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High dietary methionine induces secondary bile acids dysmetabolism and promotes the development of colorectal cancer in mice

Wanhua Li, Aiying Shi, Xiaoxia Liu, Yunhui Zhang, Yingshuang Lu, Lu Dong, Jin Wang, Yan Zhang*, Shuo Wang*

Tianjin Key Laboratory of Food Science and Health, School of Medicine, Nankai University, Tianjin 300071, China

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ABSTRACT

Methionine, an essential amino acid, is abundant in animal protein. High dietary methionine intake is associated with the promotion of colorectal cancer (CRC); however, the mechanisms remain unclear. This study aimed to investigate the underlying mechanisms of high dietary methionine promoting CRC and evaluate the effect of high dietary methionine on healthy intestine. Our results demonstrated that high dietary methionine intake exhibited a higher incidence and invasion of tumors in azoxymethane/dextran sulfate sodium-induced mice. Meanwhile, the gut microbiota were disturbed, consequently fostering the metabolism of secondary bile acids. The contents of lithocholic acid and deoxycholic acid significantly increased ($P < 0.01$), which further activated the bile acid membrane receptors TGR5, and then the activated TGR5 promoted tumor proliferation through STAT3 and YAP pathways. Pseudo-germ-free mice validated the role of gut microbiota and secondary bile acids in promoting CRC by high dietary methionine. Notably, similar disturbances in gut microbiota and bile acid metabolism were observed in the intestine of healthy mice with high dietary methionine intake. In conclusion, dysregulation of bile acid metabolism and activation of the corresponding receptor TGR5 were mechanisms promoting CRC associated with high dietary methionine intake.

1. Introduction

Dietary nutrition exerts crucial effects on health. Over the past few decades, epidemiological studies have suggested that dietary habits play an essential role in developing colorectal cancer (CRC) as a risk factor[1-3]. Methionine, as an essential amino acid, is a considerable component of dietary protein. As an important amino acid involved in one-carbon metabolism, methionine is essential for protein synthesis, sulfur metabolism, DNA, RNA, and histone methylation[4]. Animal-derived foods contain significantly higher amounts of methionine compared to plant-derived foods, including soybean products[5]. The methionine intake recommended by the WHO is 10.4 mg/(kg·day). The diet style dominated by animal-derived foods, such as the Western diet, tends to consume more than 2 times the recommended amount of methionine[6-7]. Recent study has shown that the daily methionine intake was around 17.6 mg/(kg·day)[8]. Besides, high protein intake is important for improving nutritional status indices in cancer patients due to the need to prevent weight and muscle loss[9]. However, high protein intake is commonly accompanied by excess methionine intake. Clarifying the effects of excess methionine on cancer patients is extremely important for the development of an appropriate dietary strategy. Recent studies have found that tumor cells are dependent on exogenous methionine uptake. Controlling methionine intake can

* Corresponding authors at: Tianjin Key Laboratory of Food Science and Health, School of Medicine, Nankai University, Tianjin 300071, China.
E-mail address: yzhang@nankai.edu.cn (Y. Zhang); wangshuo@nankai.edu.cn (S. Wang)
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inhibit tumor progression and increase chemotherapy sensitivity[8,10]. At the same time, previous studies have found that methionine supplementation promotes CRC progression by promoting aberrant crypt proliferation and increasing gut-specific *Cdx-1* expression[11]. However, as an important component of the tumor microenvironment, effector T cells are crucial for tumor immunity. Several studies have found that sufficient methionine plays a vital role in T cell activation and differentiation[12-13]. Therefore, the role and underlying mechanisms of methionine in tumor progression remain unresolved.

Studies have shown that the micromolecules produced by the metabolism of methionine have a profound impact on the cross-talk between gut microorganisms and their interactions with the host[14]. Accumulating evidence has indicated that dysbiosis of the gut microbiota is a considerable factor in the development of CRC[15]. Invasive bacterial biofilms were found in the colonic mucosa of approximately 50% of sporadic CRC patients and 13% of healthy control subjects. Numerous intestinal commensal bacteria promote CRC progression, including *Streptococcus*, *Eubacterium*, *Clostridium*, *Bacteroides fragilis*, *Enterococcus faecalis*, and *Escherichia coli*[16-19]. Moreover, an elevated level of commensal bacteria was noted in CRC patients compared with healthy volunteers[20]. Besides, gut microbial dysbiosis damages the intestinal barrier function, which increases intestinal permeability. Disruption of gut barrier integrity results in the translocation of bacteria or inflammatory molecules from the intestine, which can activate inflammation and enhance the initiation and advancement of CRC[21].

Recently, it has been revealed that bile acids play an important part in the control of CRC[22]. Primary bile acids are synthesized in the liver and excreted into digestive tract. Subsequently, primary bile acids are transformed into secondary bile acids by various reactions, including deconjugation and 7-dehydroxylation. Many intestinal bacteria have been involved in this process, mainly including *Bacteroides, Clostridium*, *Enterococcus*, *Lactobacillus* and *Bifidobacterium*[23-26]. Secondary bile acids, especially lithocholic acid (LCA) and deoxycholic acid (DCA), are more cytotoxic due to their strong hydrophobicity. They accelerate tumor progression by promoting mitochondrial damage and cell membrane disruption leading to increased levels of reactive oxygen[27-29]. In addition, recent studies have reported that bile acids can also act as signaling molecules by binding to bile acid receptors such as TGR5, which influence tumor proliferation by affecting the tumor initiation potential and epithelial mesenchymal transition process of tumor stem cells[30-31]. Thus, we hypothesized that excessive methionine intake may affect the development of CRC by influencing the gut microbiota and bile acid metabolism.

To verify whether high dietary methionine has the constitutive effect on intestinal tumorigenesis, azoxymethane/dextran sulfate sodium (AOM/DSS) -induced CRC mice model were used to evaluate mice's carcinogenesis-related indicators after excessive methionine intake. In addition, the variations of bile acid metabolome and the corresponding recepter TGR5 were investigated to reveal the involved potential mechanisms. Taken together, it is important to clarify the effect of methionine on intestinal health for revealing the gastrointestinal toxicity of methionine and formulating clinical nutrition strategy.

2. Materials and methods

2.1 Materials

All reagents and materials are shown in Supplementary Information.

2.2 Animals and experimental design

A total of 48 specific-pathogen-free (SPF) Balb/c mice (male, 6 weeks old) were obtained from Charles River (Biotechnology Co., Ltd., Beijing, China). All animal experiments were approved by the Animal Care and Use Committee of Nankai University (permit number: SYXK 2019-0001). Mice were housed at (22 ± 2) ℃ with (50 ± 5)% relative humidity and a standard 12/12 h light/dark cycle. Food and water were provided *ad libitum*. The methionine doses used in the current study were based on those used in previous studies[32-37]. The experimental diets were purchased from Xietong Inc. (Nanjing, China). The ingredients of the experimental diets are provided in Table S1. Firstly, 48 mice were randomly divided into 4 groups: control group (0.86% methionine, CTRL), high methionine group (1.5% methionine, HM), AOM/DSS group (0.86% methionine, AOM/DSS), and high methionine + AOM/DSS group (1.5% methionine, HM + AOM/DSS), each group contained 12 mice. Antibiotic mixtures were used to eliminate the effects of gut microbiota in AOM/DSS-treated CRC mice. Mice were given the antibiotic cocktail 7 days before the beginning of the experiment to ensure gut microbiota elimination and continued until the end of the experiment. The antibiotic cocktail was performed as previously described (1 g/L streptomycin, 0.5 g/L ampicillin, 1 g/L gentamicin and 0.5 g/L vancomycin)[38]. Then, 16 mice were randomized into 2 groups (n = 8): control chow group (0.86% methionine, anti-MOD) and high methionine chow group (1.5% methionine, anti-HM).

AOM/DSS induced the CRC model and the protocol is shown in Fig. 1A. On week 1, calculate the injection volume of AOM (1 mg/mL) to achieve a dose of 10 mg/kg (i.p.). Then, AOM/DSS-treated mice were given 3 cycles of 2.5% DSS for 7 days in weeks 2, 5, and 8. Meanwhile, the mice in CTRL group and HM group received the equivalent volume of water. Water intake in DSS treatment periods are provided in Supplementary Information. All mice were euthanized at the end of the experiment. After dissection, blood, spleen, and colon were collected. The spleen weight, length of the colon, and number of tumors were examined. The tumor-bearing colorectal tissues of the mice were fixed with 4% paraformaldehyde. The blood, remaining colorectal tissues and colonic contents were stored at −80 ℃ for further analysis.

2.3 Disease activity index (DAI) measurement

DAI was calculated weekly for each mouse according to the following criteria weight loss (0, no loss; 1, 1%–3% loss; 2, 3%–6% loss; 3, 6%–9% loss; 4, > 9% loss); stool (0, normal; 2, loose stool; 4, diarrhea), and fecal blood (0, none; 2, blood visible in stool; 4, gross bleeding), as described previously[39].

2.4 Hematoxylin-eosin (H&E) and immunohistochemistry (IHC) staining

Colon tissues were fixed in 4% paraformaldehyde and then dehydrated and paraffin-embedded. 4 μm sections of colon tissue segments were stained with H&E and IHC. The experimental procedures were shown in the Supplementary Information.

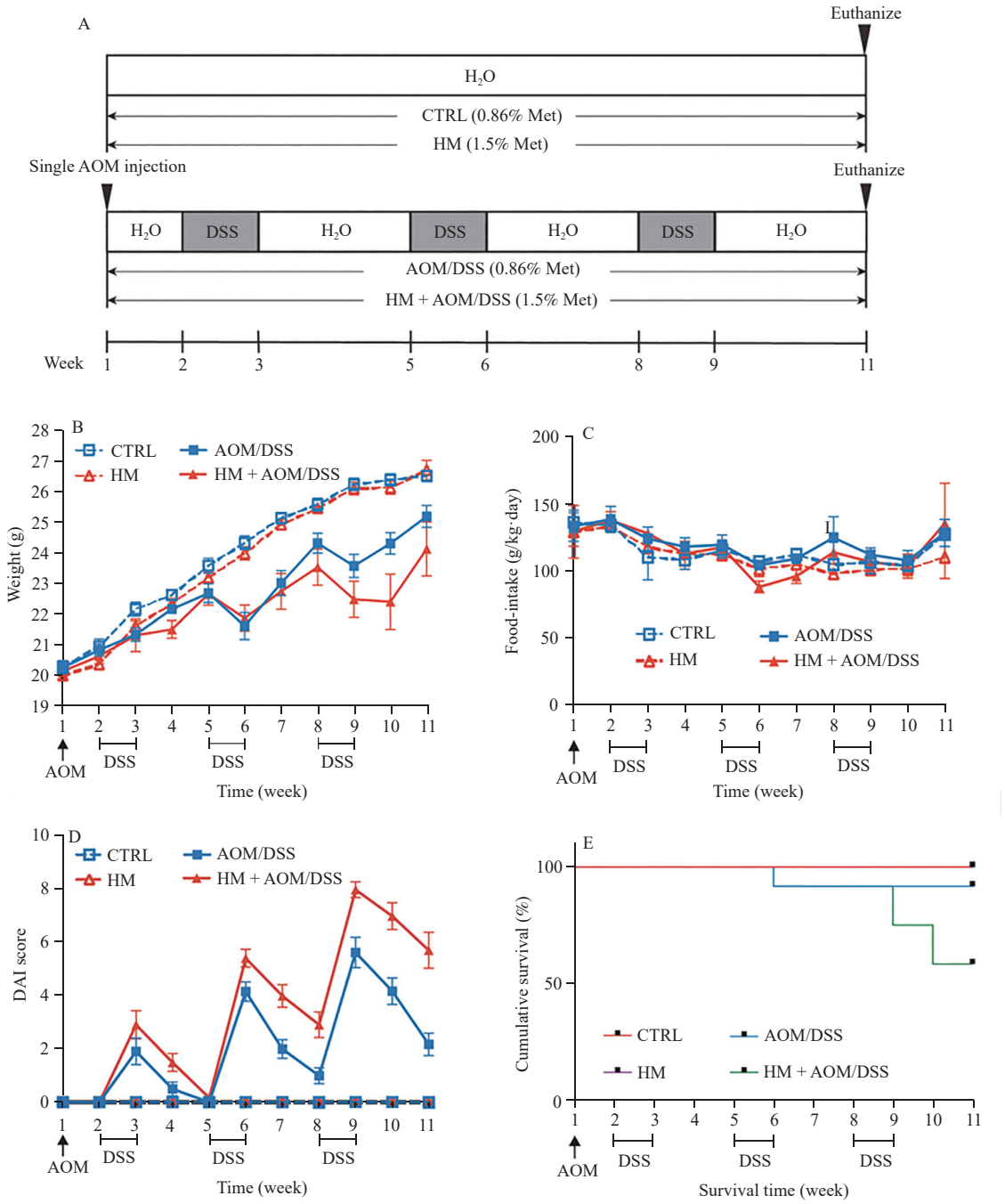

Fig. 1 Effect of high dietary methionine on the physiological indexes of AOM/DSS-induced CRC mice. (A) Schematic timeline for AOM/DSS-based CRC development. (B) Body weight was monitored during AOM/DSS treatment. (C) Food intake of mice. (D) DAI score. (E) Survival curve. Data are shown as means ± SEM.

2.5 *Intestinal permeability assay*

Fluorescein isothiocyanate (FITC)-dextran (MW = 4 kDa; Sigma-Aldrich, MO, USA) was employed to assess intestinal permeability. Mice were given intragastric administration of 500 mg/mL of FITC-dextran (0.3 mL) after 4-h fasting. Following a 90-min interval, mice were anesthetized with isoflurane and subjected to live imaging using the IVIS Spectrum system (Perkin Elmer, Waltham, Massachusetts, USA). After 4 h, all mice were sacrificed under anesthesia and the serum was collected for determination of fluorescence intensity. The fluorescence was measured using Tecan-Spark Multimode Microplate Reader (Tecan, Männedorf, Switzerland).

2.6 *Gut microbiota analysis*

The colonic content samples were used to extract microbial DNA using the E.Z.N.A.® soil DNA Kit according to the manufacturer's instructions. The detailed procedures are shown in the Supplementary Information.

2.7 *Bile acid analysis*

Bile acids were extracted from feces and measured according to the published protocol[40-41]. Briefly, feces samples were collected and lyophilized. Freeze-dried feces samples were homogenized in water

and the homogenate was mixed with ice-cold methanol (1:3, *V*/*V*) to denature the protein. The mixture was then centrifuged at 12 000 × *g* at 4 ℃ for 10 min. The supernatant was collected and filtered using a 0.22 μm nylon filter for UPLC-MS/MS analysis. The experimental procedures are shown in the Table S2.

2.8 Quantitative analysis of gene expression in colon tissue by reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from colon tissue and then converted to cDNA for RT-qPCR analysis. The RT-PCR analysis was carried out using SYBR Green Master MIX on the CFX96 RT-qPCR system of Bio-Rad. The housekeeping gene (*β-actin*) was used for normalization, the target genes were quantified by relative quantification ($2^{-\Delta\Delta Ct}$). The gene-specific primers used in this study are provided in Table S3.

2.9 Quantification of cytokines in serum by ELISA

After the mice were euthanized, blood was collected followed by centrifugation at 3 000 × *g* for 15 min to collect the supernatant to obtain serum. The levels of tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β) in serum were assessed by commercial detection kits according to the manufacturer's instructions.

2.10 Western blot analysis

Proteins were extracted from colon tissue followed by homogenized in RIPA solution with 1% protease inhibitor (HY-K0010) and 1% phosphatase inhibitor (HY-L081; MedChemExpress, Shanghai, China). Protein concentrations were quantified by the Pierce™ BCA protein assay kit (#23227; Thermo Fisher Scientific, MA, USA). The samples preparation, experimental procedures, and antibodies used in this study are shown in the Supplementary Information.

2.11 Statistical analysis

The SPSS v. 26 software was used for conducting one-way ANOVA and post Hoc Multiple Comparisons LSD or Dunnett's T3 test when the quantitative data were normally distributed for all analysis. Otherwise, statistical analysis was performed by Kruskal-Wallis Test, $P < 0.05$ is considered statistically significant. Data for independent experiments are presented as means ± standard error mean (SEM).

3. Results

3.1 Effect of high dietary methionine on physiological indexes of AOM/DSS-induced CRC mice

Similar to colitis associated CRC patients, AOM/DSS-treated mice showed obvious symptoms such as the loss of body weight, diarrhea, bloody stool, and colon atrophy. Body weight changes were shown in Fig. 1B, as indicated by significantly decreased body weight during 3 cycles of DSS treatment. High methionine intake effectively increased DSS-induced weight loss during the experiment. However, the administration of high methionine diet alone to mice without AOM/DSS-treated did not induce changes in body weight. Meanwhile, no significant differences were found among all groups regarding food intake and water intake (Figs. 1C and S1). Notably, compared with AOM/DSS group, HM + AOM/DSS group showed a higher DAI score, leading to mortality in 42% in comparison with 8% of the AOM/DSS group (Figs. 1D and E).

3.2 High dietary methionine promoted athological symptoms and colonic inflammation of AOM/DSS-induced CRC mice

As shown in Figs. 2A and B, the depletion of AOM/DSS damages the colonic tissue, and the derived cumulative pro-inflammatory response is manifested by increased splenic metabolic rate and accumulation of immune cells, leading to splenomegaly. In addition, colon tissue samples were homogenized and RNA was extracted to assess the relative expression of proinflammatory cytokines *IL-1β* and *TNF-α*. As shown in Fig. 2C, similar changes were found in AOM/DSS group and HM + AOM/DSS group. Compared with the CTRL group, *IL-1β* ($P = 0.073$) and *TNF-α* ($P < 0.01$) were increased in AOM/DSS-treaded group. However, compared with AOM/DSS group, HM + AOM/DSS group showed a significant increase in the relative mRNA expression of *TNF-α* ($P < 0.05$). Consistently, the formation of IL-1β and TNF-α ($P < 0.01$) in serum were significantly accumulated in AOM/DSS-treated mice. Excessive methionine intake significantly increased the forming of the inflammatory cytokines. Notably, the administration of high methionine diet alone to mice without AOM/DSS intervention did not induce changes in the above indices.

To further analyze colon tissue damage, colon tissues were analyzed histologically using the H&E method (Fig. 2D). Colon tissues in the CTRL group appeared normal, as indicated by the presence of an intact colonic mucosa and well-organized glandular cells within the crypts, while high methionine diet alone did not cause an obvious infiltration of inflammatory cell. Colon tissues exhibited inflammatory signs, such as surface epithelium erosion, crypt destruction, destroyed muscular, infiltrating immune cells, mucosae, and submucosa edema in the AOM/DSS-treated group. Furthermore, these inflammatory signs were exacerbated when mice were given a high methionine diet.

3.3 High dietary methionine promoted tumorigenesis of AOM/DSS-induced CRC mice

As shown in Figs. 3A-C, AOM/DSS-treated mice showed colon atrophy, dysplasia, and neoplasia. The location of tumors was mostly in the distal parts of the colon, which is similar to the clinical CRC patients. All of the AOM/DSS-treated mice developed tumors after 3 DSS cycles. Compared with AOM/DSS group, the total number of tumors was significantly larger ($P < 0.05$) and the colon length was shorter in HM + AOM/DSS group. Moreover, the expression of tumor stem cell signature genes in colon tumor tissue was also significantly increased in the HM + AOM/DSS group (Fig. 3D, $P < 0.05$). To demonstrate the proliferation of tumor cells, the expression of proliferative protein Ki67 was analyzed by IHC staining. As shown in Fig. 3G, the

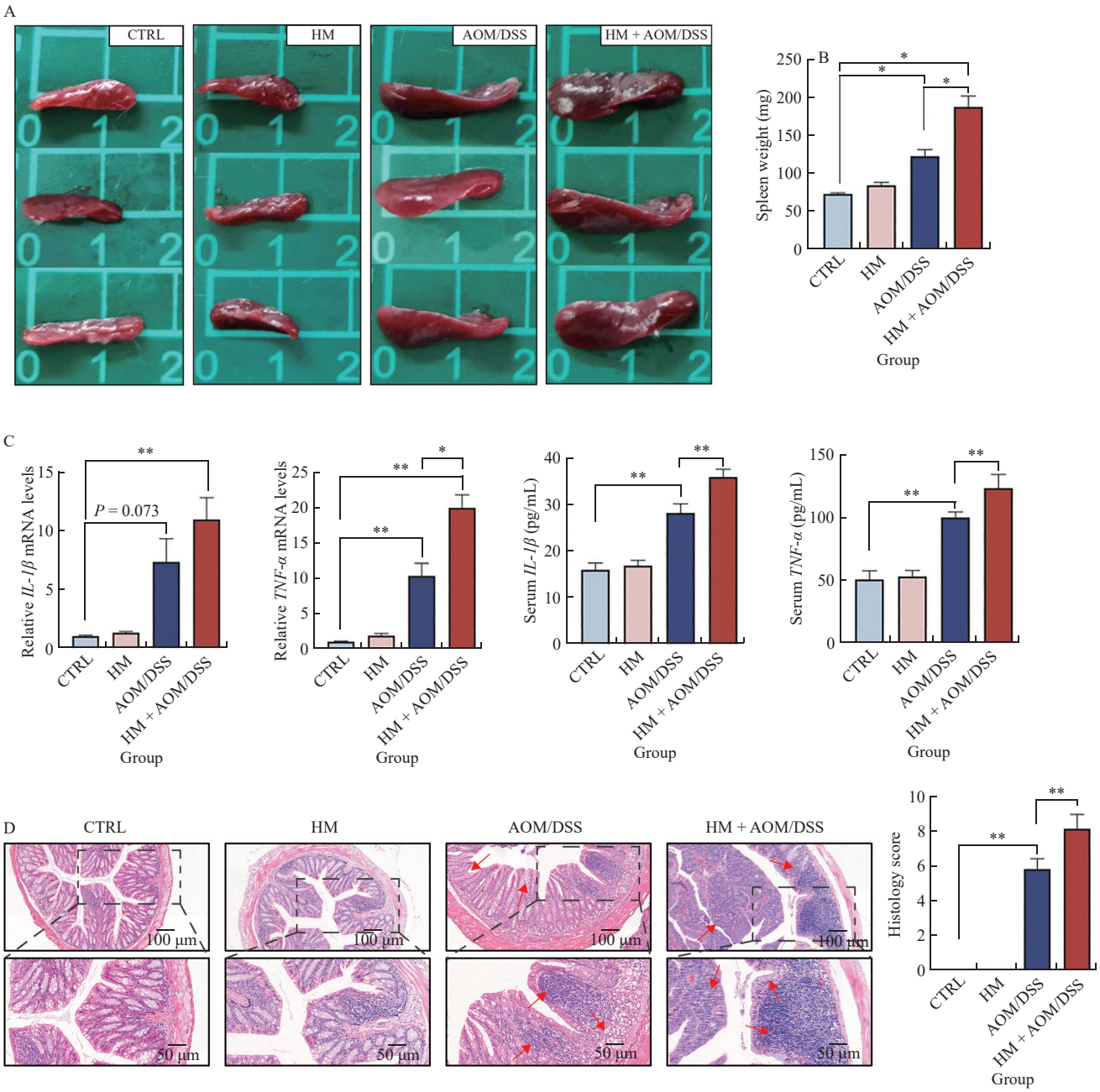

Fig. 2 Effect of high dietary methionine on inflammatory response in CRC mice. (A) Representative image of spleens. (B) Average spleen weight. (C) Relative expression of *IL-1β* and *TNF-α* in colon tumor tissue and the serum. (D) Representative images of H&E staining pathological sections of the colon tissues and quantitative histopathology score. Scale bar, 100 μm (upper panel) and 50 μm (lower panel). Data are shown as means ± SEM. *$P < 0.05$, **$P < 0.01$.

number of cells in the active proliferation increased significantly after the AOM/DSS treatment. Furthermore, high methionine diet promoted the expression of *Ki67* in the HM + AOM/DSS group compared to the AOM/DSS group (Fig. 3E, $P < 0.05$). At the same time, we analyzed the expression of vimentin, a marker of epithelial-mesenchymal transition in colon tissue. The expression of vimentin was significantly increased after AOM/DSS treatment (Fig. 3G). The high methionine diet further promoted the expression of *Vimentin*, implying that the tumors were more aggressive (Fig. 3F, $P < 0.05$). In conclusion, high methionine diet advance the development of colorectal carcinogenesis in AOM/DSS-treated CRC.

3.4 High dietary methionine exacerbated intestinal epithelial damage of AOM/DSS-induced CRC mice

AOM/DSS treatment induced harm to intestinal epithelial cells and cause colitis. To evaluate the integrity of the intestinal barrier, an intestinal epithelial assay using FITC-dextran was conducted. As shown in Fig. 4A, AOM/DSS disrupts the integrity of the intestinal barrier in mice using imaging of FITC-dextran leakage. However, mice fed with high methionine diet had significantly increased intestinal permeability and doubled plasma FITC-conjugated dextran levels compared to the AOM/DSS group. However, high methionine diet did not significantly disrupt intestinal barrier integrity in mice without AOM/DSS treatment. The tight junction protein occludin and

ZO-1 immunostaining has been used to mark the integrity of the colon barrier (Figs. 4B and C). There was no significant difference in the expression of *occludin* and *ZO-1* between CTRL group and HM group. However, the expression of *occludin* and *ZO-1* were significantly decreased after AOM/DSS treatment, and the high methionine diet exacerbated intestinal epithelial damage. These data demonstrated that a high methionine diet could aggravate the intestinal barrier dysfunction induced by AOM/DSS.

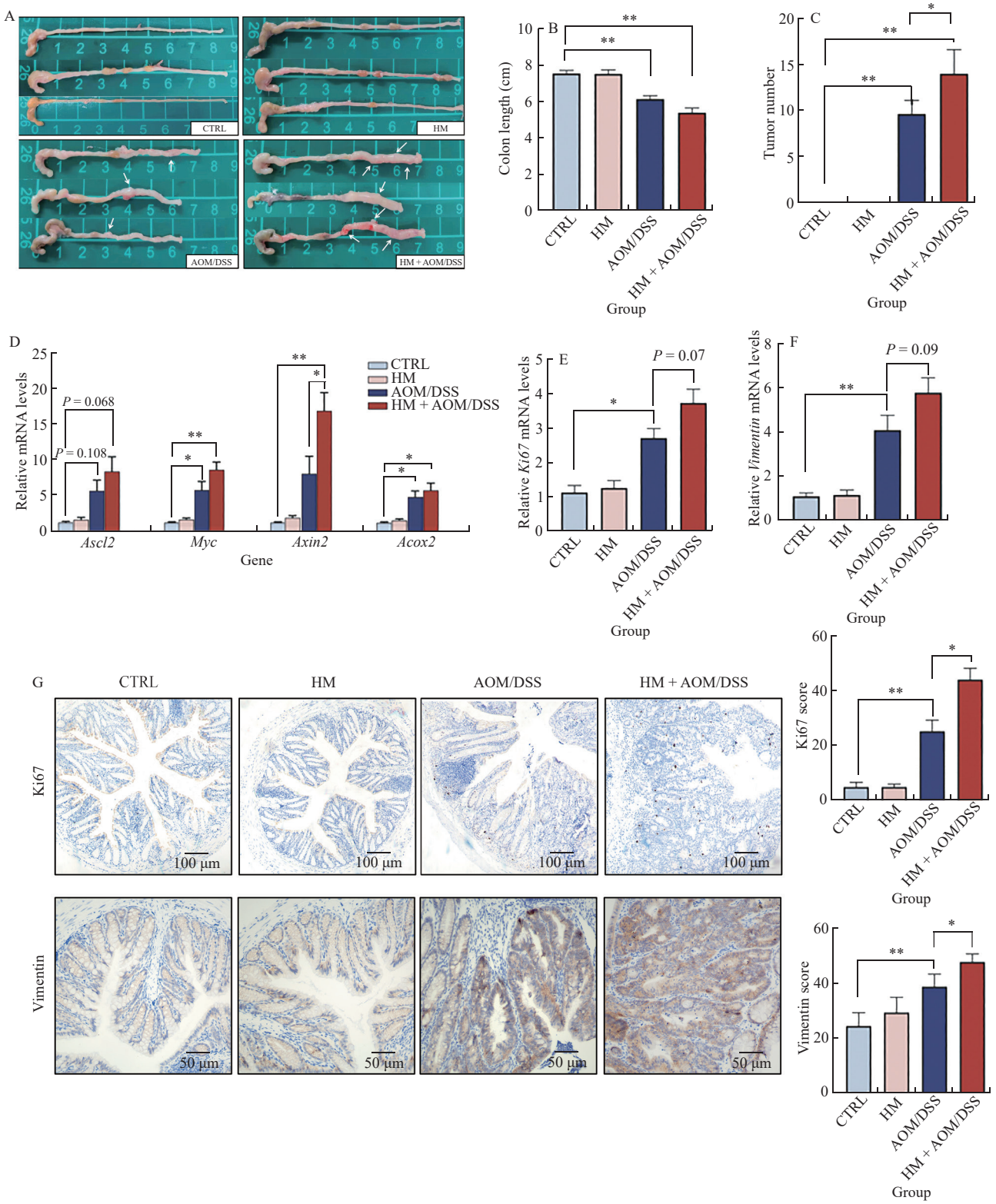

Fig. 3 Evaluation of AOM/DSS-induced colorectal tumors in mice. (A) Representative image of colons. (B) Average colon length. (C) Tumor number. (D) Relative expressions of tumor stem cell marker genes in colon tumor tissue. (E-F) Relative expression of *Ki67* and *Vimentin* in colon tumor tissue. (G) Representative IHC images and quantification of colon tissue staining Ki67 and vimentin. Data are shown as means ± SEM. *$P < 0.05$, **$P < 0.01$.

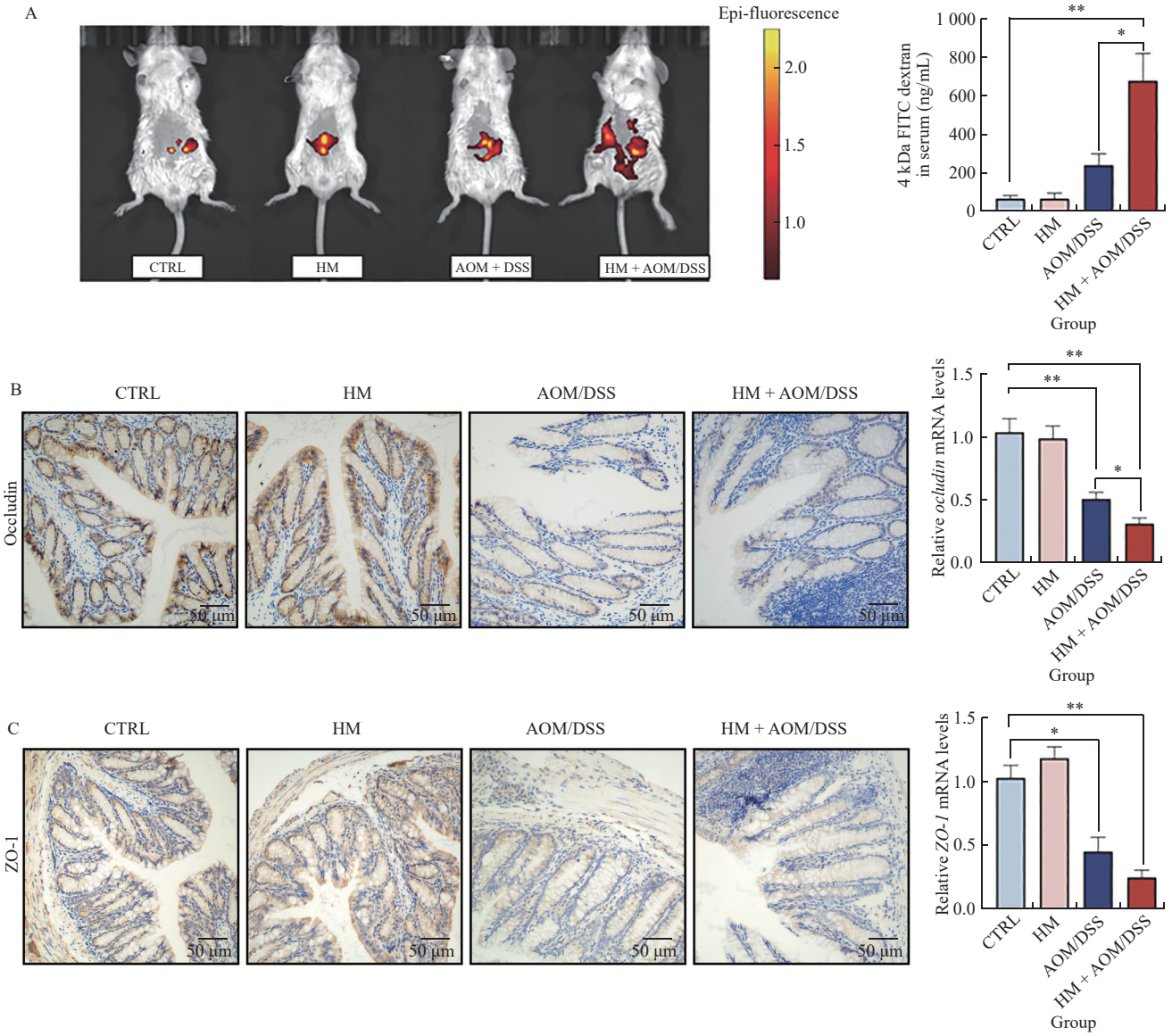

Fig. 4 Evaluation of barrier function in AOM/DSS-induced mice. (A) Fluorescence intensity of FICT-Dextran in living imaging and serum. (B) Representative IHC images of colon tissue staining occludin and the relative expression of *occludin* in colon tumor tissue. (C) Representative IHC stain images of colon tissue staining ZO-1 and the relative expression of *ZO-1* in colon tumor tissue. Data are shown as means ± SEM. *$P < 0.05$, **$P < 0.01$.

3.5 High dietary methionine promoted gut microbiota dysbiosis in AOM/DSS-induced CRC mice

We next explored the effect of the high methionine diet on gut microbiota composition. 16S rDNA sequencing was applied to investigate the structure of microoganisms in previous studies[42-43]. Effect of high methionine diet on the composition of gut microbiota was shown in Fig. S2. In this study, 16S rDNA results showed that AOM/DSS treatment decreased bacterial diversity (Sobs and Shannon indices), compared with the CTRL group. High methionine diet significantly affected α-diversity, leading to reduced Sobs and Shannon effective diversity in AOM/DSS-treated mice (Figs. 5A and B). β-Diversity was estimated using the principal co-ordinates analysis (PCoA), which revealed that samples clustered by diets and treatments (Fig. 5C). The PCoA plot showed the distinct separation of samples into 4 distinct clusters, which indicated that the high methionine diet and AOM/DSS treatment both had a significant effect on the composition of the gut microbiome. To determine whether observed differences in diversity levels were associated with changes in specific microbes, we tested differences in taxonomic groups and individual operational taxonomic units (Figs. 5D-F). At the phylum level, AOM/DSS treatment increased the relative abundance of Verrucomicrobiota. Interestingly, when mice were fed high methionine chow, their gut microbiome changed dramatically. Compared with CTRL group, the HM group decreased Bacteroidota ($P < 0.05$). At the genus level, AOM/DSS treatment decreased the relative abundance of Lachnospiraceae, *Colidextribacter*, *Anaerotruncus*, *Oscillibacter*, *Bilophila*, *Lachnoclostridium*, and *Muribaculum*, but increased the relative abundance of *Lactobacillus*, *Erysipelatoclostridium* and *Eubacterium* compared to CTRL group ($P < 0.05$). Compared with AOM/DSS group, the HM + AOM/DSS group decreased *Alistipes*, *Eubacterium* and *Bacteroides*, but increased the relative abundance of *Faecalibaculum*, *Enterococcus*, *Clostridium*, *Streptococcus* and *Escherichia-Shigella* at the genus level ($P < 0.05$, Figs. 5E and F).

Taken together, these observations suggest that AOM/DSS treatment and methionine supplementation significantly affect the gut microbial composition and structure. In addition, we found that microorganisms with increased relative abundance in the HM + AOM/DSS group (such as *Enterococcus* and *Clostridium*) have the function of bile acid metabolism. Therefore, we calculated the abundance of microorganisms containing bile salt hydrolase (including *Lactobacillus*, *Enterococcus*, *Clostridium*, *Bacteroides* and Coriobacteriaceae). Compared with the AOM/DSS group, the relative abundance of microorganisms containing bile salt hydrolase in the HM + AOM/DSS group was higher ($P = 0.05$, Fig. S3).

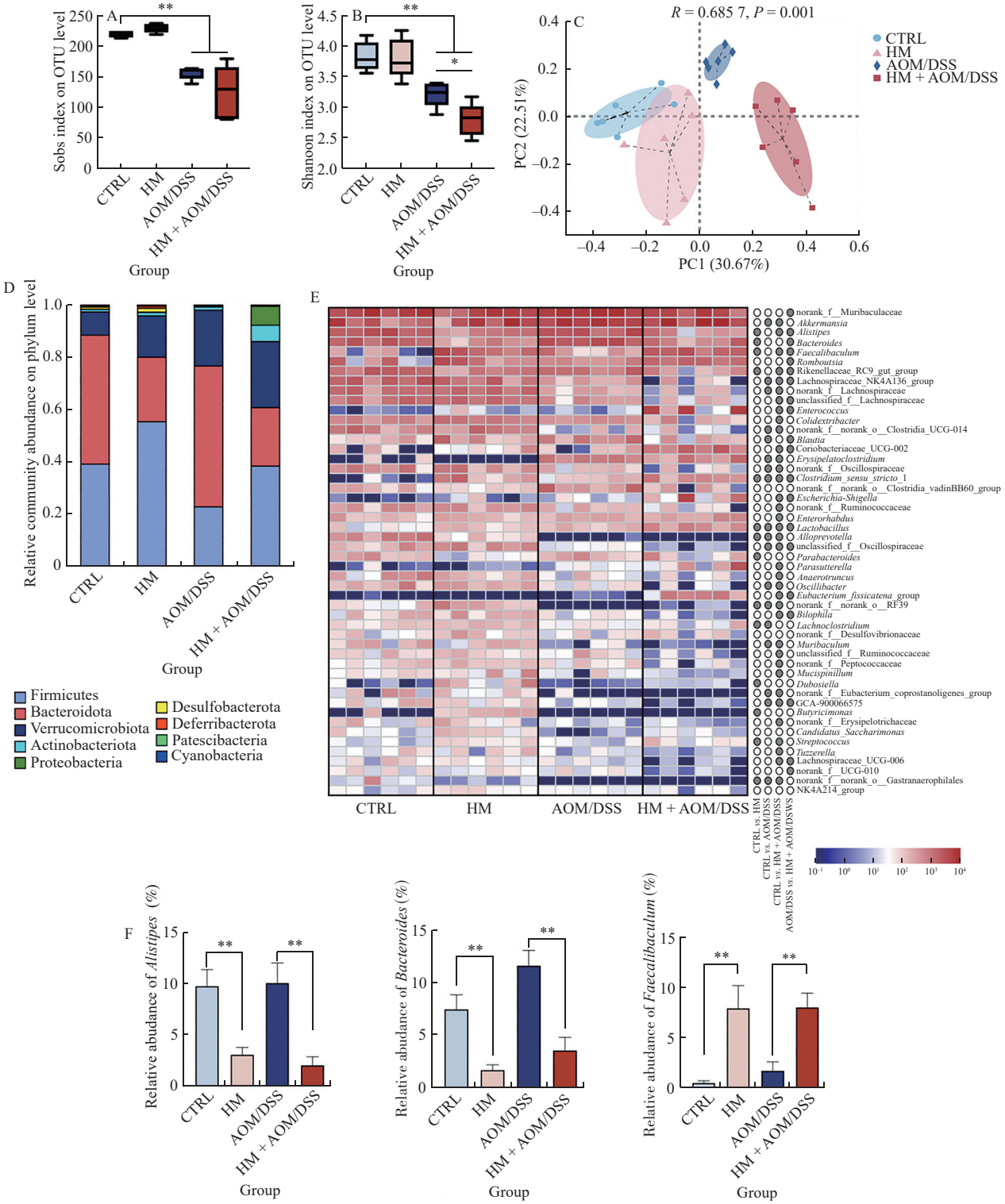

Fig. 5 High dietary methionine modulated the composition of gut microbiota. (A-B) α-Diversity was presented as the index of Sobs and Shannon. (C) PCoA analysis of the β-diversity of gut microbiota. (D) The composition of gut microbiota at the phylum level. (E) The heatmap of microbiota with significant differences in the top 50 at the genus level. (F) Effects of high dietary methionine on the level of the representative bacteria at genus level. Data are shown as means ± SEM. $^{*}P < 0.05$ and $^{**}P < 0.01$.

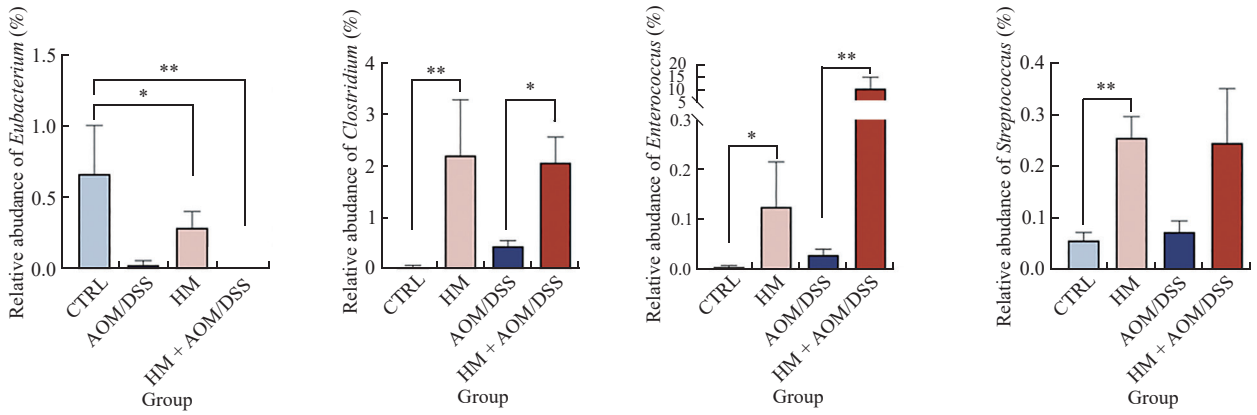

Fig. 5 (*Continued*)

3.6 *High dietary methionine promoted bile acid dysmetabolism in AOM/DSS-induced CRC mice*

Analysis of gut microbial results revealed significant changes in a variety of bacteria involved in bile acid metabolism. Subsequently, we performed the metabolomic analysis of bile acids to elucidate how excessive methionine intake affects the metabolism of bile acids, including primary and secondary bile acids. Compared with the CTRL group, AOM/DSS and HM + AOM/DSS groups both had considerably greater levels of conjugated bile acids and a significantly lower ratio of unconjugated to conjugated bile acids (Fig. 6A). Following this, we discovered significant differences in the distribution of primary *vs*. secondary bile acid content, with the AOM/DSS intervention leading to a higher primary/secondary bile acid ratio in the mouse feces (Fig. 6B). These results showed that AOM/DSS treatment resulted in a decrease in gut microorganisms linked to metabolic bile acid metabolism. In terms of individual bile acid changes, we identified a total of 20 bile acids in all groups (Figs. 6C and D). Compared with CTRL group, AOM/DSS treatment led to a decrease in DCA, LCA, hyodeoxycholic acid (HDCA), ursodeoxycholic acid (UDCA), *α*-muricholic acid (αMCA), and ωMCA ($P < 0.05$). In addition, AOM/DSS treatment increased levels of βMCA, taurodeoxycholic acid (TDCA), taurolithocholic acid (TLCA, $P < 0.05$). However, in this case, excessive methionine intake resulted in a significant increase in LCA, DCA, HDCA, TDCA, TLCA, and TUDCA. Among them, the most significant increase appeared in LCA, with more than 2-fold increase ($P < 0.001$), which is the most potent natural agonist of the bile acid receptor TGR5. Subsequently, Spearman's correlation analysis was performed to examine the relationship between fecal bile acids and the genera of gut microbiota. As shown in Fig. 6E, microbiota enriched for excessive methionine intake were significantly positively correlated with the synthesis of secondary bile acids, especially LCA. *Turicibacter*, *Eubacterium*, *Enterococcus*, *Streptococcus*, *Butyricimonas*, *Clostridium*, and *Faecalibaculum* were positively associated with LCA and HDCA ($P < 0.05$). Furthermore, *Bacteroides* and *Alistipes*, whose abundance were reduced under excessive methionine intake, were significantly negatively correlated with secondary bile acids, especially LCA and HDCA ($P < 0.05$).

In summary, fecal bile acids were discovered to be dramatically altered by excessive methionine intake, which were significantly associated with dysbiosis of the gut microbiome.

3.7 *High dietary methionine intake increases TGR5 expression and activates STAT3 and YAP program*

TGR5 is a G-coupled bile acid-responsive membrane receptor that is ubiquitously expressed in several tissues. However, the function of TGR5

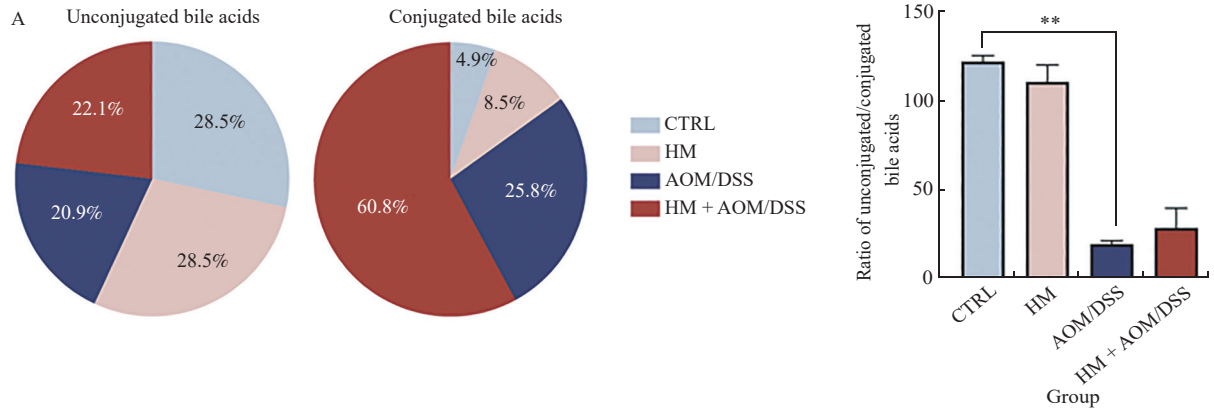

Fig. 6 High dietary methionine modulated bile acid metabolism. (A) Percent ratio of conjugated bile acids and unconjugated bile acids varies in the different groups. (B) Percent ratio of primary bile acids and secondary bile acids varies in the different groups. (C) Content of unconjugated bile acids in feces. (D) Content of conjugated bile acids in feces. (E) Spearman correlation analysis between different gut microbiota and different bile acids in control diet and high methionine diet. Data are shown as means ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

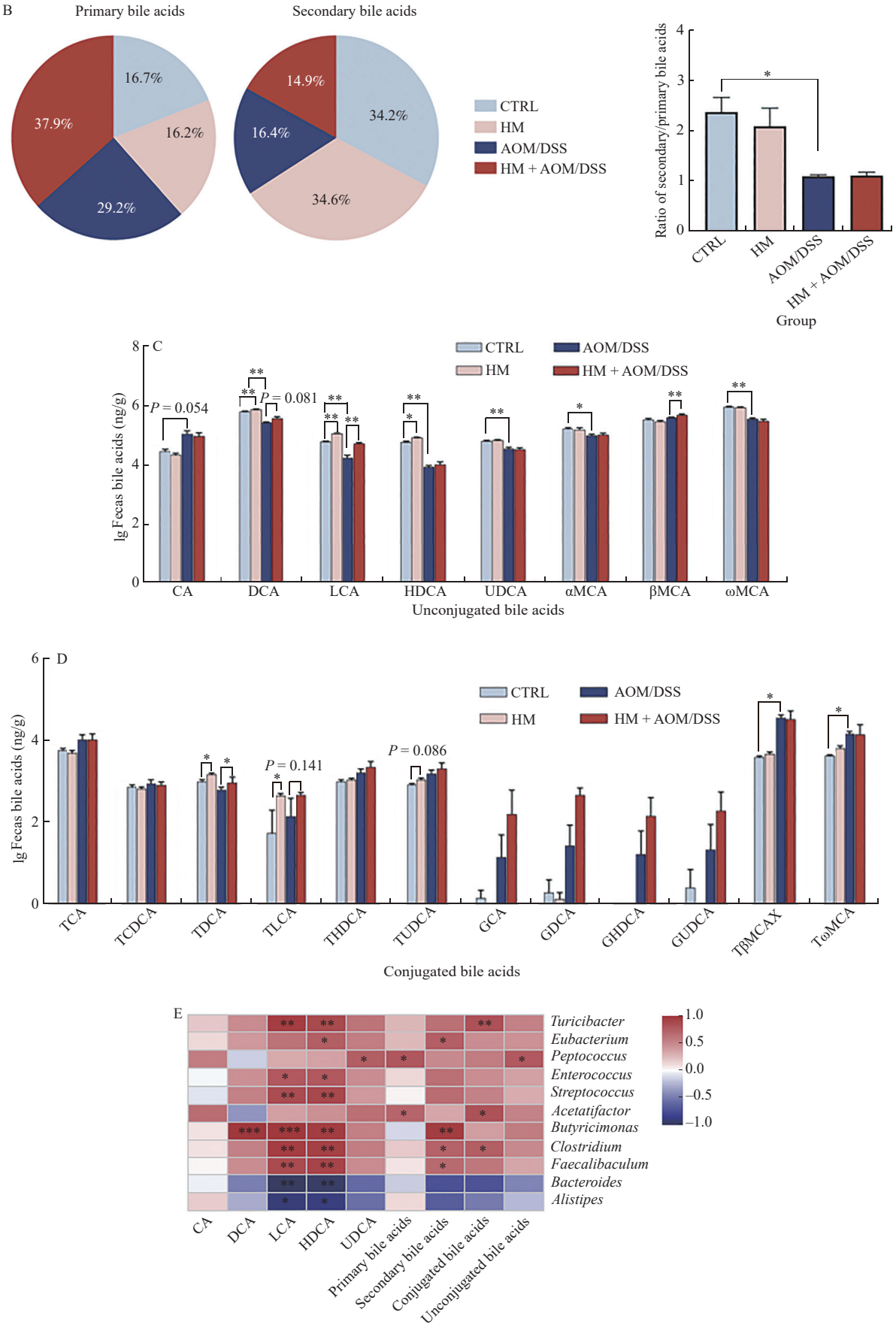

Fig. 6 (*Continued*)

in CRC development is unclear. In our experiments, we found the expression of TGR5 protein was significantly increased in colon tissues following AOM/DSS treatment (Fig. 7A). Compared to CTRL group, AOM/DSS group and HM + AOM/DSS group significantly increased the expression of TGR5 ($P < 0.05$). It has been shown that TGR5 can drive cancer cell to growth and migration through the activation of the signal transducer and activator of the transcription 3 (STAT3) signaling pathway in lung cancer[44]. Therefore, we examined the expression of phosphorylated JAK2, total JAK2, phosphorylated STAT3 and total STAT3 in colon tissues and found that p-STAT3 expression was significantly up-regulated after AOM/DSS treatment. Moreover, compared with the AOM/DSS group, the expression of p-JAK2 and p-STAT3 in the colon tissue of the HM + AOM/DSS group were higher, but there was no significant difference in total STAT3 expression (Fig. 7B). Furthermore, a trend consistent with phosphorylated STAT3 was also observed in the detection of phosphorylated YAP protein, which is probably a result of the high expression of TGR5 efficiently stimulating YAP signaling (Fig. 7C). The increased expression of TGR5 significantly induced a series of typical YAP target genes and further contributed to tumor proliferation (Fig. 7D).

Taken together, these results demonstrate that high methionine intake contributes significantly to the progression of CRC and may induce tumor progression by stimulating JAK2/STAT3 and YAP signaling *via* activating TGR5.

3.8 High dietary methionine promoted CRC progression via gut microbiota and bile acids

To verify the role of gut microbiota in CRC, we used pseudo-germ-free PGF CRC mice to explore the effects of high methionine. As shown in Figs. 8A-C, the food intake, body weight and DAI score

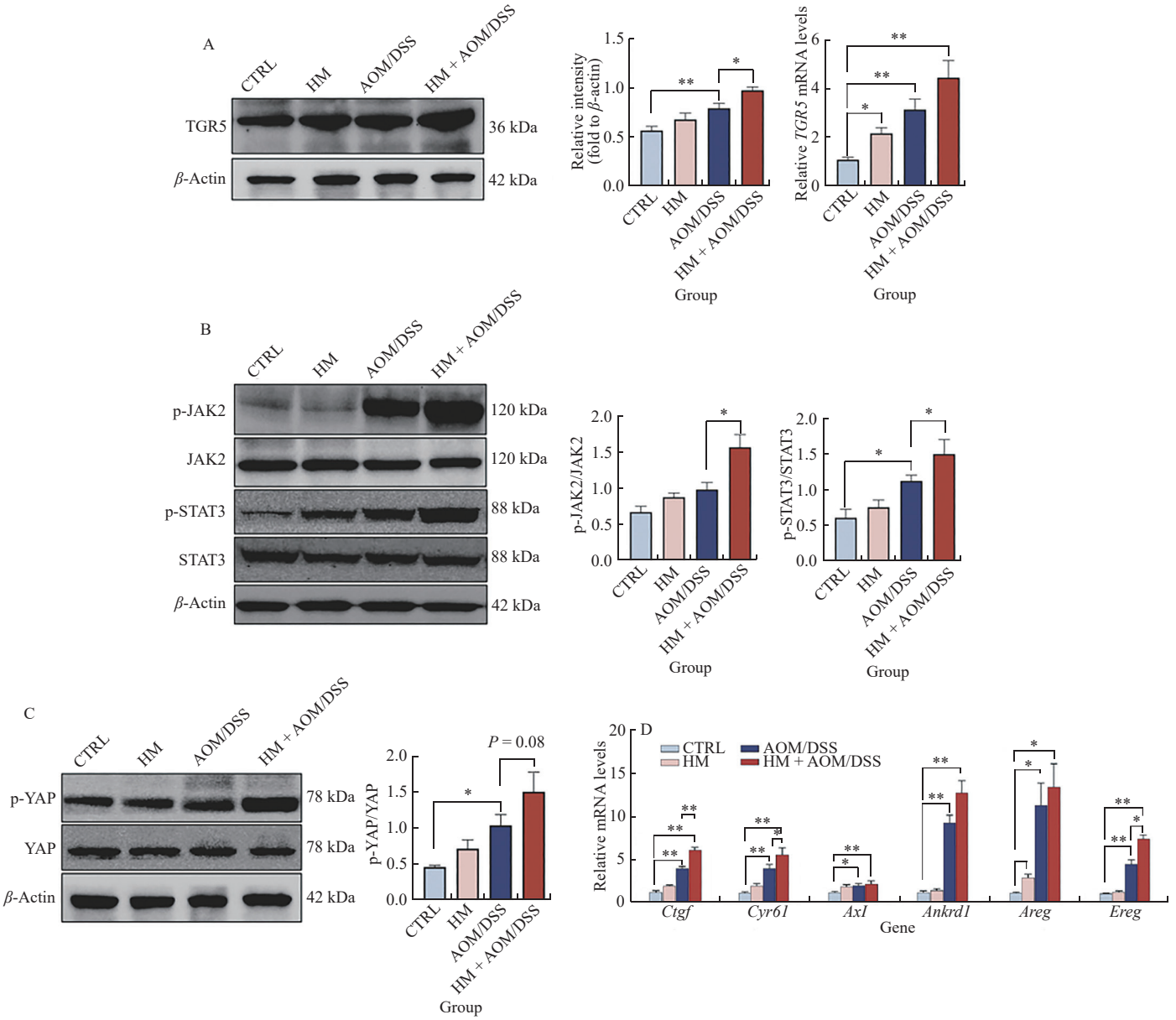

Fig. 7 High dietary methionine promotes tumor development through JAK2/STAT3 and YAP signaling. (A) The expression levels of TGR5 were measured in intestinal tumor tissue of CRC mice by RT-qPCR and Western blot. (B) Western blotting of JAK2 and STAT3. (C) Western blotting of YAP. (D) mRNA level of YAP target genes in intestinal tumor tissue. Data are shown as means ± SEM. *$P < 0.05$ and **$P < 0.01$.

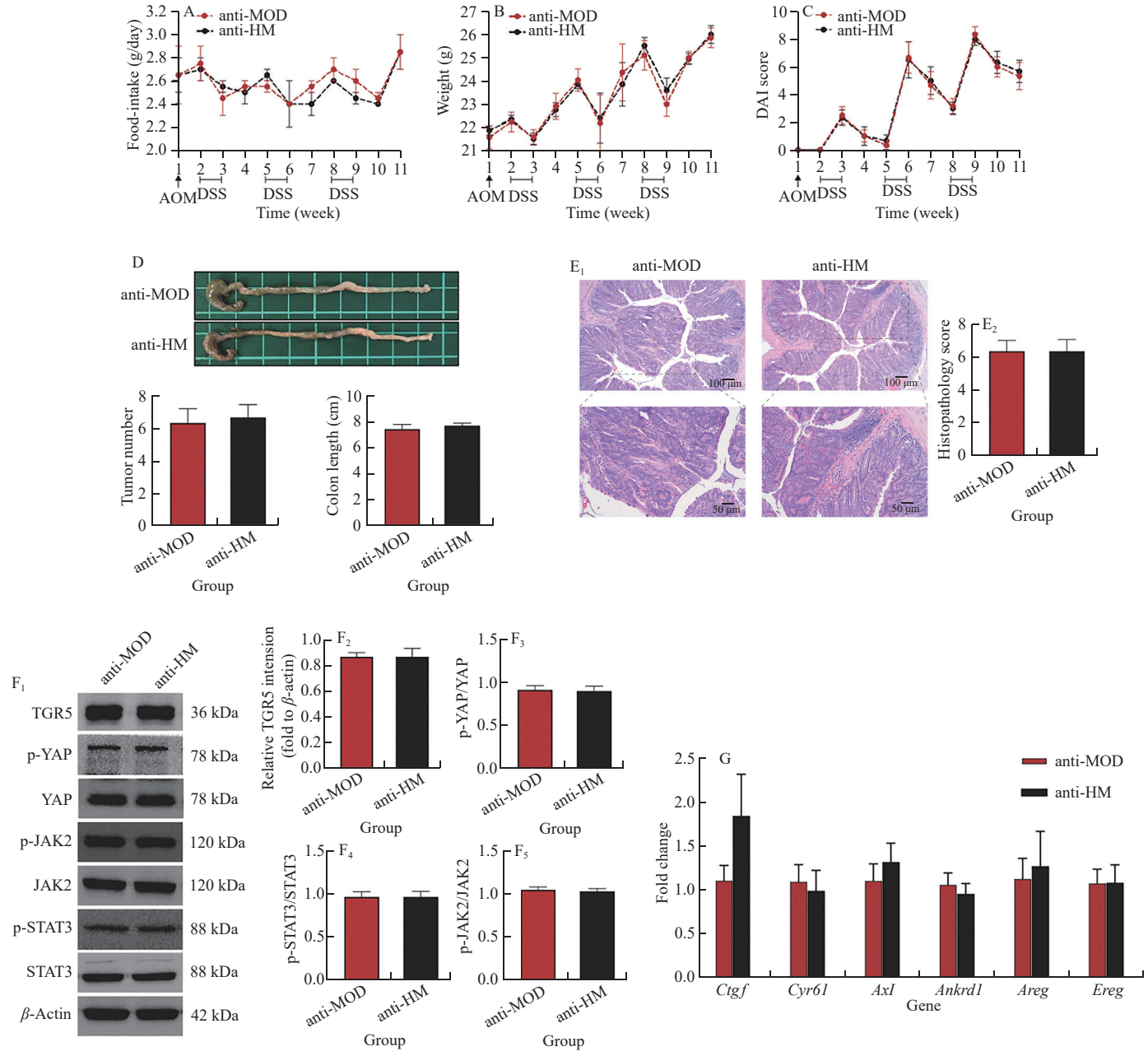

Fig. 8 High dietary methionine promoted CRC progression *via* gut microbiota. (A) Food intake. (B) Body weight. (C) DAI score. (D) Representative image of colons, total number of tumors and average colon length. (E) Representative images of H&E staining pathological sections of the colon tissues and quantitative histopathology score. (F) Expression levels of TGR5, JAK2, STAT3 and YAP were measured in intestinal tumor tissue of CRC mice by Western blot. (G) mRNA level of YAP target genes in intestinal tumor tissue. Scale bar, 100 μm (upper panel) and 50 μm (lower panel).

of CRC mice fluctuated along with the AOM/DSS administration cycle. However, there was no significant difference between anti-MOD group and anti-HM group. As shown in Fig. 8D, the total number of tumors and colon length were no significant difference between anti-MOD group and anti-HM group. There was no significant difference between 2 groups in colon structural damage caused by AOM/DSS depletion (Fig. 8E). Besides, we examined the expression of TGR5, phosphorylated YAP, total YAP, phosphorylated JAK2, total JAK2, phosphorylated STAT3, total STAT3 and a series of typical YAP target genes. As shown in Figs. 8F and G, there was no significant difference between anti-MOD group and anti-HM group.

4. Discussion

The role of dietary nutrition in cancer therapy and prolonged the survival time of cancer patients has received increasing attention. Determination of efficacious and safe quantities of specific dietary amino acids intake is necessary for appropriate clinical nutrition strategies. Methionine is an essential amino acid involved in a variety of biological processes. However, the health effects of methionine are two-sided. Excessive intake of methionine may produce gastrointestinal toxicity.

In our experiment, we found that the administration of excessive methionine intake to CRC mice significantly increased the number of tumors in the colorectum, exacerbated symptoms such as weight

loss and intestinal atrophy, and directly led to increased mortality (Figs. 1B-C, and 3A-C). The overexpression of Ki-67, a marker of cell proliferation, and Vimentin, a marker of epithelial interstitial transformation, in CRC tissues predicted a higher degree of tumor malignancy (Figs. 3E-G). Besides, excessive intake of methionine aggravated the epithelial structural damage of colonic tissue, exacerbated the dysfunction of the mucosal barrier and decreased the expression of tight junction proteins ZO-1 and occludin. The destroyed intestinal barrier led to an even greater infiltration of harmful bacteria and immune triggers, exacerbating the inflammatory response.

Colon has an anatomical location with the most abundant collection of microorganisms in the human body. Over the recent decades, more studies have concentrated on exploring the relationship between microbial dysbiosis and CRC pathogenesis. Many experiments have demonstrated that gut microbial dysbiosis plays a significant role in CRC development. Certain microorganisms and metabolites can drive and accelerate the development of CRC[45-47]. Diet is a major environmental risk factor that can directly influence gut microbiota composition[48]. As an important dietary component, amino acids are actively involved in the composition and metabolism of gut microorganisms. Previous studies have found that dietary amino acids, including methionine, are not completely absorbed by the small intestine. Some amino acids can enter the cecum and colon. Amino acids are absorbed through the colon epithelium and also participate in intestinal microbial metabolism[14]. Recent investigations have unveiled that the diminutive molecules arising from the intricate metabolic orchestration of methionine exert a discernible influence upon the intricate interplay amongst microorganisms, as well as the dynamic interrelationship between bacteria and their host within the precincts of the intestinal milieu[14].

In our study, compared with AOM/DSS group, methionine further decreased the richness and diversity of microbiota composition. Recent research suggests that the dysbiosis of the gut microbiota plays a crucial role in CRC process[49]. Although the causal relationship between microbes and intestinal disease is not yet fully understood, we already know that when the dynamic balance of microbes in the gut is disrupted, the number of pathogenic bacteria may increase the susceptibility to tumorigenesis[50]. Our data suggest that the abundances of several bacteria, including *Escherichia-Shigella*, *Streptococcus*, *Eubacterium* and *E. faecalis*, increased in CRC models and under the influence of excessive methionine feeding (Figs. 5E and F). There is an indication that *Escherichia-Shigella* is positively correlated with high expression of pro-inflammatory cytokines that disrupt intestinal barrier integrity[51]. In previous studies, there is substantial evidence that *Streptococcus* has a tumor-promoting effect on colon cells. *Streptococcus bovis* stimulates the advancement of preneoplastic lesions by enhancing cellular proliferation and the production of IL-8 in a rat model[52]. At the same time, the CRC environment is conducive to the colonization of *Streptococcus*. And bile acids, especially secondary bile acids, enormously amplify the effectiveness of this bacteriocin, resulting in heightened colonization of SGG bacteria in mice[53]. Several reports have shown that *Eubacterium* drives colorectal carcinogenesis by regulating the immune response of colonic epithelial cells[18]. *E. faecalis* stimulates CRC growth through proliferative and angiogenic effects on tumors[54]. According to these studies, excessive intake of methionine may promote tumor growth in mice by increasing the abundance of pathogenic bacteria, like *Escherichia-Shigella*, *Streptococcus*, *Eubacterium* and *E. faecalis*.

Accumulating evidence suggests that the metabolites produced by the microbiota might impact host health and the development of diseases[55-57]. We know that bile acid metabolism disorder caused by dysbiosis is a risk factor for CRC. Past studies have found high concentrations of bile acids in the feces of CRC patients and corresponding high-risk populations[58-60]. Some studies have confirmed the strong association of CRC with bile acids, especially secondary bile acids[61]. An interesting fact was found that some bacteria elevated under excessive methionine feeding such as *Clostridium*, *Enterococcus*, *Lactobacillus*. These bacteria mediate enzymatic changes from primary to secondary bile acids, which exhibit characteristics associated with inflammation and potential carcinogenesis[62-64]. Our data found that high methionine intake significantly increased the abundance of *Streptococcus*, which may be caused by the increased content of the secondary bile acid LCA. Overall, we found that changes in the bile acid profile in the gut resulted from changes in the bacteria. In our experiment, data proved that excessive intake of methionine increases levels of secondary bile acids LCA, DCA and HDCA in the intestine. Moreover, the damage of intestinal barrier and the distortion of fold structure increased the exposure of cells to bile acid and promoted the progression from adenoma to adenocarcinoma in mice. Previous studies have shown that the abundance of secondary bile acids in the large intestine may lead to an increase in intestinal permeability[65]. These results suggest that the accelerated tumor progression in the HM + AOM/DSS group may be due to the abundance of secondary bile acid caused by changes in the microbiota. Pseudo-germ-free mice validated the role of gut microbiota and secondary bile acids in promoting CRC by high dietary methionine.

Recent studies implied a pivotal function of STAT3 in inflammation-associated tumorigenesis. Continuously activated STAT3 contributes to heightened proliferation, survival, and invasion of tumor cells, concurrently dampening immune responses against tumors through the inhibition of anti-tumor T helper 1 (TH1) cytokines like IL-12 and interferon-γ. It is well known that TGR5 is a bile acid membrane receptor, which is recognized for its participation in multiple bile acid-regulated biological and pathological mechanisms. TGR5 is widely distributed throughout the body, including the gallbladder, fat, lung, colon, and other parts. Excessive intake of methionine increases contents of LCA and DCA (Fig. 6C), which are considered to be the most effective natural agonists of TGR5[66]. Although the function of TGR5 may vary in different disease models, previous studies have found that TGR5 can promote non-small cell lung cancer, cholangiocarcinoma and gastric cancer progression[67-69]. Several G-protein-coupled receptors (GPCRs) have been identified to trigger the activation of STAT3 *via* JAKs, resulting in the advancement of cancer[69-70]. As a member of GPCRs, it has been reported that TGR5 promotes the proliferation of lung cancer by activating the JAK2-STAT3 signaling pathway[71]. Our data shown secondary bile acids promote CRC progression by activating the TGR5-mediated JAK2-STST3 signaling pathway. YAP is a transcriptional coactivator, which is critical for cancer initiation and progression. The activation of YAP is widespread in many human cancers[72-73]. Elevated YAP expression levels are associated with poor prognosis in dataset of CRC patients[74]. Recent evidence suggests TGR5 can

effectively activate YAP in the intestine to promote the growth of stem cells[75]. Consistently, similar results were observed in our study, YAP was significantly activated in the HM + AOM/DSS group, which the high expression of TGR5 may cause. It is worth to further verified in TGR5 knockout mice, which was a limitation of our study.

Compared with CTRL group, the administration of high dietary methionine alone to mice without AOM/DSS-treated did not significantly affect the body weight, food intake, inflammatory response, colon length and intestinal barrier of mice within 11 weeks. However, the HM group showed similar results to the HM + AOM/DSS group in regulating the gut microbiota composition and bile acid metabolism. Overall, the disturbed gut microbiota led to an increase in the abundance of pathogenic bacteria. Then, the changes of the gut microbiota composition affected the contents of bile acids, leading to an increase in the content of cytotoxic secondary bile acids. Therefore, excessive methionine intake may cause long-term health hazards by affecting the composition of the gut microbiota and bile acids, such as promoting proliferation of tumor cells. Therefore, it is extremely important to control the intake of high methionine foods for clinical CRC patients and high-risk populations.

5. Conclusion

The current study demonstrated the role of excessive methionine intake in promoting tumor development from the perspective of regulating microbial composition and bile acid metabolism. Excessive methionine intake exacerbated the inflammatory response, intestinal barrier impairment, and gut microflora dysbiosis. Accordingly, bile acid profile in the intestinal environment changes dramatically, further exacerbating tumor development by activating bile acid receptors and STAT3/YAP program. Taken together, our study revealed the risks of excessive methionine intake on intestinal health and the underlying mechanisms, further emphasizing the necessity of regulating dietary intake of specific amino acids in the maintenance of intestinal health and cancer treatment.

Conflict of interest

Shuo Wang is an editorial board member for *Food Science and Human Wellness* and was not involved in the editorial review or the decision to publish this article. 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.

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Appendix A. Supplementary information

Supplementary data associated with this article can be found, in the online version, at http://doi.org/10.26599/FSHW.2024.9250237.

References

[1] S.A. Bingham, N.E. Day, R. Luben, et al., Dietary fibre in food and protection against colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC): an observational study, Lancet 361 (2003) 1496-1501. https://doi.org/10.1016/s0140-6736(03)13174-1.

[2] A.T. Kunzmann, H.G. Coleman, W.Y. Huang, et al., Dietary fiber intake and risk of colorectal cancer and incident and recurrent adenoma in the prostate, lung, colorectal, and ovarian cancer screening trial, Am. J. Clin. Nutr. 102 (2015) 881-890. https://doi.org/10.3945/ajcn.115.113282.

[3] S.C. Larsson, E. Giovannucci, L. Bergkvist, et al., Whole grain consumption and risk of colorectal cancer: a population-based cohort of 60,000 women, Br. J. Cancer 92 (2005) 1803-1807. https://doi.org/10.1038/sj.bjc.6602543.

[4] S.M. Sanderson, X. Gao, Z. Dai, et al., Methionine metabolism in health and cancer: a nexus of diet and precision medicine, Nat. Rev. Cancer 19 (2019) 625-637. https://doi.org/10.1038/s41568-019-0187-8.

[5] G.P. Ables, J.E. Johnson, Pleiotropic responses to methionine restriction, Exp. Gerontol. 94 (2017) 83-88. https://doi.org/ 10.1016/j.exger.2017.01.002.

[6] Joint W.H.O.F.A.O.U.N.U.E.C., Protein and amino acid requirements in human nutrition. World Health Organ. Tech. Rep. Ser. (2007) 1-265, back cover. https://doi.org/10.1016/j.jnutbio.2019.108321.

[7] J. Gomez, P. Caro, I. Sanchez, et al., Effect of methionine dietary supplementation on mitochondrial oxygen radical generation and oxidative DNA damage in rat liver and heart, J. Bioenerg. Biomembr. 41 (2009) 309-321. https://doi.org/10.1007/s10863-009-9228-4.

[8] X. Gao, S.M. Sanderson, Z. Dai, et al., Dietary methionine influences therapy in mouse cancer models and alters human metabolism, Nature 572 (2019) 397-401. https://doi.org/10.1038/s41586-019-1437-3.

[9] N.E. Deutz, E.M. Matheson, L.E. Matarese, et al., Readmission and mortality in malnourished, older, hospitalized adults treated with a specialized oral nutritional supplement: a randomized clinical trial, Clin. Nutr. 35 (2016) 18-26. https://doi.org/10.1016/j.clnu.2015.12.010.

[10] Z. Wang, L.Y. Yip, J.H.J. Lee, et al., Methionine is a metabolic dependency of tumor-initiating cells, Nat. Med. 25 (2019) 825-837. https://doi.org/10.1038/s41591-019-0423-5.

[11] B. Duranton, J.N. Freund, M. Galluser, et al., Promotion of intestinal carcinogenesis by dietary methionine, Carcinogenesis 20 (1999) 493-497. https://doi.org/10.1093/carcin.

[12] Y. Bian, W. Li, D.M. Kremer, et al., Cancer SLC43A2 alters T cell methionine metabolism and histone methylation, Nature 585 (2020) 277-282. https://doi.org/10.1038/s41586-020-2682-1.

[13] M. Ji, X. Xu, Q. Xu, et al., Methionine restriction-induced sulfur deficiency impairs antitumour immunity partially through gut microbiota, Nat. Metab. 5 (2023) 1526-1543. https://doi.org/10.1038/s42255-023-00854-3.

[14] N. Ma, X. Ma, Dietary amino acids and the gut-microbiome-immune axis: physiological metabolism and therapeutic prospects, Compr. Rev. Food Sci. Food Saf. 18 (2019) 221-242. https://doi.org/10.1111/1541-4337.12401.

[15] W.S. Garrett, The gut microbiota and colon cancer, Science 364 (2019) 1133-1135. https://doi.org/10.1126/science.aaw2367.

[16] T.A. D'Asheesh, B.M. Hussen, A.H. Al-Marzoqi, et al., Assessment of oncogenic role of intestinal microbiota in colorectal cancer patients, J. Gastrointest Cancer 52 (2021) 1016-1021. https://doi.org/10.1007/s12029-020-00531-8.

[17] X. Wang, Y. Yang, M.M. Huycke, Commensal bacteria drive endogenous transformation and tumour stem cell marker expression through a bystander effect, Gut 64 (2015) 459-468. https://doi.org/10.1136/gutjnl-2014-307213.

[18] Y. Wang, X. Wan, X. Wu, et al., *Eubacterium rectale* contributes to colorectal cancer initiation *via* promoting colitis, Gut Pathog. 13 (2021) 2. https://doi.org/10.1186/s13099-020-00396-z.

[19] Y.H. Xie, Q.Y. Gao, G.X. Cai, et al., Fecal *Clostridium* symbiosum for noninvasive detection of early and advanced colorectal cancer: test and validation studies, EBioMedicine 25 (2017) 32-40. https://doi.org/10.1016/j.ebiom.2017.10.005.

[20] T.M. Karpinski, M. Ozarowski, M. Stasiewicz, Carcinogenic microbiota and its role in colorectal cancer development, Semin Cancer Biol. 86 (2022) 420-430. https://doi.org/10.1016/j.semcancer.2022.01.004.

[21] M. Song, A.T. Chan, J. Sun, Influence of the gut microbiome, diet, and environment on risk of colorectal cancer, Gastroenterology 158 (2020) 322-340. https://doi.org/10.1053/j.gastro.2019.06.048.

[22] T. Liu, X. Song, S. Khan, et al., The gut microbiota at the intersection of bile acids and intestinal carcinogenesis: an old story, yet mesmerizing. Int. J. Cancer 146 (2020) 1780-1790. https://doi.org/10.1002/ijc.32563.

[23] Y. Xiao, J. Zhao, H. Zhang, et al., Mining genome traits that determine the different gut colonization potential of *Lactobacillus* and *Bifidobacterium* species, Microb. Genom. 7 (2021) 000581. https://doi.org/10.1099/mgen.0.000574.

[24] A. Bhagwat, U.S. Annapure, *In vitro* assessment of metabolic profile of *Enterococcus* strains of human origin, J. Genet. Eng. Biotechnol. 17 (2019) 11. https://doi.org/10.1186/s43141-019-0011-6.

[25] J.P. Coleman, L.L. Hudson, Cloning and characterization of a conjugated bile acid hydrolase gene from *Clostridium perfringens*, Appl. Environ. Microbiol. 61 (1995) 2514-2520. https://doi.org/10.1128/aem.61.7.2514-2520.1995.

[26] A.A. Adhikari, T.C.M. Seegar, S.B. Ficarro, et al., Development of a covalent inhibitor of gut bacterial bile salt hydrolases, Nat. Chem. Biol. 16 (2020) 318-326. https://doi.org/10.1038/s41589-020-0467-3.

[27] Y. Liu, S. Zhang, W. Zhou, et al., Secondary bile acids and tumorigenesis in colorectal cancer, Front. Oncol. 12 (2022) 813745. https://doi.org/10.3389/fonc.2022.813745.

[28] W. Jia, G. Xie, W. Jia, Bile acid-microbiota crosstalk in gastrointestinal inflammation and carcinogenesis, Nat. Rev. Gastroenterol. Hepatol. 15 (2018) 111-128. https://doi.org/10.1038/nrgastro.2017.119.

[29] L. Farhana, P. Nangia-Makker, E. Arbit, et al., Bile acid: a potential inducer of colon cancer stem cells, Stem Cell Res. Ther. 7 (2016) 181. https://doi.org/10.1186/s13287-016-0439-4.

[30] T. Fu, S. Coulter, E. Yoshihara, et al., FXR regulates intestinal cancer stem cell proliferation, Cell 176 (2019) 1098-1112 https://doi.org/10.1016/j.cell.2019.01.036.

[31] A. Carino, L. Graziosi, C. D'Amore, et al., The bile acid receptor GPBAR1 (TGR5) is expressed in human gastric cancers and promotes epithelial-mesenchymal transition in gastric cancer cell lines, Oncotarget 7 (2016) 61021-61035. https://doi.org/10.18632/oncotarget.10461.

[32] L. Wang, B. Ren, Q. Zhang, et al., Methionine restriction alleviates high-fat diet-induced obesity: involvement of diurnal metabolism of lipids and bile acids, Biochim. Biophys. Acta Mol. Basis Dis. 1866 (2020) 165908. https://doi.org/10.1016/j.bbadis.2020.165908.

[33] K.P. Stone, D. Wanders, M. Orgeron, et al., Mechanisms of increased *in vivo* insulin sensitivity by dietary methionine restriction in mice, Diabetes 63 (2014) 3721-3733. https://doi.org/10.2337/db14-0464.

[34] J.D. Finkelstein, J.J. Martin, Methionine metabolism in mammals. Adaptation to methionine excess, J.Biol.Chem. 261 (1986) 1582-1587. https://doi.org/10.1016/S0021-9258(17)35808-4.

[35] M. Regina, V.P. Korhonen, T.K. Smith, et al., Methionine toxicity in the rat in relation to hepatic accumulation of *S*-adenosylmethionine: prevention by dietary stimulation of the hepatic transsulfuration pathway. Arch. Biochem. Biophys. 300 (1993) 598-607. https://doi.org/10.1006/abbi.1993.1082.

[36] E.K. Lees, E. Krol, L. Grant, et al., Methionine restriction restores a younger metabolic phenotype in adult mice with alterations in fibroblast growth factor 21, Aging Cell. 13 (2014) 817-827. https://doi.org/10.1111/acel.12238.

[37] A. Kumar, R. Pathak, H.A. Palfrey, et al., High levels of dietary methionine improves sitagliptin-induced hepatotoxicity by attenuating oxidative stress in hypercholesterolemic rats, Nutr. Metab. 17 (2020) 2. https://doi.org/10.1186/s12986-019-0425-9.

[38] X. Liu, Y. Zhang, W. Li, et al., Differential responses on gut microbiota and microbial metabolome of 2′-fucosyllactose and galactooligosaccharide against DSS-induced colitis, Food Res. Int. 162 (2022) 112072. https://doi.org/10.1016/j.foodres.2022.112072.

[39] C. Quach, Y. Song, H. Guo, et al., A truncating mutation in the autophagy gene UVRAG drives inflammation and tumorigenesis in mice, Nat. Commun. 10 (2019) 5681. https://doi.org/10.1038/s41467-019-13475-w.

[40] B. Amplatz, E. Zohrer, C. Haas, et al., Bile acid preparation and comprehensive analysis by high performance liquid chromatography-high-resolution mass spectrometry, Clin. Chim. Acta 464 (2017) 85-92. https://doi.org/10.1016/j.cca.2016.11.014.

[41] T. Yang, T. Shu, G. Liu, et al., Quantitative profiling of 19 bile acids in rat plasma, liver, bile and different intestinal section contents to investigate bile acid homeostasis and the application of temporal variation of endogenous bile acids, J. Steroid Biochem. Mol. Biol. 172 (2017) 69-78. https://doi.org/10.1016/j.jsbmb.2017.05.015.

[42] L. Wrzosek, D. Ciocan, C. Hugot, et al., Microbiota tryptophan metabolism induces aryl hydrocarbon receptor activation and improves alcohol-induced liver injury, Gut 70 (2021) 1299-1308. https://doi.org/10.1136/gutjnl-2020-321565.

[43] J. Huang, D. Liu, Y. Wang, et al., Ginseng polysaccharides alter the gut microbiota and kynurenine/tryptophan ratio, potentiating the antitumour effect of antiprogrammed cell death 1/programmed cell death ligand 1 (anti-PD-1/PD-L1) immunotherapy, Gut 71 (2022) 734-745. https://doi.org/10.1136/gutjnl-2020-321031.

[44] X. Liu, B. Chen, W. You, et al., The membrane bile acid receptor TGR5 drives cell growth and migration *via* activation of the JAK2/STAT3 signaling pathway in non-small cell lung cancer, Cancer Lett. 412 (2018) 194-207. https://doi.org/10.1016/j.canlet.2017.10.027.

[45] A. Gupta, R. Madani, H. Mukhtar, *Streptococcus bovis* endocarditis, a silent sign for colonic tumour, Colorectal. Dis. 12 (2010) 164-171. https://doi.org/10.1111/j.1463-1318.2009.01814.x.

[46] A. Bashir, A.Y. Miskeen, A. Bhat, et al., *Fusobacterium nucleatum*: an emerging bug in colorectal tumorigenesis, Eur. J. Cancer Prev. 24 (2015) 373-385. https://doi.org/10.1097/CEJ.0000000000000116.

[47] X. Wang, M.M. Huycke, Extracellular superoxide production by *Enterococcus faecalis* promotes chromosomal instability in mammalian cells, Gastroenterology 132 (2007) 551-561. https://doi.org/10.1053/j.gastro.2006.11.040.

[48] M.J. Claesson, I.B. Jeffery, S. Conde, et al., Gut microbiota composition correlates with diet and health in the elderly, Nature 488 (2012) 178-184. https://doi.org/10.1038/nature11319.

[49] S. Majumder, U.N. Shivaji, R. Kasturi, et al., Inflammatory bowel disease-related colorectal cancer: Past, present and future perspectives, World J. Gastrointest. Oncol. 14 (2022) 547-567. https://doi.org/10.4251/wjgo.v14.i3.547.

[50] S.A. Fobofou, T. Savidge, Microbial metabolites: cause or consequence in gastrointestinal disease? Am. J. Physiol. Gastrointest Liver Physiol. 322 (2022) G535-G552. https://doi.org/10.1152/ajpgi.00315.2021.

[51] J.Xu, N. Chen, Z. Wu, et al., 5-Aminosalicylic acid alters the gut bacterial microbiota in patients with ulcerative colitis, Front Microbiol. 9 (2018) 1274. https://doi.org/10.3389/fmicb.2018.01274.

[52] S. Ellmerich, M. Scholler, B. Duranton, et al., Promotion of intestinal carcinogenesis by *Streptococcus bovis*, Carcinogenesis 21 (2000) 753-756. https://doi.org/10.1093/carcin/21.4.753.

[53] L. Aymeric, F. Donnadieu, C. Mulet, et al., Colorectal cancer specific conditions promote *Streptococcus gallolyticus* gut colonization, PNAS 115 (2018) E283-E291. https://doi.org/10.1073/pnas.1715112115.

[54] L. Zhang, J. Liu, M. Deng, et al., *Enterococcus faecalis* promotes the progression of colorectal cancer *via* its metabolite: biliverdin, J. Transl. Med. 21 (2023) 72. https://doi.org/10.1186/s12967-023-03929-7.

[55] S.L. Long, C.G.M. Gahan, S.A. Joyce, Interactions between gut bacteria and bile in health and disease, Mol. Aspects Med. 56 (2017) 54-65. https://doi.org/10.1016/j.mam.2017.06.002.

[56] X. Gao, J. Zhao, H. Zhang, et al., Modulation of gut health using probiotics: the role of probiotic effector molecules, J. Fut. Food 2 (2022) 1-12. https://doi.org/10.1016/j.jfutfo.2022.03.011.

[57] C. Wang, J. Bai, B. Wang, et al., Stachyose modulates gut microbiota and alleviates DSS-induced ulcerative colitis in mice, Food Sci. Hum. Wellness 12 (2023) 2211-2220. https://doi.org/10.1016/j.fshw.2023.03.041

[58] B.S. Reddy, E.L. Wynder, Large-bowel carcinogenesis: fecal constituents of populations with diverse incidence rates of colon cancer, J. Natl. Cancer Inst. 50 (1973) 1437-1442. https://doi.org/10.1093/jnci/50.6.1437.

[59] N. Turjman, G.T. Goodman, B. Jaeger, et al., Diet, nutrition intake, and metabolism in populations at high and low risk for colon cancer. Metabolism of bile acids, Am. J. Clin. Nutr. 40 (1984) 937-941. https://doi.org/10.1093/ajcn.

[60] M.J. Hill, B.S. Drasar, R.E. Williams, et al., Faecal bile-acids and clostridia in patients with cancer of the large bowel, Lancet 1 (1975) 535-539. https://doi.org/10.1016/s0140-6736(75)91556-1.

[61] S. Ocvirk, S.J.D. O'Keefe, Dietary fat, bile acid metabolism and colorectal cancer, Semin. Cancer Biol. 73 (2021) 347-355. https://doi.org/10.1016/j.semcancer.2020.10.003.

[62] J.P. Chae, V.D. Valeriano, G.B. Kim, et al., Molecular cloning, characterization and comparison of bile salt hydrolases from *Lactobacillus johnsonii* PF01, J. Appl. Microbiol. 114 (2013) 121-133. https://doi.org/10.1111/jam.12027.

[63] A. Mukherjee, C. Lordan, R.P. Ross, et al., Gut microbes from the phylogenetically diverse genus *Eubacterium* and their various contributions

to gut health, Gut Microbes 12 (2020) 1802866. https://doi.org/10.1080/19490976.2020.1802866.

[64] A. Wijaya, A. Hermann, H. Abriouel, et al., Cloning of the bile salt hydrolase (*bsh*) gene from *Enterococcus faecium* FAIR-E 345 and chromosomal location of *bsh* genes in food enterococci, J Food Prot. 67 (2004) 2772-2778. https://doi.org/10.4315/0362-028x-67.12.2772.

[65] Y. Murakami, S. Tanabe, T. Suzuki, High-fat diet-induced intestinal hyperpermeability is associated with increased bile acids in the large intestine of mice, J. Food Sci. 81 (2016) H216-H222. https://doi.org/10.1111/1750-3841.13166.

[66] T. Maruyama, Y. Miyamoto, T. Nakamura, et al., Identification of membrane-type receptor for bile acids (M-BAR), Biochem. Biophys. Res. Commun. 298 (2002) 714-719. https://doi.org/10.1016/s0006-291x(02)02550-0.

[67] O. Erice, I. Labiano, A. Arbelaiz, et al., Differential effects of FXR or TGR5 activation in cholangiocarcinoma progression, Biochim. Biophys. Acta Mol. Basis Dis. 1864 (2018) 1335-1344. https://doi.org/10.1016/j.bbadis.2017.08.016.

[68] Z. Ni, Y. Min, C. Han, et al., TGR5-HNF4α axis contributes to bile acid-induced gastric intestinal metaplasia markers expression, Cell Death Discov. 6 (2020) 56. https://doi.org/10.1038/s41420-020-0290-3.

[69] H. Lee, J. Deng, M. Kujawski, et al., STAT3-induced S1PR1 expression is crucial for persistent STAT3 activation in tumors, Nat. Med. 16 (2010) 1421-1428. https://doi.org/10.1038/nm.2250.

[70] H. Xin, R. Lu, H. Lee, et al., G-protein-coupled receptor agonist BV8/prokineticin-2 and STAT3 protein form a feed-forward loop in both normal and malignant myeloid cells, J. Biol. Chem. 288 (2013) 13842-13849. https://doi.org/10.1074/jbc.M113.450049.

[71] L. Zhao, H. Zhang, X. Liu, et al., TGR5 deficiency activates antitumor immunity in non-small cell lung cancer *via* restraining M2 macrophage polarization, Acta Pharm. Sin B 12 (2022) 787-800. https://doi.org/10.1016/j.apsb.2021.07.011.

[72] S. Piccolo, S. Dupont, M. Cordenonsi, The biology of YAP/TAZ: hippo signaling and beyond, Physiol. Rev. 94 (2014) 1287-1312. https://doi.org/10.1152/physrev.00005.2014.

[73] F. Zanconato, M. Forcato, G. Battilana, et al., Genome-wide association between YAP/TAZ/TEAD and AP-1 at enhancers drives oncogenic growth, Nat. Cell Biol. 17 (2015) 1218-1227. https://doi.org/10.1038/ncb3216.

[74] K.W. Lee, S.S. Lee, S.B. Kim, et al., Significant association of oncogene YAP1 with poor prognosis and cetuximab resistance in colorectal cancer patients, Clin. Cancer Res. 21 (2015) 357-364. https://doi.org/10.1158/1078-0432.CCR-14-1374.

[75] G. Sorrentino, A. Perino, E. Yildiz, et al., Bile acids signal *via* TGR5 to activate intestinal stem cells and epithelial regeneration, Gastroenterology 159 (2020) 956-968. https://doi.org/10.1053/j.gastro.2020.05.067.