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Chemopreventive potential of kimchi, an ethnic food from Korea, against colorectal carcinogenesis associated with red meat intake

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Abstract

Kimchi, the traditional fermented vegetables with seasonings from Korea, is globally renowned as healthy food with anticancer properties. Colorectal cancer is the world's third leading cancer with an increasing incidence rate over the past years. High red meat intake is strongly associated with colorectal cancer, and the World Health Organization has identified red meat as probably carcinogenic to humans (Group 2A). This study aimed to investigate the chemopreventive potential of cabbage kimchi against the promotion of colorectal cancer by red meat in vivo using carcinogen-injected rats. Rats were fed by AIN-76 diet enriched in freeze-dried beef sirloin supplemented or not with kimchi powder for 120 days prior to killing. Kimchi supplementation at moderate (5%) and high (10%) concentration significantly suppressed the formation of precancerous lesions in the colon of red meat-fed rats. Using fecal water, the aqueous extract of feces, we found out that kimchi supplementation limited iron-mediated oxidation and reduced lipid peroxidation in the feces of rats. Furthermore, kimchi supplementation reduced the toxicity of fecal water of red meat-fed rats toward human colonic epithelial cells in vitro by suppressing the formation of cellular reactive oxygen species (ROS). The chemopreventive effects of kimchi were associated with the acidification of fecal matrix and increase in fecal lactic acid bacteria (LAB). Gene expression analysis in the colon of rats demonstrated that kimchi supplementation prevented colorectal carcinogenesis by up-regulating the expression of tumor-suppressor genes and antioxidant enzymes, as well as by down-regulating the expression of proinflammatory proteins. Taken together, our findings suggested that kimchi consumption is correlated with lower promotion of colorectal cancer associated with red meat intake.

Keywords Colorectal cancer, Red meat, Kimchi, Cancer prevention

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Introduction

Kimchi is a broad term defining traditional lactic acid-fermented vegetable dish originating from Korea [1]. *Baechu* kimchi made from napa cabbage (*Brassica rapa* subsp. *pekinensis*) fermented in a mixture of seasonings (garlic, ginger, red chili powder, fish sauce, etc.) is the most popular type of kimchi and is often addressed as simply kimchi [1]. In Korea, kimchi is considered as staple food besides rice. It has become an integral part of Korean food culture for thousands of years [2]. Kimchi is always present in Korean diet on a daily basis, and a Korean traditional meal would be not complete without kimchi. Every year, a Korean is estimated to eat 40–57 pounds of kimchi [3] or about 50–70 g kimchi daily. Today, kimchi is globally renowned as healthy food rich in dietary fiber, vitamins, minerals, phytochemicals, lactic acid bacteria (LAB) and other compounds suggested to promote human health [4, 5]. Studies have reported that kimchi exerts beneficial properties, including antioxidant, anticancer, antimutagenic, antiobesity, antiaging, antiatherogenic and antidiabetic activities [6]. This study highlights the anticarcinogenic potential of kimchi.

Cancer is a leading cause of death worldwide, accounting for nearly 10 million deaths in 2020, or nearly 1 in 6 deaths [7]. Colorectal cancer is the primary cause of global mortality by cancer in non-smokers, particularly in affluent countries [8]. In terms of global incidence rate, colorectal cancer is the third most diagnosed cancer (10.7%) behind breast cancer (12.5%) and lung cancer (12.2%) [9]. Epidemiological studies identified several risk factors strongly associated with colorectal cancer development, among which diet appeared as an essential and modifiable environmental risk factor [8]. Regarding diet, the World Health Organization (WHO) categorized red meat in Group 2A as probably carcinogenic to humans [10]. The term "red meat" usually refers to all mammalian muscle meat, including beef, veal, lamb, pork, mutton, horse and goat [10]. Red meat is characterized by its red color due to the presence of myoglobin, an iron-bound hemoprotein, in the muscular tissue [11]. The American Institute for Cancer Research (AICR) and the World Cancer Research Fund (WCRF) established a recommendation for limiting red meat consumption to 350–500 g per week [12].

In Korea, consuming red meat is a tradition, habit and trend that have continuously soared over the past recent years. Many Koreans consume red meat on a daily basis, particularly in the context of communal Korean barbecue during which red meat (mostly beef and pork) is consumed in relatively high amount. In 2021, the annual consumption per capita of beef and pork in South Korea stood at 13.8 kg and 27.6 kg, respectively [13, 14], thus higher than the recommended amount of 350–500 g/week (about 18.2–26.0 kg/year). Koreans were also the biggest meat consumers in Asia (51.3 kg per capita per year), in front of China (47.1 kg per capita per year) and Japan (35.5 per capita per year) [15]. In addition, Koreans usually consume very little to no dairy products in their diet, and consuming dairy products is known to decrease the risk of colorectal cancer [16, 17]. Indeed, colorectal cancer has emerged as a major public health concern in Korea, and the incidence of colorectal cancer in Korea is among the highest in the world with the estimated rate of 44.5 cases per 100,000 persons per year [18]. The incidence rate of colorectal cancer in Korea has seen a continuous increase with an increase rate of 5.7% in men and 4.3% in women between 1999 and 2012 [19]. In Korea, where cancer is the major cause of death, colorectal cancer appeared to be the third leading cause of cancer death after lung cancer and liver cancer [20].

The main objective of this study was to investigate the chemopreventive potential of kimchi with regard to colorectal carcinogenesis associated with red meat intake in rats. We fed carcinogen-injected rats with standard diet supplemented with freeze-dried beef together with freeze-dried kimchi, and then, we observed whether kimchi consumption would prevent the early development of colorectal cancer in these rats. Two concentrations of kimchi in the diet were established to represent moderate kimchi consumption (5%) and high kimchi consumption (10%). Furthermore, through gene expression analysis, we also analyzed several possible mechanisms that could be involved in the chemoprevention by kimchi. Several previous studies reported the chemopreventive activities of kimchi against colorectal carcinogenesis in animal models [21–23], but without incorporating red meat in the studies. Another study also showed the anticancer effects of kimchi in human colon cancer cells [24]. Therefore, taking into account the anticancer potential of kimchi, we hypothesized that kimchi consumption would inhibit the promotion of colorectal carcinogenesis in rats. The originality of this study relied on our focus on the role of kimchi in preventing colorectal carcinogenesis driven by red meat consumption. To our knowledge, such a topic has not been previously published in any scientific media. This study would allow to support the development of kimchi as a functional food beneficial for human health, in particular with regard to the prevention of colorectal cancer. Furthermore, this study would help establish a diet-related recommendation for colorectal cancer prevention through kimchi consumption.

Methodology

Animal experiments

Seventy male albino Wistar rats aged 3–4 weeks old were purchased from the Central Animal House of IPB

University (Bogor, West Java, Indonesia). The rats were housed with a 12-h light/dark cycle at room temperature and had access to food and water ad libitum. The rats were given control AIN-76 diet (standard diet) for 1 week prior to injections using carcinogenic 1,2-dimethylhydrazine (DMH) to induce the development of colitis-associated colorectal cancer.

To incorporate red meat in the ratios, beef sirloin purchased from a local supermarket in Central Jakarta, Indonesia, was ground using meat grinder, freeze-dried and powdered. This powder was then used as a single protein source for the modified AIN-76 diet (red meat-based diet) containing 20% protein from beef powder. Therefore, two kinds of ratios were used in the animal experiments: a standard diet and a red meat-based diet containing beef powder (32% w/w of beef dry matter). A package of commercial kimchi (brand Ommason Mat Kimchi SKU 01574579) aged 2 months old from the production date and constantly kept at 4 °C was purchased from a local supermarket in Central Jakarta, Indonesia. The kimchi was ground, freeze-dried and powdered to form kimchi powder that was further used as additional ingredients in either standard or red meat-based diet.

The rats were then randomly divided into 7 groups, each of which consisted of 10 rats. The rats in group 1 (Saline) were injected with 0.9% saline solution, while the others in group 2–7 were injected with DMH (20 mg/kg body weight). Following the injections, the rats were given access to food as follows: group 1 (Saline) and group 2 (DHM) received standard diet, group 3 (RM) received red meat-based diet, group 4 (K-5%) received standard diet with supplementation of 5% kimchi powder, group 5 (RM + K-5%) received red meat-based diet with supplementation of 5% kimchi powder, group 6 (K-10%) received standard diet with supplementation of 10% kimchi powder and group 7 (RM + K-10%) received red meat-based diet with supplementation of 10% kimchi powder. The concentration of supplemented kimchi powder in the diet was based on our preliminary observation regarding the mass proportion of kimchi consumed in a standard Korean meal (unpublished data). The supplementation of 5% kimchi powder represented moderate kimchi consumption, while the supplementation of 10% kimchi powder represented high kimchi consumption in Korean diet.

All rats were euthanized by CO_2 asphyxiation at day 120. Their colons were collected for further analyses, including aberrant crypt foci (ACF) and mucin-depleted foci (MDF) scoring as previously described [25]. Both ACF and MDF are putative early biomarkers of colon cancer [26]. Briefly, the colons were stained in methylene blue solution. The number of ACF per colon was determined under light microscope (Olympus CX31) at 40 × magnification. After being scored for ACF, the colons were stained with a high iron diamine-Alcian blue (HID-AB) solution for 18 h to evaluate mucin production. After rinsing, the colons were counterstained in 1% AB solution for 30 min. The number of MDF per colon was counted under light microscope (Olympus CX31) at 40 × magnification. The protocol for our animal study has been approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (Jakarta, Indonesia).

Preparation and analysis of fecal water

Fecal water was prepared as previously described [27] with some modifications by extracting 2 g of rat feces obtained from days 60–64 with 5 mL of distilled water. The feces all individuals in the same treatment group were pooled together and treated as a sample. The mixture of feces and water was then ground and centrifuged (10,000*g*, 10 min), and the supernatant was collected. The supernatant was then diluted 10 × in Cell Biologics' culture complete epithelial cell medium (Cell Biologics, Chicago, IL, USA) and sterilized using microfilter (pore size 0.2 μm) for further assays. The pH of fecal waters was determined using pH-meter (Aquasearcher AB41PH, Ohaus, NJ, USA). The concentration of iron and thiobarbituric acid reactive substances (TBARS) in the fecal water was analyzed by colorimetric assay [28, 29]. TBARS concentration was expressed in malondialdehyde (MDA) equivalent. The microbial load analysis of fecal water was done by diluting fecal water in peptone water containing 10% NaCl in serial tenfold steps prior to application onto agar. The total plate count (TPC) and lactic acid bacteria (LAB) count were determined using plate count agar (PCA) and MRS agar, respectively [30]. All the inoculated agars were incubated for 5 days at 35 °C prior to counting.

Cell culture and cellular assays

Human primary colonic epithelial cells were purchased from Cell Biologics (Chicago, IL, USA) and were grown in Cell Biologics' culture complete epithelial cell medium according to the manufacturer's instructions prior to assays. These cells were isolated from normal human colon tissue. During the actual experiments, the cells were treated with fecal water for different durations. The cellular assays, including reactive oxygen species (ROS) assay (4 h-treatment), caspase-3 activity (6 h-treatment) and cell viability assay (8 h-treatment), were done as previously described [31]. All the assays were performed by fluorimetry according to the instructions of their respective manufacturer using a fluorescence spectrometer (Cary Eclipse, Agilent, Santa Clara, CA, USA). Cellular ROS were quantified using a fluorogenic dye DCFDA/H2DCFDA-Cellular ROS detection kit (Abcam,

Cambridge, UK). Cellular caspase-3 activity was analyzed using caspase-3 activity assay kit containing a fluorogenic substance Ac-DEVD-AMC (Cell Signaling Technology, Danvers, MA, USA). Cell viability was quantified with resazurin cell viability kit (Cell Signaling Technology, Danvers, MA, USA). The RFUs (relative fluorescence units) of all the samples were normalized to control (untreated cells at $t = 0$ h).

RT-qPCR analysis

The mRNA expression of several genes in the rat colonic tissue was quantified using RT-qPCR following tissue lysis and mRNA extraction using mRNA isolation kit (Roche, Mannheim, Germany) according to manufacturer's instructions. The synthesis of cDNA was done using High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA, USA) with 1 µg RNA, and the quantitative PCR was conducted using AriaMx Real-Time PCR systems (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instructions. RPL37A (Ribosomal Protein L37A) was chosen as housekeeping gene. The thermocycling conditions were set as follows: initial denaturation at 95 °C (5 min), denaturation at 94 °C (30 s), annealing at 56 °C (30 s), extension at 72 °C (40 s) and final extension at 72 °C (5 min). The sequences of the primers (5′–3′) used are listed below: TNF-α (F-GGG GCC ACC ACG CTC TTC TGT C, R-TGG GCT ACG GGC TTG TCA CTC G), COX-2 (F-GAT TGA CAG CCC ACC AAC TT, R-CGG GAT GAA CTC TCT CCT CA), iNOS (F-CAC CAC CCT CCT TGT TCA AC, R-CAA TCC ACA ACT CGC TCC AA), p53 (F-CCT ATC CGG TCA GTT GTT GGA, R-TTG CAG AGT GGA GGA AAT GG), p21 (F-TGT TCC ACA CAG GAG CAA AG, R-AAC ACG CTC CCA GAC GTA GT), catalase (F-CAT TGA GCC CAG CCC G, R-GGC GGT GAG TGT CTG GGT AA), SOD-1 (F-CCG GTG CAG GGC GTC, R-TCC TGT AAT CTG TCC TGA CAC CA), SOD-2 (F-AGC TGC ACC ACA GCA AGC AC, R-TCC ACC ACC CTT AGG GCT CA), GPx-1 (F-AGT TCG GAC ATC AGG AGA ATG GCA, R-TCA CCA TTC ACC TCG CAC TTC TCA), HO-1 (F-ACA GGG TGA CAG AAG AGG CTA A, R-CTG TGA GGG ACT CTG GTC TTT G) and RPL37A (F-TTG AAA TCA GCC AGC ACG C, R-TGC CAA CGC CTC GTC TCT).

Statistical analysis

All data ($n \geq 5$) were analyzed using analysis of variance (ANOVA) followed by Tukey's post hoc test when the results showed significant differences among the samples ($p < 0.05$). The heatmap illustrating the induction or repression of gene expression was generated using online application Displayr.

Results and discussion

Effects of kimchi supplementation on the appearance of preneoplastic lesions promoted by red meat intake in the colon of rats

Aberrant crypt foci (ACF) and mucin-depleted foci (MDF) are microscopic precancerous lesions representing the early stages of colorectal cancer [32]. They are probable precursors of adenomas in the colon and are extensively used as biomarkers to neoplasia and precancerous stage [32]. ACF have been described as early lesions in carcinogen-treated animal models and humans at risk, and their formation is regarded as a necessary step in colorectal cancer development [33]. MDF possess dysplastic features similar to those encountered in colon tumors, and their number and multiplicity are strongly correlated with carcinogenesis [34].

In this study, the colon of saline-injected rats instead of DHM contained neither ACF nor MDF, thus confirming the carcinogenesis initiation by DMH (Fig. 1A, B). When compared to the DMH group, kimchi supplementation in standard diet effectively reduced the appearance of ACF and MDF in the colon of rats. Red meat intake strongly promoted the formation of ACF and MDF in the colon of rats, as reported in previous studies [25, 35, 36]. In this study, the average daily red meat intake of the rats receiving red meat-based diet was 1.62 g/kg body weight (equivalent to 113 g of red meat per day or 791 g of red meat per week for a human weighing 70 kg), which was considered as high when compared to the recommendation (less than 350–500 g of red meat per week). Interestingly, kimchi supplementation significantly reduced the formation of ACF and MDF in the colon of rats consuming red meat. Higher kimchi supplementation (10%) resulted in lower amount of ACF and MDF in the colon compared to lower kimchi supplementation (5%). These findings demonstrated the potential of kimchi in inhibiting colorectal carcinogenesis promoted by red meat intake. In a previous study [21], kimchi intake was reported to restore the colon shortening and suppress the number of tumors in the colon of carcinogen-induced mice.

The mechanism by which kimchi prevents colorectal carcinogenesis associated with red meat intake is proposed to involve kimchi's antioxidant properties. Kimchi is rich in antioxidants, including vitamin A, vitamin C, vitamin E, sulforaphane from cabbage, capsaicin from red chili powder, allicin from garlic and many other antioxidant compounds present in kimchi ingredients [37]. Oxidation and formation of free radicals are suggested to be one of the early events transforming normal colon cells into precancerous ones since these phenomena could lead to mutation and genetic impairment [38]. Heme iron present in

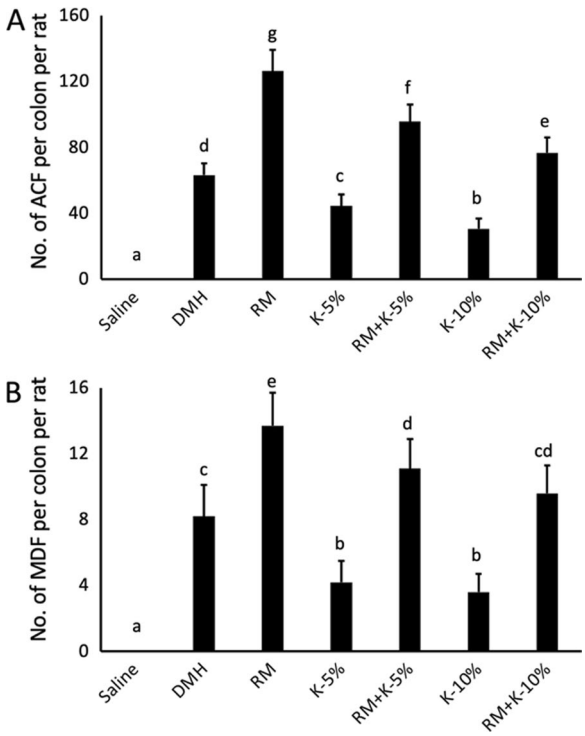

Fig. 1 Effects of standard diet and red meat-based diet with or without kimchi supplementation on the number of (**A**) aberrant crypt foci (ACF) and (**B**) mucin-depleted foci (MDF) in the colon of rats 120 days following the injection of 1,2-dimethylhydrazine (DMH, 20 mg/kg body weight). Data ($n = 10$) are expressed in mean ± SD. Different lettering indicates statistically significant difference among samples ($p < 0.05$). Saline: group of rats injected with NaCl solution (0.9%) and given standard diet. DMH: group of rats injected with DMH and given standard diet. RM: group of rats injected with DMH and given red meat-based diet. K-5%: group of rats injected with DMH and given standard diet with kimchi supplementation (5%). RM + K-5%: group of rats injected with DMH and given red meat-based diet with kimchi supplementation (5%). K-10%: group of rats injected with DMH and given standard diet with kimchi supplementation (10%). RM + K-10%: group of rats injected with DMH and given red meat-based diet with kimchi supplementation (10%)

red meat is a potent prooxidant that could provoke oxidation in colonic environment [39]. Antioxidants in kimchi are expected to counteract iron-driven oxidation in the context of red meat consumption. Other food products with strong antioxidant activities that have been demonstrated to suppress early colorectal carcinogenesis associated with red meat intake include olive oil, red wine and pomegranate [35, 40]. In addition, calcium and chlorophyll havs also been shown to prevent early colorectal carcinogenesis in heme-fed animal models through heme-trapping mechanism [41].

Characteristics and toxicity of fecal waters of red meat- and kimchi-fed rats

Fecal water is the aqueous extract of feces containing common fecal matters and by-products of food digestion, including bile acid, bilirubin, lysophospholipids and undigested food components [42]. It is a representative tool to study colorectal carcinogenesis since feces are in direct contact with colon cells in the colon lumen. In previous studies, fecal water has been analyzed to investigate diet-related promotion or inhibition of colorectal carcinogenesis [27, 43].

Figure 2 recapitulates the chemical and microbial characteristics of fecal water extracted from the feces of rats at days 60–64. Red meat consumption increased the pH of fecal water (Fig. 2A). The increase in the alkalinity of fecal water might be associated with the formation of basic nitrogenous compounds derived from the microbial degradation of red meat protein, such as ammonia [44]. The fecal pH of patients suffering from colorectal cancer was shown to be higher compared to the fecal pH of healthy individuals [45], thus suggesting that the increase of fecal alkalinity could be associated with colorectal cancer development. Interestingly, kimchi supplementation decreased the pH of fecal water independently on the type of diet (Fig. 2A). Iron, a potent prooxidant, is suggested to promote colorectal carcinogenesis by provoking oxidation, generation of free radicals and DNA damage in colon cells [46]. Indeed, iron is present in high amount in red meat in the form of myoglobin, the red pigment in red meat [47]. Excess iron in the colorectal environment increased the risk of colorectal cancer, and the level of iron in the colon is a determinant factor governing cancer development [46, 48]. Figure 2B demonstrates that iron was present in high amount particularly in the fecal water of rats consuming red meat-based diet. Kimchi supplementation did not seem to alter iron concentration in fecal water. TBARS are known as by-products generated from lipid peroxidation [49]. Fecal TBARS have been used in previous studies to examine the involvement of oxidation in colorectal carcinogenesis [27, 42]. Red meat consumption increased significantly TBARS concentration in fecal water (Fig. 2C) as confirmed by previous studies [27] Kimchi supplementation decreased the formation of TBARS in the fecal water of red meat-fed rats (Fig. 2C) despite the similar level of fecal iron among the three groups receiving red meat-based diet. The decrease in TBARS was higher in the group receiving 10% kimchi supplementation compared to the other group with 5% kimchi supplementation. This finding suggested that kimchi was able to exert antioxidant activities against iron-promoted oxidation in the colon lumen. With regard to the microbial

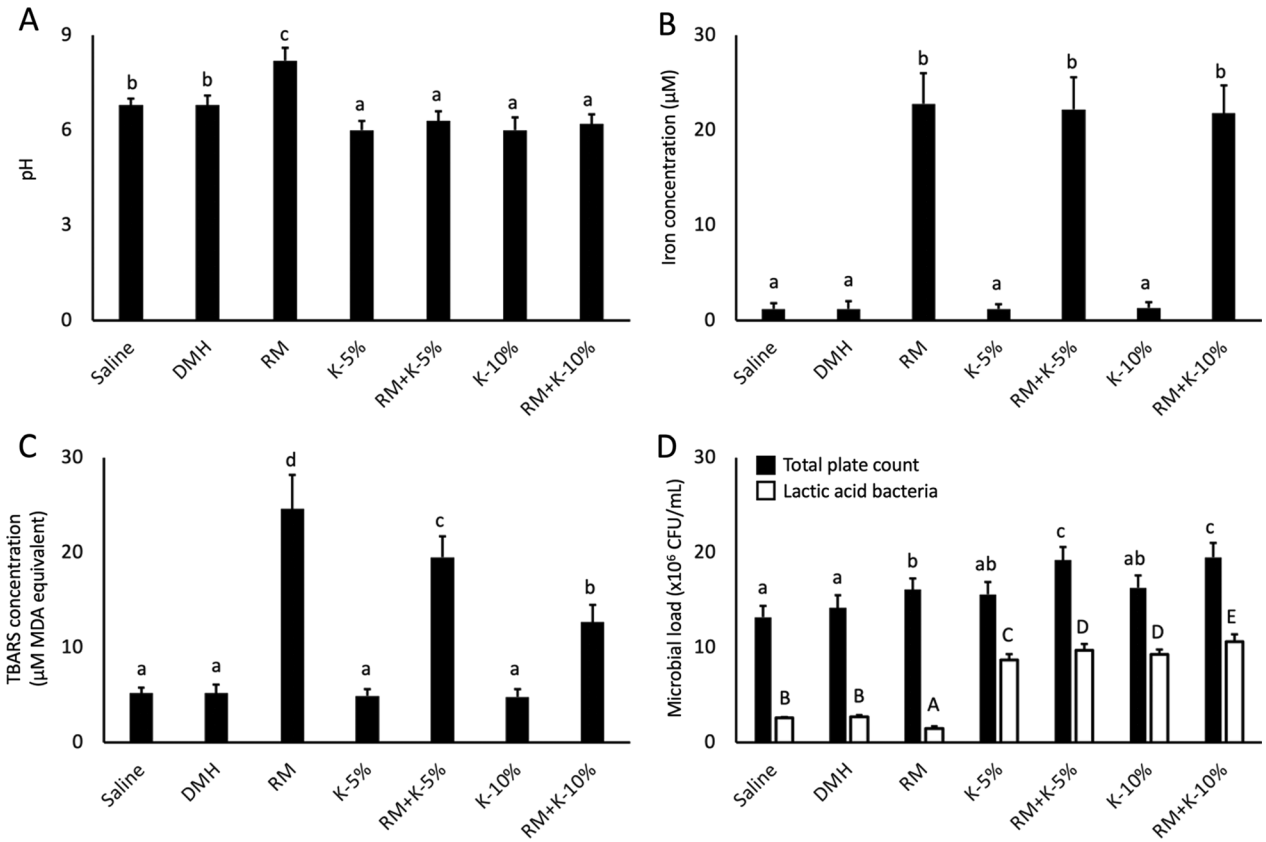

Fig. 2 Characteristics of fecal water extracted from the feces of rats consuming standard diet and red meat-based diet with or without kimchi supplementation. Feces were collected on days 60–64 following the injection of 1,2-dimethylhydrazine (DMH, 20 mg/kg body weight). The characteristics included (**A**) pH, (**B**) iron concentration, (**C**) thiobarbituric acid reactive substances (TBARS) concentration and (**D**) microbial load of fecal water (total plate count and lactic acid bacteria count). Data ($n = 10$) are expressed in mean ± SD. Different lettering indicates statistically significant difference among samples ($p < 0.05$). Saline: group of rats injected with NaCl solution (0.9%) and given standard diet. DMH: group of rats injected with DMH and given standard diet. RM: group of rats injected with DMH and given red meat-based diet. K-5%: group of rats injected with DMH and given standard diet with kimchi supplementation (5%). RM + K-5%: group of rats injected with DMH and given red meat-based diet with kimchi supplementation (5%). K-10%: group of rats injected with DMH and given standard diet with kimchi supplementation (10%). RM + K-10%: group of rats injected with DMH and given red meat-based diet with kimchi supplementation (10%). *MDA* malondialdehyde. *CFU* colony forming unit

load of fecal water, Fig. 2D displays that red meat and kimchi consumption increased the total microorganisms in the fecal water. Red meat intake decreased the LAB present in the fecal water, while kimchi supplementation significantly increased the LAB in the fecal water. The concentration of LAB in the fecal water was in accordance with the pH of fecal water (Fig. 2A). The decrease of fecal LAB in red meat-fed rats was associated with increasing alkalinity of the fecal water. In the fecal water of rats consuming kimchi, the decrease in pH was associated with the increasing LAB count. LAB are able to produce organic acids, such as lactate, acetate, citrate, formate and succinate [27]. An acidic colonic environment could be toxic toward colorectal cancer cells, thus potentially inhibiting colorectal carcinogenesis [50]. In addition, as a fermented food, kimchi is naturally rich in LAB. Some abundant LAB commonly found in kimchi include, but not limited to *Bacillus mycoides, B. subtilis, Lactobacillus brevis, Lb. plantarum, Lb. kimchi, Lactococcus lactis, Lc. carnosum, Leuconostoc mesentroides, Ln. carnosum, Ln. citreum, Ln. kimchi, Serratia marcescens, Weissella cibaria, W. kimchi, W. koreensis* and *W. soli* [51]. Several LAB are also known as probiotics, which are defined as beneficial live microorganisms intended to provide health benefits when consumed. Indeed, kimchi has been considered as a probiotics functional food due to its abundance in LAB [52]. In colorectal carcinogenesis, LAB from fermented food products are able to reduce procarcinogen load in the intestine by

lowering the concentration of enzymes converting procarcinogens into carcinogens, including β-glucosidase, β-glucuronidase, nitroreductase and azoreductase [53].

To study the cellular response upon exposure to fecal matter in the colon, we conducted in vitro experiments using human colonic epithelial cells. The cells were treated with the fecal water of red meat- and kimchi-fed rats. Figure 3 demonstrates the toxicity of fecal water toward human colonic epithelial cells. Fecal water induced oxidation and the formation of reactive oxygen species (ROS) in the cells (Fig. 3A), particularly in the presence of iron from red meat-based diet. Kimchi supplementation lowered the formation of cellular ROS, supposedly due to the antioxidants present in kimchi. The inhibition was stronger when kimchi was supplemented in a higher amount (10%) compared to 5%. In accordance with this finding, the cells exposed to the fecal water of red meat-fed rats with kimchi supplementation exhibited a lower mortality rate compared to those exposed to the fecal water of red meat-fed rats without kimchi supplementation, as shown by the caspase-3 activity and cell viability assay (Fig. 3B, C). The higher kimchi supplementation (10%) gave a better survival compared to the lower kimchi supplementation (5%). Fecal water toxicity toward healthy colon cells was previously reported to correlate with colorectal carcinogenicity [42, 54]. Previous studies also hypothesized that healthy colon cells had a higher sensitivity toward the toxicity of fecal water of heme- and beef-fed rats when compared to precancerous colon cells, thus suggesting a mechanism for colorectal

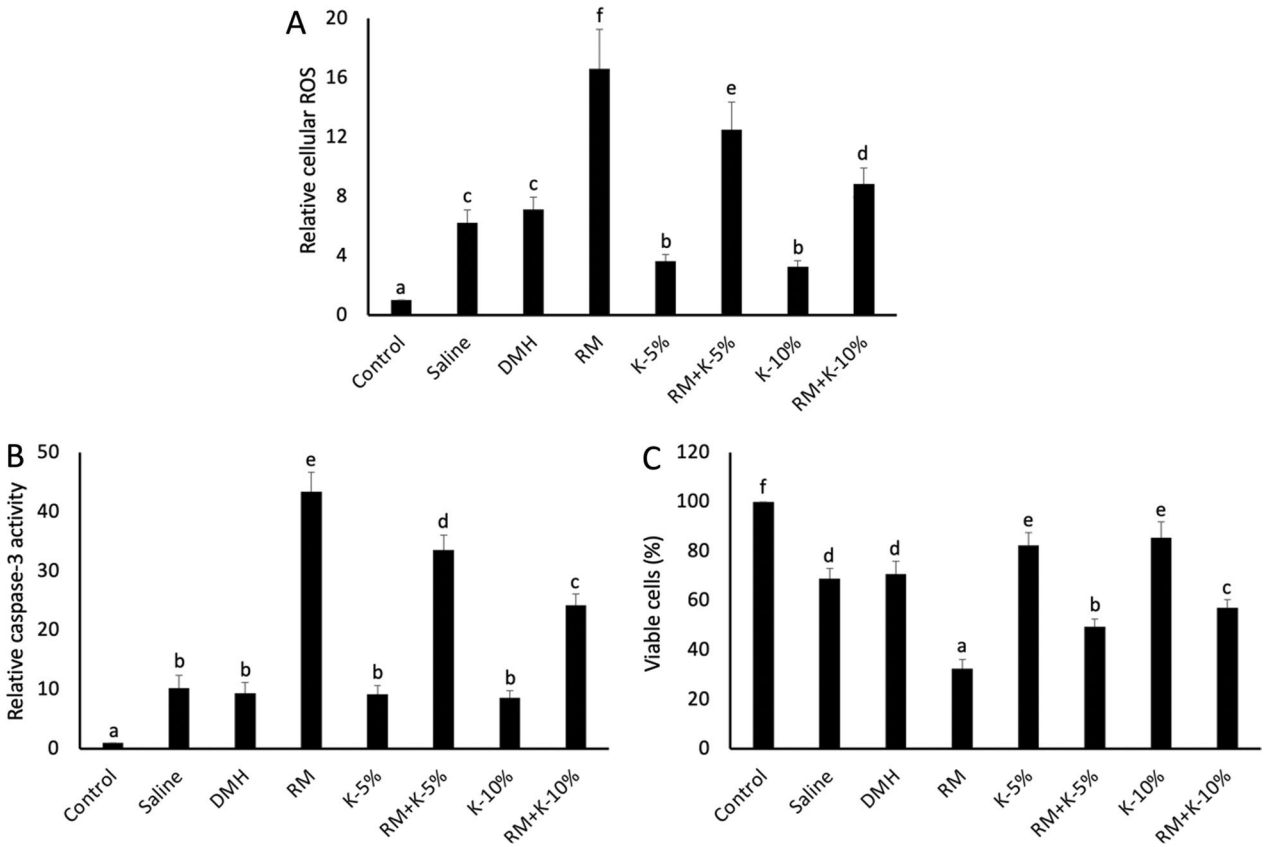

Fig. 3 Effects and toxicity of fecal water toward human colonic epithelial cells. Fecal water was extracted from the feces of rats consuming standard diet and red meat-based diet with or without kimchi supplementation. Feces were collected on days 60–64 following the injection of 1,2-dimethylhydrazine (DMH, 20 mg/kg body weight) and pooled based on the treatment groups. The observed effects of fecal water on the cells included **A** level of cellular reactive oxygen species (ROS), **B** level of caspase-3 activity and **C** cell viability. Data ($n = 5$) are expressed in mean ± SD. Different lettering indicates statistically significant difference among samples ($p < 0.05$). Control: cell medium. Saline: fecal water of rats injected with NaCl solution (0.9%) and given standard diet. DMH: fecal water of rats injected with DMH and given standard diet. RM: fecal water of rats injected with DMH and given red meat-based diet. K-5%: fecal water of rats injected with DMH and given standard diet with kimchi supplementation (5%). RM + K-5%: fecal water of rats injected with DMH and given red meat-based diet with kimchi supplementation (5%). K-10%: fecal water of rats injected with DMH and given standard diet with kimchi supplementation (10%). RM + K-10%: fecal water of rats injected with DMH and given red meat-based diet with kimchi supplementation (10%)

carcinogenesis similar to Darwin's natural selection theory in favor of precancerous cells over normal cells upon red meat consumption [55, 56].

Therefore, it is suggested that kimchi supplementation could protect colon cells from the toxicity of fecal water resulting from red meat-based diet. This latter is characterized by high level of iron and lipid peroxidation by-products as reported in previous studies [25, 27, 35, 36]. The mechanism by which the fecal water is toxic toward colon cells would involve oxidation and the formation of cellular ROS. The protective effects of kimchi in the prevention of colorectal carcinogenicity were associated with the increase in fecal LAB and acidification of fecal matrix.

Modulation of gene expression in the colon cells of red meat- and kimchi-fed rats

To investigate the molecular events that would be involved in red meat-associated colorectal carcinogenesis and the protective effects of kimchi, we analyzed the expression of several genes in the colon of rats following the killing. Those genes included: (1) TNF-α, COX-2 and iNOS involved in inflammation [57], (2) p53 and p21 regulating cell cycle and known as tumor-suppressor genes [58], (3) catalase, SOD-1, SOD-2 and GPx1 expressing cellular antioxidant enzymes [59] and (4) HO-1 involved in heme metabolism [60]. Figure 4 recapitulates the induction or suppression of the genes in the colon of all groups of rats.

The expression of inflammation-related genes (TNF-α, COX-2 and iNOS) increased strongly in the colon of red meat-fed rats (Fig. 4). This suggests that red meat intake provoked inflammatory responses in the colon. Overexpression of these genes was previously reported to increase the risk of colorectal cancer [57]. Kimchi supplementation suppressed the expression of such genes, thus preventing colorectal carcinogenesis by helping reduce the inflammation associated with red meat intake.

The tumor-suppressor protein p53 regulates cell proliferation and induces the activation of p21 (a cyclin-dependent kinase inhibitor) to arrest cell cycle, inhibit abnormal cell proliferation and reduce tumor growth [58]. Kimchi supplementation increased the expression of p53 and p21 in the colon of standard diet-fed rats (Fig. 4), as reported in a previous study [61]. In contrast, red meat intake reduced the expression of p53 and p21, thus suggesting the cancer promoting effects of red meat. Interestingly, kimchi intake along with red meat could restore the expression level of p53 and p21 to a similar level as shown in the colon of standard diet-fed rats.

Catalase, SOD and GPx are the main antioxidant enzymes in the cell first-line antioxidant defense system against oxidative insults [62]. Red meat intake was shown

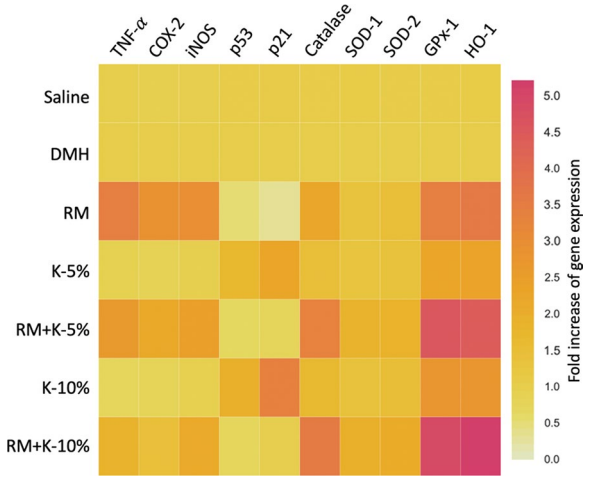

Fig. 4 Heatmap representing relative expression level of genes in the colon of rats consuming standard diet and red meat-based diet with or without kimchi supplementation. The colons were collected 120 days following the injection of 1,2-dimethylhydrazine (DMH, 20 mg/kg body weight). Gene expression in all groups was normalized relatively to DMH group. Saline: group of rats injected with NaCl solution (0.9%) and given standard diet. DMH: group of rats injected with DMH and given standard diet. RM: group of rats injected with DMH and given red meat-based diet. K-5%: group of rats injected with DMH and given standard diet with kimchi supplementation (5%). RM + K-5%: group of rats injected with DMH and given red meat-based diet with kimchi supplementation (5%). K-10%: group of rats injected with DMH and given standard diet with kimchi supplementation (10%). RM + K-10%: group of rats injected with DMH and given red meat-based diet with kimchi supplementation (10%). *TNF-α* Tumor Necrosis Factor alpha, *COX-2* Cyclooxygenase-2, *iNOS* inducible Nitric Oxide Synthase 2, *SOD-1* Superoxide Dismutase-1, *SOD-2* Superoxide Dismutase-2, *GPx-1* Glutathione Peroxidase-1, *HO-1* Heme Oxygenase-1

to induce the expression of catalase, SOD-1, SOD-2 and GPx1 in the colon of rats (Fig. 4), as observed previously [27, 58]. We suggest that the expression of these enzymes was promoted due to the presence of iron (Fig. 2B) and the ROS formation (Fig. 3A) associated with red meat intake. The colon cells would respond to oxidative insults by up-regulating the expression of antioxidant enzymes. Kimchi intake potentiated the expression of antioxidant enzymes in rats consuming red meat-based diet. The expression of antioxidant enzymes is regulated by the nuclear factor erythroid 2-related factor 2 (Nrf2), a transcription factor that regulates the cellular defense through the expression of genes involved in oxidative stress response and drug detoxification [63]. We hypothesized that Nrf2 could be involved in the antioxidant response observed in the colon cells of red meat- and kimchi-fed rats. To support our hypothesis, we show in Fig. 4 that the expression of HO-1, an antioxidant enzyme regulated by Nrf2 [63], was also induced by red meat and kimchi intake. HO-1 could be involved in the

heme detoxification in the colon cells by degrading heme from red meat to free iron, carbon monoxide and biliverdin [64].

Conclusions

Taken together, our findings in this study highlighted the potential of kimchi in preventing the promotion of colorectal carcinogenesis by red meat intake. Such a chemopreventive potential of kimchi was associated with acidification of fecal matrix, increase in fecal lactic acid bacteria (LAB), limitation of cellular reactive oxygen species (ROS) formation, reduction of fecal water toxicity toward colon cells, down-regulation of the expression of proinflammatory genes and up-regulation of the expression of tumor-suppressor genes and cellular antioxidant enzymes. Therefore, we drew a conclusion that kimchi possesses antioxidant activity that could reduce the formation of toxic compounds in feces and stimulate the expression of antioxidant enzymes in colon cells. Lactic acid bacteria (LAB) present in kimchi would also contribute in the prevention of colorectal cancer promotion by producing organic acids that acidify fecal matter, thus creating an environment that support the inhibition of cancer cell growth.

This study provides novel insights into the health benefits of kimchi, particularly regarding its anticancer properties. For medical practitioners, this study suggests a possibility for public health recommendation regarding regular kimchi consumption to prevent colorectal cancer related to high red meat intake. For ethnic food practitioners, this study gives strong evidence for further development of kimchi as functional food. As limitation, this study focused only on the role of kimchi consumption in the early development of colorectal cancer promoted by red meat, not on the later stage of colorectal cancer where the cancer would be fully developed. In addition, it is noteworthy that there are very few studies about the role of kimchi in colorectal cancer that could support our study. For further studies, we suggest to analyze the chemopreventive potential of kimchi during the later stage of colorectal cancer. To study the mechanisms by which kimchi could inhibit the promotion of colorectal cancer, an in vivo study can be performed using a precancerous colon cell line. It would also be interesting to investigate whether the probiotics in kimchi are involved in the chemopreventive potential of kimchi against the promotion of colorectal cancer associated with red meat intake.

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Author contributions
RS, ES, JSO and RB collaborated in the project and conceptualized the study. RS, ES, AR and DN were involved in data collection and data analysis. WPR, NK and RB helped in manuscript review and editing. RS, ES and DN were the principal writers of this manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The data and materials related to this study are available upon request.

Declarations

Ethics approval and consent to participate
The protocol for animal experiments in this study has been approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (Jakarta, Indonesia).

Consent for publication
All the authors have read and approved the content of this manuscript for a publication.

Competing interests
The authors declare no competing interests.

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