Research Communication

Curcumin Combined with Turmerones, Essential Oil Components of Turmeric, Abolishes Inflammation-Associated Mouse Colon Carcinogenesis

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Abstract

Curcumin (CUR), a yellow pigment in turmeric, has marked potential for preventing colon cancer. We recently reported that ar-turmerone (ATM) suppressed nitric oxide (NO) generation in macrophages. In the present study, we explored the molecular mechanisms by which ATM attenuates NO generation and examined the anti-carcinogenesis activity of turmerones (TUR, a mixture of 5 sesquiterpenes including ATM). Both CUR and ATM inhibited lipopolysaccharide (LPS)-induced expression of inducible forms of both nitric oxide synthase and cyclooxygenase (iNOS and COX-2, respectively). A chase experiment using actinomycin D revealed that ATM accelerated the decay of iNOS and COX-2 mRNA, suggesting a post-transcriptional mechanism. ATM prevented LPS-induced translocation of HuR, an AU-rich element-binding protein that determines mRNA stability of certain inflammatory genes. In a colitis model, oral administration of TUR significantly suppressed 2% dextran sulfate sodium (DSS)-induced shortening of the large bowel by 52–58%. We also evaluated the chemopreventive effects of oral feeding of TUR, CUR, and their combinations using a model of dimethylhydradine-initiated and DSS-promoted mouse colon carcinogenesis. At the low dose, TUR markedly suppressed adenoma multiplicity by 73%, while CUR at both doses suppressed adenocarcinoma multiplicity by 63–69%. Interestingly, the combination of CUR and TUR at both low and high doses abolished tumor formation. Collectively, our results led to our hypothesis that TUR is a novel candidate for colon cancer prevention. Furthermore, we consider that its use in combination with CUR may become a powerful method for prevention of inflammation-associated colon carcinogenesis. © 2012 BioFactors, 00(00):000–000, 2013

Keywords: *chemoprevention; turmeric; colorectal cancer; colitis; mRNA stability*

Abbreviations: *CUR, curcumin; ATM, ar-turmerone; NO, nitric oxide; TUR, turmerones; LPS, lipopolysaccharide; iNOS, inducible nitric oxide synthase; COX, cyclooxygenase; DSS, dextran sulfate sodium; UC, ulcerative colitis; CRC, colorectal cancer; AOM, azoxymethane; DMH, dimethylhydradine; DMEM, Dulbecco's modified eagle medium; FBS, fetal bovine serum; RT-PCR, Reverse transcription-polymerase chain reaction; DMSO, dimethyl sulfoxide.*

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1. Introduction

Inflammatory bowel disease such as ulcerative colitis (UC) and Chrohn's disease has been reported to have some association with the onset of colorectal cancer (CRC). For example, patients with UC are 10 times more likely to develop colorectal dysplasia and adenocarcinoma than the general population [1]. Although the mechanisms underlying inflammation-associated colon carcinogenesis are not fully understood, dysregulated activation of immune cells and resultant oxidative stress may play some pivotal roles in the progression of its pathology [2,3]. Recently, one of the authors and coworkers established a novel animal model in which mice are initiated with azoxymethane (AOM) or dimethylhydrazine (DMH), then intestinal tumor development with elevated expressions of both inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX)-2 proteins is promoted by administration of dextran sulfate sodium (DSS) [4]. Those proinflammatory molecules have been reported to contribute to the development of CRC [5], thus this animal model may have some advantages for the discovery of novel chemopreventive agents.

Curcumin (CUR) has long attracted the attention of investigators because of its pronounced chemopreventive efficacy, together with its no or low level of toxicity in many organs, especially the colon [6]. In fact, several independent research groups have shown that CUR prevents chemically and genetically induced intestinal tumorigenesis in mice and rats [7–11]. Although the mechanisms of action underlying the chemopreventive effects of CUR have not been fully elucidated, iNOS and COX-2 may be critical target molecules of CUR [12]. In support of that speculation, Camacho-Barquero et al. demonstrated that oral administration of CUR suppressed trinitrobenzenesulfonic acid-induced colitis by down-regulating both iNOS and COX-2 in rodents [13].

Turmeric (*Curcuma longa* L.) is used as a yellow pigment in curry and is one of the most well-known botanical sources of curcuminoids, a group of diarylheptanoid compounds that includes CUR. On the other hand, it is less known that its essential oil contains abundant mono- and sesquiterpenoids [14], whose physiological and biological functions are largely unknown. Turmerones (TUR), which consist of five structurally related sesquiterpenes (Fig. 1), were originally identified as an anti-bacterial byproduct of CUR manufacturing [15]. Interestingly, Kuroda et al. recently reported that an ethanol extract from turmeric containing CUR, demethoxycurcumin, bisdemethoxycurcumin, and the sesquiterpene, ar-turmerone (ATM) significantly suppressed increases in blood glucose in type 2 diabetic KK-A(y) mice [16]. In addition, other authors have shown that TUR are capable of suppressing endotoxin-induced nitric oxide (NO) generation by mouse macrophage, with an efficacy comparable to that of CUR [17]. Although the *in vitro* apoptosis-inducing properties of TUR components have been recently documented [18], no *in vivo* studies in regard to cancer prevention have been reported. In the present study, we investigated the molecular mechanisms by which ATM and CUR attenuate iNOS/COX-2 expression in macrophages, and found that ATM promotes mRNA decay via post-transcriptional mechanisms. In addition, we examined the effects of TUR on DSS-induced mouse colitis and the cancer preventive activities of TUR, CUR, and those in combination, in a DMH-initiated and DSS-promoted mouse colon carcinogenesis model.

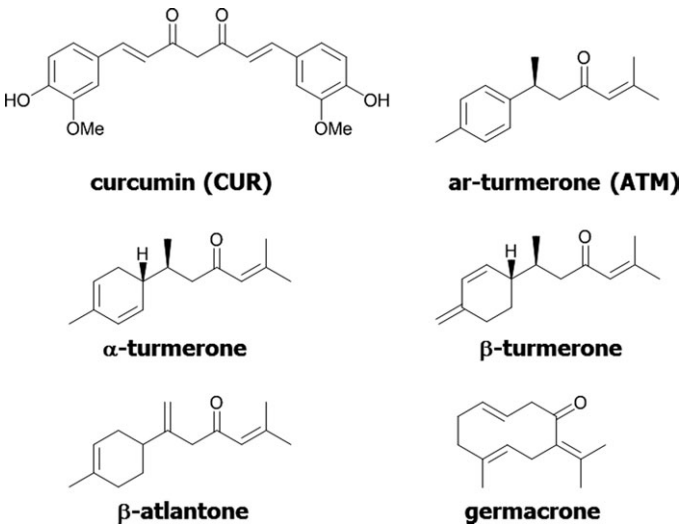

FIG 1 *Chemical structures of CUR and five sesquiterpenes of TUR.*

2. Materials and Methods

2.1. Cells, Animals and Reagents

RAW264.7 murine macrophages were obtained from American Type Culture Collection (Manassas, VA). Cytotoxicity was determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and found to be greater than 90% in each (data not shown). Female ICR mice at 7 weeks of age were obtained from Japan SLC (Shizuoka, Japan), housed five per cage, and given fresh tap water *ad libitum* and commercial rodent pellets (MF: Oriental Yeast Co., Kyoto, Japan), which were freshly changed twice a week. The mice were treated in accordance with the Guidelines for Animal Experimentation of Kyoto University and the experimental protocols in the present study were certified by the Animal Research Committee of the Graduate School of Agriculture, Kyoto University (no. 19–35). The mice were maintained in a controlled room at (24 ± 2) °C with a relative humidity of (60 ± 5)% and a 12-h light/dark cycle (06:00 to 18:00). Dulbecco's modified eagle medium (DMEM) and fetal bovine serum (FBS) were purchased from Invitrogen (Carlsbad, CA). Lipopolysaccharide (LPS) (*Escherichia coli* serotype 0127, B8) came from Difco Labs (Detroit, MI). ATM was purified as previously reported [17]. CUR used in the *in vivo* (purity: 75%) and *in vitro* (purity: 90%) experiments was from Cayman Chemical (Ann Arbor, MI). All other chemicals were obtained from Wako Pure Chemical Industries (Osaka, Japan) unless specified otherwise.

TABLE 1 *List of PCR primers used*

Name	*Sequence*	*Conditions*	*Cycles*
COX-1	5′-CTTTGCACAACACTTCACCCACC-3′	94 °C for 45 sec	25
	5′-AGCAACCCAAACACCTCCTGG-3′	65 °C for 45 sec	
		72 °C for 2 min	
COX-2	5′-GCATTCTTTGCCCAGCACTT-3′	95 °C for 30 sec	22
	5′-AGACCAGGCACCAGACCAAAGA-3′	55 °C for 25 sec	
		72 °C for 45 sec	
iNOS	5′-CTGCAGCACTTGGATCAGGAACCTG-3′	95 °C for 20 sec	22
	5′-GGGAGTAGCCTGTGTGCACCTGGAA-3′	61 °C for 25 sec	
		72 °C for 45 sec	
β-actin	5′-TGTGATGGTGGGAATGGGTCAG-3′	94 °C for 1 min	23
	5′-TTTGATGTCACGCACGATTTC-3′	54 °C for 1 min	
		72 °C for 1 min	

2.2. Preparation of TUR

TUR mixtures were prepared from commercially available turmeric oil (KANCOR, Kerala, India) and used in the mouse colitis and colon carcinogenesis experiments. Turmeric oil (2 g) was subjected to silica gel column chromatography (3 × 50 cm), with the column loaded with Wakogel C-200 (Wako Pure Chemical Industries) suspended in *n*-hexane. Increasing amounts of ethyl acetate (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0% in *n*-hexane) were used as the elutes and TUR (960 mg) were recovered in a 2.5% ethyl acetate fraction. The purity of the TUR was 95% or greater and the ratio of each component was as follows: *β*-atlantone:germacrone:ATM:*β*-turmerone:*α*-turmerone = 1.0:2.1:2.4:2.4:6.9 as detected by ^{1}H-nuclear magnetic resonance (ARX500, 500 MHz, Brucker, Billerica, MA, data not shown).

2.3. Reverse Transcription-Polymerase Chain Reaction

RAW 264.7 cells (1×10^6) were grown in 5 mL of DMEM with 10% FBS on 6-well plate, and incubated in an atmosphere containing 5% CO_2 at 37 °C for 13 h. The cells were washed with phosphate-buffered saline (PBS) twice, after which the media were exchanged with FBS- and phenol-red-free media (5 mL) containing samples (0–100 μM) dissolved in 25 μL of dimethyl sulfoxide (DMSO). After 30 min of pre-incubation, the cells were treated with LPS (100 ng/mL). Following a 6-h incubation, the cells were lysed and total RNA was extracted using kits (RNeasy® mini kit and QIAshredder®, Qiagen, Valencia, CA). One microgram of total RNA was reverse transcribed using an RNA polymerase chain reaction (PCR) kit® (Takara, Kyoto, Japan) with an oligo dT-adaptor primer, as recommended by the supplier. Then, cDNAs for COX-1, COX-2, iNOS, and *β*-actin were amplified with primers (Table 1) synthesized by Proligo (Boulder, CO). PCR was done on a thermal cycler (PTC-0100; MJ Research, Watertown, MA) using 1 μL of a cDNA preparation, 45 μL of Platinum® PCR SuperMix (Invitrogen), and 0.5 μL of each primer (100 nM). The PCR products were separated on 2% NuSieve® 3:1 agarose (BioWhittaker Molecular Applications, Rockland, ME) and each band was visualized using 0.01% SYBR Gold® stain (Molecular Probes, Leiden, The Netherlands). The amplified products were photographed with a digital camera. The number of PCR cycles was optimized in such a way so that the intensity of each band increased proportionally as the amount of cDNA increased. Each experiment was done at least three times.

2.4. Reporter Assays

Reporter assays were done using a Mercury® Pathway Profiling System (Clontech Laboratories, Mountain View, CA). RAW 264.7 cells (3×10^5) were preincubated in 1 mL of DMEM with 10% FBS on 24-well plates for 13 h in an atmosphere containing 5% CO_2 at 37 °C. Then, 625 μL of OPTI-MEM® (Invitrogen) and 37.5 μL of LipofectAMINE Reagent® (Invitrogen) were mixed in a tube, and 4 μg of pNF*κ*B-, pAP-1-, or pCREB-luciferase vector, provided in the kit, and 4 μg of pRL-TK vector (Promega, Madison, WI), which served as the internal standard, were added. The transfection mixture was allowed to stand at room temperature for 30 min, after which 5 mL of OPTI-MEM was added. After washing the cells twice with Hanks' buffer, 250 μL of transfection mixture was added to each well and the cells were incubated at 37 °C for 6 h. After

washing, the cells were incubated in 1 mL of DMEM containing 10% FBS for 12 h, washed twice with Hanks' buffer, then exposed to 5 μL of DMSO or the sample dissolved in DMSO in serum-free DMEM for 30 min. After stimulation of the cells with LPS (100 ng/mL) for 12 h, luciferase activity in the cell lysate was determined using a Dual-Luciferase Reporter Assay Kit® (Promega, Madison, WI).

2.5. Chase Experiment

RAW 264.7 cells (1×10^6) were grown in 1 mL of DMEM with 10% FBS in 12-well plates for 13 h in an atmosphere containing 5% CO_2 at 37 °C. After washing twice with PBS, the cells were exposed to LPS (100 ng/mL) and incubated in serum-free DMEM for 24 h for iNOS and COX-2 induction. Thirty minutes after adding actinomycin D (500 ng/mL) to the cell culture media, the cells were treated with 5 μL of DMSO, ATM (100 μM), or SB203580 (20 μM) for 0, 1, 3, or 6 h for Reverse transcription (RT)-PCR analyses, which were performed as described above.

2.6. HuR Translocation

RAW264.7 cells (5×10^4) were cultured in 200 μL of DMEM with 10% FBS on 1-chamber slides (GLASS/PS, 9 × 9, IWAKI, Osaka, Japan) for 13 h in an atmosphere containing 5% CO_2 at 37 °C. After washing twice with PBS, the cells were suspended in 200 μL of FBS-free DMEM and treated with 1 μL of DMSO or ATM (100 μM) for 30 min followed by exposure to LPS (100 ng/mL). After 1 h, the cells were washed with PBS three times and fixed with 4% paraformaldehyde/PBS, then allowed to stand for 20 min at room temperature. After washing with PBS three times, the cells were permeated with 0.1% Triton X-100 in PBS and allowed to stand for 30 min at room temperature. The cells were washed with PBS three times and treated with BlockAce for 1 h at room temperature. After washing again with PBS three times, the cells were incubated with the primary antibody (mouse anti-HuR, 1:50 dilution, Santacruz) at 4 °C overnight. Next, the cells were washed with PBS five times, and incubated with the secondary antibody (anti-mouse IgG, 1:40 dilution, DAKO, Kyoto, Japan) for 1 h at room temperature. After washing with PBS five times, the cells were incubated with DAPI (2 μg/mL) for 5 min at room temperature. After washing again with PBS three times, the cells were mounted with 50% glycerol in PBS and observed under a fluorescent microscope, with the results shown at the original ×400 magnification.

2.7. DSS-Induced Mouse Colitis

After 1-week quarantine, 36 mice were divided into four groups composed of 9 mice each. In the DSS group (group 1), 2% DSS (molecular weight: 36,000–50,000, ICN Biomedicals, Aurora, OH) in tap water was given 1 week after the beginning of experiment to induce colitis, and the mice were fed with MF pellets. In the control group (group 4), mice were given fresh tap water *ad libitum* and MF pellets, freshly changed twice a week, for 2 weeks. In the other two groups, a 0.01% TUR (group 2) or 0.05% TUR (group 3) mixture was added to the MF pellets for 2 weeks. One week after beginning the experiment, 2% DSS drinking water was given to all mice, except for the control (group 4). The body weight of each mouse was recorded every 2 days after the start of the experiment, and the intake of food and water was measured daily. After the end of the experiment (week 2), all mice were killed by cervical dislocation and the large intestines without the cecum were removed. After washing in ice-cold PBS, they were placed on filter papers to measure their length (from the ileocecal junction to the anal verge). Then, the large bowels were cut open longitudinally along the main axis, and gently washed with saline, and the colonic mucosa was scraped off for mRNA isolation to examine iNOS expression by RT-PCR analysis. In addition, the livers, kidneys, and spleens were removed and weighed.

2.8. DMH/DSS-Induced Mouse Colon Carcinogenesis

After 1-week quarantine, a total of 72 male ICR mice were divided into 10 experimental and 1 control group. Mice in groups 1–7 were given double intraperitoneal injections of DMH (20 mg/kg body weight) at the beginning of weeks 1 and 2. One week after the second injection, the mice received 2% DSS in drinking water for 7 days. Mice in those groups were fed diets containing TUR at 0 (group 1), 20 (group 2), 100 TUR (group 3) ppm, CUR at 1,000 (group 4), 5,000 (group 5) ppm, TUR at 20 ppm + CUR at 1,000 ppm (group 6), and TUR at 100 ppm + CUR at 5,000 ppm (group 7) for 16 weeks. Groups 8, 9, and 10 were fed a diet containing 100 ppm of TUR, 5,000 ppm of CUR, and 100 ppm of TUR + 5,000 ppm of CUR, respectively, and received no further treatments. Group 11 was an untreated control. Following euthanasia, the large bowels were flushed with saline and excised, then the length was measured, and they were cut open longitudinally along the main axis and gently washed with saline. The whole large bowel was macroscopically inspected for the presence of tumors, then cut along the vertical axis and fixed in 10% buffered formalin for at least 24 h. Histopathological examinations were performed by one of the authors (T.T.) using paraffin-embedded sections after hematoxylin and eosin (H&E) staining.

2.9. Immunohistochemistry

Immunohistochemistry for COX-2 and iNOS expression was performed using 4-mm-thick paraffin-embedded sections, which were obtained from the colons of mice in all groups and prepared using a labeled streptavidin biotin method with a LSAB KIT (DAKO Japan,), with microwave accentuation [19]. The paraffin-embedded sections were heated for 30 minutes at 65 °C, then deparaffinized in xylene and rehydrated through a graded ethanol series at room temperature. A 0.05 M Tris HCl buffer (pH 7.6) was used for washing between the steps. Incubation was performed in a humidified chamber. Sections were treated for 40 min at room temperature, with 2% BSA and incubated overnight at 4 °C with the primary antibodies; anti-COX-2 rabbit polyclonal (diluted 1:50, IBL Co., Gunma, Japan)

and anti-iNOS rabbit polyclonal (diluted 1:1,000, Wako Pure Chemical Industries, Osaka, Japan) antibodies. To reduce nonspecific staining of mouse tissue by mouse antibodies, a Mouse On Mouse IgG blocking reagent (Vector Laboratories, Burlingame, CA) was applied for 1 h. Horseradish peroxidase activity was visualized by treatment with H_2O_2 and 3,3′-diaminobenzidine for 5 min. For the final step, the sections were weakly counterstained with Mayer's hematoxylin (Merck, Tokyo, Japan). For each specimen, serial sections were used a negative control and incubation with the primary antibodies was omitted.

2.10. Statistical Analysis

Each experiment was done at least three times unless specified otherwise, with the values shown as the mean ± standard deviation ($m \pm SD$). The statistical significance of differences between groups in each assay was assessed using a Student's *t*-test (two-sided) that assumed unequal variance. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Both ATM and CUR Suppressed LPS-Induced iNOS and COX-2 Expression in Macrophages

Inflammatory cells, which are known to induce excess NO generation, play pivotal roles in the development of inflammation-associated colon carcinogenesis [20]. On the other hand, we previously reported that TUR including ATM decreased NO generation in LPS-stimulated RAW264.7 mouse macrophages, though the mechanism of action was not addressed [17]. In the present study, we pretreated RAW264.7 cells with the vehicle, ATM, or CUR for 30 min then exposed them to LPS for 6 h, after which mRNA expressions of iNOS and COX-2 were examined by RT-PCR. The concentrations of those agents were determined based on their cytotoxicity, and each experiment was done under non-toxic conditions (data not shown). As depicted in Fig. 2A, the turmeric components suppressed both COX-2 and iNOS mRNA expressions in concentration-dependent manners, and abolished them at the highest concentrations (100 μM for ATM, 20 μM for CUR). In contrast, COX-1 and β-actin, both of which are expressed in a constitutive manner, remained unchanged.

LPS is well known to activate MAPKs including ERK1/2 and p38 MAPK which are transducers of extracellular signals for inducing transactivation of the *inos* and *cox-2* genes through transcription factors such as NFκB and AP-1 [21]. In addition, the mRNA stability of some oncogenic and inflammatory molecules, including both COX-2 and iNOS, is dependent upon the p38 MAPK pathway [22]. However, LPS-induced ERK1/2 and p38 MAPK activation and IκB degradation were not significantly affected by CUR and ATM (data not shown). Although CUR is considered to be a potent suppressor of AP-1 and NFκB, the effects of ATM are yet to be reported. Thus we examined the transcription activities of AP-1, NFκB, and CREB using a luciferase reporter assay. RAW264.7 cells were transiently transfected with those vectors and exposed to the vehicle, ATM (100 μM), or CUR (20 μM) followed by exposure to LPS for 12 h. LPS treatment led to marked increases in the

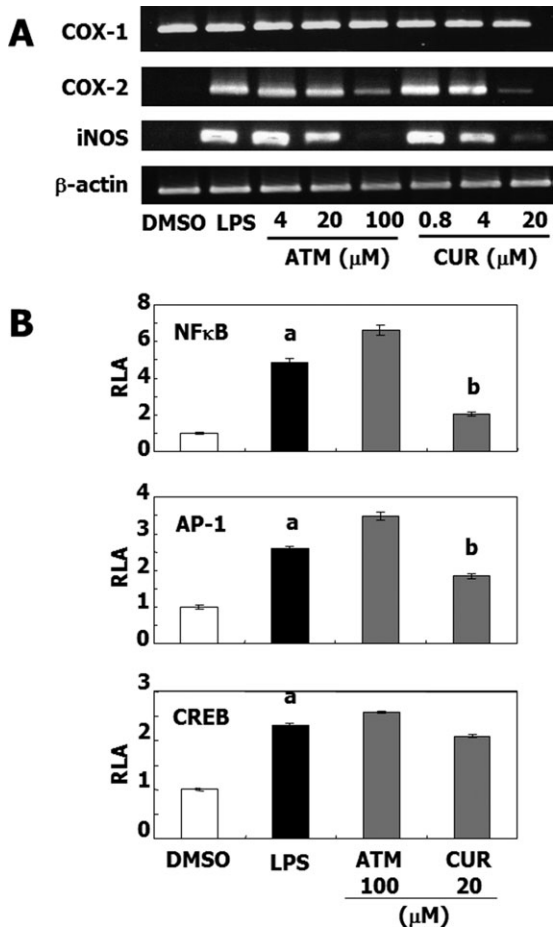

FIG 2

ATM and CUR attenuated LPS-induced iNOS and COX-2 expression and promoter activities of NFκB and AP-1, but not of CREB. A: RAW 264.7 cells were incubated with the samples (0–100 μM) for 30 min, then treated with LPS. Following 6-h incubation, the cells were lysed and total RNA was extracted. One microgram of total RNA was reverse-transcribed and cDNA for COX-1, COX-2, iNOS, and β-actin were amplified as described in Materials and Methods section. The PCR products were separated on 2% NuSieve® 3:1 agarose and each band was visualized using 0.01% SYBR Gold®. Each experiment was done at least three times, with one representative image from each shown. B: Luciferase reporter assays were done using a Mercury® Pathway Profiling System. RAW 264.7 cells were transfected with a pNFκB-pAP-1- or pCREB-luciferase vector using OPTI-MEM® and LipofectAMINE Reagent®, while a pRL-TK vector served as the internal standard. The cells were exposed to the vehicle or samples for 30 min. After stimulation of the cells with LPS for 12 h, luciferase activity in the cell lysate was determined using a Dual-Luciferase Reporter Assay Kit®. [a]P < 0.005 versus DMSO, and [b]P < 0.05 versus LPS in Student's t*-test.*

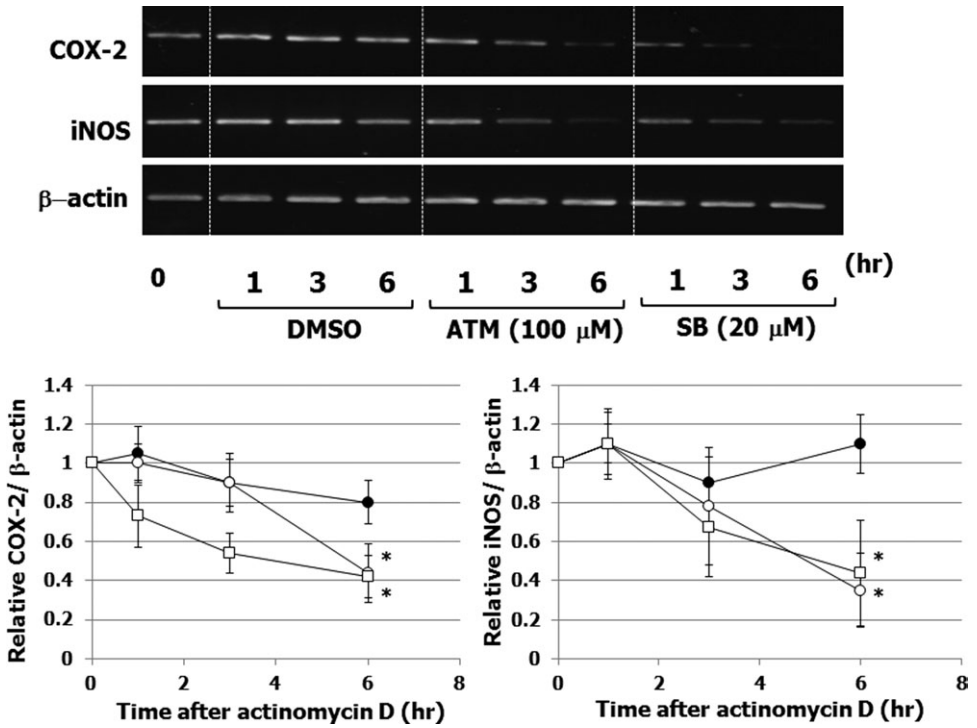

FIG 3

ATM and SB203580 (p38 MAPK inhibitor) accelerated the time-dependent decay of iNOS and COX-2 mRNAs. RAW 264.7 cells were grown on 12-well plates, then incubated for 12 h. After washing, the cells were exposed to LPS (100 ng/mL) and incubated in serum-free DMEM for 24 h for iNOS and COX-2 induction. Thirty minutes after adding actinomycin D (500 ng/mL) to the cell culture media, the cells were treated with the vehicle (0.5% DMSO, closed circles), ATM (100 μM, open circles), or SB203580 (20 μM, open triangles) for 0, 1, 3, and 6 h for RT-PCR analyses. [a]P < 0.05 versus vehicle control in Student's t*-test.*

activities of AP-1 (2.6-fold), NFκB (4.9-fold), and CREB (2.3-fold) as compared with the non-treated control ($P < 0.0005$ each, Fig. 2B). It is notable that CUR decreased the activities of AP-1 (by 48%) and NFκB (by 73%, $P < 0.005$ each), but not that of CREB, whereas ATM was virtually inactive toward disrupting those transcription factors. These results suggest that ATM targets iNOS and COX-2 mRNA expression via post-transcriptional mechanisms.

3.2. ATM Accelerated Actinomycin D-Induced iNOS and COX-2 mRNA Decay

The above results led us to perform a "chase experiment," in which iNOS/COX-2 mRNA decay was evaluated by blocking *de novo* synthesis of mRNA by actinomycin D. RAW264.7 cells were pretreated with LPS for 12 h to induce those mRNAs, then exposed to the transcription inhibitor actinomycin D. Thereafter, the vehicle, ATM (100 μM), or SB203580 (20 μM, specific p38 MAPK inhibitor used as positive control), was added to the cells, and RT-PCR was performed after 0, 1, 3, and 6 h of culturing. As shown in Fig. 3, those agents significantly accelerated actinomycin D-induced degradation of both COX-2 and iNOS mRNA after 6 h as compared with the vehicle ($P < 0.05$ each). In contrast, the expression levels of β-actin mRNA were stable with all treatments until 6 h.

3.3. ATM Attenuated LPS-Induced Translocation of HuR from Nucleus into Cytoplasm

The stability of many, if not all, mRNAs with the AU-rich element (ARE) at the 3′-untranslated region is dependent upon the association and dissociation of ARE-binding proteins (ARE-BP) including HuR [22]. HuR, which is localized in the nucleus in normal conditions, is known to be phosphorylated by PKC-α and then translocate to the cytoplasm for mRNA stabilization in human mesangial cells [23]. As shown in Fig. 4, constitutive and exclusive localization of HuR was detected in the nucleus, and when RAW cells were stimulated with LPS for 1 h, a significant portion was distributed over into the cytoplasm. Pretreatment with ATM (100 μM) notably suppressed this HuR shuttling.

3.4. Oral Feeding of TUR Suppressed DSS-Induced Mouse Colitis

It was not practical to purify large amounts of ATM for rodent experiments because turmeric oil consists of at least five closely related sesquiterpenes including ATM. Thus, we evaluated the *in vivo* anti-inflammatory effect of a mixture of β-atlantone, germacrone, ATM, β-turmerone, and α-turmerone (molar ratio = 1.0:2.1:2.4:2.4:6.9). This sesquiterpene mixture, designated as TUR, was prepared from commercially available turmeric oil using a 1-step purification process with column

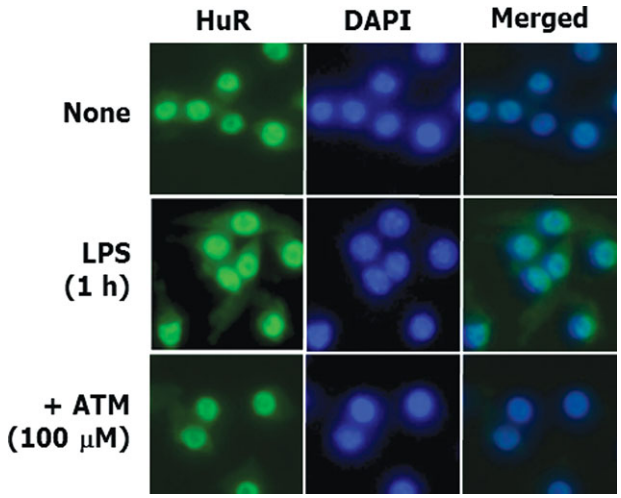

FIG 4

ATM inhibited LPS-induced HuR nucleus-cytoplasm shuttling in RAW264.7 cells. RAW264.7 cells were cultured on one-chamber slides. After washing with PBS twice, the cells were treated with the vehicle or ATM (100 μM) for 30 min followed by LPS exposure (100 ng/mL). After 1 h, the cells were washed with PBS three times and fixed with 4% paraformaldehyde/PBS, then allowed to stand for 20 min at room temperature. After washing with PBS three times, cells were permeated with 0.1% Triton X-100 in PBS and stood for 30 min at room temperature. Next, the cells were washed with PBS three times and treated with BlockAce for 1 h at room temperature. After washing with PBS three times, cells were incubated with the primary antibody (mouse anti-HuR, 1:50 dilution) at 4 °C overnight. The cells were then washed with PBS five times, and incubated with the secondary antibody (anti-mouse IgG, 1:40 dilution). After washing, the cells were incubated with DAPI (2 μg/mL) for 5 min at room temperature and mounted with 50% glycerol in PBS and observed under a fluorescent microscope (original magnification, 400×).

chromatography. TUR had potency for iNOS/COX-2 suppression as well as action mechanisms similar to those of ATM (data not shown).

We examined the anti-inflammatory efficacy of TUR in a DSS-induced colitis model using ICR mice. Acute colitis was induced by 2% DSS in drinking water for 10 days, during which time TUR was given at a dose of 100 or 500 ppm in the basal diet. There were no significant differences in regard to food and drinking water intake among the groups, and no detectable toxicity was observed (data not shown). As summarized in Table 2, both the body and liver weights of DSS-exposed mice (group 1) showed reduction tendencies as compared to the non-treated control (group 4), while the spleen weight of group 1 was significantly larger (23%) than that of group 4 ($P < 0.05$), indicating a typical symptom of colitis. Oral administration of TUR at both 100 and 500 ppm for 10 days did not affect the spleen weight increase, whereas it markedly suppressed DSS-induced shortening of the large bowel length due to intestinal inflammation (58% and 53%, $P < 0.002$ and $P < 0.05$, respectively). In addition, RT-PCR analysis revealed that TUR tended to suppress DSS-up-regulated iNOS mRNA expression in the colonic mucosa.

3.5. Combined TUR and CUR Abolished DMH-Initiated and DSS-Promoted Colon Carcinogenesis

We also investigated the ability of TUR to prevent colitis-induced tumor development in the colon of ICR mice. For this experiment, we combined TUR with CUR based on our findings that these turmeric phytochemicals have different modes of actions for suppressing iNOS and COX-2 (Fig. 2B), which implied additive or synergistic effects from their combination [24]. The doses of TUR (20 and 100 ppm) used were the same or lower than those (100 and 500 ppm) used in the colitis experiment, because oral feeding of TUR at 500 ppm for 20 weeks resulted in a significant decrease in body weight in a preliminary experiment (data not shown). On the other hand, the doses of CUR were within a similar range reported in previous studies [7,11].

Male ICR mice were initiated by an *i.p.* administration of DMH and were then exposed to a colon tumor promoter (2% DSS in drinking water, for 1 week) and sacrificed after 20 weeks. This experimental protocol has been reported to rapidly and effectively yield colon tumor formation [25]. As summarized in Table 3, body weights and colon lengths in each group did not change significantly except in the group that received 5,000 ppm of CUR without DMH/DSS treatment (group 9), which exhibited a 12% decrease in body weight ($P < 0.01$) as compared to the non-treatment group (group 11). As for liver, kidney, and spleen weights, mice that received 100 ppm of TUR (group 3) showed significant decreases (17–34%) as compared with those who received DMH/DSS treatment (group 1).

The DMH/DSS treatment (group 1) showed formations of colonic adenomas and adenocarcinomas (multiplicity: 4.29 ± 3.3 in total and 88% tumor incidence, Table 4). TUR at a low dose (group 2) reduced the multiplicity of adenomas by 73% ($P < 0.05$ versus group 1), while CUR at both low and high doses suppressed that of adenocarcinoma by 69% and 63%, respectively, ($P < 0.05$ each versus group 1). Strikingly, the combination of those turmeric components at low (20 ppm TUR plus 1,000 ppm CUR) and high (100 ppm TUR plus 5,000 ppm CUR) doses abolished colon tumor formation. There was no tumor formation in the control groups (groups 8–11).

Next, we then semi-quantified the expression levels of both COX-2 and iNOS protein in normal colonic mucosa in each group by immunohistochemical scoring (Fig. 5). Mice who received DMH/DSS treatment (group 1) had immunohistochemical scores for those proteins increased by 5.3- and 2.6-fold, respectively, as compared to the non-treatment group (group 11). On the other hand, the combination treatment groups (groups 6 and 7) showed notable suppression of those proinflammatory proteins (reduction of 65% and 67%, respectively, for COX-2, and of 95% and 95%, respectively, for iNOS, $P <$

TABLE 2 ***Body, liver, kidney, and spleen weights, length of large bowel, and iNOS expression in mouse colitis experiment***

Group no.	*Treatment*	*No. mice*	*Body weight (g)*	*Liver weight (g)*	*Kidney weight (g)*	*Spleen weight (g)*	*Length of large bowel (cm)*	*iNOS/HPRT mRNA*
1	DSS	9	35.9 ± 5.5	1.75 ± 0.30	0.55 ± 0.10[a]	0.18 ± 0.05[a]	7.02 ± 1.1[b]	1.00 ± 0.16
2	+ 100 ppm TUR	9	34.5 ± 4.6	1.77 ± 0.34	0.62 ± 0.06	0.19 ± 0.10	8.81 ± 1.2[c]	0.85 ± 0.40
3	+ 500 ppm TUR	9	35.5 ± 3.9	1.89 ± 0.23	0.58 ± 0.10	0.21 ± 0.07	8.48 ± 1.1[d]	0.60 ± 0.39
4	None	9	38.9 ± 2.4	1.94 ± 0.17	0.64 ± 0.04	0.13 ± 0.02	10.1 ± 0.56	0.42 ± 0.52

[a] $P < 0.05$ *(versus group 4) in Student's* t*-test.*
[b] $P < 0.001$ *(versus group 4) in Student's* t*-test.*
[c] $P < 0.002$ *(versus group 1) in Student's* t*-test.*
[d] $P < 0.05$ *(versus group 1) in Student's* t*-test.*

0.005 for each). In addition, individual administrations of CUR (groups 4 and 5), but not TUR (groups 2 and 3), led to significant iNOS suppression (48% and 51%, respectively, $P < 0.005$ each).

4. Discussion

CRC has become a great threat in most developed countries over recent decades, and is the third most common cancer diagnosed in men and women and the second leading cause of death from cancer in the United States [26]. In Japan as well, the number of male and female has been predicted to increase by 12.3 and 10.5 times, respectively, by 2020 from the 1975 baseline [27]. There is a large body of evidence showing that lifestyle, dietary habits in particular, plays some predominant role in the onset of many malignancies, including CRC [28]. On the other hand, cancer prevention through the use of food phytochemicals obtained from biologically active plants is an

TABLE 3 ***Body, liver, kidney, and spleen weights and length of large bowel in DMH/DSS-induced mouse colon carcinogenesis experiment***

Group no.	*Treatment*	*No. of surviving mice*	*Body weight (g)*	*Liver weight (g)*	*Kidney weight (g)*	*Spleen weight (g)*	*Length of large bowel (cm)*
1	DMH/DSS	9/10	52.1 ± 11	2.70 ± 0.38	0.91 ± 0.19	0.23 ± 0.06	12.3 ± 1.6
2	+ 20 ppm TUR	8/10	49.9 ± 8.3	2.45 ± 0.18	0.86 ± 0.11	0.20 ± 0.05	12.6 ± 1.3
3	+ 100 ppm TUR	8/10	53.6 ± 6.9	2.23 ± 0.40b	0.74 ± 0.14[b]	0.15 ± 0.03[c]	12.5 ± 1.3
4	+ 1,000 ppm CUR	8/10	49.1 ± 4.4	2.88 ± 0.35	0.85 ± 0.11	0.24 ± 0.09	12.5 ± 1.4
5	+ 5,000 ppm CUR	9/10	50.8 ± 6.5	3.00 ± 0.39	0.88 ± 0.10	0.24 ± 0.08	13.3 ± 1.4
6	+ 20 ppm TUR/1,000 ppm CUR	8/10	49.0 ± 4.0	2.59 ± 0.29	0.84 ± 0.13	0.16 ± 0.02[c]	12.6 ± 1.4
7	+100 ppm TUR/5,000 ppm CUR	9/10	51.3 ± 6.1	2.91 ± 0.49	0.88 ± 0.11	0.19 ± 0.02	13.1 ± 0.89
8	100 ppm TUR alone	5/5	54.0 ± 7.5	2.55 ± 0.28	0.91 ± 0.11	0.19 ± 0.04	13.2 ± 0.74
9	5,000 ppm CUR alone	5/5	47.1 ± 2.4[a]	2.51 ± 0.33	0.80 ± 0.07	0.21 ± 0.08	12.2 ± 0.73
10	100 ppm TUR/5,000 ppm CUR	5/5	47.9 ± 2.7	2.65 ± 0.23	0.92 ± 0.10	0.16 ± 0.03	12.8 ± 0.36
11	None	5/5	53.7 ± 1.9	2.53 ± 0.11	0.93 ± 0.09	0.17 ± 0.03	13.5 ± 0.99

[a] $P < 0.01$ *(versus group 11) in Student's* t*-test.*
[b] $P < 0.05$ *(versus group 1) in Student's* t*-test.*
[c] $P < 0.02$ *(versus group 1) in Student's* t*-test.*

TABLE 4 *Incidence and multiplicity of colonic neoplasia in DMH/DSS-induced mouse colon carcinogenesis experiment*

Group no.	Treatment	No. of surviving mice	Incidence (%)			Multiplicity (% inhibition)		
			Total (%)	Adenoma	Adeno-carcinoma	Total	Adenoma	Adenocarcinoma
1	DMH/DSS	8/8	7/8 (88)	7/8 (88)	7/8 (88)	4.29 ± 3.3	1.21 ± 1.3	2.71 ± 2.3
2	+ 20 ppm TUR	6/8	5/6 (83)	2/6 (22)	4/6 (67)	1.33 ± 1.0[a] (69)	0.33 ± 0.52[b] (73)	1.17 ± 1.2 (57)
3	+ 100 ppm TUR	6/8	6/6 (100)	3/6 (50)	6/6 (100)	2.50 ± 1.6 (42)	0.67 ± 0.82 (45)	1.83 ± 0.75 (32)
4	+ 1,000 ppm CUR	6/8	4/6 (67)	2/6 (22)	3/6 (50)	1.33 ± 1.9[b] (69)	0.50 ± 0.84 (59)	0.83 ± 1.2[b] (69)
5	+ 5,000 ppm CUR	8/8	6/8 (75)	4/8 (50)	4/8 (50)	1.50 ± 1.6[c] (65)	0.50 ± 0.53 (59)	1.00 ± 1.4[b] (63)
6	+ 20 ppm TUR/ 1,000 ppm CUR	7/8	0	0	0	0[d](100)	0[a](100)	0[d](100)
7	+ 100 ppm TUR/ 5,000 ppm CUR	8/8	0	0	0	0[d](100)	0[a](100)	0[d](100)
8	100 ppm TUR alone	4/4	0	0	0	0	0	0
9	5,000 ppm CUR alone	4/4	0	0	0	0	0	0
10	100 ppm TUR/ 5,000 ppm CUR	4/4	0	0	0	0	0	0
11	None	4/4	0	0	0	0	0	0

[a] $P < 0.01$ *(versus group 1) in Student's* t*-test.*
[b] $P < 0.05$ *(versus group 1) in Student's* t*-test.*
[c] $P < 0.02$ *(versus group 1) in Student's* t*-test.*
[d] $P < 0.001$ *(versus group 1) in Student's* t*-test.*

attractive and reasonable strategy, especially when considering traditional uses in local remedies as well as the unique dietary habits of indigenous populations [17]. Indeed, the active constituents of spices and herbs have been shown to have marked potentials for cancer prevention [29]. In particular, turmeric as well as CUR, ingredients in common Indian curry spices, have drawn the attention of various investigators because of their promising efficacy for cancer prevention and tolerable toxicity. CUR exhibited minimal toxicity in healthy volunteers who were administered escalating doses from 500 to 12,000 mg, while its tolerance when given high single oral doses has been reported to be excellent [30]. There is also substantial evidence that CUR has preventive effects toward chemically and genetically induced intestinal tumorigenesis in rodents [7–11].

On the other hand, there are few or no studies regarding the cancer preventive capabilities of turmeric components other than CUR. To the best of our knowledge, the present results are the first to demonstrate that TUR, the major constituent in the essential oil of turmeric, have powerful cancer preventive effects when combined with CUR (Table 4). However, it should be kept in mind that the highest dose of TUR given (100 ppm, Table 3) for 20 weeks decreased liver, kidney, and spleen weights via unknown mechanisms. Along a similar line, the tumor suppressive potency of the low dose (20 ppm) was superior to that of the highest dose (Table 4), thus the optimal dose of TUR may be 20 ppm or less in the present animal model.

DSS is a sulfate-containing polysaccharide recognized by immune cells such as dendric cells and macrophages. Recently we reported that DSS binds to CXCL16 thereby generating reactive oxygen species that activated both ERK1/2 and p38 MAPK for inducing IL-1β release from mouse peritoneal macrophages [31]. It is of importance to note that IL-1β is a potent inducer of both iNOS and COX-2 in many cell types including resident macrophages [32], suggesting that these inducible isoforms play some critical roles in the onset of inflammatory tumorigenesis in the colon. In support of this notion, one of the authors and coworkers reported that DSS potentiated the intensity of both iNOS and COX-2 immunostaining of colonic mucosa from DMH-initiated mice [33]. Therefore, together with our present results (Fig. 2A and Table 2), it is speculated

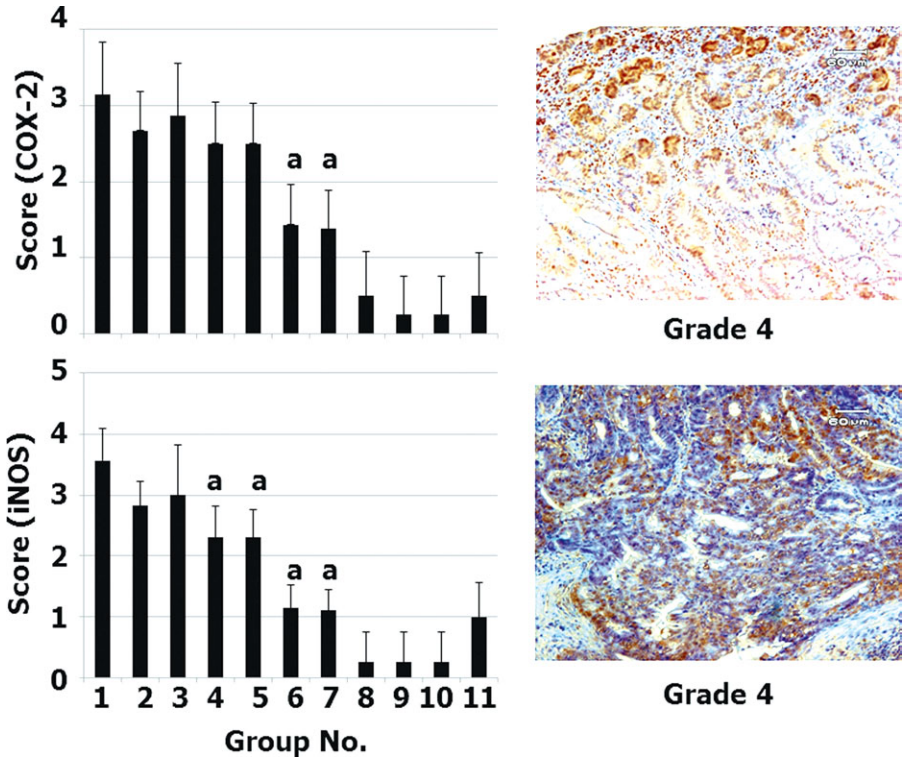

FIG 5

Suppressive effects of ATM and CUR on DSS-induced iNOS and COX-2 protein expressions in ICR mice. Immunohistochemistry for COX-2 and iNOS was performed using 4-mm-thick paraffin-embedded sections prepared from colons of mice in each group by a labeled streptavidin biotin method, using a LSAB KIT with microwave accentuation. The paraffin-embedded sections were heated for 30 min at 65 °C, then deparaffinized in xylene and rehydrated through graded ethanol at room temperature. A 0.05 M Tris HCl buffer (pH 7.6) was used to prepare the solutions and for washes between the various steps. Incubations were performed in a humidified chamber. Sections were treated for 40 min at room temperature with 2% BSA, and incubated overnight at 4°C with the primary antibodies, anti-COX-2 rabbit polyclonal, and anti-iNOS rabbit polyclonal antibodies. To reduce nonspecific staining of mouse tissue by the mouse antibodies, a Mouse On Mouse IgG blocking reagent was applied for 1 h. Horseradish peroxidase activity was visualized by treatment with H_2O_2 and 3,3′-diaminobenzidine for 5 min. For the final step, the sections were weakly counterstained with Mayer's hematoxylin. For each case, negative control staining procedures were performed with serial sections, during which incubation with the primary antibodies was omitted. [a]*P < 0.005 versus group 1 in Mann–Whitney U-test. Representative pictures of grade 4 are shown.*

that TUR and CUR prevent experimental colon carcinogenesis through, at least in part, down-regulation of those inducible inflammatory proteins.

Combinations of synthetic drugs or natural compounds, which have different modes of actions, may amplify the efficacy of each agent and reduce toxicity, in contrast to a strategy that utilizes individual agents at high doses [24,34]. Although the mechanism related to the synergistic cancer preventive effects provided by a combination of CUR and TUR remains to be elucidated, the differences in molecular mechanisms underlying iNOS and COX-2 suppression may play a key role. In the present study, those turmeric components attenuated mRNA expressions of both iNOS and COX-2 in LPS-stimulated macrophages (Fig. 2A), while CUR also down-regulated the transcriptional activities of NFκB and AP-1, but not of CREB (Fig. 2B). These results are consistent with those presented in previous reports, which showed that the molecular targets of CUR include NFκB, the master transcriptional factor for numerous inflammatory and oncogenic genes such as iNOS and COX-2 [35]. In contrast, ATM did not suppress their transcriptional factors, though results of a chase experiment that investigated mRNA decay revealed that this agent targets iNOS and COX-2 *via* post-transcriptional mechanisms.

HuR is a nuclear protein that binds to the ARE in the 3′-UTR of many mRNAs of inflammatory genes and oncogenes present in several types of cells [36]. The ARE forms stem loops in its secondary structure and mRNA with this unique moiety is less susceptible to exonuclease, leading to initiation of stable and rapid translation processes. Increased expression levels of ARE-BPs have recently emerged as reliable tumor markers in several types of cancerous tissues for determination of cancer invasion, metastasis, and recurrence, thus they can also indicate patient prognosis [37–40]. Recently, Young et al. presented important evidence that increased expression of HuR and loss of the decay factor tristetraprolin may have some association with COX-2 over-expression during the early

stages of colorectal tumorigenesis [41]. Once cells are stimulated with inflammatory and oncogenic agents, several signaling molecules, including PKCs and p38 MAPK [22,23], are rapidly and transiently activated to export nucleus HuR into the cytoplasm. p38 MAPK is known to activate MAPK-activated protein kinase-2, which may play a role in HuR methylation via arginine methyltransferase, leading to the promotion of nucleo-cytoplasmic shuttling of HuR [42]. In the present study, ATM disrupted HuR shuttling (Fig. 4), but did not affect p38 MAPK activation (data not shown), thus its molecular target is presumably other signaling molecule(s) involved in HuR shuttling. To our best knowledge, this is the first report on the cancer preventive agent which targets HuR shuttling and activation.

In conclusion, our *in vitro* and *in vivo* results have demonstrated that a combination of CUR and TUR may be a powerful strategy for preventing colon tumorigenesis. ATM, one of the constituents of TUR, was shown to target HuR, a nucleus protein responsible for mRNA stabilization of iNOS and COX-2. Therefore, turmeric may be designated as an intriguing and remarkable spice that concomitantly contains different categories of chemopreventive agents in regard to not only chemical types but also mechanisms of cancer preventive actions.

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References

[1] Lashner, B., Provencher, K., Bozdech, J., and Brzezinski, A. (1995) Worsening risk for the development of dysplasia or cancer in patients with chronic ulcerative colitis. Am. J. Gastroenterol. 90, 377–380.

[2] Akgun, E., Caliskan, C., Celik, H., Ozutemiz, A., Tuncyurek, M., et al. (2005), Effects of *N*-acetylcysteine treatment on oxidative stress in acetic acid-induced experimental colitis in rats. J. Int. Med. Res. 33, 196–206.

[3] Damiani, C., Benetton, C., Stoffel, C., Bardini, K., Cardoso, V., et al. (2007) Oxidative stress and metabolism in animal model of colitis induced by dextran sulfate sodium. J. Gastroenterol. Hepatol. 22, 1846–1851.

[4] Suzuki, R., Kohno, H., Sugie, S., and Tanaka, T. (2004) Sequential observations on the occurrence of preneoplastic and neoplastic lesions in mouse colon treated with azoxymethane and dextran sodium sulfate. Cancer Sci. 95, 721–727.

[5] Bing, R., Miyataka, M., Rich, K., Hanson, N., Wang, X., et al. (2001) Nitric oxide, prostanoids, cyclooxygenase, and angiogenesis in colon and breast cancer. Clin. Cancer Res. 7, 3385–3392.

[6] Surh, Y. and Chun, K. (2007) Cancer chemopreventive effects of curcumin. Adv. Exp. Med. Biol. 595, 149–172.

[7] Huang, M., Lou, Y., Ma, W., Newmark, H., Reuhl, K., et al. (1994) Inhibitory effects of dietary curcumin on forestomach, duodenal, and colon carcinogenesis in mice. Cancer Res. 54, 5841–5847.

[8] Kawamori, T., Lubet, R., Steele, V., Kelloff, G., Kaskey, R., et al. (1999) Chemopreventive effect of curcumin, a naturally occurring anti-inflammatory agent, during the promotion/progression stages of colon cancer. Cancer Res. 59, 597–601.

[9] Kim, J., Araki, S., Kim, D., Park, C., Takasuka, N., et al. (1998) Chemopreventive effects of carotenoids and curcumins on mouse colon carcinogenesis after 1,2-dimethylhydrazine initiation. Carcinogenesis 19, 81–85.

[10] Mahmoud, N., Carothers, A., Grunberger, D., Bilinski, R., Churchill, M., et al. (2000) Plant phenolics decrease intestinal tumors in an animal model of familial adenomatous polyposis. Carcinogenesis 21, 921–927.

[11] Rao, C., Rivenson, A., Simi, B., and Reddy, B. (1995) Chemoprevention of colon cancer by dietary curcumin. Ann. NY Acad. Sci. 768, 201–204.

[12] Surh, Y., Chun, K., Cha, H., Han, S., Keum, Y., et al. (2001) Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF-kappa B activation. Mutat. Res. 480–481, 243–268.

[13] Camacho-Barquero, L., Villegas, I., Sánchez-Calvo, J., Talero, E., Sánchez-Fidalgo, S., et al. (2007) Alarcón de la Lastra, Curcumin, a Curcuma longa constituent, acts on MAPK p38 pathway modulating COX-2 and iNOS expression in chronic experimental colitis. Int. Immunopharmacol. 7, 333–342.

[14] Ma, X. and Gang, D. (2006) Metabolic profiling of turmeric (*Curcuma longa* L.) plants derived from in vitro micropropagation and conventional greenhouse cultivation. J. Agric. Food Chem. 54, 9573–9583.

[15] Negi, P., Jayaprakasha, G., Jagan Mohan Rao, L., and Sakariah, K. (1999) Antibacterial activity of turmeric oil: a byproduct from curcumin manufacture. J. Agric. Food Chem. 47, 4297–4300.

[16] Kuroda, M., Mimaki, Y., Nishiyama, T., Mae, T., Kishida, H., et al. (2005) Hypoglycemic effects of turmeric (*Curcuma longa* L. *rhizomes*) on genetically diabetic KK-Ay mice. Biol. Pharm. Bull. 28, 937–939.

[17] Murakami, A., Ishida, H., Kobo, K., Furukawa, I., Ikeda, Y., et al. (2005) Suppressive effects of Okinawan food items on free radical generation from stimulated leukocytes and identification of some active constituents: implications for the prevention of inflammation-associated carcinogenesis. Asian Pac. J. Cancer Prev. 6, 437–448.

[18] Ji, M., Choi, J., Lee, J., and Lee, Y. (2004) Induction of apoptosis by ar-turmerone on various cell lines. Int. J. Mol. Med. 14, 253–256.

[19] Kohno, H., Suzuki, R., Curini, M., Epifano, F., Maltese, F., et al. (2006) Dietary administration with prenyloxycoumarins, auraptene and collinin, inhibits colitis-related colon carcinogenesis in mice. Int. J. Cancer 118, 2936–2942.

[20] van der Woude, C., Kleibeuker, J., Jansen, P., and Moshage, H. (2004) Chronic inflammation, apoptosis and (pre-)malignant lesions in the gastrointestinal tract. Apoptosis 9, 123–130.

[21] Divya, C. S. and Pillai, M. R. (2006) Antitumor action of curcumin in human papillomavirus associated cells involves downregulation of viral oncogenes, prevention of NFkB and AP-1 translocation, and modulation of apoptosis. Mol. Carcinog. 45, 320–332.

[22] Dean, J. L., Sully, G., Clark, A. R., and Saklatvala, J. (2004) The involvement of AU-rich element-binding proteins in p38 mitogen-activated protein kinase pathway-mediated mRNA stabilisation. Cell Signal. 16, 1113–1121.

[23] Doller, A., Huwiler, A., Müller, R., Radeke, H., Pfeilschifter, J., et al. (2007) Protein kinase C alpha-dependent phosphorylation of the mRNA-stabilizing factor HuR: implications for posttranscriptional regulation of cyclooxygenase-2. Mol. Biol. Cell 18, 2137–2148.

[24] Ohigashi, H. and Murakami, A. (2004) Cancer prevention with food factors: alone and in combination. Biofactors 22, 49–55.

[25] Tanaka, T., Kohno, H., Suzuki, R., Yamada, Y., Sugie, S., et al. (2003) A novel inflammation-related mouse colon carcinogenesis model induced by azoxymethane and dextran sodium sulfate. Cancer Sci. 94, 965–973.

[26] Levin, B., Lieberman, D., McFarland, B., Andrews, K., Brooks, D., et al. (2008) Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. Gastroenterology 134, 1570–1595.

[27] Kuriki, K. and Tajima, K. (2006) The increasing incidence of colorectal cancer and the preventive strategy in Japan. Asian Pac. J. Cancer Prev. 7, 495–501.

[28] Kim, Y. S. and Milner, J. A. (2007) Dietary modulation of colon cancer risk. J. Nutr. 137, 2576S–2579S.

[29] Aggarwal, B. B., Kunnumakkara, A. B., Harikumar, K. B., Tharakan, S. T., Sung, B., et al. (2008) Potential of spice-derived phytochemicals for cancer prevention. Planta. Med. 74, 1560–1569.

[30] Lao, C. D., Ruffin, M. T. t., Normolle, D., Heath, D. D., Murray, S. I., et al. (2006) Dose escalation of a curcuminoid formulation. BMC Complement Altern. Med. 6, 10.
[31] Kwon, K., Ohigashi, H., and Murakami, A. (2007) Dextran sulfate sodium enhances interleukin-1 beta release via activation of p38 MAPK and ERK1/2 pathways in murine peritoneal macrophages. Life Sci. 81, 362–371.
[32] Ohama, T., Hori, M., Momotani, E., Elorza, M., Gerthoffer, W., et al. (2007) IL-1beta inhibits intestinal smooth muscle proliferation in an organ culture system: involvement of COX-2 and iNOS induction in muscularis resident macrophages. Am. J. Physiol. Gastrointest. Liver Physiol. 292, G1315–G1322.
[33] Kohno, H., Suzuki, R., Sugie, S., and Tanaka, T. (2005) Beta-Catenin mutations in a mouse model of inflammation-related colon carcinogenesis induced by 1,2-dimethylhydrazine and dextran sodium sulfate. Cancer Sci. 96, 69–76.
[34] Xiao, H. and Yang, C. (2008) Combination regimen with statins and NSAIDs: a promising strategy for cancer chemoprevention. Int. J. Cancer 123, 983–990.
[35] Aggarwal, B. B. and Shishodia, S. (2004) Suppression of the nuclear factor-kappaB activation pathway by spice-derived phytochemicals: reasoning for seasoning. Ann. NY Acad. Sci. 1030, 434–441.
[36] Brennan, C. and Steitz, J. (2001) HuR and mRNA stability. Cell Mol. Life. Sci. 58, 266–277.
[37] Ortega, A., Sala, S., Espinosa, E., González-Barón, M., and Cuezva, J. (2008) HuR and the bioenergetic signature of breast cancer: a low tumor expression of the RNA-binding protein predicts a higher risk of disease recurrence. Carcinogenesis 29, 2053–2061.
[38] Wang, J., Zhao, W., Guo, Y., Zhang, B., Xie, Q., et al. (2009) The expression of RNA-binding protein HuR in non-small cell lung cancer correlates with vascular endothelial growth factor-C expression and lymph node metastasis. Oncology 76, 420–429.
[39] Yi, X., Zhou, Y., Zheng, W., and Chambers, S. (2009) HuR expression in the nucleus correlates with high histological grade and poor disease-free survival in ovarian cancer. Aust. NZ J. Obstet. Gynaecol. 49, 93–98.
[40] Yoo, P., Sullivan, C., Kiang, S., Gao, W., Uchio, E., et al. (2009) Tissue microarray analysis of 560 patients with colorectal adenocarcinoma: high expression of HuR predicts poor survival. Ann. Surg. Oncol. 16, 200–207.
[41] Young, L., Sanduja, S., Bemis-Standoli, K., Pena, E., Price, R., et al. (2009) The mRNA binding proteins HuR and tristetraprolin regulate cyclooxygenase 2 expression during colon carcinogenesis. Gastroenterology 136, 1669–1679.
[42] Doller, A., Pfeilschifter, J., and Eberhardt, W. (2008) Signalling pathways regulating nucleo-cytoplasmic shuttling of the mRNA-binding protein HuR. Cell Signal. 20, 2165–2173.