Quercetin downregulates the protein expression of cyclin D_1 in SW480 cells in a dose-dependent manner ($\bar{x} \pm s$, $n = 3$). $^bP < 0.05$, $^cP < 0.01$ vs. control group.

Quercetin induced a delay in the G_2/M phase of the cell cycle. These arresting effects of quercetin were similar to the traditional anticancer drugs, vinorelbine and vincristine, in colonic cancer cells. Moreover, for ovarian cancer, a positive correlation between G_2/M-phased fraction and the response to anticancer agents has been documented (28). Hence, the recruitment of cancer cells to the G_2/M phase of the cell cycle by quercetin might sensitize colonic cancer cells to reinforced response to

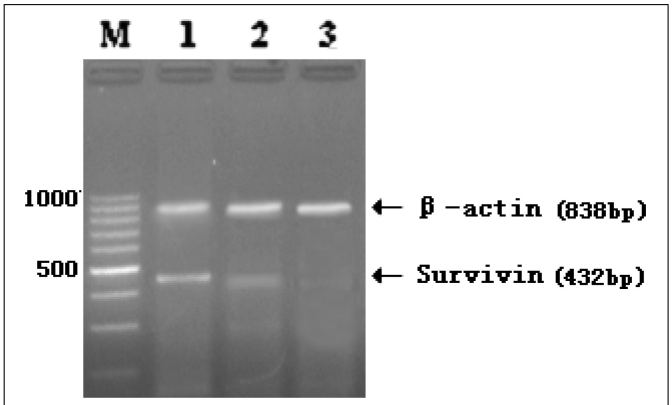

Figure 8. Survivin mRNA expression in SW480 cells treated with quercetin was detected with RT-PCR analysis. M, marker; 1, control; 2, 60 μmol/L quercetin; 3, 120 μmol/L quercetin. The picture is representative of three independently repeated experiments.

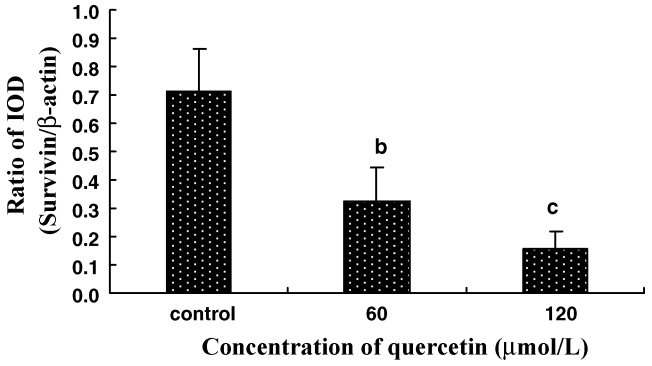

Figure 9. Results of survivin mRNA expression in SW480 cells treated with quercetin was detected by semiquantitative analysis of RT-PCR ($\bar{x} \pm S$, $n = 3$). $^bP < 0.05$, $^cP < 0.01$ vs. control group.

established chemotherapeutic drugs. In addition to the antiproliferative action of quercetin on its own, possible synergistic effects of quercetin and anticancer drugs will be interesting to investigate.

Besides its cell-cycle-interfering effects, quercetin was shown to induce apoptosis in SW480 cells and clone 26 cells, as assessed by flow cytometry. The precise molecular modes of action of quercetin in altering the death program of colonic cells remain to be elucidated. The apoptosis-inducing action of quercetin has been also observed *in vivo* in regenerating liver after partial hepatectomy (29) and in cells of disparate lineages, including colonic adenocarcinoma cell lines (30, 31). Wei et al.

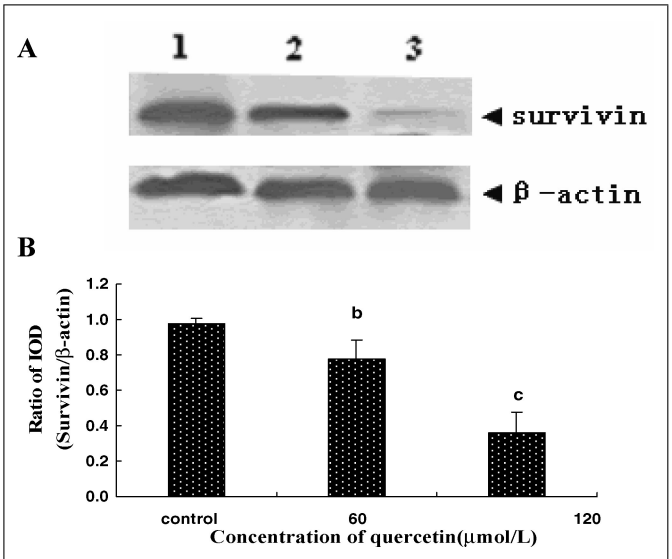

Figure 10. β-catenin/Tcf downstream protein, survivin expression in SW480 cells treated with quercetin, was detected by Western blot. (**A**) lane 1. control, lane 2∼3. Quercetin at 60 and 120 μmol/L, respectively. (**B**) Relative expression level of survivin protein analyzed with Gel-Pro Analyzer Software (Bethesda, MD, USA). Quercetin downregulates the protein expression of survivin in SW480 cells in a dose-dependent manner ($\bar{x} \pm s$, $n = 3$). $^bP < 0.05$, $^cP < 0.01$ vs. control group.

(32) and Nagasaka and Nakamura (33) have observed that the apoptotic action of the flavonoid on tumor cells is related to the inhibition of the synthesis of heat-shock proteins, a class of bioactive molecules known to play an important role in cell survival. Further, since quercetin was shown to be an effective protein kinase inhibitor (33), an alternative possibility is that the death-inducing action of this compound resides, at least partly, in promoting the dephosphorylation of key proteins involved in the control of apoptotic events, which is needed to be further studied in the future.

The elevated β-catenin/Tcf signaling is an important event in the genesis of a number of malignancies, such as colon cancer. Mutations in the regulatory region of β-catenin or loss of APC function have been identified in human colon cancers (10). Activation of an abnormal APC/β-catenin/Tcf signaling pathway and alterations in cellular adhesion mediated through changes in β-catenin homeostasis within the colonic epithelium are initiating factors in the development of the majority of colorectal cancers (10, 31). Here, we hypothesized that the antitumor effects of quercetin in colon cancer are mediated by its ability to downregulate β-catenin/Tcf signaling. Studies on the inhibitory agent against β-catenin/Tcf signaling in cancer cell lines have been performed. Dashwood et al. (34) reported that EGCG inhibited β-catenin/Tcf activity in HEK293 cells transiently transfected with a constitutively active mutant β-catenin gene. In addition, it was revealed by Jaiswal et al. (35) that curcumin inhibited the transcriptional activity of β-catenin/Tcf so as to induce growth arrest and apoptosis in HCT116 colon cancer cells. Recently, Orner et al. (36) suggested that a combination of tea plus sulindac was highly effective at inhibiting intestinal neoplasia male Apc^{min} mice via direct or indirect effects on the β-catenin/APC pathway. As the importance of β-catenin as a cause of tumorigenesis increases, many more studies on the β-catenin inhibitor and its inhibitory mechanism are being conducted. Our data on luciferase activity show that quercetin inhibits the transcriptional activity of β-catenin/Tcf in SW480 colon cancer cells in a concentration-dependent manner. Further, it is well known that the cyclin D_1 and survivin are β-catenin/Tcf target genes (18, 19). Therefore, we investigated whether quercetin affects the expression of the β-catenin/Tcf target gene in SW480 cells. As we expected, quercetin downregulated cyclin D_1 and survivin mRNA expression and protein expression by inhibiting β-catenin/Tcf signaling. This is an intriguing observation, since altered cyclin D_1 expression and activity have been related, although not consistently, to early stages of colonic tumorigenesis. It may be that the sustained *in vitro* exposure of colonic cells to quercetin was a promoting factor. Taken together, these results strongly suggest that the inhibitory mechanism of quercetin is related to β-catenin itself or the downstream components of the β-catenin/Tcf pathway. This paper reveals the molecular mechanism underlying the antitumor effect of quercetin by suppressing the Wnt/β-catenin/Tcf signal pathway via inhibiting the transcriptional activity of β-catenin/Tcf in SW480 colon cancer cells.

Although the present results show a significant suppression of tumor growth *in vitro* by quercetin, it should be pointed

out that the concentrations of quercetin used in this study are very high, which will later make the *in vivo* application be a little difficulty. However, compared with other *in vitro* reports, the similar high concetrations were used in colorectal cancer cells. For example, Kim et al. reported that quercetin at 0–100 μmol/L inhibited HT-29 colon cancer cell growth (37). Recently, clinical trials exploring different schedules of administration of quercetin have been hampered by its extreme water insolubility requiring dissolution in DMSO. There are concerns about using higer doses of DMSO, as it causes dose-dependent hemolysis, it is harmful to the liver and kidneys (38). A potential solution to this problem is to improve its solubility by pharmaceutical modification; it was revealed by Yuan et al. (38) that liposomal quercetin is a perfect type of preparation, which could overcome this limitation. In addition, perhaps the additive induction by the combination with other anticancer drugs at lower doses will be achieved, which suggest that quercetin may cooperatively protect against colorectal cancer through conjoint action (27). Quercetin has also been used in clinical trials in cancer patients (39). Since cancer is a major public health issue and flavonoids are consumed daily, further clarification of the full biological and biochemical effects of quercetin in humans is warranted.

CONCLUSION

To conclude, our data show that quercetin inhibits proliferation, induces apoptosis, and causes cell-cycle arrest of SW480 cells and clone 26 cells. Cyclin D_1 and survivin expression could be downregulated markedly by quercetin at both the mRNA and protein level. The antitumor mechanism of quercetin may be related to the inhibited expression of cyclin D_1 and survivin via the Wnt/β-catenin signaling pathway. Therefore, we think that the Wnt/β-catenin signaling pathway may qualify as promising targets for innovative treatment strategies of colorectal cancer.

DECLARATION OF INTEREST

The authors report no conflict of interest. The authors alone are responsible for the content and writing of this paper.

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