Original Article

Dietary intake of fermented kimchi prevented colitis-associated cancer

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Kimchi is composed of various chemopreventive phytochemicals and profuse probiotics, defining kimchi as probiotic foods. Concerns had increased on the modulation of intestinal microbiota on various kinds of systemic diseases. Under the hypothesis that dietary intake of kimchi can be ideal intervention for either ameliorating colitis or preventing colitic cancer, we performed the study to validate the efficolitic cancery of fermented kimchi on preventing colitic cancer. Using azoxymethane-initiated and dextran sulfate sodium-promoted colitic cancer models, we have administrated fermented or non-fermented kimchi to modulate colitic cancer preemptively. Detailed molecular mechanisms were explored. Preemptive administration of fermented kimchi significantly afforded colitic cancer prevention through attenuating inflammasomes (IL-18, IL-1β, caspase-1), enhancing antioxidative (NQO1, GST-π), imposing anti-proliferative (Bax, caspase-3, β-catenin), and affording cytoprotective actions (HSP70, 15-PGDH), while non-fermented kimchi did not prevent colitic cancer. Special recipe cancer preventive kimchi (cpkimchi) was more effective compared to standard recipe fermented kimchi ($p<0.01$), while non-fermented kimchi (kimuchi) worsened colitic cancer development, telling the importance of fermentation in cancer prevention. Repression of NF-kB p65, induction of tumor suppressive 15-PGDH, and inactivation of ERK1/2 by cpkimchi contributed to colitic cancer prevention. Dietary intake of cpkimchi ameliorated colitis and prevented colitic cancer via concerted anti-inflammatory, antioxidative, and anti-mutagenic actions.

***Key Words*:** **kimchi, colitis-associated cancer, preemptive administration, prevention**

Patients with inflammatory bowel disease (IBD) have an increased risk of developing colitis-associated colorectal cancer (colitic cancer), influenced by colitis duration, greater extent of inflammation, a family history of colorectal cancer (CRC), severity of bowel inflammation, and co-existent of primary sclerosing cholangitis.[1,2] Different with carcinogenic pathways of sporadic CRC, carcinogenic evolution of colitic cancer in IBD with different evolutionary pathway might be essential for prevention.[3] Representative molecular pathogenesis of colitic cancer includes inter-relative pathways such as increased mucosal inflammatory pathways, changes in the expression of receptors on the epithelial cells, and oxidative stress, after which so diverse targeted trials including anti-inflammatory, antioxidative, and anti-mutagenic agents had been reported. Though diverse trials including anti-inflammatory agents or drugs had been tried in addition to endoscopic surveillance.[4–6]

The fact that agent that cause colitis in healthy rodents or genetically engineered cancer-prone mice accelerates the development of so called colitic cancer, after which administration of carcinogen AOM initiated followed with DSS promoted especially in the presence of bacterial colonization led investigators to challenge anti-inflammatory agents or modification of intestinal microbiota for the prevention of colitic cancer.[7–11] There has been a surge of interest in studying the relation between microbiota, inflammation, and colitic cancer. Since the alteration of microbiota can influence the development of both colitic cancer and CRC,[12] many studies had been investigated. For instance, the estrogen receptor beta (ER-β) can attenuate colitic cancer/CRC development through favorable microbiome,[13] chemopreventive effects of epigallocatechin gallate through structural shift of gut microbiota,[14] colitic cancer prevention with isoliquiritigenin by modulating the intestinal microbiota.[15] Taken together these two backgrounds for colitic cancer, attention had been paid to develop "dietary intervention" either to ameliorate colitis or to block promoting colitic cancer.

Since kimchi is well-known representative probiotic food, we put hypothesis long-term dietary intake of kimchi can attenuate inflammation-associated gastrointestinal cancer. As similar events with colitic cancer, we have documented dietary prevention of *Helicobacter pylori* (*H. pylori*)-associated gastric cancer with kimchi, in which daily dietary intake of kimchi was an effective way either to rejuvenate *H. pylori*-atrophic gastritis or to prevent tumorigenesis supported with the concerted actions of antioxidative, anti-inflammatory, and anti-mutagenic mechanisms.[16–18] Though microbiota contained in the kimchi were proven to be essential with documented phytochemicals, comparison of special recipe cancer preventive kimchi (named as "cpkimchi") with standard folk-recipe kimchi (named as "skimchi") and non-fermented Japanese style kimchi (named as "kimuchi") seems to be essential in emphasizing the value of microbiota in the prevention of GI cancer. In this study, under the hypothesis that dietary intake of kimchi can either ameliorate colitis or prevent colitic cancer, we have compared the efficolitic cancery of fermented special recipe kimchi (cpkimchi), fermented standard recipe kimchi (skimchi), and non-fermented Japanese-style kimchi (kimuchi) against DSS-induced acute colitis and colitis promoted colitic cancer with exploration of molecular mechanisms.

Materials and Methods

Reagents. The following materials were obtained from commercial sources: all chemical reagents from Sigma (St. Louis, MO).

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Antibodies for Western blotting were purchased as follows: β-Actin, NF-κB p65, NF-κB p50, Lamin B, 15-hydroxyprostaglandin dehydrogenase (15-PGDH), NACHT, LRP and PYD domains-containing protein 3 (NLRP3), IL-18, IL-1β, caspase-3, heme oxygenase-1 (HO-1), β-catenin, Bcl-2, Bax, cleaved PARP, cleaved caspase-3, and cleaved caspase-8 from Santa Cruz Biotechnology (Santa Cruz, CA), iNOS from BD Biosciences (San Jose, CA), COX-2 from Thermo Scientific (Seoul, Korea), HSP70, HSP60, HSP27, NQO-1, GST(pi), glutathione peroxidase (GPX), ERK1/2, and p-ERK1/2 from Abcam (Cambridge, MA). Horseradish peroxidase-conjugated anti-rat/rabbit/mouse IgG was purchased from Thermo Scientific Pierce (Rockford, IL).

Kimchi preparation and pellet diet containing kimchi. Preparation of kimchi was based on the standardized kimchi recipe of the Kimchi (sKimchi) CJ Food Research Center, Suwon, Korea. First of all, skimchi is made of birned baechu cabbage (a kind of Chinese cabbage), red pepper powders, garlic, ginger, anchovy juice, sliced redish, green onion, some sugar, then fermented for some periods yielding lactobacillus like *Lactobacillus plantarum* (*L. plantarum*). In addition to these ingredients necessary for sKimchi production, additional supplements such as mustard leaf, Chinese pepper, pear, mushroom, and sea tangle juice instead of anchovy juice were included in cpkimchi. Japanese style non-fermented kimchi, kimuchi, was also prepared according to recipe without process of fermentation and fermentating procedure. In order to administer to mice, we prepared pellet diet containing each kimchi, for which all of the kinds of kimchi were freeze-dried and grounded into a fine powder. The kimchi powder underwent an extraction process with 20 times of methanol by stirring overnight. Finally, the kimchi methanol extracts were concentrated by heat evaporation (Büchi RE 111 rotavapor, Switzerland) and stored at 4°C. Since the usual serving dose of kimchi in Korea is approximately 30–100 g/day upon individual taste and about of 90% of general kimchi is composed of water, we could reduce the volume of kimchi through lyophilization and vaporization. For animal dietary intake, 1.7 g/kg/day and 5.0 g/kg/day equivalent with usual general Korean intake dose of kimchi.

Animals. Animals were handled in an accredited animal facility in accordance with Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International) guidelines under the facility named colitic cancerU (The Center of Animal Care and Use) of CHA University Laboratory Animal Research Center after IRB approval. Two experimental protocols were followed and conducted separately (Fig. 1A and 4A).

Preemptive intake of kimchi before dextran sulfate sodium (DSS)-induced colitis (Fig. 1A). Germ-free male C57BL/6 mice (5 weeks of age, Orient Bio, Seongnam, Korea) were used for the experiments. A total of 60 mice were divided into 6 groups, 10 mice per each group, respectively; a non-colitic group that received no drug treatment and distilled water without DSS (Normal, Group 1); a colitic control group that received 4% DSS (molecular weight 36,000–50,000; MP Biomedicals) in tap water ingestion for 1 week alone (Group 2); preemptive administration of fermented cpkimchi, 1.7 g/kg, two weeks before 4% DSS administration (Group 3); preemptive administration of fermented cpkimchi, 5.1 g/kg, two weeks before 4% DSS administration (Group 4); preemptive administration of fermented skimchi, 5.1 g/kg, two weeks before 4% DSS administration (Group 5); preemptive administration of fermented cpkimchi, 1.7 g/kg, three weeks without DSS administration (kimchi control, Group 6).

Preemptive intake of kimchi before AOM-initiated, DSS-promoted colitic cancer (Fig. 4A). Germ-free male C57BL/6 mice (5 weeks of age, Orient Bio, Seongnam, Korea) were used for the experiments. A total of 60 mice were divided into 6 groups, 10 mice per each group, respectively; a non-colitic group that received no drug treatment and distilled water without DSS (Normal, Group I); a colitic cancer group that received 5 mg/kg AOM, *i.p.* followed with 2.5% DSS in tap water ingestion for 1 week, then followed up 13 weeks (Group II); colitic cancer modeling with administration of fermented cpkimchi, 1.7 g/kg up to 13 weeks (Group III); colitic cancer modeling with administration of fermented cpkimchi, 5.1 g/kg up to 13 weeks (Group IV); colitic cancer modeling with administration of fermented skimchi, 5.1 g/kg up to 13 weeks (Group V); colitic cancer modeling with administration of non-fermented kimchi, kimuchi, 5.1 g/kg up to 13 weeks (Group VI).

Disease Activity Index (DAI), colon length, and bowel preparation. Clinical phenotypes such as hematochezia, rectal prolapse and body weight were investigated and charted daily. After 7 days after the first DSS administration, all mice were killed and colons were removed, opened longitudinally, and rinsed with PBS. The lengths of colon were measured, and isolated tissues were subjected to a histologic examination and extraction of protein.

Assessment of colonic damage. Animal body weight, the presence of gross blood in the feces, and stool consistency were recorded daily for each rat by an observer unaware of the treatment. None of the mice were died in all the groups. After 7 days of DSS ingestion. Once mice were sacrificed, their colons were immediately removed and rinsed with ice-cold phosphate-buffered saline. The excised colonic segments were placed on an ice-cold plate, cleaned of fat and mesentery, and blotted on filter paper. Each specimen was weighed and its length measured under a constant load (2 g). The colon was longitudinally opened, and a cross section from the distal diseased area was immediately fixed in 3.7% formaldehyde and embedded in paraffin for histological analysis. Afterward, it was sectioned into different longitudinal fragments to be used for biochemical determination and Western blotting.

Histopathological examinations. The paraffin sections were stained with hematoxylin and eosin (H&E) or saved for immunohistochemical staining. Pathologic index was graded according to criteria. Pathologic data and slides were blindly reviewed by two independent gastrointestinal specialists (Kim KJ and Hahm KB). For Periodic acid and Schiff's staining, histochemical staining of glycoconjugates was carried out as per the method of Pandurangan, using 2% periodic acid and Schiff (PAS)'s reagent in dark for 20 min.

Western blot analysis. The colon tissues were homogenized with ice-cold cell lysis buffer (Cell Signaling Technology, Danvers, MA) containing 1 mM phenylmethylsulfonyl fluoride (PMSF). After 20 min of incubation, samples were centrifuged at 12,000 *g* for 15 min. Supernatants were then collected. Total protein-equivalents for each sample were separated by SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes, which were incubated with which were incubated with appropriate antibodies and then visualized using West-zol Plus (Intron Biotechnology, Seongnam, Korea).

Statistical analysis. The data are presented as means ± SD. The data were analyzed by ONE-WAY ANOVA, and the statistical significance between groups was determined by Student's *t* test. Statistical significance was accepted when $p<0.05$. The survival curves between the groups were compared using log-rank test.

Results

Cancer preventive special recipe kimchi, cpkimchi, significantly ameliorated DSS-induced colitis through anti-inflammatory, antioxidative, and cytoprotective actions. In order to know whether kimchi can ameliorate colitis, 4% DSS-induced colitis model was generated, in which model 1.7 g/kg cancer preventive fermented kimchi (cpkimchi, Group 3), 5.1 g/kg cpkimchi (Group 4), 5.1 g/kg standard recipe fermented kimchi

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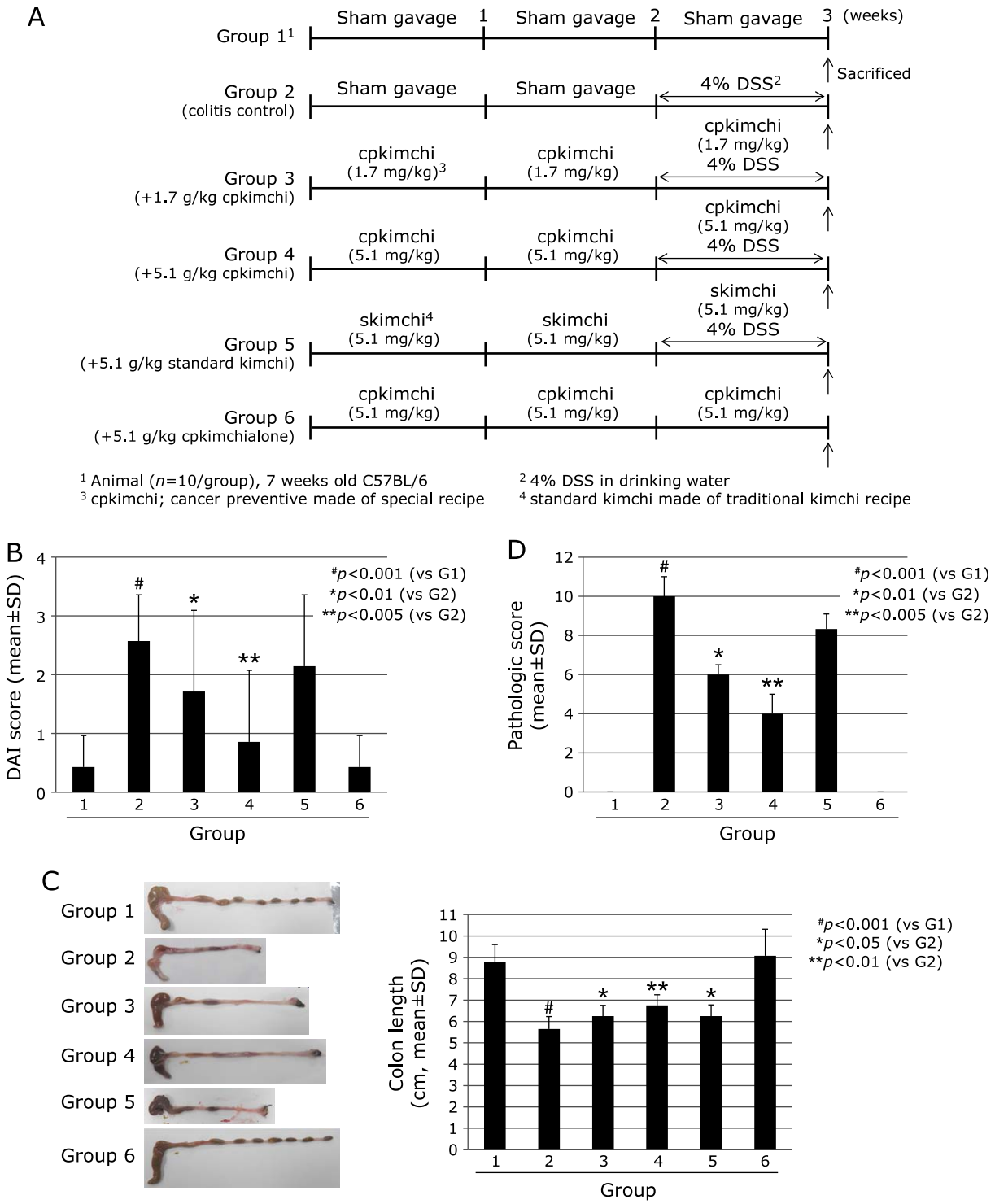

Fig. 1. Preemptive intake of kimchi ameliorated DSS-induced colitis. (A) Study agenda for DSS-colitis. In order to check whether probiotic kimchi can ameliorate colitis, 4% DSS-induced colitis model was generated, before which 1.7 g/kg cancer preventive kimchi (special recipe kimchi named as cpkimchi, Group 3), 5.1 g/kg cpkimchi (Group 4), 5.1 g/kg standard kimchi (folk marketed standard recipe kimchi named as skimchi, Group 5) were preemptively administered. Group 6 was mice only administered with cpkimchi without DSS challenge. (B) Disease activity index (DAI). Mean disease activity index assessed with diarrhea, hematochexia, and abdominal pain according to group. These DAIs were significantly decreased in Group 3 and Group 4 ($p<0.01$). (C) Representation gross photo of whole colon and mean length of colon according to group. On measuring colon length according to group, 4% DSS challenge led to significant decreases in colon length ($p<0.01$), while colon length was significantly increased in Group 3 and Group 4 ($p<0.05$). (D) Mean pathological score according to group. 4% DSS administration significantly provoked extensive ulcerations, inflammatory cell infiltrations and submucosal edema. However, group pretreated with cpkimchi showed significantly decreased pathological indices ($p<0.01$).

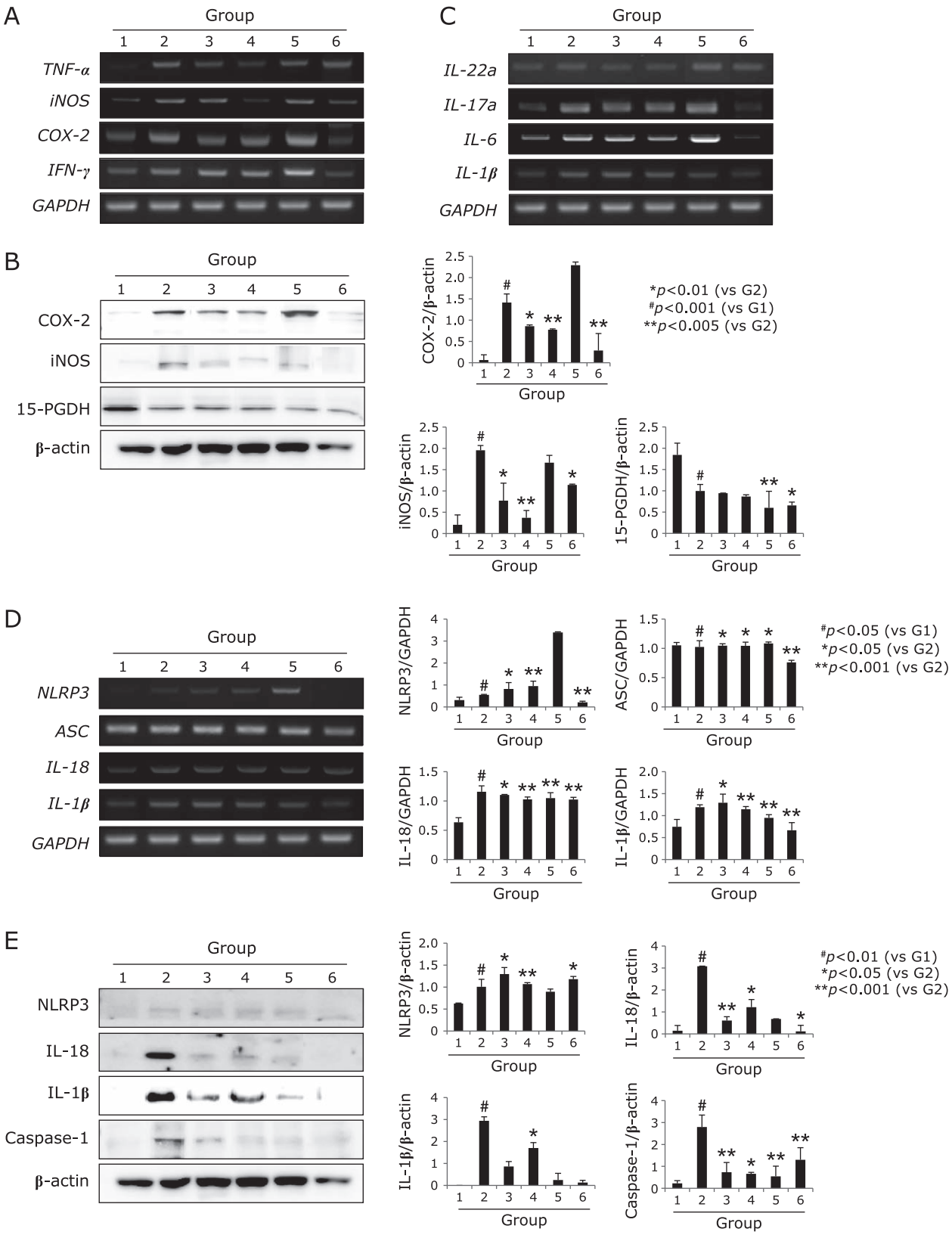

Fig. 2. Comparative changes of inflammatory mediators according to group. (A) RT-PCR for *TNF-α*, *iNOS*, *COX-2*, and *IFN-γ* mRNA according to group (B) Western blot for COX-2, iNOS, and 15-PGDH according to group. Western blot for COX-2 and iNOS showed significant decreases in their expressions in Group 3 and Group 4 ($p<0.001$), while the expressions of tumor suppressive gene 15-PGDH were significantly decreased in control Group 2, but significantly preserved in Group 3 and Group 4 ($p<0.01$). (C) RT-PCR for *IL-22a*, *IL-17a*, *IL-6*, and *IL-1β*. (D) RT-PCR for *NLRP3*, *ASC*, *IL-18*, and *IL-1β* mRNA. (E) Western blot for inflammasomes, including NLRP3, IL-18, IL-1β, and Caspase-3. Western blot for inflammasomes including NLRP3, IL-1β, IL-18, and caspase-1 were all significantly increased in Group 2, of which levels were all significantly decreased in group pretreated with any kinds of kimchi ($p<0.01$).

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(skimchi, Group 5) were preemptively administered. Group 6 was mice only administered with cpkimchi without DSS challenge (Fig. 1A). As result, Group 2 disease control group showed significant bout of diarrhea, hematochexia, and abdominal pain, whereas these disease activity indices were significantly decreased in Group 3 and Group 4 (p<0.01, Fig. 1B). Interestingly skimchi did not show any benefit of symptom change. On measuring colon length according to group, as shown in Fig. 1C, 4% DSS administration led to significant decreases in colon length (p<0.01), but colon length was significantly increased in Group 3 and Group 4 (p<0.05), signifying that only cpkimchi significantly ameliorated DSS-induced colitis. These gross findings were further investigated through pathological analysis. As shown in Fig. 1D, 4% DSS administration significantly provoked extensive ulcerations, inflammatory cell infiltrations and submucosal edema. However, group pretreated with cpkimchi showed significantly decreased pathological indices (p<0.01). Although same kimchi, skimchi did not show significant ameliorating action, signifying that special recipe cpkimchi exerted these rescuing actions against colitis since standard kimchi did not benefits. These results were explained with molecular changes according to group (Fig. 2). On measuring inflammatory cytokines mRNA such as *TNF-α*, *iNOS*, *COX-2*, *γ-IFN*, and IL-6, significantly increased expressions of these cytokines were noted after DSS administration (p<0.001). On comparing the expressions of these inflammatory cytokines, as shown in Fig. 2A, cpkimchi showed significantly decreasing action of inflammatory cytokines, whereas not in group treated with skimchi. On Western blot for COX-2 and iNOS proteins, the expressions of COX-2 and iNOS were significantly decreased on Group 3 and Group 4, not in Group 5 (p<0.01). 15-PGDH known as significant antagonizing enzyme of inflammatory and mutagenic PGE_2 was significantly decreased in Group 2, but 15-PGDH was significantly preserved in Group 3 and Group 4 (p<0.05, Fig. 2B). Another set of inflammatory cytokines such as IL-22a, IL-17a, IL-6, and IL-1β mRNA were measured. AS seen in Fig. 2C, IL-17a, IL-6, and IL-1β mRNA was significantly increased in control group, but cpkimchi significantly attenuated IL-17a, IL-6, and IL-1β. Inflammasome is a complex orchestrating colitis. Under the hypothesis cpkimchi can modulate colitis-associated inflammasome, we extended the measurements of *NLRP3*, *IL-18*, *IL-1β*, and *capsase-1* mRNA. As seen in Fig. 2D, increased levels of *IL-18*, *IL-1β*, and *capsase-1* mRNA after DSS administration were all significantly decreased in all group treated with cpkimchi (p<0.01). These changes of inflammasome were further validated with Western blot for NLRP3, IL-18, IL-1β, and capsase-1 (Fig. 2E). As far as inflammasome in concerned, skimchi was better than cpkimchi in suppressing in DSS-induced inflammasome formations. Based on the preliminary study that cpkimchi exerted significant actions of cytoprotection against *H. pylori* infection (data not shown), we continued to measure heat shock proteins (HSPs) and phase II antioxidative enzymes according to group. As shown in Fig. 3A, the most significant elevated expressions of HSP70 and HSP27 were noted in Group 4 (p<0.01). When we added the measurement of NQO1, GST(pi),

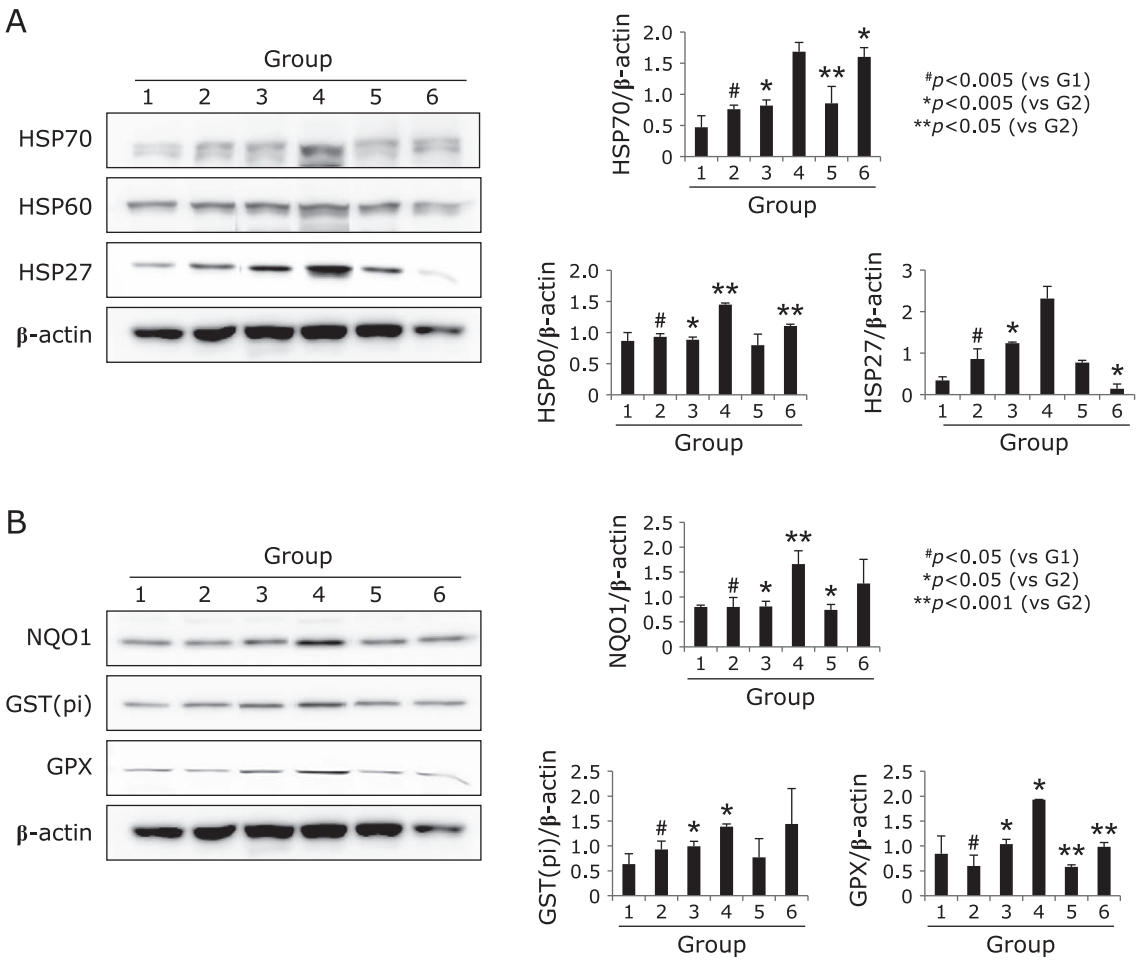
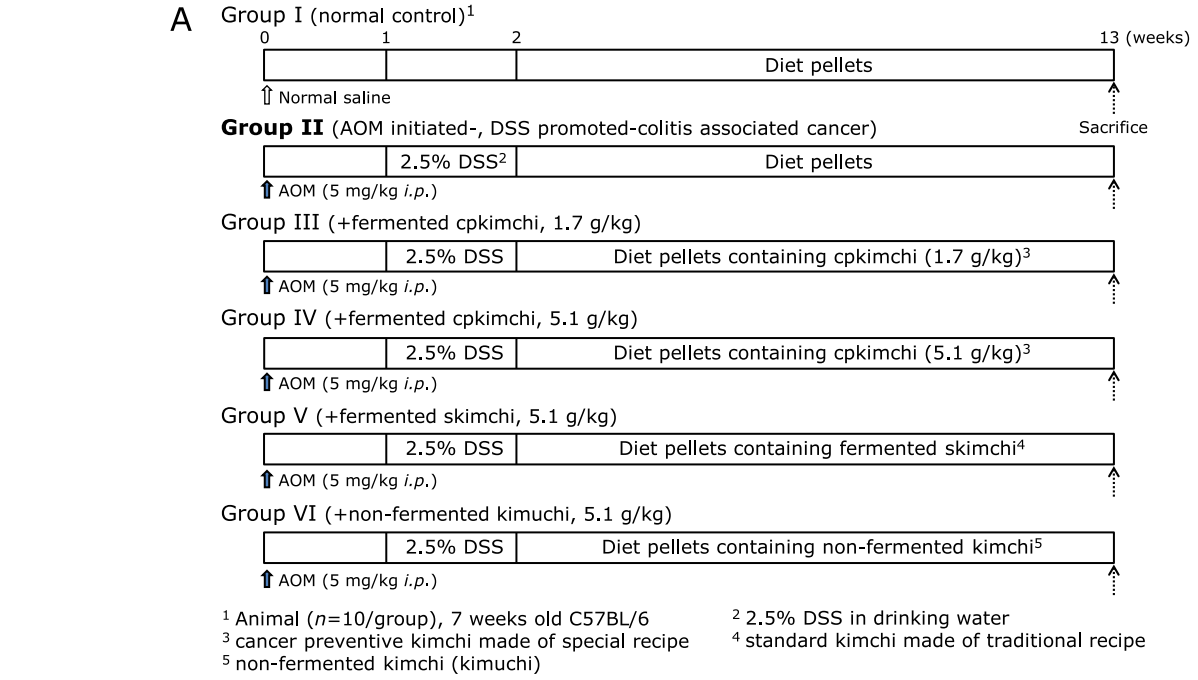
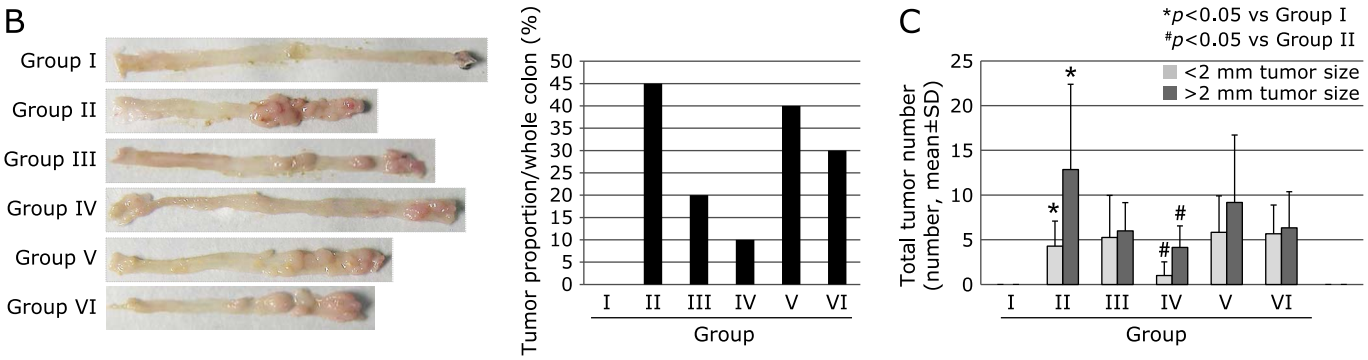


Fig. 3. Comparative changes of host cytoprotective proteins according to group. (A) Western blot for HSP70, HSP60, and HSP27. (B) Western blot for NQO1, GST9p), and GPX according to group.





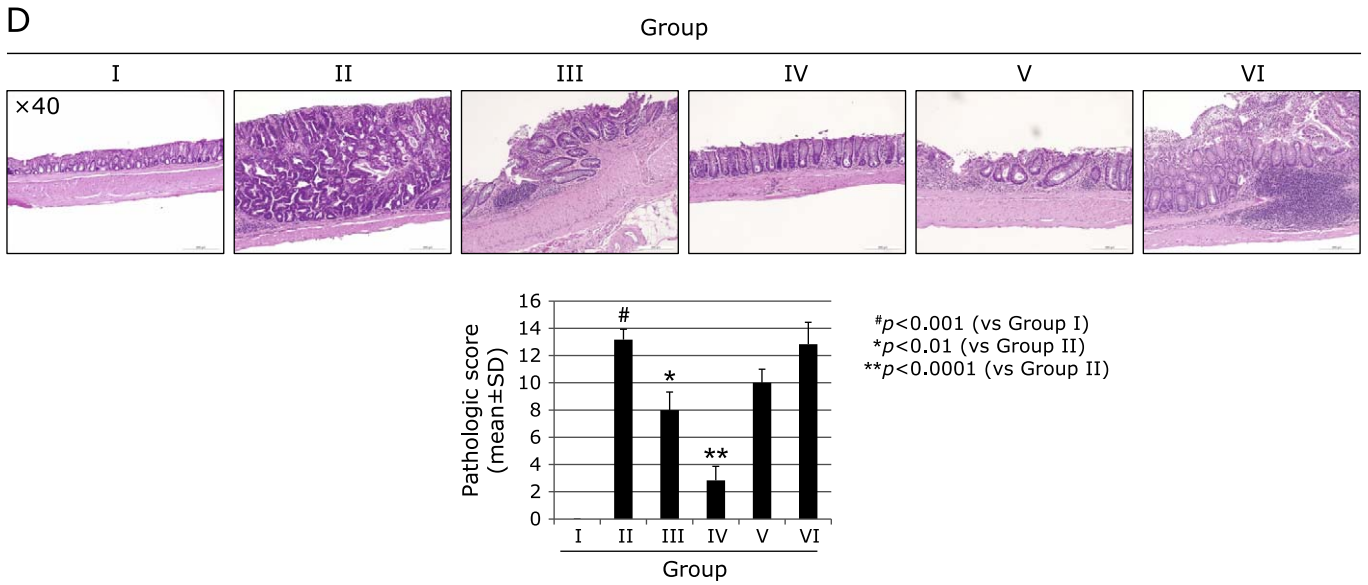


Fig. 4. Preemptive intake of kimchi mitigated colitis-associated cancer. (A) Study design for colitis-associated carcinogenesis (colitic cancer), for which AOM, 5 mg/kg, *i.p.* initiated carcinogenesis and 2.5% DSS promoted colitic cancer was used. (B) Mean gross morphology of colon according to group developing colitic cancer. (C) Mean tumor numbers of colitic cancer according to group and tumor size (2 mm). (D) Pathological feature of proximal rectum according to group and mean pathological score according to group.

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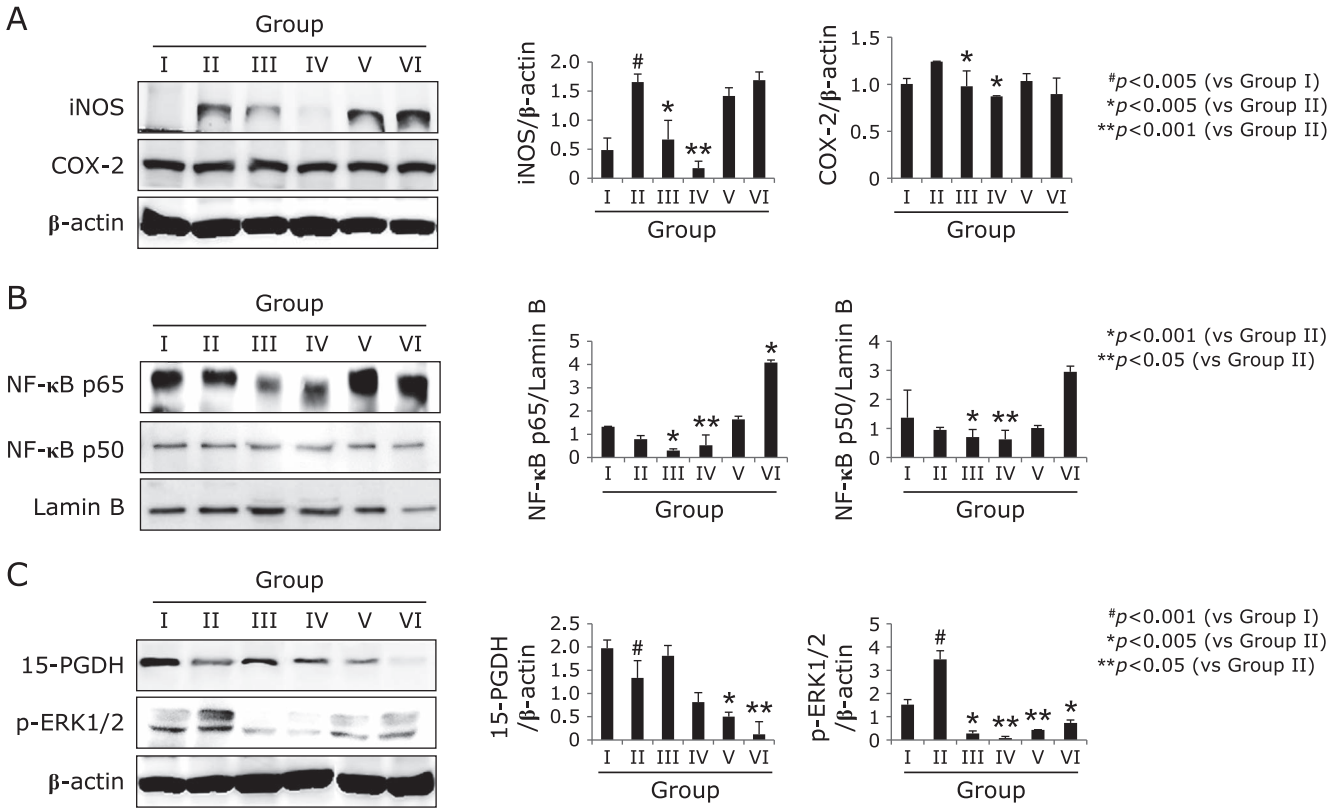

Fig. 5. Molecular changes regarding inflammation and proliferation according to group. (A) Western blot for iNOS and COX-2. (B) Western blot for NF-κB p65 and p50 according to group. Western blot for NF-κB P65 and p50 was done in nuclear fractions according to group. (C) Western blot for 15-PGDH and p-ERK1/2. Western blot for tumor suppressive enzyme responsible for mutagenic PGE_2 degradation and p-ERK1/2 among MAPKs was done according to group.

and GPX, also highest expressions of these antioxidative proteins were noted in group treated with 5.1 g/kg cpkimchi. Conclusively, fermented cpkimchi consistently showed anti-inflammatory, antioxidative, and cytoprotective actions to ameliorate DSS-induced colitis.

Fermented cancer preventive specific recipe kimchi, cpkimchi, significantly prevented AOM-initiated, DSS-promoted colitic cancer. After the above experiment that cpkimchi significantly ameliorated acute colitis, under the hypothesis that the chronic offering of anti-inflammatory, antioxidative, and cytoprotective actions with special recipe kimchi, cpkimchi, can lead to rescuing from colitis-associated cancer (colitic cancer) and another curiosity whether fermentation is essential, we proceed the additional experiments (Fig. 4A). As shown in Fig. 4A, in this experiment for documenting the efficolitic cancery of cpkimchi to prevent colitic cancer, we set the following 6 group, Group I as normal control, Group II as colitic cancer control group, Group III as colitic cancer administered with 1.7 g/kg fermented cpkimchi, Group IV as colitic cancer administered with 5.1 g/kg fermented cpkimchi, Group V as colitic cancer administered with 5.1 g/kg fermented skimchi, and Group VI as colitic cancer administered with 5.1 g/kg non-fermented kimchi (Japanese style kimchi known as kimuchi). All the kinds of kimchi were administered as diet pellet containing each kimchi formula for 11 weeks of duration. All mice developed significant tumors with AOM-initiated, DSS-promoted colitic cancer (Fig. 4B). When we counted and measured tumors according to group, significant decreases of colitic cancers were noted in Group III and Group IV, while not in Group V and Group VI, signifying that only fermented cpkimchi was effective in preventing colitic cancer (p<0.01, Fig. 4B and C). Similar results showing significant tumor preventing action of cpkimchi were noted on histological analysis (Fig. 4D). In detail, all mice in Group II developed significant invasive cancers as shown in Fig. 4D. Also, on pathological analysis of Group V and Group VI, extensively invading cancers were noted after AOM-initiated, DSS-promoted carcinogenesis. However, significantly decreased carcinogenesis was noted in Group III and Group IV, of which colitic cancer tumor was smaller in size and lesser invasive in nature. The efficolitic cancery of fermented cpkimchi, different with contribution of skimchi and non-fermented kimchi, can be explained with special recipe kimchi than skimchi and the fermentation than non-fermentation. On exploring cancer preventive mechanisms of fermented cpkimchi, first we measured the expressions of iNOS and COX-2 implicated in colitic cancer according to group. As shown in Fig. 5A, iNOS expressions were significantly increased in Group II, Group V, and Group VI, but significantly decreased in Group III and Group IV. Different with above DSS-induced acute colitis model, COX-2 expressions were not differed among groups. However, looking at 15-PGDH, the 15-PGDH expressions were significantly decreased in Group II, whereas preserved in Group III and Group IV, telling significant cancer preventive action via 15-PGDH was operated with fermented cpkimchi. NF-κB as major redox sensitive transcription factor involved in colitic cancer was significantly repressed in Group III and Group IV, especially NF-κB p65 (p<0.01, Fig. 5B). These transcriptional repression of redox sensitive, inflammation mediated NF-κB was associated with significant inactivation of ERK1/2 transduction signaling (p<0.001, Fig. 5C). Lastly, cancer preventive effects of fermented cpkimchi were associated with significant induction of

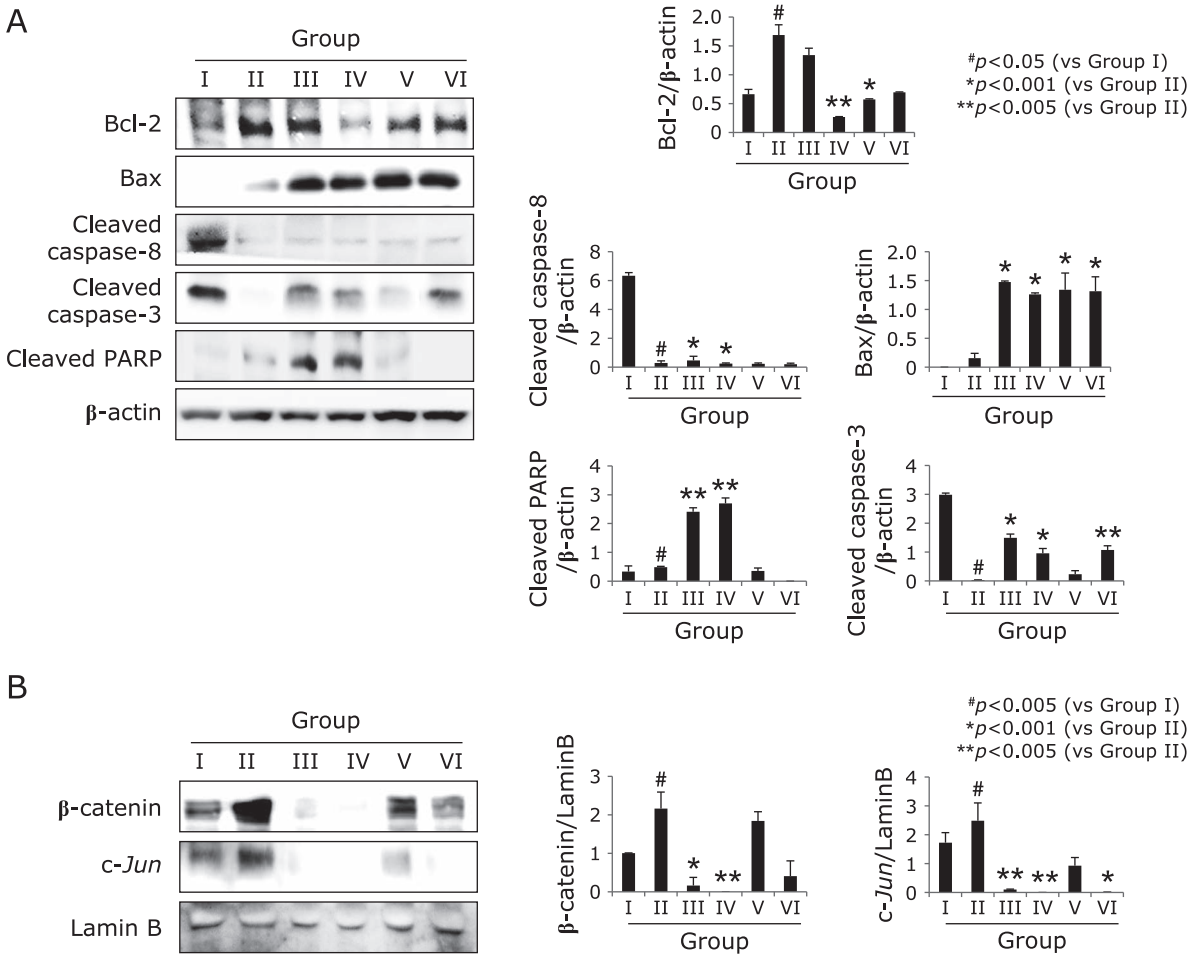

Fig. 6. Apoptotic executor and nuclear expressions of β-catenin and c-*Jun* according to group. (A) Western blot for apoptotic markers. Western blot for Bcl-1, Bax, cleaved capspase-8, cleaved capspase-3, and cleaved PARP was done according to group. (B) Western blot for β-catenin and c-*Jun* in nuclear fractions according to group. Western blot for β-catenin and c-*Jun* was done in each nuclear fractions according to group.

apoptotic execution suppressing tumorous proliferations. As seen in Fig. 6A, either increased cleavages of capsase-3 and PARP or decreased expression of Bcl-2 were noted in Group III and Group IV ($p<0.01$). Also pertinent to proliferation, significant inhibition of proliferation-engaged β-catenin nuclear translocation and c-*Jun* was only noted in Group III and Group IV, while not in Group V and Group VI ($p<0.01$, Fig. 6B), all signifying the significant antiproliferating action was imposed with cpkimchi.

Discussion

From the current study dealing with either administration of fermented kimchi on ameliorating colitis or the preemptive inhibition of colic cancer with special recipe kimchi, we could conclude the continuous intake of fermented cpkimchi can be an ideal way to tackle troublesome colitic cancer as dietary intervention of cancer prevention. As mode of action of kimchi's chemoprevention, cpkimchi significantly afforded anti-inflammation through attenuating inflammasome, antioxidative through phase 2 antimutagenesis and cytoprotective actions, and anti-proliferating through apoptosis, all mechanisms implicated in colitic cancer. Though long-term prescription of anti-inflammatory agent such as sulfasalazine, mesalazine, and some biologics has been attributed to cancer preventive outcome,[8,19–22] dietary intake of nutrients possessing all of these beneficiary mechanisms, kimchi in this case, can be of rationale and of safety. Conclusively, preemptive administration of kimchi exerted time-appropriate mechanisms, such as initially and continuously anti-inflammation, inducing tumor suppressive and anti-mutagenic actions of which concerted influence resulted in significant prevention of colitic cancer.

As shown in both models of acute colitis and colitic cancer, fermented cpkimchi afforded beneficiary mechanisms of cancer preventive actions based on anti-inflammation. As shown in former model of acute colitis (Fig. 1A), only cpkimchi administration exerted significant ameliorating action against DSS-induced colitis better than standard market kimchi, signifying that though dietary fermented kimchi was effective, our action-fortifying cpkimchi was proven to be better in either anti-inflammatory action documented with lowering inflammatory cytokines as well as inflammasome or adding cytoprotection such as HSP70, HSP27, and some phase 2 antioxidative proteins against DSS administration. A failure of balance between inflammation assault and the immune system can lead to chronic intestinal inflammation and increase the chances to develop colorectal cancer significantly,[23] during which one of critical molecular platforms linking intestinal homeostasis might be "inflammasome", which is molecule complexes assembled in response to pro-inflammatory cytokines.[24] As shown in our investigation, one inflammasome, in particular NLRP3, has been analyzed extensively in its contribution to colitis and has been shown to be associated with

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the development of colitic cancer,[25] but fermented cpkimchi intake significantly ameliorated cancer-prone inflammasome as much better than agents by others.[26] Phase 2 antioxidative and antimutagenic enzyme contributed to either cytoprotection or cancer prevention. For instance, Inoue *et al.*[27] reported geranylgeranylacetone (teprenon), professional inducer of HSPs, could suppress colitic cancer in mice and nimesulide through inhibiting COX significantly prevented colitic cancer in experimental murine colitis-associated cancer,[28] signifying modulation of oxidative stress and provision of cytoprotection can be essential in colitic cancer prevention.[29] We propose kimchi intake satisfied these two conditions against colitis and colitic cancer development much better than specific drug administration.

From further documentations dealing with colitic cancer model, we could successfully verify only fermented kimchi showed significant protection. Unexpectedly non-fermented kimchi called Japanese style-kimuchi worsened colitic cancer, signifying both the component of special recipe kimchi and fermentation might determine the efficacy of cancer prevention. As shown in Fig. 4, fermented cpkimchi only exerted significant rescuing action against colitic cancer, while non-fermented kimchi aggravated colitic cancer. These outcomes of colitic cancer were quite compatible with the molecular explorations that iNOS and its transcription factor NF-κB was significantly decreased on group treated with fermented cpkimchi only (Fig. 5). Looking at the levels of tumor suppressive 15-PGDH, cpkimchi only significantly increased its expression in accordant with significant inactivation of ERK1/2. 15-PGDH as an prostaglandin-degrading enzyme, has been known as tumor suppressive[30] since PGE_2 has been responsible for CRC and colitic cancer. Though 15-PGDH inhibition potentiates tissue regeneration in benign condition,[31,32] synthetic triterpenoid suppressed colitic cancer through induction of 15-PGDH.[33] In our investigation, fermented kimchi intake induced 15-PGDH, while significantly suppressed iNOS under the transcriptional repression of NF-κB against colitic cancer, of which actions were proven to be much better than our other reports in this matter.[9,10]

While cellular proliferation condition was pivotally implicated in both CRC and colitic cancer, tracing apoptotic genes such as Bax, caspase and PARP cleavage, these apoptotic executive and substrate genes were significantly increased in group treated with cpkimchi. The ultimate condition of colon proliferation was inferred with the expression of β-catenin and c-*Jun*. As shown in Fig. 6B, the nuclear expressions of β-catenin and c-*Jun* were significantly increased in colitic cancer model ($p<0.005$, Group II vs Group I). As shown in our investigation, fermented kimchi afforded significant inhibiting action of NF-κB, in which persistent NF-κB activation in intestinal epithelial cells may accelerate loss of heterozygocity by enhancing nitrosative DNA damage and exhibited more β-catenin accumulation, leading to tumorigenesis.[34] Reversely, β-catenin can regulate NF-κB activities.[35] Before our investigation, several agents such as isoquercitrin,[36] vitamin D,[37] omeprazole,[38] and omega-3 polyunsaturated fatty acids[11] could prevent colitic cancer via the significant inhibition of colon proliferation manifested with β-catenin inaction, current study showed prominent action of fermented kimchi in β-catenin inactivation.

In the current study, only fermented, probiotic cpkimchi significantly contributed to cancer preventive as well was mitigating action against colitis because non-fermented kimchi, so called kimuchi, did not affect at all in this matter. Though skimchi is a traditional Korean fermented side-dish containing rather diverse strains of lactic acid bacilli such as *L. plantarum*, *L. acidophilus*, *L. curvatus*, *L. brevis*, *L. sakei*, *Leuconostoc mesenteroides*, *Enterococcus faecium*, and *Weissella cibaria*[39–41] in addition to several prebiotics and the presence of vitamins, minerals, and dietary fibers,[42] our study might be the first document to prove the probiotic food contributed to beneficial outcome in either mitigating colitis or preventing colitic cancer. Although specific probiotics are beneficial in some GI problems, but still many of the new publications did not report clear benefits of probiotic. For instance, some probiotics can relieve GI symptoms in irritable bowel syndrome, prevent antibiotics-associated diarrhea, and *H. pylori* eradication therapy, but still of limited significance.[43,44] Furthermore, recent some publications thrown dread question about the risk of indiscernible intake of probiotics against disease. For instance, probiotic prophylaxis in severe acute pancreatitis, double-blind, placebo-controlled trial showed probiotic prophylaxis resulted in an increased risk of mortality,[45] while children who received a 5-day course of *Lactobacillus rhamnosus* GG did not have better outcomes than those who received placebo,[46] and basic immunologic study showing some probiotic can drive inflammatory Th17 cell accumulation in intestine.[47] Therefore, until answered in future, safe way to modulate microbiota-cooperated homeostasis can be through "probiotic food" and through "dietary intake". Lastly, we are under investigation whether fermented cpkimchi can afford probiotic-induced *O*-linked β-*N*-acetylglucosamine (*O*-GlcNAc) modification (*O*-GlcNAcylation) supported with publication by Hirata *et al.*[48] that *O*-GlcNAcylation prevented colon carcinogenesis through reducing acute maximum inflammation in *O*-GlcNAc transferase-transgenic (*Ogt*-Tg) mice and some strain of probiotics can modify Ogt.[49]

Our current investigation consistently showed dietary intake of fermented and special recipe kimchi, cpkimchi, can be ideal way for prevention of colitic cancer. While we studied the timing of cpkimchi administration in separate experiment, though co-incidental intake of cpkimchi also showed significant suppression of colitic cancer, the best outcome was noted in group with preemptive intake of cpkimchi. Conclusively, we could conclude that long-term intake of fermented cpkimchi can be feasible, ideal, and effective way of colitic cancer prevention in addition to ameliorating the course of colitis. However, since we should prove these results with well-documented clinical trials, RCT of randomized, double blind clinical trials showed quite beneficial outcome that 10 weeks of cpkimchi led to either significant inhibition of microbiota implicated in advancement of colon adenoma or enrichment of beneficial strains of microbiota implicated in inhibition of colon adenoma. Conclusively, we found dietary intake of cpkimchi can be of potential way for patients with high risk of colitic cancer, long history, extensive involvement, and family history of CRC.

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Author Contributions

Study concept and design: JYO and KBH; acquisition of data: YMH, EAK, and JMP; analysis and statistical analysis: JMP and YMH; Preparation of kimchi and materials: JYO, DYL, and SHC; interpretation of data: YMH, JMP, JYO; drafting of manuscript: JYO and KBH.

Abbreviations

AOM	azoxymethane
colitic cancer	colitis associated cancer
cpkimchi	cancer preventive special recipe kimchi
CRC	colorectal cancer
DSS	dextran sulfate sodium
H. pylori	*Helicobacter pylori*
HO-1	heme oxygenase-1
HSP	heat shock protein
NLRP3	NACHT, LRP and PYD domains-containing protein 3
15-PGDH	15 hydroxyprostaglandin dehydrogenase
skimchi	standard recipe kimchi

Conflict of Interest

No potential conflicts of interest were disclosed.

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