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Review Article

Microbiome and Colorectal Cancer in Humans: A Review of Recent Studies

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Abstract
The tumor microenvironment has recently been well-studied in various gastrointestinal cancers, including colorectal cancer (CRC). The gut microbiota, a collection of microorganisms in the human gastrointestinal tract, is one of the microenvironments associated with colon carcinogenesis. It has been challenging to elucidate the mechanisms by which gut microbiota contributes to carcinogenesis and cancer progression due to complex interactions with the host, including its metabolites and immune and inflammatory responses. Various studies described the influence of diet on reported changes in the composition and microbiota of gut bacteria and its association with CRC. In recent years, metagenomic techniques such as shotgun sequencing and genome-wide association studies focused on understanding the role of the microbiota and the metabolome on early CRCs and colon carcinogenesis to determine if there are modifiable or intervenable targets for CRC. In this review, we will attempt to provide an overview of gut microbiota related to CRC, with particular attention to the findings of recent studies.

Keywords
microbiota, metabolome, colorectal cancer, metagenomic analysis

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Introduction

The human gastrointestinal tract is a series of organs with barrier and immune functions. The human gut microbiota is an ecosystem populated by bacteria, fungi, viruses, archaea, and parasites whose cells in total typically outnumber that of the host and may interact with the immune system[1].

A diverse and large number of microorganisms, or bacteria, live in the human gut. These complex microenvironments, including a colony of microorganisms, are called microbiota. Studies have demonstrated that alterations in microbiota are associated with human health and disease. However, it is challenging to explain disease associations by analyzing a single bacterium, species, or genus alone. Recently, many researchers recognized the complex association of microbiota with disease but the mechanisms and details are still unclear.

Two technical developments has provided an approach to better understanding the interactions between the gut microbiota and the gastrointestinal system. One of these techniques is 16S ribosomal RNA (rRNA) gene amplicon sequencing[2]. The 16S rRNA is only present in gut bacteria and not in human cells, making it possible to analyze information on gut bacteria alone. Another technology is shotgun sequencing made possible by next-generation sequencing development.

High-throughput technologies facilitated the identification of bacteria associated with human colorectal cancer (CRC)

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Table 1. Bacteria in the Human Gut Related to Colorectal Cancer.

Bacteria related to colorectal cancer
Fusobacterium nucleatum
Parvimonas micra
Peptostreptococcus stomatis
Peptostreptococcus anaerobius
Atopobium parvulum
Actinomyces odontolyticus

by use of shotgun sequencing, named so due to the process by which all genomic DNA of bacteria, including human genes, are cut into short fragments by restriction enzymes. In the whole genome shotgun method, the overlaps of the DNA sequences are assembled by a high-power computer.

Next-generation sequencing technologies provided a method of analyzing this large amount of metagenomic data and led to CRC-associated bacteria identification (Table 1)[3]. Furthermore, the shotgun sequence had the advantage of analyzing the function of the bacteria compared with 16S rRNA gene amplicon sequences[3-5].

Previous studies showed that a diverse population of bacteria inhabiting the human gut, with estimates suggesting approximately 3,000-5,000 species or more. Most bacterial species within the gut microbiota are anaerobes, such as commonly found indigenous microflora including *Bifidobacterium*, *Enterococcus*, *Clostridium*, and *Lactobacilli* species[6]. Swidsinski et al. reported an association between disease and gut microbiota changes in chronic diarrhea[7]. Alterations of gut microbiota were reported in inflammatory bowel disease[8]. Until recently, there were no clear reports on gut microbiota changes associated with CRC. In recent years, however, several novel studies reported an association between CRC and certain gut bacteria.

In this review, we will provide an overview of gut microbiota associated with CRC and introduce a novel hypothesis of how changes in the microbiota are associated with carcinogenesis and early precursor lesions, as well as future research areas in the field.

Carcinogenesis of Colorectal Cancer and the Gut Microbiota

Carcinogenesis of CRC is associated with genetic and environmental factors such as pathogenic genomic variants, genomic DNA methylation, diet, or smoking. In addition, it is becoming clear that the gut microbiota is also associated with CRC carcinogenesis. Two landmark papers published in 2012 brought the relationship between the gut microbiota and CRC into the spotlight[9,10]. Castellarin et al. examined frozen tumor and mucosal tissue in 99 pairs of CRC and non-CRC patients. A quantitative PCR of bacteria revealed that most bacteria related genes were similar in the two groups, while only *Fusobacterium nucleatum* varied between patients with and without CRC. Kostic et al. analyzed 95 pairs of tumor cells and nontumor cells using whole genome sequencing to determine the microbiota composition in CRC. The enrichment of *F. nucleatum* in CRC tissues was found by comparing the bacterial species or domains identified by quantitative PCR and 16S rDNA amplicon sequencing. These studies demonstrated that the gut microbiota profile differs between CRC and non-CRC patients or that bacterial species or domain spectrum varied in CRC patients. Since then, many additional studies reported the association between CRC and gut microbiota.

Mechanism of Carcinogenesis of CRC Associated with Gut Microbiota

The mechanisms of CRC carcinogenesis are diverse. There may be several factors involved in addition to pathogenic genetic variants and DNA methylations, such as inflammation of the gastrointestinal tract as well as dietary influences. Irrazábal et al. reviewed the association between various factors and gut microbiota in the development of CRC[11]. CRC is fundamentally considered to be a disease caused by genetic abnormalities. In general, CRC results from uncontrollable cell proliferation due to gene abnormalities such as APC, TP53, and MMR[12-14]. Microbial abnormalities in the human gut caused by inflammation or aberrant signaling pathways can result in component changes of the various bacteria, leading to dysbiosis thought to be associated with colorectal carcinogenesis.

Colorectal Carcinogenesis Associated with Inflammation Caused by Dysbiosis

Recent studies increasingly clarified the link between dysbiosis and inflammation causing CRC. However, it remains unclear whether dysbiosis is the cause or result of inflammation. The association of IL-6, a proinflammatory cytokine, with colorectal carcinogenesis via abnormalities in signaling pathways was also investigated[15]. It is also unclear whether abnormalities in IL-6 cause CRC in the context of inflammation or whether the cytokine has carcinogenic potential in the absence of inflammation. The mechanism by which dysbiosis causes colorectal carcinogenesis is thought to be mediated by several abnormalities triggering genetic abnormalities. Possible intervening mechanisms are shown in Table 2. One suspected mechanism is that CRC occurs when the barrier of the gut mucosa is compromised by dysbiosis. This allows toxic substances that could damage DNA to invade the human body. Another proposed mechanism is that dysbiosis-related enzymes of reactive oxygen species (ROS) metabolism lead to DNA damage

Table 2. Various Mechanisms of Carcinogenesis of Colorectal Cancer by Dysbiosis.

Change of function	Cause substance/signal	Mechanism of carcinogenesis
Loss of barrier function	Various toxic substance	DNA damage
Change in enzyme activity	Increase of ROS	DNA damage
Product of ROS by immune cells	Increase of ROS	DNA damage
Change of microbiome	Increase of cytokine	Cell proliferation
Increase of IL-6 and/or IL-1b	Induction of DNMT and methylation of promoter	Silencing of tumor suppressor genes
Cytokine		Downregulation of tumor suppressor genes

from an increase in ROS[16].

Dysbiosis may also produce various cytokines that induce aberrant cell proliferation. As mentioned above, dysbiosis may increase IL-6 and IL-1b, resulting in the silencing of tumor suppressor genes by DNA promoter methylation[17].

Furthermore, it was been suggested that dysbiosis may express microRNAs via cytokine production and inflammatory pathways leading to the downregulation of cancer suppressor genes[18].

Associations of Dysbiosis with CRC without Inflammation

Another mechanism of dysbiosis that causes CRC is downstream alterations in microbiota product metabolites. This may be due to a different bacterial distribution from the individual's normal bacterial flora. For example, hydrogen sulfide (H_2S), nitrosamines, and heterocyclic amines were considered candidates. H_2S does not cause DNA damage directly but is associated with colon carcinogenesis via induction of apoptosis[19]. Nitrosamines and heterocyclic amines are known to be risk factors for the development of CRC. These metabolites are produced by the gut bacterial metabolism from meat-rich diets. These findings provided a way to reconcile dysbiosis within the gut microbiome with metabolites linked to diet-induced CRC. However, the relationship between inflammatory dysbiosis and diet remains unclear. Further research is needed to elucidate the role and mechanisms of the gut microbiota.

Metagenomic Changes in Early CRC

Most previous studies analyzing the gut microbiota focused on advanced CRC, i.e., CRC of T2 or deeper in depth. Sporadic CRC is known to develop in multistage carcinogenesis from normal colonic tissue to hyperproliferative epithelium to adenoma and finally to carcinoma[20]. Recent studies reported that the gut microbiota and metabolomic changes were observed in early CRC or adenoma. Yachida et al. reported that *F. nucleatum* is increased in early CRC and this change continues in advanced CRC[21]. Furthermore, *Atopobium parvulum* and *Actinomyces odontolyticus* are increased in adenomas and early CRC, while this change is absent in advanced CRC. These findings suggest that changes in gut microbiota related to CRC were already present in early-stage CRC or precursor lesions. In addition, metabolomics, i.e., including analysis of products and substances produced by gut microbiota metabolism, was performed. Gut metabolites such as branched-chain amino acids, phenylalanine, and deoxycholic acid were increased only in the adenomas and not advanced CRC. Based on the results, Yachida et al. developed a diagnostic model for early CRC carcinogenesis using the gut microbiome and metabolome. This suggests its potential as an early detection marker and treatment target.

Validation and Meta-analysis of the Studies of Gut Microbiota

The report by Yachida et al. provided new ideas and findings. Using shotgun sequencing to understand the metagenomics of the gut microbiota and CRC required further validation and exploration. Wirbel et al. conducted a meta-analysis of eight studies using shotgun sequencing to analyze the microbiome. A total of 368 patients with CRC and 392 healthy controls were extracted. This meta-analysis supported an association between CRC and common gut microbiota and metabolome in different regions and countries. A core set of 29 CRC-associated bacteria was found in this meta-analysis (Table 3). A further metabolome analysis was also performed. The *Clostridium* genus is involved in the bile acid-inducible (bai) operon gene related to secondary bile acid production as bile acid metabolism[22]. Deoxycholic and lithocholic acids, products of the bile acid pathway, are also thought to promote CRC[23]. These factors or substances are not included in databases commonly used for metagenome annotation. Therefore, Wirbel et al. developed a metagenomic annotation workflow based on hidden Markov models to identify and quantify the factors and pathways related to CRC[4]. In addition, they estimated associations between multiple gene operons. The bai operon was analyzed in metagenomes in human fecal samples. However, significant differences were seen concerning consistency between studies. However, there could be no significant differences

Table 3. Twenty-Nine Species Associated with CRC after Meta-Analysis of Metagenomics Studies Using Shotgun Sequences.

Parvimonas micra
Gemella morbillorum
Peptostreptococcus stomatis
Fusobacterium nucleatum subspecies *animalis*
Unknown *Dialister*
Unknown *Porphyromonas*
Solobacterium moorei
Porphyromonas uenonis
Clostridium symbiosum
Unknown *Clostridiales*
Hungatella hathewayi
Prevotella intermedia
Porphyromonas somerae
Porphyromonas asaccharolytica
Fusobacterium nucleatum subspecies *nucleatum*
Parvimonas species
Prevotella nigrescens
Unknown *Porphyromonas*
Ruminococcus torques
Fusobacterium nucleatum subspecies *vincentii*
Fusobacterium species
Unknown *Peptostreptococcaceae*
Anaerococcus obesiensis/vaginalis
Unknown *Anaerotruncus*
Porphyromonas uenonis
Unknown *Clostridiales*
Unknown *Porphyromonas*
Clostridium bolteae/clostridioforme
Subdoligranulum species

detected in the metagenomic analysis despite reported carcinogenic effects in patients with CRC. These studies supported the potential of microbiota and metabolome-based CRC diagnosis, including early-stage CRC. These results suggest gut metagenomic and metabolomic markers could establish the basis for future CRC diagnosis.

Change of Microbiome and Metabolome before and after Colorectal Surgery

As previously mentioned, the association between bile acids and CRC was already known, however, several studies reported controversial associations with CRC. Therefore, Shiroma et al. analyzed changes in gut microbiota and metabolomes in 170 preoperative and postoperative fecal samples from 85 CRC patients[24]. The distribution of gut microbiota differed at the genus level among healthy controls, early-stage CRC, and invasive or advanced CRC. Similarly, the distribution of metabolomes was found to be different in each patient. *Parvimonas micra*, *F. nucleatum*, and *Peptostreptococcus stomatis* decreased after surgery, while *Bifidobacterium longum* and *Clostridium scindens* increased in both cohorts. *Bifidobacterium longum* and *C. scindens* were found to increase after surgery. Many CRC-related metabolites including serine, urocanic acid, and glycylleucine decreased, while deoxycholate (DCA), and total bile acids showed significant increases in patients after surgery. These findings suggested an association between the change in the microbiome and the bai operon gene cluster. Comparing the gene sequences related to the bai operon revealed its relative increase after surgery, strongly suggesting an effect of *C. scindens*. Next, they compared the patients with right hemicolectomy to left hemicolectomy to examine the effects of bile acid metabolism and reabsorption. DCA, cholic acid, and total bile acids were significantly increased after right and left hemicolectomy. However, *C. scindens*, thought to be associated with the bai operon, was significantly increased only after left hemicolectomy. Thus, the gut microbiota and metabolomes are altered not only by CRC removal but also by the difference in surgical methods. Another study of patients who had surgery for gastric cancer showed similar changes in their intestinal environment[25]. The changes in the gut microenvironment are becoming more evident.

Discussions

We reviewed several recent studies on the association between the gut microbiota and CRC. One of the latest findings is that dysbiosis of the gut microbiota in CRC carcinogenesis is observed from the very early-stage of CRC development and changes cancer progression. In addition, dynamic changes in the gut microbiome and bacterial-derived metabolites were found according to the cancer progression from adenoma to intramucosal carcinoma, submucosal invasive carcinoma, and advanced carcinoma. This may represent a paradigm shift from clarifying the relationship between the gut microbiota and CRC to targeting the changes in the microbiota during each phase of CRC carcinogenesis. It remains unclear whether metagenomic changes are a cause or a result of CRC, although the association with CRC is becoming increasingly evident. While CRC was long thought to be the result of genomic mutations from the activation of oncogenes and inactivation of tumor suppressor genes, it can also be considered to be a disease associated with bacterial changes in the colonic environment[21]. The meta-analysis reviewed suggests that statistical methods are not a limiting factor in developing clinically applicable predictive models to diagnose CRC. Both gut microbiota and metabolome were shown to have the potential for robust diagnostic methods in the future. Further studies may be necessary to evaluate the diagnostic sensitivity and cost-effectiveness for widespread clinical application. We should view CRC as a multifaceted disease influenced not only by

genetic, epigenomic, and environmental factors but also by the symbiotic relationship with the gut microbiota. When we study cancer genomics with regards to early detection, prevention, and treatment of CRC, we need genomic information from the microbiome as well as genetic abnormalities such as mutations, pathogenetic variants, and aberrant methylations and anticipate the microbiome will play an important role in the future.

Conflicts of Interest

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Disclaimer

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References

1. Sender R, Fuchs S, Milo R. Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. Cell. 2016 Jan; 164(3): 337-40.
2. Liao M, Xie Y, Mao Y, et al. Comparative analyses of fecal microbiota in Chinese isolated Yao population, minority Zhuang and rural Han by 16sRNA sequencing. Sci Rep. 2018 Jan; 8(1): 1142.
3. Mizutani S, Yamada T, Yachida S. Significance of the gut microbiome in multistep colorectal carcinogenesis. Cancer Sci. 2020 Mar; 111(3): 766-73.
4. Wirbel J, Pyl PT, Kartal E, et al. Meta-analysis of fecal metagenomes reveals global microbial signatures that are specific for colorectal cancer. Nat Med. 2019 Apr; 25(4): 679-89.
5. Mori H, Maruyama T, Yano M, et al. VITCOMIC2: visualization tool for the phylogenetic composition of microbial communities based on 16S rRNA gene amplicons and metagenomic shotgun sequencing. BMC Syst Biol. 2018 Mar; 12(suppl 2): 30.
6. Dethlefsen L, Huse S, Sogin ML, et al. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. PLOS Biol. 2008 Nov; 6(11): e280.
7. Swidsinski A, Loening-Baucke V, Verstraelen H, et al. Biostructure of fecal microbiota in healthy subjects and patients with chronic idiopathic diarrhea. Gastroenterology. 2008 Aug; 135(2): 568-79.
8. Swidsinski A, Loening-Baucke V, Vaneechoutte M, et al. Active Crohn's disease and ulcerative colitis can be specifically diagnosed and monitored based on the biostructure of the fecal flora. Inflamm Bowel Dis. 2008 Feb; 14(2): 147-61.
9. Castellarin M, Warren RL, Freeman JD, et al. Fusobacterium nucleatum infection is prevalent in human colorectal carcinoma. Genome Res. 2012; 22(2): 299-306.
10. Kostic AD, Gevers D, Pedamallu CS, et al. Genomic analysis identifies association of Fusobacterium with colorectal carcinoma. Genome Res. 2012 Feb; 22(2): 292-8.
11. Irrazábal T, Belcheva A, Girardin SE, et al. The multifaceted role of the intestinal microbiota in colon cancer. Mol Cell. 2014 Feb; 54(2): 309-20.
12. Groden J, Thliveris A, Samowitz W, et al. Identification and characterization of the familial adenomatous polyposis coli gene. Cell. 1991 Aug; 66(3): 589-600.
13. Poulogiannis G, Frayling IM, Arends MJ. DNA mismatch repair deficiency in sporadic colorectal cancer and Lynch syndrome. Histopathology. 2010 Jan; 56(2): 167-79.
14. Lakatos PL, Lakatos L. Risk for colorectal cancer in ulcerative colitis: changes, causes and management strategies. World J Gastroenterol. 2008 Jul; 14(25): 3937-47.
15. Hu B, Elinav E, Huber S, et al. Microbiota-induced activation of epithelial IL-6 signaling links inflammasome-driven inflammation with transmissible cancer. Proc Natl Acad Sci U S A. 2013 Jun; 110(24): 9862-7.
16. Shaked H, Hofseth LJ, Chumanevich A, et al. Chronic epithelial NF-κB activation accelerates APC loss and intestinal tumor initiation through iNOS up-regulation. Proc Natl Acad Sci U S A. 2012 Aug; 109(35): 14007-12.
17. Grady WM, Carethers JM. Genomic and epigenetic instability in colorectal cancer pathogenesis. Gastroenterology. 2008 Oct; 135 (4): 1079-99.
18. Pekow JR, Dougherty U, Mustafi R, et al. miR-143 and miR-145 are downregulated in ulcerative colitis: putative regulators of inflammation and protooncogenes. Inflamm Bowel Dis. 2012 Jan; 18(1): 94-100.
19. Strocchi A, Furne J, Ellis C, et al. Methanogens outcompete sulphate reducing bacteria for H2 in the human colon. Gut. 1994 Aug; 35(8): 1098-101.
20. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell. 1990 Oct; 61(5): 759-67.
21. Yachida S, Mizutani S, Shiroma H, et al. Metagenomic and metabolomic analyses reveal distinct stage-specific phenotypes of the gut microbiota in colorectal cancer. Nat Med. 2019 Jun; 25(6): 968-76.
22. Ridlon JM, Harris SC, Bhowmik S, et al. Consequences of bile salt biotransformations by intestinal bacteria. Gut Microbes. 2016 Jan; 7(1): 22-39.
23. Ajouz H, Mukherji D, Shamseddine A. Secondary bile acids: an underrecognized cause of colon cancer. World J Surg Oncol. 2014 Dec; 12: 164.
24. Shiroma H, Shiba S, Erawijantari PP, et al. Surgical treatment for colorectal cancer partially restores gut microbiome and metabolome traits. mSystems. 2022 Apr; 7(2): e0001822.
25. Erawijantari PP, Mizutani S, Shiroma H, et al. Influence of gastrectomy for gastric cancer treatment on faecal microbiome and metabolome profiles. Gut. 2020 Aug; 69(8): 1404-15.