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Differential Effects of Theaflavin Monogallates on Cell Growth, Apoptosis, and *Cox-2* Gene Expression in Cancerous *versus* Normal Cells[1]

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ABSTRACT

Theaflavin (TF-1), theaflavin-3-monogallate and theaflavin-3′-monogallate mixture (TF-2), and theaflavin-3,3′-digallate (TF-3) are the major black tea polyphenols. Here we compared the effects of these polyphenols on cell growth, apoptosis, and gene expression in normal and cancerous cells. We showed that TF-2 (10–50 μM) inhibited the growth of SV40 transformed WI38 human cells (WI38VA) and Caco-2 colon cancer cells but had little effect on the growth of their normal counterparts. The IC_{50}s of TF-2 for the growth inhibition of WI38 and WI38VA cells were, respectively, 300 and 3 μM. The other two black tea polyphenols, TF-1 and TF-3, did not exhibit such differential growth-inhibitory effect. TF-2, but not TF-1 or TF-3, induced apoptosis in transformed WI38VA cells but not in normal WI38 cells, suggesting that apoptosis was responsible, at least in part, for the differential growth-inhibitory effect of TF-2. Cox-2 has been implicated in intestinal carcinogenesis. Among the tea polyphenols tested, TF-2 and, to a lesser degree, (−)-epigallocatechin gallate inhibited *cyclooxygenase (Cox)-2* gene expression. TF-2 at 50 μM completely blocked the serum-induced *Cox-2* gene expression at both mRNA and protein level. Other genes, including c-*fos,* c-*myc, thymidine kinase, proliferating cell nuclear antigen, BRCA1, BRCA2,* and *Cox-1,* were not significantly affected by TF-2. These findings suggest that TF-2 may be responsible, at least in part, for the chemopreventive activity in black tea extracts.

INTRODUCTION

Epidemiological studies suggest that tea may have a protective role against certain human cancers (reviewed in Refs. 1–3). Catechin polyphenols in green tea have been shown to inhibit the proliferation of cultured mammalian cells including colon carcinoma, lung carcinoma, breast carcinoma, melanoma, and leukemic cells (4, 5). We have reported that EGCG, a major green tea catechin polyphenol, inhibits the growth of human tumor cells, including Caco-2 colorectal cancer cells, Hs578T breast cancer cells, and SV40-transformed WI38 cells but has little or no inhibitory effect on the growth of their normal counterparts (6). Black tea extract has been shown to be potent in inhibiting tumorigenesis in animal model systems, including skin (7), lung (8), colon (9, 10), esophagus (11), and mammary gland (10, 12). The major black tea polyphenols, TF-1, TF-2, and TF-3, are biochemical oxidation products derived from green tea polyphenols and are responsible for the characteristic color, fragrance, and taste of black tea (1–3). TF-3 has been shown to be as potent as EGCG in inhibiting the growth of human A431 carcinoma cells (13). The biological effects of each individual black tea polyphenol have not been compared or studied in detail at the molecular level. In this study, we compared the effects of TF-1, TF-2, and TF-3 on cell proliferation, apoptosis, and gene expression in cancerous human cells (WI38VA and Caco-2 colon cancer cells) and in their normal counterparts (WI38 diploid fibroblasts, CCD-33Co, and FHC colorectal cells). We found that, among the three black tea polyphenols tested, only TF-2 exhibited a clear differential growth-inhibitory and apoptotic effect toward cancerous cells.

The two isoforms of cyclooxygenase, constitutive Cox-1 and inducible Cox-2, are key enzymes for prostaglandin biosynthesis (reviewed in Ref. 14). An elevation of the Cox-2 activity has been associated with certain pathological processes, including colon cancer (14–17). A direct link between Cox-2 expression level and polyps formation has been demonstrated in APC knockout mice, suggesting that Cox-2 plays an important role in colon cancer formation (15). In light of the important role of the *Cox-2* gene in intestinal carcinogenesis and other inflammatory processes (14–17), we have also examined whether any of the tea polyphenol may affect the expression of Cox-2 and other growth-related genes in colon cancer cells. We found that TF-2 specifically inhibited *Cox-2* gene expression at both the mRNA and protein level.

Materials and Methods

Materials. DMEM and fetal bovine serum were obtained from Life Technologies, Inc. (Gaithersburg, MD). Other chemicals were from Sigma Chemical Co. (St. Louis, MO). [α-^{32}P]dATP (>3000 Ci/mmol) was purchased from ICN Chemical (Radioisotope Division, Irvine, CA). Theaflavin polyphenols were isolated and purified from black tea powder as described previously (18). The structures of these compounds are shown in Fig. 1. The TF-2 used in this study contains two theaflavin monogallate isomers.

Tissue Culture. Normal human WI38 (cell strain AG06814E; PDL=16) and the SV40 virally transformed WI38 cells (cell strain AG07217) were obtained from Coriell Institute for Medical Research (Camden, NJ). Human colon cancer cell, Caco-2 (ATCC HTB-37), normal human colon cells CCD-33Co (ATCC CRL-1539), and FHC (ATCC CRL-1831) were purchased from American Type Culture Collection (Rockville, MD). Cells were cultured in Dulbecco's medium containing 10% fetal bovine serum at 37°C, 5% CO_2. For proliferation assay, cells were plated at about 2×10^5 cells per 35-mm dish, with or without tea polyphenol, and the number of viable cells, as determined by trypan blue dye exclusion, was counted under a phase contrast microscope. To alleviate the concern of the degradation of theaflavins during incubation, we also replenished the culture with fresh growth medium containing the tea chemical once every other day. We did not find any significant difference in the results obtained by either method. Proliferation of human fibroblasts was also estimated by a crystal violet staining method. Cells were seeded in a standard 24-well tissue culture plate at about 1×10^5 cells/ml in the presence of black tea polyphenol. On the 4th or 5th day after plating, cells were fixed with 5% trichloroacetic acid and stained with Bacto Gram Crystal Violet solution (Difco, Detroit, MI). The staining intensity of the fibroblast culture correlated well with the number of viable cells in the culture as determined by cell counting (data not shown).

TUNEL Assay. Apoptosis was analyzed by TUNEL assay as described previously (6). Briefly, cultures at ~90% confluency were treated with TF-2 (100 μM) for 18 h. Cells were fixed with 4% of formaldehyde solution, washed, and incubated in a buffer containing fluorescein-12-dUTP and terminal deoxynucleotidyl transferase for 1 h. The nuclei of apoptotic cells exhibited green fluorescence using an FITC filter under fluorescent microscope.

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[3] The abbreviations used are: EGCG, (−)-epigallocatechin gallate; COX, cyclooxygenase; TF-1, theaflavin; TF-2, theaflavin-3-monogallate and theaflavin-3′-monogallate mixture; TF-3, theaflavin-3,3′-digallate; ATCC, American Type Culture Collection; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling; RT-PCR, reverse transcription-PCR; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; TK, thymidine kinase; PCNA, proliferating cell nuclear antigen.

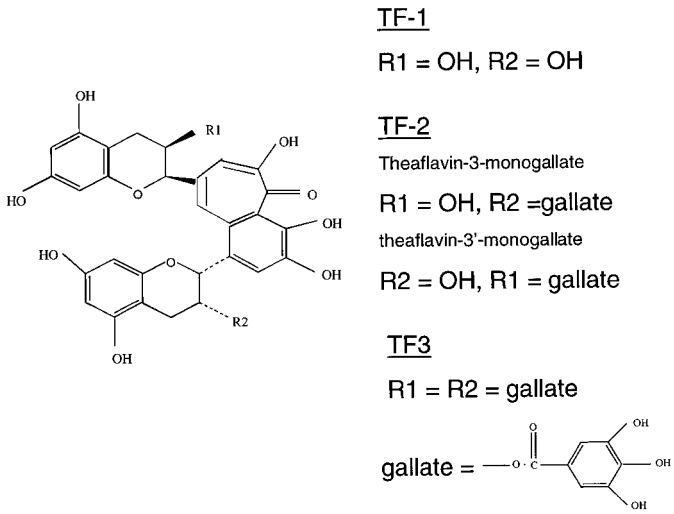

Fig. 1. Chemical structures of TF-1, TF-2, and TF-3.

DNA Fragmentation Analysis. Confluent cultures were treated with tea chemicals at different concentrations for various times. Cells were harvested and suspended in a lysis buffer [10 mM Tris-HCl (pH 8.0), 100 mM NaCl, 25 mM EDTA, 0.5% SDS, and 100 μg/ml proteinase K] for 20 h at 37°C. DNA was extracted with a phenol-chloroform mixture, precipitated by ethanol, dried, and dissolved in a TE buffer. RNA was digested with 2 μg/ml of RNase Cocktail (Ambion, Austin, Texas). The DNA samples were analyzed by electrophoresis on a 1% agarose gel containing ethidium bromide (0.5 μg/ml).

Northern Blot Analysis. Total RNA samples were resolved by electrophoresis on 1% agarose-formaldehyde gel (6 μg/lane) and transferred onto a nylon membrane. Northern blot analysis was performed as described previously (19).

RT-PCR. Serum, growth factors, cytokines, or phorbol esters can induce *Cox-2* gene expression (20, 21). In this study, fresh serum was used to induce *Cox-2* and other growth-related genes in quiescent cultures. Confluent cultures were serum-deprived for 48 h to become quiescent and then stimulated with 10% fresh fetal bovine serum as described previously (6). Tea chemicals at various concentrations were added to the culture immediately after serum stimulation. No significant morphological change of Caco-2 cells was observed, indicating that Caco-2 cells did not undergo differentiation during serum deprivation (data not shown). Cells were harvested at indicated times for total RNA preparation using RNeasy Total RNA kit (Qiagen, Chatsworth, CA). Total RNA (1 μg) was reverse transcribed by incubating with SuperScript RNase H reverse transcriptase (Life Technologies, Inc., Grand Island, NY) using Oligo(dT)$_{12-18}$ as primer. For PCR amplification, gene-specific primers used are listed below: GAPDH sense, 5′-TGAAGGTCGGAGTCAACGGATTTGGT-3′; GAPDH antisense, 5′-CATGTGGGCCATGAGGTCCACCAC-3′; BRCA1 sense, 5′-CTCTGGGAAAGTATCGCTGTCATG-3′; BRCA1 antisense, 5′-AGAGGCATCCAGAAAAGTATCAGG-3′; BRCA2 sense, 5′-TGCTGCCAGTAGAAATTCTC-3′; BRCA2 antisense, 5′-CTTTGTCCAAAGATTCCTTTG-3′; ODC sense, 5′-AATCAACCCAGCGTTGGACAA-3′; ODC antisense, 5′-ACATCACATAGTAGATCGTCG-3′; TK sense, 5′-AGCACAGAGTTGATGAGACGC-3′; TK antisense, 5′-GCTTCCTCTGGAAGGTCCCAT-3′; PCNA sense, 5′-ACGTCTCTTTGGTGCAGCTC; PCNA antisense, 5′-CAAGTTGTTCAACATCTAAATCCATC-3′; COX1 sense, 5′-GTTCAACACCTCCATGTTGGTGGAC-3′; COX1 antisense, 5′-TGGTGTTGAGGCAGACCAGCTTC-3′; COX-2 sense, 5′-TTCAAATGAGATTGTGGGAAAAT-3′; COX-2 antisense, 5′-AGATCATCTCTGCCTGAGTATCTT-3′; c-myc sense, 5′-CAGGATCCGTGCATCGACCCCTCGGTG-3′; c-myc antisense, 5′-CGCCTAAGCTTTGACATTCTCCTCGGTG-3′; c-jun sense, 5′-CCAAGATCCTGAAACAGAGCATG-3′; c-jun antisense, 5′-TCCGAGTTCTGAGCTTTCAAGGT-3′; c-fos sense, 5′-ATGATGTTCTCGGGCTTCAACGCAG-3′; and c-fos antisense, 5′- CCGAAGAAGCCAGGCTCTAGTTAGCG-3′.

PCR was performed under conditions that allowed the amounts of PCR products to be proportional to the amounts of input RNA. GAPDH was used as an internal control. The PCR products were analyzed by electrophoresis on 1% agarose gel containing 0.5 μg/ml ethidium bromide.

Western Blot Analysis. Cells after various treatment were harvested in a lysis buffer [150 mM NaCl, 100 mM Tris (pH 8.0), 1% Tween 20, 1 mM EDTA, 50 mM DDT, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, and 10 μg/ml leupeptin]. The cell lysates were sonicated and centrifuged at 11,000 $\times$ *g* for 10 min. The supernatant containing 30 μg of protein was analyzed on a 10% SDS-PAGE under reducing conditions. The gel was transferred onto a nitrocellulose membrane, and the membrane was probed with anti-Cox-2 antibody (Cayman Chemical, Ann Arbor, MI) at 1:1000 dilution. The affinity purified goat antirabbit IgG conjugated to horseradish peroxidase (Bio-Rad Laboratories, Hercules, CA) was used as secondary antibody. The hybridized protein bands were detected using the ECL kit (Amersham Pharmacia, Piscataway, NJ).

RESULTS

Differential Inhibitory Effect of TF-2 on the Growth of WI38 and WI38VA Cells. WI38 human diploid fibroblasts have a finite life span (22), whereas WI38VA, the SV40 virally transformed WI38 cells, are immortal and cancerous in nude mice (23). The dose-response effects of three black tea polyphenols on the growth of these two cell types were qualitatively compared by a crystal violet staining method. As shown in Fig. 2, only TF-2 exhibited a differential growth-inhibitory effect. Thus, TF-2 at 10 μM prominently inhibited the proliferation of WI38VA cells but had little or no inhibitory effect on the growth of normal WI38 cells. TF-1 at 50 μM inhibited the growth of both WI38 and WI38VA cells to the same extent, and TF-3 at 50 μM inhibited the growth of WI38 cells but not the growth of WI38VA cells. We then examined the effect of TF-2 on the growth kinetics and the morphology of WI38 and WI38VA cells. Fig. 3*A* shows clearly that TF-2 affected the growth rate of WI38 and WI38VA cells differently. Although the growth of WI38 cells was not significantly affected by TF-2 at 50 μM, TF-2 at 10 μM completely blocked the growth of WI38VA cells, consistent with the crystal violet staining data as shown in Fig. 2. The IC_{50} of TF-2 for the growth inhibition was estimated to be 300 μM for WI38 and 3 μM for WI38VA cells, a difference of two orders of magnitude. Fig. 3*B* shows that although TF-2 did not affect the growth and viability of WI38 cells, it did cause a slight morphological change in the treated

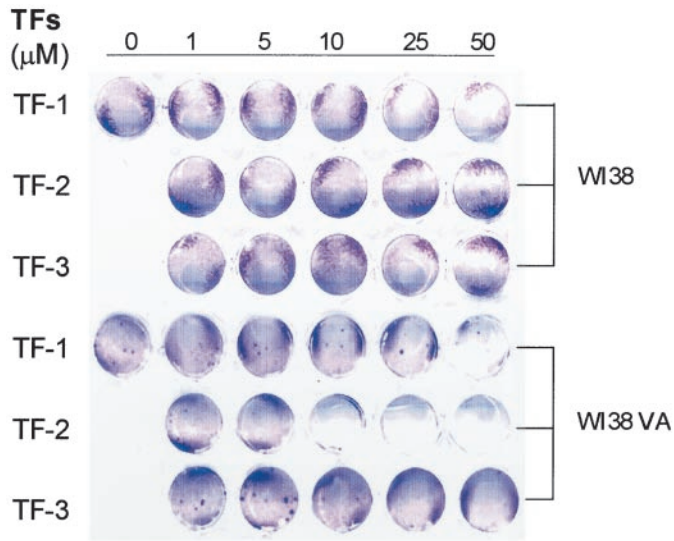

Fig. 2. Comparison of the effect of black tea polyphenols, TF-1, TF-2, and TF-3, on the proliferation of WI38 and WI38VA cells. Cells were seeded at 1 $\times$ 10^5 cells/ml in a standard 24-well tissue culture plate and then treated with 0, 1, 5, 10, 25, and 50 μM TF-1, TF-2, or TF-3. Cells were fixed with trichloroacetic acid and stained with crystal violet 4 days after plating.

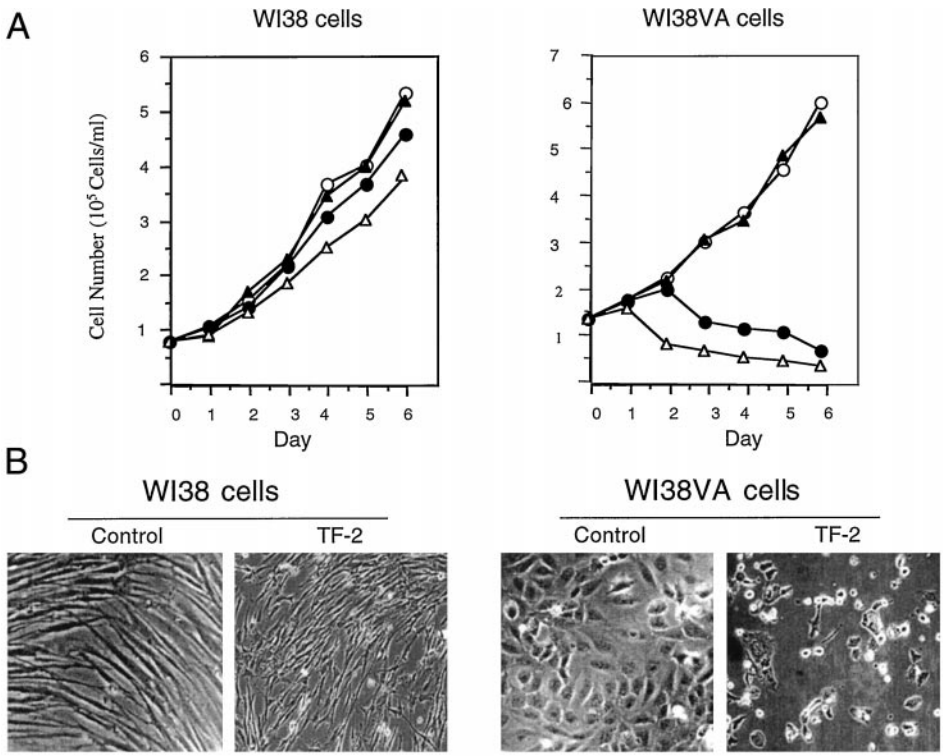

Fig. 3. *A*, effect of TF-2 on the growth rate of normal WI38 and SV40 transformed WI38VA cells. Cells were plated in a 35-mm dish on day 1 in the absence (○) or presence of 1 μM (▲), 10 μM (●), and 50 μM (△) of TF-2. The viable cells were counted at the indicated times. Each *point* represents an average of three separate dishes. *B*, WI38 and WI38VA cells were cultured in the absence (control) or presence 10 μM TF-2. Phase contrast micrographs from representative fields were taken 5 days after the treatment.

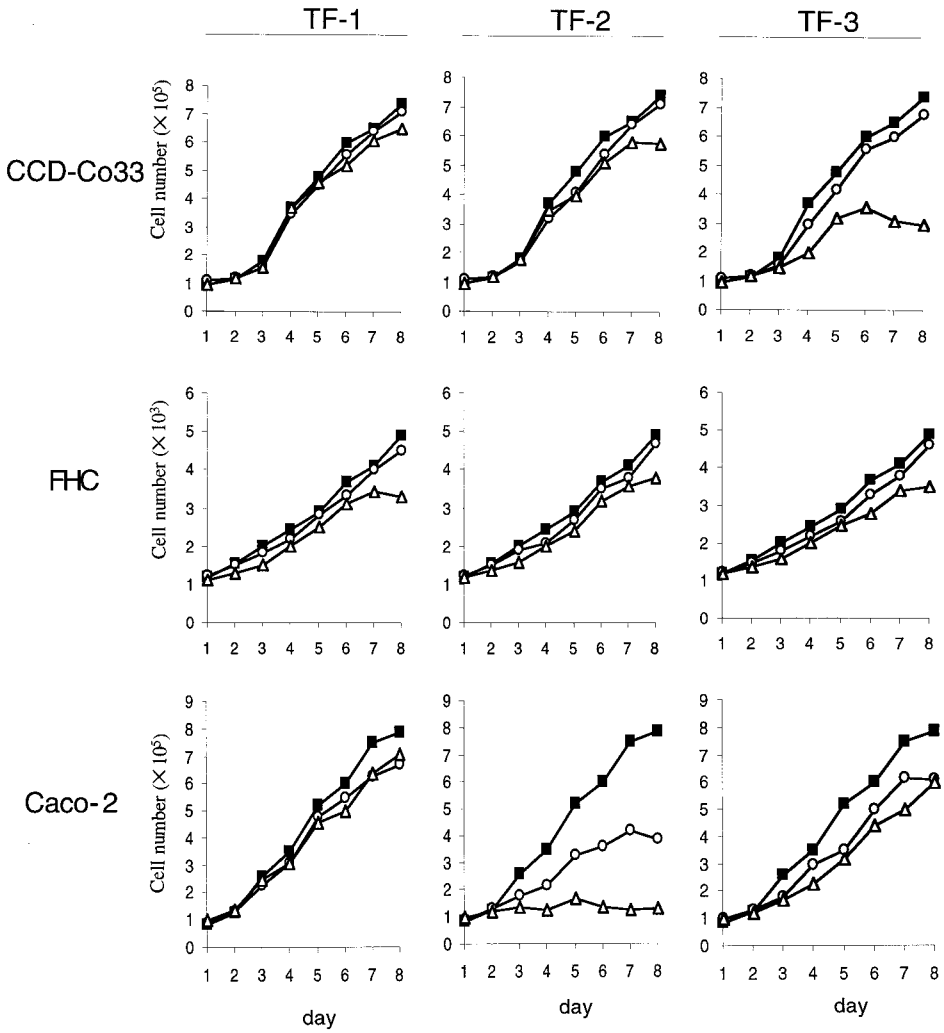

Fig. 4. Effect of three black tea polyphenols, TF-1, TF-2, and TF-3, on the growth kinetics of CCD-33Co colon cells, FHC colon cells, and Caco-2 colon cancer cells. Cells were plated in a 35-mm dish on day 1 in the absence (■) or presence of 10 μM (○) and 50 μM (△) of TF-1, TF-2, or TF-3. The viable cells were counted at indicated times. Each *point* represents an average of two separate dishes.

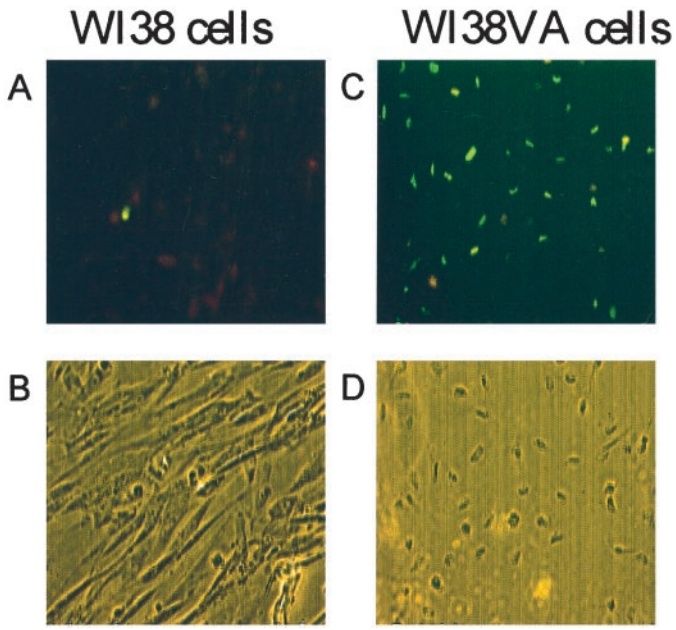

Fig. 5. TUNEL assay of the effect of TF-2 on apoptosis. WI38 and WI38VA cells at 90% confluency were treated with TF-2 at 100 μM for 18 h. Apoptotic cells were detected by labeling with fluorescein-12-dUTP using terminal deoxynucleotidyl transferase. The labeled cells were detected by a fluorescence microscope using an FITC filter (*A* and *C*). All cells, including apoptotic ones, in the cultures were also stained by propidium iodide and detected by a fluorescence microscope using a rhodamine filter (*B* and *D*).

cells (Fig. 3*B, left panels*). In the WI38VA cultures treated with TF-2, very few viable cells could be detected (Fig. 3*B, right panels*).

Effect of Black Tea Theaflavins on the Growth of Normal and Cancerous Colon Cells. Caco-2 colon cancer cells and CCD-33Co colorectal cells have been used as a normal and cancerous pair to study the biological effects of EGCG (6). Because CCD-33Co cells appear to be fibroblastic (data not shown), we also included the FHC normal human colon cell strain (epithelial origin; Ref. 24) in the present study. Fig. 4 shows the effects of the three black tea theaflavins on the growth of normal (CCD-33Co and FHC) and cancerous colorectal cells. TF-2 at 50 μM inhibited the growth of Caco-2 but had little effect on the growth of CCD-33Co or FHC cells. TF-1 and TF-3 did not exhibit such a differential growth-inhibitory effect.

Differential Effect of TF-2 on the Induction of Apoptosis. Because apoptosis could be a major cause for growth inhibition, we examined whether TF-2 may induce apoptosis differently in normal and transformed cells. We first used the TUNEL assay to examine this possibility. Fig. 5 shows that TF-2 caused almost every cell in the WI38VA culture to become apoptotic, as indicated by the green fluorescence attributable to fluorescein-12-dUTP labeling (Fig. 5, *C versus D*). In contrast, almost no cells in the normal WI38 culture exhibited green fluorescence after TF-2 treatment (Fig. 5, *A versus B*). We next compared the effects of the three black tea polyphenols on apoptosis using DNA fragmentation analysis. Fig. 6 shows that both TF-1 and TF-3 did not induce any appreciable DNA fragmentation in either WI38 or WI38VA cells (Fig. 6, *A* and *C*). In contrast, TF-2 caused an extensive DNA fragmentation in transformed WI38VA cells but not in WI38 cells (Fig. 6*B*). The propensity of transformed WI38VA for undergoing apoptosis in the presence of TF-2 could explain, at least in part, why TF-2 preferentially inhibited the growth of transformed cells. Because TF-1 and TF-3 have been reported to be capable of inducing apoptosis in human lymphoid leukemia cells and stomach tumor cells (25), the efficacy of tea polyphenol on apoptosis may be cell type dependent.

Effect of TF-2 on *Cox-2* Gene Expression. In light of the potential role of the *Cox-2* gene in colon cancer carcinogenesis, we have examined the effect of TF-2 and other tea polyphenols on *Cox-2* gene expression. Fig. 7*A* shows that TF-2 at 50–100 μM prominently suppressed the *Cox-2* gene expression in Caco-2 cells. TF-1 and TF-3 did not appear to have any significant effect on *Cox-2* gene expression. EGCG, a green tea polyphenol, was less potent than TF-2 in suppressing *Cox-2* gene expression.

We next examined the effect of TF-2 on the time course of *Cox-2* gene expression. Fig. 7*B* shows that the *Cox-2* mRNA was detectable in quiescent Caco-2 cells, consistent with the notion that colon cancer cells have elevated *Cox-2* gene expression (Fig. 7*B, Lane 1*). TF-2 not

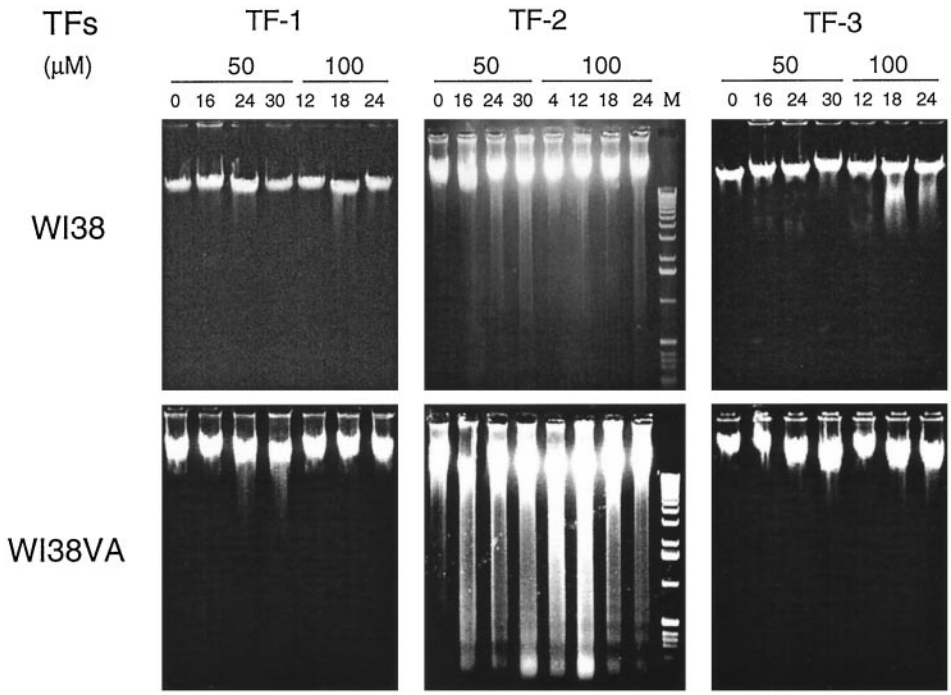

Fig. 6. DNA fragmentation analysis. WI38 and WI38VA cells at were treated with TF-1 (*A*), TF-2 (*B*), and TF-3 (*C*) at 50 or 100 μM. The cells were harvested at the indicated times, and the formation of a DNA internucleosomal ladder was monitored by agarose gel electrophoresis. *M*, DNA size markers.

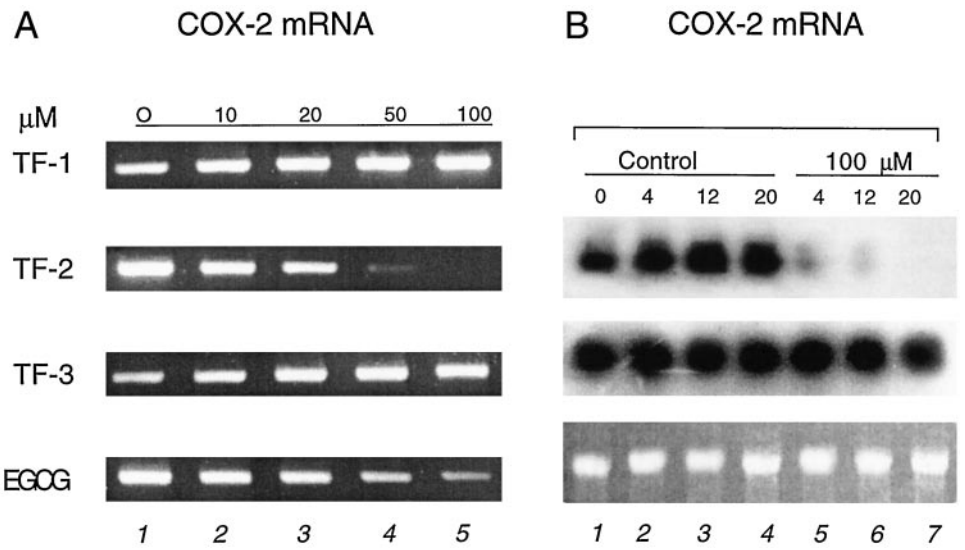

Fig. 7. *A*, effects of theaflavin chemicals and EGCG on the expression of the *Cox-2* gene in Caco-2 cells. Confluent cultures of Caco-2 cells were serum deprived for 48 h and then stimulated with 10% of fetal bovine serum in the presence of various tea polyphenol at indicated concentration for 4 h. *Cox-2* gene expression was determined by RT-PCR as described in "Materials and Methods." *B*, time course of *Cox-2* gene expression in Caco-2 cells. Cells at 90% confluency were serum-deprived for 36 h and then replenished with complete growth medium containing 10% fetal bovine serum, without (control) or with TF-2 at 100 μM. The cells were harvested at the indicated times for total RNA preparation. RNA samples were analyzed by Northern blot analysis as described in "Materials and Methods." GAPDH and 28S rRNA were used as internal standards.

only blocked the serum-induced increase in *Cox-2* gene expression but also abolished the basal level of *Cox-2* mRNA (Fig. 7*B*, *Lanes 5–7 versus Lanes 2–4*). Unlike Caco-2 cells, *Cox-2* mRNA was not detectable in quiescent WI38 or WI38VA cells (Fig. 8*A*, *Lanes 1* and *8*). The addition of fresh serum to these two fibroblastic cultures prominently induced the appearance of a 4.5-kb *Cox-2* transcript (Fig. 8*A*, *Lane 2 versus Lane 1* and *Lane 9 versus Lane 8*). However, the levels of induced *Cox-2* mRNA in WI38VA cells were much higher and more sustained than that in WI38 cells, suggesting that *Cox-2* mRNA may be more stable in transformed WI38VA cells (Fig. 8*A*, *Lane 10 versus Lane 3*). Again, TF-2 blocked the serum-induced increase in *Cox-2* gene expression in both WI38 and WI38VA cells (Fig. 8*A*, *Lanes 4–7* and *Lanes 11–14*). Consistent with the notion that *Cox-2* is transcriptionally regulated, Fig. 8*B* shows that TF-2 at 40 μM reduced the Cox-2 protein level in WI38 cells by ~50% (Fig. 8*B*, *Lane 4 versus Lane 1*) and completely eliminated Cox-2 protein in WI38VA cells (Fig. 8*B*, *Lane 9 versus Lane 6*).

Effect of TF-2 on the Expression of Growth-related Genes. To determine whether the effect of TF-2 on *Cox-2* gene expression could be a part of global suppression of serum-inducible genes, we examined the effect of TF-2 on the expression of other important genes. We included in this study growth-related genes such as c-*fos*, c-*myc*, *TK*, and PCNA. We also included *Cox-1* and the breast cancer-related tumor suppressor genes, *BRCA1* and *BRCA2*. Fig. 9 shows that among all of these genes, the only one that was dramatically attenuated by TF-2 was *Cox-2*. The constitutive *Cox-1* gene was completely insen-

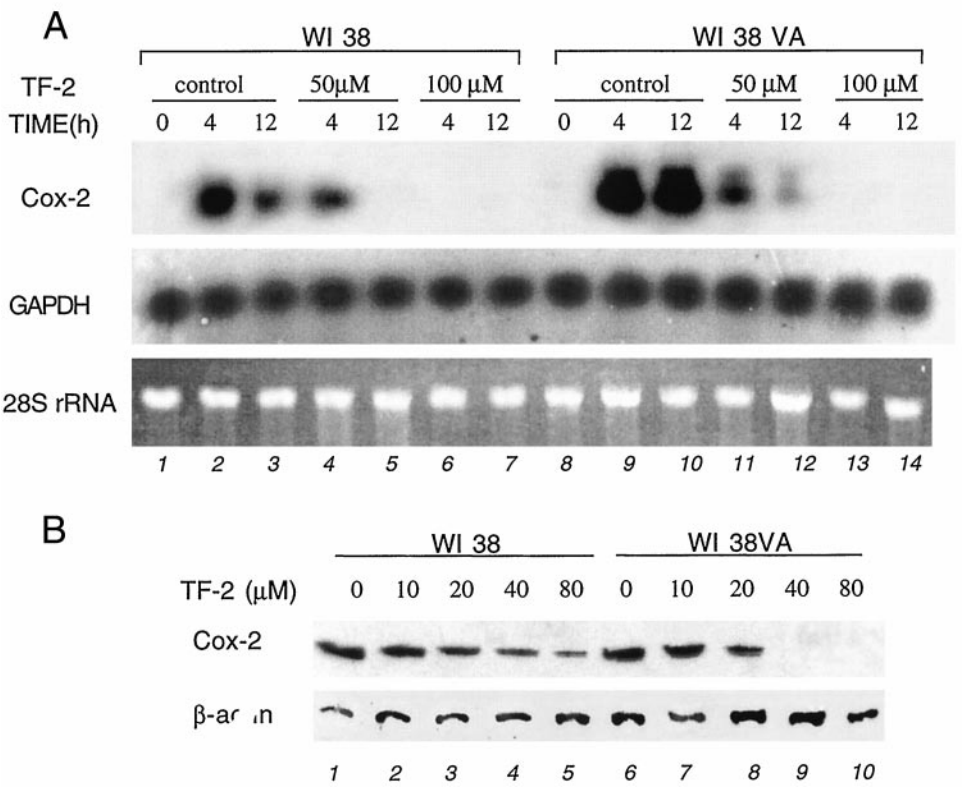

Fig. 8. Effects of TF-2 on *Cox-2* gene expression in WI38 and WI38VA cells. *A*, Northern blot analysis. Cells at 90% confluency were serum-deprived for 48 h and then replenished with complete growth medium containing 10% fetal bovine serum without (control) or with TF-2 at various concentrations. The cells were harvested at the indicated time for total RNA preparation. RNA samples were used for Northern blot analysis as described in "Materials and Methods." The levels of GAPDH mRNA and 28S rRNA were used as internal standards. *B*, Western blot analysis. Confluent cultures of WI38 and WI38VA cells were serum-deprived for 48 h and then serum stimulated with 10% fetal bovine serum for 8 h in the presence of TF-2 at the indicated concentrations. Cells were harvested, and whole-cell extracts were prepared for Western blot analysis using anti-Cox-2 antibody and anti-actin antibody as described in "Materials and Methods." Each lane contained 30 μg of proteins. The actin was used as an internal standard.

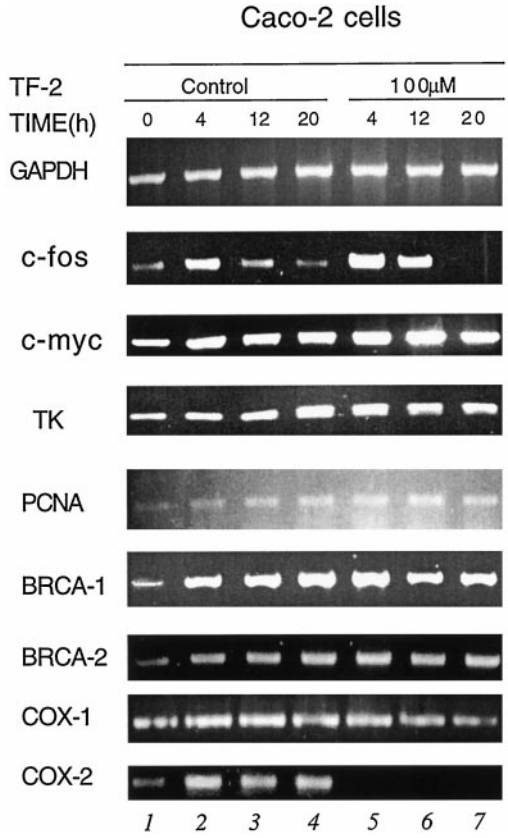

Fig. 9. Effect of TF-2 on the expression of growth-related genes. Caco-2 cells at 90% confluency were serum-deprived for 48 h and then stimulated with 10% fetal bovine serum in the absence (*Lanes 1–4, Control*) or presence of TF-2 (*Lanes 5–7, 100 μM*). The cells were harvested at the indicated time for total RNA preparation. The relative level of mRNA of each gene was analyzed by RT-PCR as described in "Materials and Methods." The mRNA level of housekeeping gene, *GAPDH*, was used as an internal standard.

sitive to TF-2, indicating that the action of TF-2 on the suppression of *Cox-2* gene expression is highly specific.

DISCUSSION

Epidemiological studies suggest that tea may reduce cancer risk (1–3). In addition, chemopreventive effects of tea extracts have been demonstrated in animal models for cancers of the skin, lung, esophagus, mammary glands, and colon (1–11). To understand the molecular basis underlying the biological effects of tea extracts, we have investigated the effects of three black tea polyphenols on growth, apoptosis, and gene expression in normal and cancerous human cells.

Among the three black tea polyphenols tested, TF-2 exhibited a striking differential growth-inhibitory effect for at least two cancerous cell lines, WI38VA and Caco-2 (Figs. 2–4). The fact that TF-2 was potent in inducing apoptosis in WI38VA cells but not in WI38 cells (Figs. 5 and 6) suggests that apoptosis may contribute to the differential growth-inhibitory effect of TF-2. Because TF-1 and TF-3 did not induce apoptosis in either WI38 or WI38VA cells (Fig. 6, *A* and *C*), we suspect that TF-2 may target specifically some components involved in apoptotic pathways in cancerous cells.

Inhibition of Cox enzyme by nonsteroidal anti-inflammatory drugs can reduce the risk of colon cancer (26, 27). Specific inhibition of *Cox-2* gene expression could be used as an alternative means for treating inflammation and diseases that are associated with *Cox-2* elevation (28, 29). The finding that TF-2 inhibited *Cox-2* gene expression is interesting in several regards: (*a*) other black tea theaflavins, TF-1 and TF-3 at 100 μM, did not inhibit *Cox-2* gene expression (Fig. 7); (*b*) the IC_{50} of TF-2 in inhibiting *Cox-2* gene expression was about 20–40 μM, comparable with that of nonsteroidal anti-inflammatory drugs (Figs. 7 and 8); and (*c*) TF-2 did not inhibit the expression of the constitutive *Cox-1* gene and other growth-related genes including c-*fos*, c-*myc*, *TK*, and *PCNA* (Fig. 9). Nonetheless, much work still needs to be done to assess the potential therapeutic promise of TF-2 *in vivo*.

Tea polyphenols generally exhibit antioxidative effects (30, 31), inhibit the AP-1 binding activity (32), and block the autophosphorylation of the epidermal growth factor and platelet-derived growth factor receptors (13). However, these biological effects are unlikely to be involved in *Cox-2* gene regulation, because neither TF-1 nor TF-3 shared the inhibitory action of TF-2 on *Cox-2* gene expression (Fig. 7*A*). Because the *Cox-2* gene is controlled primarily at the transcription level (33, 34), TF-2 may specifically affect the binding of certain *trans*-acting factors such as CCAAT/enhancer-binding proteins or nuclear factor-κB to the Cox-2 promoter. This possibility is currently under investigation.

In summary, we showed that, among the black tea polyphenols, TF-2 was unique in that it was a potent inhibitor of cancer cell growth, it differentially induced apoptosis in transformed cells, and it specifically inhibited *Cox-2* gene expression. These features make TF-2 a useful tea compound for further evaluation as a potential therapeutic reagent.

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