Research Article

Alterations of the Predominant Fecal Microbiota and Disruption of the Gut Mucosal Barrier in Patients with Early-Stage Colorectal Cancer

Xia Liu,1 Yiwen Cheng,2 Li Shao,3,4 and Zongxin Ling2

1Department of Intensive Care Unit, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, Zhejiang 310003, China
2Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, Zhejiang 310003, China
3Hangzhou Normal University, Hangzhou, Zhejiang 311121, China
4Institute of Translational Medicine, The Affiliated Hospital of Hangzhou Normal University, Hangzhou, Zhejiang 310015, China

Correspondence should be addressed to Li Shao; 0011334@zju.edu.cn and Zongxin Ling; lingzongxin@zju.edu.cn

Received 17 January 2020; Revised 18 February 2020; Accepted 9 March 2020; Published 21 March 2020

Guest Editor: Yan Huang

Copyright © 2020 Xia Liu et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Growing evidence indicated that the gut microbiota was the intrinsic and essential component of the cancer microenvironment, which played vital roles in the development and progression of colorectal cancer (CRC). In our present study, we investigated the alterations of fecal abundant microbiota with real-time quantitative PCR and the changes of indicators of gut mucosal barrier from 53 early-stage CRC patients and 45 matched healthy controls. We found that the traditional beneficial bacteria such as Lactobacillus and Bifidobacterium decreased significantly and the carcinogenic bacteria such as Enterobacteriaceae and Fusobacterium nucleatum were significantly increased in CRC patients. We also found gut mucosal barrier dysfunction in CRC patients with increased levels of endotoxin (LPS), D-lactate, and diamine oxidase (DAO). With Pearson’s correlation analysis, D-lactate, LPS, and DAO were correlated negatively with Lactobacillus and Bifidobacterium and positively with Enterobacteriaceae and F. nucleatum. Our present study found dysbiosis of the fecal microbiota and dysfunction of the gut mucosal barrier in patients with early-stage CRC, which implicated that fecal abundant bacteria and gut mucosal barrier indicators could be used as targets to monitor the development and progression of CRC in a noninvasive and dynamic manner.

1. Introduction

Colorectal cancer (CRC) is one of the leading causes of cancer-related mortality, accounting for the fifth most commonly diagnosed cancer and the fifth most common cause of death by cancer in China [1]. The overall decline or stabilization in the incidence of CRC was noted in several high-income countries [2]; however, the incidence and mortality rates of CRC in China still showed an increasing trend in the past decades, with approximately 376,300 new cases and 191,000 deaths per year [3]. Recently, many previous studies have verified that the human intestinal microbiota, including microbiome, mycobiome, and virome, was one of the important factors associated with CRC [4–10]. Intestinal microbiota, one part of the tumor microenvironment, has attracted increasing attention, as it can affect CRC growth and spread in many ways. A variety of intestinal commensal bacteria and their metabolites are known for triggering inflammatory cascades and oncogenic signaling, thereby promoting genetic and epigenetic alterations in CRC development [11].

Increasing evidence indicated that intestinal dysbiosis plays vital roles in CRC initiation, progression, and metastasis. Our previous study has found that the structures of the intestinal microbiota altered significantly in CRC patients compared to healthy individuals, which found that Fusobacterium, Porphyromonas, Peptostreptococcus, and...
2.2. Sampling. Prior to bowel cleansing for scheduled colonoscopy, fecal samples and blood samples were collected from these participants. We collected approximately 2 g of fresh feces into a sterile plastic cup from these participants and kept these samples in an icebox. Samples for bacterial genomic DNA extraction were transferred immediately to the laboratory and stored at -80°C after preparation within 15 min until use. In addition, blood samples were also collected simultaneously from these participants for intestinal mucosal barrier function analysis. Plasma was also stored at -80°C after preparation within 15 min (Table 1 shows the details of the samples).

2.3. Bacterial Genomic DNA Extraction. Fecal genomic DNA was extracted using a QIAamp® DNA Stool Mini Kit (QIAGEN, Hilden, Germany) according to our previous studies [4, 19]. The amount of bacterial genomic DNA was analyzed using a NanoDrop ND-1000 spectrophotometer; the integrity and size of bacterial genomic DNA were checked by electrophoresis. All bacterial genomic DNA was stored at -80°C for further use.

2.4. Fecal Abundant Bacteria Analysis. For fecal abundant bacteria analysis, real-time quantitative PCR (qPCR) was performed on ABI Prism 7900HT real-time PCR system (Applied Biosystems, Carlsbad, CA) with a Power SYBR Green PCR Master Mix (Takara, Dalian, China) according to the manufacturer’s instructions. The bacterial primer sets and the reaction conditions are shown in Table 2 [19, 20]. SDS 2.4 was used for the data analysis. Triplicate repeats were carried out for all reactions in every analysis with a nontemplate included. The quantity of these bacteria was presented as log10 bacteria per gram of feces (wet weight).

2.5. Intestinal Mucosal Barrier Function Analysis. The plasma from each participant was collected for intestinal mucosal barrier function analysis. The parameters of gut mucosal barrier function such as endotoxin (LPS), D-lactate, and diamine oxidase (DAO) were detected by a dry chemical method using the Intestinal Mucosal Barrier Biochemical Index Analysis System (JY-DLT, Beijing Zhongsheng Jinyu Diagnostic Technology Co., Ltd., China) [19, 21, 22]. The experiments were undergone according to the protocols suggested by the manufacturer and conducted within 4 h after plasma extraction.

2.6. Statistical Analysis. The quantitative data of fecal abundant bacteria and other parameters in our study are presented as the mean ± standard deviation (SD), the differences between the two groups were evaluated by Student’s t test, and the correlations between variables were tested by Pearson correlation using SPSS version 20.0 (SPSS Inc., Chicago, IL), respectively. p < 0.05 was considered statistically significant for all analyses.

3. Results

3.1. Characteristics of the Early-Stage CRC Patients. In our present study, these participants were all newly diagnosed early-stage CRC patients in our hospital, who were not...
treated with antibiotics, prebiotics, probiotics, and synbiotics in the previous month. Before sampling, these participants have been performed a complete physical examination such as colonoscopy and pathology, serological markers detection, and radiological examination. Most of the CRC patients (50/53) were found to be positive in fecal occult blood testing, while several patients (8/53) occurred severe diarrhoea. In our study, the BMI values and the serum liver function, kidney function, lipid levels, blood sugar, and blood pressure in the CRC patients were not different from healthy controls significantly (p > 0.05). However, the serological marker such as carcinoembryonic antigen (CEA) was increased significantly in CRC patients when compared with healthy controls (p < 0.05). Our study demonstrated that no any CRC patients were found to be positive in tumor metastasis.

3.2. Abnormal Fecal Microbiota in CRC Patients. In our present study, we investigated the abundant bacteria in the fecal microbiota by real-time quantitative PCR (Figure 1). The abundant bacteria represented the higher relative abundance of bacteria in the fecal microbiota, which would determine the overall structure and composition of the fecal microbiota. Ten abundant bacteria, which accounted for more than 90% of the total bacteria, were selected to explore the alterations of fecal microbiota in these CRC patients. We found that the copy numbers of the total bacteria were not significantly different between healthy controls and CRC patients. However, the traditional beneficial bacteria such as Lactobacillus and Bifidobacterium decreased significantly in CRC patients. In addition, the gut-indigenous Clostridium included several different clusters, including abundant clusters such as Clostridium cluster I, cluster XI, and cluster XIVb. Our data indicated that Clostridium cluster I was significantly decreased in CRC patients when compared with healthy controls, while Clostridium cluster XI and cluster XIVb were not significantly different between healthy controls and CRC patients. Another bacterium, Enterobacteriaceae, was considered as gut harmful bacteria, which could produce lipopolysaccharide into peripheral blood. We observed that Enterobacteriaceae was significantly increased in CRC patients when compared with healthy controls. F. nucleatum was the confirmed carcinogenic bacterium that associated with CRC development. Consistent with the previous studies, our results demonstrated that F. nucleatum was significantly increased in CRC patients when compared with healthy controls. Our data indicated that dysbiosis of fecal microbiota was found in CRC patients, which might be contributed to CRC initiation, progression, and metastasis.

3.3. Changed Intestinal Mucosal Barrier Function. The three parameters such as LPS, D-lactate, and DAO could be used to evaluate the integrity of the gut mucosal barrier. The increased permeability of the gut mucosal barrier was associated with increased systemic and local inflammation, which was closely correlated with the initiation and development of CRC. In our present study, we found obviously disturbed gut mucosal barrier in CRC patients when compared with healthy controls. Our data indicated that plasma D-lactate, a byproduct of bacterial metabolism, increased significantly in CRC patients when compared with healthy controls (p < 0.01; Figure 2(a)). LPS, a structural component of the cell wall of Gram-negative bacteria, was a potent inducer of proinflammatory cytokines that activate a systemic inflammatory response syndrome. Elevated LPS level has been reported to be an early marker of impaired mucosal barrier function. In our study, we also observed that the levels of LPS were significantly higher in the CRC patients than that in the healthy controls (p < 0.01; Figure 2(b)). In addition, DAO, an enzyme which deaminates histamine and polyamines, has its highest activity in the intestinal mucosa in most mammalian species, including humans. DAO activity has been shown to reflect changes associated with the gut mucosal barrier. Our data indicated that significant differences of DAO levels were observed between CRC patients and healthy controls (p < 0.01; Figure 2(c)). Taken together, our results showed that the loss of gut mucosal barrier integrity and function was found in CRC patients, which indicated that the impairment of gut mucosal barrier function might be one of the important factors for the initiation and development of CRC.

4. Discussion

The development of human CRC is influenced by various risk factors, such as diets, genetic, epigenetic, environmental, and metabolic factors, while environmental factors are the predominant trigger for CRC [23]. A growing body of evidence has indicated that human gut microbiota may be an important environmental factor that promotes CRC development [15, 24]. With the advent of high-throughput

| Summary information of the samples. |
|------------------------------------|
| Sample size | 53 | 45 |
| Age (years): mean ± SD | 52.4 ± 18.8 | 53.7 ± 16.7 |
| Gender (male/female) | 33/20 | 25/20 |
| BMI | 24.8 ± 3.58 | 23.7 ± 3.67 |
sequencing platforms “omics” technologies and advanced bioinformatics approaches, the changes of the composition and function of the gut microbiota have been identified in the last decades, and the suspected causative roles and molecular mechanisms of gut microbiota in CRC development have been established by many previous studies. Recently, several bacterial species such as *Fusobacterium nucleatum* and *Peptostreptococcus anaerobius* have been confirmed in the CRC development and progression [14, 25]. These fecal bacteria have been considered as one of the most important elements of the tumor microenvironment. Now, fecal candidate bacteria can be used as novel biomarkers with existing methods, such as real-time qPCR and fecal immunochemical test, for noninvasive diagnosis of CRC [7].

With real-time qPCR, we found that the abundant fecal bacteria changed significantly in these early-stage CRC patients, which indicated that the dysbiosis of fecal microbiota might participate in CRC development and progression. In our present study, ten abundant bacteria were investigated to illustrate the alterations of fecal microbiota in CRC patients. In our present study, the two traditional beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* (belonged to the lactic acid bacteria category) decreased significantly in these CRC patients, which were consistent with previous study [26]. Mendes et al. has found that the abundance of the genera *Lactobacillus* and *Bifidobacterium* increased significantly after microbiota modification by probiotic supplementation [27]. Increasing reports in recent years have shown that these bacteria have demonstrated a host of properties in preventing CRC development by inhibiting initiation or progression through multiple pathways, such as apoptosis, antioxidant DNA damages, immune responses, and epigenetics [28–30]. Although *Lactobacillus* and *Bifidobacterium* may only act before carcinogenesis and have little inhibitory impact on established cancer, these two beneficial bacteria were the most frequently used as probiotics to prevent colon carcinogenesis in animal models or patients, which might help regulate the CRC-related tumor microenvironment. Different from *Lactobacillus* and *Bifidobacterium*, Enterobacteriaceae was often considered as harmful bacteria in the fecal microbiota, which could induce inflammation in the host gut epithelium [31]. As inflammation has been a well-documented risk factor for various form of cancer [32], it was implicated that Enterobacteriaceae participated actively in the development and progression of CRC. Previous studies showed that the actions of Enterobacteriaceae are similar to the prolonged inflammatory response induced by enterotoxigenic *Bacteroides fragilis* [33, 34]. Sun et al. has showed significant enrichment of Enterobacteriaceae in the DMH-induced CRC animal model [35]. They also found that Enterobacteriaceae showed higher relative abundance in the early stages of tumor formation, while it was gradually replaced with other bacteria such as Rikenellaceae, Lachnospiraceae, Ruminococcaceae, and Streptococcaceae

**Table 2:** Bacterial-specific primer sets for detection of fecal microbiota by qPCR.

| PCR specificity       | Primer | Sequence (5′→3′) | Annealing temperature (°C) | Amplicon size (bp) |
|-----------------------|--------|------------------|----------------------------|-------------------|
| **Total bacteria**    | Uni331F| TCCTACGGGAGGCAGCAGT | 58                          | 466               |
|                       | Uni797R| GGACTACCAGGGTATCTCATTCGTT | 56                          | 418               |
| **Bacteroides-Prevotella group** | Bac303F| GAAGGTCGCCCAACATTG | 58                          | 341               |
|                       | Bac708R| CAACTCGAGGATTCTCG   | 58                          | 500               |
| **Lactobacillus spp.** | Lac-F  | AGGAGTAGGAAATCCTCCA | 58                          | 341               |
|                       | Lac-R  | CACCCGTACACATGGAAG | 58                          | 500               |
| **Bifidobacterium spp.** | Bifid-F| CTCCTGGAGACGGGGTG   | 55                          | 550               |
|                       | Bifid-R| GGTGTGCTTCCCGGATATCTACA | 58                          | 700               |
| **Clostridium cluster I** | CG1-F  | TACCRRAGGAGGAAGGCCAC | 63                          | 1000              |
|                       | CG1-R  | GTCCTCTCTAAATCTCAGCAT | 63                          | 1000              |
| **Clostridium cluster XI** | CG2-F  | AGGCTACTTGGAGGAGGA   | 58                          | 141               |
|                       | CG2-R  | GAGGCGTAGGCTTTCAT   | 58                          | 141               |
| **Clostridium cluster XIVab** | CG3-F  | GAWGAGATATYTCCGGATATG | 52                          | 152               |
|                       | CG3-R  | CTACGCGGCTTCTTAC     | 52                          | 152               |
| **Faecalibacterium prausnitzii** | Fpra-F | GATGGCCTCGCGTGAGGAGTAG | 58                          | 199               |
|                       | Fpra-R | CGGAGGACCTTCTTCCTCC  | 58                          | 199               |
| **Fusobacterium nucleatum** | Fn-F   | CAACCACTTATTCATCTACATGGTTCA | 60                          | 112               |
|                       | Fn-R   | GTTGACTTTACAGAAGGAGATTATGAAAATTC | 63                          | 195               |
| **Enterobacteriaceae** | Eco-F  | CATTAGCSTTACGGGAGAAGAGC | 63                          | 195               |
|                       | Eco-R  | CTCTACGAGCTCAAGGTTGC | 63                          | 195               |
| **Enterococcus spp.**  | ENco-F | CCCATTATTGATTGTCGCCATATT | 61                          | 144               |
|                       | ENco-R | ACTCGTGGTJACTTCCCATGT | 61                          | 144               |
However, the genus *Bacteroides* reduction of certain bacterial genera like CRC tissues have decreased microbial diversity, including a cluster I decreased significantly in CRC patients when compared with healthy controls. Kostic et al. found that CRC tissues have decreased microbial diversity, including a reduction of certain bacterial genera like *Clostridium* and *Bacteroides* [42]. However, the genus *Clostridium* includes a diverse group of Gram-positive, spore-forming anaerobes [43]. In general, clostridial fermentative metabolism functions by the conversion of hexose sugars to butyrate, acetate, and CO₂ [44]. Our study firstly found that *Clostridium* cluster I was negatively correlated with CRC development. The present study indicated that the abundant bacteria in fecal microbiota participated actively in the development and progression of CRC.

The gut mucosal barrier is a functional unit organized as a multilayer system, and its multiple functions are crucial for maintaining gut homeostasis. The inherent property of the gut to act as a semipermeable barrier is crucial for the maintenance of health. Dysfunction of the gut mucosal barrier leads to increased translocation of commensal bacteria and their metabolites locally and systemically, triggering the inflammatory response. Gut inflammation results in excess production of proinflammatory cytokines that can in turn increase mucosal permeability by altering intercellular tight junction structure and induce apoptosis of intestinal epithelial cells. Numerous scientific evidences showed a significant association between impaired gut mucosal barrier and gastrointestinal/extraintestinal diseases [45–48]. Previous study has found that progression of colorectal neoplasia has been linked to alterations of tumor microenvironment and gut mucosal barrier function, which facilitate the interaction of microbial products with host pathways [11]. D-lactate, LPS, and DAO were three accepted and convenient indicators to evaluate the integrity of the gut mucosal barrier. The increased permeability of the gut mucosal barrier would lead to bacterial translocation that was accompanied by increasing levels of D-lactate, LPS, and DAO, which finally contribute to a number of intestinal diseases such as CRC. D-lactate, a hydroxycarboxylic acid produced by bacterial fermentation, is a useful indicator of increased gut permeability and gut barrier dysfunction. Our present study found that the level of D-lactate was increased significantly in CRC patients, which was correlated with *Lactobacillus*, *Bifidobacterium*, and *Clostridium* cluster I negatively and with *Fusobacterium* and Enterobacteriaceae positively, confirming the existence of gut mucosal barrier dysfunction. LPS is large molecules found in the outer membrane of Gram-negative bacteria. The increase in LPS is associated with bacterial translocation due to the impairment of intestinal epithelial cell [49]. Previous study has found that gut microbiota dysbiosis alters the intestinal barrier function, increases plasma LPS levels, which promotes endotoxia, and contributes to the onset and development of CRC [50]. Our study also found that the concentration of LPS increased significantly in CRC patients, which was correlated with *Lactobacillus*, *Bifidobacterium*, and *Clostridium* cluster I negatively and with *Fusobacterium* and Enterobacteriaceae positively. The results suggest that there was an increased intestinal permeability in these CRC patients. DAO is an enzyme mainly produced in the small intestine involved in the histamine metabolism [51]. Yee et al. has found that an established first-line treatment for patients in T2DM, metformin, inhibits DAO activity [52]. Previous study also found that serum DAO activity decreased step-by-step significantly during anticancer drug therapy in human, which may be to serve as a useful predictor of gastrointestinal toxicity due to anticancer drug [53]. Our present data found that the levels of DAO also increased significantly
in CRC patients, which was correlated with *Lactobacillus*, *Bifidobacterium*, and *Clostridium* cluster I negatively and with *F. nucleatum* and Enterobacteriaceae positively. Taken together, the low levels of D-lactate, LPS, and DAO in normal conditions increased significantly in CRC patients, suggesting a breakdown of the gut mucosal barrier.

In summary, dysbiosis of the fecal microbiota, which was featured by altered abundant fecal bacteria, and dysfunction of the gut mucosal barrier, which was characterized by increased levels of D-lactate, LPS, and DAO, occurred in patients with early-stage CRC. The abundant bacteria in the feces and the indicators of the gut mucosal barrier in the plasma could be used as targets to monitor the development and progression of CRC in the future.

### Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

### Ethical Approval

All research conformed to the Helsinki Declaration and was approved by the Ethics Committee of the First Affiliated Hospital, School of Medicine, Zhejiang University (China), and was implemented in accordance with the approved guidelines.

### Consent

Informed written consent was obtained from each of the patients before enrollment.

### Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

### Authors’ Contributions

XL and ZXL conceived and designed the experiments. XL, YWC, LS, and ZXL performed the experiments and analyzed the data. XL and ZXL wrote the paper and edited the manuscript. The final manuscript was read and approved by all authors. Xia Liu and Yiwen Cheng contributed equally to this work.

### Acknowledgments

The authors thank all of the participants who recruited patients in this study. This present work was funded by the grants from the National Natural Science Foundation of China under Grant Nos. 31700800, 81771724, 81790631, and 31870839 and National S&T Major Project of China.
under Grant No. 2018YFC2000500 and the Nutrition and Care of Maternal & Child Research Fund Project of Guangzhou BioTime Institute of Nutrition & Care (2019BINC9CMC045).

References

[1] W. Chen, K. Sun, R. Zheng et al., “Cancer incidence and mortality in China, 2014,” Chinese Journal of Cancer Research, vol. 30, no. 1, pp. 1–12, 2018.

[2] M. Araghi, I. Soerjomataram, A. Bardot et al., “Changes in colorectal cancer incidence in seven high-income countries: a population-based study,” Lancet Gastroenterol Hepatol, vol. 4, no. 7, pp. 511–518, 2019.

[3] W. Chen, R. Zheng, P. D. Baade et al., “Cancer statistics in China, 2015,” CA: a Cancer Journal for Clinicians, vol. 66, no. 2, pp. 115–132, 2016.

[4] W. Chen, F. Liu, Z. Ling, X. Tong, and C. Xiang, “Human intestinal lumen and mucosa-associated microbiota in patients with colorectal cancer,” PLoS One, vol. 7, no. 6, article e39743, 2012.

[5] O. O. Coker, G. Nakatsu, R. Z. Dai et al., “Enteric fungal microbiota dysbiosis and ecological alterations in colorectal cancer,” Gut, vol. 68, no. 4, pp. 654–662, 2019.

[6] S. H. Wong, L. Zhao, X. Zhang et al., “Garage of fecal samples from patients with colorectal cancer promotes intestinal carcinogenesis in germ-free and conventional mice,” Gastroenterology, vol. 153, no. 6, pp. 1621–1633.e6, 2017.

[7] Q. Liang, J. Chiu, Y. Chen et al., “Fecal bacteria act as novel biomarkers for noninvasive diagnosis of colorectal cancer,” Clinical Cancer Research, vol. 23, no. 8, pp. 2061–2070, 2017.

[8] G. Nakatsu, X. Li, H. Zhou et al., “Gut mucosal microbiome across stages of colorectal carcinogenesis,” Nat Commun, vol. 6, no. 1, 2015.

[9] J. Yu, Q. Feng, S. H. Wong et al., “Metagenomic analysis of faecal microbiome as a tool towards targeted non-invasive biomarkers for colorectal cancer,” Gut, vol. 66, no. 1, pp. 70–78, 2016.

[10] G. Nakatsu, H. Zhou, W. K. K. Wu et al., “Alterations in enteric virome are associated with colorectal cancer and survival outcomes,” Gastroenterology, vol. 155, no. 2, pp. 529–541.e5, 2018.

[11] T. Irrazabal, A. Belcheva, S. E. Girardin, A. Martin, and D. J. Philpott, “The multifaceted role of the intestinal microbiota in colon cancer,” Molecular Cell, vol. 54, no. 2, pp. 309–320, 2014.

[12] H. Wei, L. Dong, T. Wang et al., “Structural shifts of gut microbiota as surrogate endpoints for monitoring host health changes induced by carcinoemogen exposure,” FEMS Microbiology Ecology, vol. 73, no. 3, pp. 577–586, 2010.

[13] T. Wang, G. Cai, Y. Qiu et al., “Structural segregation of gut microbiota between colorectal cancer patients and healthy volunteers,” The ISME Journal, vol. 6, no. 2, pp. 320–329, 2012.

[14] H. Tsoi, E. S. H. Chu, X. Zhang et al., “Peptostreptococcus anaerobius Induces Intracellular Cholesterol Biosynthesis in Colon Cells to Induce Proliferation and Causes Dysplasia in Mice,” Gastroenterology, vol. 152, no. 6, pp. 1419–1433.e5, 2017.

[15] G. Zeller, J. Tap, A. Y. Voigt et al., “Potential of fecal microbiota for early-stage detection of colorectal cancer,” Molecular Systems Biology, vol. 10, no. 11, p. 766, 2014.

[16] T. N. Y. Kwong, X. Wang, G. Nakatsu et al., “Association between bacteremia from specific microbes and subsequent diagnosis of colorectal cancer,” Gastroenterology, vol. 155, no. 2, pp. 383–390.e8, 2018.

[17] A. J. Scott, J. L. Alexander, C. A. Merrifield et al., “International Cancer Microbiome Consortium consensus statement on the role of the human microbiome in carcinogenesis,” Gut, vol. 68, no. 9, pp. 1624–1632, 2019.

[18] R. W. Burt, J. S. Barthel, K. B. Dunn et al., “NCCN clinical practice guidelines in oncology. Colorectal cancer screening,” Journal of the National Comprehensive Cancer Network, vol. 8, no. 1, pp. 8–61, 2010.

[19] X. Xia, J. Chen, J. Xia et al., “Role of probiotics in the treatment of minimal hepatic encephalopathy in patients with HBV-induced liver cirrhosis,” Journal of International Medical Research, vol. 46, no. 9, pp. 3596–3604, 2018.

[20] C. Tan, Z. Ling, Y. Huang et al., “Dysbiosis of intestinal microbiota associated with inflammation involved in the progression of acute pancreatitis,” Pancreas, vol. 44, no. 6, pp. 868–875, 2015.

[21] Z. Ling, X. Liu, S. Guo et al., “Role of probiotics in mycoplasma pneumoniae pneumonia in children: a short-term pilot project,” Frontiers in Microbiology, vol. 9, 2019.

[22] L. Shen, L. Ao, H. Xu et al., “Poor short-term glycemic control in patients with type 2 diabetes impairs the intestinal mucosal barrier: a prospective, single-center, observational study,” BMC Endocrine Disorders, vol. 19, no. 1, p. 29, 2019.

[23] E. Nistal, N. Fernández-Fernández, S. Vivas, and J. L. Olcoz, “Factors determining colorectal cancer: the role of the intestinal microbiota,” Frontiers in Oncology, vol. 5, p. 220, 2015.

[24] L. Sobbani, J. Tap, F. Roudot-Thoraval et al., “Microbial dysbiosis in colorectal cancer (CRC) patients,” PLoS One, vol. 6, no. 1, article e16393, 2011.

[25] T. Yu, F. Guo, Y. Yu et al., “Fusobacterium nucleatum Promotes Chemoresistance to Colorectal Cancer by Modulating Autophagy,” Cell, vol. 170, no. 3, pp. 548–563.e16, 2017.

[26] S. J. D. O’Keefe, “Diet, microorganisms and their metabolites, and colon cancer,” Nature Reviews Gastroenterology & Hepatology, vol. 13, no. 12, pp. 691–706, 2016.

[27] M. C. S. Mendes, D. S. Paulino, S. R. Brambilla, J. A. Camargo, G. F. Persinoti, and J. B. C. Carvalheira, “Microbiota modification by probiotic supplementation reduces colitis associated colon cancer in mice,” World Journal of Gastroenterology, vol. 24, no. 18, pp. 1995–2008, 2018.

[28] L. Zhong, X. Zhang, and M. Covasa, “Emerging roles of lactic acid bacteria in protection against colorectal cancer,” World Journal of Gastroenterology, vol. 20, no. 24, pp. 7878–7886, 2014.

[29] S. Danese, “Inflammatory bowel disease and inflammation-associated colon cancer: partners in crime,” Current Drug Targets, vol. 9, no. 5, p. 360, 2008.

[30] A. Nowak, A. Paliwoda, and J. Blasiak, “Anti-proliferative, pro-apoptotic and anti-oxidative activity of Lactobacillus and Bifidobacterium strains: a review of mechanisms and therapeutic perspectives,” Critical Reviews in Food Science and Nutrition, vol. 59, no. 21, pp. 3456–3467, 2019.

[31] C. Lupp, M. L. Robertson, M. E. Wickham et al., “Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of Enterobacteriaceae,” Cell Host & Microbe, vol. 2, no. 2, pp. 119–129, 2007.

[32] E. Elinav, R. Nowarski, C. A. Thaiss, B. Hu, C. Jin, and R. A. Flavell, “Inflammation-induced cancer: crosstalk between...
tumours, immune cells and microorganisms,” *Nature Reviews Cancer*, vol. 13, no. 11, pp. 759–771, 2013.

[33] F. Housseau and C. L. Searls, “Enterotoxigenic *Bacteroides fragilis* (ETBF)-mediated colitis in Min (Apc(-/-)) mice: a human commensal-based murine model of colon carcinogenesis,” *Cell Cycle*, vol. 9, no. 1, pp. 3–5, 2010.

[34] P. R. Mangan, L. E. Harrington, D. B. O’Quinn et al., “Transforming growth factor-β induces development of the Tg17 lineage,” *Nature*, vol. 441, no. 7090, pp. 231–234, 2006.

[35] T. Sun, S. Liu, Y. Zhou et al., “Evolutionary biologic changes of gut microbiota in an ‘adenoma-carcinoma sequence’ mouse colorectal cancer model induced by 1, 2-dimethylhydrazine,” *Oncotarget*, vol. 8, no. 1, pp. 444–457, 2017.

[36] J. M. Cavaillon, “Exotoxins and endotoxins: inducers of inflammatory cytokines,” *Toxicon*, vol. 149, pp. 45–53, 2018.

[37] A. Gonzalez-Sarrias, M. A. Nunez-Sanchez, M. A. Avala-Galvez et al., “Consumption of pomegranate decreases plasma lipopolysaccharide-binding protein levels, a marker of metabolic endotoxemia, in patients with newly diagnosed colorectal cancer: a randomized controlled clinical trial,” *Food & Function*, vol. 9, no. 5, pp. 2617–2622, 2018.

[38] L. Zhao, L. Luo, W. Jia et al., “Serum diamine oxidase as a hemorraghic shock biomarker in a rabbit model,” *PLoS One*, vol. 9, no. 8, article e102285, 2014.

[39] S. W. Yee, L. Lin, M. Merski et al., “Prediction and validation of enzyme and transporter off-targets for metformin,” *Journal of Pharmacokinetics and Pharmacodynamics*, vol. 42, no. 5, pp. 463–475, 2015.

[40] J. Miyoshi, H. Miyamoto, T. Goji et al., “Serum diamine oxidase activity as a predictor of gastrointestinal toxicity and malnutrition due to anticancer drugs,” *Journal of Gastroenterology and Hepatology*, vol. 30, no. 11, pp. 1582–1590, 2015.