Dietary Corn Oil Promotes Colon Cancer by Inhibiting Mitochondria-Dependent Apoptosis in Azoxymethane-Treated Rats

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How dietary corn oil is involved in colon carcinogenesis and cancer development is poorly understood. The aim of this study was to investigate whether long-term dietary corn oil promotes colon cancer by inhibiting the tumor suppressor gene *p53*-mediated mitochondria-dependent apoptosis in azoxymethane (AOM)-treated rats. Male Sprague-Dawley rats were injected with AOM or with saline and fed on a basal diet or basal diet supplemented with 10% corn oil for 48 weeks. Colonic aberrant crypt foci (ACF) and tumors, including adenomas and carcinomas, were examined. Colonic apoptosis and cell proliferation were evaluated. Wild type (*wt*) *p53* was analyzed using reverse transcription–polymerase chain reaction (RT-PCR) and Western blotting. In addition, Bcl-2, Bcl-xL, Bax, and Bak localized in the mitochondria were detected. Long-term dietary corn oil increased ACF in AOM-treated rats at 12 weeks and promoted colon cancer invasion at 48 weeks. Cancer invasion was not observed in the AOM-treated rats without dietary corn oil, although colon adenomas and cancers were detected. Apoptosis was decreased and cell proliferation was increased in the AOM-treated rats with dietary corn oil, compared with the AOM-treated rats with dietary basal diet. In these rats, mitochondrial *wt p53* was significantly inhibited through decreased mitochondrial localization of *wt p53* and increased cytosolic *p53*, resulting in the upregulation of Bcl-2 and Bcl-xL and the downregulation of Bak in the mitochondria. Results suggest that long-term dietary corn oil promotes AOM-induced colon cancer development partly by inhibiting the tumor suppressor gene *p53*-mediated mitochondria-dependent apoptosis. Exp Biol Med 229:1017–1025, 2004

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Colon cancer is one of the leading causes of cancer death in both men and women in Western countries (1). Epidemiological studies have suggested a positive association between dietary fat and colon cancer (2, 3). Furthermore, animal model studies have shown that the colon tumor–promoting effect of a high-fat diet depends not only on the amount consumed but also on the fatty acid composition. A high-fat diet rich in corn oil (*n*-6 polyunsaturated fatty acid [*n*-6 PUFA]) promotes colon carcinogenesis, particularly in the postinitiation or promotional phases or both (3–5), whereas a diet rich in fish oil (*n*-3 PUFA) or olive oil (*n*-9 PUFA) decreases colon tumor incidence in both the initiation and postinitiation phases (4, 6).

Colon carcinogenesis is a multistep processor that requires considerable time for these chance events to accumulate. Several observations have confirmed the putative association between aberrant crypt foci (ACF) and colon cancer in animals (7–9) and have suggested that ACF might be a high risk factor for colon cancer in humans (10, 11). Quantification of the formation and growth of colonic ACF has been used as a short-term bioassay to evaluate the roles of nutritive components at a very early stage of colon carcinogenesis in animals and humans (7–11). It has been proposed that potentially tumorigenic clones might be eliminated through apoptotic targeting of damaged intestinal epithelial stem cells and that inhibition of this apoptosis causes aberrant cell survival and contributes to oncogenesis (12–14).

Wild type (*wt*) *p53* is thought to function as a gene-transcription factor, and the active *wt p53* can induce the expression of a large number of genes, many of which evoke either cell cycle arrest or apoptosis (15–17). *Wild type p53* induces cell death through a multitude of molecular pathways involving transcription-dependent or independent functions or both (18, 19). It can mediate apoptosis via

transcriptional activation of proapoptotic genes such as BH3 proteins (e.g., Noxa and Puma) and Bcl-2 family members (e.g., Bax and Bak) (20–22). In addition, it is a potent inhibitor of cell growth and tumor growth, and its inactivation or mutation or both is considered a prerequisite for tumor formation (23). Furthermore, evidence for transcription-independent *wt p53*–mediated apoptosis has been accumulated, and suggests that *wt p53* has a direct apoptotic genetic role in the mitochondria (24).

Previous studies revealed that dietary corn oil rich in *n*-6 PUFA promoted colon cancer (3–5). However, how dietary corn oil is involved in colon carcinogenesis and cancer development is poorly understood. Mitochondria play a key role in cellular survival and death, and *wt p53*–mediated mitochondria-dependent apoptosis (24, 25), but it is unclear whether dietary corn oil inhibits *wt p53*–mediated mitochondria-dependent apoptosis during colon carcinogenesis and cancer development. Azoxymethane (AOM), a standard chemical carcinogen, was used in this study. AOM treatment increases ACF formation in rats, which leads to the induction of colon adenomas and cancers (26–28). The aim of the present study was to investigate whether long-term dietary corn oil promotes colon cancer development by inhibiting the tumor suppressor gene *p53*-mediated mitochondria-dependent apoptosis in AOM-treated rats.

Materials and Methods

Animals and Experimental Procedures. Male Sprague-Dawley rats were used in this study. At 5 weeks of age, the rats were divided into four groups: (i) basal diet plus vehicle rats were fed on a basal diet (Clea, Tokyo, Japan) and given intraperitoneal injections of 1 ml physiological saline once a week for 2 weeks; (ii) corn oil plus vehicle rats were fed on a basal diet supplemented with 10% corn oil (Clea) consisting of 8.1% (w/w) *n*-6 PUFA in the total diet and given intraperitoneal injections of 1 ml physiological saline once a week for 2 weeks; (iii) basal diet plus AOM rats were fed on a basal diet and given intraperitoneal injections of AOM (Sigma, St. Louis, MO) dissolved in 1 ml physiological saline once a week for 2 weeks at a dose of 15 mg/kg body wt; and (iv) corn oil plus AOM rats were fed on a basal diet supplemented with 10% corn oil and given injections of AOM as described in Group 3. The basal diet included 8.9% moisture, 25.4% crude protein, 4.4% crude fat, 4.1% crude fiber, and 6.9% crude ash. The corn oil included 56.8% linoleic acid, 29.0% oleic acid, 10.5% palmitic acid, and 1.9% stearic acid. At 12 weeks, after the second injection of AOM, colonic ACF formation was analyzed, and at 12, 24, 36, and 48 weeks colon carcinogenesis and cancer development were investigated.

Collection of Intestinal Tissue Samples. The entire colon was carefully removed and placed on ice-cold glass except for the cecum. Each segment was rinsed thoroughly with physiological saline and opened longitudinally on its antimesenteric border to expose the mucosa. The mucosa and tumors were carefully harvested, respectively.

DNA Fragmentation Assay. At 48 weeks, the colonic mucosa and tumors of all four groups of rats were examined. The amount of fragmented DNA was determined as previously described (29). Tissues were homogenized in 10 volumes of a lysis buffer (pH 8.0) consisting of 5 m*M* Tris-HCl, 20 m*M* EDTA (Sigma), and 0.5% (w/v) t-octylphenoxypolyethoxyethanol (Triton X-100, Sigma). One-milliliter aliquots of each sample were centrifuged for 20 mins at 27,000 *g* to separate the intact chromatin (pellet) from the fragmented DNA (supernatant). The supernatant was decanted and saved, and the pellet was resuspended in 1 ml of Tris buffer (pH 8.0) consisting of 10 m*M* Tris-HCl and 1 m*M* EDTA. The pellet and supernatant fractions were assayed for DNA content using a diphenylamine reaction. The results were expressed as the percentage of fragmented DNA divided by the total DNA. Six rats were studied in each group.

DNA Ladders Assay. At 48 weeks, the colonic mucosa (basal diet plus vehicle and corn oil plus vehicle rats) and the colon tumors were examined. The total DNAs were extracted sequentially using a phenol-chloroform-isoamyl alcohol mixture (25:24:1, v/v/v) to remove proteins and then purified as previously described (29). Resolving agarose gel electrophoresis was performed using a 1.5% gel containing 1.0 μg/ml ethidium bromide. DNA standards were included to identify the sizes of the DNA fragments. The DNA ladders were observed under ultraviolet fluorescent light.

ACF Analysis. For ACF assessment, the colon was removed at 12 weeks, after the second injection of AOM, opened longitudinally, rinsed in ice-cold physiological saline, placed flat mucosal side up between wet filter papers, and fixed in 10% buffered formalin for 24 hrs. The colon was then stained with 0.2% methylene blue dissolved in the same formalin solution for 5 mins and rinsed in physiological saline. After staining, the entire colonic mucosa was observed using a stereoscopic microscope. Crypt multiplicity was determined as the number of crypts in each colon and was categorized as containing <4 or ≥ 4 aberrant crypts. Six rats were examined in each group.

Histological Analyses. At 12 weeks, after the assessment as described above, the ACF were embedded in paraffin. At 24, 36, and 48 weeks, after the second injection of AOM, colon tumors over 5 mm were collected and fixed in 10% buffed formalin and embedded in paraffin. Four-micrometer sections were cut and stained with hematoxylin and eosin (H&E) and periodic acid-schiff (PAS) and subjected to histological analysis. Six rats were examined in each group at each time point.

Total RNA Extraction and Reverse Transcription–Polymerase Chain Reaction (RT-PCR). At 48 weeks, after the second injection of AOM, the colonic mucosa (basal diet plus vehicle and corn oil plus vehicle rats) and the colon tumors were examined. Total RNA was

extracted using Isogen (Nippon Gene, Tokyo, Japan) according to the manufacturer's instructions. RT-PCR specific for the rat *wt p53* mRNA was amplified using a primer specific for the leader sequence of exon 6–7 of the *wt p53* allele (5′-GGCTCCTCCCCAACATCTTATC-3′) and the downstream primer (5′-TCTCCCAGGACAGGCA-CAAAC-3′). For the internal control, the expression of the glyceraldehyde 3-phosphate dehydrogenase (*G3PDH*) gene in each sample was also quantified using the primer (5′-TCCACCACCCTGTTGCTGTA-3′) and the downstream primer (5′-ACCACAGTCCATGCCATCAC-3′). RT-PCR was performed on 1 μg total RNA using a RT-PCR kit (Toyobo Co., Osaka, Japan). The PCR products were electrophoresed in 2% agarose gels and visualized under ultraviolet fluorescent light after staining with ethidium bromide. Densitometric assessment of the autoradiogram bands was conducted using Image Gauge Version 3.12 (Fujifilm, Tokyo, Japan). Band intensity was quantified by measuring the absolute integrated optical intensity, which estimates the volume of the band in the lane profile. Six rats were studied in each group. The results were expressed as a ratio to G3PDH densitometry units.

Western Blotting Analysis. At 48 weeks, after the second injection of AOM, the colonic mucosa (basal diet plus vehicle and corn oil plus vehicle rats) and the colonic mucosa and tumors were examined. Total proteins, cytosolic fractions, and mitochondrial fractions were purified as previously described (29–32). Equal quantities of protein were electrophoresed in a sodium dodecyl sulfate polyacrylamide gel and electroblotted onto a nitrocellulose membrane (Trans-Blot Bio-Rad; Hercules, CA). After blocking with phosphate-buffered saline containing 0.1% polyoxyethylene sorbitan monolaurate (Tween-20, Sigma) and 5% skimmed milk at 4°C overnight, the membrane was respectively incubated with rabbit polyclonal anti–proliferating cell nuclear antigen (PCNA) antibody (1:1000), mouse monoclonal anti–*wt p53* antibody (1:1000), mouse monoclonal anti–Bcl-2 antibody (1:500), mouse monoclonal anti–Bcl-xL antibody (1:500), mouse monoclonal anti-Bax antibody (1:500), rabbit polyclonal anti-Bak antibody (1:1000), rabbit polyclonal anti–cytochrome-*c* antibody (1:1000), and rabbit polyclonal anti–caspase-3 antibody (1:1000, all from Santa Cruz Biotech, Santa Cruz, CA) for 1 hr. Antigen-antibody complexes were detected with horseradish peroxidase–conjugated anti-mouse IgG or anti-rabbit IgG (1:1000, Santa Cruz Biotech). Detection of the chemiluminescence was carried out using Western blotting detection reagents (Amersham Pharmacia Biotech, Buckinghamshire, UK). Densitometric assessment of the autoradiogram bands was conducted using Image Gauge Version 3.12 (Fujifilm). Band intensity was quantified by measuring the absolute integrated optical intensity, which estimates the volume of the band in the lane profile. Six rats were studied in each group. The results were expressed as a ratio to β-actin densitometry units.

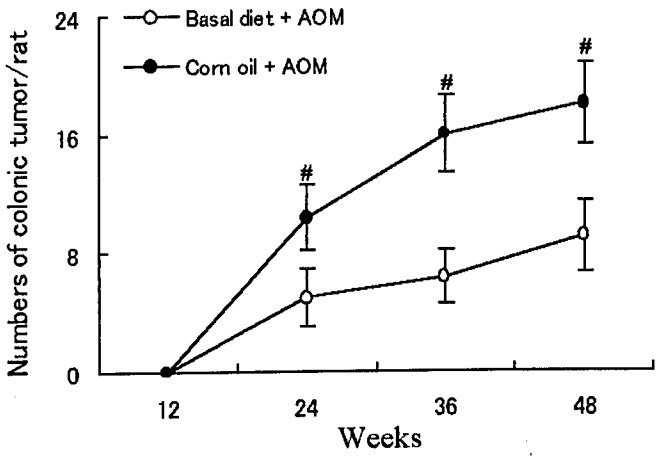

Figure 1. Long-term dietary corn oil ingestion promoted azoxymethane (AOM)-induced colon carcinogenesis and cancer development. The number of colon tumors is significantly increased in the AOM-treated rats with dietary corn oil compared with the AOM-treated rats with dietary basal diet. Values are the means ± SD, six rats were examined in each group at each time point. #$P < 0.01$ compared with the AOM-treated rats with basal diet.

Table 1. Long-Term Dietary Corn Oil Ingestion Promoted Colonic Aberrant Crypt Foci (ACF) Formation in Azoxymethane (AOM)-Treated Rats at 12 Weeks[a]

	Total ACF/colon		≥4 ACF/colon	
	Vehicle	AOM	Vehicle	AOM
Basal diet	1.2 ± 0.3	41.0 ± 4.8*	0	16.3 ± 2.1*
Corn oil	1.3 ± 0.4	73.3 ± 9.9**	0	32.3 ± 3.6**

[a] Values are means ± SD, six rats were examined in each group.
* $P < 0.01$ compared with the vehicle-treated rats.
** $P < 0.01$ compared with the vehicle-treated rats and with the AOM-treated rats with basal diet.

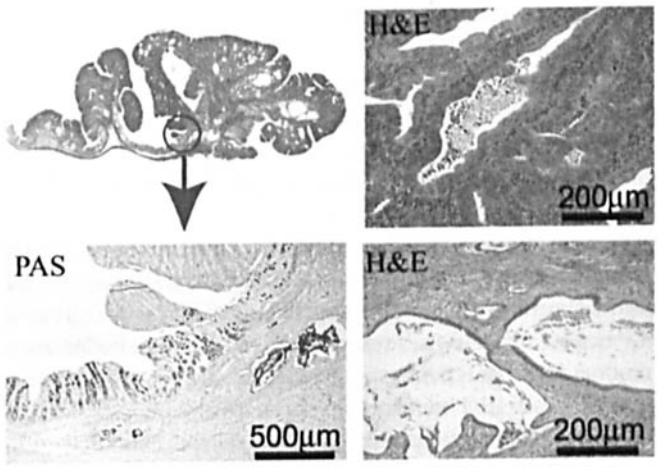

Figure 2. Histological findings of invasive colon cancers. A well-differentiated adenocarcinoma with cancer invasion was observed in the azoxymethane-treated rats with dietary corn oil at 48 weeks.

Table 2. Long-Term Dietary Corn Oil Ingestion Promoted Colon Cancer Invasion in azoxymethane (AOM)-Treated Rats at 48 Weeks

	Cancer incidence[a] (%)		Mucosal cancer (%)		Invasive cancer (%)	
	Vehicle	AOM	Vehicle	AOM	Vehicle	AOM
Basal diet	0 (0/6)	100 (6/6)*	0 (0/6)	100 (6/6)*	0 (0/6)	0 (0/6)
Corn oil	0 (0/6)	100 (6/6)*	0 (0/6)	100 (6/6)*	0 (0/6)	100 (6/6)**

[a] Cancer incidence is expressed as the ratio of rats with colon cancer to total rats. Results were analyzed statistically using the chi-square test.
* $P < 0.01$ compared with the vehicle-treated rats.
** $P < 0.01$ compared with the vehicle-treated rats and with the AOM-treated rats with basal diet.

Statistical Analysis. Results are expressed as mean ± SD. Data were evaluated by analysis of variance (ANOVA) in which multiple comparisons were performed by the method of least significant difference. Colon cancer incidence is expressed as the percentage of animals with cancer, and the results were analyzed statistically using the chi-square test. Differences were considered significant if the probability of the difference occurring by chance was less than 5 in 100 ($P < 0.05$).

Results

Long-Term Dietary Corn Oil Promoted Colon Carcinogenesis and Cancer Invasion in AOM-Treated Rats. At 12 weeks, after the second injection of AOM, the entire colonic mucosa was stained with 0.2% methylene blue to evaluate ACF formation using stereoscopic microscopy and they then were histologically examined. The ACF had dilated irregular luminal openings, thicker epithelial linings, and protrusions towards the lumen (data not shown). Few ACF were found in both the dietary basal diet and the dietary corn oil rats without AOM treatment. A large number of ACF (especially ≥4 ACF) were observed in the AOM-treated rats. Compared with AOM-treated rats with dietary basal diet, the number of total ACF and ≥4 ACF were about 2-fold higher in the AOM-treated rats with dietary corn oil as shown in Table 1. ANOVA revealed that corn oil (total ACF, $F_{1,20} = 8.7$; ≥4 ACF, $F_{1,20} = 14.6$), AOM (total ACF, $F_{1,20} = 102.5$; ≥4 ACF, $F_{1,20} = 134.9$), and interaction of the two factors (total ACF, $F_{1,20} = 8.5$; ≥4 ACF, $F_{1,20} = 14.6$) significantly induced ACF formation in the colonic mucosa ($P < 0.01$ in each).

Colon tumors were not observed in rats without AOM treatment throughout the study period. No tumors were observed in AOM-treated rats at 12 weeks. At 24 weeks, colon tumors were found in the AOM-treated rats, and all tumors were tubular adenomas (data not shown). At 36 and 48 weeks, the tumors showed well-differentiated adenocarcinomas with polypoid growth. The number of colon tumors was significantly increased in the corn oil plus AOM rats compared with the basal diet plus AOM rats as indicated in Figure 1 ($F_{1,46} = 12.2$, $P < 0.01$). At 48 weeks, cancer invasion was only observed in the corn oil plus AOM rats (Fig. 2). As shown in Table 2, all AOM-treated rats fed on

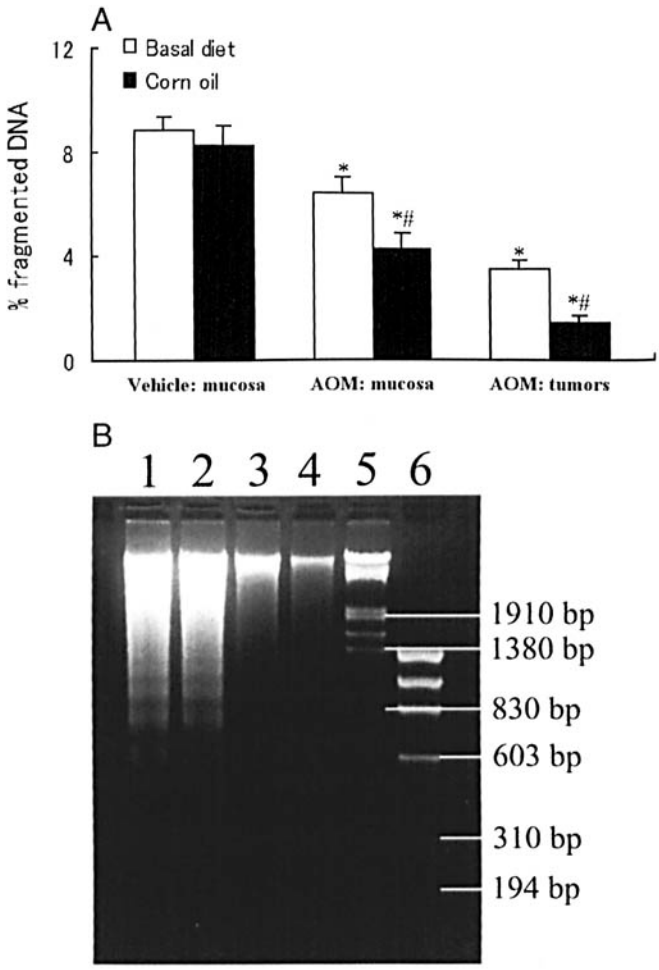

Figure 3. Long-term dietary corn oil ingestion enhanced the inhibitory effect of azoxymethane (AOM) on apoptosis in colonic mucosa and cancer. At 48 weeks, the colonic mucosa (basal diet plus vehicle and corn oil plus vehicle rats) and the colonic mucosa and tumors (basal diet plus AOM rats and corn oil plus AOM rats) were examined. (A) The percentage of fragmented DNA. Values are means ± SD, six rats were studied in each group. *$P < 0.01$ compared with the vehicle-treated rats. #$P < 0.05$ compared with the AOM-treated rats with basal diet. (B) DNA ladders. Lanes 1–4, basal diet plus vehicle rat, corn oil plus vehicle rat, basal diet plus AOM rat, and corn oil plus AOM rat, respectively. Lanes 5 and 6 contain marker DNA.

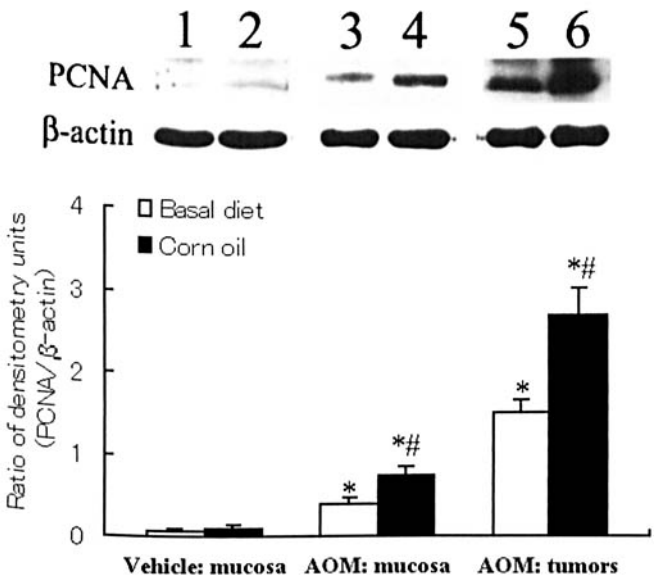

Figure 4. Long-term dietary corn oil ingestion promoted cell proliferation in azoxymethane (AOM)-induced colonic mucosa and cancer. At 48 weeks, the colonic mucosa (basal diet plus vehicle and corn oil plus vehicle rats) and the colonic mucosa and tumors (basal diet plus AOM and corn oil plus AOM rats) were examined. PCNA was evaluated in the total protein. Lanes 1–6: mucosa in basal diet plus vehicle rats, mucosa in corn oil plus vehicle rats, mucosa in basal diet plus AOM rats, mucosa in corn oil plus AOM rats, tumors in basal diet plus AOM rats, and tumors in corn oil plus AOM rats, respectively. Values are means ± SD, six rats were studied in each group. $^*P < 0.01$ compared with the vehicle-treated rats. $\#P < 0.01$ compared with the AOM-treated rats with basal diet.

dietary corn oil (100%) had invasive colon cancers, but no invasive cancer was found in the AOM-treated rats with dietary basal diet. Histologically, poorly differentiated or undifferentiated adenocarcinomas were not found in the colons of these rats. These results suggest that long-term dietary corn oil might promote not only colon carcinogenesis but also cancer invasion.

Long-Term Dietary Corn Oil Inhibited Cell Apoptosis and Promoted Cell Proliferation in AOM-Induced Colonic Mucosa and Colon Cancer. Apoptosis was evaluated using a DNA fragmentation assay (Fig. 3A). After AOM treatment, the percentage of fragmented DNA was significantly inhibited in the colonic mucosa and tumors ($P < 0.01$ in each). This inhibitory effect of AOM was significantly enhanced by dietary corn oil in the mucosa and tumors ($P < 0.05$ in each). The DNA ladders assay also showed that colon tumor apoptosis was significantly inhibited in the AOM-treated rats at 48 weeks, and the inhibitory effect was enhanced by dietary corn oil (Fig. 3B).

Cell proliferation was evaluated by assessing PCNA as shown in Figure 4. After AOM treatment, the colonic mucosal and tumors PCNA significantly increased at 48 weeks ($P < 0.01$ in each). This effect of AOM was significantly enhanced by dietary corn oil in the mucosa and tumors ($P < 0.01$ in each).

Long-Term Dietary Corn Oil Enhanced the Inhibitory Effects of AOM on Mitochondrial *wt p53*. The mRNA of *wt p53* was confirmed by RT-PCR, and *wt p53* protein expression was determined by Western blotting analysis. As shown in Figure 5, *wt p53* mRNA and total protein were not inhibited by AOM treatment at 48 weeks, but cytosolic *wt p53* significantly increased in AOM-treated rats, and this was enhanced by a corn oil diet. At 48 weeks, compared with the rats with vehicle treatment, mitochondrial *wt p53* was decreased in the rats with AOM treatment, although total *wt p53* was not inhibited. The reduction in mitochondrial *wt p53* was significantly enhanced by dietary corn oil.

Long-Term Dietary Corn Oil Enhanced the Inhibitory Effects of AOM on Mitochondrial Apoptotic Signaling. We have shown that mitochondrial localization of *wt p53* is inhibited by AOM treatment, and that dietary corn oil enhanced this inhibitory effect at 48 weeks. Furthermore, we investigated mitochondrial apoptotic signaling. The results are shown in Figure 6. Inhibition of mitochondrial *wt p53* localization caused by AOM treatment resulted in upregulation of the mitochondrial antiapoptotic members, Bcl-2 and Bcl-xL. Long-term dietary corn oil promoted Bcl-xL accumulation in the mitochondria by enhancing the inhibitory effect of AOM on mitochondrial *wt p53* localization. AOM treatment with a corn oil diet induced the downregulation of mitochondrial proapoptotic Bak members at 48 weeks but without any influence on Bax. Further, AOM treatment significantly inhibited procaspase-3 formation. The release of cytochrome-*c* from the mitochondria into the cytosol was decreased, resulting in the reduction of cleaved caspase-3 at 48 weeks in the AOM-induced tumors, and these effects were also enhanced by a corn oil diet. These results suggest that long-term dietary corn oil enhances the inhibitory effect of AOM on *wt p53*–mediated mitochondrial apoptotic signaling.

Discussion

Several observations have confirmed the putative association between ACF and cancer in the colon and AOM-induced ACF formation in animals (7–9). In this study, the results show that colonic ACF was about 2-fold higher in corn oil plus AOM rats compared with basal diet plus AOM rats and suggest that long-term dietary corn oil might be involved in the induction of colon cancer with ACF formation. Furthermore, our data demonstrate that long-term dietary corn oil promotes colon cancer invasion after AOM treatment. Previous studies have reported that corn oil rich in *n*-6 PUFA involved colon carcinogenesis occurring in the postinitiation or promotional phase or both (4, 5). The present results suggest that long-term dietary corn oil is involved in colon cancer in both ACF formation and cancer invasion. Tumor growth is determined not only by increased cell proliferation but also by decreased tumor-cell apoptosis (12, 13). Our results showed that AOM

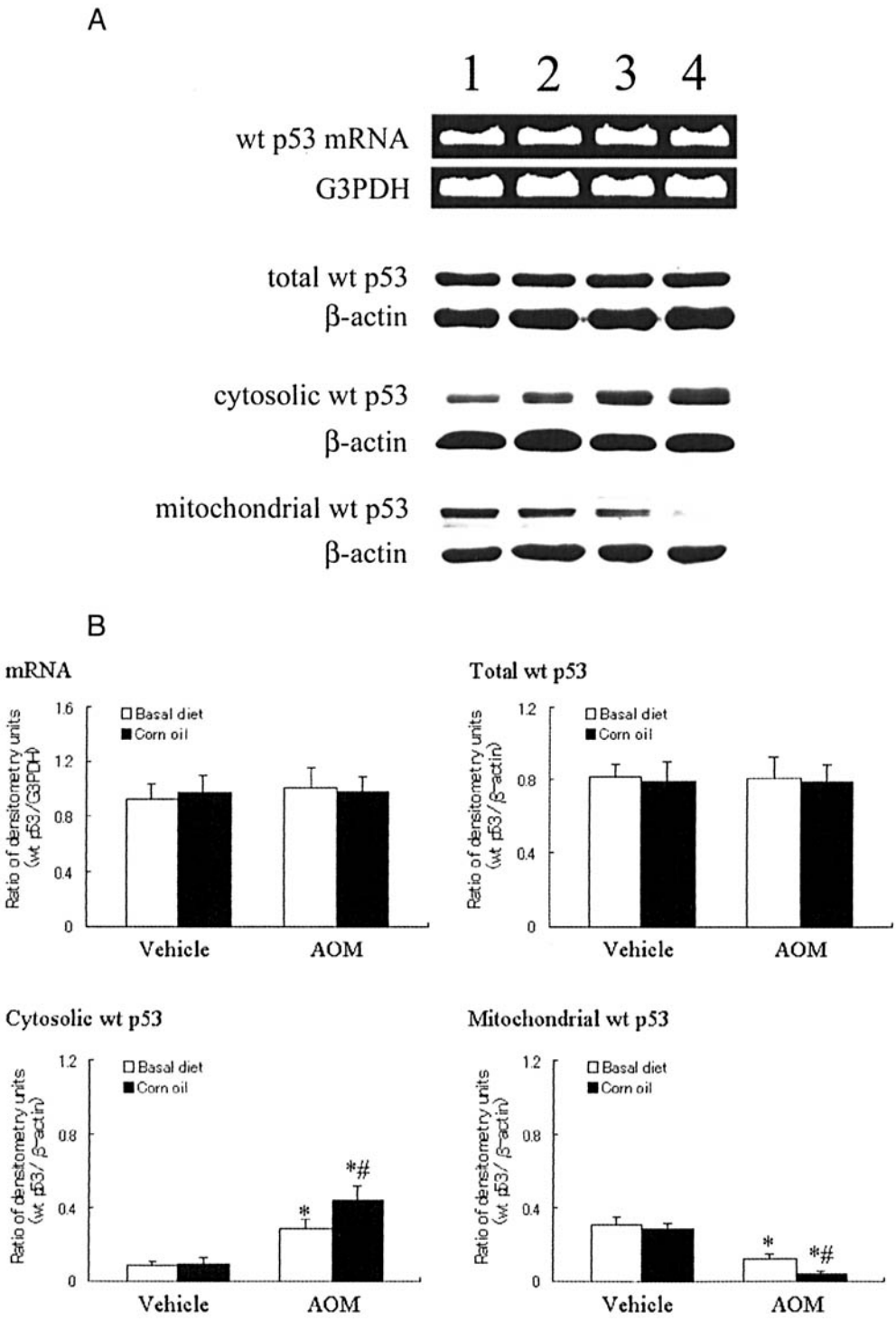

Figure 5. Long-term dietary corn oil ingestion enhanced the inhibitory effect of azoxymethane (AOM) on mitochondrial *wt p53* in colon cancer. At 48 weeks, the colonic mucosa (basal diet plus vehicle and corn oil plus vehicle rats) and tumors (basal diet plus AOM and corn oil plus AOM rats) were assessed. Total, cytosolic, and mitochondrial *wt p53* were measured using Western blotting. Lanes 1–4, basal diet plus vehicle rats, corn oil plus vehicle rats, basal diet plus AOM rats, and corn oil plus AOM rats, respectively. (A) *Wt p53* determined using RT-PCR and Western blotting. (B) The band quantified. Values are means ± SD, six rats were studied in each group. $^*P < 0.01$ compared with the vehicle-treated rats. $\#P < 0.01$ compared with the AOM-treated rats with basal diet.

treatment significantly inhibited cell apoptosis and promoted cell proliferation leading to malignant cell growth in both the colonic mucosa and tumors, and this effect was significantly enhanced by long-term dietary corn oil.

Wild type p53 has multiple functions as a tumor suppressor, including cell cycle arrest in response to DNA damage, induction of apoptosis, and DNA repair (33–36). In its normal state, the tumor suppressor action of *wt p53* is

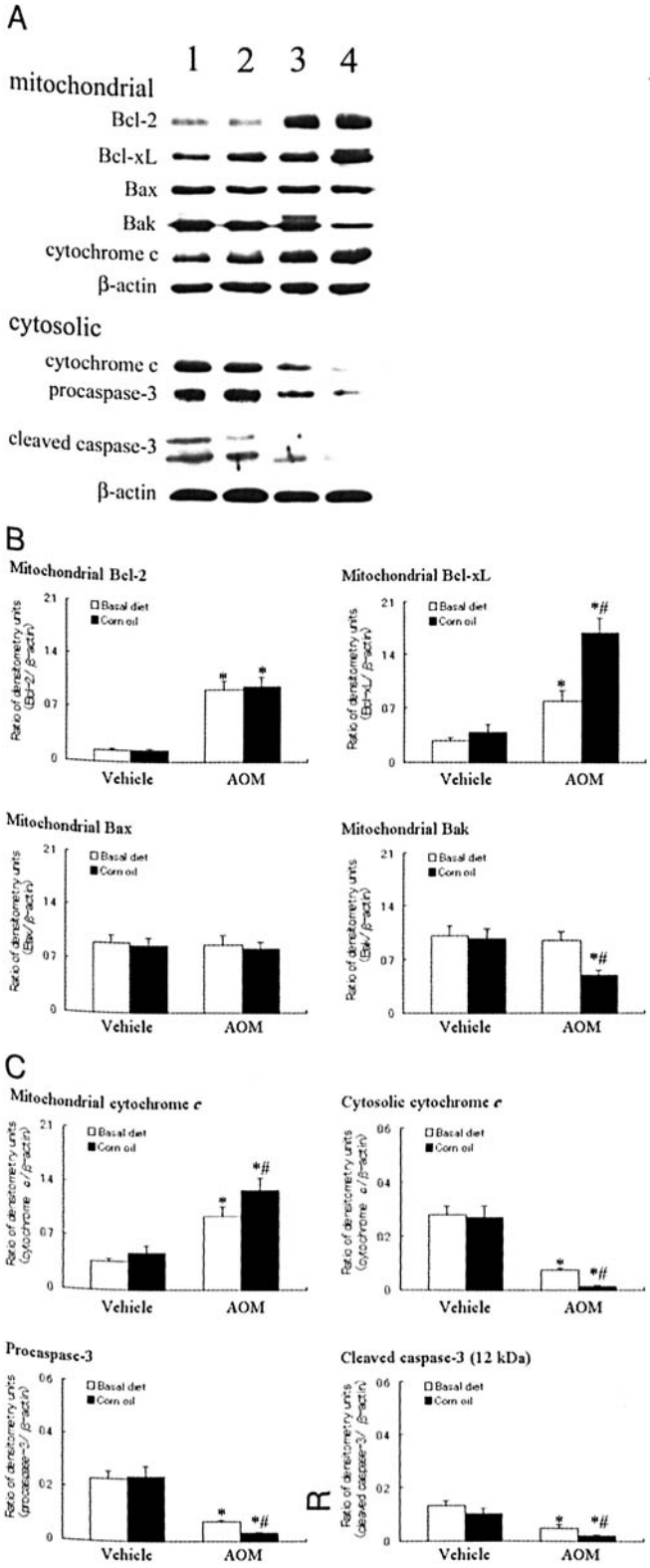

Figure 6. Long-term dietary corn oil ingestion enhanced the inhibitory effects of azoxymethane (AOM) on mitochondrial apoptotic signaling in colon cancer. At 48 weeks, the colonic mucosa (basal diet plus vehicle and corn oil plus vehicle rats) and tumors (basal diet plus AOM and corn oil plus AOM rats) were assessed. Lanes 1–4, basal diet plus vehicle rats, corn oil plus vehicle rats, basal diet plus AOM rats, and corn oil plus AOM rats, respectively. (A) The image of Western blotting. (B and C) The band quantified. Values are means ± SD, six rats were studied in each group. *$P < 0.01$ compared with the vehicle-treated rats. #$P < 0.01$ compared with the AOM-treated rats with basal diet.

present in the nucleus and mediated by specific DNA binding and protein-protein interactions within the nucleus, although sometimes a secondary event occurs in which *wt p53* is lost (37). Abnormal cytosolic *wt p53* localization has been observed in a number of cancer cell lines, and this cytosolic *wt p53* is stable and inactive (38). Our data indicate that an increase in cytosolic *wt p53* localization occurs after AOM treatment leading to reduced *wt p53* activity in colon cancer, although *wt p53* mRNA and total proteins were not inhibited by AOM. Dietary corn oil enhanced this AOM-induced cytosolic *wt p53* localization. These results suggest that long-term dietary corn oil might enhance colon cancer development after AOM treatment via *wt p53* inactivation.

Cytosolic *wt p53* is inactivated, but when it is localized in the mitochondria it can contribute toward apoptosis. Previous evidence has shown that *wt p53* has an apoptotic role in mitochondria (39–41), but how mitochondrial *wt p53* induces apoptosis has not been clearly demonstrated. Our data revealed that mitochondrial *wt p53* induces apoptosis by regulating mitochondrial Bcl-2 family proteins. The Bcl-2 family proteins are central regulators of the mitochondria-dependent apoptotic pathway and can be subdivided into two classes: antiapoptotic members, such as Bcl-2 and Bcl-xL, which protect cells from apoptosis, and proapoptotic members, such as Bax and Bak, which trigger or sensitize cells for apoptosis. The ratio of antiapoptotic and proapoptotic Bcl-2 members determines the susceptibility of cells to death signals. Following multiple death stimuli, Bax and Bak induce mitochondrial dysfunction and cell death, while Bcl-2 and Bcl-xL prevent Bax- and Bak-mediated mitochondrial apoptosis (42). Bcl-2 family members are integral proteins located mainly on the outer membrane of mitochondria, and their overexpression prevents the efflux of cytochrome-*c* from the mitochondria and the initiation of apoptosis (30, 43). We previously demonstrated that the release of cytochrome-*c* from the mitochondria to the cytosol is important for apoptosis in intestinal mucosa (29, 44, 45). Cytosolic cytochrome-*c* forms a complex with Apaf-1 and caspase-9, resulting in the activation of caspase-9 and caspase-3 (46). Caspase-3 possesses a small prodomain and participates in the execution phase of apoptosis. In this study, the results show that long-term dietary corn oil enhanced the inhibitory effects of AOM on mitochondrial cytochrome-*c* release, procaspase-3 formation, and caspase-3 cleavage. This result suggests that inhibition of mitochondrial cytochrome-*c* release might be involved in inhibition of caspase-3 in both procaspase-3 synthesis and caspase-3 activation. Our results revealed

that AOM treatment significantly inhibited the mitochondrial localization of *wt p53*, preventing both the mitochondrial cytochrome-*c* release and the caspase-3 activation partly through the regulation of Bcl-2 family members. Long-term dietary corn oil significantly enhanced this inhibitory effect, resulting in inhibited mitochondrial apoptotic signaling.

In conclusion, colon cancer invasion was developed in AOM-treated rats after long-term dietary corn oil ingestion, suggesting that dietary corn oil can enhance AOM-induced colon carcinogenesis and cancer development. The reason why cancer development was enhanced by long-term dietary corn oil in this model might be in part explained by the inhibition of the tumor suppressor gene *p53*-mediated mitochondria-dependent apoptosis. These results warrant further exploration including the effect of diet oil with other carcinogens and the effect of other diet oils on colon cancer development.

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