Protective Effects of Epigallocatechin Gallate on Colon Preneoplastic Lesions Induced by 2-Amino-3-Methylimidazo(4,5-*f*)Quinoline in Mice

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Epigallocatechin gallate (EGCG), a key active ingredient in green tea, has multiple anticarcinogenic effects. The aim of the present study was to investigate if EGCG could prevent the formation of colon aberrant crypt foci (ACF) induced by 2-amino-3-methylimidazo(4,5-*f*)quinoline (IQ) and to explore possible mechanisms for resultant effects. Sixty male BALB/cA nude, immunodeficient mice were divided into six groups including a normal unexposed control, mice induced with IQ alone, three groups treated with varying doses of EGCG post–IQ induction, and a EGCG-treated control population. Six weeks later, the mice were killed, and tissues subjected to hematoxylin-eosin (H&E) and 0.2% methylene blue staining to observe histopathological alterations of colon mucus and the formation of ACF, respectively. Protein expression of NF-E2–related factor 2 (Nrf2) was assessed via immunohistochemistry (IHC) and Western analysis, and mRNA levels of Nrf2 and uridine 5′-diphosphate-glucuronosyltransferase (UGT)1A10 were determined in colon tissues. Our results demonstrate that, compared with IQ-induced controls, the degree of atypical hyperplasia decreased and the number of total ACF and total AC also decreased significantly ($P < 0.05$ and $P < 0.01$, respectively) in mice belonging to all EGCG dosing groups. At the same time, the protein levels of Nrf2 detected by IHC and Western blotting increased (both $P < 0.01$ compared with IQ group), and the mRNA levels of Nrf2 and UGT1A10 increased (both $P < 0.01$ compared with IQ group). In conclusion, EGCG had preventive effects on preneoplastic lesions induced by IQ. Our observations suggest that this effect may be the result of activation of the Nrf2-UGT1A10 signaling pathway.

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INTRODUCTION

Colorectal cancer is a significant cause of mortality in Western countries and is the second most common type of fatal cancer (1). The risk factors for developing colorectal cancer include hereditary considerations as well as environmental factors, which can include genotoxins and cocarcinogens ingested in alimentation (2). Some of these genotoxic chemicals are formed during food preparation, such as heterocyclic aromatic amines (HAA) produced during the cooking of food with high creatine, free amino acid, and sugar content (3–5). Among all the components of HAA, 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) is commonly found in the human diet (6–9). IQ, one kind of HAA, demonstrates high mutagenicity (6) and was found to induce tumors in the liver, colon, and lung of rats (10,11) along with hepatocellular carcinomas in cynomolgus monkeys (12) with chronic feeding. Various experimental protocols with immunocompetent mice have shown that IQ can induce liver, lung, and stomach tumors (13,14). In colon, IQ can induce the formation of colonic aberrant crypt foci (ACF). ACF are believed to be the earliest morphological alterations during the development of colonic mucosal neoplasia (15–17) and are used extensively to identify modulators of colon carcinogenesis. Indeed, the morphological and molecular features of ACF support the contention that ACF are putative preneoplastic lesions that can serve as biomarkers of colon cancer (18,19).

The identification of epigallocatechin gallate (EGCG) is one important outcome of studies performed to search for effective chemopreventive substances with limited side effects in humans (20,21). EGCG, the major polyphenol in green tea, was found to effectively and broadly inhibit colon carcinogenesis through var-

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ious mechanisms (22–25). It remained unclear, however, whether EGCG could inhibit the formation of colonic aberrant crypt foci and its possible mechanisms.

Our previous studies showed that sulforaphane (26) and EGCG (27) could induce the expression of UGT1A family members *in vitro*. We demonstrated that this upregulation was due to the actions of transcription factor NF-E2–related factor 2 (Nrf2). UDP-glucuronosyltransferases (UGTs), the phase II drug metabolizing enzymes, mediate the transfer of glucuronic acid—from UDP glucuronic acid to predominantly hydrophobic xeno- and endobiotic compounds—thus facilitating their detoxification and excretion. UGT genes are classified into UGT1A and UGT2B superfamilies, with each superfamily and its isoforms exhibiting tissue-specific distribution patterns. The isoforms of UGT1A8 and UGT1A10 are expressed exclusively in extrahepatic tissues, notably in the gastrointestinal tract (28). UGT, an important enzyme in the detoxification of HAA (29–32), can be induced by dietary constituents (33). The induction of phase II enzyme genes is regulated by their *cis*-acting antioxidant response element (ARE) or electrophile responsive element (EpRE) (34–36). Nrf2 binds to and regulates transcription through the ARE/EpRE after heterodimerizing with one of the small Maf proteins (37,38). Hence, Nrf2 is an essential regulator of the inducible expression of detoxifying enzyme genes by chemopreventive agents.

In the current study, we employed a mouse model with preneoplastic lesions of ACF induced by IQ to determine whether EGCG can serve as a chemopreventive substance to delay, slow, or prevent colon carcinogenesis. We propose one mechanism where the protective effects of EGCG on IQ-induced colonic aberrant crypt foci would involve induction of isoform UGT1A10 in mice gastrointestinal tract via Nrf2 signal pathway. We demonstrate that EGCG might protect colon from IQ-induced aberrant crypt foci through the Nrf2-UGT1A10 signaling pathway.

MATERIALS AND METHODS

Animals

Sixty male BALB/cA nude mice (Shanghai SLAC Laboratory Animal Co. Ltd., Shanghai, China), 4–6 wk old, weighing 16–22 g at the beginning of the experiment, were used in this study. Mice were housed under specific pathogen–free (SPF) conditions. Experiments were performed in accordance with the Laboratory Animal Care and Use Regulations of Shandong University. The temperature was maintained at 21° to 22°C with 60%–65% humidity. Ambient light was maintained on a 12-h light and 12-h dark schedule. Sterilized food and water were available *ad libitum*, and the animals were allowed to adapt to the laboratory housing conditions for at least 1 wk before the start of experiments.

According to a previous study (39), we randomly divided the mice into six groups of 10 animals each. IQ (Toronto Research Chemicals, Toronto, Canada) and EGCG (Sigma, St. Louis, MO, USA) were dissolved in 0.2 mL corn oil (Knorr GmbH, Wels, Austria) and 0.2 mL sterilized water. The treatment schedule was carefully designed (Figure 1) and the total experiment was carried out over 6 wks. Group 1 mice (negative control) were treated with 0.2 mL corn oil every 2 d by oral gavage for 2 wks from the beginning of the third wk. The experimental group (group 2) was treated with IQ (50 mg/kg) dissolved in 0.2 mL corn oil for 2 wks. The mice of groups 3–5 were treated with three doses of EGCG (5, 10, and 20 mg/kg) 1 wk before and during the 2-wk treatment with the same dose of IQ as group 2. As an additional control, mice in group 6 were treated with medium-dose EGCG (10 mg/kg) by oral gavage in the preceding 4 wks. Body weights of mice were measured every 2–3 d.

Hematoxylin-Eosin (H&E) Staining

At the time of necropsy, colon tissue was immediately fixed in 10% buffered formalin and processed for histological examination including routine H&E staining and immunohistochemical staining. Another sample of colon tissues was collected, frozen in liquid nitrogen, and stored at –80°C for subsequent molecular analyses. Formalin-fixed and paraffin-embedded colon specimens were cut into 5-µm-thick sections for routine histopathological observation.

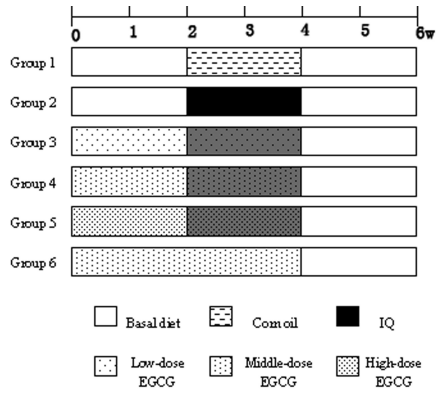

Figure 1. Experimental treatment of nude mice in six groups. The total experiment lasted for 6 wks. Group 1 was defined as the normal control. At the first and last 2 wks, the mice were given a basal diet. From the beginning of the third wk, the mice were treated with 0.2 mL corn oil every 2 d by oral gavage for 2 wks. Group 2 was the model group and mice were treated with IQ (50 mg/kg) dissolved in 0.2 mL corn oil for 2 wks. Groups 3–5 were treated with different doses of EGCG before and during the 2-wk treatment with the same dose of IQ as group 2. Doses of EGCG were 5, 10, and 20 mg/kg dissolved in 0.2 mL sterilized pure water every d. The group 6 EGCG control was treated with medium doses of EGCG (10 mg/kg) in the preceding 4 wks.

Identification and Quantification of ACF

To characterize ACF, the colon was removed, cleaned with Ringer's solution, cut open along the longitudinal median, and fixed flat in 10% buffered formalin (pH 7.5) for 24 h. The samples were stained in 0.2% methylene blue, and the number of ACF per colon and the number of aberrant crypts (AC) per focus were identified using the following published set of criteria which distinguishes these lesions from normal

crypts: *a*) larger size; *b*) an increased pericryptal area; *c*) greater staining intensity due to the thickened layer of epithelial cells; *d*) microscopically elevated above the adjacent normal crypts; and *e*) abnormally shaped lumina, often slit-shaped in appearance (40).

Table 1. Primers used for reverse transcription-polymerase chain reaction.

Gene name	Source	Forward primer (5′→3′)	Reverse primer (5′→3′)	Product size (bp)
β-Actin	NM_007393	TGTCCCTGTATGCCTCTG	GATGTCACGCACGATTTC	218
Nrf2	NM_008685	TTGGCAGAGACATTCCCAT	GCTGCCACCGTCACTGGG	501
UGT1A10	NM_201641	GAAGCCTATGTCAACGCC	GCATCATCACCATCGGAA	304

Immunohistochemical Detection of Nrf2 Protein

Samples of the colon of each mouse were fixed in 10% buffered formalin for 24 h, embedded in paraffin, and sectioned. The sections were deparaffinized in xylene and rehydrated before analysis, immersed in 3% H_2O_2 (vol/vol) to quench endogenous peroxidase activity, and microwaved in 10 mM sodium citrate (pH 6.0) for 15 min for antigen retrieval. Five percent normal goat serum was applied to eliminate nonspecific background staining. The sections were then incubated overnight with primary rabbit antihuman Nrf2-specific antibody (1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4°C in a high-humidity chamber. Then HRP-conjugated goat antirabbit IgG (Santa Cruz Technology) was added to the sections and incubated for 20 min at room temperature. The sections were stained with DAB solution, counterstained with hematoxylin, washed for 1 h with PBS, air-dried, and covered with mounting medium. A known sample from a patient with breast cancer was used as a positive control, and negative control slides were processed with PBS in place of primary antibody. All samples were evaluated blindly by the same pathologist. The results of immunohistochemical staining were analyzed using Image-Pro plus (IPP) 5.0.

Western Blotting Analysis for Nrf2

For the Nrf2 Western blotting analysis, extracts were prepared from frozen colon tissue samples. Equal amounts of protein (100 μg) were loaded in each lane and separated by 10%–15% SDS-PAGE. The proteins were then transferred to a nitrocellulose membrane blocked with 5% skim milk in TBST (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.15% Tween-20) and incubated at room temperature for 2 h. The membrane was washed three times with TBS-T and incubated overnight at 4°C in the presence of primary rabbit antihuman Nrf2-specific antibody (1:150; Santa Cruz Technology). The membrane was washed and stained with HRP-conjugated goat antirabbit secondary antibody at a 1:1000 dilution at room temperature for 1 h. The membrane was washed three times with TBS-T again and stained with DAB solution. The intensities of acquired bands were measured by computerized image analysis (IPP 5.0) and normalized to β-actin as the internal control.

Analysis of Nrf2 and UGT1A10 mRNA Expression in Colon Mucosa by Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Total RNA was isolated from colon tissue using Trizol reagent (Sigma). Reverse transcription of RNA was performed using the RT-PCR kit (Gibco, Gaithersburg, MD, USA). The primers used in the PCR reactions are summarized in Table 1. PCR amplification was performed in 50-μL volumes, containing 30 to 60 ng RNA, 0.5 units Taq DNA polymerase, 200 μmol of each dNTP, and 2 pmol of each primer. PCR conditions were as follows: 94°C for 5 min (predenaturation), 30 cycles at 94°C for 45 s (denaturation), 58°C for 45 s (annealing), and 72°C for 45 s (extension), and 72°C for 7 min (postextension). PCR reactions were performed on the GeneAmp PCR system 9700.

The amplified products were subjected to electrophoresis on 1.5% agarose gel. The gel was then stained with ethidium bromide and photographed. Negative controls for PCR were run under the same conditions without RNA or reverse transcriptase. Expression of β-actin was used to examine the integrity of RNA in each sample and to standardize the amount of cDNA added to each of the PCR reactions. The intensities of bands were measured by computerized image analysis (Image J, Sun Microsystems, Inc., Santa Clara, CA, USA).

Statistical Analysis

Data are presented as the mean ± SD. The significance of these differences among five groups was analyzed by means of one-way ANOVA using SPSS 13.0 for Windows. *P* values less than 0.05 were considered statistically significant.

RESULTS

Effect of EGCG on the Body Weights of Mice

At the beginning of the experiment, the mean body weights across all six groups were the same. During the first 2 wks after the beginning of the experiment, all groups gained weight at nearly the same rate. From the third week, along with administration with IQ by oral gavage, changes in body weight

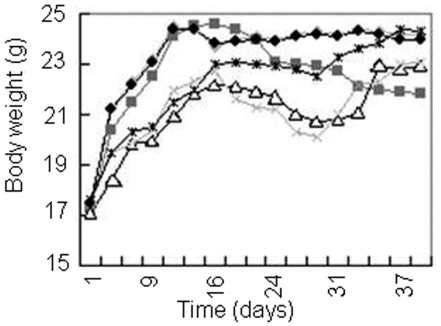

Figure 2. Body weight time course of mice in all six groups. ◆, normal control; ■, IQ; △, low dose (EGCG + IQ); ×, middle dose (EGCG + IQ); ✱, high dose (EGCG + IQ); ◆, EGCG.

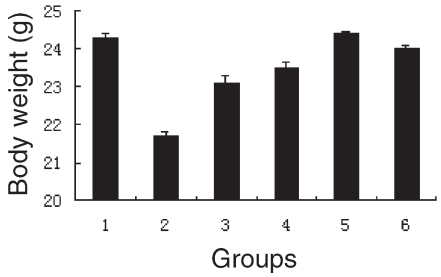

Figure 3. Comparison of the body weights of mice at the end of the experiment. Data presented as mean ± SD.

were noted in all six groups (Figure 2). The body weights of the mice in the IQ group decreased until the experiment was completed. Body weights of the mice in the other three EGCG groups also decreased concurrently. But the degrees of body weight loss were fairly low. After stopping IQ administration by oral gavage the body weights of the mice in the three EGCG groups gradually increased, with the greatest difference observed in mice in the high-dose EGCG group, which recovered to the level of normal controls. At the end of the experiment, we compared the body weights of all six groups. The body weights of mice treated with EGCG alone were less when compared with normal controls, but this difference was not statistically significant. However, mice exposed to EGCG after IQ induction showed significant differences in body weight compared with the IQ group (Figure 3, all $P < 0.01$). Dose-dependent changes in body weight were also observed in these mice exposed to EGCG, resulting in significant differences between dose groups (Figure 3, $P < 0.01$).

Effect of EGCG on Colon Histology

Following acute, large-dose carcinogen exposure (IQ) for 2 wks, mice in group 2 exhibited obvious karyomegaly and anachromasis, as well as increased karyoplasmic ratio in some epithelial cells of the colon tissue. The colon histopathological alterations in these mice presented a high degree of atypical hyperplasia (Figure 4-2). The mice in groups 3–5 also showed mild to high degrees of atypical hyperplasia (Figure 4-3, -4). The histopathology of the colon tissue in group 5 approached that of normal controls (Figure 4-5) demonstrating high-dose EGCG (20 mg/kg) could reverse the histopathological change of colon tissue to normal. There were no observable colon histopathological alterations in mice treated with EGCG as a normal control (Figure 4-6).

Effect of EGCG on ACF Formation

Based on prior observations, we postulated that EGCG could mitigate the carcinogenic effects of IQ in the ACF model of colorectal cancer development. To determine whether the effects of EGCG were specific to IQ-induced ACF formation, we examined ACF burden in mice that were unexposed or only exposed to EGCG. We did not observe the formation of ACF and AC in the mice of groups 1 and 6. In contrast, short-term, flushing-dose oral administration of the carcinogen IQ to mice in group 2 produced a large amount of ACF in the intestinal mucosa of colon specimens stained with 0.2% methylene blue (64.20 ± 45.18). As a function of increasing dose of EGCG by oral gavage, the number of ACF in the intestinal mucosa decreased (45.00 ± 31.56, 32.00 ± 21.34, and 18.00 ± 7.51), and the shape of colon crypts gradually improved. This effect was most striking in colon tissue samples derived from mice in the high-dose EGCG group, which approached the appearance of normal crypts (Figure 5).

The total number of ACF (along with total AC) in the IQ group was the highest among the six groups (Table 2), with the number of ACF containing seven aberrant crypts registering as the highest among five groups. We observed significant effects of EGCG on both the total number of ACF and AC. The number of total ACF, total AC, and ACF with more than seven aberrant crypts had all shown significant EGCG dose-dependent decreases, compared with those in the IQ group (18.00 ± 7.51 versus 64.20 ± 45.18, $P < 0.05$; 63.90 ± 18.56 versus 168.80 ± 35.34, $P < 0.01$; 0.50 ± 0.45 versus 9.60 ± 3.14, $P < 0.01$).

Effect of EGCG on Nrf2 Protein Expression

We next sought to determine what role Nrf2 might play in mediating the affects of EGCG on decreased ACF/AC incidence. We observed a large amount of Nrf2-positive cells (brown staining) in endochylema of colon endothelial cells of mice in group 6, but there was no statistical significance in comparison with the normal control. A small quantity of Nrf2-positive cells were observed in the mice of the model group (Figure 6A-2). In contrast, a large quantity of Nrf2-positive cells were identified in groups 3–5 treated with different doses of EGCG (Figure 6A-3, -4, and -5). Nrf2-positive cells were also observed in the nuclei of colon endothelial cells. Results analyzed by the image processing software of IPP 5.0 indicated that the mean optical density values [IOD (Sum)/Area (Sum)] of Nrf2 in three different doses of EGCG groups increased significantly in comparison with the IQ group (Figure 6B, all $P < 0.01$). Additionally, the mean optical density value of Nrf2 across the three EGCG dose groups exhibited significant pair-wise differences (Figure 6B, all $P < 0.01$).

We then carried out a Western analysis as a second means of assessing Nrf2 protein levels (Figure 7A). Nrf2 protein expression increased significantly in groups 3–5 compared with values observed in the model group (Figure 7B, all $P < 0.01$), which is consistent with IHC staining patterns. This increase in Nrf2 expression had a positive correlation with increasing doses of EGCG, with significant differences in protein amount observed in all three EGCG groups (Figure 7B, all $P < 0.01$).

Effect of EGCG on Nrf2 and UGT1A10 mRNA Expression

To determine whether UGT1A10, the downstream gene of Nrf2, would exhibit

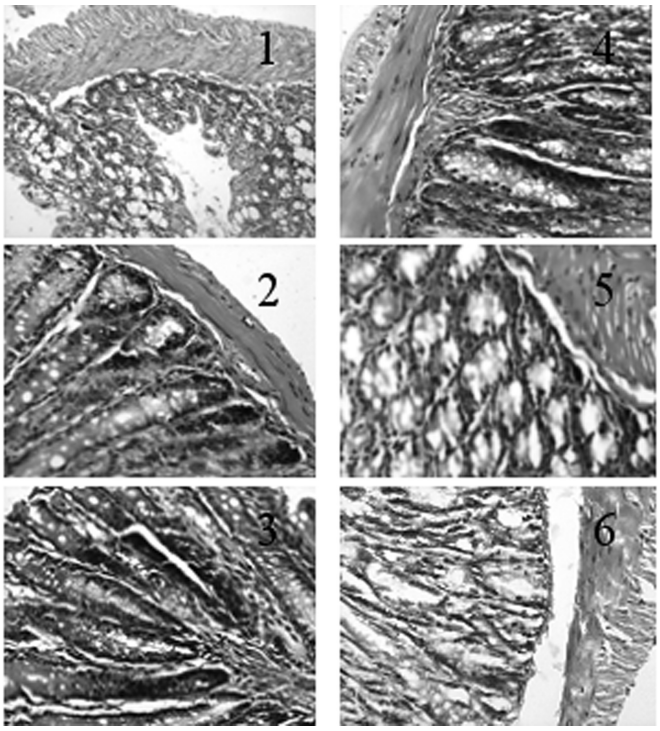

Figure 4. Colon tissues histology from five groups following H&E staining (1, original magnification 200×; 2–6, original magnification 400×).

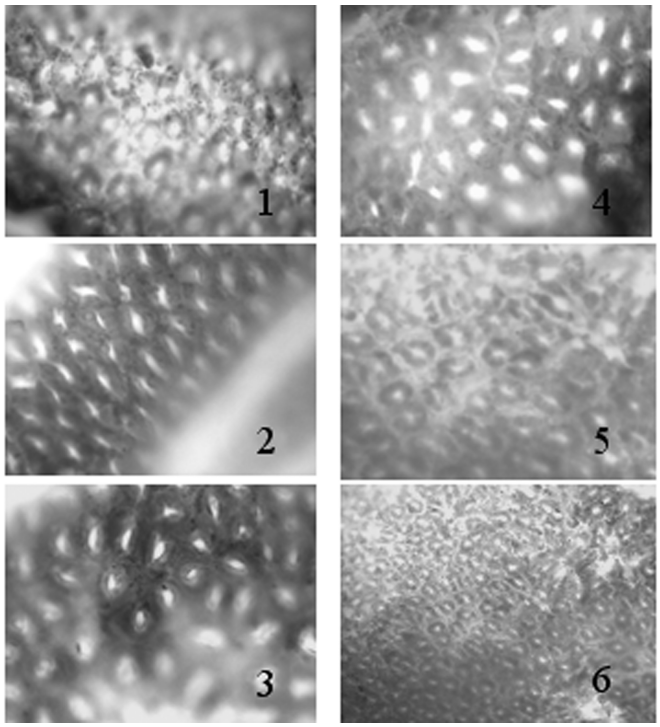

Figure 5. Observations of ACF by 0.2% methylene blue staining (1–5, original magnification 200×; 6, original magnification 100×).

dose-dependent increases in expression after EGCG administration, we compared the mRNA levels of Nrf2 and UGT1A10 by RT-PCR. The mRNA expression of these two genes in the mice of group 1 and group 6 were comparable. The same expression levels of the two genes in group 2 mice were the lowest among the five experimental groups (Figure 8A). The mRNA levels of Nrf2 and UGT1A10 increased by different degrees in the mice administered with three different doses of EGCG. The levels of the two genes in the three EGCG groups had significant differences when compared with those in group 2 (Figure 8B, all $P < 0.01$). EGCG groups also showed significant differences (Figure 8B, $P < 0.01$).

DISCUSSION

In previous studies (15,41–43), an animal model with preneoplastic lesions of colon cancer could be induced by short-term and flushing-dose IQ. In the current work, we found treatment with IQ for 2 wks to be significantly more effective in inducing ACF than the same amount of carcinogen fractionated over longer periods of time (43). Additionally, we used BALB/cA nude immunodeficient mice, which lack functional T cells and are therefore highly susceptible to specific induction of colon ACF. These two changes to the published approach allowed us to model induced preneoplastic lesions of colon cancer on nude mice using short-term and high-dose IQ by oral gavages every 2 d for 2 wks.

Histological analysis revealed a high degree of atypical hyperplasia in colon epithelial cells derived from mice in the IQ group after H&E staining. EGCG groups showed varying degrees of atypical hyperplasia, with the histopathology of colon tissue in the high-dose EGCG group approaching normal. ACF analysis indicated that the number of total ACF and total AC, especially the number of

Table 2. Comparison of the number of ACF among different groups.

Group	ACF, *n*			Total ACF	Total AC
	1–3 AC	4–7 AC	>7 AC		
1	0	0	0	0	0
2	32.20 ± 8.78	15.00 ± 4.35	9.60 ± 3.14	64.20 ± 45.18	168.80 ± 35.34
3	20.30 ± 8.54	10.20 ± 3.61	5.90 ± 1.56[a]	45.00 ± 31.56	102.40 ± 23.75[b]
4	15.40 ± 5.42	9.70 ± 4.89	3.40 ± 3.67[a]	32.00 ± 21.34	98.40 ± 21.79[a]
5	8.40 ± 3.55	5.10 ± 2.11	0.50 ± 0.45[a]	18.00 ± 7.51[b]	63.90 ± 18.56[a]
6	0	0	0	0	0

Data are given as means ± SD, and the values with different superscripts differ significantly (by one-way ANOVA) ([a]$P < 0.01$ and [b]$P < 0.05$) and compared with group 2. Group 1 and group 6 had no ACF information.

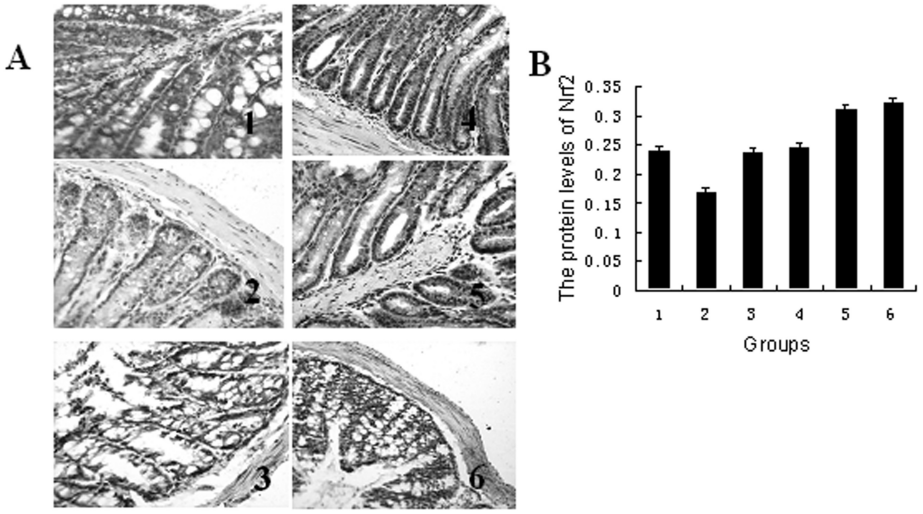

Figure 6. Immunohistochemical staining for Nrf2 in colon tissues. (A) Representative observations of Nrf2 staining in six groups (original magnification 400×). (B) The mean optical density values of Nrf2 in colon tissues. Data presented as mean ± SD.

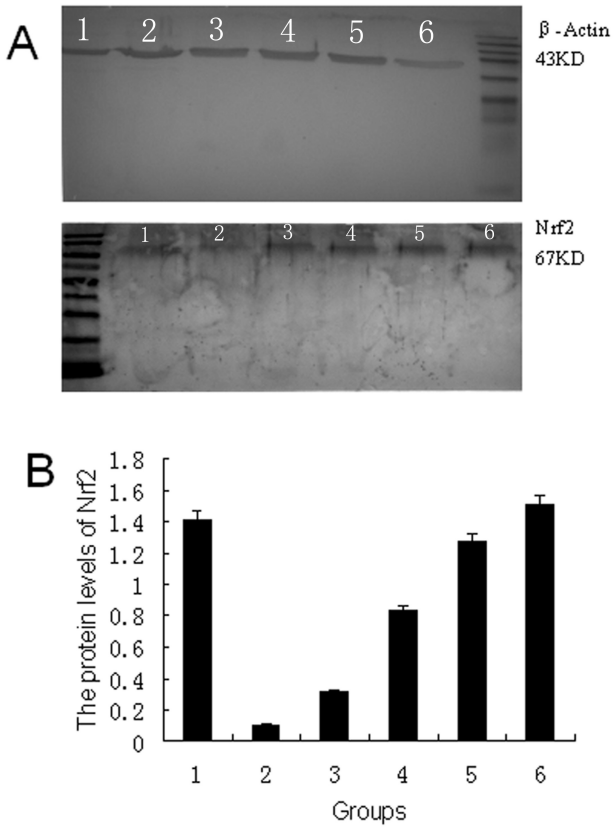

Figure 7. Representative Western blotting for Nrf2 protein expression in the colon tissues of mice in six groups. (A) The bands of Nrf2 are represented by different gray scales in six groups. (B) Specific bands were quantified by scanning densitometry and normalized to the signal of β-actin. Data presented as mean ± SD.

ACF with more than seven aberrant crypts, significantly increased in the mice of the IQ group in comparison with those of the three EGCG groups. ACF containing more than seven aberrant crypts are more significant in identifying colonic carcinogenesis (44–47). Taken together, the data suggest that the H&E staining results are consistent with the ACF analysis of colon epithelial cells in five experimental groups. It is likely that these precursor lesions progress to colon cancer in the presence of chronic IQ exposure.

Prior work has focused on the chemopreventive attributes of many agents including Brussels sprouts, red cabbage (41,48), and beer (49) that decrease the total number of ACF, including large ACF, induced by HAA. In current study, we used different doses of EGCG, a major ingredient in the widely consumed green tea beverage, to prevent preneoplastic lesions induced by IQ.

Some studies have reported that EGCG can inhibit the formation of colonic aberrant crypts, as well as the growth and metastasis of cancer cells, via inhibiting cell proliferation or promoting apoptosis in colorectal cancers (50–52). However, we focused our work on a mechanism of suppression of early lesions (ACF) through upregulation of the Nrf2-UGT1A signaling pathway after EGCG administration. To assess whether UDP-glucuronosyltransferases (UGTs) would increase after different doses of EGCG administration, we compared mRNA levels of UGTs by RT-PCR. UGTs are members of the phase II biotransformation enzyme superfamily and catalyze the transfer of the glucuronyl group from 5′-disphosphoglucuronic acid to endogenous molecules and exogenous substrates (53), producing less toxic and more easily excreted molecules. UGTs play an important role in the maintenance of steady-state levels of endogenous compounds and the metabolism of both toxic and beneficial exogenous compounds. Although liver microsomes are the major site of conjugation between glucuronic acid and other materials, ex-

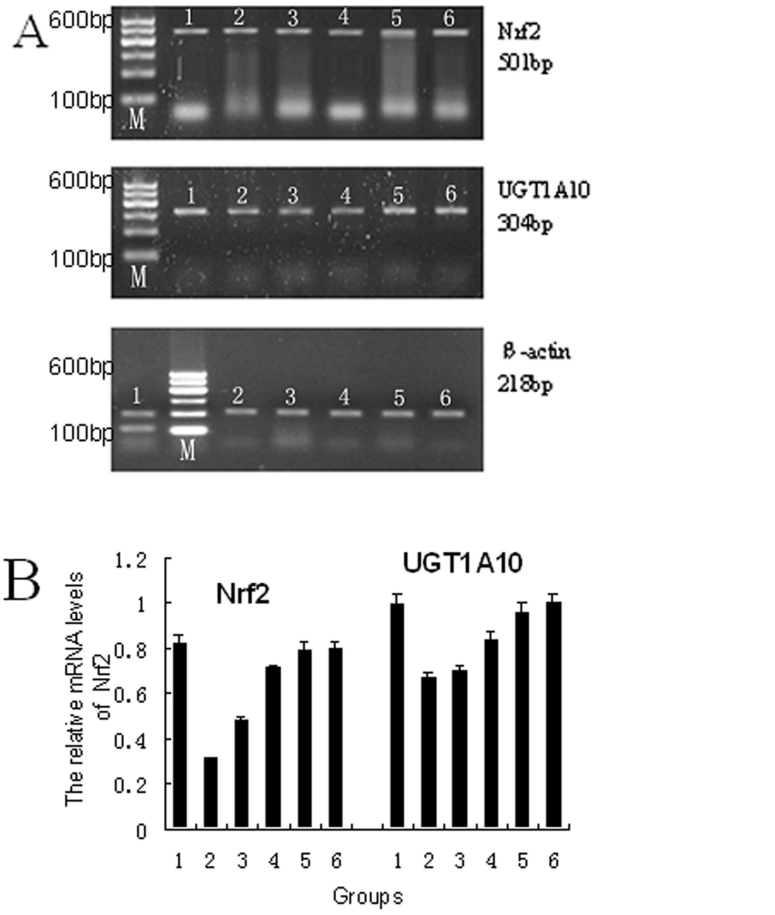

Figure 8. Nrf2 and UGT1A10 mRNA expressions in the colon tissues of mice in all six groups. (A) The electrophoretic bands of Nrf2 and UGT1A10 are represented on agarose gel by different gray scales. (B) Specific bands were quantified by scanning densitometry and normalized to the signal of β-actin. Data presented as mean ± SD.

pression of both UGT1A8 and UGT1A10 was detected specifically in the gastrointestinal (GI) tract (28).

We initially wanted to assess mRNA expressions of UGT1A8 and UGT1A10 of colon mucosa after EGCG administration in mice, because these two genes are specifically expressed in human GI tract. The full-length sequence of UGT1A8 of mice was not available from NCBI, however, so we followed mRNA expression of UGT1A10 as one measure of EGCG effect. We found that UGT1A10 was upregulated by varying doses of EGCG. This effect was especially prominent at the high dose (20 mg/kg). This increase in UGT1A10 expression in colon cancer could promote the combination of IQ and glycuronic acid and increase its water solubility. IQ could then be eliminated through urine and bile, thereby reducing or mitigating the carcinogenicity of IQ in colon tissue.

Next, we asked if EGCG administered by oral gavages could upregulate the expression of UGT1A10 in mouse colon tissue via the transcription factor Nrf2 signaling pathway. We found that as the levels of Nrf2 increased following varying doses of EGCG, the levels of UGT1A10 increased concurrently. As a result, we found that the expression patterns of UGT1A10 correlated with that of Nrf2.

To further elucidate the role of this signaling pathway, we determined protein and mRNA levels of Nrf2 in colon tissue by immunohistochemical staining (IHC), Western blotting, and RT-PCR. Our results show that the protein and mRNA levels of Nrf2 increased after EGCG dosing before and during IQ treatment. Furthermore, we observed nuclear translocation of Nrf2 by immunohistochemical staining, whereupon Nrf2 signal transferred from cytoplasm to nuclei of colonic epithelial cells. Our findings are consistent with those of other studies (33,54,55).

Nrf2, a bZIP transcription factor, is sequestered in the cytoplasm by Kelch-like ECH-associated protein 1 (Keap1). Exposure of cells to antioxidant response element (ARE) inducers results in the dissociation of Nrf2 from Keap1 and facilitates translocation of Nrf2 to the nucleus. Once there, Nrf2 heterodimerizes with the small Maf protein and binds to the ARE, eventually resulting in the transcriptional regulation of target genes including both phase I (oxidation and reduction) and phase II biotransformation (conjugation). Some agents exert cancer chemoprevention by strong induction of phase II enzymes including UGTs via activation of Nrf2 (32,33,56–59).

In the current study, we showed that varying doses of EGCG can prevent IQ-induced colon formation of ACF. Further, we showed that expression of Nrf2 and UGT1A10 had a positive correlation with doses of EGCG administered by oral gavage, with high-dose EGCG exhibiting the most striking effect of preventing carcinogenesis in this model. Further studies on a large number of animals are needed to verify our findings.

In summary, varying doses of EGCG had a different preventive effect on preneoplastic lesions induced by IQ. This effect might result from the activation of the Nrf2 and UGT1A10 signaling pathway. Our results suggest that the Nrf2-UGT1A10 signaling pathway may contribute to an EGCG-induced mechanism of colon cancer prevention. EGCG, the major polyphenol in green tea, may offer an effective and inexpensive preventive

strategy for patients predisposed to or suffering from colon cancer.

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