***Lactobacillus rhamnosus* FLRH93 protects against intestinal damage in mice induced by 5-fluorouracil**

Miaomiao Hu,¹ Xiaoli Wu,² Meng Luo,¹ Hua Wei,^{1,3} Di Xu,^{1*} and Feng Xu^{3*}

¹State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang, 330047, China

²College of Basic Medicine, Jiangxi University of Traditional Chinese Medicine, Nanchang, 330004, China

³Jiangxi-OAI Joint Research Institute, Nanchang University, 235 Nanjing East Road, Nanchang, 330047, China

ABSTRACT

5-Fluorouracil (5-FU) is widely used as a chemotherapeutic drug for the treatment of cancer but it has toxic side effects. It can induce severe intestinal damage and even lead to death. The purpose of this study was to investigate whether milk fermented with *Lactobacillus rhamnosus* FLRH93 could alleviate intestinal damage induced by 5-FU. The results of injury intervention in a mouse model showed that milk fermented with *Lb. rhamnosus* FLRH93 significantly ameliorated intestinal injury caused by 5-FU. The results of hematoxylin and eosin staining showed that mice fed *Lb. rhamnosus* FLRH93 preserved the villus/crypt ratio and reduced the loss of goblet cells in ileum sections of 5-FU-treated animal. Further, administration of fermented milk up-regulated expression of Bcl-2 in the intestinal tract and downregulated the expression of NLRP3, thus reducing the production of inflammatory factors interleukin 1- β and tumor necrosis factor- α . The survival rate of mice treated with fermented milk was twice that of mice not fed fermented milk after continuous oral administration of 5-FU. In conclusion, *Lb. rhamnosus* FLRH93 has positive effects on body injury and could be used to prevent intestinal damage caused by cancer chemotherapy.

Key words: probiotics, cancer chemotherapy, intestinal damage and permeability, inflammatory factor

INTRODUCTION

The morbidity and mortality associated with malignant tumors rank highest among all diseases and continue to rise. Worldwide in 2018, there were approximately 18.1 million new cancer cases and 9.6 million cancer deaths (Bray et al., 2018). Chemotherapy is

the principal treatment used in several types of cancer (Sonis, 1998; Longley et al., 2003; Wang et al., 2018). 5-Fluorouracil (**5-FU**) is widely used for the clinical treatment of colon, rectal, gastric, breast, chorionic epithelial, and liver cancers and malignant hydatidiform mole, among others (Anonymous, 1977; Liu et al., 2018). The drug interferes with DNA synthesis and induces apoptosis by blocking the conversion of deoxyribouridine to thymidylate by intracellular thymidylate synthetase (Pinedo and Peters, 1988; Miura et al., 2010).

However, treatment with 5-FU not only destroys neoplastic cells, it also affects cell populations of healthy tissue throughout the body (Duncan and Grant, 2003; Chen et al., 2018). More than 50% of patients treated with 5-FU suffer from oral and intestinal mucositis, which are accompanied by pain, bacteremia, and malnutrition (Decker-Baumann et al., 1999; Liu et al., 2012; Tang et al., 2017). These complications are caused by the proliferation of intestinal cells, decreased migration, and increased apoptosis, which together destroy the normal function of intestinal barrier (Yeung et al., 2015; Abalo et al., 2017). The destruction of the intestinal mucosa induces a decrease in nutrient absorption and an increase in susceptibility to infection and affects the quality of life of the patient (Duncan and Grant, 2003). In severe cases, the chemotherapy dosage has to be reduced or the treatment discontinued, which ultimately affects the treatment of cancer (Soveri et al., 2014).

Local anesthetics, analgesics, and antibiotics can be used to treat these side effects. However, these only alleviate the side effects of chemotherapy (Herbers et al., 2014) and do not reduce the duration or severity (Daniele et al., 2001). In recent years, clinical consensus supports the use of probiotics to mitigate intestinal mucosal damage because probiotics can alter the composition of intestinal microbial communities and their ability to improve epithelial barrier function (Shigemori et al., 2015; Bastos et al., 2016) and immune regulation (Justino et al., 2015; Zhang et al., 2016; Oh et al.,

Received November 2, 2019.

Accepted January 7, 2020.

*Corresponding authors: oai308@163.com and ncuskxudi@163.com

2017). Thus, the use of probiotic microecological agents can promote recovery from clinical radiotherapy and chemotherapy and reduce complications. Currently, the use of probiotics in this way is one of the most promising developments in the treatment of cancer.

Probiotics are living microorganisms that benefit the health of host when they are supplied in sufficient quantities (Hill et al., 2014). Recent studies have demonstrated that probiotics can improve antioxidant capacity, inhibit the activity of intestinal reactive oxygen species, and protect the function of intestinal epithelial barrier. Probiotics can improve intestinal barrier, regulate the inflammatory process, promote immune response, maintain intestinal health, inhibit the proliferation of pathogens, and reduce the colonization by pathogens in the intestine (Ewaschuk et al., 2008; Seth et al., 2008; Mennigen et al., 2009). More specifically, lactic acid bacteria and bifidobacteria can produce short-chain fatty acids, which are involved in the metabolism of vitamins and promote the absorption of nutrients (Salminen and Salminen, 1997; Kociubinski et al., 1999). In addition, probiotics are helpful to maintain the integrity of intestinal mucosal barrier (De Jesus et al., 2019).

Thus, probiotics may have a potential therapeutic effect on intestinal injury induced by chemotherapy. The specific repair mechanism of probiotics on intestinal injury remains unknown. Therefore, the aim of this study was to explore the protective effect of probiotics on intestinal injury induced by 5-FU in a BALB/c mice model and its possible mechanism.

MATERIALS AND METHODS

Screening of Probiotic Strains by Cell Injury Model

In this study, 87 probiotics preserved in our laboratory were screened to identify those with antioxidant capacity in co-incubation experiments. Caco-2 cells (100 μ L/well) were cultured in Dulbecco's modified Eagle medium (DMEM, Solarbio, Beijing, China) supplemented with 10% fetal bovine serum and antibiotics (100 U/mL penicillin, Solarbio; 100 μ g/mL streptomycin, Solarbio) for 24 h at 37°C under 5% CO₂ in 96-well plate and harvested at a density of 1×10^5 cells/mL. The supernatant was removed. Probiotics were added, cells were resuspended in DMEM at a density of 1×10^7 cfu/mL, and incubated for 0.5 h as the experimental group. Cells in the control group and H₂O₂ group were incubated with sterilized DMEM medium for 0.5 h. Then, H₂O₂ at a final concentration of 1,000 μ M was added and incubated for 1.5 h (H₂O₂ group), and the control group was incubated in DMEM without

H₂O₂ for 1.5 h. After incubation, the supernatant was obtained, and the leakage rate of lactate dehydrogenase (LDH) was measured using an LDH kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) to evaluate the level of cell damage.

Preparation of Milk Fermented with *Lactobacillus rhamnosus* FLRH93

Frozen bacterial suspension (−80°C) of *Lb. rhamnosus* FLRH93 was reactivated in de Man, Rogosa, and Sharpe (MRS) broth (Solarbio) at 37 °C for 16 h. Next, 1 mL of the culture was inoculated into 100 mL of skim milk (10% wt/vol) and incubated under anaerobic conditions at 37°C for 24 h to a final density of 2×10^9 cfu/mL to obtain fermented milk.

Mouse Model of Intestinal Injury

All experiments were conducted on female BALB/c mice (6–8 wk old, BW 21–24 g) that were obtained from Animal Experimental Center of Nanchang University Medical College (Nanchang, China). Mice were kept in polycarbonate boxes under controlled conditions: $\sim 22 \pm 2^\circ\text{C}$, humidity of $65\% \pm 5\%$, and a 12-h light/dark photoperiod. All animal care and experimental procedures used for the care and use of laboratory animals were in accordance with the guidance of the Department of Animal Science in Nanchang University. Mice were randomly divided into 4 groups (10 per group): control group (CTL), control + probiotic (FLRH93), model group (5-FU), and model + probiotic group (5-FU+FLRH93). The CTL and 5-FU groups were intragastrically administered 100 μ L of skim milk per day. The FLRH93 and 5-FU+FLRH93 groups were intragastrically administered with 100 μ L of *Lb. rhamnosus* FLRH93 fermented milk per day. Gastrointestinal mucositis was induced on d 10 by a single intraperitoneal (i.p.) injection of 5-FU (300 mg/kg) administered in the 5-FU and 5-FU+FLRH93 groups, according to previous reports (Carvalho et al., 2017). The CTL and FLRH93 groups were injected i.p. with 0.9% NaCl (wt/vol). Animals were euthanized with anhydrous ether on d 14 of the experiment (Figure 1A). Blood and small intestine were collected for further analysis. The weight and food intake were counted daily.

Intestinal Histology and Morphological Analysis

After the mice were euthanized, the entire small intestine was taken out and measured, after which 2 to 3 cm of ileum was taken for histological analysis. Tissue samples were placed in 10% buffered formaldehyde

for 24 h and then embedded in paraffin wax. Sections (4 μ m) were mounted on glass slides and stained with hematoxylin and eosin (HE) or periodic acid Schiff (PAS). In HE-stained slides, alterations of the mucosal architecture and polymorphonuclear cells infiltrate were analyzed using a histopathological grading system (Soares et al., 2008). Ten images per specimen were captured using a microscope (Eclipse E100, Nikon, Tokyo, Japan), and digital images were processed using Image-Pro Express 4.0 (Media Cybernetics, Bethesda, MD) for morphological evaluation. Images were taken using a 40 $\times$ magnification objective. Villus height (VH) measurements (from villus tip to villus-crypt junction) and crypt depth (CD) (defined as invagination depth between adjacent villi) per small intestinal tissue section (10 sections/mouse) were measured and values presented as averages. The VH:CD ratio of the intestinal epithelium was also calculated. The study of goblet cells was done in PAS-stained samples.

Intestinal Permeability

Two complementary methods were used to evaluate intestinal permeability. In both, 3 to 4 cm of small intestine was cut from intestine of mice in all 4 groups. The intestinal contents were washed 3 times with sterilized cooled PBS (Solarbio). Fifty microliters of blue dextran 2000 (20 mg/mL; Solarbio) (method 1) or dextran-fluorescein isothiocyanate (dextran-FITC, 20 mg/mL; Sigma, St. Louis, MO) (method 2) was added into the small intestine by pipette and both ends of the intestine segment were sealed with sterilized cotton thread. Intestines were placed into the wells of a 6-well plate. Then, colorless DMEM containing 100 U/mL of penicillin and 100 μ g/mL streptomycin was added to a final volume of 4 mL and incubated at 37°C under 5% CO₂ for 2 h. For method 1, 100 μ L of culture was obtained from each well, and absorbance at 610 nm (method 1) or fluorescence values (method 2) at

Figure 1. The scheme of animal experiments. (A) Intervention effect of *Lactobacillus rhamnosus* FLRH93 fermented milk on injured mice induced by 5-fluorouracil (5-FU): BALB/c mice (n = 10 in each group) were treated with milk fermented with *Lb. rhamnosus* FLRH93 for 13 d. On d 10, intestinal injury was induced by intraperitoneal (i.p.) injection of 5-FU (300 mg/kg). The control group received 0.9% (wt/vol) NaCl (i.p.). (B) Protective effect of *Lb. rhamnosus* FLRH93 fermented milk on injured mice induced by 5-FU: BALB/c mice (n = 10 in each group) were treated with milk fermented with *Lb. rhamnosus* FLRH93 every day. On d 10 and 17, injury was induced by i.p. injection of 300 mg/kg 5-FU. The control group received 0.9% (wt/vol) NaCl (i.p.), and the survival rate of each group was counted after 22 d.

excitation and emission wavelengths at 490 and 530 nm, respectively, were determined to evaluate intestinal permeability.

Quantitative Real-Time PCR Analysis

Total RNA from intestine was extracted with Trizol according to the manufacturer's instruction (Sigma). Then, RNA was reverse transcribed into cDNA using the RT 2 First Strand Kit (Sigma). Quantitative real-time (q)PCR was performed on an ABI 7900 HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA) with RT 2 SYBR Green qPCR Mastermix (Sigma). Two primers for genes related to intestinal damage and housekeeping genes were purchased from Gen-eray Biotech (Sangon Biotech, China), including *Nlrp3* (reverse: 5'-GCAGCCCTGCTGTTTCAGCAC-3'; forward: 5'-CTGTGTGTGGGACTGAAGCAC-3') and *Bcl2* (reverse: 5'-TACTGCTTTAGTGAACCTTTT-3'; forward: 5'-TTGTGGCCTTCTTTGAGTTTCG-3'). The housekeeping gene *Gapdh* (reverse: 5'-CTGAACGGGAAGCTCACTGG-3'; forward: 5'-ATGCCTGCTTCACCACCTTC-3') was used for standardization. Differential expression was calculated as threshold cycle ($2^{-\Delta\Delta C_t}$).

Determination of Cytokines

Serum obtained from mice in all 4 groups was placed in a refrigerator at 4°C for 12 h and then centrifuged at $2,800 \times g$ for 10 min. The supernatant of serum

was collected for the determination of tumor necrosis factor- α (TNF- α) and IL-1 β by biotin-labeled double-antibody sandwich ELISA. The specific procedure was carried out using ELISA kits (Mouse IL-1 β ELISA kit and Mouse TNF- α ELISA kit, Solarbio).

Differential Analysis of Caco-2 Cells at the Transcriptional Level

Colon cancer Caco-2 cells were cultured to 1×10^5 cells/mL according to the requirements of transcriptome sequencing and medium was discarded. Then, 1×10^8 cfu/mL of *Lb. rhamnosus* FLRH93 was resuspended in DMEM without double antibody and incubated for 2 h. Total RNA was extracted by the Trizol method, and DNA impurities were digested with DNase I. Then, cDNA was synthesized using mRNA as a template. The cDNA was sent to BGI Company (Shenzhen, China) for high-throughput sequencing of the transcriptome to determine changes in Caco-2 cells at the transcription level induced by *Lb. rhamnosus* FLRH93.

Immunohistochemical Detection of Bcl-2 and NLRP3

Immunohistochemical staining of Bcl-2 and neutrophilic alkaline phosphatase (NLRP3) was performed on ileum cross-sections. Paraformaldehyde-fixed, paraffin-embedded intestinal sections were dried on polylysine-treated glass slides. Slides were deparaffinized and rehydrated. Endogenous peroxidase was quenched in 3% H_2O_2 and methanol solution. The slides were

Figure 2. We investigated oxidative stress in Caco-2 cells induced by H_2O_2 and evaluated the protective effects of different probiotics. The leakage of lactate dehydrogenase (LDH) induced by H_2O_2 was significantly decreased in the presence of 28 of the 87 probiotics tested; strain *Lactobacillus rhamnosus* FLRH93 demonstrated the best protection against H_2O_2 and was thus selected for further study. Data were presented as means $\pm$ SD of 3 independent experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; compared with the H_2O_2 group.

covered with normal goat serum for 20 min and then treated with mouse monoclonal antibodies against Bcl-2 and NLRP3 (1:100; Boster Biological Technology, Wuhan, China) overnight. After washing, the slides were incubated with a biotin-conjugated goat-anti-mouse antibody and then incubated by horseradish peroxidase-conjugated streptavidin (Boster Biological Technology) for 30 min. Diaminobenzidine was used as the immunodetection substrate. Stained slides were observed using a light microscope. Quantification of im-

munohistochemical staining was processed by an Image Pro-Plus program (Media Cybernetics Inc., Rockville, MD) and measured as average optical density (Brey et al., 2003).

Western Blotting

Mice ileum samples (CTL, FLRH93, 5-FU, 5-FU+FLRH93 groups) were rinsed with PBS and ground into powder under liquid nitrogen. Then, the

Figure 3. Food intake (A) and percentage BW change of mice (B). CTL = control group, fed 100 μ L/d of skim milk; FLRH93 = group fed 100 μ L/d of *Lactobacillus rhamnosus* FLRH93-fermented milk; 5-FU = group fed 100 μ L/d of skim milk and injected with 300 mg/kg 5-fluorouracil (5-FU) on d 10; 5-FU+FLRH93 = group fed 100 μ L/d of *Lb. rhamnosus* FLRH93-fermented milk and injected with 300 mg/kg 5-FU on d 10. The results represent the average of 3 independent experiments $\pm$ SD; * P < 0.05, ** P < 0.01, *** P < 0.001: compared with the CTL group; # P < 0.05: 5-FU+FLRH93 group compared with the 5-FU group.

powder was dissolved in protein loading buffer at a ratio of 1:10 (tissue/buffer) and boiled for 5 min. After cooling to room temperature, the supernatant was used. Western blotting and data analysis were performed according to Chen et al. (2018). Antibodies used (all from Solarbio) were anti-NLRP3 polyclonal antibody (rabbit, 1:1,000), anti-Bcl2 polyclonal antibody (rabbit, 1:1,000) and anti- β -actin polyclonal antibody (rabbit, 1:1,000) and goat anti-rabbit IgG/horseradish peroxidase antibody (rabbit, 1:5,000).

Protective Effect of *Lb. rhamnosus* FLRH93 on Mice Injured by Chemotherapy

To examine the potential protective effect of *Lb. rhamnosus*, mice were divided into the same groups as before and administered 100 μ L of skim milk per day (CTL and 5-FU groups) or 100 μ L of fermented milk per day (FLRH93 and 5-FU+FLRH93 groups). Gastrointestinal mucositis was induced on d 10 by a single i.p. injection of 5-FU (300 mg/kg) administered in the 5-FU and 5-FU+FLRH93 groups, and mice in the CTL and FLRH93 groups were injected i.p. with 0.9% NaCl (wt/vol). On d 17, mice in the 5-FU and 5-FU+FLRH93 groups were given a second i.p. injection of 5-FU, and those in the CTL and FLRH93 groups were injected with 0.9% NaCl (wt/vol). Survival was assessed daily (Figure 1B) until mice were euthanized on d 22.

Statistical Analysis

All experiments were performed 3 times, and statistical analysis was performed using SPSS 13.0 (IBM Corp., Armonk, NY). GraphPad Prism 5 (GraphPad Inc., San Diego, CA) was used to draw plots, and *t*-tests were used to determine differences between experimental and control groups. Differences between groups were compared using one-way ANOVA. $P < 0.05$ was considered statistically significant.

RESULTS

Screening Probiotic Strains by Cell Damage Model

We investigated oxidative stress in Caco-2 cells induced by H_2O_2 and the protective effects of probiotics. The leakage of LDH induced by hydrogen peroxide was significantly decreased in the presence of 28 of 87 probiotics tested (Figure 2). Thus, these 28 probiotic strains could effectively protect Caco-2 cells from oxidative stress induced by hydrogen peroxide. Notably, *Lb. rhamnosus* FLRH93 decreased the amount of LDH leakage at the highest level (about 53% of the group

treated with H_2O_2); therefore, this strain was chosen for the further animal injury experiment.

Milk Fermented with *Lb. rhamnosus* FLRH93 Attenuated BW Loss

Before the induction of intestinal injury, all groups of mice consumed the same amount of food (about 6.3 g/d per animal), with no significant difference between groups. There was no significant change in food intake before and after induction of mucositis in the 5-FU+FLRH93 group, but food intake in the 5-FU group was significantly reduced (4.9 ± 0.906 g, $P < 0.01$; Figure 3A). Analysis of daily BW showed no significant change in mice of the CTL and FLRH93 groups after 11 d, but BW of mice administered 5-FU (5-FU and 5-FU+FLRH93 groups) decreased ($P < 0.05$). After 15 d, the BW of mice in the 5-FU group was lower than that in the CTL group ($P < 0.001$), and the percentage BW loss in the 5-FU+FLRH93 group (about 3.5%) was lower than that in the 5-FU group ($P < 0.05$; Figure 3B). These results indicate that treatment with FLRH93 fermented milk can prevent BW loss induced by chemotherapy.

Figure 4. The effect of milk fermented with *Lactobacillus rhamnosus* FLRH93 on the length of intestine. (A) Appearance of small intestine; (B) measurement of small intestine length. CTL = control group, fed 100 μ L/d of skim milk; FLRH93 = group fed 100 μ L/d of *Lb. rhamnosus* FLRH93-fermented milk; 5-FU = group fed 100 μ L/d of skim milk and injected with 300 mg/kg 5-fluorouracil (5-FU) on d 10; 5-FU+FLRH93 = group fed 100 μ L/d of *Lb. rhamnosus* FLRH93-fermented milk and injected with 300 mg/kg 5-FU on d 10. The results represent the average of 3 independent experiments $\pm$ SD; * $P < 0.05$; compared with the CTL; # $P < 0.05$; 5-FU+FLRH93 group compared with 5-FU.

Milk Fermented with *Lb. rhamnosus* FLRH93 Protects the Integrity of Intestine

It can be seen from Figure 4A that the length of small intestine of mice in the 5-FU group was shorter than that of mice in other groups. As shown in Figure 4B, the length of small intestine of mice in the 5-FU group was significantly less than that of mice in the CTL group ($P < 0.05$). However, compared with that of the 5-FU group, the length of small intestine of mice in the 5-FU+FLRH93 group was significantly longer ($P < 0.05$).

Change in intestinal permeability is another side effect of 5-FU. To determine whether the application of milk fermented with *Lb. rhamnosus* FLRH93 could prevent mucosal damage, blue dextran was chosen as a marker to evaluate intestinal permeability (Cserr and Ostrach, 1974). As shown in Figure 5A, absorbance (measured as optical density at 610 nm, OD_{610}) values were significantly higher in the 5-FU group than in the CTL group, and values were significantly lower in the 5-FU+FLRH93 group than in the 5-FU group. Compared with that of other groups, intestine in the 5-FU group was stained blue (Figure 5B). These results confirmed that intestinal cells were damaged by 5-FU

and the intestinal structure was incomplete, resulting in penetration of blue dextran 2000 into the intestine. Intestinal cells were not stained blue after oral administration of *Lb. rhamnosus* FLRH93, indicating that milk fermented with *Lb. rhamnosus* FLRH93 alleviated the intestinal injury caused by intestinal mucositis.

Dextran FITC is a marker molecule commonly used to evaluate tissue barrier leakage using a combination of Evans blue and high-molecular-weight FITC dextran (Hoffmann et al., 2011). A higher fluorescence value indicates more serious intestinal leakage. As shown in Figure 5C, only the fluorescence values of the 5-FU group were significantly higher ($P < 0.01$) than those of the CTL group. The fluorescence values in the 5-FU+FLRH93 group were significantly lower ($P < 0.05$) than those of the 5-FU group. Thus, we showed that milk fermented with *Lb. rhamnosus* FLRH93 could prevent the decrease in intestinal length and increase of intestinal permeability caused by treatment with 5-FU.

Effect of *Lb. rhamnosus* FLRH93 on Changes in Intestinal Morphology Induced by 5-FU

The HE staining (Figure 6A) showed that intestinal tissue structure in mice of the CTL and FLRH93

Figure 5. The effects of milk fermented with *Lactobacillus rhamnosus* FLRH93 on the length and permeability of small intestine. (A) Small intestine after gastric administration of blue dextran 2000; (B) small intestine washed with PBS after incubation of blue dextran 2000 for 2 h; (C) optical density at 610 nm (OD_{610nm}) value of incubation medium in blue dextran 2000; (D) fluorescence value of incubation medium in dextran-FITC. CTL = control group, fed 100 μ L/d of skim milk; FLRH93 = group fed 100 μ L/d of *Lb. rhamnosus* FLRH93-fermented milk; 5-FU = group fed 100 μ L/d of skim milk and injected with 300 mg/kg 5-fluorouracil (5-FU) on d 10; 5-FU+FLRH93 = group fed 100 μ L/d of *Lb. rhamnosus* FLRH93-fermented milk and injected with 300 mg/kg 5-FU on d 10. The results represent the average of 3 independent experiments $\pm$ SD; * $P < 0.05$, ** $P < 0.01$: compared with CTL; # $P < 0.05$: 5-FU+FLRH93 compared with 5-FU.

Figure 6. (A) Histopathological sections of stained mucosal (20× objective, scale bar = 100 µm); (B) morphometric analysis of villus height; (C) morphometric analysis of crypt depth; (D) ratio of villus height to crypt depth; (E) Alcian blue-periodic acid Schiff staining of ileum; the blue color indicates goblet cells (20× objective, scale bar = 100 µm); (F) number of goblet cells/field for experimental groups. CTL = control group, fed 100 µL/d of skim milk; FLRH93 = group fed 100 µL/d of *Lactobacillus rhamnosus* FLRH93-fermented milk; 5-FU = group fed 100 µL/d of skim milk and injected with 300 mg/kg 5-fluorouracil (5-FU) on d 10; 5-FU+FLRH93 = group fed 100 µL/d of *Lb. rhamnosus* FLRH93-fermented milk and injected with 300 mg/kg 5-FU on d 10. The results represent the average of 3 independent experiments ± SD; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; compared with the CTL; # $P < 0.05$, ## $P < 0.01$; 5-FU+FLRH93 compared with 5-FU.

groups was intact, but that of the 5-FU group was seriously damaged. After administration of fermented milk (5-FU+FLRH93 group), the morphology of intestinal tissue showed fewer signs of damage. In addition, mice in the 5-FU+FLRH93 group showed less intestinal damage and lesser changes in CD, VH, and VH:CD ratio compared with mice in the 5-FU group (shown in Figure 6B,C, and D).

Significantly fewer goblet cells were found in intestines of mice in the 5-FU group (36 ± 5 cells/field) than in mice of the CTL group (63.5 ± 4.5 cells/field; $P < 0.01$), as shown by Alcian blue-PAS staining. However, compared with the 5-FU group, mice in the 5-FU+FLRH93 group had more ($P < 0.05$) goblet cells (46 ± 3 cells/field; Figure 6E,F).

Effect of Milk Fermented with *Lb. rhamnosus* FLRH93 on Expression of Cytokines

The expression level of cytokines in serum are shown in Figure 7A,B. Compared with that in the CTL group, expression of TNF- α and IL-1 β in serum (163.9 and 159.8%, respectively) was significantly higher ($P < 0.05$ and $P < 0.001$, respectively) in the 5-FU group. Expression of TNF- α and IL-1 β was significantly lower ($P < 0.05$ and $P < 0.001$, respectively) in the 5-FU+FLRH93 group than in the 5-FU group (Figure 7A,B).

Differential Analysis of Caco-2 Cells at the Transcription Level

We assessed differential expression of genes among samples. As shown in Figure 8A, transcriptome analysis revealed that 1,743 genes were differentially expressed in mice treated with *Lb. rhamnosus* compared with the control group. Of these, 917 were upregulated and 826 downregulated. In addition, expression of NLRP3 decreased by 22.5% and that of Bcl-2 increased by 175% compared with the control group. Different genes coordinate with each other to perform their biological functions in organisms. Differentially expressed genes involved in the main metabolic pathway and signal transduction pathway were depicted through enrichment of pathways in Kyoto Encyclopedia of Genes and Genomes (Figure 8B). The differentially expressed genes were abundant in toll-like receptor signal pathway and transcription regulation.

Effect of Fermented Milk on Expression and Activity of NLRP3 and Bcl-2 Protein

Expression of Bcl-2 decreased significantly and that of NLRP3 increased significantly in mice treated with

5-FU, as shown by reverse transcription-qPCR and Western blotting. Interestingly, the increase in *Nlrp3* expression and decrease in Bcl-2 expression were changed slightly after treatment with *Lb. rhamnosus* (Figure 9A, B, C, D, and E). Further, the immunohistochemistry results showed that milk fermented with *Lb. rhamnosus* increased the expression of Bcl-2 and decreased that of NLRP3 relative to that induced by 5-FU (Figure 9F, G, H, and I).

Milk Fermented with *Lb. rhamnosus* FLRH93 Has a Protective Effect on Mice Injured by Chemotherapy

The above experimental results confirmed that *Lb. rhamnosus* FLRH93 had a protective effect on mice

Figure 7. Results from ELISA of (A) tumor necrosis factor (TNF)- α , and (B) IL-1 β in serum. CTL = control group, fed 100 μ L/d of skim milk; FLRH93 = group fed 100 μ L/d of *Lactobacillus rhamnosus* FLRH93-fermented milk; 5-FU = group fed 100 μ L/d of skim milk and injected with 300 mg/kg 5-fluorouracil (5-FU) on d 10; 5-FU+FLRH93 = group fed 100 μ L/d of *Lb. rhamnosus* FLRH93-fermented milk and injected with 300 mg/kg 5-FU on d 10. The results represent the average of 3 independent experiments $\pm$ SD; * $P < 0.05$, *** $P < 0.001$: compared with the CTL; # $P < 0.05$, ### $P < 0.001$: 5-FU+FLRH93 compared with 5-FU.

Figure 8. Transcriptomics analysis (Li et al., 2019) showing differentially expressed genes and pathways in Caco-2 cells treated with *Lactobacillus rhamnosus* FLRH93: (A) scatter diagram analysis of differentially expressed genes involved in the metabolic pathway; (B) scatter diagram of differentially expressed genes in different pathways.

treated with 5-FU, a drug often used in chemotherapy. However, cancer patients generally receive multiple chemotherapy drugs rather than a single drug. To investigate whether *Lb. rhamnosus* FLRH93 has an effect on repeated doses of chemotherapy, mice received chemotherapy twice as shown in Figure 1B. We observed no deaths in the CTL and FLRH93 groups (controls). After 22 d, the survival rate (60%) of mice in the 5-FU+FLRH93 group was numerically higher than that of the 5-FU group (30%; Figure 10), indicating that milk fermented with *Lb. rhamnosus* FLRH93 by gavage had a protective effect on injury induced by chemotherapy.

DISCUSSION

A longer small intestine provides more absorption area for nutrition intake and reduces the loss of water and electrolytes. Thus, it plays a positive role in the homeostasis of the internal environment. Compared with mice in the CTL group, mice in the 5-FU group had small intestines that were 6.3% shorter and mice in the 5-FU+FLRH93 group had small intestines that were 1.6% shorter. A reduction in nutrient absorption in the small intestine is likely to lead to BW loss in mice, which is consistent with the results from Vieira et al. (2012) and de Barros et al. (2018). We showed that

Figure 9. The effect of *Lactobacillus rhamnosus* FLRH93 on the expression of NLRP3 and Bcl-2 proteins in mice with intestinal injury induced by 5-fluorouracil (5-FU). Quantitative PCR of (A) Bcl-2, and (B) NLRP3; (C) Western blotting of Bcl-2 and NLRP3; quantitative values of (D) Bcl-2, (E) NLRP3 from Western blotting; immunohistochemical section (F) and quantitative value (G) of Bcl-2; immunohistochemical section (H) and quantitative value (I) of NLRP3. CTL = control group, fed 100 μ L/d of skim milk; FLRH93 = group fed 100 μ L/d of *Lb. rhamnosus* FLRH93-fermented milk; 5-FU = group fed 100 μ L/d of skim milk and injected with 300 mg/kg 5-fluorouracil (5-FU) on d 10; 5-FU+FLRH93 = group fed 100 μ L/d of *Lb. rhamnosus* FLRH93-fermented milk and injected with 300 mg/kg 5-FU on d 10. The results represent the average of 3 independent experiments $\pm$ SD; * P < 0.05, ** P < 0.01, *** P < 0.001: compared with the CTL; # P < 0.05, ## P < 0.01, ### P < 0.001: 5-FU+FLRH93 compared with 5-FU. Scale bar = 25 μ m.

mice injured by 5-FU treatment had significant BW loss compared with mice in the CTL group. However, mice in the 5-FU+FLRH93 group showed lesser BW loss than mice in the 5-FU group, similar to the results of Maioli et al. (2014).

As reported by Sonis et al. (2004), intestinal damage increases intestinal permeability, leading to a loss of epithelial barrier function (Daniele et al., 2001; Song et al., 2013). In the current study, we observed that mice injected with 5-FU and treated with FLRH93 fermented milk changed intestinal permeability, as detected by blue dextran 2000 and dextran FITC. We observed that 5-FU-treated mice had decreased intestinal permeability when also treated with fermented milk

(5-FU+FLRH93). This is consistent with results showing that probiotics can reduce intestinal permeability induced by chemotherapy drugs (Favaro-Trindade and Grosso, 2000; Justino et al., 2014; Bastos et al., 2016).

Another important feature of intestinal injury is the change in intestinal structure and integrity, resulting in flattening of villi, infiltration of inflammatory cells into the lamina propria, and cell damage. These effects reduced the ratio of villi/crypt (Duncan and Grant, 2003; Lee et al., 2014; Sonis, 2004) and also increased the production of proinflammatory cytokines. In this study, mice who were injected with 5-FU and not fed with lactobacillus showed severe structural damage to the ileal mucosa. Mice from the 5-FU+FLRH93 group

Figure 9 (Continued). The effect of *Lactobacillus rhamnosus* FLRH93 on the expression of NLRP3 and Bcl-2 proteins in mice with intestinal injury induced by 5-fluorouracil (5-FU). Quantitative PCR of (A) Bcl-2, and (B) NLRP3; (C) Western blotting of Bcl-2 and NLRP3; quantitative values of (D) Bcl-2, (E) NLRP3 from Western blotting; immunohistochemical section (F) and quantitative value (G) of Bcl-2; immunohistochemical section (H) and quantitative value (I) of NLRP3. CTL = control group, fed 100 μ L/d of skim milk; FLRH93 = group fed 100 μ L/d of *Lb. rhamnosus* FLRH93-fermented milk; 5-FU = group fed 100 μ L/d of skim milk and injected with 300 mg/kg 5-fluorouracil (5-FU) on d 10; 5-FU+FLRH93 = group fed 100 μ L/d of *Lb. rhamnosus* FLRH93-fermented milk and injected with 300 mg/kg 5-FU on d 10. The results represent the average of 3 independent experiments $\pm$ SD; * P < 0.05, ** P < 0.01, *** P < 0.001: compared with the CTL; # P < 0.05, ## P < 0.01, ### P < 0.001: 5-FU+FLRH93 compared with 5-FU. Scale bar = 25 μ m.

showed less intestinal damage and had villi length and crypt depth similar to that of controls. In addition, application of 5-FU reduces the number of goblet cells (Stringer et al., 2009; Yeung et al., 2015; Ciobanu et al., 2016). These cells are responsible for mucin secretion and, together with trefoil factor, are important components for epithelial protection (van Vliet et al., 2010). In the current study, the number of goblet cells decreased dramatically in 5-FU-treated mice. However, mice administered FLRH93 (5-FU+FLRH93 group) showed significantly higher numbers of goblet cells than mice administered only 5-FU. Thus, our results show that oral administration of FLRH93-fermented milk was able to attenuate mucosal damage in inflamed mice.

The immune system of the small intestine is connected to the systemic immune system through lymph and blood circulation (Deak and Csáky, 1984; Liang et al., 2016). Cytokines secreted by the intestinal immune system are important components of intestinal homeostasis (Schnoor, 2015; Hegazy et al., 2017). Overexpression of proinflammatory cytokines damage the immune system and aggravate intestinal lesions (Allam et al., 2012; Xie et al., 2016). Therefore, regulation of inflammatory mediators is very important for the prevention of intestinal injury.

The Bcl-2 protein plays an important role in the regulation of inflammation; Bcl-2 family proteins regulate the oligomerization of mitochondrial membrane, which leads to the release of cytochrome C and the inhibition of apoptosis. They also play an important role in

mitochondrion redox metabolism. In addition to the classic role of antiapoptotic proteins, Bcl-2 family proteins are also involved in redox regulation. According to Hockenbery et al. (1990), overexpression of Bcl-2 can protect cells from oxidative damage induced by H_2O_2 or menadione, and Bcl-2 can reduce the occurrence of inflammation by inhibiting apoptosis and antioxidant function (Hockenbery et al., 1990; Adams and Cory, 1998; Renault et al., 2017). Our results showed that milk fermented with *Lb. rhamnosus* FLRH93 upregulated expression of Bcl-2 protein in intestinal tissue of mice treated with 5-FU.

The inflammasome is a cellular solute protein system that responds to various external and internal stimuli. It contains the NOD-like receptor, apoptosis-related adaptor protein, and the precursor form of caspase-1 (Franchi et al., 2009; Bauer et al., 2010). The NLRP3 inflammatory corpuscle is the main representative of the NLRP family. It is an intracellular receptor that can regulate the inflammatory reaction and innate immune receptors inside the cell (Bauer et al., 2010; Neudecker et al., 2017). It mainly promotes the maturation and secretion of IL-1 β by regulating the activity of caspase-1 and thus participates in the regulation of the innate immune response. Interleukin-1 β can promote the infiltration of leukocytes, activate lymphocytes in an injured or infected site, and induce local and systemic inflammatory responses (Coccia et al., 2012). In addition, NLRP3 can stimulate macrophages to produce a large number of inflammatory cytokines, such as IL-1 β , IL-6, and TNF- α , thus triggering a strong

Figure 10. Survival curves of mice after intragastric administration of milk fermented with *Lactobacillus rhamnosus* FLRH93. CTL = control group, fed 100 μ L/d of skim milk; FLRH93 = group fed 100 μ L/d of *Lb. rhamnosus* FLRH93-fermented milk; 5-FU = group fed 100 μ L/d of skim milk and injected with 300 mg/kg 5-fluorouracil (5-FU) on d 10; 5-FU+FLRH93 = group fed 100 μ L/d of *Lb. rhamnosus* FLRH93-fermented milk and injected with 300 mg/kg 5-FU on d 10. Mice in the CTL and FLRH93 groups had the same survival status (curves overlap).

inflammatory response (Siegmund et al., 2001; Gao et al., 2017). Downregulation of these proinflammatory cytokines can reduce the occurrence and development of intestinal diseases. Our results indicated that *Lb. rhamnosus* FLRH93 could ameliorate the intestinal injury induced by chemotherapy. We identified 20 differentially expressed genes involved in the metabolic pathway that could have a regulatory effect in preventing cancer and antagonizing pathogens (Figure 8B). Thus, we speculate that *Lb. rhamnosus* FLRH93 not only controls inflammation but also participates in cell repair, regeneration, and recovery from damage to the intestinal tract. However, no related gene or molecular mechanisms has been identified; thus, further experiments need to be performed.

CONCLUSIONS

We showed that milk fermented with *Lb. rhamnosus* FLRH93 had a protective effect on intestinal injury induced by the chemotherapeutic agent 5-FU. In addition, *Lb. rhamnosus* FLRH93 reduced intestinal damage in mice by regulating the expression of Bcl-2 and NLRP3. This study expands the application of this strain, and supports the use of probiotic therapy and anticancer therapy in repair of body damage.

ACKNOWLEDGMENTS

This project was sponsored by the National Natural Science Foundation of China (NSF31260363, 31000048, 31260263, 31170091, Beijing), Hundred People Overseas Training Project of Jiangxi Association for Science and Technology (2016-07), and Natural Science Foundation of JiangXi (2019BAB205120). The authors have not stated any conflicts of interest.

REFERENCES

- Abalo, R., J. A. Uranga, I. Pérez-García, R. de Andrés, R. Girón, G. Vera, A. E. López-Pérez, and M. I. Martín-Fontelles. 2017. May cannabinoids prevent the development of chemotherapy-induced diarrhea and intestinal mucositis? Experimental study in the rat. *Neurogastroenterol. Motil.* 29:e12952–e12966. <https://doi.org/10.1111/nmo.12952>.
- Adams, J. M., and S. Cory. 1998. The Bcl-2 protein family: Arbiters of cell survival. *Science* 281:1322–1326. <https://doi.org/10.1126/science.281.5381.1322>.
- Allam, R., C. R. Scherbaum, M. N. Darisipudi, S. R. Mulay, H. Hägele, J. Lichtnekert, J. H. Hagemann, K. V. Rupanagudi, M. Ryu, C. Schwarzenberger, B. Hohenstein, C. Hugo, B. Uhl, C. A. Reichel, F. Krombach, M. Monestier, H. Liapis, K. Moreth, L. Schaefer, and H.-J. Anders. 2012. Histones from dying renal cells aggravate kidney injury via TLR2 and TLR4. *J. Am. Soc. Nephrol.* 23:1375–1388. <https://doi.org/10.1681/ASN.201111077>.
- Anonymous. 1977. Highlighting cancer treatment reports. *CA: A Cancer Journal for Clinicians* 27:188–189.
- Bastos, R. W., S. H. S. P. Pedrosa, A. T. Vieira, L. M. C. Moreira, C. S. França, C. T. Cartelle, R. M. E. Arantes, S. V. Generoso, V. N. Cardoso, M. J. Neves, J. R. Nicoli, and F. S. Martins. 2016. *Saccharomyces cerevisiae* UFMG A-905 treatment reduces intestinal damage in a murine model of irinotecan-induced mucositis. *Benef. Microbes* 7:549–557. <https://doi.org/10.3920/BM2015.0190>.
- Bauer, C., P. Duewell, C. Mayer, H. A. Lehr, K. A. Fitzgerald, M. Dauer, J. Tschoop, S. Endres, E. Latz, and M. Schnurr. 2010. Colitis induced in mice with dextran sulfate sodium (DSS) is mediated by the NLRP3 inflammasome. *Gut* 59:1192–1199. <https://doi.org/10.1136/gut.2009.197822>.
- Bray, F., J. Ferlay, I. Soerjomataram, R. L. Siegel, L. A. Torre, and A. Jemal. 2018. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* 68:394–424. <https://doi.org/10.3322/caac.21492>.
- Brey, E. M., Z. Lalani, C. Johnston, M. Wong, L. V. McIntire, P. J. Duke, and C. W. Patrick Jr.. 2003. Automated selection of dab-labeled tissue for immunohistochemical quantification. *J. Histochem. Cytochem.* 51:575–584. <https://doi.org/10.1177/002215540305100503>.
- Carvalho, R. D., N. Breyner, Z. Menezes-Garcia, N. M. Rodrigues, L. Lemos, T. U. Maioli, D. da Gloria Souza, D. Carmona, A. M. C. de Faria, P. Langella, J.-M. Chatel, L. G. Bermúdez-Humarán, H. C. P. Figueiredo, V. Azevedo, and M. S. de Azevedo. 2017. Secretion of biologically active pancreatitis-associated protein i (pap) by genetically modified dairy *Lactococcus lactis* NZ9000 in the prevention of intestinal mucositis. *Microb. Cell Fact.* 16:27–38. <https://doi.org/10.1186/s12934-017-0624-x>.
- Chen, X.-X., K. K.-H. Lam, Y.-B. Feng, K. Xu, S. C.-W. Sze, S. C.-W. Tang, G. P.-H. Leung, C. K.-F. Lee, J. Shi, Z.-J. Yang, S.-T. Li, Z.-J. Zhang, and K. Y.-B. Zhang. 2018. Ellagitannins from pomegranate ameliorates 5-fluorouracil-induced intestinal mucositis in rats while enhancing its chemotoxicity against HT-29 colorectal cancer cells through intrinsic apoptosis induction. *J. Agric. Food Chem.* 66:7054–7064. <https://doi.org/10.1021/acs.jafc.8b02458>.
- Ciobanu, L., M. Tantau, S. Valean, A. Parau, I. Bedecian, and R. Mirleneanu. 2016. Rifaximin modulates 5-fluorouracil-induced gastrointestinal mucositis in rats. *Eur. Rev. Med. Pharmacol. Sci.* 20:4993–5001.
- Coccia, M., O. J. Harrison, C. Schiering, M. J. Asquith, B. Becher, F. Powrie, and K. J. Maloy. 2012. IL-13 γ mediates chronic intestinal inflammation by promoting the accumulation of IL-17A secreting innate lymphoid cells and CD4⁺ Th17 cells. *J. Exp. Med.* 209:1595–1609. <https://doi.org/10.1084/jem.20111453>.
- Cserr, H. F., and L. H. Ostrach. 1974. Bulk flow of interstitial fluid after intracranial injection of blue dextran 2000. *Exp. Neurol.* 45:50–60. [https://doi.org/10.1016/0014-4886\(74\)90099-5](https://doi.org/10.1016/0014-4886(74)90099-5).
- Daniele, B., M. Secondulfo, R. De Vivo, S. Pignata, L. De Magistris, P. Delrio, R. Palaia, E. Barletta, R. Tambaro, and R. Carratù. 2001. Effect of chemotherapy with 5-fluorouracil on intestinal permeability and absorption in patients with advanced colorectal cancer. *J. Clin. Gastroenterol.* 32:228–230. <https://doi.org/10.1097/00004836-200103000-00010>.
- de Barros, P. A. V., M. E. Rabelo Andrade, S. de Vasconcelos Generoso, S. E. Mendes Miranda, D. C. dos Reis, P. C. Lacerda Leocádio, É. L. de Sales e Souza, F. dos Santos Martins, M. A. S. da Gama, G. D. Cassali, J. I. Alvarez Leite, S. O. Antunes Fernandes, and V. N. Cardoso. 2018. Conjugated linoleic acid prevents damage caused by intestinal mucositis induced by 5-fluorouracil in an experimental model. *Biomed. Pharmacother.* 103:1567–1576. <https://doi.org/10.1016/j.biopha.2018.04.133>.
- De Jesus, L. C. L., M. M. Drumond, A. de Carvalho, S. S. Santos, F. S. Martins, É. Ferreira, R. S. Fernandes, A. L. B. de Barros, F. L. R. do Carmo, P. F. Perez, V. Azevedo, and P. Mancha-Agresti. 2019. Protective effect of *Lactobacillus delbrueckii* ssp. *lactis* cidca 133 in a model of 5 fluorouracil-induced intestinal mucositis. *J. Funct. Foods* 53:197–207. <https://doi.org/10.1016/j.jff.2018.12.027>.
- Deak, S. T., and T. Z. Csáky. 1984. Factors regulating the exchange of nutrients and drugs between lymph and blood in the small intestine. *Microcirc. Endothelium Lymphatics* 1:569–588.
- Decker-Baumann, C., K. Buhl, S. Frohmüller, A. V. Herbay, M. Dueck, and P. M. Schlag. 1999. Reduction of chemotherapy-induced side-

- effects by parenteral glutamine supplementation in patients with metastatic colorectal cancer. *Eur. J. Cancer* 35:202–207. [https://doi.org/10.1016/S0959-8049\(98\)00389-X](https://doi.org/10.1016/S0959-8049(98)00389-X).
- Duncan, M., and G. Grant. 2003. Oral and intestinal mucositis—Causes and possible treatments. *Aliment. Pharmacol. Ther.* 18:853–874. <https://doi.org/10.1046/j.1365-2036.2003.01784.x>.
- Ewaschuk, J. B., H. Diaz, L. Meddings, B. Diederichs, A. Dmytrash, J. Backer, M. Looijer-van Langen, and K. L. Madsen. 2008. Secreted bioactive factors from *Bifidobacterium infantis* enhance epithelial cell barrier function. *Am. J. Physiol. Gastrointest. Liver Physiol.* 295:G1025–G1034. <https://doi.org/10.1152/ajpgi.90227.2008>.
- Favaro-Trindade, C., and F. Grosso. 2000. The effect of the immobilisation of *Lactobacillus acidophilus* and *Bifidobacterium lactis* in alginate on their tolerance to gastrointestinal secretions. *Milchwissenschaft* 55:496–505.
- Franchi, L., T. Eigenbrod, R. Muñoz-Planillo, and G. Nuñez. 2009. The inflammasome: A caspase-1-activation platform that regulates immune responses and disease pathogenesis. *Nat. Immunol.* 10:241–247. <https://doi.org/10.1038/ni.1703>.
- Gao, H., L. Liu, Y. Zhao, H. Hara, P. Chen, J. Xu, J. Tang, L. Wei, Z. Li, D. K. C. Cooper, Z. Cai, and L. Mou. 2017. Human IL-6, IL-17, IL-1 β , and TNF- α differently regulate the expression of pro-inflammatory related genes, tissue factor, and swine leukocyte antigen class I in porcine aortic endothelial cells. *Xenotransplantation* 24:e12291–e12304. <https://doi.org/10.1111/xen.12291>.
- Hegazy, A. N., N. R. West, M. J. T. Stubbington, E. Wendt, K. I. M. Suijker, A. Datsi, S. This, C. Danne, S. Champion, S. H. Duncan, B. M. J. Owens, H. H. Uhlig, A. McMichael, A. Berghaler, S. A. Teichmann, S. Keshav, and F. Powrie. 2017. Circulating and tissue-resident CD4⁺T cells with reactivity to intestinal microbiota are abundant in healthy individuals and function is altered during inflammation. *Gastroenterology* 153:1320–1337.e16. <https://doi.org/10.1053/j.gastro.2017.07.047>.
- Herbers, A. H. E., A. F. J. de Haan, W. J. F. M. van der Velden, J. P. Donnelly, and N. M. A. Blijlevens. 2014. Mucositis not neutropenia determines bacteremia among hematopoietic stem cell transplant recipients. *Transpl. Infect. Dis.* 16:279–285. <https://doi.org/10.1111/tid.12195>.
- Hill, C., F. Guarner, G. Reid, G. R. Gibson, D. J. Merenstein, B. Pot, L. Morelli, R. B. Canani, H. J. Flint, S. Salminen, P. C. Calder, and M. E. Sanders. 2014. Expert consensus document: The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat. Rev. Gastroenterol. Hepatol.* 11:506–514. <https://doi.org/10.1038/nrgastro.2014.66>.
- Hockenbery, D., G. Nuñez, C. Milliman, R. D. Schreiber, and S. J. Korsmeyer. 1990. Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. *Nature* 348:334–336. <https://doi.org/10.1038/348334a0>.
- Hoffmann, A., J. Bredno, M. Wendland, N. Derugin, P. Ohara, and M. Wintermark. 2011. High and low molecular weight fluorescein isothiocyanate (FITC)-dextrans to assess blood-brain barrier disruption: Technical considerations. *Transl. Stroke Res.* 2:106–111. <https://doi.org/10.1007/s12975-010-0049-x>.
- Justino, P. F. C., L. F. M. Melo, A. F. Nogueira, J. V. G. Costa, L. M. N. Silva, C. M. Santos, W. O. Mendes, M. R. Costa, A. X. Franco, A. A. Lima, R. A. Ribeiro, M. H. L. P. Souza, and P. M. G. Soares. 2014. Treatment with *Saccharomyces boulardii* reduces the inflammation and dysfunction of the gastrointestinal tract in 5-fluorouracil-induced intestinal mucositis in mice. *Br. J. Nutr.* 111:1611–1621. <https://doi.org/10.1017/S0007114513004248>.
- Justino, P. F. C., L. F. M. Melo, A. F. Nogueira, C. M. Morais, W. O. Mendes, A. X. Franco, E. P. Souza, R. A. Ribeiro, M. H. L. P. Souza, and P. M. G. Soares. 2015. Regulatory role of *Lactobacillus acidophilus* on inflammation and gastric dysmotility in intestinal mucositis induced by 5-fluorouracil in mice. *Cancer Chemother. Pharmacol.* 75:559–567. <https://doi.org/10.1007/s00280-014-2663-x>.
- Kociubinski, G., P. Pérez, and G. De Antoni. 1999. Screening of bile resistance and bile precipitation in lactic acid bacteria and bifidobacteria. *J. Food Prot.* 62:905–912. <https://doi.org/10.4315/0362-028X-62.8.905>.
- Lee, C. S., E. J. Ryan, and G. A. Doherty. 2014. Gastro-intestinal toxicity of chemotherapeutics in colorectal cancer: The role of inflammation. *World J. Gastroenterol.* 20:3751–3761. <https://doi.org/10.3748/wjg.v20.i14.3751>.
- Li, X., H. Sun, B. Li, X. Zhang, J. Cui, J. Yun, Y. Yang, L. Zhang, Q. Meng, S. Wu, J. Duan, H. Yang, J. Wu, Z. Sun, Y. Zou, and R. Chen. 2019. Probiotics ameliorate colon epithelial injury induced by ambient ultrafine particles exposure. *Adv. Sci. (Weinh)* 6:1900972. <https://doi.org/10.1002/advs.201900972>.
- Liang, G., N. Malmuthuge, H. Bao, P. Stothard, P. J. Griebel, and L. L. Guan. 2016. Transcriptome analysis reveals regional and temporal differences in mucosal immune system development in the small intestine of neonatal calves. *BMC Genomics* 17:602–618. <https://doi.org/10.1186/s12864-016-2957-y>.
- Liu, S., X. Li, Q. Li, H. Liu, Y. Shi, H. Zhuo, C. Li, and H. Zhu. 2018. Silencing Livin improved the sensitivity of colon cancer cells to 5-fluorouracil by regulating crosstalk between apoptosis and autophagy. *Oncol. Lett.* 15:7707–7715. <https://doi.org/10.3892/ol.2018.8282>.
- Liu, W., X. Li, Y. S. Wong, W. Zheng, Y. Zhang, W. Cao, and T. Chen. 2012. Selenium nanoparticles as a carrier of 5-fluorouracil to achieve anticancer synergism. *ACS Nano* 6:6578–6591. <https://doi.org/10.1021/nn202452c>.
- Longley, D. B., D. P. Harkin, and P. G. Johnston. 2003. 5-Fluorouracil: Mechanisms of action and clinical strategies. *Nat. Rev. Cancer* 3:330–338. <https://doi.org/10.1038/nrc1074>.
- Maioli, T. U., B. de Melo Silva, M. N. Dias, N. C. Paiva, V. N. Cardoso, S. O. Fernandes, C. M. Carneiro, F. dos Santos Martins, and S. de Vasconcelos Generoso. 2014. Pretreatment with *Saccharomyces boulardii* does not prevent the experimental mucositis in Swiss mice. *J. Negat. Results Biomed.* 13:6–14. <https://doi.org/10.1186/1477-5751-13-6>.
- Mennigen, R., K. Nolte, E. Rijcken, M. Utech, B. Loeffler, N. Senninger, and M. Bruewer. 2009. Probiotic mixture vsl#3 protects the epithelial barrier by maintaining tight junction protein expression and preventing apoptosis in a murine model of colitis. *Am. J. Physiol. Gastrointest. Liver Physiol.* 296:G1140–G1149. <https://doi.org/10.1152/ajpgi.90534.2008>.
- Miura, K., M. Kinouchi, K. Ishida, W. Fujibuchi, T. Naitoh, H. Oga-wa, T. Ando, N. Yazaki, K. Watanabe, S. Haneda, C. Shibata, and I. Sasaki. 2010. 5-FU metabolism in cancer and orally-administrable 5-FU drugs. *Cancers (Basel)* 2:1717–1730. <https://doi.org/10.3390/cancers2031717>.
- Neudecker, V., M. Haneklaus, O. Jensen, L. Khailova, J. C. Masters-son, H. Tye, K. Biette, P. Jedlicka, K. S. Brodsky, M. E. Gerich, M. Mack, A. A. B. Robertson, M. A. Cooper, G. T. Furuta, C. A. Dinarello, L. A. O'Neill, H. K. Eltzschig, S. L. Masters, and E. N. McNamee. 2017. Myeloid-derived mir-223 regulates intestinal inflammation via repression of the NLRP3 inflammasome. *J. Exp. Med.* 214:1737–1752. <https://doi.org/10.1084/jem.20160462>.
- Oh, N. S., J. Y. Lee, J. M. Lee, K. W. Lee, and Y. Kim. 2017. Mulberry leaf extract fermented with *Lactobacillus acidophilus*, A4 ameliorates 5-fluorouracil induced intestinal mucositis in rats. *Lett. Appl. Microbiol.* 64:459–468. <https://doi.org/10.1111/lam.12741>.
- Pinedo, H. M., and G. F. Peters. 1988. Fluorouracil: Biochemistry and pharmacology. *J. Clin. Oncol.* 6:1653–1664. <https://doi.org/10.1200/JCO.1988.6.10.1653>.
- Renault, T. T., L. M. Dejean, and S. Manon. 2017. A brewing understanding of the regulation of Bax function by Bcl-xL and Bcl-2. *Mech. Ageing Dev.* 161(Pt B):201–210. <https://doi.org/10.1016/j.mad.2016.04.007>.
- Salminen, S., and E. Salminen. 1997. Lactulose, lactic acid bacteria, intestinal microecology and mucosal protection. *Scand. J. Gastroenterol.* 32(sup222):45–48. <https://doi.org/10.1080/00365521.1997.11720717>.
- Schnoor, M. 2015. E-cadherin is important for the maintenance of intestinal epithelial homeostasis under basal and inflammatory conditions. *Dig. Dis. Sci.* 60:816–818. <https://doi.org/10.1007/s10620-015-3622-z>.

- Seth, A., F. Yan, D. B. Polk, and R. K. Rao. 2008. Probiotics ameliorate the hydrogen peroxide-induced epithelial barrier disruption by a PKC- and MAP kinase-dependent mechanism. *Am. J. Physiol. Gastrointest. Liver Physiol.* 294:G1060–G1069. <https://doi.org/10.1152/ajpgi.00202.2007>.
- Shigemori, S., T. Watanabe, K. Kudoh, M. Ihara, S. Nigar, Y. Yamamoto, Y. Suda, T. Sato, H. Kitazawa, and T. Shimosato. 2015. Oral delivery of *Lactococcus lactis* that secretes bioactive heme oxygenase-1 alleviates development of acute colitis in mice. *Microb. Cell Fact.* 14:189–201. <https://doi.org/10.1186/s12934-015-0378-2>.
- Siegmund, B., H. A. Lehr, G. Fantuzzi, and C. A. Dinarello. 2001. IL-1 beta-converting enzyme (caspase-1) in intestinal inflammation. *Proc. Natl. Acad. Sci. USA* 98:13249–13254. <https://doi.org/10.1073/pnas.231473998>.
- Soares, P. M. G., J. M. S. C. Mota, A. S. Gomes, R. B. Oliveira, A. M. S. Assreuy, G. A. C. Brito, A. A. Santos, R. A. Ribeiro, and M. H. L. P. Souza. 2008. Gastrointestinal dysmotility in 5-fluorouracil-induced intestinal mucositis outlasts inflammatory process resolution. *Cancer Chemother. Pharmacol.* 63:91–98. <https://doi.org/10.1007/s00280-008-0715-9>.
- Song, M. K., M. Y. Park, and M. K. Sung. 2013. 5-Fluorouracil-induced changes of intestinal integrity biomarkers in BALB/c mice. *J. Cancer Prev.* 18:322–329. <https://doi.org/10.15430/JCP.2013.18.4.322>.
- Sonis, S. T. 1998. Mucositis as a biological process: A new hypothesis for the development of chemotherapy-induced stomatotoxicity. *Oral Oncol.* 34:39–43. [https://doi.org/10.1016/S1368-8375\(97\)00053-5](https://doi.org/10.1016/S1368-8375(97)00053-5).
- Sonis, S. T. 2004. The pathobiology of mucositis. *Nat. Rev. Cancer* 4:277–284. <https://doi.org/10.1038/nrc1318>.
- Soveri, L. M., K. Hermunen, A. De Gramont, T. Poussa, E. Quinaux, P. Bono, T. André, and P. Österlund. 2014. Association of adverse events and survival in colorectal cancer patients treated with adjuvant 5-fluorouracil and leucovorin: Is efficacy an impact of toxicity? *Eur. J. Cancer* 50:2966–2974. <https://doi.org/10.1016/j.ejca.2014.08.017>.
- Stringer, A. M., R. J. Gibson, R. M. Logan, J. M. Bowen, A. S. Yeoh, J. Hamilton, and D. M. K. Keefe. 2009. Gastrointestinal microflora and mucins may play a critical role in the development of 5-fluorouracil-induced gastrointestinal mucositis. *Exp. Biol. Med.* (Maywood) 234:430–441. <https://doi.org/10.3181/0810-RM-301>.
- Tang, Y., Y. Wu, Z. Huang, W. Dong, Y. Deng, F. Wang, M. Li, and J. Yuan. 2017. Administration of probiotic mixture dm#1 ameliorated 5-fluorouracil-induced intestinal mucositis and dysbiosis in rats. *Nutrition* 33:96–104. <https://doi.org/10.1016/j.nut.2016.05.003>.
- van Vliet, M. J., H. J. M. Harmsen, E. S. J. M. De Bont, and W. J. E. Tissing. 2010. The role of intestinal microbiota in the development and severity of chemotherapy-induced mucositis. *PLoS Pathog.* 6:e1000879–e1000887. <https://doi.org/10.1371/journal.ppat.1000879>.
- Vieira, E. L. M., A. J. Leonel, A. P. Sad, N. R. M. Beltrão, T. F. Costa, T. M. R. Ferreira, A. C. Gomes-Santos, A. M. C. Faria, M. C. G. Peluzio, D. C. Cara, and J. I. Alvarez-Leite. 2012. Oral administration of sodium butyrate attenuates inflammation and mucosal lesion in experimental acute ulcerative colitis. *J. Nutr. Biochem.* 23:430–436. <https://doi.org/10.1016/j.jnutbio.2011.01.007>.
- Wang, J., L. Luo, D. Wang, B. Guo, J. Li, Z. Yang, and D. Tang. 2018. Combination adjuvant chemotherapy with targeted drugs for treatment of colorectal cancer: A network meta-analysis. *J. Cell. Biochem.* 119:1521–1537. <https://doi.org/10.1002/jcb.26312>.
- Xie, T. Y., W. Yan, J. Lou, and X. Y. Chen. 2016. Effect of ozone on vascular endothelial growth factor (VEGF) and related inflammatory cytokines in rats with diabetic retinopathy. *Genet. Mol. Res.* 15:15027558–15027569. <https://doi.org/10.4238/gmr.15027558>.
- Yeung, C.-Y., W.-T. Chan, C.-B. Jiang, M.-L. Cheng, C.-Y. Liu, S.-W. Chang, J.-S. Chiang Chiau, and H.-C. Lee. 2015. Amelioration of chemotherapy-induced intestinal mucositis by orally administered probiotics in a mouse model. *PLoS One* 10:e0138746–e0138762. <https://doi.org/10.1371/journal.pone.0138746>.
- Zhang, Z., X. Tao, N. P. Shah, and H. Wei. 2016. Antagonistics against pathogenic *Bacillus cereus* in milk fermentation by *Lactobacillus plantarum* ZDY2013 and its anti-adhesion effect on Caco-2 cells against pathogens. *J. Dairy Sci.* 99:2666–2674. <https://doi.org/10.3168/jds.2015-10587>.

ORCIDS

Hua Wei <https://orcid.org/0000-0003-4341-8290>
 Feng Xu <https://orcid.org/0000-0002-6963-5798>