Kimchi Protects Against Azoxymethane/Dextran Sulfate Sodium-Induced Colorectal Carcinogenesis in Mice

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ABSTRACT The chemopreventive effects of different types and quantities of kimchi prepared with different subingredients, including commercial kimchi (CK), standardized kimchi (SK), cancer-preventive kimchi (CPK), and anticancer kimchi (ACK), on colorectal carcinogenesis in mice were evaluated. The development of colon cancer was induced in male BALB/c mice with a single intraperitoneal injection of azoxymethane (AOM, 10 mg/kg body weight) and subsequent treatment with 2% dextran sulfate sodium (DSS) in drinking water for 7 days for two cycles. After exposure to AOM and DSS, treatment with the methanolic extracts from different kimchis, particularly 1.89 g/kg of ACK, significantly increased colon length, decreased the ratio of colon weight/length, and resulted in the lowest number of tumors compared with the other kimchi-treated groups. Histological observation revealed that ACK was able to suppress AOM- and DSS-induced colonic mucosal damage and neoplasia. ACK also significantly decreased the mRNA levels of proinflammatory cytokines (*TNF- α* , *IL-6*, and *IFN- γ*) as well as the mRNA and protein expression of inducible nitric oxide synthase and cyclooxygenase-2 (COX-2). In addition, the mRNA and protein expression of p53 and p21 was elevated in colon tissues from the ACK-treated mice compared with the other kimchi-treated groups. Our results suggest that kimchi exerted a suppressive effect on AOM- and DSS-induced colorectal carcinogenesis in the BALB/c mice. The anticancer effects of ACK were particularly potent. Thus, it is possible that the health-promoting subingredients added to ACK might be used to prevent colon carcinogenesis in humans.

KEY WORDS: • colorectal carcinogenesis • COX-2 • iNOS • kimchi • proinflammatory cytokines • p53 • p21

INTRODUCTION

KIMCHI IS A TRADITIONAL Korean fermented food prepared from various vegetables and other subingredients. It is well known that kimchi is a healthy food and rich in dietary fiber, vitamin C, β -carotene, β -sitosterol, chlorophylls, phenols, lactic acid bacteria (LAB), minerals, and other compounds that promote human health.^{1,2} Current studies have reported that kimchi has many beneficial properties, such as antimutagenic,³ anticancer,^{3,4} antiobesity,^{5,6} antioxidant,^{7,8} antiaging,⁹ antiatherogenic,^{10,11} and antidiabetic¹² activities. It has also been noted that the addition of several subingredients (e.g., red pepper powder, garlic, mustard leaf, mistletoe, and chitosan) is able to improve the antioxidant, antimutagenic, and anticancer effects of kimchi.^{13–16}

Colorectal cancer (CRC) is a serious disease associated with inflammatory bowel diseases (IBDs), which include ulcerative colitis (UC) and Crohn's disease (CD).¹⁷ Patients

with both UC and CD are at increased risk for developing CRC compared with the general population. In particular, ~25–30% of patients with long-term cases of colitis will develop CRC.¹⁸ The etiology of CRC is still unclear. However, chronic inflammation is known as an important predisposing factor for the pathological process of CRC development. High levels of proinflammatory cytokines, such as tumor necrosis factor (TNF)- α , interleukin (IL)-6, IL-12, and interferon (IFN)- γ , have been reported to promote the development of CRC.^{19,20} Overexpression of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 is also associated with colorectal carcinogenesis.^{21,22} In addition to IBD, several other risk factors, including ages over 50 years, a family history of CRC, lifestyle factors (diet, exercise, tobacco use, and alcohol consumption), and colorectal polyp formation as well as the mutation of certain tumor suppression genes such as p53, adenomatous polyposis coli (APC), and Rb, play an important role in CRC development.^{23,24} In particular, the loss of p53 function is believed to be the defining event that drives the transformation of adenoma to carcinoma during CRC progression.²⁴

Epidemiologically, poor dietary habits, such as high-energy intakes and red meat along with a low intake of vegetables and

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dietary fiber, increase the risk of CRC.²⁵ The consumption of kimchi does not increase CRC risk. Conversely, kimchi has been found to help prevent CRC due to high LAB levels and beneficial properties of the ingredients (red pepper, garlic, and ginger).^{3,4} Over the past 60 years, the lifestyle and eating habits of South Koreans have been Westernized. Consequently, the incidences of CRC have increased rapidly.²⁶ However, more than 70% of CRC cases could be prevented by nutritional intervention because diet is the most important exogenous lifestyle-related risk factor of CRC etiology.²⁷

In the present study, we assessed the chemopreventive effect of different subingredients added to kimchi, especially those that are used to make anticancer kimchi (ACK), on colon carcinogenesis induced by azoxymethane (AOM) and dextran sulfate sodium (DSS) in BALB/c male mice, which exhibited many histological and biological alterations similar to those found in human CRC cases.²⁸ In addition, we also evaluated the potential mechanism underlying the effects of the different types of kimchi against AOM- and DSS-induced CRC in the mice.

MATERIALS AND METHODS

Kimchi preparation

Standardized kimchi (SK), cancer-preventive kimchi (CPK), and ACK were prepared according to recipes previously developed by our research group²⁹ as shown in Table 1. Organically cultivated *Baechu* cabbage (*Brassica campestris* L. ssp. *pekinensis* Rupr.) that was purchased from Kanglim Co. (Milyang, Gyeongsang-do, South Korea) was used to prepare CPK and ACK. *Baechu* cabbage (for preparing SK), garlic, ginger, red pepper powder, radish, green onion, dried *Lentinus edodes*, sea tangle, and anchovy juice were purchased from a local market in Busan, South Korea. The Korean mistletoe extract was purchased from Mistle Biotech Co. (Pohang, Gyeongsang-do, South Korea).

TABLE 1. INGREDIENTS AND COMPOSITION (%) OF KIMCHI SAMPLES

Ingredients (%)	SK	CPK	ACK
Brined <i>Baechu</i> cabbage	100.0	100.0	100.0
Red pepper powder	3.5	5.0	2.5
Crushed garlic	1.4	2.8	2.8
Crushed ginger	0.6	0.6	0.6
Anchovy juice	2.2	2.2	—
Radish	13.0	11.0	11.0
Green onion	2.0	2.0	2.0
Sugar	1.0	1.0	1.0
Mustard leaf	—	5.0	7.5
Chinese pepper	—	0.1	0.1
Pear	—	2.8	2.8
Mushroom (shiitake) and sea tangle juice	—	2.5	5.0
Mistletoe extract	—	—	0.05
Final salt concentration (%)	2.5	2.5	2.2

SK, kimchi prepared with standard kimchi recipe; CPK, kimchi prepared with cancer preventive kimchi recipe; ACK, kimchi prepared with anticancer kimchi recipe.

The juice of *L. edodes* and sea tangle was prepared as follows. Five pieces of *L. edodes* and five of sea tangles (2×3 cm²) in 1 L of water were left overnight, and the extract was boiled for 5 sec. *Baechu* cabbage was brined in a 10% salt (solar salt without bitter; Shinan, Cheonnam, South Korea) solution for 10 h and then rinsed with fresh water three times at room temperature; thereafter, *Baechu* cabbage and all subingredients were mixed. SK, CPK, and ACK samples were fermented at 5°C for 5 weeks to reach optimal ripeness (pH 4.3). Commercial kimchi (CK; D Co.; pH 4.3) that was used as a positive control was purchased from a local market (Busan, South Korea). All of the kimchi samples were freeze-dried and ground into a fine powder. The kimchi powder underwent an extraction process with 20 volumes of methanol by stirring overnight. Finally, the kimchi methanol extracts were concentrated by heat evaporation (RE 111 rotavapor; Büchi, Flawil, Switzerland) and stored at 4°C.

Chemicals and reagents

TriZOL, oligodT₁₈ primer, reverse transcriptase buffer, dNTPs, murine maloney leukemia virus (MMLV) reverse transcriptase, RNase inhibitor, agarose, and RIPA buffer were obtained from Invitrogen Life Technologies (Carlsbad, CA, USA). AOM and ethidium bromide (EtBr) were purchased from Sigma (St. Louis, MO, USA). Dextran sulfate sodium (DSS; molecular weight=36,000–50,000) was obtained from MP Biomedical (Solon, OH, USA). All the chemicals used were of analytical grade.

Animal studies

Male BALB/c mice (5-week old) were purchased from Samtako, Bio Korea (Kyung-ki-do, South Korea). The mice were housed with a 12-h light/dark cycle at room temperature, and had access to food (DooYeol Biotech, Seoul, South Korea) and water *ad libitum*. The animals were randomly divided into 10 groups of 10 mice each: group 1 (normal) was treated with 0.9% normal saline; group 2 (control) was treated with AOM and DSS; and groups 3–10 included AOM- and DSS-treated animals fed 0.63 g/kg or 1.89 g/kg doses of CK (groups 3 and 4), SK (groups 5 and 6), CPK (groups 7 and 8), or ACK (groups 9 and 10) extracts, respectively. The kimchi extracts and vehicle were intragastrically administered daily (0.2 mL/mouse) from the third week until the end of sixth week in the total experimental periods. To induce the development of colitis-associated CRC, the mice were given a single intraperitoneal (i.p.) injection of AOM (10 mg/kg body weight) on the first day of the experiment. Two weeks later, the animals were given DSS [2% (w/v)] in drinking water for the third and sixth week of the experiment. At the end of the experiment (eighth week), the mice were euthanized using CO₂ and sacrificed. Colon length and weight as well as the number of tumors formed were determined. The protocol used for this study was approved by the Institutional Animal Care and Use Committee (PNU-IACUC; approval number PNU-2011-000408) of Pusan National University (Busan, South Korea).

Histological evaluation

Distal colon tissues from each animal were subjected to histological examination. The colon tissues were fixed in 10% (v/v) neutral buffered formalin, dehydrated in ethanol, and embedded in paraffin. Sections (4- μ m thick) were cut and stained with hematoxylin and eosin (H&E). Images were acquired using a Zeiss Axioskop 2 Plus microscope (Carl Zeiss MicroImaging, Thornwood, NY, USA) equipped with an AxioCam MRc5 CCD camera (Carl Zeiss MicroImaging).

Reverse transcription-PCR assay

mRNA expression of *TNF- α* , *IL-6*, *IFN- γ* , *iNOS*, *COX-2*, *p53*, and *p21* in the colon mucosa was measured with a reverse transcription (RT)-PCR assay. Total RNA was isolated from the colonic tissue (100 mg) using TriZOL reagent according to the manufacturer's recommendations and centrifuged at 12,000 *g* for 15 min at 25°C before chloroform (0.2 mL) was added. Isopropanol was then added to the supernatant at a 1:1 ratio and the RNA was pelleted by centrifugation (12,000 *g* for 15 min). After washing the pellet with ethanol, the RNA was solubilized in diethyl-pyrocyanate-treated RNase-free water and quantified by measuring the absorbance at 260 nm using a UV-2401PC spectrophotometer (Shimadzu, Kyoto, Japan). Equal amounts of RNA (1 μ g) were reverse transcribed in a master mix containing 1 $\times$ reverse transcriptase buffer, 1 mM dNTPs, 500 ng of each oligoDT₁₈ primer, 140 U MMLV reverse transcriptase, and 40 U RNase inhibitor for 45 min at 42°C. PCR was then carried out in an automatic thermocycler (Bioneer, Daejeon, South Korea) for 25 cycles (94°C for 30 sec, 55°C for 30 sec, and 72°C for 40 sec) followed by an 8-min final incubation at 72°C. The PCR products were separated in 2% agarose gels and visualized by EtBr staining. β -Actin was used for normalization. Gene expression was quantified using ImageJ software (<http://rsbweb.nih.gov/ij/>).

Protein extraction and western blot analysis

Colon tissue samples (100 mg) were first washed with ice-cold PBS, homogenized with ice-cold RIPA buffer, and then

centrifuged at 12,000 *g* for 15 min at 4°C. Protein concentrations were determined with a Bio-Rad protein assay kit (Hercules, CA, USA). For western blot analysis, aliquots of the homogenate containing 50 μ g of protein were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then electrotransferred onto a nitrocellulose membrane (Schleicher and Schuell, Keene, NH, USA). The blots were incubated with antibodies against iNOS, COX-2, p53, and p21 obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA), and then incubated with horseradish-peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology) for 1 h at room temperature. The blots were washed three times with PBS containing 0.05% v/v Tween 20 (PBS-T) and antibody binding was visualized by enhanced chemiluminescence (GE Healthcare Life Sciences, Little Chalfont, United Kingdom).

Statistical analysis

All data are presented as the mean $\pm$ SD. Differences between the mean values for individual groups were assessed using a one-way ANOVA with Duncan's multiple-range tests. *P*-values < .05 were considered statistically significant. The SAS v9.1 software package (SAS Institute, Inc., Cary, NC, USA) was used to perform the statistical analysis.

RESULTS

Effects of kimchi extracts on colonic length and colon weight/length ratio

As shown in Table 2, mice treated with AOM and DSS had significantly decreased colon lengths (7.1 $\pm$ 0.6 cm) compared with that of the normal animals (7.7 $\pm$ 0.6 cm; *P* < .05). In the presence of AOM and DSS, both concentrations of the ACK extract significantly prevented AOM- and DSS-induced colonic shortening (7.4 $\pm$ 0.5 and 7.7 $\pm$ 0.4 cm for 0.63 and 1.89 g/kg, respectively). At the same doses, CPK prevented the decrease in colon length (7.3 $\pm$ 0.2 and 7.4 $\pm$ 0.7 cm for 0.63 and 1.89 g/kg, respectively) by AOM/DSS treatment to a greater extent than SK and CK. The colon weight/length ratio was used as an indicator of AOM- and DSS-induced intestinal

TABLE 2. EFFECT OF KIMCHI EXTRACTS ON COLON LENGTH, COLON WEIGHT/LENGTH RATIO, AND TUMOR DEVELOPMENT IN THE COLON OF BALB/C MICE WITH AOM- AND DSS-INDUCED COLITIS-ASSOCIATED COLORECTAL CANCER

Treatment (g/kg)	Colon length (cm)		Colon weight/length (mg/cm)		Number of tumors	
	0.63	1.83	0.63	1.83	0.63	1.83
Norm.	7.68 $\pm$ 0.83 ^a		22.21 $\pm$ 6.42 ^c		—	
AOM + DSS	7.10 $\pm$ 0.57 ^b		43.45 $\pm$ 5.54 ^a	24.6 $\pm$ 2.5 ^a		
AOM + DSS + CK	7.26 $\pm$ 0.33 ^b	7.19 $\pm$ 0.23 ^b	42.0 $\pm$ 7.2 ^a (5)*	43.0 $\pm$ 6.6 ^a (2)	22.8 $\pm$ 3.9 ^{ab} (7)	20.5 $\pm$ 3.8 ^a (17)
AOM + DSS + SK	7.31 $\pm$ 0.21 ^{ab}	7.30 $\pm$ 0.27 ^{ab}	39.8 $\pm$ 4.5 ^{ab} (10)	42.6 $\pm$ 4.9 ^a (4)	23.5 $\pm$ 4.7 ^{ab} (4)	18.4 $\pm$ 4.9 ^a (25)
AOM + DSS + CPK	7.34 $\pm$ 0.19 ^{ab}	7.43 $\pm$ 0.70 ^{ab}	39.1 $\pm$ 3.0 ^{ab} (10)	38.3 $\pm$ 4.4 ^{ab} (24)	20.4 $\pm$ 5.0 ^{bc} (17)	17.7 $\pm$ 4.5 ^{cde} (28)
AOM + DSS + ACK	7.41 $\pm$ 0.47 ^{ab}	7.69 $\pm$ 0.38 ^a	37.2 $\pm$ 7.3 ^{ab} (15)	35.2 $\pm$ 5.2 ^b (39)	15.6 $\pm$ 3.1 ^{de} (37)	14.1 $\pm$ 2.8 ^e (43)

Data represent mean $\pm$ SD.

^{abcde}Means with the different letters are significantly different (*P* < .05) by Duncan's multiple-range test.

*The values in parentheses are the inhibition rates (%).

—Means not detected.

AOM, azoxymethane; CK, commercial kimchi; DSS, dextran sulfate sodium.

wall thickening, severity of inflammation, and neoplasia development. Mice treated with AOM and DSS had a significantly increased colon weight/length ratio (43.5 ± 5.5 mg/cm) compared with that of the normal mice (22.2 ± 6.4 mg/cm). Treatment with 1.89 g/kg of the CK, SK, CPK, and ACK extracts resulted in colonic weight/length ratios of 43.0 ± 6.6 , 42.6 ± 4.9 , 38.3 ± 4.4 , and 35.2 ± 5.2 mg/cm, respectively. Mice treated with the ACK extract showed the lowest weight/length ratio among CPK-, SK-, and CK-treated mice at the same dose. Similar results were observed for the groups treated with the low dose of the kimchi extracts.

Effect of kimchi extracts on colonic tumor development

As shown in Table 2, mice treated with AOM and DSS all developed colon adenocarcinoma with a multiplicity of 24.6 ± 2.5 . Following treatment with the CK, SK, CPK, and ACK extracts, particularly at 1.89 g/kg dose, the incidences of colonic adenocarcinoma were decreased by 17%, 25%, 28%, and 43%, respectively, compared with the mice treated with AOM and DSS alone. Treatment with 0.63 g/kg of the CK, SK, CPK, and ACK extracts resulted in colonic tumor numbers of 22.8 ± 3.9 , 23.5 ± 4.7 , 20.4 ± 5.0 , and 15.6 ± 3.1 , respectively.

Histological observations

AOM and DSS resulted in significant histological alterations of the colonic mucosa, including infiltration of inflammatory cells into the lamina propria, loss of crypts, and adenocarcinoma development (Fig. 1). Treatment with CK (0.63 or 1.89 g/kg) was able to reduce the occurrence of AOM- and DSS-induced adenocarcinoma compared with the

control mice. In the SK- and CPK-treated groups, only dysplasia was observed in the colon tissue. The area of dysplasia in the CPK-treated mice was smaller than that of the SK-treated mice. It was noted that 1.89 g/kg of the kimchi extracts more effectively prevented dysplasia than 0.63 g/kg. Only low-grade dysplasia was observed in the colon tissue of 0.63 g/kg ACK-treated mice. However, treatment with 1.89 g/kg of the ACK extract restored the colonic epithelium to a greater degree than administration of 0.63 g/kg ACK extract.

Effects on the expression of proinflammatory cytokines in colonic tissues

mRNA expression of $TNF-\alpha$, $IL-6$, and $IFN-\gamma$ in the colon. Colonic levels of $TNF-\alpha$, $IL-6$, and $IFN-\gamma$ mRNA are presented in Figure 2. Treatment with AOM and DSS significantly increased the mRNA expression of $TNF-\alpha$, $IL-6$, and $IFN-\gamma$ compared with that found in the normal mice ($P < .05$). At the 1.89 g/kg dose, the ACK extract reduced the mRNA expression of $TNF-\alpha$ (56%) and $IL-6$ (68%) more significantly than the CPK (39% and 33%, respectively), SK (22% and 32%, respectively), or CK (8% and 40%, respectively) extracts. However, the CPK extract attenuated the colonic mRNA expression of $IFN-\gamma$ to a greater extent than the other kimchi extracts.

mRNA and protein levels of iNOS and COX-2 in the colon tissues. Overexpression of iNOS and COX-2 results in an increased risk of colorectal carcinogenesis.^{21,22} As shown in Figure 3, AOM and DSS significantly increased the mRNA and protein expression of iNOS and COX-2 in the colorectal tissue. At a dose of 1.89 g/kg, ACK significantly reduced the mRNA levels of iNOS and COX-2 by 54.9% and

FIG. 1. Histological images of colonic mucosa from BALB/c mice with AOM- and DSS-induced colitis-associated colon cancer treated with different kimchi extracts. ACK, kimchi prepared with anticancer kimchi recipe; AOM, azoxymethane; CK, commercial kimchi; CPK, kimchi prepared with cancer preventive kimchi recipe; DSS, dextran sulfate sodium; SK, kimchi prepared with standard kimchi recipe. Tumors are shown as $\rightarrow$ and the inflammatory regions are shown as $\rightarrow$. Color images available online at www.liebertpub.com/jmf

FIG. 2. Effects of extracts from different types of kimchi (1.89 g/kg) on the mRNA levels of *TNF-α*, *IL-6*, and *IFN-γ* in colon tissue from BALB/c mice with AOM- and DSS-induced colitis-associated colon cancer. Band intensities were measured with a densitometer and expressed as fold of the control. Fold increase = gene expression/ β -actin $\times$ control value (control fold increase = 1). Mean values with different letters over the bars are significantly different ($P < .05$) according to Duncan's multiple-range test.

59.6%, respectively, and lowered the protein levels of iNOS and COX-2 by 65.3% and 50.2%, respectively, compared with those found in the AOM- and DSS-treated control mice. In addition, ACK also effectively reduced the mRNA and protein levels of iNOS and COX-2 compared with those of CPK-, SK-, and CK-treated mice.

mRNA and protein levels of p53 and p21 in the colon tissue. p53 is a tumor suppressor that regulates cell proliferation and induces the activation of p21 (a cyclin-dependent kinase inhibitor) to arrest the cell cycle, inhibit abnormal cell

proliferation, and reduce tumor growth.^{23,24} Following AOM and DSS administration, mRNA and protein levels of p53 and p21 were significantly decreased compared with the normal mice. At a concentration of 1.83 g/kg, ACK significantly increased the mRNA and protein levels of p53 and p21 compared with those found in the AOM- and DSS-treated control mice as well as those of the CPK-, SK-, and CK-treated animals. The mRNA expression levels of p53 and p21 in mice given the ACK extract were 14.4 and 2.6 times higher, respectively, compared with the AOM- and DSS-treated control animals. Additionally, the protein levels of p53 and p21

FIG. 3. Effects of extracts from different types of kimchi (1.89 g/kg) on the mRNA (A) and protein (B) levels of iNOS and COX-2 in colon tissue from BALB/c mice with AOM- and DSS-induced colitis-associated colon cancer. Band intensities were measured with a densitometer and expressed as fold of the control. Fold increase = gene expression/ β -actin $\times$ control value (control fold increase = 1). Mean values with different letters over the bars are significantly different ($P < .05$) according to Duncan's multiple-range test.

FIG. 4. Effects of extracts from different types of kimchi (1.89 g/kg) on the mRNA (A) and protein (B) levels of p53 and p21 in colon tissue from BALB/c mice with AOM- and DSS-induced colitis-associated colon cancer. Band intensities were measured with a densitometer and expressed as fold of the control. Fold increase = gene expression/ β -actin $\times$ control value (control fold increase = 1). ^{a,b,c,d,e}Mean values with different letters over the bars are significantly different ($P < .05$) according to Duncan's multiple-range test.

found in the ACK-extract-treated mice were 3.0 and 2.0 times higher, respectively, than those observed in the control group (mice treated with AOM and DSS alone) (Fig. 4).

DISCUSSION

Traditional kimchi is generally prepared with brined *Bae-chu* cabbage, salt, sugar, red pepper powder, garlic, ginger, fermented anchovy juice, and other ingredients. The beneficial properties of kimchi are associated with the type and amount of subingredients that possess health-promoting properties.³ Therefore, the anticancer effects of kimchi have been improved by modulating the combination of subingredients.^{13–16} In addition to the raw materials and ingredients, other factors including fermentation temperature and time, and the type of LAB are also associated with the quality of kimchi and associated health benefits. In particular, a low temperature (5°C), suitable pH (4.3), and the use of Korean pots (*onggi*) or Mirafresh (permeability-controlled polyethylene) containers have been reported to increase the quality and beneficial characteristics of kimchi.^{30,31}

In the present study, we evaluated the chemopreventive effects of kimchi prepared with different subingredients fermented under optimal conditions (5°C and pH 4.3) in a Mirafresh container. Compared with CK and SK, CPK and ACK contained more mustard leaf, Chinese pepper, mushroom, sea tangle, and mistletoe extracts, which have high levels of antioxidant and anticancer activities.^{32–34} The results of the present study clearly indicated that kimchi extracts, especially 1.89 g/kg doses of the ACK extract, effectively inhibited AOM- and DSS-induced colorectal carcinogenesis in mice.

All mice treated with AOM and DSS developed colorectal adenocarcinoma. Further, the ratio of colon weight/

colon length (an indicator of diseases associated intestinal wall thickening, inflammation severity, and neoplasia) was increased in these animals. However, subsequent administration of the different kimchi extracts, particularly the ACK extract, significantly decreased the colon weight/length ratio and suppressed tumor formation induced by AOM and DSS.

Multiple pathways have been implicated in colon cancer carcinogenesis. IBD lesions are considered precancerous in humans.¹⁷ The oncogenesis of IBD-associated CRC involves a multistep progression from chronic inflammation through low- to high-grade dysplasia, and finally to adenocarcinoma. Results from the current study have demonstrated the dose-dependent effects of different types of kimchi on CRC development. In particular, the colon epithelium of ACK-extract-treated mice was nearly normalized. Animals receiving the CPK or SK extracts developed precancerous lesions and dysplasia, indicating that these two types of kimchi could delay carcinogenesis in the colon. It was also noted that the 0.63 g/kg dose of ACK extract was able to more effectively suppress the AOM- and DSS-induced neoplasia compared with 1.89 g/kg doses of the CK and SK extracts. Thus, the addition of cancer-preventive subingredients during kimchi preparation is very important.

It is widely accepted that increased expression of proinflammatory cytokines (*e.g.*, TNF- α , IL-6, and IFN- γ) amplifies inflammatory cascade signaling, causes intestinal tissue damage, and increases the risk of colorectal carcinogenesis in humans as well as animal models of CRC.^{19,20} Downregulating or abolishing the activity of proinflammatory cytokines (*e.g.*, with specific monoclonal antibodies) has been found to greatly suppress colitis-associated colorectal carcinogenesis in mice.¹⁹ In the previous study, ACK extract was able to decrease the levels of TNF- α , IL-6,

and IFN- γ in the serum compared with mice treated with AOM and DSS alone, and it also decreased the production of these cytokines compared with CPK-, SK-, and CK-treated mice.³⁵ Overactivation of iNOS and COX-2 promotes colorectal carcinogenesis in humans and AOM-induced colon tumor formation in rodents.³⁶ Several studies have provided evidence of interplay between iNOS and COX-2 expression and cell system activities as well as CRC progression. For example, nitric oxide (NO) production due to iNOS overactivation increases COX-2 activity, resulting in the generation of prostaglandin E2 (PGE2) that promotes the inflammatory process, increases cell proliferation, and reduces the tumor cell apoptosis in the CRC.^{22,37–40} Treatment with iNOS-specific inhibitors is able to reduce the activation of iNOS and COX-2 to help control AOM-induced colorectal carcinogenesis.^{41,42} It was found that more than 50% of adenoma cases and 85% of adenocarcinoma cases of the colon are accompanied by elevated COX-2 expression.⁴³ Activation of COX-2 promotes inflammation, abnormal cell proliferation, angiogenesis, and metastasis while preventing apoptosis in cases of colon cancer.⁴⁴ Inhibition of COX-2 activity using celecoxib, a specific COX-2 inhibitor, suppresses AOM-induced colorectal carcinogenesis in mice.⁴⁵ Previous studies have also reported that kimchi extract significantly reduces the mRNA and protein levels of iNOS and COX-2 in HT-29 human colon carcinoma cells¹³ and WI-38 human fibroblasts.⁹ We also observed in the present investigation that extracts from kimchi, especially ACK, were able to reduce the mRNA and protein expression of iNOS and COX-2 in colon tissues from AOM- and DSS-treated mice.

Loss of function of several tumor suppressor genes such as p53 is an important step in the progression of colitis-associated colon cancer.⁴⁶ In particular, the loss of p53 function is an early critical event in the transformation of dysplasia to neoplasia, and facilitates the development of colitis-associated colon cancer.^{17,47} The maintenance of p53 activity is able to inhibit AOM- and DSS-induced colorectal carcinogenesis.^{48,49} Additionally, initiation of p53 upregulated modulator of apoptosis (PUMA)-mediated apoptosis suppresses intestinal tumorigenesis in mice.⁵⁰ p21^{waf1/cip1}, a cyclin-dependent kinase (CDK) inhibitor, is tightly controlled by p53. This factor is able to inhibit the CDK/cyclin complex, influence the G1 to S phase transition of the cell cycle, and suppress abnormal intestinal cell proliferation during CRC development.^{51,52} Disruption of the p21 gene enhances colon tumor formation in mice with an APC mutation,⁵³ and also leads to a high frequency of colorectal tumorigenesis in AOM-treated mice.⁵⁴ In contrast, enhancing p21 activation decreases AOM- and DSS-induced colorectal carcinogenesis in mice.⁵⁵ We also observed that ACK extract increased the mRNA and protein levels of p53 and p21 in the colon tissues of AOM- and DSS-treated mice than those of CPK-, SK-, and CK-treated mice. These results suggested that the mechanisms underlying the ability of kimchi to protect against AOM- and DSS-induced colon carcinogenesis were associated with the activation of p53 and p21.

In addition, we found that both CPK and ACK were rich in isorhamnetin that was not detected in CK and SK.³⁵ Isorhamnetin is a representative flavonoid found in mustard

leaf,⁵⁶ and it showed antioxidant⁵⁷ and anti-inflammatory effects⁵⁸ and anticarcinogenic activity in AOM/DSS-treated mice.^{59,60} Fucoidan from sea tangle, lentinan in *L. edodes* and lectin in mistletoe extracts, and phytochemicals in organically cultivated *Baechu* cabbage^{61–64} might contribute an important preventive role in ACK in the AOM/DSS-induced carcinogenesis in mice. Importantly, the taste of ACK was similar to SK.

In conclusion, we demonstrated that the administration of kimchi extracts could effectively suppress AOM- and DSS-induced colitis-associated colorectal carcinogenesis in BALB/c mice. These results indicate that kimchi, especially types (such as ACK) made with high levels of health-promoting subingredients, significantly reduces AOM- and DSS-induced neoplasia formation and suppresses increases in colon weight/length ratio caused by this condition. The chemopreventive effects of ACK and the other types of kimchi against AOM- and DSS-induced CRC in mice appear to be mediated through decreased TNF- α , IL-6, and IFN- γ expression; reduced iNOS and COX-2 expression; and upregulated p53 and p21 activity in the colorectal tissues.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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