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Soluble Dietary Fiber Ameliorates Radiation-Induced Intestinal Epithelial-to-Mesenchymal Transition and Fibrosis

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Abstract

Background: Intestinal fibrosis is a late complication of pelvic radiotherapy. Epithelial-to-mesenchymal transition (EMT) plays an important role in tissue fibrosis. The aim of this study was to examine the effect of soluble dietary fiber on radiation-induced intestinal EMT and fibrosis in a mouse model. *Materials and Methods:* Apple pectin (4% wt/wt in drinking water) was administered to wild-type and pVillin-Cre-EGFP transgenic mice with intestinal fibrosis induced by a single dose of abdominal irradiation of 10 Gy. The effects of pectin on intestinal EMT and fibrosis, gut microbiota, and short-chain fatty acid (SCFA) concentration were evaluated. *Results:* Intestinal fibrosis in late radiation enteropathy showed increased submucosal thickness and subepithelial collagen deposition. Enhanced green fluorescent protein $(EGFP)^+$/$vimentin^+$ and $EGFP^+$/α–smooth muscle actin $(SMA)^+$ coexpressing cells were most clearly observed at 2 weeks after irradiation and gradually decreased at 4 and 12 weeks. Pectin significantly attenuated the thickness of submucosa and collagen deposition at 12 weeks (24.3 vs 27.6 μm in the pectin + radiation-treated group compared with radiation-alone group, respectively, $P < .05$; 69.0% vs 57.1%, $P < .001$) and ameliorated EMT at 2 and 4 weeks. Pectin also modulated the intestinal microbiota composition and increased the luminal SCFA concentration. *Conclusion:* The soluble dietary fiber pectin protected the terminal ileum against radiation-induced fibrosis. This effect might be mediated by altered SCFA concentration in the intestinal lumen and reduced EMT in the ileal epithelium. *(JPEN J Parenter Enteral Nutr. XXXX;xx:xx-xx)*

Keywords

radiation; intestinal fibrosis; epithelial-to-mesenchymal transition; soluble dietary fiber

Clinical Relevancy Statement

Intestinal fibrosis is a late complication of pelvic radiotherapy and the main reason for surgery. Despite the high prevalence of radiation-induced intestinal fibrosis, few antifibrotic pharmacological approaches have been successfully used in clinical practice. We found that dietary pectin protected the terminal ileum against radiation-induced epithelial-to-mesenchymal transition and fibrosis, possibly through modulating gut microbiota compositions and luminal short-chain fatty acid concentration.

These findings are clinically relevant for guiding clinicians to prophylactic use of soluble dietary fiber to protect against radiation-induced late gastrointestinal complications.

Introduction

Radiotherapy is an important treatment modality for abdominal and pelvic malignancy that is widely applied in more than half of all patients with cancer. Despite ongoing technological progress in radiotherapy strategies, the incidence of chronic radiation enteropathy (CRE) is increasing, which occurs in about one-fifth of patients receiving pelvic radiotherapy.[1] Approximately one-third of patients with CRE require surgery for late intestinal complications, which are potentially life threatening and seriously affect quality of life. Radiation-induced intestinal fibrosis, which causes complete or incomplete digestive obstruction, is the most common symptom and surgical indication. Radiation-induced intestinal obstruction, secondary to strictures, accounts for nearly three-quarters of

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patients with CRE who require surgery.[2] The terminal ileum is the most frequently affected site of radiation-induced intestinal obstruction.[3] Hence, prevention or treatment of radiation-induced intestinal fibrosis is of clinical significance.

Intestinal fibrosis after radiotherapy is a complex process that is characterized by the sustained production and accumulation of extracellular matrix. Radiation-induced intestinal fibrosis follows a mechanism of fibrogenesis similar to other organs for the purpose of wound healing.[4] Although emerging evidence has demonstrated that epithelial-to-mesenchymal transition (EMT) is associated with fibrogenesis, such as in the kidney, lung, and liver, few studies have examined the role of EMT in inflammation-associated intestinal fibrosis, especially as induced by radiation.[5] During EMT, epithelial cells lose their epithelial phenotype and functional characteristics (E-cadherin and β-catenin expression) and instead exhibit a mesenchymal phenotype (α–smooth muscle actin [α-SMA], vimentin, and fibroblast-specific protein 1 expression).[6] Previous studies have reported that EMT participated in fistula formation in human Crohn's disease and contributed to the pathogenesis of intestinal fibrosis in a trinitro-benzene-sulfonic acid induced murine model.[7,8] To date, the role of EMT in radiation-induced intestinal fibrosis has not been investigated.

Despite the high prevalence of radiation-induced intestinal fibrosis, few antifibrotic pharmacological approaches have been successfully used in clinical practice.[9,10] Recently, several lines of evidence have emerged that link gut microbiota metabolism of dietary fiber to beneficial effects in both intestinal and extra-intestinal inflammatory disorders. Short-chain fatty acids (SCFAs), the most abundant gut microbial fermentation products of nondigestible polysaccharides, play a crucial role in modulating intestinal microbiota and gut immunity.[11,12] In addition to serving as energy sources for intestinal epithelial cells, SCFAs, in particular butyrate, have been shown to have anticarcinogenic, anti-inflammatory, antioxidant, and antifibrogenic effects.[13–15] Pacheco et al[16] reported that butyrate enemas attenuated inflammation and fibrosis in experimental diversion colitis, highlighting the potential antifibrotic effects of butyrate in chronic inflammation–dominated diseases.

Pectin, a highly fermentable soluble dietary fiber present in fruit and vegetables, has potential effects of decreasing food intake, body weight gain, and body fat mass and increasing intestinal weight and intestinal mucosal cell proliferation.[17] Sureban et al[18] demonstrated that dietary pectin could serve as a radioprotective agent in the distal ileum by preventing ionizing radiation–induced intestinal stem cell depletion and facilitating crypt regeneration. Bernard et al[19] showed that dietary pectin-derived acidic oligosaccharides could modify the intestinal microbiota by stimulating the growth of species involved in immunity development and at the same time increased the production of butyrate and propionate. Jiang et al[20] revealed that apple-derived pectin modulated gut microbiota in rats with diet-induced obesity. However, the effects of dietary pectin as a protector against radiation-induced intestinal fibrosis and its impact on radiation-induced intestinal microbiota alteration have not been studied.

The purpose of the present study was to investigate the effect of oral dietary pectin on intestinal fibrosis and to examine the role of EMT in the pathogenesis of intestinal fibrosis in a mouse model after irradiation. Moreover, the influence of radiation and dietary pectin treatment on gut microbiota composition and SCFA concentration was investigated.

Materials and Methods

Ethical Approval

All experimental protocols were performed in accordance with the guidelines for the care and use of laboratory animals established by Jinling Hospital and approved by the Ethics Committee of Jinling Hospital.

Animals

Wild-type and pVillin-Cre; B6.129(Cg) and Gt(ROSA) 26Sor$^{tm4(ACTB-tdTomato,-EGFP)\ Luo}$ transgenic mice on a C57BL/6 background (Jackson Laboratory, Bar Harbor, ME) were used. The latter 2 transgenic mice were crossed to generate pVillin-Cre-EGFP double-transgenic mice and genotyped according to the protocol of the provider.[21,22] In the double-transgenic mice, the stop cassette was removed by Cre recombinase in villin-positive intestinal epithelial cells, resulting in irreversible activation of enhanced green fluorescent protein (EGFP) in all intestinal epithelium-derived cells. All mice were housed in plastic-bottomed wire-lidded cages and kept at 25°C with a 12-hour/12-hour light/dark cycle and with ad libitum access to pelleted chow and water.

Experimental Design

Mice were divided into 3 groups: (1) control, (2) radiation, and (3) radiation + pectin group (n = 4 at each time point). Apple pectin (cat no. 76282; Sigma, St Louis, MO) with a molecular weight of 30,000–100,000 and 70%–75% esterification was added to the drinking water at a concentration of 4% from 2 weeks before to 2 weeks after irradiation.

For irradiation, mice were anesthetized with intraperitoneal pentobarbital sodium (0.05 mg/g) and received a single abdominal dose of 10 Gy gamma-irradiation (^{60}Co source, dose rate 2 Gy/min).[23] Mice in each group were separately euthanized 1 day prior to irradiation and 2, 4, and 12 weeks postirradiation.

Histopathology

Terminal ileum was fixed in 4% buffered paraformaldehyde and embedded in paraffin. Sections (5 μm) were stained with hematoxylin and eosin (H&E) to evaluate histological changes and

Masson's trichrome staining (MTS) to assess the severity of collagen deposition as previously reported.[24] The thickness of the submucosa was evaluated randomly in 5 fields in every sample. The severity of fibrosis was quantified by measuring the number of blue pixels in 5 points in every specimen at 400× magnification using Adobe Photoshop Magic Wand (Adobe Systems, San Jose, CA). All analyses were performed on coded slides by 2 pathologists who were blinded to the experimental design.

Immunofluorescence

Immunofluorescence staining was performed as described previously.[8] Frozen sections were cut at 5 μm, transferred onto coated slides, and fixed in 4°C acetone for 10 minutes. Sections from double-transgenic mice were incubated overnight with primary antibodies against vimentin (1:1000; Abcam, Cambridge, MA) or α-SMA (1:200; Abcam). After incubation, the sections were washed with phosphate buffered saline with tween and conjugated with Alexa Fluor 647 (red) (1:400; Cell Signaling Technology, Danvers, MA). Sections from wild-type mice were incubated with E-cadherin (1:2000; R&D Systems, Minneapolis, MN), vimentin, or α-SMA and conjugated with Alexa Fluor 647 (red) and Alexa Fluor 488 (green) (1:400; Abcam). The nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) (Sigma). Images were captured using a confocal microscopy (Olympus, Tokyo, Japan). The extent of EMT was quantified by calculating the number of cells showing coexpression (yellow) at 5 high-magnification fields (400×) in each section.

Intestinal Microbiota Analysis

Cecal fecal samples from mice housed in separate cages were harvested under sterile conditions in a tube at 2 weeks after irradiation. Feces were stored immediately in liquid nitrogen at −80°C until processing for DNA isolation. The microbiota were analyzed using 16S ribosomal RNA (rRNA) pyrosequencing technology at the BGI laboratory (Beijing Genomic Institute, Shenzhen, China), as previously described.[25] After bacterial DNA isolation, sample analyses were performed, including concentration testing and sample integrity. Concentration was detected by fluorometer or microplate reader, and sample integrity was detected by 1% agarose gel electrophoresis (voltage, 150 V; electrophoresis time, 40 minutes). To construct the library, genomic DNA was normalized to 30 ng per polymerase chain reaction (PCR), including V4 Dual-index Fusion PCR Primer Cocktail and PCR Master Mix (NEB Phusion High-Fidelity PCR Master Mix; NEB, Ipswich, MA). The PCR products were then purified with AMPure XP beads (Agencourt; Beckman Coulter, Beverly, MA) to remove unspecific products. The library validation was quantified by real-time quantitative PCR (QPCR) (EvaGreen; Biotium, Fremont, CA). The qualified libraries were sequenced pair end on the Illumina MiSeq platform (Illumina, San Diego, CA) with the sequencing strategy PE250 (PE251+8+8+251) (MiSeq Reagent Kit; Illumina). The data were analyzed using the Mothur pipeline and QIIME pipeline.

SCFA Analysis

The feces in the cecum were excised immediately and stored at −80°C. SCFA concentration in cecal feces was measured by propyl chloroformate derivatization procedures and gas chromatography–mass spectrometry (GC-MS) analysis, as described previously.[26] Briefly, feces samples (50–150 mg) in 1000 μL of 0.005 M aqueous NaOH containing internal standard (5 μg/mL caproic acid–d3) were homogenized and centrifuged. A 500-μL aliquot of supernatant fecal water was transferred into a 10-mL Corning (Corning, NY) disposable glass centrifuge tube, and then 300 μL of water, 500 μL of PrOH/Py mixture solvent (3:2, v/v), and 100 μL of propyl-chloroformate were added and the sample was briefly vortexed. The resultant mixture was analyzed using an Agilent 7890A gas chromatography system coupled to an Agilent 5975C inert XL EI/CI mass spectrometric detector (MSD; Agilent Technologies, Santa Clara, CA).

Statistical Analysis

Statistical analysis was performed using SPSS version 19.0 (SPSS, Inc, an IBM Company, Chicago, IL). The data were expressed as the mean ± standard error (SEM). Nonparametric Mann-Whitney *t* tests were used to test the difference between the study groups with $P < .05$ as statistically significant.

Results

Dietary Pectin Ameliorates Radiation-Induced Intestinal Fibrosis

The radiation group had marked thickening of the submucosal layer compared with controls ($P < .05$, $P < .01$, and $P < .001$ at 2, 4, and 12 weeks, respectively) (Figure 1). Dietary pectin significantly ameliorated the radiation-induced increase in submucosa thickness at 12 weeks compared with the radiation-alone group ($P < .05$).

In the irradiated intestine, marked collagen deposition in the submucosa was observed at 4 weeks after irradiation compared with controls ($P < .01$). The most severe lesion occurred at 12 weeks (Figure 2). However, compared with mice in the irradiation group, there was a significant reduction in blue-stained areas in the submucosa at 12 weeks in the pectin-treated group ($P < .05$).

EMT Is a Source of Intestinal Fibrosis Induced by Irradiation

To investigate the role of EMT in radiation-induced intestinal fibrosis, double-labeling experiments were performed to

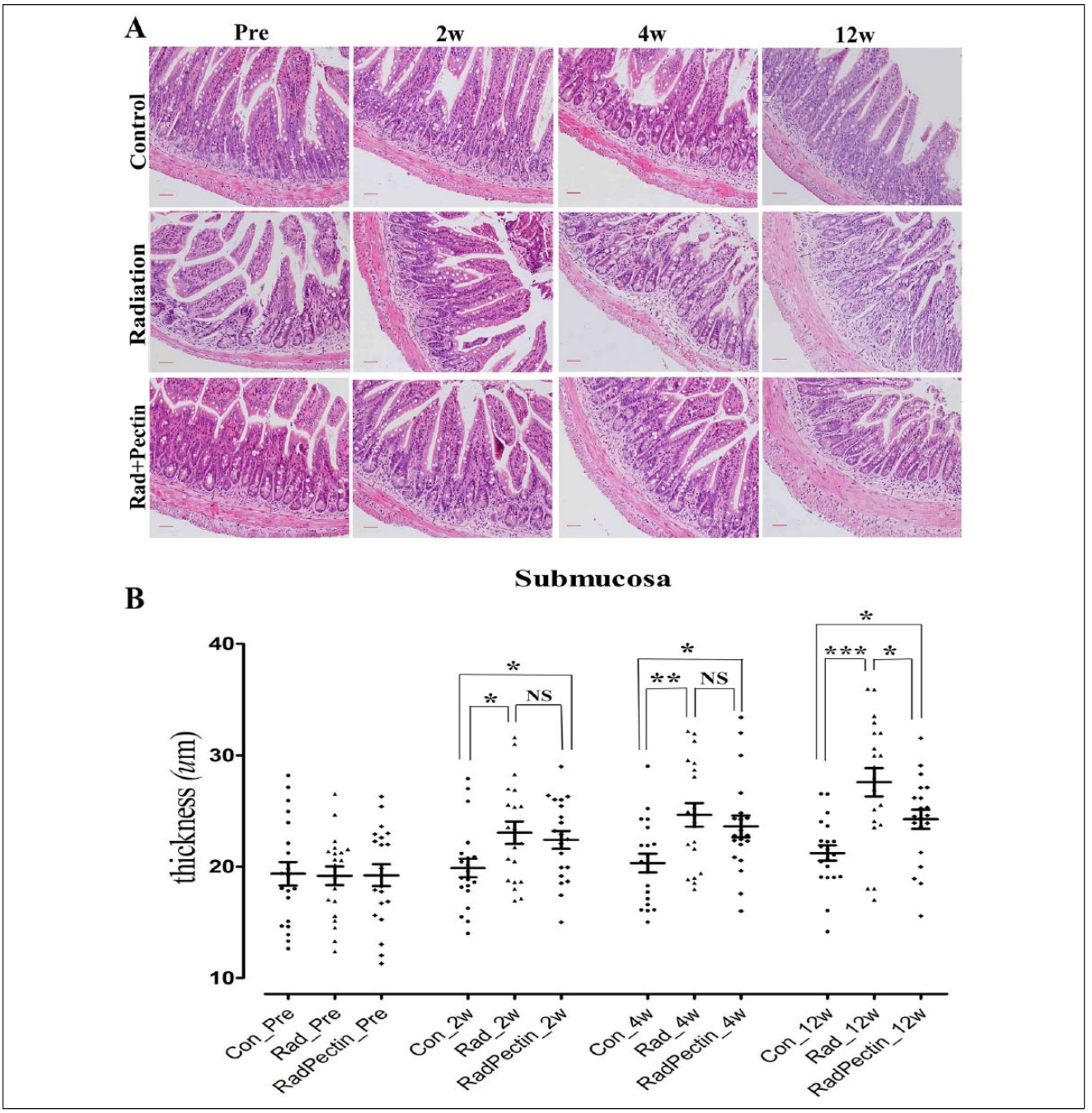

Figure 1. Dietary pectin ameliorates radiation-induced thickened submucosa in the ileum. Hematoxylin and eosin staining (A) and quantitative analysis (B) showed thickened submucosa after irradiation and pectin reduced the increased submucosal thickness at 12 weeks (n = 4 each). The black line in each image indicates the thickness of submucosa. The results are shown as means ± SEMs. **P* < .05. ***P* < .01. ****P* < .001. Con, control; NS, not significant; Pre, preirradiation; Rad, radiation; RadPectin, radiation + pectin. Bars represent 50 μm.

assess the coexpression of the epithelial cell adhesion marker E-cadherin and fibroblast markers vimentin or α-SMA. E-cadherin$^+$/vimentin$^+$ and E-cadherin$^+$/α-SMA$^+$ coexpressing cells were clearly visible in mice after irradiation, while no coexpression was detected in the control group (Figure 3).

To further examine the role of EMT in ileal fibrosis, pVillin-Cre-EGFP double-transgenic mice were used. As described previously, the EGFP gene is activated in all intestinal epithelial-originated cells regardless of any further phenotypic changes.[8] Cells that coexpressed α-SMA$^+$/EGFP$^+$ or vimentin$^+$/

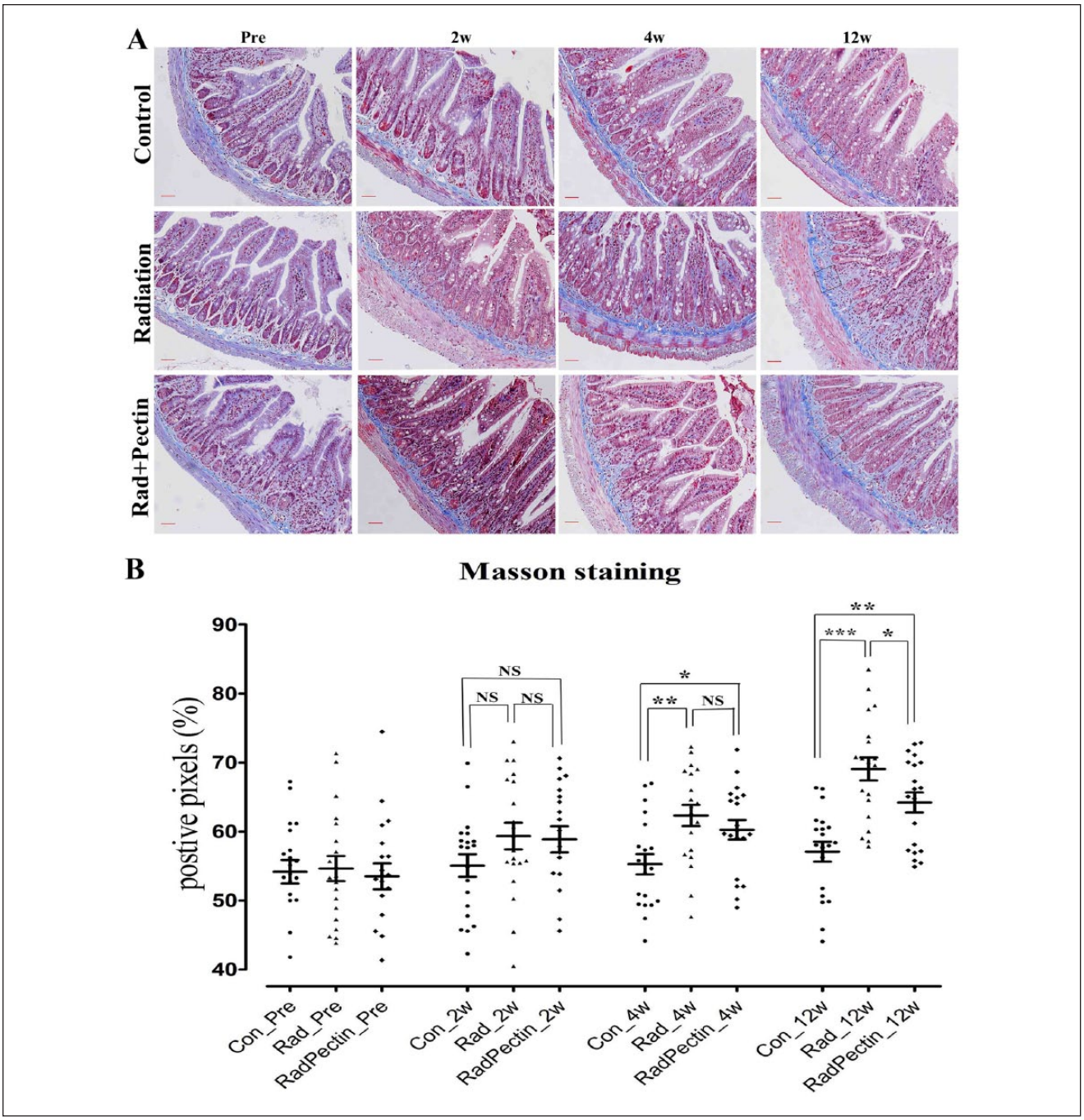

Figure 2. Dietary pectin ameliorates radiation-induced increased collagen deposition. (A) Masson's Trichrome staining showed increased collagen deposition after irradiation. (B) Quantitative analysis showed that pectin reduced collagen deposition induced by radiation at 12 weeks (n = 4 each). The results are shown as means ± SEMs. *$P < .05$. **$P < .01$. ***$P < .001$. Con, control; NS, not significant; Pre, preirradiation; Rad, radiation; RadPectin, radiation + pectin. Bars represent 50 μm.

EGFP$^+$ were observed in mice treated with radiation, as shown in Figures 4 and 5. Both vimentin$^+$/EGFP$^+$ and α-SMA$^+$/EGFP$^+$ coexpressing cells in the irradiated mice were most obvious at 2 weeks, and cell numbers decreased at 4 and 12 weeks. However, few α-SMA$^+$/EGFP$^+$ and vimentin$^+$/EGFP$^+$ coexpressing cells were seen in the control group and in mice prior to irradiation.

Dietary Pectin Modulates EMT Process After Irradiation

To evaluate the effect of dietary pectin on EMT, we next quantified the number of EMT cells in double-transgenic mice at different times after radiation (Figures 4 and 5). The number

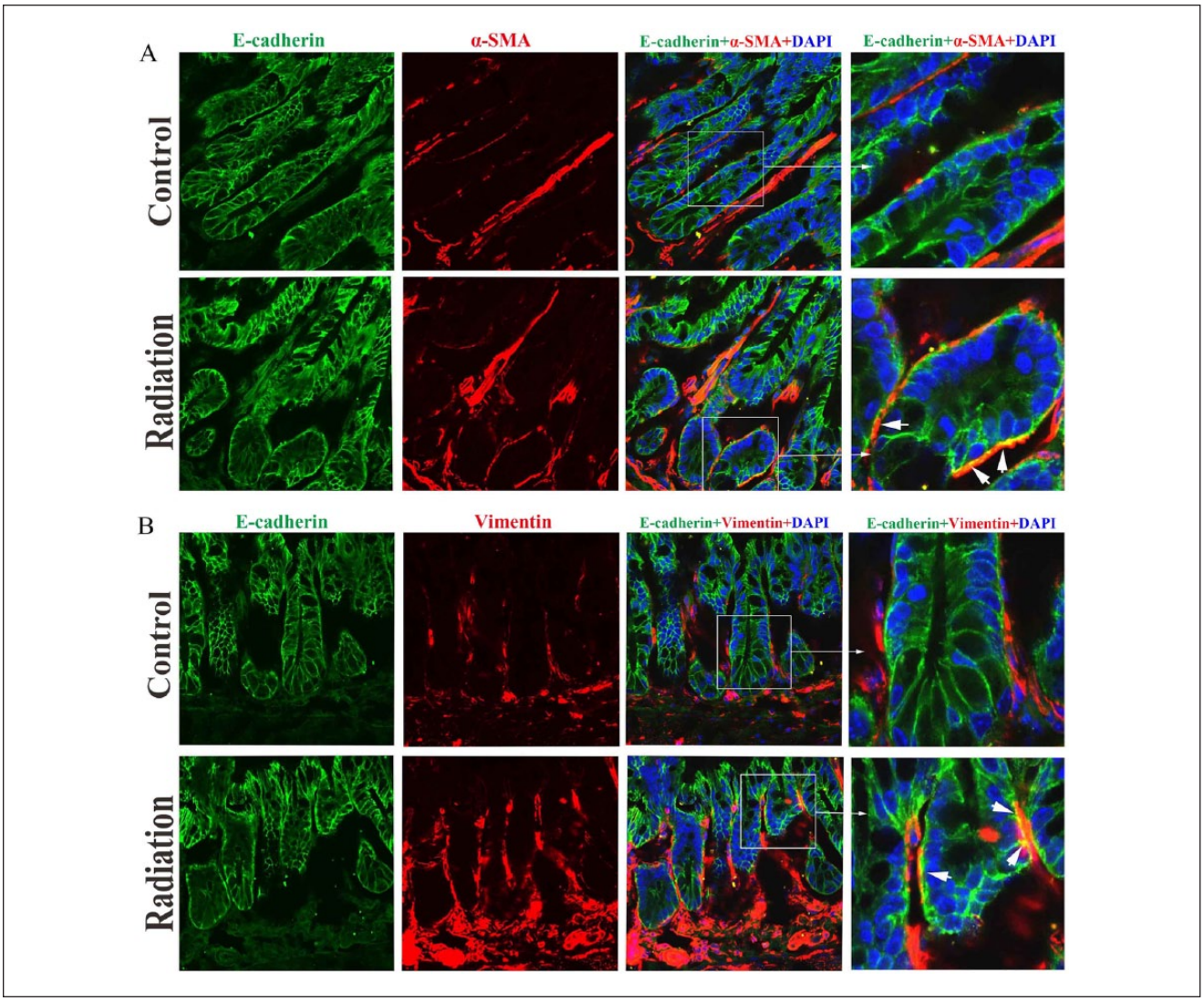

Figure 3. Evidence for the epithelial-to-mesenchymal transition in radiation-induced intestinal fibrosis. Frozen sections of terminal ileum tissue from mice (n = 4 each) were stained with antibodies to E-cadherin (green) and α–smooth muscle actin (SMA) (A) or vimentin (B) (red). The nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI). In the merged images, double-positive cells appear yellow. Representative images from each group are displayed. Con, control; NS, not significant; Pre, preirradiation; Rad, radiation; RadPectin, radiation + pectin.

of double-stained cells per visual field significantly decreased in the pectin-treated group at 2 and 4 weeks after irradiation compared with mice in the radiation group ($P < .001$ and $P < .05$ for vimentin$^+$/EGFP$^+$ cells; $P < .05$ and $P < .05$, for α-SMA$^+$/EGFP$^+$ cells, respectively).

Dietary Pectin Shapes Gut Microbiota

Recent studies have demonstrated that irradiation modifies the intestinal microbiota.[27–30] Thus, we next analyzed the effect of dietary pectin on the bacterial community in mice 2 weeks after irradiation. Stool was collected from 4 mice in each group, but DNA integrity was compromised in 2 samples, and therefore 3 mice in each group were examined. Irradiation markedly decreased the diversity of intestinal microbiota, while dietary pectin treatment decreased this downward trend (Figure 6A). Weighted UniFrac cluster analysis of gut microbiota profiles also indicated separation of the control, radiation, and pectin-treated mice (Figure 6B).

We next analyzed the gut microbiota composition at various taxonomic levels. At the phylum level, the proportion of Firmicutes decreased and those of Verrucomicrobia and Bacteroidetes increased after irradiation, while dietary pectin reversed this effect to some extent (Figure 6C). At the family level, Alcaligenaceae, Erysipelotrichaceae, Prevotellaceae, and Verrucomicrobiaceae markedly increased and Lactobacillaceae and Bifidobacteriaceae decreased after irradiation, while dietary pectin prevented these changes to some extent (Figure 6D).

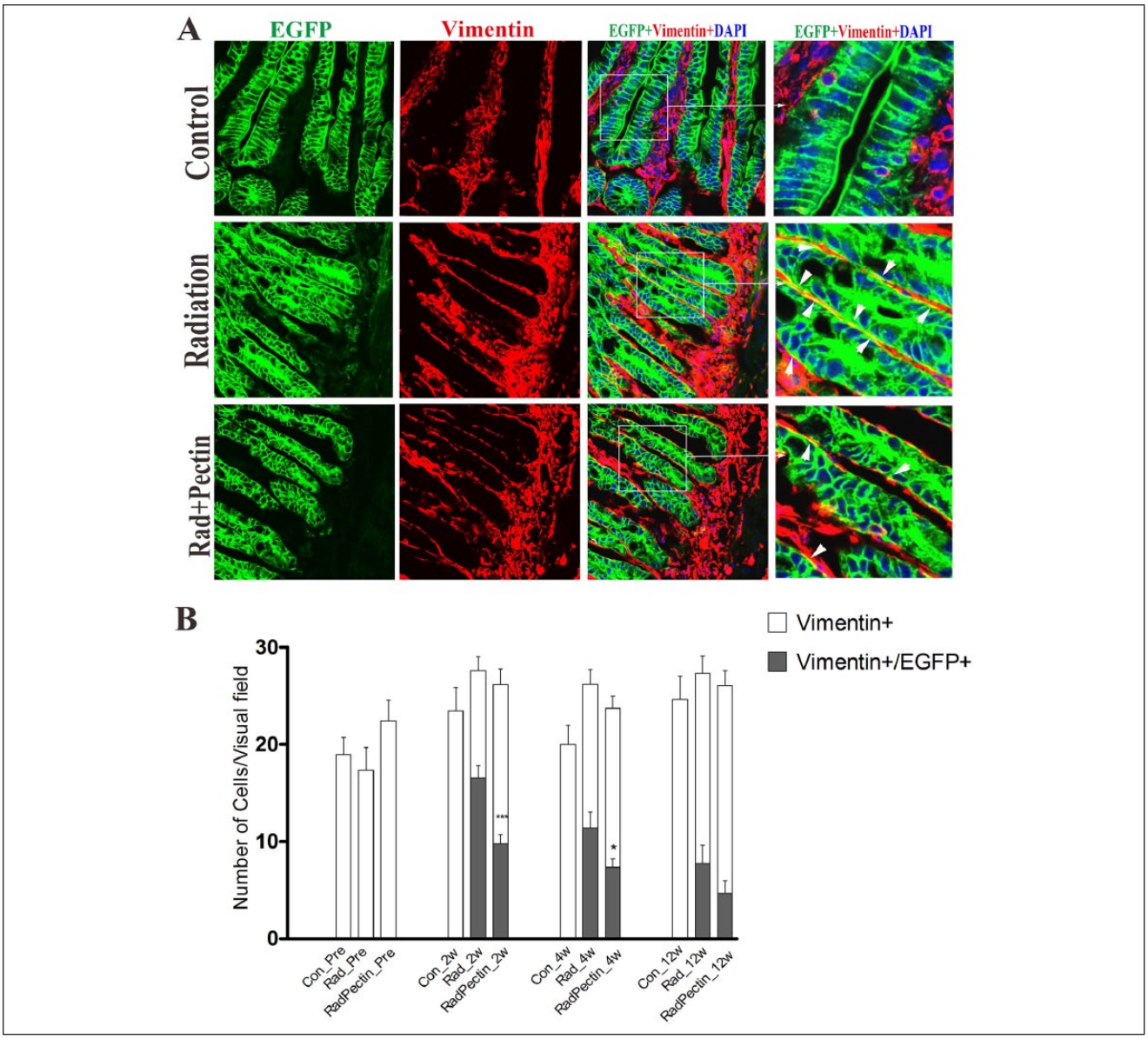

Figure 4. Pectin ameliorates radiation-induced epithelial-to-mesenchymal transition in double-transgenic mice. (A) Frozen terminal ileum sections from double-transgenic mice (n = 4 each) were stained with antibodies to vimentin (red) and enhanced green fluorescent protein (EGFP) (green). The nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI). Double-positive cells appear yellow. Representative immunofluorescent images from each group are displayed. (B) Data are presented as means ± SEMs. Con, control; NS, not significant; Pre, preirradiation; Rad, radiation; RadPectin, radiation + pectin. *$P < .05$. ***$P < .001$.

Linear discriminant analysis effect size revealed that the phylum Verrucomicrobia was significantly increased in irradiated mice, while the class Clostridia and genus *Ruminococcus* in Firmicutes phylum were overrepresented in control and pectin-treated groups, respectively (Figure 6E,F).

SCFA Concentrations

To characterize the changes in SCFAs after irradiation and investigate the effect of pectin treatment on these alterations, we analyzed the concentration of SCFAs in cecal feces. The concentrations of total SCFAs, acetate, and butyrate in mice after 2 weeks' irradiation were markedly reduced compared with those in control mice ($P = .057$, $P < .05$, and $P < .05$, respectively). However, dietary pectin treatment markedly increased the level of total SCFA, acetate, propionate, and butyrate compared with mice in the radiation group (all $P < .05$). The systemic ratio of the major SCFAs, including acetic, propionic, and butyrate, was 56:24:20, which is similar to those of previous studies.[12] The alterations of cecal SCFA concentration are shown in Figure 7.

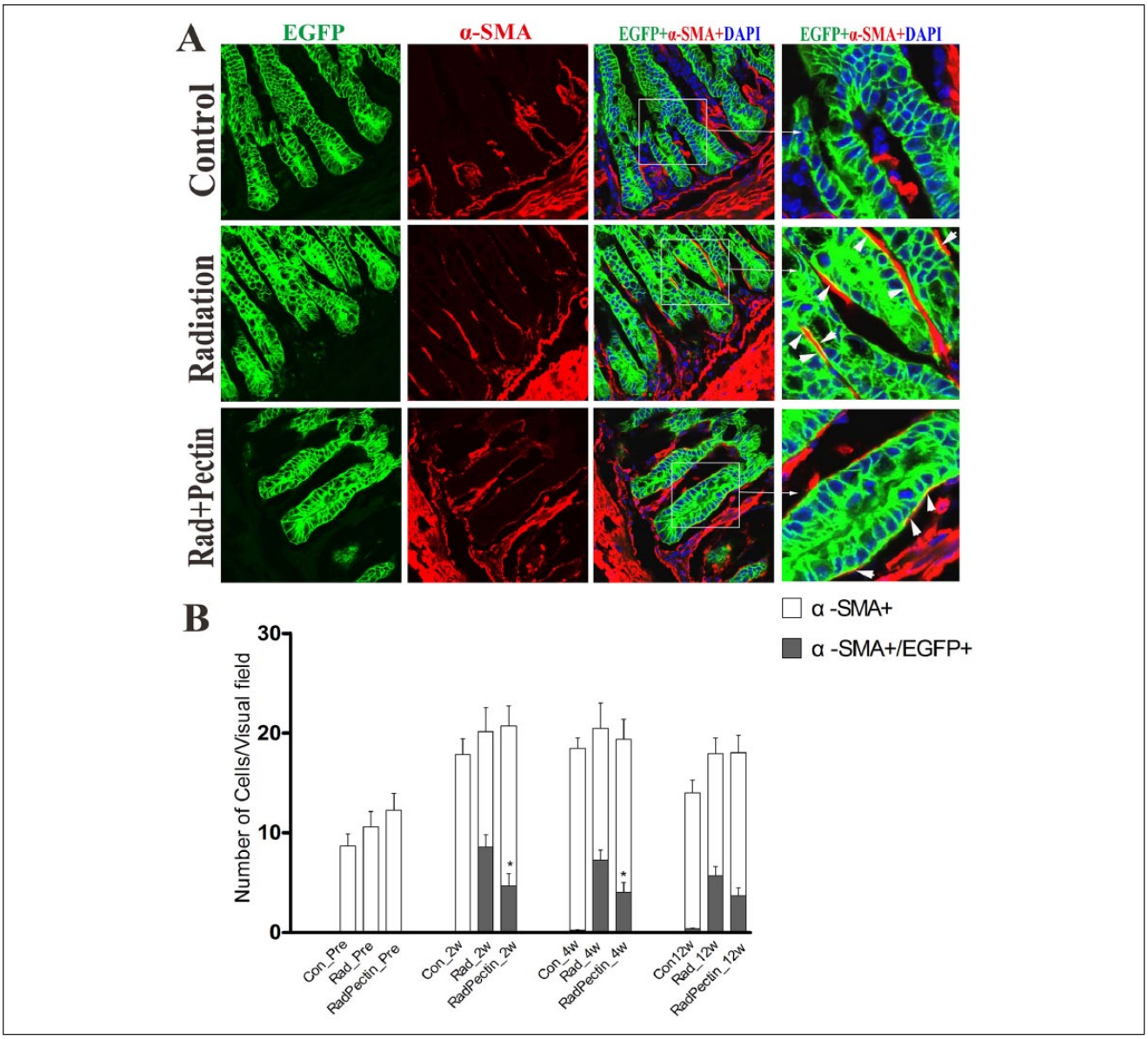

Figure 5. Pectin ameliorates radiation-induced epithelial-to-mesenchymal transition in double-transgenic mice. (A) Frozen terminal ileum sections from double-transgenic mice (n = 4 each) were stained with antibodies to α–smooth muscle actin (SMA) (red) and enhanced green fluorescent protein (EGFP) (green). The nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI). Double-positive cells appear yellow. Representative immunofluorescent images from each group are displayed. (B) Data are presented as means ± SEMs. Con, control; NS, not significant; Pre, preirradiation; Rad, radiation; RadPectin, radiation + pectin. *$P < .05$.

Discussion

In the present study, we first examined the impact of gut microbiota metabolism, SCFA, on radiation-induced intestinal fibrosis. Our results showed that prophylactic use of pectin ameliorated the late intestinal fibrotic lesions of the ileum after irradiation and demonstrated the involvement of EMT in radiation-induced intestinal fibrosis.

Gut microbiota metabolism of dietary pectin, SCFA, plays a crucial role in maintaining the intestinal homeostasis and overall health status.[11,12,19,31] Sureban et al[18] reported that dietary pectin had a beneficial protective effect on the intestine in a mouse model of acute intestinal injury after irradiation by stimulating crypt proliferation. Kerem et al[32] reported that either oral dietary fiber or trans-rectal administration of SCFA improved colonic anastomosis healing and decreased matrix metalloproteinase-2 activity in rats after irradiation. However, the effect of SCFA on radiation-induced intestinal fibrosis has not been studied. We showed that dietary pectin treatment significantly ameliorated the intestinal fibrotic lesions from radiation.

Until now, several mouse models have been used in defining the mechanism of radiation-induced intestinal fibrosis, including

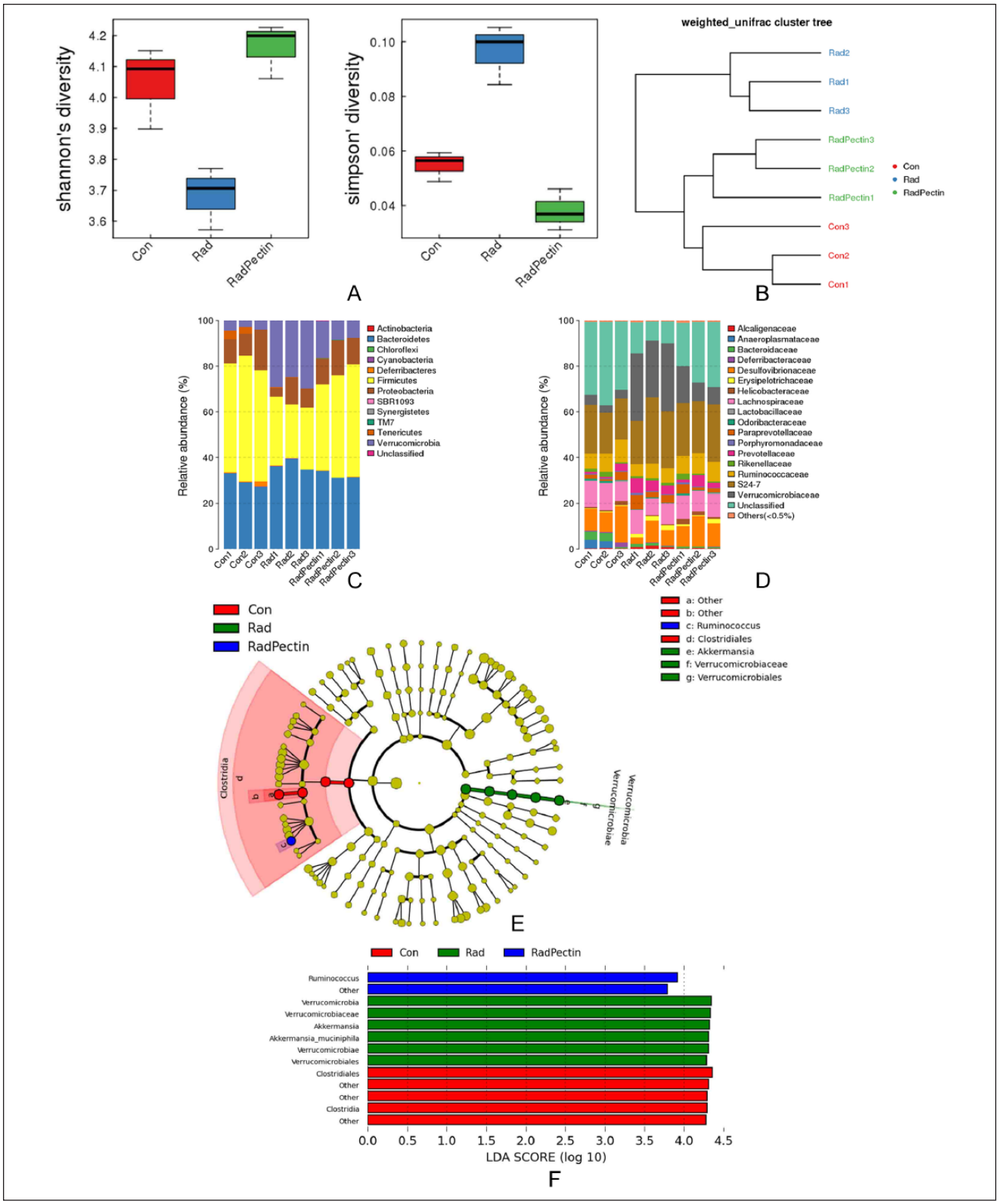

Figure 6. Pectin modifies intestinal microbiota composition at week 2 after irradiation. Microbiota diversity is expressed by Shannon's index and Simpson's index (n = 3 each) (A). Weighted UniFrac cluster analysis of gut microbiota in the 3 groups (B). 16S ribosomal RNA gene sequencing analysis of the fecal microbiota composition from mice at the phylum (C) and family (D) levels. Differentially abundant bacterial groups based on linear discriminant analysis (LDA) and effect size (LEfSe) (E, F). Data are presented as means ± SEMs (n = 3 each). Con, control; NS, not significant; Pre, preirradiation; Rad, radiation; RadPectin, radiation + pectin.

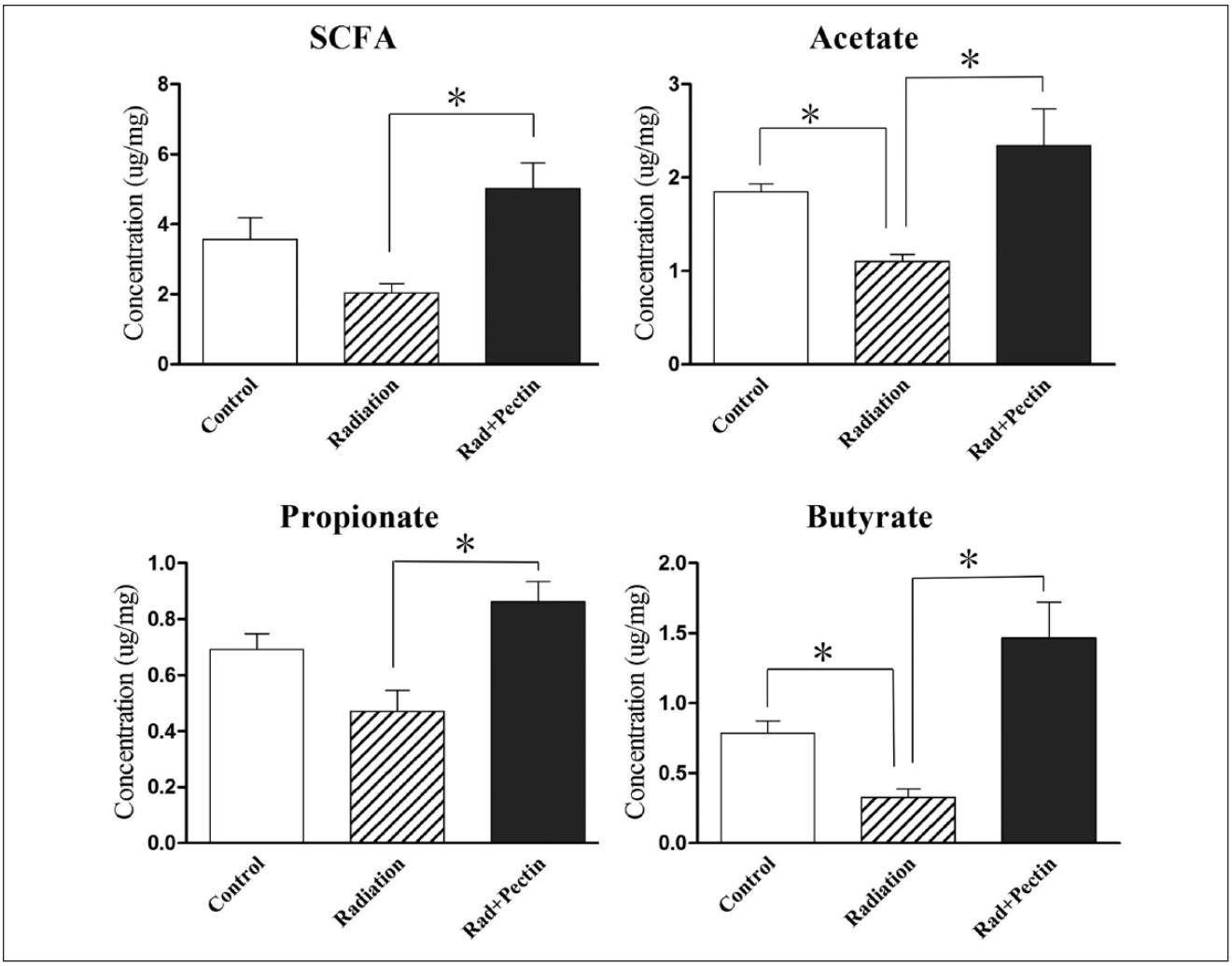

Figure 7. Pectin increases concentration of cecal short-chain fatty acid (SCFA) after irradiation. Gas chromatography–mass spectrometry analysis of total SCFA (A), acetate (B), propionate (C), and butyrate (D) concentration in cecum feces (n = 4 each). Data are presented as means ± SEMs. Con, control; NS, not significant; Pre, preirradiation; Rad, radiation; RadPectin, radiation + pectin. *$P < .05$.

irradiation of exteriorized small intestine, abdominal irradiation, and whole-body irradiation.[33–35] Yeh et al[36] showed that 10 Gy whole-body irradiation caused chronic intestinal fibrosis after irradiation. Linard et al[23] and Okoshi et al[24] established a murine model of late radiation-induced intestinal fibrosis with 10 and 15 Gy abdominal irradiation, respectively. In the current study, we established an intestinal fibrosis model after a single dose of 10 Gy abdominal irradiation. As previously described, a 10-Gy dose did not induce acute radiation enteritis or colitis.[23] In clinical settings, external irradiation of a total dosage of 45 Gy with 20–25 fractions is the common treatment.[37] Although the mouse model in the study cannot completely mimic the clinical scenario, it demonstrated the prophylactic effect of dietary pectin against late intestinal fibrosis after irradiation.

EMT is a common process associated with fibrosis in several organs, including kidney, liver, and lung.[5,38,39] During EMT, epithelial cells lose their polarized phenotype and convert into mesenchymal-like myofibroblasts, which are characterized by colocalization of epithelial markers, such as E-cadherin, and mesenchymal markers, such as α-SMA or vimentin. Recent studies have demonstrated the role of EMT in radiation-induced kidney and lung fibrosis.[38,40] However, the process of EMT in radiation-induced intestinal fibrosis has yet to be investigated. In our study, immunofluorescence staining of irradiated ileum showed cells coexpressing $EGFP^+/\alpha\text{-}SMA^+$ or $EGFP^+/vimentin^+$ in double-transgenic mice and $E\text{-}cadherin^+/\alpha\text{-}SMA^+$ or $E\text{-}cadherin^+/vimentin^+$ in wild-type mice. Our data implied that epithelial cells had gained a mesenchymal-like phenotype, which suggests a role for EMT in radiation-induced intestinal fibrosis. In addition, the numbers of EMT cells were proportionately decreased by dietary pectin treatment, which suggests that SCFA plays a role in inhibiting EMT.

The beneficial effects of SCFA are multiple and involve at least 2 mechanisms, including activation of G-protein-coupled

receptors (GPR41, GPR43, and GPR109A) and inhibition of histone deacetylases (HDACs).[14,31,41] In functioning as an HDAC inhibitor, SCFAs can operate as modulators of gene expression by inducing protein hyperacetylation, chromatin remodeling, transcriptional activation, and repression, resulting in cell cycle arrest and cell death.[15,38] Recent studies reported that HDAC inhibitors ameliorate organ fibrosis by suppressing extracellular matrix production, inhibiting myofibroblast activation, blocking EMT, and reducing proinflammatory cytokine production.[42,43] Chung et al[44] reported that SCFAs, as HDAC inhibitors, promote wound healing induced by irradiation and ameliorate skin fibrosis and tumorigenesis by suppressing expression of radiation-induced transforming growth factor (TGF)–β and tumor necrosis factor (TNF)–α. Wang et al[45] showed that sodium butyrate attenuated TGF-β–induced EMT and inhibited cell migration and invasion in hepatocarcinoma cells in vitro. Taken together, the effect of SCFA on the process of EMT and intestinal fibrosis might partly be attributed to their modulatory effect as HDAC inhibitors. The role of G-protein-coupled receptor activation in reducing the process of EMT is largely unknown and warrants further investigation. Previous studies demonstrated that irradiation induces preferential differentiation of T helper cells into Th2 polarization.[23,46] Linard et al[23] reported that the fibrotic response to radiation was more severe in a Th1-deficient mouse model compared with that in wild-type mice. This indicates that changes in the Th1/Th2 immune balance play a role in potentiating radiation-induced intestinal fibrosis. Bernard et al[19] found that oral administration of dietary pectin-derived acidic oligosaccharides shifts the Th1/Th2 balance toward a Th1 response through modifying the intestinal microbiota composition and attenuating the lung infection induced by *Pseudomonas aeruginosa*. Trompette et al[12] found that mice treated with dietary fiber showed improved allergic inflammation and inhibition of dendritic cell activation of Th2 effector cells in the lungs. SCFAs are not restricted to the intestinal tract but can be disseminated systemically and exert an influence on peripheral tissues, which could shift the Th1/Th2 balance to a Th1 response and stimulate Treg cells.[12,19,41,47] In light of these findings, research is under way on the effect of dietary fiber on immune balance during intestinal fibrosis in radiation-induced mouse models.

A further observation in our study was that gut microbial compositions were changed after irradiation and that dietary pectin had the potential to modify the intestinal community. The phyla Firmicutes markedly decreased and Verrucomicrobia and Bacteroidetes increased after irradiation, while dietary pectin restored the bacteria to control levels. Thus, similar to previous studies,[27,30,48,49] the decreased diversity of intestinal microbiota induced by irradiation could be reversed after dietary pectin treatment. Moreover, the concentration of SCFA and its main products, such as acetic, propionic, and butyrate, significantly decreased after irradiation, which may largely result from a decrease in gut microbiota caused by radiation injury.[29] Given the fact that dietary pectin can act as a prebiotic and has a potential benefit in modulating gut microbial composition, we speculate that the protective effect of dietary pectin against intestinal fibrosis is associated with its immunomodulation effect of SCFAs. However, the dynamic interaction between gut microbiota and host is a complex process, and future studies remain to be conducted.

In conclusion, we demonstrated that EMT plays a role in the process of radiation-induced intestinal fibrosis. Soluble dietary fiber reduced radiation-induced EMT and intestinal fibrosis possibly through modulating gut microbiota compositions and luminal SCFA concentration. As a consequence, prophylactic use of soluble dietary fiber prior to radiotherapy can be used in clinical practice to protect against radiation-induced late gastrointestinal complications.

Statement of Authorship

J. Gong, J. Yang, and C. Ding contributed to the conception and design of the research; W. Zhu, N. Li, and J. Li contributed to the design of the research; J. Yang, C. Ding, X. Dai, T. Lv, T. Xie, T. Zhang, and W. Gao contributed to the acquisition, analysis, and interpretation of the data; and J. Yang, J. Gong, and C. Ding drafted the manuscript. All authors critically revised the manuscript, agree to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved the final manuscript.

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