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Original article

Role of the global gut microbial community in the development of colitis-associated cancer in a murine model

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ABSTRACT

The gut microbiota has been implicated in the development of colitis-associated cancer (CAC). We investigated how the gut microbiota affects the development of CAC when the composition of the microbial community is altered by the administration of various antibiotics in a murine model. C57BL/6 mice were given intraperitoneal injection of 12.5 mg/kg azoxymethane (AOM), followed by two rounds of 2.0 % dextran sodium sulfate (DSS) exposure. Antibiotics, including ampicillin, neomycin, metronidazole, and/or vancomycin, were administered 14 days prior to AOM injection until the end of the experiment. High-throughput sequencing of mice feces was conducted to evaluate alterations of the gut microbiota. Tumorigenesis and inflammation were most markedly suppressed in the mice treated with an antibiotic cocktail therapy consisting of ampicillin, neomycin, metronidazole, and vancomycin. Individual antibiotic treatments had different effects on tumorigenesis and inflammation. Metronidazole attenuated both tumorigenesis and inflammation. Neomycin suppressed tumorigenesis but did not alleviate inflammation. Ampicillin and vancomycin did not significantly attenuate either tumorigenesis or inflammation. Antimicrobial therapy differentially altered the diversity and composition of the gut microbiota depending on antibiotic type. The phyla Proteobacteria and Tenericutes were positively correlated with tumor burden. Colon tumorigenesis was attenuated through various antibiotics in the AOM/DSS-induced CAC model. Individual antibiotics differentially altered the gut microbial composition and showed different effects on tumor suppression; however, the degree of tumor suppression was less pronounced than that relative to the antibiotic cocktail therapy, suggesting that the global gut microbial community plays an important role in the development of CAC.

1. Introduction

Colorectal cancer (CRC) is the third most common cancer in men and the second in women, and is the leading cause of cancer-related deaths worldwide [1]. Colitis-associated cancer (CAC) is a type of CRC that occurs in association with preceding chronic inflammatory bowel disease (IBD) [2]. Patients with IBD have a 60 % increase in the incidence of CRC compared with the general population [3].

CAC is preceded by prolonged gut inflammation, and mutations in the p53 gene occur as an early event [4]. Chronic inflammation also causes direct DNA damage and contributes to colon carcinogenesis [5]. The gut microbiota interacts with the host immune system, and an excessive immune response to commensal microbiota causes gut inflammation [6]. Gut microbiota contribute to carcinogenesis by sustaining inflammation, impairing host immunity, directly damaging DNA with genotoxin, and altering metabolic activity [6]. Gut microbial composition of CRC patients is different from that of healthy individuals [7]. In addition, mucosa-associated microbiota in CAC patients is also different from those in sporadic CRC patients [8].

Attempts have been made to suppress colon tumorigenesis by manipulating the gut microbiota with antimicrobial agents. Colon tumorigenesis was attenuated with antibiotic treatment in a murine CAC model [9]. Depletion of the gut microbiome using antibiotics reduced tumor burden in multiple xenograft cancer models [10]. Specific bacterial species, including *Escherichia coli* carrying the polyketide synthase (pks) island, enterotoxigenic *Bacteroides fragilis*, and *Fusobacterium nucleatum*, have been implicated in the pathogenesis of CRC [11]. However, the gut microbiota consists of a complex community of more than 100 trillion microbes, and it is unclear how gut microbial composition should be changed to effectively suppress colon tumorigenesis. We

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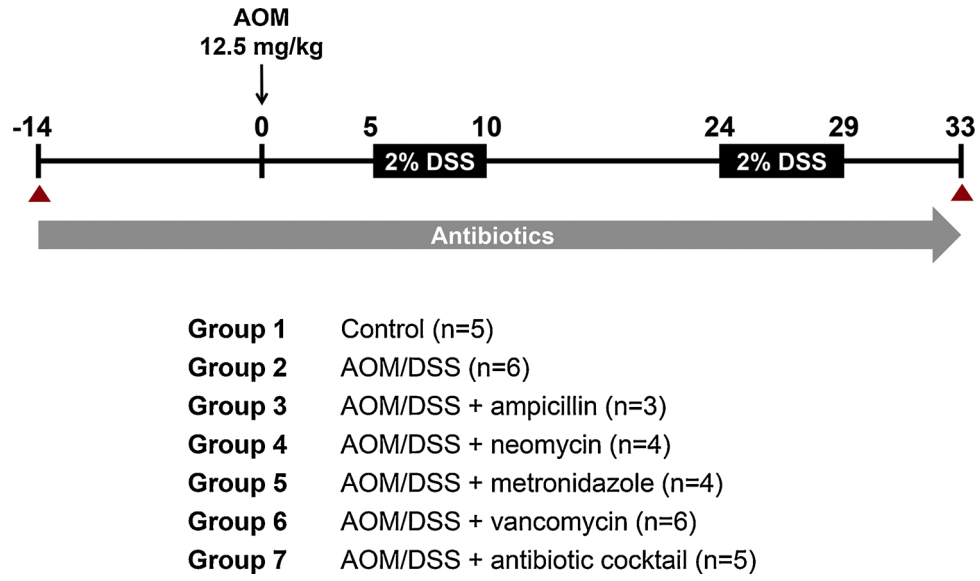

Fig. 1. Study protocol. The antibiotic cocktail included ampicillin, neomycin, metronidazole, and vancomycin. AOM, azoxymethane; DSS, dextran sodium sulfate.

therefore aimed to investigate how composition of the gut microbial community affects the development of CAC when it is altered by administering antibiotics targeting different species in the microbial community using the azoxymethane (AOM)/dextran sodium sulfate (DSS)-induced murine CAC model. We aimed to identify the specific gut microbial community involved in CAC development and to obtain information on the potential prevention and treatment strategy for CAC.

2. Materials and methods

2.1. Animals

We used C57BL/6 mice (female, 6-weeks-old) obtained from Orient Bio (Seongnam, Korea) for this study. A total of 35 mice were used for the experiment. Mice were co-housed in groups and housed at 23 ± 3 °C, 50 ± 20 % humidity, in a 12/12 -h light/dark cycle, and free access to food and water under specific pathogen-free conditions in an accredited animal facility at Hanyang University. All mice were fed a standard mouse chow (LabDiet 5053, Orient Bio, Korea). To minimize animal suffering and to determine humane endpoints, mice were monitored daily for the following signs of distress: weight change, hair loss, abnormal eye opening, reduced physical activity, and abnormal posture. The criteria for determining the humane endpoint are shown in Table A1 in supplementary material. All experimental procedures were performed according to the guidelines outlined and approved by the Animal Experimental Ethics Committee of Hanyang University (approval number: HY-IACUC-17−0007).

2.2. Induction of colitis-associated cancer

The mice received a single intraperitoneal injection of 12.5 mg/kg AOM. On the fifth day after AOM injection, the mice were fed with water containing 2.0 % DSS for five days, and then untreated water for 14 days. This process was repeated once again, resulting in a total of two rounds of DSS administration. The control group was given phosphate buffered saline intraperitoneally and normal drinking water without DSS. The mice were euthanized by CO2 asphyxiation followed by cervical dislocation 4 days after the last round of DSS administration.

2.3. Antibiotic treatment

Antibiotics were mixed with drinking water and administered 14 days prior to AOM injection until the end of the experiment. Four types of antibiotics were used: ampicillin, neomycin, metronidazole, and vancomycin. The mice were divided into seven groups according to antibiotic treatment: (1) control group (n = 5), no AOM/DSS and no antibiotics; (2) AOM/DSS group (n = 6), AOM/DSS without antibiotics; (3) ampicillin group (n = 4), ampicillin (500 mg/L) administration; (4) neomycin group (n = 4), neomycin (1 g/L) administration; (5) metronidazole group (n = 4), metronidazole (1 g/L) administration; (6) vancomycin group (n = 6), vancomycin (250 mg/L) administration; and (7) antibiotic cocktail group (n = 6), mixture of ampicillin, neomycin, metronidazole, and vancomycin. One mouse in the ampicillin treatment group and one in the antibiotic cocktail treatment group were euthanized early because they met the humane endpoint before the end of the experiment. No mice were found dead during the experiment. Finally, five mice in the control group, six in the AOM/DSS group, three in the ampicillin treatment group, four in the neomycin treatment group, four in the metronidazole treatment group, six in the vancomycin treatment group, and five in the antibiotic cocktail treatment group completed the experiment and were analyzed. The study protocol is shown in Fig. 1.

2.4. Gross and histological analysis

After euthanasia, mice colons were extracted to measure tumor burden and tissue inflammation. Digital photographs were taken, colon length and the number of tumors in the entire colon were measured. Colon tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and stained with hematoxylin and eosin for histological evaluation. Three of the authors, blinded to the slide information, measured histological scores by summing the following scores as described in a previous study: degree of inflammation (0–4), epithelial defects (0–4), crypt atrophy (0–4), degree of dysplasia/neoplasia (0–4), and extent of dysplasia/neoplasia (0–4) (Table A2 in supplementary material) [5].

2.5. Reverse transcription PCR (RT-PCR)

Total RNA was extracted from colon tissues using the Hybrid-R™ Total RNA Isolation Kit (GeneAll Biotechnology, Seoul, Korea) and quantified using a Biospec-nano spectrophotometer (Life Science, Columbia, MD, USA). RT-PCR was performed with an initial denaturation at 95 °C for 2 min, 30 times with denaturation at 95 °C for 30 s, 55−65 °C for 30 s for primer annealing, extension at 72 °C for 1 min, and final elongation at 72 °C for 5 min. The PCR products were resolved

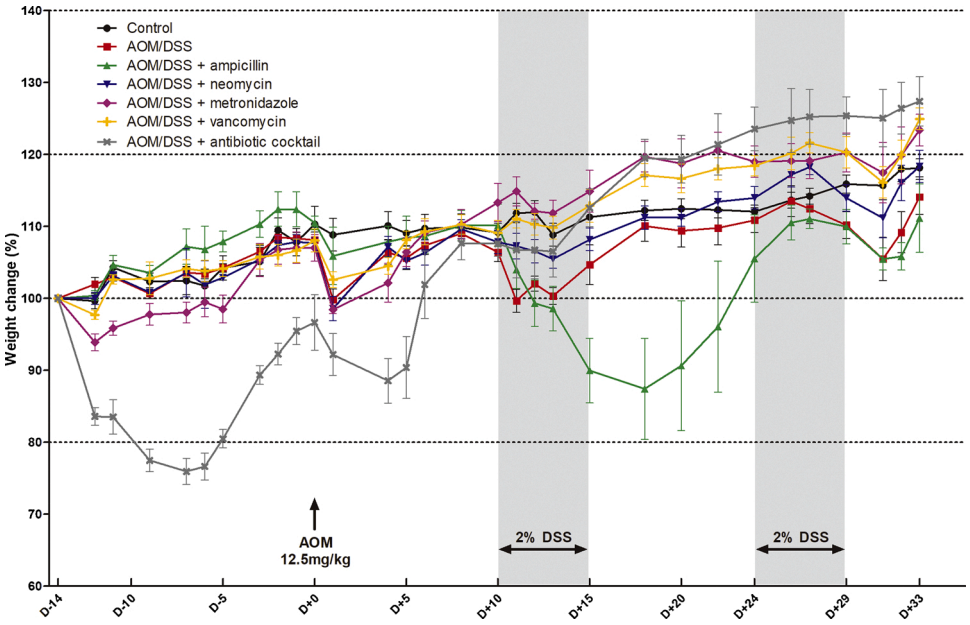

Fig. 2. Changes in mouse body weight during the study period. Body weight is expressed as the percentage change over initial body weight. Body weight decreased at the beginning of antibiotic treatment in the antibiotic cocktail group and during the first round of DSS administration in the ampicillin group. There was no significant difference in body weight between groups at the end of the experiment. AOM, azoxymethane; DSS, dextran sodium sulfate.

by electrophoresis on 1.5 % agarose gels that contained Safe-Pinky DNA gel staining solution (GenDEPOT) and bands were visualized using a ChemiDoc™ XRS + System (Bio-Rad, CA, USA). The primer sequences used for PCR are shown in Table A.3 in supplementary material.

2.6. DNA extraction and 16S rRNA gene sequencing

Mouse fecal samples were collected at baseline and before the mice were sacrificed and immediately stored at −80 °C. To extract bacterial DNA, bacterial walls were pulverized using phenol/chloroform extraction and a bead beating method, and purified DNA was extracted using a PowerFecal DNA Isolation Kit (MOBIO Laboratories, USA).

To amplify the extracted DNA, primers for the V3-V4 region of the 16S rRNA gene were used as follows: forward, 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3′; reverse, 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3′. Gene amplification conditions were initial denaturation at 95 °C for 5 min, denaturation 35 times at 95 °C for 40 s, primer annealing at 57 °C for 40 s, extension at 72 °C for 60 s, and a final elongation at 72 °C for 60 s. Amplified 16S rRNA PCR products were quantified, purified, and sequenced using the MiSeq platform (Illumina). The short or extra-long reads in the sequences were trimmed, and the filtered sequence was classified using CD-HIT-DUP. Chimeric reads were identified, and small noise sequences were removed. Sequences with 97 % accordance among the remaining representative readings were classified as operational taxonomic units (OTUs). A taxonomy for each OTU representative sequence was assigned using the QIIME pipeline. Alpha diversity was measured using the Chao 1 richness index, Shannon index, Simpson index, and the observed number of OTUs. Principal coordinate analysis was performed to evaluate structural changes of the gut microbial community. The complete genome sequence dataset has been deposited in the NCBI Sequence Read Archive under BioProject accession number PRJNA635665.

2.7. Statistical analysis

Continuous and categorical variables were compared between groups using the Mann-Whitney test and Chi-square or Fisher's exact tests, respectively. A p-value <0.05 was considered statistically significant. All statistical procedures were performed using R (version 3.5.2; R Foundation for statistical calculations in Vienna, Austria).

3. Results

3.1. Antimicrobial therapy differentially suppressed colon tumorigenesis depending on antibiotic type

Body weight of the mice rapidly decreased at the beginning of antibiotic administration and then gradually recovered in the antibiotic cocktail treatment group. In the ampicillin treatment group, body weight decreased when DSS was administered and then recovered thereafter (Fig. 2).

Figs. 3 and 4 show the impact of various antimicrobial therapies on colon tumorigenesis and inflammation. Colitis and tumorigenesis were induced by AOM/DSS injection, resulting in severe inflammation and higher tumor numbers in the AOM/DSS group than in the control group. Tumorigenesis was most markedly suppressed in the mice treated with the antibiotic cocktail. Mice receiving antibiotic cocktail treatment with AOM/DSS (cocktail group) had longer colon length, fewer tumor numbers, and lower histological inflammation scores than those receiving AOM/DSS alone. Tumorigenesis was also suppressed in the individual antibiotic treatment groups, but not as markedly as in the antibiotic cocktail treatment group. In addition, individual antibiotic treatment had different effects on colon tumorigenesis and inflammation. Metronidazole treatment attenuated both tumorigenesis and colon inflammation. Neomycin treatment suppressed tumorigenesis but did not alleviate inflammation. Ampicillin and vancomycin treatments did not significantly attenuate either tumor formation or colon inflammation. The degree of histological inflammation was positively correlated with tumorigenesis.

Antimicrobial therapy altered the expression of various inflammatory cytokines. The expression of IL-6, TNF-α, IFN-γ, IL-17A, IL-10, IL-22, and SOCS3 was significantly increased in the AOM/DSS group compared with the control group. The antibiotic cocktail group showed significantly decreased relative expression of TNF-α, IFN-γ, IL-17A, IL-10, and IL-22 as compared with the AOM/DSS group. Individual antibiotic treatment groups each showed differential expression of cytokines. The expression of IL-10 and IL-22 was significantly decreased in

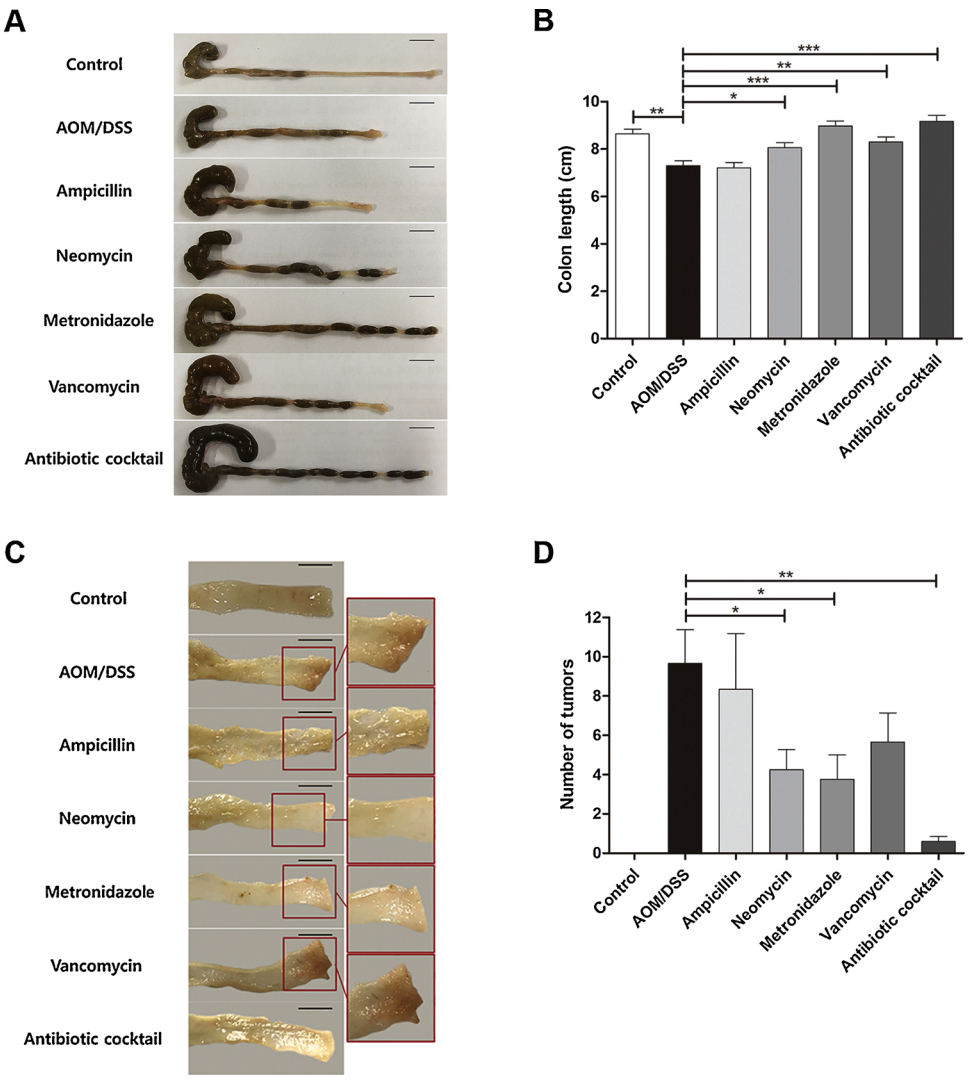

Fig. 3. Colon tumorigenesis was differentially attenuated according to the type of antibiotic treatment. (A) Representative images of harvested colons. Scale bar, 1 cm. (B) Colonic length was significantly shorter in the AOM/DSS group than in the control group. The neomycin, metronidazole, vancomycin, and antibiotic cocktail treatment groups had longer colon length compared with the AOM/DSS group. (C) Representative images of colonic tumors. Scale bar, 1 cm. (D) The number of tumors was significantly lower in the neomycin, metronidazole, and antibiotic cocktail treatment groups than in the AOM/DSS group. AOM, azoxymethane; DSS, dextran sodium sulfate.
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

all the antibiotic-treated groups. The expression of IL-18 was increased in the neomycin, metronidazole, and antibiotic cocktail treatment groups relative to the AOM/DSS group (Fig. 5).

Taken together, tumorigenesis was most markedly suppressed in the antibiotic cocktail treatment group, but it was also significantly suppressed in the neomycin and metronidazole treatment groups. Histologically confirmed colon inflammation was significantly alleviated in the metronidazole and antibiotic cocktail treatment groups.

3.2. *Antimicrobial therapy differentially altered the diversity and composition of the gut microbiota depending on the type of antibiotic treatment*

Fig. 6 shows the gut microbial diversity of each antibiotic treatment group. Rarefaction curves show that the observed OTUs were significantly decreased in the antibiotic cocktail, vancomycin, and ampicillin treatment groups. Observed OTUs of the neomycin treatment group did not decrease significantly compared with the AOM/DSS group. Similarly, the Chao 1 richness, Shannon, and Simpson indices were most markedly reduced in the ampicillin, vancomycin, and antibiotic cocktail treatment groups.

In addition, principal coordinate analysis showed that the gut microbial composition of each antibiotic treatment group was significantly different from each other at the end of the study. Changes in gut microbiota at the phylum level demonstrated a decrease in the relative abundance of Bacteroidetes in the AOM/DSS group, and an increase in the relative abundance of the Proteobacteria in the antibiotic cocktail treatment group. In the ampicillin treatment group, the relative abundance of Bacteroidetes and Firmicutes decreased, whereas the relative abundance of Proteobacteria and Tenericutes increased. In the neomycin treatment group, the relative abundance of Deferribacteres decreased and that of Verrucomicrobia increased. The relative abundance of Actinobacteria and Verrucomicrobia increased in the metronidazole treatment group. In the vancomycin treatment group, the relative abundance of Bacteroidetes was decreased and the abundance of Proteobacteria and Verrucomicrobia increased significantly (Fig. 7).

3.3. *Gut microbial diversity and community structure were associated with tumorigenesis*

Antibiotic cocktail therapy led to gut bacterial depletion and was associated with suppression of tumorigenesis. The median number of OTUs was reduced from 186 to 9 after administration of the antibiotic cocktail. The number of OTUs was generally positively correlated with the number of tumors. However, in the ampicillin and vancomycin treatment groups, tumorigenesis was not suppressed although the number of OTUs was significantly reduced. Meanwhile, in the metronidazole and neomycin treatment groups, both the number of OTUs and the number of tumors were moderately reduced compared with the AOM/DSS group.

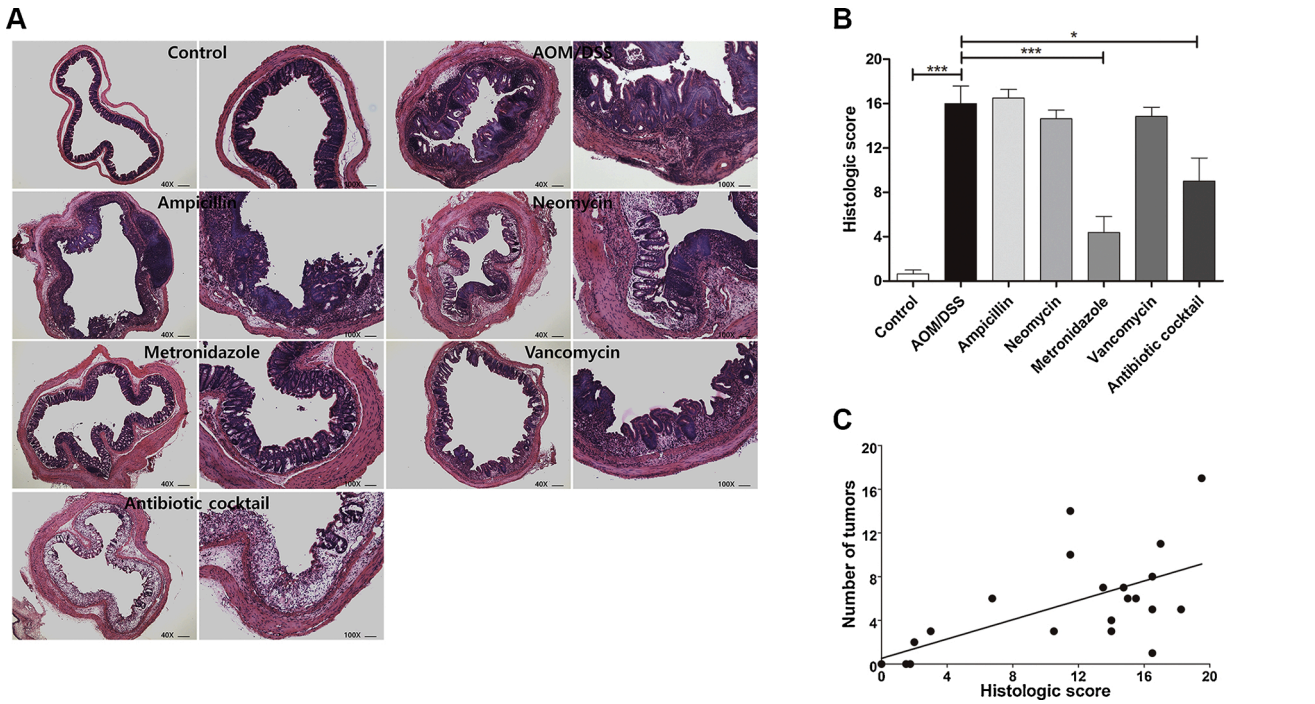

Fig. 4. Colon inflammation was suppressed to varying degrees and was positively correlated, but not completely consistent, with tumorigenesis. (A) Representative histological findings. Images were taken from the distal part of the colon at 40x and 100x magnifications. Scale bar, 200 μm. (B) Histological score for colon inflammation was significantly lower in the metronidazole and antibiotic cocktail treatment groups than in the AOM/DSS group. (C) Histological score and number of tumors were positively correlated.

In an analysis of the correlation between specific bacterial population and tumor burden, the phyla Proteobacteria and Tenericutes were positively correlated with tumor burden. The genera *Enterobacter* and *Proteus* were also positively correlated with tumor burden. The phyla Bacteroidetes plus Firmicutes and the order Clostridiales were negatively correlated with tumor burden (Fig. 8).

4. Discussion

In this study, we demonstrated that antibiotic therapy induces changes in gut microbial composition and attenuates colon tumorigenesis in the AOM/DSS-induced murine CAC model. Antibiotic cocktail therapy resulted in the depletion of gut bacterial community and a marked reduction in tumor burden. Individual antibiotic treatments differentially altered the gut microbial composition and showed different effects on tumor suppression, but the degree of tumor suppression was less pronounced than that relative to the antibiotic cocktail therapy. The phyla Proteobacteria and Tenericutes were found to be positively correlated with tumor burden.

In our results, colonic inflammation and tumorigenesis were positively correlated overall. However, the degree of colitis is not always consistent with the degree of tumorigenesis. In the neomycin treatment group, tumorigenesis was significantly suppressed although colon inflammation was not alleviated. These results suggest that the gut microbiota plays a certain role in tumorigenesis in addition to inflammation. A previous study demonstrated that colonization with *E. coli* or *Enterococcus faecalis* differentially affects colon tumorigenesis without affecting inflammation in AOM-treated interleukin-10 deficient germ-free mice, suggesting that the microbes contributing to the development of colonic inflammation and CAC are not identical [12]. In addition, colon tumorigenesis is a sequential process in which several factors work together, and a substantial body of literature suggests that microbes, inflammation, and tumorigenesis are intimately connected and influenced by each other [11,13]. Several specific microorganisms are known to be implicated in the development of CRC, including *Streptococcus gallolyticus*, *E. faecalis*, colibactin-producing *E. coli*, enterotoxigenic *B. fragilis*, and *F. nucleatum* [11]. However, not only individual microbes but also the gut microbial community as a whole may contribute to colon tumorigenesis [9].

The results of this study show that the administration of ampicillin and vancomycin did not significantly attenuate tumorigenesis. After administration of ampicillin and vancomycin, relative abundance of the phyla Proteobacteria and Tenericutes was increased, which was associated with high tumor burden. On the other hand, tumorigenesis was suppressed in the neomycin and metronidazole treatment groups, where the relative abundance of phyla Proteobacteria and Tenericutes was not increased and the abundance of phyla Bacteroidetes and Firmicutes was relatively maintained. In a previous study using human colorectal tumors, the relative abundance of phyla Bacteroidetes and Firmicutes was decreased and the abundance of phyla Proteobacteria and Tenericutes was increased in colorectal adenoma tissue relative to normal colon tissue [14]. The phylum Proteobacteria occupies a minor portion of the normal human gut microbiome, but the relative abundance of Proteobacteria increases in metabolic or inflammatory disorders and cancer [15]. The Proteobacteria and its metabolites have been reported to be associated with CRC [16]. The phylum Firmicutes is the most dominant phylum in the normal gut microbiome and reductions in the abundance or diversity of Firmicutes has been associated with IBD [17]. Our results support previous results that the overall gut microbial community plays an important role in colon tumorigenesis.

Antibiotic cocktail therapy reduced tumor burden most significantly among the various antibiotic treatments and also led to a depletion of the gut microbiota. The antibiotic-induced microbiome depletion model is different from the germ-free mouse model in that the gut microbiome has not been completely eradicated and the immune system has developed normally [18]. These mice have shown increased insulin sensitivity, altered bile acid metabolism, and alleviation of inflammation [18]. In addition, gut microbial depletion has been shown to reduce tumor burden in multiple xenograft cancer models as well as in the intraluminal CRC model [10]. Moreover, an increased load of gut

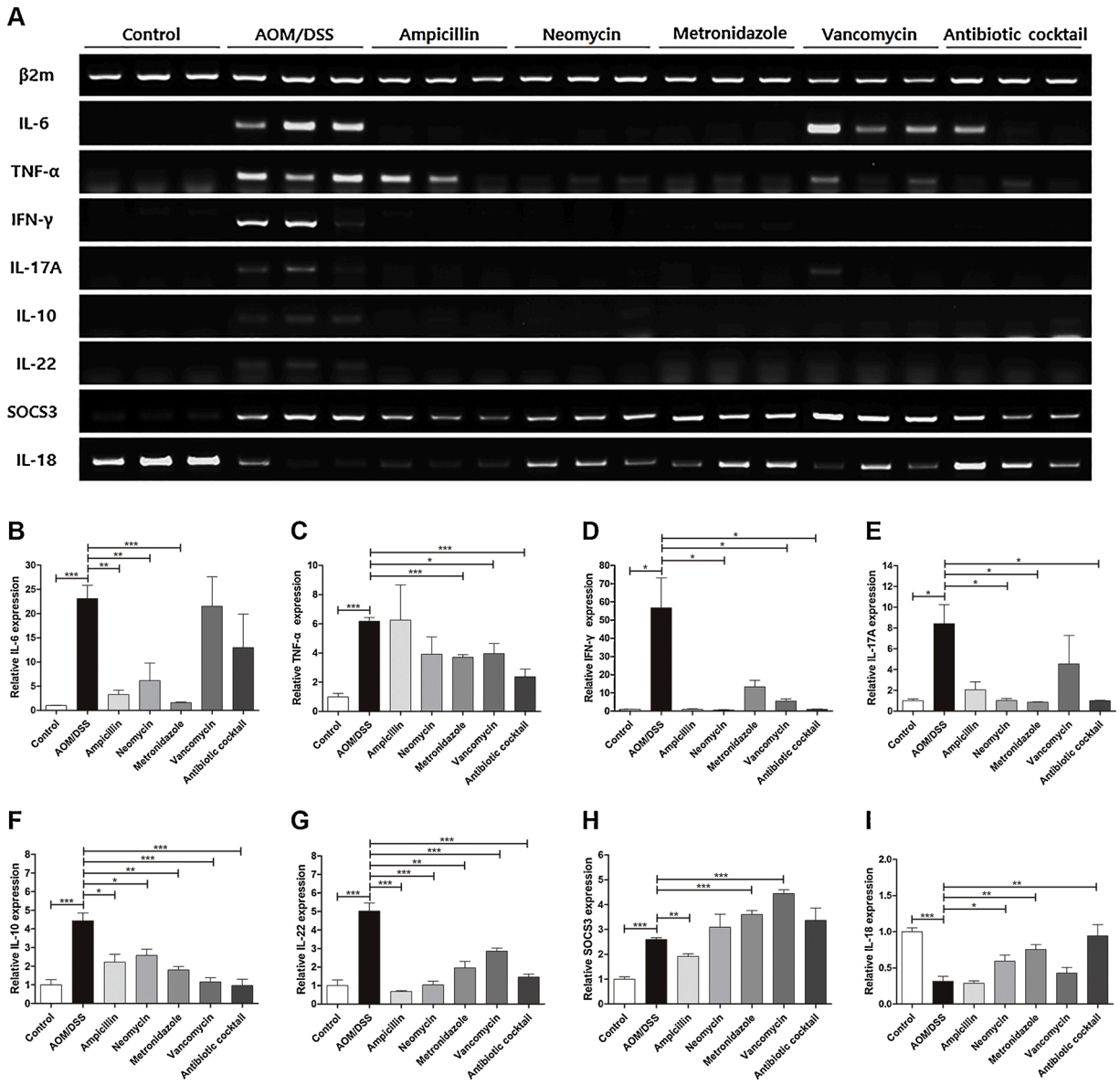

Fig. 5. Cytokine expression was altered by antibiotic treatment. (C, D, E) The expression of TNF-α, IFN-γ, and IL-17A was significantly increased in the AOM/DSS group compared with the control group, and was decreased in the antibiotic cocktail treatment group. (F, G) The expression of IL-10 and IL-22 was significantly decreased in all antibiotic treatment groups. (I) The expression of IL-18 was increased in the neomycin, metronidazole, and antibiotic cocktail treatment groups relative to the AOM/DSS group. AOM, azoxymethane; DSS, dextran sodium sulfate.
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

bacteria has been reported to be associated with colonic inflammation and tumorigenesis [19]. Our results, which led to eradication of the gut microbiota and suppression of colon tumorigenesis with antibiotic cocktail therapy, are consistent with previous literature, suggesting that total colitogenic microbiota load and the interaction between the gut microbiota and the immune system are important factors in colon tumorigenesis.

Several specific bacterial species have been implicated in the pathogenesis of CRC. However, certain microbial species may be interchangeable in terms of functionality due to the functional redundancy of gut microbiota. Certain microorganisms of different composition can act similarly and produce similar protein and metabolite profiles [20,21]. Therefore, even if only a single strain associated with CRC is eliminated, other strains can contribute to tumorigenesis by taking a similar role. This data suggests that no one single pathogenic strain plays an absolute role in the development of CRC, and plenty of gut microbiota species will be involved in a complex manner, and the interaction and community function of the gut microbiota plays an important role in colon tumorigenesis.

Antibiotics are used to eradicate specific pathogenic bacteria in clinical practice, but antibiotics cause heavy collateral damage on gut commensal bacteria and long-term metabolic consequences [22,23]. On the other hand, certain antimicrobial agents have anti-inflammatory and immunomodulatory effects [24,25]. Antimicrobial therapy has been effective in inducing clinical remission in patients with IBD [26–28], and depletion of gut microbiota using antibiotics has led to an improvement in metabolic profiles and a suppression of tumorigenesis in mouse models. [10,18]. In addition, it has been reported that antibiotics suppress colon tumorigenesis by inhibiting aberrant DNA methylation [29]. Furthermore, antibiotic treatment can remove bacterial biofilms that create conditions conducive to colon carcinogenesis in patients with CRC [30]. From this point of view, gut microbiota modulation through antimicrobial therapy may emerge as a potential treatment strategy for CRC.

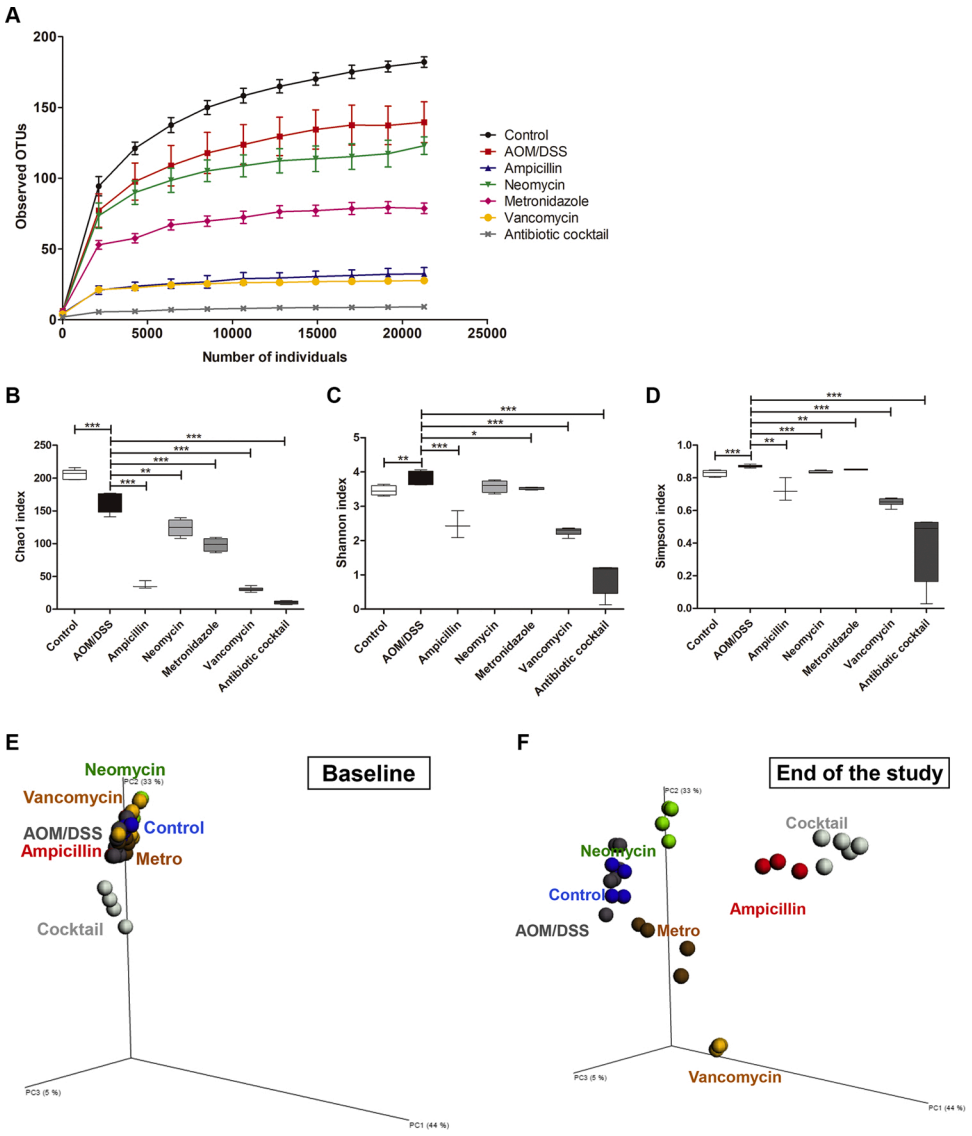

Fig. 6. Antibiotic treatments altered gut microbial diversity and composition. (A) Rarefaction curves show that the observed OTUs were significantly decreased in the antibiotic cocktail, vancomycin, and ampicillin treatment groups. (B, C, D) Chao 1 richness, Shannon, and Simpson indices were most markedly reduced in the ampicillin, vancomycin, and antibiotic cocktail treatment groups. (E, F) Principal coordinate analysis demonstrated that the gut microbial composition of each antibiotic treatment group was significantly different from each other at the end of the study. AOM, azoxymethane; DSS, dextran sodium sulfate. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

In conclusion, colon tumorigenesis was attenuated through various antimicrobial therapies as assessed in the AOM/DSS-induced mouse CAC model. Individual antimicrobial therapy suppressed colon tumorigenesis to various levels by differentially altering the diversity and composition of gut microbiota. Since the degree of tumor reduction by individual antibiotics is lower than that by antibiotic cocktail treatment, interaction in the global gut microbial community seems to play a more important role in the development of CAC than individual bacterial species.

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CRediT authorship contribution statement

Jae Gon Lee: Formal analysis, Writing - original draft, Writing - review & editing. **Yu-ra Lee:** Data curation, Methodology, Writing - review & editing. **A-reum Lee:** Data curation, Methodology, Writing - review & editing. **Chan Hyuk Park:** Formal analysis, Writing - review & editing. **Dong Soo Han:** Conceptualization, Writing - review & editing. **Chang Soo Eun:** Conceptualization, Methodology, Writing - review & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biopha.2020.111206.

References

[1] L.A. Torre, F. Bray, R.L. Siegel, J. Ferlay, J. Lortet-Tieulent, A. Jemal, Global cancer statistics, 2012, CA Cancer J. Clin. 65 (2) (2015) 87–108.
[2] D.C. Rubin, A. Shaker, M.S. Levin, Chronic intestinal inflammation: inflammatory bowel disease and colitis-associated colon cancer, Front. Immunol. 3 (2012) 107.
[3] L.J. Herrinton, L. Liu, T.R. Levin, J.E. Allison, J.D. Lewis, F. Velayos, Incidence and mortality of colorectal adenocarcinoma in persons with inflammatory bowel disease from 1998 to 2010, Gastroenterology 143 (2) (2012) 382–389.
[4] T.A. Brentnall, D.A. Crispin, P.S. Rabinovitch, R.C. Haggitt, C.E. Rubin, A. C. Stevens, G.C. Burmer, Mutations in the p53 gene: an early marker of neoplastic progression in ulcerative colitis, Gastroenterology 107 (2) (1994) 369–378.
[5] L.B. Meira, J.M. Bugni, S.L. Green, C.W. Lee, B. Pang, D. Borenshtein, B. H. Rickman, A.B. Rogers, C.A. Moroski-Erkul, J.L. McFaline, D.B. Schauer, P.

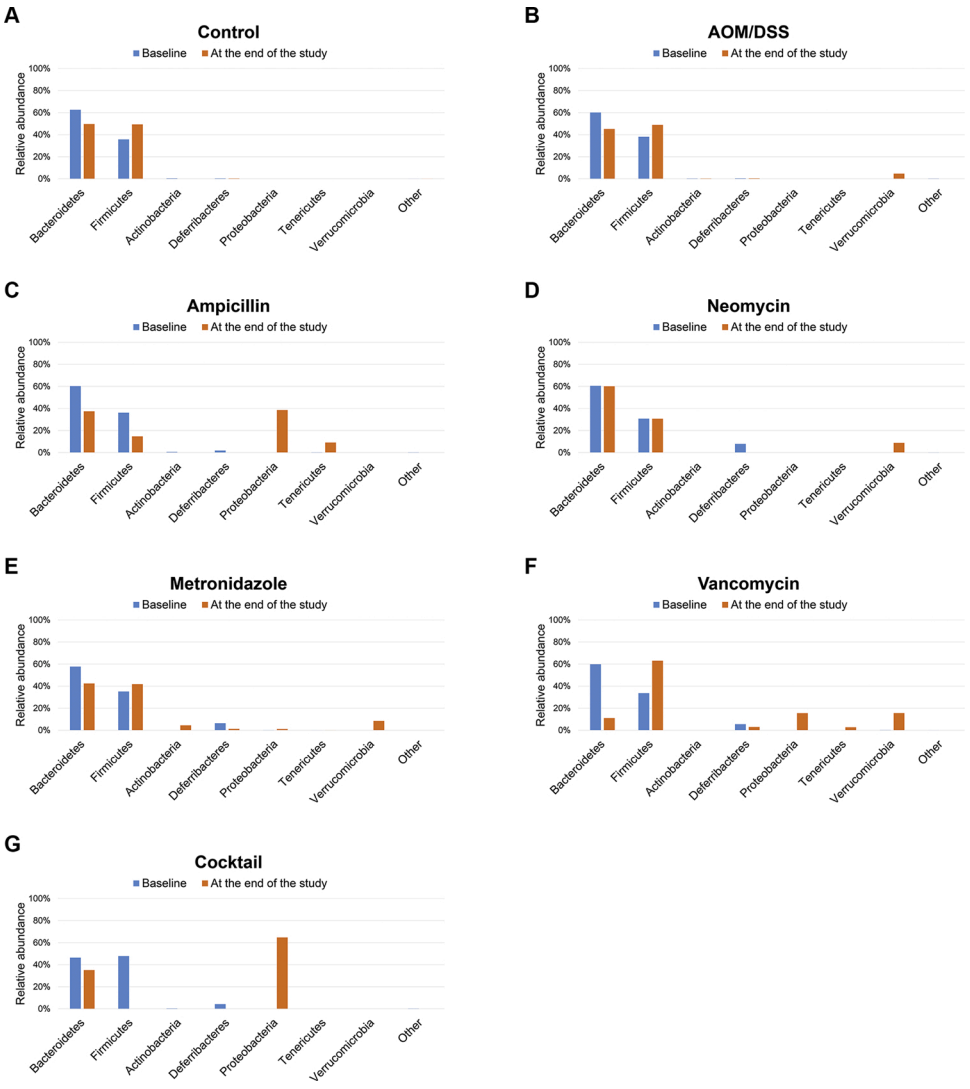

Fig. 7. Changes in the composition of gut microbiota at the phylum level. (B) In the AOM/DSS group, the relative abundance of Bacteroidetes was decreased. (C) In the ampicillin treatment group, the relative abundance of Bacteroidetes and Firmicutes decreased, whereas the relative abundance of Proteobacteria and Tenericutes increased. (D) In the neomycin treatment group, the relative abundance of Deferribacteres decreased and that of Verrucomicrobia increased. (E) In the metronidazole treatment group, the relative abundance of Actinobacteria and Verrucomicrobia increased. (F) In the vancomycin treatment group, the relative abundance of Bacteroidetes was decreased and the abundance of Proteobacteria and Verrucomicrobia increased. (G) In the antibiotic cocktail treatment group, the relative abundance of Proteobacteria was increased. AOM, azoxymethane; DSS, dextran sodium sulfate.

C. Dedon, J.G. Fox, L.D. Samson, DNA damage induced by chronic inflammation contributes to colon carcinogenesis in mice, J. Clin. Invest. 118 (7) (2008) 2516–2525.
[6] M. Kang, A. Martin, Microbiome and colorectal cancer: unraveling host-microbiota interactions in colitis-associated colorectal cancer development, Semin. Immunol. 32 (2017) 3–13.
[7] T. Wang, G. Cai, Y. Qiu, N. Fei, M. Zhang, X. Pang, W. Jia, S. Cai, L. Zhao, Structural segregation of gut microbiota between colorectal cancer patients and healthy volunteers, ISME J. 6 (2) (2012) 320–329.
[8] M.L. Richard, G. Liguori, B. Lamas, G. Brandi, G. da Costa, T.W. Hoffmann, M. Pierluigi Di Simone, C. Calabrese, G. Poggioli, P. Langella, M. Campieri, H. Sokol, Mucosa-associated microbiota dysbiosis in colitis associated cancer, Gut Microbes 9 (2) (2018) 131–142.
[9] J.P. Zackular, N.T. Baxter, G.Y. Chen, P.D. Schloss, Manipulation of the gut microbiota reveals role in colon tumorigenesis, mSphere 1 (1) (2016).
[10] V. Sethi, S. Kurtom, M. Tarique, S. Lavania, Z. Malchiodi, L. Hellmund, L. Zhang, U. Sharma, B. Giri, B. Garg, A. Ferrantella, S.M. Vickers, S. Banerjee, R. Dawra, S. Roy, S. Ramakrishnan, A. Saluja, V. Dudeja, Gut microbiota promotes tumor growth in mice by modulating immune response, Gastroenterology 155 (1) (2018) 33–37, e6.
[11] C.A. Brennan, W.S. Garrett, Gut microbiota, inflammation, and colorectal cancer, Annu. Rev. Microbiol. 70 (2016) 395–411.
[12] J.C. Arthur, E. Perez-Chanona, M. Muhlbauer, S. Tomkovich, J.M. Uronis, T.J. Fan, B.J. Campbell, T. Abujamel, B. Dogan, A.B. Rogers, J.M. Rhodes, A. Stintzi, K. W. Simpson, J.J. Hansen, T.O. Keku, A.A. Fodor, C. Jobin, Intestinal inflammation targets cancer-inducing activity of the microbiota, Science 338 (6103) (2012) 120–123.
[13] A. Lasry, A. Zinger, Y. Ben-Neriah, Inflammatory networks underlying colorectal cancer, Nat. Immunol. 17 (3) (2016) 230–240.
[14] Y. Lu, J. Chen, J. Zheng, G. Hu, J. Wang, C. Huang, L. Lou, X. Wang, Y. Zeng, Mucosal adherent bacterial dysbiosis in patients with colorectal adenomas, Sci. Rep. 6 (2016) 26337.
[15] N.R. Shin, T.W. Whon, J.W. Bae, Proteobacteria: microbial signature of dysbiosis in gut microbiota, Trends Biotechnol. 33 (9) (2015) 496–503.
[16] R. Sinha, J. Ahn, J.N. Sampson, J. Shi, G. Yu, X. Xiong, R.B. Hayes, J.J. Goedert, Fecal microbiota, fecal metabolome, and colorectal cancer interrelations, PLoS One 11 (3) (2016) e0152126.
[17] H. Sokol, P. Seksik, L. Rigottier-Gois, C. Lay, P. Lepage, I. Podglajen, P. Marteau, J. Dore, Specificities of the fecal microbiota in inflammatory bowel disease, Inflamm. Bowel Dis. 12 (2) (2006) 106–111.
[18] A. Zarrinpar, A. Chaix, Z.Z. Xu, M.W. Chang, C.A. Marotz, A. Saghatelian, R. Knight, S. Panda, Antibiotic-induced microbiome depletion alters metabolic homeostasis by affecting gut signaling and colonic metabolism, Nat. Commun. 9 (1) (2018) 2872.
[19] M. Kim, L. Friesen, J. Park, H.M. Kim, C.H. Kim, Microbial metabolites, short-chain fatty acids, restrain tissue bacterial load, chronic inflammation, and associated cancer in the colon of mice, Eur. J. Immunol. 48 (7) (2018) 1235–1247.
[20] A. Moya, M. Ferrer, Functional redundancy-induced stability of gut microbiota subjected to disturbance, Trends Microbiol. 24 (5) (2016) 402–413.
[21] A.E. Perez-Cobas, M.J. Gosalbes, A. Friedrichs, H. Knecht, A. Artacho, K. Eismann, W. Otto, D. Rojo, R. Bargiela, M. von Bergen, S.C. Neulinger, C. Daumer, F. A. Heinsen, A. Latorre, C. Barbas, J. Seifert, V.M. dos Santos, S.J. Ott, M. Ferrer, A. Moya, Gut microbiota disturbance during antibiotic therapy: a multi-omic approach, Gut 62 (11) (2013) 1591–1601.
[22] I. Cho, S. Yamanishi, L. Cox, B.A. Methe, J. Zavadil, K. Li, Z. Gao, D. Mahana, K. Raju, I. Teitler, H. Li, A.V. Alekseyenko, M.J. Blaser, Antibiotics in early life alter the murine colonic microbiome and adiposity, Nature 488 (7413) (2012) 621–626.
[23] L.M. Cox, S. Yamanishi, J. Sohn, A.V. Alekseyenko, J.M. Leung, I. Cho, S.G. Kim, H. Li, Z. Gao, D. Mahana, J.G. Zarate Rodriguez, A.B. Rogers, N. Robine, P. Loke, M.J. Blaser, Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences, Cell 158 (4) (2014) 705–721.
[24] S.P. Sadarangani, L.L. Estes, J.M. Steckelberg, Non-anti-infective effects of antimicrobials and their clinical applications: a review, Mayo Clin. Proc. 90 (1) (2015) 109–127.

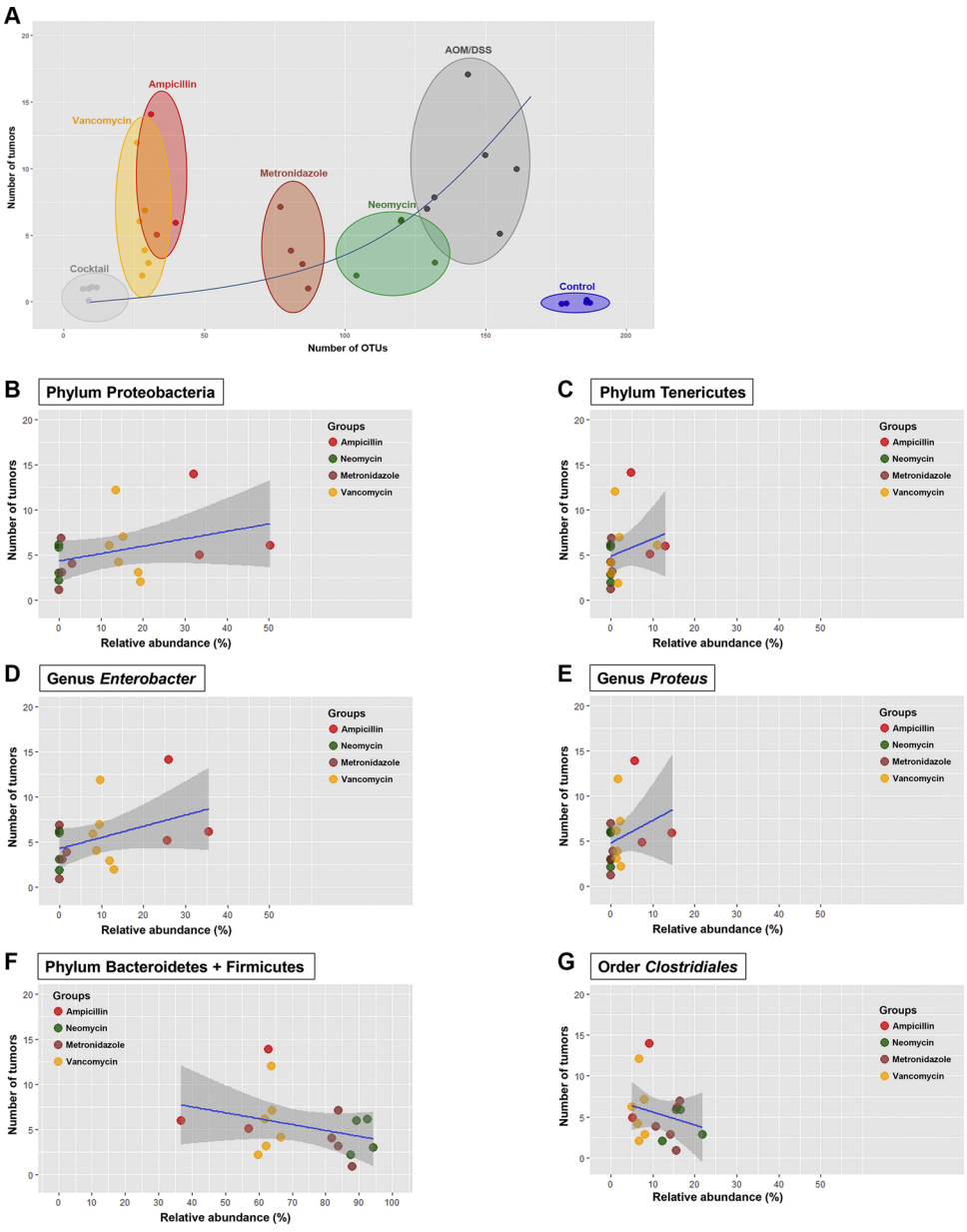

Fig. 8. Gut microbial diversity and community structure are associated with tumorigenesis. (A) Overall, number of OTUs was positively correlated with the number of tumors. (B, C) The phyla Proteobacteria and Tenericutes were positively correlated with tumor burden. (D, E) The genera *Enterobacter* and *Proteus* were positively correlated with tumor burden. (F, G) The phyla Bacteroidetes plus Firmicutes and the order Clostridiales were negatively correlated with tumor burden. OTU, operational taxonomic unit; AOM, azoxymethane; DSS, dextran sodium sulfate.

[25] J. Altenburg, C.S. de Graaff, T.S. van der Werf, W.G. Boersma, Immunomodulatory effects of macrolide antibiotics - part 1: biological mechanisms, Respiration 81 (1) (2011) 67–74.

[26] C. Prantera, H. Lochs, M. Campieri, M.L. Scribano, G.C. Sturniolo, F. Castiglione, M. Cottone, Antibiotic treatment of Crohn's disease: results of a multicentre, double blind, randomized, placebo-controlled trial with rifaximin, Aliment. Pharmacol. Ther. 23 (8) (2006) 1117–1125.

[27] A. Levine, D. Turner, Combined azithromycin and metronidazole therapy is effective in inducing remission in pediatric Crohn's disease, J. Crohns Colitis 5 (3) (2011) 222–226.

[28] J.W. Su, J.J. Ma, H.J. Zhang, Use of antibiotics in patients with Crohn's disease: a systematic review and meta-analysis, J. Dig. Dis. 16 (2) (2015) 58–66.

[29] N. Hattori, T. Niwa, T. Ishida, K. Kobayashi, T. Imai, A. Mori, K. Kimura, T. Mori, Y. Asami, T. Ushijima, Antibiotics suppress colon tumorigenesis through inhibition of aberrant DNA methylation in an azoxymethane and dextran sulfate sodium colitis model, Cancer Sci. 110 (1) (2019) 147–156.

[30] C.H. Johnson, C.M. Dejea, D. Edler, L.T. Hoang, A.F. Santidrian, B.H. Felding, J. Ivanisevic, K. Cho, E.C. Wick, E.M. Hechenbleikner, W. Uritboonthai, L. Goetz, R.A. Casero Jr., D.M. Pardoll, J.R. White, G.J. Patti, C.L. Sears, G. Siuzdak, Metabolism links bacterial biofilms and colon carcinogenesis, Cell Metab. 21 (6) (2015) 891–897.