Biochemical Pharmacology 182 (2020) 114279

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

17β-Estradiol strongly inhibits azoxymethane/dextran sulfate sodium-induced colorectal cancer development in Nrf2 knockout male mice

Chin-Hee Song [a], Nayoung Kim [a,b,*], Ryoung Hee Nam [a], Soo In Choi [a], Joo Hee Son [a], Jeong Eun Yu [a], Eun Shin [c], Ha-Na Lee [d], Do-Hee Kim [e], Young-Joon Surh [e]

[a] Department of Internal Medicine, Seoul National University Bundang Hospital, Seongnam, Gyeonggi-do, South Korea
[b] Department of Internal Medicine and Liver Research Institute, Seoul National University College of Medicine, Seoul, South Korea
[c] Department of Pathology, Hallym University Dongtan Sacred Heart Hospital, Hwaseong, Gyeonggi-do, South Korea
[d] Laboratory of Immunology, Division of Biotechnology Review and Research-III, Office of Biotechnology Products, Center for Drug Evaluation and Research, Food and Drug Administration, Silver Spring, MD 20993, USA
[e] Tumor Microenvironment Global Core Research Center, Seoul National University College of Pharmacy, Seoul, South Korea

ARTICLE INFO

Keywords:
Nrf2 knockout
AOM/DSS mouse model
17β-Estradiol
Colitis-associated colon cancer
Colitis

ABSTRACT

Nuclear factor erythroid 2-related factor 2 (Nrf2) has dual effects on inflammation and cancer progression depending on the microenvironment. Estrogens have a protective effect on colorectal cancer (CRC) development. The aim of this study was to investigate CRC development in Nrf2 knockout (KO) mice. Azoxymethane (AOM) and dextran sulfate sodium (DSS)-treated wild-type (WT) and Nrf2 KO male mice were sacrificed at weeks 2 and 16 after AOM injection with/without 17β-estradiol (E2) treatment during week 1. Disease activity index and colon tissue damage at week 2 showed strong attenuation following E2 administration in WT mice but to a lesser extent in Nrf2 KO male mice. At week 16, E2 significantly diminished AOM/DSS-induced adenoma/cancer incidence at distal colon in the Nrf2 KO group, but not in the WT. Furthermore, mRNA or protein levels of NF-κB-related mediators (i.e., iNOS, TNF-α, and IL-1β) and Nrf2-related antioxidants (i.e., NQO1 and HO-1) were significantly lower in the Nrf2 KO group regardless of E2 treatment compared to the WT. The expression of estrogen receptor beta (ERβ) was higher in the Nrf2 KO group than in the WT. In conclusion, estrogen further inhibits CRC by upregulating ERβ-related alternate pathways in the absence of Nrf2.

1. Introduction

Colorectal cancer (CRC) is estimated to be the third leading cause of cancer-related death in the United States in 2020 [1]. In Korea, the incidence rate of CRC is higher in men than in women in all age groups, although CRC remains a main cause of death in elderly women over 65 years of age [2]. Generally, colon tumors develop more frequently in men than in women [3], and CRC shows sex-specific differences worldwide [4]. Azoxymethane (AOM) and dextran sulfate sodium (DSS) treatments [5–7] are the most widely used protocols for the establishment of animal models of colon carcinogenesis [8–10] because they result in the well-developed multistep colon carcinogenesis based on the aberrant crypt foci (ACF)-adenoma-carcinoma sequence with the relevant molecular alterations [9]. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor that consists of a basic leucine zipper protein that regulates adaptive cellular defense mechanism in response to various stimuli such as oxidative, proteotoxic, and metabolic stresses as well as inflammation [11,12]. Under stress conditions, Nrf2 becomes dissociated from Kelch-like ECH-associated protein 1 (KEAP1, a negative regulator of Nrf2 in the cytoplasm), followed by translocation into the nucleus [11], binds to a consensus antioxidant response element (ARE) binding sequence (5′-TGACnnnGC-3′), and regulates antioxidant and phase II carcinogen detoxifying enzymes, such as heme oxygenase-1 (HO-1), nicotinamide adenine dinucleotide phosphate: quinone dehydrogenase 1 (NQO1), glutamate-cysteine ligase catalytic subunit (GCLC), and glutamate-cysteine ligase modifier subunit (GCLM) [13]. The activation of these enzymes maintains cellular homeostasis, and exerts anti-inflammatory and anti-tumorigenic activity [14,15]. Furthermore, anti-inflammatory mechanisms of Nrf2 include suppression of lipopolysaccharide (LPS)-induced expression of proinflammatory

* Corresponding author at: Department of Internal Medicine, Seoul National University Bundang Hospital, 82 Gumi-ro, 173 beon-gil, Bundang-gu, Seongnam-si, Gyeonggi-do 13620, South Korea.
E-mail addresses: nakim49@snu.ac.kr, nayoungkim49@empas.com (N. Kim).

https://doi.org/10.1016/j.bcp.2020.114279
Received 24 July 2020; Received in revised form 18 September 2020; Accepted 7 October 2020
Available online 15 October 2020

cytokines such as Interleukin (IL)-6 and IL-1β [16], and reduction of DSS-induced expression of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and IL-6 through blocking of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling [17].

Recently, emerging evidences have revealed the dual effect of Nrf2 as anti-carcinogenic and pro-carcinogenic. Activated Nrf2 not only protects normal cells from transforming into cancer cells, but also promotes the survival of cancer cells under detrimental conditions [18,19]. In addition, *meta*-analysis showed that cancer patients with high Nrf2 expression exhibit worse clinical outcomes compared to patients with low Nrf2 expression [20]. Nrf2 overexpression is associated with chemotherapy resistance in many cancers including gastric cancer [21]. Furthermore, overexpression of HO-1 and NQO1 (Nrf2 target genes) has been reported in prostate [22] and colon cancers [23], respectively. Nrf2 KO ($Nrf2^{-/-}$) and WT ($Nrf2^{+/+}$) male and female AOM/DSS mouse models represent a useful tool to clarify the role of Nrf2 in colitis and CRC development.

The protective role of estrogen in colon carcinogenesis [24,25], neurodegenerative diseases [26], and cardiovascular diseases [27] has been reported in both males and females. The administration of 17β-estradiol (E2) in an ovariectomized retinal degeneration model reduces reactive oxygen species (ROS) production through Nrf2 activation mediated by the PI3K/AKT- and estrogen receptor (ER)-dependent pathways [28]. In addition, phytoestrogens (such as resveratrol and quercetin) and ER agonists (such as genistein) modulate the activity of NF-κB and Nrf2 [29]. Resveratrol induces P53 expression and cleavage

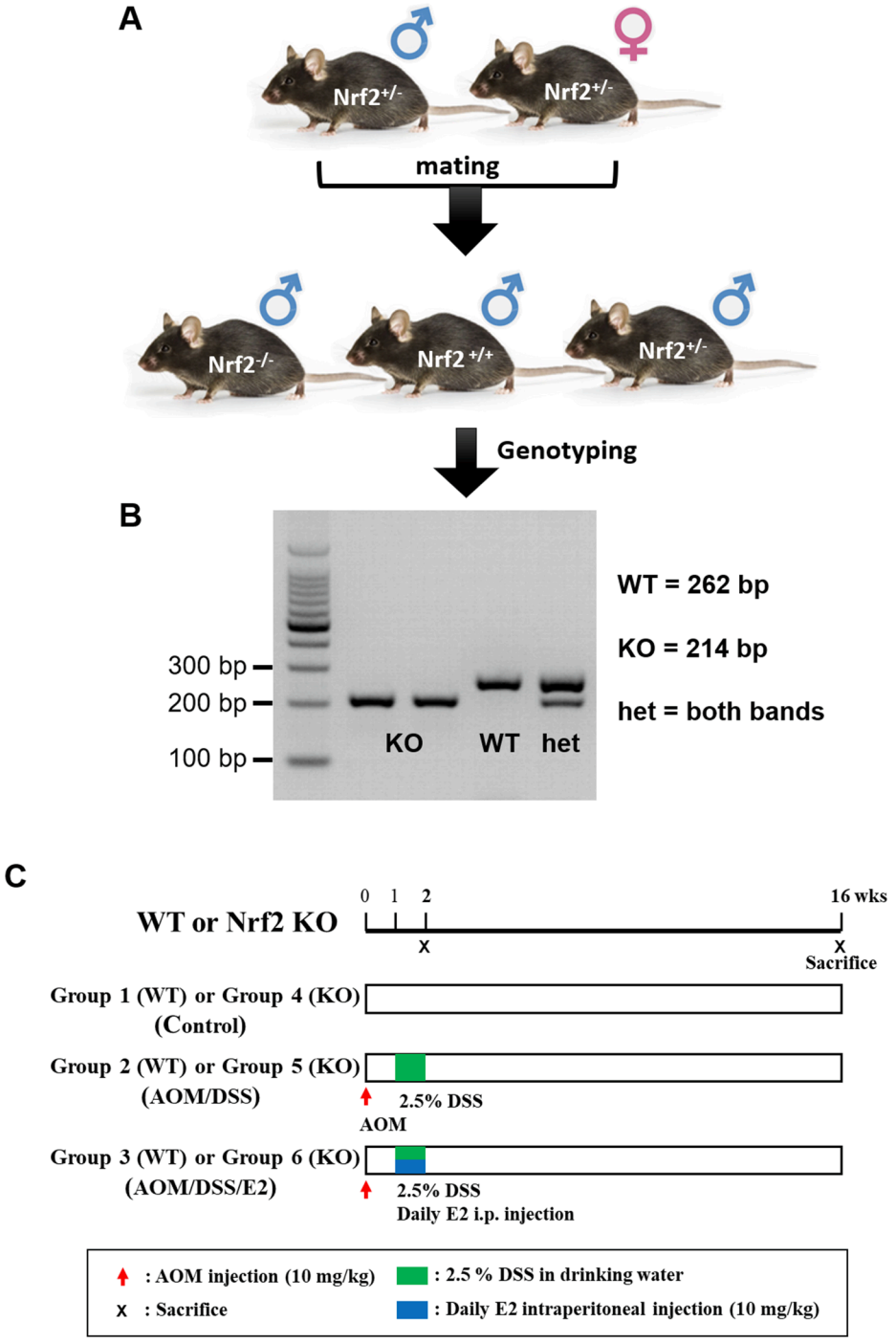

Fig. 1. Selection of Nrf2 KO mice using genotyping and a schematic illustration of the experiment. (A) Heterogeneous Nrf2 KO ($Nrf2^{+/-}$) male and female mice were mated and then homogeneous Nrf2 KO ($Nrf2^{-/-}$) male and female mice were selected. (B) Genotyping of WT and Nrf2 KO mice. Representative agarose gel showing the PCR products. Nrf2 WT allele produces a 262-bp band, whereas the targeted allele produces a 214-bp band. Heterogeneous Nrf2 KO allele produces both, 214-bp and 262-bp bands. (C) Experimental scheme. WT and Nrf2 KO male mice were used in the AOM/DSS-induced colitis-associated CRC protocol. AOM (10 mg/kg) was injected to the mice on Day 0. One week after, DSS (2.5%) was provided in the drinking water for one week. E2 (10 mg/kg) was administrated by daily intraperitoneal injection for one week during DSS treatment. The mice were sacrificed at week 2 and 16 after AOM injection. ♂, male; ♀, female; +/+ and WT, wild-type; +/- and het, heterogeneous knockout; -/- and KO, homogeneous knockout; AOM, azoxymethane; DSS, dextran sodium sulfate; E2, 17β-estradiol.

of PARP in an Nrf2-dependent manner in MCF-7 breast cancer cells [30]. We previously reported that E2 (10 mg/kg) suppresses the initiation of CRC by activating Nrf2 in AOM/DSS-treated male mice although Nrf2 promotes CRC at the tumorigenesis stage [31]. In addition, E2 (10 nM) showed anti-inflammatory effects by inhibiting NF-κB and COX-2, and by increasing antioxidant proteins in TNF-α-treated CCD841CoN cells, originating from normal human female epithelial cells [32]. We further verified the role of E2 (10 nM) as anti-inflammatory agent through activation of Nrf2 in mouse embryonic fibroblasts (MEF). In WT MEFs, E2 (10 nM) inhibited TNF-α-induced nuclear translocation of NF-κB and the expression of its target protein inducible Nitric Oxide Synthase (iNOS). However, its effect was diminished in Nrf2 KO MEFs [33]. Based on these data, we hypothesized that the anti-CRC activity of E2 may be altered in the absence of Nrf2. To validate the hypothesis, we investigated the development of colitis and colitis-associated CRC in AOM/DSS-treated Nrf2 KO and WT male mice.

2. Materials and methods

2.1. Chemicals

AOM (Cat no A5486) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and stored at −20 °C. AOM stock solution was prepared after dissolving of 10 mg AOM in 10 mL Phosphate-buffered saline (PBS), pH 7.4 (Cat no 10010-023, Gibco BRL, Gaithersburg, MD, USA). DSS (36,000–50,000 M.W, MP grade, Cat no 160110) was purchased from MP Biomedicals (Aurora, OH, USA) and stored at room temperature. 2.5% DSS solution was prepared after dissolving of 25 g DSS into 1 L water. E2 (Cat no E8876) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and stored at room temperature. E2 solution was prepared after dissolving in olive oil (Cat no O1514, Sigma-Aldrich, St. Louis, MO, USA).

2.2. Animals and genotyping

Heterozygous Nrf2 KO ($Nrf2^{+/-}$) mice in a C57BL6/129SV background [34] were kindly provided by Prof. Y-J Surh. WT ($Nrf2^{+/+}$) and homozygous Nrf2 KO ($Nrf2^{-/-}$) mice were obtained by crossing the Nrf2 heterozygous ($Nrf2^{+/-}$) mice (Fig. 1A). The mice were housed in cages at 23 °C with a 12/12-hour light/dark cycle under specific pathogen-free conditions. Genomic DNA (gDNA) obtained using DNeasy® Blood & Tissue Kit (Qiagen, Gmbh, Hilden, Germany) was used as the template DNA (listed in Table 1) and visualized under a ChemiDoc™ Imaging System (Bio-Rad Laboratories, Inc.) (Fig. 1B). All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the Seoul National University Bundang Hospital (BA1705-223/043-01) and performed in accordance with the ARRIVE (Animals Research: Reporting *In Vivo* Experiments) protocol.

Table 1
List of oligonucleotide sequence and their characteristics.

Gene	Sequence (5′→3′)	Purpose
Nrf2 WT	F: GGA ATG GAA AAT AGC TCC TGC C R: GCC TGA GAG CTG TAG GCC	Genotyping
Nrf2 KO	R: GGG TTT TCC CAG TCA CGA	Genotyping
iNOS	F: TGG TGG TGA CAA GCA CAT TT R: AAG GCC AAA CAC AGC ATA CC	qRT-PCR
TNF-α	F: ACG GCA TGG ATC TCA AAG AC R: GTG GGT GAG GAG CAC GTA GT	qRT-PCR
NQO1	F: GCG AGA AGA GCC CTG ATT GTA CTG R: TCT CAA ACC AGC CTT TCA GAA TGG	qRT-PCR
HO-1	F: CCT CAC TGG CAG GAA ATC ATC R: CCT CGT GGA GAC GCT TTA CAT A	qRT-PCR
GAPDH	F: TTC ACC ACC ATG GAG AAG GC R: GGC ATG GAC TGT GGT CAT GA	qRT-PCR

Nrf2, nuclear factor erythroid-derived 2-related factor 2; WT, wild type; KO, knockout; F, forward; R, reverse; iNOS, inducible nitric oxide synthase; TNF-α, tumor necrosis factor-alpha; NQO1, NAD(P)H dehydrogenase (quinone) 1; HO-1, heme oxygenase 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

2.3. Experimental design

WT and Nrf2 KO male mice were randomized into the following three groups each. Group 1: WT control mice, Group 2: AOM/DSS-treated WT mice, Group 3: AOM/DSS-treated WT mice administrated with E2, Group 4: Nrf2 KO control mice, Group 5: AOM/DSS-treated Nrf2 KO mice, and Group 6: AOM/DSS-treated Nrf2 KO mice administrated with E2. The number of mice of each group was 9–12. For the induction of colitis, 2.5% (w/v) DSS was supplied in the drinking water for 7 days, one week following the injection of AOM (10 mg/kg) counted as Day 0 [35]. E2 was dissolved in olive oil, and administered once daily as an intraperitoneal injection for 7 days (E2-supplemented mice), while olive oil was utilized on all other groups. The animals were euthanized by CO_2 asphyxiation at week 2 and 16 after AOM injection (Fig. 1C).

2.4. Evaluation of clinical symptoms

Clinical symptoms were evaluated using the disease activity index (DAI), which includes loss of body weight, stool characterization, and hematochezia [36,37]. The DAI was scored by two researchers in a blinded manner.

2.5. Enumeration of lesions

The colons were opened longitudinally, and stool was washed out with phosphate-buffered saline (PBS). The length of the colon was measured from the cecum to the rectum using a ruler. Polypoid lesions with a diameter $\leq$2 mm or >2 mm were independently counted by two researchers in a blinded manner [36,37].

2.6. Tissue processing and histopathology

The extracted colon was divided into proximal and distal portions. The proximal colon up to 1.5 cm from the ileocecal valve, the rectum up to 1.5 cm from the anal verge, and colonic segments containing any gross polyps were fixed with phosphate-buffered formalin and embedded in paraffin. The sections (5 mm) were stained with hematoxylin and eosin (H&E). The classification of adenoma and adenocarcinoma, and the specification of the depth of adenocarcinoma invasion into the colonic tissues as mucosal or submucosal invasion was performed in a blinded manner [38]. The histological severity was assessed using a microscopic damage score reflecting colonic epithelial damage and depth of inflammatory cell infiltration [39].

2.7. Measurement of inflammatory cytokines

The level of myeloperoxidase (MPO), IL-6, and IL-1β in the colonic tissues was measured using a mouse MPO enzyme linked immunosorbent assay (ELISA) kit (HK210, Hycult Biotechnology, The Netherlands), a mouse IL-6 Quantikine ELISA kit (R&D Systems Inc., Minneapolis, MN), and a mouse IL-1β/IL-1F2 Quantikine ELISA kit (R&D Systems Inc., Minneapolis, MN), respectively, according to the manufacturer's instructions. All assays were performed in triplicate.

2.8. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from the colon tissues using TRIzol® reagent (Invitrogen, Carlsbad, CA, USA). For qRT-PCR, 2 μg of total RNA was reverse transcribed using High Capacity cDNA Reverse Transcription kit according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). The cDNA was used to perform qRT-PCR using specific primers (listed in Table 1) in a ViiA7 instrument (Applied Biosystems, Foster City, CA, USA). The expression levels were

normalized to that of GAPDH.

2.9. Western blot analysis

Colon tissue was lysed with RIPA buffer (Cell Signaling Technology, Beverly, MA, USA) containing protease and phosphatase inhibitors. Total protein was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene difluoride (PVDF) membrane (Cat no 10600023, GE Healthcare Life science, Germany). Western blot analysis was performed with specific primary antibodies (listed in Table 2). The signals were then detected with an enhanced chemiluminescence (ECL) kit (GE Healthcare Biosciences, UK). The band intensity was quantified by densitometric analysis using the ImageJ software (National Institutes of Health, Bethesda, MD).

2.10. Statistical analysis

Data are expressed as the mean ± standard error of mean (SEM). Statistical significance was examined with the Mann-Whitney test or Fisher's exact test. $P < 0.05$ was considered statistically significance. All statistical analyses were conducted using GraphPad Prism (GraphPad) and SPSS version 18.0 (SPSS Inc.).

3. Results

3.1. Nrf2 determines the differential effect of E2 on DAI score during inflammation and tumorigenesis.

DAI score parameters including body weight changes, stool consistency, and hematochezia were measured every week (Fig. 2A and D). Loss of body weight is a sensitive indicator of the severity of colitis [40]. The AOM/DSS-induced reduction in body weight was not reversed by E2 treatment in both WT and Nrf2 KO mice at week 2 or 16 (Fig. 2B and 2C). There was no significant difference in the DAI score between control and the E2 supplemented groups in both WT and Nrf2 KO mice (Fig. 2E). However, the inhibitory effect of E2 on DAI score was observed at week 16, which was stronger in the Nrf2 KO group than in the the WT group (Fig. 2F), although the loss of body weight was significant in Nrf2 KO compared to the WT (Fig. 2C) at week 16.

3.2. Nrf2 is critical for E2-induced suppression of colitis at week 2.

Representative histopathological images (Fig. 3A) and microscopic damage scores (Fig. 3B) showed that the E2-treated AOM/DSS group had significantly less inflammatory cell infiltration and milder cryptic damage than AOM/DSS group in both WT and Nrf2 KO mice. This inhibitory effect of E2 was significantly weaker in the Nrf2 KO group than in the WT group (Fig. 3B). Next, we performed ELISA to measure the level of MPO. At week 2, the AOM/DSS-mediated enhancement of the MPO level was significantly decreased following E2 administration, both in the WT and Nrf2 KO group. However, the inhibitory effect of E2 in the Nrf2 KO group showed only a tendency to decrease compared with WT, but there was no significance (Fig. 3C). These results indicate that the anti-inflammatory effect of estrogen is mediated through Nrf2 at the colitis stage.

Table 2
List of antibodies and their characteristics.

Antigen	Antibody	Dilution
ERβ	Abcam (ab3576)	WB (1:500)
β-Actin	Santa Cruz Biotechnology (sc-47778)	WB (1:1000)

WB, Western blot; ERβ, estrogen receptor beta; β-Actin, beta-actin.

3.3. E2 strongly attenuates colitis-associated tumorigenesis in Nrf2 KO mice at week 16

The tumors that developed in the distal region of colon following AOM/DSS treatment were strongly diminished following E2 administration regardless of the polyp size. However, the anti-tumorigenic effect of E2 was more significant in the Nrf2 KO group compared to the WT group (Fig. 4A). Representative histopathological images are shown in Fig. 4B. Table 3 summarizes the incidence of microscopic colonic neoplasms. Similar to tumor count results, AOM/DSS-mediated increment in tumor incidence rate, specifically that of mucosal invasive adenocarcinoma, at distal region of colon was sharply decreased by E2 supplementation in Nrf2 KO, but not in WT mice (Fig. 4C). These results indicate that the anti-tumorigenic effect of estrogen is more powerful in the absence of Nrf2.

3.4. Nrf2 deficiency enhances the inhibitory effect of E2 on proinflammatory gene expression at week 16

At week 2, AOM/DSS-induced mRNA expression of iNOS and TNF-α decreased following E2 administration both in WT and Nrf2 KO mice (Fig. 5A). Next, we performed ELISA to measure the concentration of the proinflammatory cytokine IL-1β. The elevation in the colonic IL-1β level following AOM/DSS treatment was significantly inhibited by E2 administration in both WT and Nrf2 KO mice to a similar extent (Fig. 5B). Surprisingly, the AOM/DSS-induced IL-1β level was strongly enhanced in the Nrf2 KO mice compared to the WT mice (Fig. 5B). Furthermore, the inhibitory effect of E2 on TNF-α and IL-1β production by AOM/DSS treatment was significantly weakened in Nrf2 KO compared with WT (Fig. 5A and 5B). At week 16, the elevated mRNA expression of iNOS and TNF-α by AOM/DSS treatment was significantly decreased in the Nrf2 KO group and further inhibited by E2 treatment (Fig. 5C). Furthermore, AOM/DSS-induced IL-1β level was significantly reduced by E2 treatment in Nrf2 KO group, but not in the WT group. (Fig. 5D).

3.5. Effect of E2 on Nrf2-mediated antioxidant enzyme genes during colitis and carcinogenesis in Nrf2 KO mice at week 2 and 16

We measured mRNA expression of the antioxidant enzyme genes using qRT-PCR at week 2 and week 16. At week 2, mRNA expression of NQO1 and HO-1 was strongly increased by E2 administration in WT, but not in Nrf2 KO mice (Fig. 6A). At week 16, AOM/DSS-induced NQO1 and HO-1 mRNA levels were significantly decreased by E2 supplementation in WT mice (Fig. 6B). However, in the Nrf2 KO group, the AOM/DSS-induced mRNA expression was significantly decreased compared to the WT group and further inhibited following E2 supplementation (Fig. 6B).

We previously investigated the molecular link between Nrf2 and estrogen signaling. In normal colonic epithelial CCD841CoN cells, ERβ expression was increased by E2 treatment, but not ERα [32]. The inhibitory effect of E2 on TNF-α-induced COX-2 or iNOS expression was removed by ERβ specific antagonist PHTPP (4-(2-phenyl-5,7-bis(trifluoromethyl) pyrazolo(1,5-a)pyrimidin-3-yl)phenol) treatment in CCD841CoN cells [32] and WT MEFs [33]. However, in Nrf2 KO MEFs, TNF-α-induced iNOS expression was not changed by E2 with or without PHTPP treatment [33]. Furthermore, in ovariectomized (OVX) female mice, during carcinogenesis stage at weeks 16, AOM/DSS-induced decrement of ERβ protein expression was increased by E2 supplementation, but not ERα [41]. In this regard, to determine the molecular link between Nrf2 and estrogen signaling, we analyzed the protein expression of estrogen receptor beta (ERβ), which is a member of the nuclear steroid receptor superfamily. At week 16 of cancer development, AOM/DSS-mediated decrease in ERβ protein expression was not rescued by E2 treatment in the WT group (Fig. 6C). Interestingly, E2-mediated induction of ERβ protein expression was stronger in Nrf2 KO mice than in WT

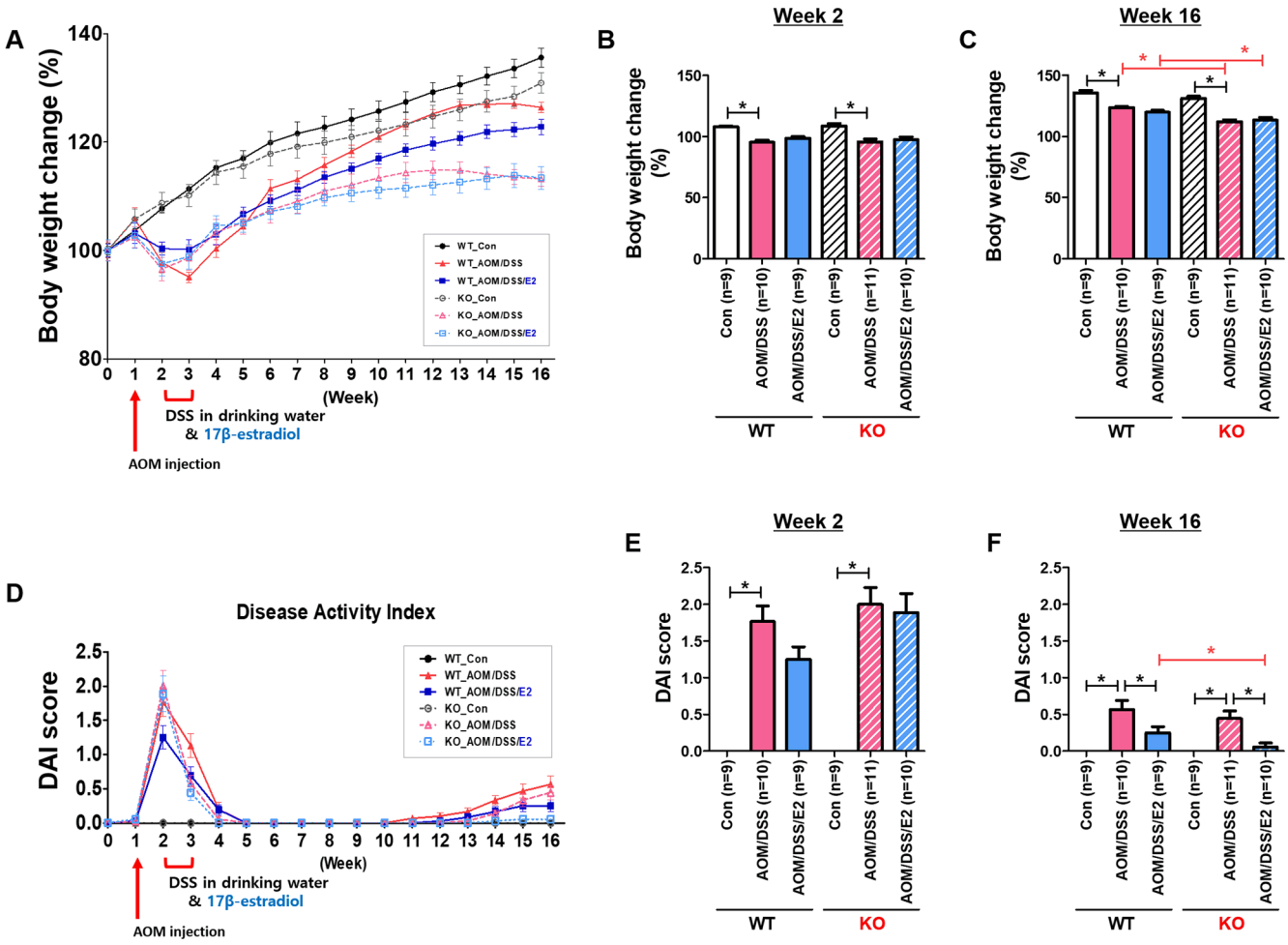

Fig. 2. Effect of E2 *via* Nrf2 on phenotypic changes of colitis. (A-C) Body weight during the experimental period (A), at week 2 (B), and at week 16 (C). (D-F) DAI score during experimental period (D), at week 2 (E), and at week 16 (F). Con, male control mice; AOM/DSS, AOM and DSS-treated male mice; AOM/DSS/E2, AOM, DSS, and 17β-estradiol-treated male mice. *$p < 0.05$ between two groups. WT, wild-type; KO, homogeneous knockout; AOM, azoxymethane; DSS, dextran sodium sulfate; E2, 17β-estradiol; DAI, disease activity index.

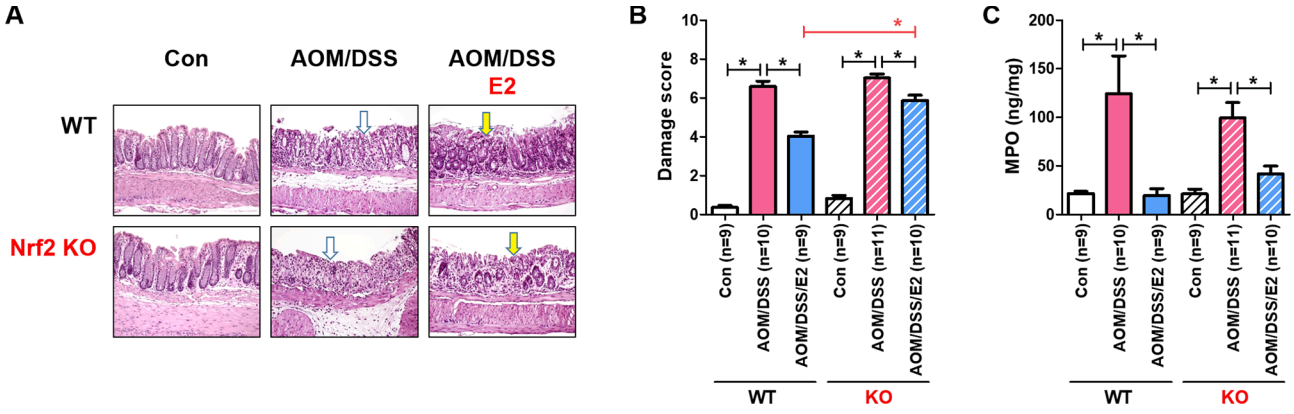

Fig. 3. Inhibitory effect of E2 *via* Nrf2 on the colitis indices. (A) Weakened inhibitory effect of E2 on AOM/DSS-treated enhanced inflammatory cell infiltration in Nrf2 KO male mice. Representative H&E staining images of colon tissues at week 2. Magnification, ×100. The crypt within colon tissues was normal in WT and Nrf2 KO control mice. However, crypt loss and strong inflammatory cell infiltration within the colon tissues (white arrow) were observed in both AOM/DSS-treated WT and Nrf2 KO male mice. In the WT group, AOM/DSS-induced histologic damage was inhibited by E2 supplementation, with only mild erosion (yellow arrow) observable. However, the inhibition was weakened in Nrf2 KO mice than in WT. (B) The inhibitory effect of E2 on the microscopic damage score was weakened in AOM/DSS-induced Nrf2 KO male mice. (C) The AOM/DSS-mediated increased MPO level in colonic tissues was inhibited following administration of E2 in both WT and Nrf2 KO male mice. Con, male control mice; AOM/DSS, AOM and DSS-treated male mice; AOM/DSS/E2, AOM, DSS, and 17β-estradiol-treated male mice. *$p < 0.05$ for comparison between two groups. WT, wild-type; KO, homogeneous knockout; AOM, azoxymethane; DSS, dextran sodium sulfate; E2, 17β-estradiol; MPO, myeloperoxidase. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

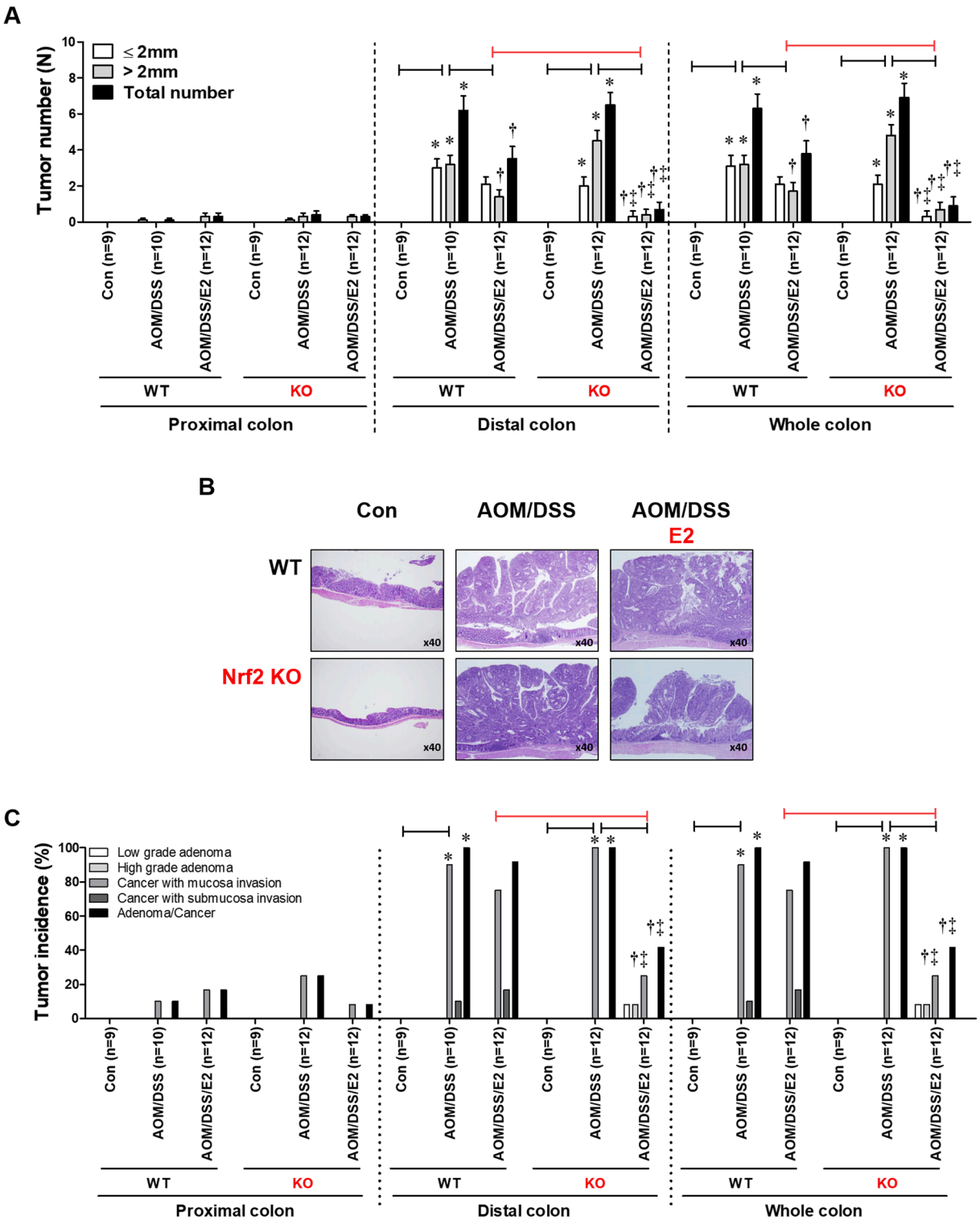

Fig. 4. Effect of E2 *via* Nrf2 on the colon tumor multiplicity at weeks 16. (A) Average tumor numbers and size distribution in the proximal, distal, and whole colon in each group sacrificed at week 16 following AOM injection. (B) Representative H&E staining images at week 16. (C) Quantification of adenoma/adenocarcinoma incidence and invasion in each group by microscopic evaluation of the colonic tissues at the carcinogenesis step (at week 16). Con, male control mice; AOM/DSS, AOM and DSS-treated male mice; AOM/DSS/E2, AOM, DSS, and 17β-estradiol-treated male mice. *$p < 0.05$ for Con. vs. AOM/DSS; †, $p < 0.05$ for AOM/DSS vs. AOM/DSS/E2; ‡, $p < 0.05$ for WT vs. Nrf2 KO. WT, wild-type; KO, homogeneous knockout; AOM, azoxymethane; DSS, dextran sodium sulfate; E2, 17β-estradiol.

mice (Fig. 6C).

4. Discussion

Our results showed strong attenuation of the disease activity index and colon tissue damage by E2 in WT mice at week 2 and to a lesser extent in the Nrf2 KO male mice. However, at week 16, E2 significantly diminished AOM/DSS-induced adenoma/cancer incidence in the distal colon in the Nrf2 KO group, but not in the WT, suggesting differential roles of Nrf2 during inflammation and carcinogenesis stages. In

Table 3
Incidence of adenoma and cancer in the colon.

	WT male				KO male				WT vs KO
	Con (n = 9)	AOM/DSS (n = 10)	AOM/DSS/E2 (n = 12)	*p*-value[a] *p*-value[b]	Con (n = 9)	AOM/DSS (n = 10)	AOM/DSS/E2 (n = 12)	*p*-value[a] *p*-value[b]	*p*-value[c] *p*-value[d]
Proximal colon									
Low grade adenoma incidence	0.0	0.0	0.0	1.000	0.0	0.0	0.0	1.000	1.000
	(0/9)	(0/10)	(0/12)	1.000	(0/9)	(0/12)	(0/12)	1.000	1.000
High grade adenoma incidence	0.0	0.0	0.0	1.000	0.0	0.0	0.0	1.000	1.000
	(0/9)	(0/10)	(0/12)	1.000	(0/9)	(0/12)	(0/12)	1.000	1.000
Cancer with mucosa invasion	0.0	10.0	16.7	1.000	0.0	25.0	8.3	0.229	0.594
	(0/9)	(1/10)	(2/12)	1.000	(0/9)	(3/12)	(1/12)	0.590	1.000
Cancer with submucosa invasion	0.0	0.0	0.0	1.000	0.0	0.0	0.0	1.000	1.000
	(0/9)	(0/10)	(0/12)	1.000	(0/9)	(0/12)	(0/12)	1.000	1.000
Adenoma/Cancer incidence	0.0	10.0	16.7	1.000	0.0	25.0	8.3	0.229	0.594
	(0/9)	(1/10)	(2/12)	1.000	(0/9)	(3/12)	(1/12)	0.590	1.000
Distal colon									
Low grade adenoma incidence	0.0	0.0	0.0	1.000	0.0	0.0	8.3	1.000	1.000
	(0/9)	(0/10)	(0/12)	1.000	(0/9)	(0/12)	((1/12)	1.000	1.000
High grade adenoma incidence	0.0	0.0	0.0	1.000	0.0	0.0	8.3	1.000	1.000
	(0/9)	(0/10)	(0/12)	1.000	(0/9)	(0/12)	(1/12)	1.000	1.000
Cancer with mucosa invasion	0.0	90.0	75.0	**<0.001**	0.0	100.0	25.0	**<0.001**	0.455
	(0/9)	(9/10)	(9/12)	0.594	(0/9)	(12/12)	(3/12)	**<0.001**	**0.039**
Cancer with submucosa invasion	0.0	10.0	16.7	1.000	0.0	0.0	0.0	1.000	0.455
	(0/9)	(1/10)	(2/12)	1.000	(0/9)	(0/12)	(0/12)	1.000	0.478
Adenoma/Cancer incidence	0.0	100.0	91.7	**<0.001**	0.0	100.0	41.7	**<0.001**	1.000
	(0/9)	(10/10)	(11/12)	1.000	(0/9)	(12/12)	(5/12)	**0.005**	**0.027**
Whole colon									
Low grade adenoma incidence	0.0	0.0	0.0	1.000	0.0	0.0	8.3	1.000	1.000
	(0/9)	(0/10)	(0/12)	1.000	(0/9)	(0/12)	(1/12)	1.000	1.000
High grade adenoma incidence	0.0	0.0	0.0	1.000	0.0	0.0	8.3	1.000	1.000
	(0/9)	(0/10)	(0/12)	1.000	(0/9)	(0/12)	(1/12)	1.000	1.000
Cancer with mucosa invasion	0.0	90.0	75.0	**<0.001**	0.0	100.0	25.0	**<0.001**	0.455
	(0/9)	(9/10)	(9/12)	0.594	(0/9)	(12/12)	(3/12)	**<0.001**	**0.039**
Cancer with submucosa invasion	0.0	10.0	16.7	1.000	0.0	0.0	0.0	1.000	0.455
	(0/9)	(1/10)	(2/12)	1.000	(0/9)	(0/12)	(0/12)	1.000	0.478
Adenoma/Cancer incidence	0.0	100.0	91.7	**<0.001**	0.0	100.0	41.7	**<0.001**	1.000
	(0/9)	(10/10)	(11/12)	1.000	(0/9)	(12/12)	(5/12)	**0.005**	**0.027**

Values are expressed as the % (number/subtotal). WT, wild-type; KO, knockout; Con, control; AOM, azoxymethane; DSS, dextran sodium sulfate; E2, 17β-estradiol. [a], control vs AOM/DSS; [b], AOM/DSS vs AOM/DSS/E2; [c], WT vs KO for AOM/DSS; [d], WT vs KO for AOM/DSS/E2 (by Fisher's exact test for a 2 × 2 table); red color, statistical significance ($p < 0.05$).

addition, the anti-cancer effect of estrogen was stronger in the absence of Nrf2 due to the nonexistence of Nrf2-induced cancer promoting effects.

The Nrf2 signaling pathway plays a pivotal role in the regulation of inflammation and oxidative stress [42–46]. It has been shown that the lack of Nrf2 in sickle cell disease (SCD) mice results in reduced expression of its target antioxidant proteins such as HO-1, leading to increased levels of ROS and proinflammatory cytokines [47]. A study using peritoneal macrophages prepared from WT and Nrf2 KO mice showed that the anti-inflammatory effects of phytochemicals such as phenethyl isothiocyanate and curcumin is mediated through Nrf2 [42]. According to the report, iNOS protein expression is more strongly inhibited by phenethyl isothiocyanate and curcumin treatment in WT macrophages than in Nrf2 KO macrophages [42]. Several studies have suggested that Nrf2 KO mice are more susceptible to inflammatory disorders [48,49]. Furthermore, Nrf2 deficiency also affects body weight regulation. According to the study by Pi et al., the body weight of adult Nrf2 KO mice is lower compared to that of WT mice under normal chow diet [50]. Nrf2 deficiency prevents high fat diet-induced weight gain and obesity due to a decrease in the expression of peroxisome proliferator-activated receptor γ [50]. Similarly, in the present study, the body weight of Nrf2 KO mice was significantly lower compared to that of the WT mice at week 16 after AOM/DSS treatment. Colitis symptoms such as disease activity index and colon tissue damage were more strongly inhibited by E2 in WT mice than in Nrf2 KO mice. Although body weight loss is a sensitive indicator of the severity of colitis, it did not show consistent results with DAI score on the inhibitory effect of E2. At the molecular level, the basal mRNA expression level of the proinflammatory mediator, iNOS, was strongly elevated in Nrf2 KO mice compared to WT mice. AOM/DSS-induced colonic IL-1β concentration was strongly enhanced in the Nrf2 KO group than in the WT group. We previously reported that the anti-inflammatory effect of E2 at the molecular levels was weakened in TNF-α-stimulated Nrf2 KO MEFs than in WT MEFs [33]. We believe that these results are complicated by the fact that multi-signal state system operates *in vivo* in animals.

Unlike its cytoprotective effect during inflammation, Nrf2 is involved in maintaining proliferation and invasion of cancer cells by metabolic reprogramming, repression of cancer cell apoptosis, and enhancement of self-renewal capacity of cancer stem cells during the cancerous stages. More importantly, aberrant activation of Nrf2 is associated with chemoresistance of cancer cells and poor prognosis (reviewed in [51]). Furthermore, we previously showed the regulatory mechanism of estrogen during colon cancer progression in terms of Nrf2 using the AOM/DSS-treated male mice model [31]. Nrf2 and its-related antioxidant enzymes were highly expressed in the AOM/DSS-treated group, which developed cancer [31]. Therefore, we expected that AOM/DSS-induced carcinogenesis would be less severe in Nrf2 KO mice than in WT mice. Although no alleviation in tumor incidence was observed in the Nrf2 KO, the mRNA and protein levels of proinflammatory mediators were significantly lower in the AOM/DSS-treated Nrf2 KO mice than in the WT mice. Unexpectedly, when E2 was administered, its anti-tumorigenic effect was stronger in the AOM/DSS-treated Nrf2 KO group than in WT. Interestingly, the expression of ERβ protein increased more strongly in the AOM/DSS-treated Nrf2 KO group

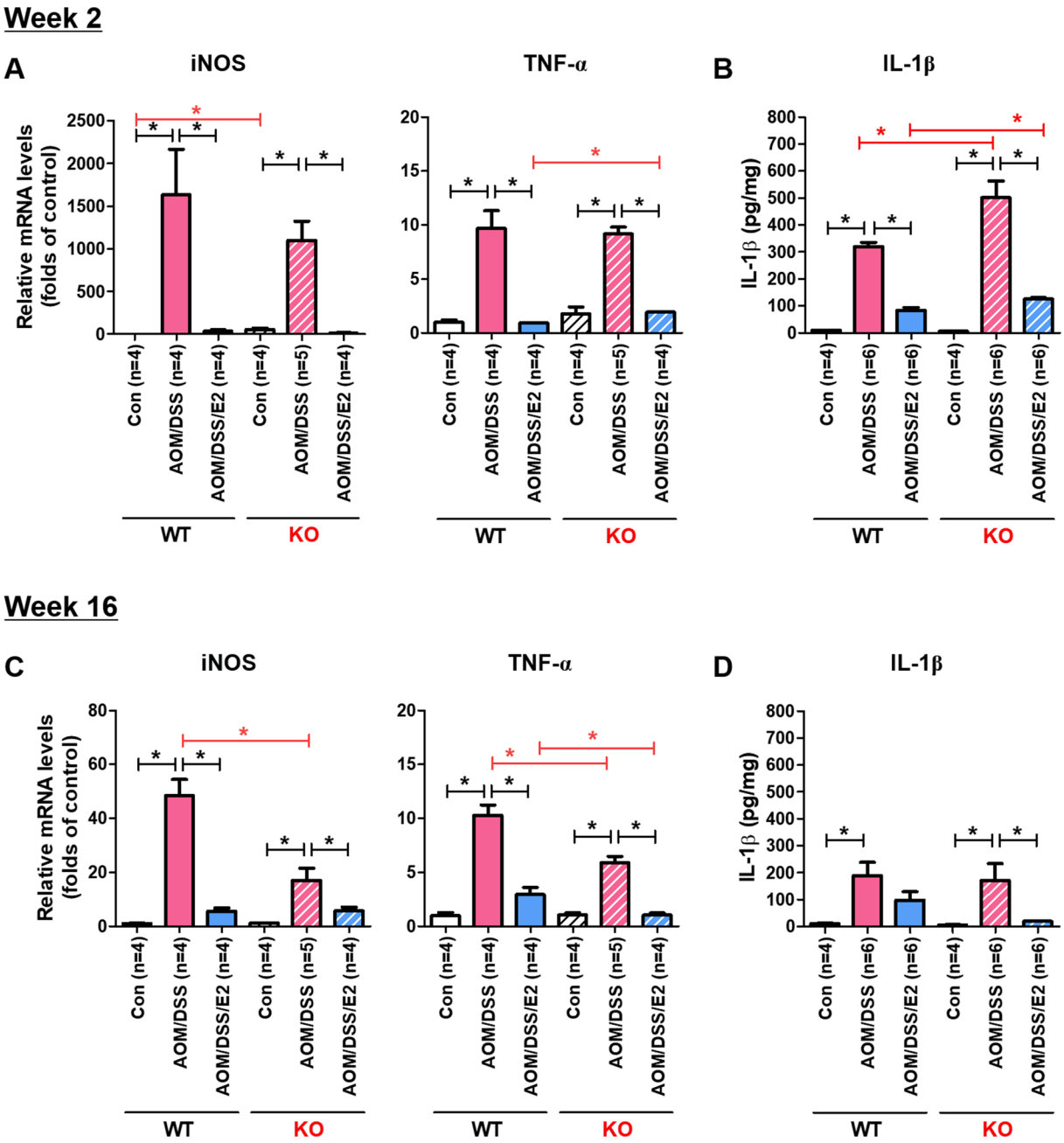

Fig. 5. Inhibitory effect of E2 *via* Nrf2 on the pro-inflammatory genes in colon tissues at weeks 2 (A and B) and 16 (C and D). (A and C) mRNA expression of iNOS and TNF-α by qRT-PCR analysis at weeks 2 (A) and 16 (C). (B and D) IL-1β level measurement by ELISA in colon tissues at weeks 2 (B) and 16 (D). Con, male control mice; AOM/DSS, AOM and DSS-treated male mice; AOM/DSS/E2, AOM, DSS, and 17β-estradiol-treated male mice. * $p < 0.05$ for comparison between two groups. WT, wild-type; KO, homogeneous knockout; AOM, azoxymethane; DSS, dextran sodium sulfate; E2, 17β-estradiol; iNOS, inducible nitric oxide synthase; TNF-α, tumor necrosis factor-alpha; IL-1β, interleukin 1 beta.

supplemented with E2 than in WT. These data suggest that in the absence of Nrf2, the anti-tumorigenic effect of estrogen may be mediated through other pathways such as ERβ-related pathway.

It is clear that there exists a compensatory mechanism of estrogen signaling which is independent on Nrf2. Estrogen receptors have both genomic and non-genomic pathways in response to estrogen signaling [52]. Non-genomic pathways are common to sex steroid hormones and are generally associated with the activation of various protein kinase cascades such as the mitogen-activated protein kinase (MAPK), c-Jun N-terminal kinase (JNK), and extracellular-signal related kinase (ERK)1/2 [53,54]. According to a recent report, ERK1/2-dependent ELK-1 activation upregulates 15-Hydroxyprostaglandin dehydrogenase (15-PGDH) in the presence of 15-deoxy-$\Delta^{12,\ 14}$-prostaglandin J2 (15d-PGJ2), which is one of the terminal products of cyclooxygenase-2 (COX-2), in the MDA-MB-231 cells [55]. Several studies have demonstrated the tumor suppressor function of 15-PGDH [56,57]. The expression of 15-PGDH is significantly reduced in various human malignances, such as colon, lung, and gastric cancers compared to normal tissues [58,59]. We selected and tested 15-PGDH as another target of estrogen in addition to ERβ. However, the level of 15-PGDH which decreased following AOM/DSS treatment was not rescued by estrogen treatment (data not shown). To overcome the limitation of our present study, we intend to perform further animal experiments in the future using an ERβ antagonist to clarify the compensatory mechanism of estrogen through ERβ in the absence of Nrf2.

We previously reported that Nrf2 plays dual roles in colitis and

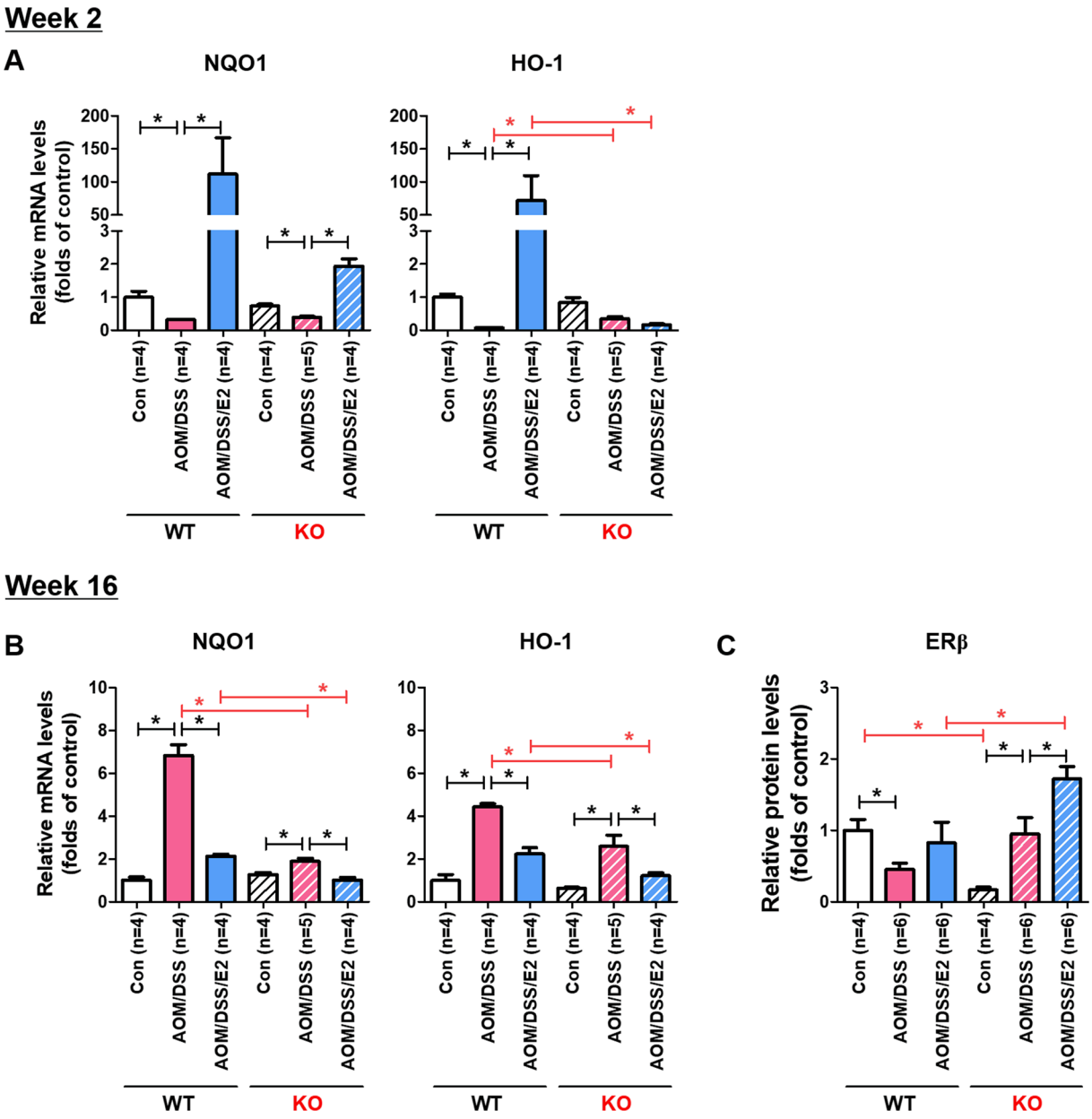

Fig. 6. Effect of E2 on mRNA and protein expression of Nrf2-mediated antioxidant enzyme genes and ERβ at weeks 2 (A) and 16 (B and C). (A and B) mRNA expression of antioxidant enzyme genes, NQO1 and HO-1, by qRT-PCR analysis at weeks 2 (A) and 16 (B). (C) ERβ protein (60 μg of proteins) expression by western blot analysis at week 16. Con, male control mice; AOM/DSS, AOM and DSS-treated male mice; AOM/DSS/E2, AOM, DSS, and 17β-estradiol-treated male mice. *$p < 0.05$ for comparison between two groups. WT, wild-type; KO, homogeneous knockout; AOM, azoxymethane; DSS, dextran sodium sulfate; E2, 17β-estradiol; NQO1, NAD(P)H dehydrogenase (quinone) 1; HO-1, heme oxygenase 1; ERβ, estrogen receptor beta.

cancer progression, and has sex differences [31]. In this study, using Nrf2 KO male mice, we verified estrogen-mediated effects through Nrf2. Based on the present and previously published [31–33,41,60–63] data, the regulatory mechanism of estrogen in colitis and colitis-associated CRC in WT and Nrf2 KO mice is proposed to be as follows (Fig. 7). At week 2, in the presence of Nrf2, estrogen signals through G protein coupled receptor (GPCR) to induce Nrf2 activation [31,60,61] leading to the subsequent expression of various Nrf2 target genes such as NQO1 and HO-1. Nrf2 inhibits NF-κB pathway and ROS production through upregulation of antioxidant enzymes [31–33]. Eventually, estrogen ameliorates the severity of colitis indicated as DAI score and microscopic damage score through Nrf2 activation (left panel at week 2). In contrast, in the absence of Nrf2, estrogen does not effectively inhibit colitis (right panel at week 2) [33]. After unsuccessful elimination of precancerous cells, inflammation progresses to cancer [62]. At week 16, in the presence of Nrf2, Nrf2 promotes tumor progression by activation of antioxidant enzymes [31,41]. Estrogen signaling through membrane estrogen receptor beta (mERβ) [41,63] inhibits antioxidant and proinflammatory enzymes. The inhibitory effect of E2 in the presence of Nrf2 is not sufficient to inhibit tumor development in the distal colon (left panel at week 16). In contrast, in the absence of Nrf2, estrogen strongly inhibits tumor development in the distal colon through Nrf2-independent ERβ-related signaling pathway (right panel at week 16).

In conclusion, our study shows that estrogen further inhibits CRC by upregulating other ERβ-related pathways in the absence of Nrf2. These findings may provide a new therapeutic strategy in which estrogen inhibits the progression of colitis-associated CRC depending on the level of Nrf2, and suggest that ERβ may be a useful target for gene therapy in CRC patients.

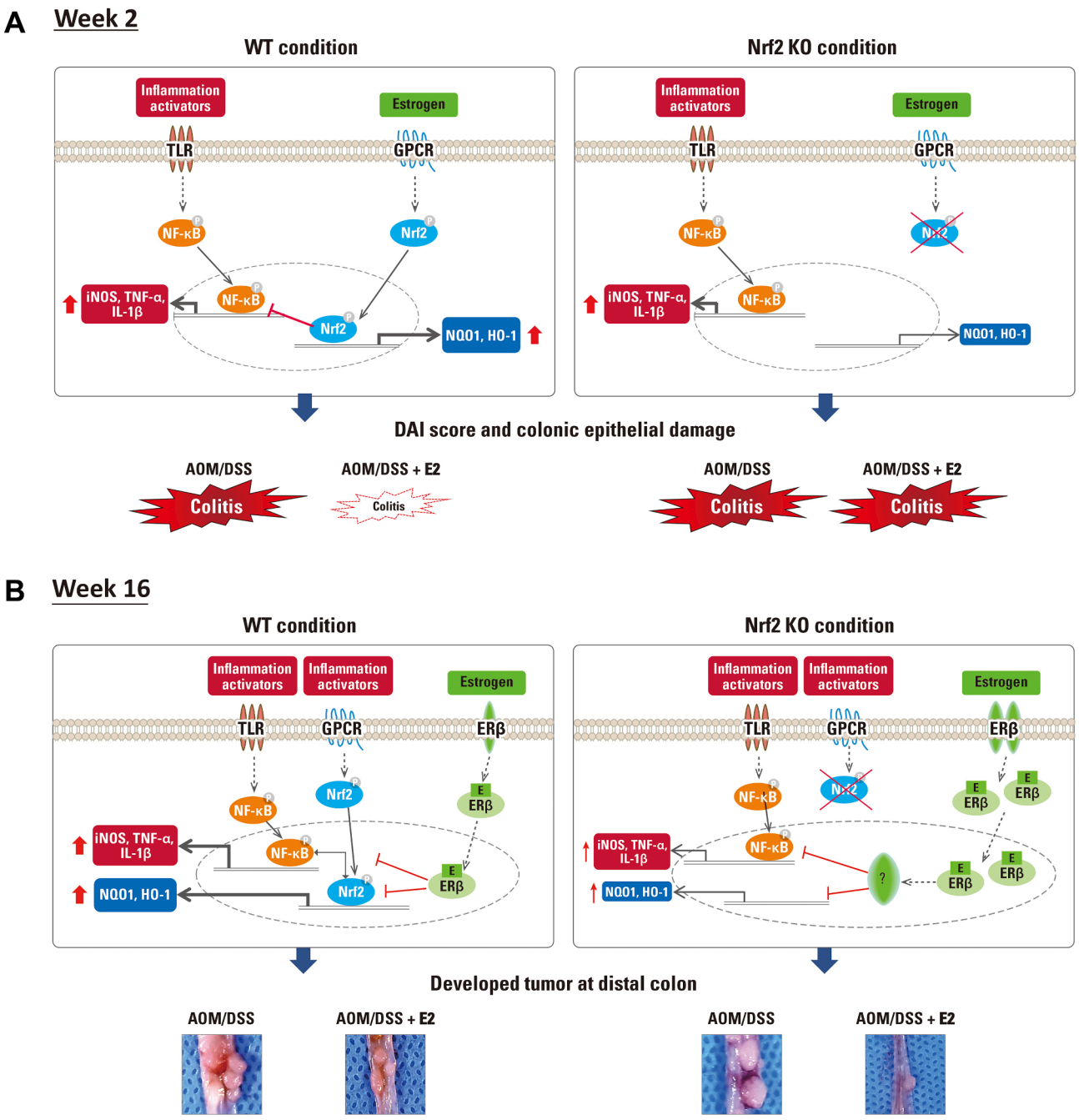

Fig. 7. Proposed regulatory mechanism of estrogen through Nrf2 in colitis-associated CRC at week 2 (A) and week 16 (B). (A) At week 2, in the presence of Nrf2, estrogen signaling through G protein coupled receptor (GPCR) induces Nrf2 activation and subsequently induces the Nrf2 target genes such as NQO1 and HO-1. Nrf2 inhibits NF-κB and ROS through the antioxidant enzymes. Eventually, colitis indices such as DAI score and microscopic damage score mediated by Nrf2 activation are inhibited by estrogen (left panel). In contrast, in the absence of Nrf2, estrogen does not effectively inhibit colitis (right panel). (B) At week 16, in the presence of Nrf2, Nrf2 promotes tumor progression by activation of antioxidant enzymes. Estrogen signaling through the membrane estrogen receptor beta (ERβ) inhibits antioxidant enzymes and proinflammatory enzymes. The inhibitory effect of E2 in the presence of Nrf2 is not sufficient to inhibit tumor development at the distal colon (left panel). In contrast, in the absence of Nrf2, estrogen strongly inhibits tumor development at distal colon through Nrf2-independent ERβ-related signaling pathway (right panel).

Author contributions

Chin-Hee Song drafted the manuscript and performed the molecular experiments; Nayoung Kim designed and supervised the experiments, and revised the manuscript; Ryoung Hee Nam, Soo In Choi, Joo Hee Son and Jeong Eun Yu performed the animal experiments; Eun Shin analyzed the histologic results; Ha-Na Lee revised the manuscript; Do-Hee Kim and Young-Joon Surh provided $Nrf2^{+/-}$ transgenic mice and revised the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by a grant from the National Research Foundation of Korea (NRF) funded by the government of the Republic of Korea (2019R1A2C2085149).

References

[1] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, CA Cancer J. Clin. 70 (2020) (2020) 7–30, https://doi.org/10.3322/caac.21590.
[2] K.W. Jung, Y.J. Won, H.J. Kong, E.S. Lee, Cancer statistics in Korea: incidence, mortality, survival, and prevalence in 2016, Cancer Res Treat 51 (2019) 417–430, https://doi.org/10.4143/crt.2019.138.
[3] T.M. McCashland, R. Brand, E. Lyden, P. de Garmo, C.R. Project, Gender differences in colorectal polyps and tumors, Am. J. Gastroenterol. 96 (2001) 882-6, 10.1111/j.1572-0241.2001.3638_a.x.
[4] S.E. Kim, H.Y. Paik, H. Yoon, J.E. Lee, N. Kim, M.K. Sung, Sex- and gender-specific disparities in colorectal cancer risk, World J. Gastroenterol. 21 (2015) 5167–5175, https://doi.org/10.3748/wjg.v21.i17.5167.
[5] T. Tanaka, H. Kohno, R. Suzuki, Y. Yamada, S. Sugie, H. Mori, A novel inflammation-related mouse colon carcinogenesis model induced by azoxymethane and dextran sodium sulfate, Cancer Sci. 94 (2003) 965–973, https://doi.org/10.1111/j.1349-7006.2003.tb01386.x.
[6] R. Suzuki, H. Kohno, S. Sugie, T. Tanaka, Sequential observations on the occurrence of preneoplastic and neoplastic lesions in mouse colon treated with azoxymethane and dextran sodium sulfate, Cancer Sci. 95 (2004) 721–727, https://doi.org/10.1111/j.1349-7006.2004.tb03252.x.
[7] A.I. Thaker, A. Shaker, M.S. Rao, M.A. Ciorba, Modeling colitis-associated cancer with azoxymethane (AOM) and dextran sulfate sodium (DSS), J. Vis. Exp. (2012) pp, https://doi.org/10.3791/4100.
[8] D. Tardieu, J.P. Jaeg, J. Cadet, E. Embvani, D.E. Corpet, C. Petit, Dextran sulfate enhances the level of an oxidative DNA damage biomarker, 8-oxo-7,8-dihydro-2'-deoxyguanosine, in rat colonic mucosa, Cancer Lett. 134 (1998) 1–5.
[9] M. De Robertis, E. Massi, M.L. Poeta, S. Carotti, S. Morini, L. Cecchetelli, et al., The AOM/DSS murine model for the study of colon carcinogenesis: From pathways to diagnosis and therapy studies, J. Carcinogenesis 10 (2011) 9, https://doi.org/10.4103/1477-3163.78279.
[10] R. Suzuki, H. Kohno, S. Sugie, H. Nakagama, T. Tanaka, Strain differences in the susceptibility to azoxymethane and dextran sodium sulfate-induced colon carcinogenesis in mice, Carcinogenesis 27 (2006) 162–169, https://doi.org/10.1093/carcin/bgi205.
[11] C. Tonelli, I.I.C. Chio, D.A. Tuveson, Transcriptional regulation by Nrf2, Antioxid. Redox Signal. 29 (2018) 1727–1745, https://doi.org/10.1089/ars.2017.7342.
[12] M. Rojo de la Vega, M. Dodson, C. Gross, H.M. Mansour, R.C. Lantz, E. Chapman, et al., Role of Nrf2 and autophagy in acute lung injury, Curr. Pharmacol. Rep. 2 (2016) 91–101, https://doi.org/10.1007/s40495-016-0053-2.
[13] J.M. Lee, M.J. Calkins, K. Chan, Y.W. Kan, J.A. Johnson, Identification of the NF-E2-related factor-2-dependent genes conferring protection against oxidative stress in primary cortical astrocytes using oligonucleotide microarray analysis, J. Biol. Chem. 278 (2003) 12029–12038, https://doi.org/10.1074/jbc.M211558200.
[14] R.K. Thimmulappa, K.H. Mai, S. Srisuma, T.W. Kensler, M. Yamamoto, S. Biswal, Identification of Nrf2-regulated genes induced by the chemopreventive agent sulforaphane by oligonucleotide microarray, Cancer Res. 62 (2002) 5196–5203.
[15] J.K. Kundu, Y.J. Surh, Nrf2-Keap1 signaling as a potential target for chemoprevention of inflammation-associated carcinogenesis, Pharm. Res. 27 (2010) 999–1013, https://doi.org/10.1007/s11095-010-0096-8.
[16] E.H. Kobayashi, T. Suzuki, R. Funayama, T. Nagashima, M. Hayashi, H. Sekine, et al., Nrf2 suppresses macrophage inflammatory response by blocking proinflammatory cytokine transcription, Nat. Commun. 7 (2016) 11624, https://doi.org/10.1038/ncomms11624.
[17] L. Yang, L. Shen, Y. Li, Y. Li, S. Yu, S. Wang, Hyperoside attenuates dextran sulfate sodium-induced colitis in mice possibly via activation of the Nrf2 signalling pathway, J. Inflamm. (Lond.) 14 (2017) 25, https://doi.org/10.1186/s12950-017-0172-5.
[18] A. Lau, N.F. Villeneuve, Z. Sun, P.K. Wong, D.D. Zhang, Dual roles of Nrf2 in cancer, Pharmacol. Res. 58 (2008) 262–270, https://doi.org/10.1016/j.phrs.2008.09.003.
[19] S. Menegon, A. Columbano, S. Giordano, The Dual Roles of NRF2 in Cancer, Trends Mol. Med. 22 (2016) 578–593, https://doi.org/10.1016/j.molmed.2016.05.002.
[20] Y. Guo, L. Shen, Overexpression of NRF2 is correlated with prognoses of patients with malignancies: a meta-analysis, Thorac. Cancer 8 (2017) 558–564, https://doi.org/10.1111/1759-7714.12462.
[21] H. Yang, W. Wang, Y. Zhang, J. Zhao, E. Lin, J. Gao, et al., The role of NF-E2-related factor 2 in predicting chemoresistance and prognosis in advanced non-small-cell lung cancer, Clin. Lung Cancer 12 (2011) 166–171, https://doi.org/10.1016/j.cllc.2011.03.012.
[22] M.D. Maines, P.A. Abrahamsson, Expression of heme oxygenase-1 (HSP32) in human prostate: normal, hyperplastic, and tumor tissue distribution, Urology 47 (1996) 727–733, https://doi.org/10.1016/s0090-4295(96)00010-6.
[23] J.J. Schlager, G. Powis, Cytosolic NAD(P)H:(quinone-acceptor)oxidoreductase in human normal and tumor tissue: effects of cigarette smoking and alcohol, Int. J. Cancer 45 (1990) 403–409, https://doi.org/10.1002/ijc.2910450304.
[24] J.M. Gierisch, R.R. Coeytaux, R.P. Urrutia, L.J. Havrilesky, P.G. Moorman, W. J. Lowery, et al., Oral contraceptive use and risk of breast, cervical, colorectal, and endometrial cancers: a systematic review, Cancer Epidemiol. Biomarkers Prevent. 22 (2013) 1931–1943, https://doi.org/10.1158/1055-9965.EPI-13-0298.
[25] R.T. Chlebowski, J. Wactawski-Wende, C. Ritenbaugh, F.A. Hubbell, J. Ascensao, R.J. Rodabough, et al., Estrogen plus progestin and colorectal cancer in postmenopausal women, N. Engl. J. Med. 350 (2004) 991–1004, https://doi.org/10.1056/NEJMoa032071.
[26] C. Behl, Oestrogen as a neuroprotective hormone, Nat. Rev. Neurosci. 3 (2002) 433–442, https://doi.org/10.1038/nrn846.
[27] S. Menazza, E. Murphy, The expanding complexity of estrogen receptor signaling in the cardiovascular system, Circ. Res. 118 (2016) 994–1007, https://doi.org/10.1161/CIRCRESAHA.115.305376.
[28] C. Zhu, S. Wang, B. Wang, F. Du, C. Hu, H. Li, et al., 17beta-Estradiol up-regulates Nrf2 via PI3K/AKT and estrogen receptor signaling pathways to suppress light-induced degeneration in rat retina, Neuroscience 304 (2015) 328–339, https://doi.org/10.1016/j.neuroscience.2015.07.057.
[29] M.M. Mocanu, P. Nagy, J. Szollosi, Chemoprevention of breast cancer by dietary polyphenols, Molecules 20 (2015) 22578–22620, https://doi.org/10.3390/molecules201219864.
[30] B. Singh, R. Shoulson, A. Chatterjee, A. Ronghe, N.K. Bhat, D.C. Dim, et al., Resveratrol inhibits estrogen-induced breast carcinogenesis through induction of NRF2-mediated protective pathways, Carcinogenesis 35 (2014) 1872–1880, https://doi.org/10.1093/carcin/bgu120.
[31] H.J. Son, S.H. Sohn, N. Kim, H.N. Lee, S.M. Lee, R.H. Nam, et al., Effect of estradiol in an azoxymethane/dextran sulfate sodium-treated mouse model of colorectal cancer: implication for sex difference in colorectal cancer development, Cancer Res. Treat. 51 (2019) 632–648, https://doi.org/10.4143/crt.2018.060.
[32] H.J. Son, N. Kim, C.H. Song, S.M. Lee, H.N. Lee, Y.J. Surh, 17beta-Estradiol reduces inflammation and modulates antioxidant enzymes in colonic epithelial cells, Korean J. Intern. Med. 35 (2020) 310–319, https://doi.org/10.3904/kjim.2018.098.
[33] C.H. Song, N. Kim, D.H. Kim, H.N. Lee, Y.J. Surh, 17-beta estradiol exerts anti-inflammatory effects through activation of Nrf2 in mouse embryonic fibroblasts, PLoS ONE 14 (2019), e0221650, https://doi.org/10.1371/journal.pone.0221650.
[34] K. Chan, R. Lu, J.C. Chang, Y.W. Kan, NRF2, a member of the NFE2 family of transcription factors, is not essential for murine erythropoiesis, growth, and development, Proc. Natl. Acad. Sci. USA 93 (1996) 13943–13948, https://doi.org/10.1073/pnas.93.24.13943.
[35] H.W. Yum, X. Zhong, J. Park, H.K. Na, N. Kim, H.S. Lee, et al., Oligonol inhibits dextran sulfate sodium-induced colitis and colonic adenoma formation in mice, Antioxid. Redox Signal. 19 (2013) 102–114, https://doi.org/10.1089/ars.2012.4626.
[36] H.S. Cooper, S.N. Murthy, R.S. Shah, D.J. Sedergran, Clinicopathologic study of dextran sulfate sodium experimental murine colitis, Lab. Invest. J. Techn. Methods Pathol. 69 (1993) 238–249.
[37] Y.H. Park, N. Kim, Y.K. Shim, Y.J. Choi, R.H. Nam, Y.J. Choi, et al., Adequate dextran sodium sulfate-induced colitis model in mice and effective outcome measurement method, J. Cancer Prev. 20 (2015) 260–267, 10.15430/JCP.2015.20.4.260.
[38] Y.J. Choi, N. Kim, R.H. Nam, S. Lee, H.S. Lee, H.N. Lee, et al., Acai berries inhibit colon tumorigenesis in azoxymethane/dextran sulfate sodium-treated mice, Gut and liver 11 (2017) 243–252, https://doi.org/10.5009/gnl16068.
[39] K. Katakura, J. Lee, D. Rachmilewitz, G. Li, L. Eckmann, E. Raz, Toll-like receptor 9-induced type I IFN protects mice from experimental colitis, J. Clin. Investig. 115 (2005) 695–702, https://doi.org/10.1172/JCI22996.
[40] B. Parang, C.W. Barrett, C.S. Williams, AOM/DSS model of colitis-associated cancer, Methods Mol. Biol. 1422 (2016) 297–307, https://doi.org/10.1007/978-1-4939-3603-8_26.
[41] C.H. Song, N. Kim, S.M. Lee, R.H. Nam, S.I. Choi, S.R. Kang, et al., Effects of 17beta-estradiol on colorectal cancer development after azoxymethane/dextran sulfate sodium treatment of ovariectomized mice, Biochem. Pharmacol. 164 (2019) 139–151, https://doi.org/10.1016/j.bcp.2019.04.011.
[42] S.S. Boyanapalli, X. Paredes-Gonzalez, F. Fuentes, C. Zhang, Y. Guo, D. Pung, et al., Nrf2 knockout attenuates the anti-inflammatory effects of phenethyl isothiocyanate and curcumin, Chem. Res. Toxicol. 27 (2014) 2036–2043, https://doi.org/10.1021/tx500234h.
[43] T.Y. Wu, T.O. Khor, C.L. Saw, S.C. Loh, A.I. Chen, S.S. Lim, et al., Anti-inflammatory/Anti-oxidative stress activities and differential regulation of Nrf2-mediated genes by non-polar fractions of tea Chrysanthemum zawadskii and licorice Glycyrrhiza uralensis, AAPS J. 13 (2011) 1–13, https://doi.org/10.1208/s12248-010-9239-4.
[44] S.C. Garzon-Castano, I.A. Lopera-Castrillon, F.J. Jimenez-Gonzalez, F. Siller-Lopez, L.A. Veloza, J.C. Sepulveda-Arias, Nrf2-Mediated Antioxidant Activity of the inner bark extracts obtained from Tabebuia rosea (Bertol) DC and Tabebuia chrysantha (JACQ) G. Nicholson, F1000Res, 7 (2018) 1937, 10.12688/f1000research.17165.1.
[45] A.L. Levonen, M. Inkala, T. Heikura, S. Jauhiainen, H.K. Jyrkkanen, E. Kansanen, et al., Nrf2 gene transfer induces antioxidant enzymes and suppresses smooth muscle cell growth in vitro and reduces oxidative stress in rabbit aorta in vivo, Arterioscler. Thromb. Vasc. Biol. 27 (2007) 741–747, https://doi.org/10.1161/01.ATV.0000258868.80079.4d.
[46] K. Itoh, M. Mochizuki, Y. Ishii, T. Ishii, T. Shibata, Y. Kawamoto, et al., Transcription factor Nrf2 regulates inflammation by mediating the effect of 15-deoxy-Delta(12,14)-prostaglandin j(2), Mol. Cell. Biol. 24 (2004) 36–45.
[47] X. Zhu, C. Xi, B. Thomas, B.S. Pace, Loss of NRF2 function exacerbates the pathophysiology of sickle cell disease in a transgenic mouse model, Blood 131 (2018) 558–562, https://doi.org/10.1182/blood-2017-10-810531.

[48] Q. Ma, L. Battelli, A.F. Hubbs, Multiorgan autoimmune inflammation, enhanced lymphoproliferation, and impaired homeostasis of reactive oxygen species in mice lacking the antioxidant-activated transcription factor Nrf2, Am. J. Pathol. 168 (2006) 1960–1974, https://doi.org/10.2353/ajpath.2006.051113.
[49] J. Kim, Y.N. Cha, Y.J. Surh, A protective role of nuclear factor-erythroid 2-related factor-2 (Nrf2) in inflammatory disorders, Mutat. Res. 690 (2010) 12–23, https://doi.org/10.1016/j.mrfmmm.2009.09.007.
[50] J. Pi, L. Leung, P. Xue, W. Wang, Y. Hou, D. Liu, et al., Deficiency in the nuclear factor E2-related factor-2 transcription factor results in impaired adipogenesis and protects against diet-induced obesity, J. Biol. Chem. 285 (2010) 9292–9300, https://doi.org/10.1074/jbc.M109.093955.
[51] S. Wu, H. Lu, Y. Bai, Nrf2 in cancers: a double-edged sword, Cancer Med. 8 (2019) 2252–2267, https://doi.org/10.1002/cam4.2101.
[52] L. Bjornstrom, M. Sjoberg, Mechanisms of estrogen receptor signaling: convergence of genomic and nongenomic actions on target genes, Mol. Endocrinol. 19 (2005) 833–842, https://doi.org/10.1210/me.2004-0486.
[53] R. Losel, M. Wehling, Nongenomic actions of steroid hormones, Nat. Rev. Mol. Cell Biol. 4 (2003) 46–56, https://doi.org/10.1038/nrm1009.
[54] P.H. Driggers, J.H. Segars, Estrogen action and cytoplasmic signaling pathways. Part II: the role of growth factors and phosphorylation in estrogen signaling, Trends Endocrinol. Metab. 13 (2002) 422–427, https://doi.org/10.1016/s1043-2760(02)00634-3.
[55] H.R. Kim, H.N. Lee, K. Lim, Y.J. Surh, H.K. Na, 15-Deoxy-Delta 12,14-prostaglandin J2 induces expression of 15-hydroxyprostaglandin dehydrogenase through Elk-1 activation in human breast cancer MDA-MB-231 cells, Mutat. Res. 768 (2014) 6–15, https://doi.org/10.1016/j.mrfmmm.2014.06.005.
[56] S.J. Myung, R.M. Rerko, M. Yan, P. Platzer, K. Guda, A. Dotson, et al., 15-Hydroxyprostaglandin dehydrogenase is an in vivo suppressor of colon tumorigenesis, Proc. Natl. Acad. Sci. USA 103 (2006) 12098–12102, https://doi.org/10.1073/pnas.0603235103.
[57] I. Wolf, J. O'Kelly, T. Rubinek, M. Tong, A. Nguyen, B.T. Lin, et al., 15-hydroxyprostaglandin dehydrogenase is a tumor suppressor of human breast cancer, Cancer Res. 66 (2006) 7818–7823, https://doi.org/10.1158/0008-5472.CAN-05-4368.
[58] M.G. Backlund, J.R. Mann, V.R. Holla, F.G. Buchanan, H.H. Tai, E.S. Musiek, et al., 15-Hydroxyprostaglandin dehydrogenase is down-regulated in colorectal cancer, J. Biol. Chem. 280 (2005) 3217–3223, https://doi.org/10.1074/jbc.M411221200.
[59] A. Thiel, A. Ganesan, J. Mrena, S. Junnila, A. Nykanen, A. Hemmes, et al., 15-hydroxyprostaglandin dehydrogenase is down-regulated in gastric cancer, Clin. Cancer Res. 15 (2009) 4572–4580, https://doi.org/10.1158/1078-0432.CCR-08-2518.
[60] M.K. Cho, W.D. Kim, S.H. Ki, J.I. Hwang, S. Choi, C.H. Lee, et al., Role of Galpha12 and Galpha13 as novel switches for the activity of Nrf2, a key antioxidative transcription factor, Mol. Cell. Biol. 27 (2007) 6195–6208, https://doi.org/10.1128/MCB.02065-06.
[61] E.R. Prossnitz, J.B. Arterburn, L.A. Sklar, GPR30: A G protein-coupled receptor for estrogen, Mol. Cell. Endocrinol. 265–266 (2007) 138–142, https://doi.org/10.1016/j.mce.2006.12.010.
[62] E.A. Miao, J.V. Rajan, A. Aderem, Caspase-1-induced pyroptotic cell death, Immunol. Rev. 243 (2011) 206–214, https://doi.org/10.1111/j.1600-065X.2011.01044.x.
[63] E.R. Levin, Plasma membrane estrogen receptors, Trends Endocrinol. Metab. 20 (2009) 477–482, https://doi.org/10.1016/j.tem.2009.06.009.