

Role of gut microbiota in the pathogenesis of colorectal cancer; a review article

Somayeh Jahani-Sherafat^{1,2}, Masoud Alebouyeh², Sharareh Moghim¹, Hamed Ahmadi Amoli³, Hajieh Ghasemian-Safaei¹

¹Microbiology Department, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

²Foodborne and Waterborne Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran

³Sina Hospital, Tehran University of Medical Sciences, Tehran, Iran

ABSTRACT

Colorectal cancer (CRC) is one of the most frequently diagnosed cancers worldwide. Lifestyle is identified as one of the most important risk factors for CRC, especially in sporadic colorectal cancer. The natural composition of the gut microbiota changes rapidly during the first decade of life. Maintaining homeostasis in the gut is essential as structural and metabolic functions of the commensal microbiota inhibit gut colonization of pathogens. Dysbiosis, imbalance in function or structure of gut microbiota, has been associated with a variety of diseases, such as colorectal cancer. The aim of this review was to investigate the possible links between the dysbiosis in gut microbiota and colorectal cancer, and the potential role of anaerobic gut microbiota in the pathogenesis of colorectal cancer. Based on this review, various studies have shown that some of the gut microbiota such as anaerobic bacteria significantly increased in CRC patients, but we suggest more investigations are required to assess the importance of these bacteria and their metabolites in the pathogenesis of CRC are required.

Keywords: Gut microbiota, Pathogenesis, Colorectal cancer.

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Introduction

Colorectal cancer (CRC) is one of the top three most frequently diagnosed cancers worldwide, with nearly 1.4 million new cases diagnosed in 2012. More than 50 percent of colorectal cancer cases were reported in developed countries with the least incidence in Africa and Asia (1). Although developing countries are low-risk countries for CRC, particularly among the older population, but the age standardized rate within the young population in some Asian countries such as Iran and Pakistan, is as the same as US inhabitants (2,3). Despite extensive research exact etiology for CRC is still unknown, but genetic and environmental factors have been implicated as disease' risk factors. The

majority of CRC cases occur sporadically and less than 25 percent of CRC cases are hereditary (4). The similar incidence in young population between developed and developing countries seems associated with variations in lifestyle. Lifestyle is one of the most important risk factors for CRC, especially in sporadic colorectal cancers. Change in lifestyle factors, such as a diet rich in processed foods, animal fat and red meat with a low intake of fiber and fruits, decrease of physical inactivity and obesity, is thought to change the gut microbiota composition and increase the risk of disease in developing countries (5).

The colon is exposed to a large number of microorganisms. Approximately more than 10^{13} bacteria harbor in the adult human colon and other parts of the large intestine. During the first year of life, quick changes occur in the variety and composition of the microbiota. This composition, shaped by contact to

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Reprint or Correspondence: Hajieh Ghasemian-Safaei, PhD. Microbiology Department, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: ghasemian@med.mui.ac.ir

environmental factors such as diet, antibiotic therapy, hospitalization, chemical exposure, and contact with the vaginal microbiota during birth, could be a connection between life style and accumulation of mutation in host (6-8).

Gut microbiota are predominantly strict anaerobes, including *Bacteroides*, *Eubacterium*, *Bifidobacterium*, *Fusobacterium*, *Peptostreptococcus*, and *Atopobium*, while facultative anaerobes, such as *Enterococci*, *Lactobacilli*, *Enterobacteriaceae*, and *Streptococci*, form a minor portion of inhabitants (9). Maintaining this structure is essential for the gut hemostasis because structural and metabolic functions of the commensal microbiota inhibit gut colonization of pathogens. Microbiota participate in the production of short chain fatty acids and proteolytic fermentation by fermentation of anaerobic carbohydrate. Short chain fatty acids elaborate butyrate, propionate and acetate, used as a source of energy in gut and helps to proliferation and differentiation of intestinal epithelial cells (10). Dysbiosis, imbalance in function or structure of gut microbiota, has been associated with a variety of diseases, such as inflammatory bowel disease, obesity, colitis, and colorectal cancer (11-15).

Although the gut microbiota have long been considered commensal residents in the gut, recent studies have demonstrated that microbiota may contribute to CRC pre-carcinogenesis (16). In certain conditions the intestinal microbiota may be linked to an increase in the risk of carcinogenesis and promote tumor growth via various mechanisms (Figure 1) (9). The relationship between cancer and microorganisms has been demonstrated in some organs, with the most well-known example which is the relationship between *Helicobacter pylori* and gastric cancer and mucosa-associated lymphoid tissue lymphoma or *papillomavirus* and cervical cancer (17, 18). Hence, there is much interest in understanding the composition of the gut microbiota in CRC patients in comparison to the healthy population as this knowledge may help develop new therapeutic methods for microbiota manipulation in benefit of the hosts health and disease prevention strategies.

The aim of this review is to present the possible links between dysbiosis in the gut microbiota and colorectal cancer, and discuss the potential role of gut microbiota in the pathogenesis of colorectal cancer.

Methods

Searches were performed in PubMed, Medline, Google scholar, for articles published in English, and other bibliographic references and appropriate sources such as SID and Magiran for Persian-language journals from 2000 to December 2017 using the following keywords alone or in combination: “anaerobic,” “microbiota,” “pathogenesis,” “colorectal cancer,” “microbiome,” “microbiota,” and “dysbiosis.” However, according to our explorer, no Persian-language papers were found. In total, 141 studies were published regarding the microbiota composition in colorectal cancer and based on the study scopes, we categorized all the papers into four major categories including gut microbiota colonization, frequency of gut microbiota, microbiota influence, and inflammatory Pathways.

Gut microbiota in colorectal cancer patients

Several studies have shown that numerous bacterial species appear to be associated with the pathogenesis of CRC and recent studies have provided a mechanism for the participation of gut microbiota in the progress of CRC (14, 19-22). Some bacterial species like *Clostridium septicum*, *Enterococcus faecalis*, *Streptococcus bovis*, *Bacteroides fragilis*, *Helicobacter pylori*, *Escherichia coli* and *Fusobacterium* spp. have been detected and supposed to play a role in colorectal pathogenesis (19-24).

For example, *Streptococcus gallolyticus* (In the past *Streptococcus bovis*) is reported in nearly 20–50% and 5% of colon tumors and normal colon respectively. In CRC patients *Ruminococcus bromii*, *Clostridium clostridioforme* and *Bifidobacterium longum* have low prevalence compared to normal population (23). Furthermore, in different studies a notably increase number of the *Bacteroides/Prevotella* and *Fusobacterium nucleatum* population is described in CRC population (24).

Frequency and pathogenesis of gut microbiota

Recent investigations have confirmed strong relations between the development of colorectal cancer and gut microbiota (25-58). According to global investigations, the most predominant species of the adult health intestinal microbiota are *Bacteroidetes* and *Firmicutes* followed by *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia* but the composition and

frequency of following microbiota changed in CRC patients (25). Intestinal microbiota can contribute to carcinogenesis through production of secondary metabolites, such as reactive oxygen intermediates that caused DNA damage, or direct effects on cell transformation through the production of genotoxin. Different bacterial species such as *Bacteroides fragilis*, *Clostridium septicum*, *Enterococcus faecalis*, *H. pylori*, *Streptococcus bovis*, *Escherichia coli*, and *Fusobacterium* spp. are supposed to play a role in colorectal carcinogenesis (table 1 and 2) (26-58). Meanwhile the mechanisms of some of these bacteria

were partly recognized.

In different studies the prevalence of *S. bovis/galloyticus* and *C. septicum* in CRC patients was reported from 33% to 100% and up to 40% respectively (33-36). In their meta-analysis study, Boleij et al confirmed the relationship between *S. bovis/galloyticus* and *C. septicum* infections and CRC (59). *C. septicum* normally grows in soil and does not represent part of the normal bowel flora but there is no clear mechanism to explain the frequent association between *C.septicum* infection and colon cancer (31,33). *S. bovis/galloyticus* bacteria were found in 2.5-15% of

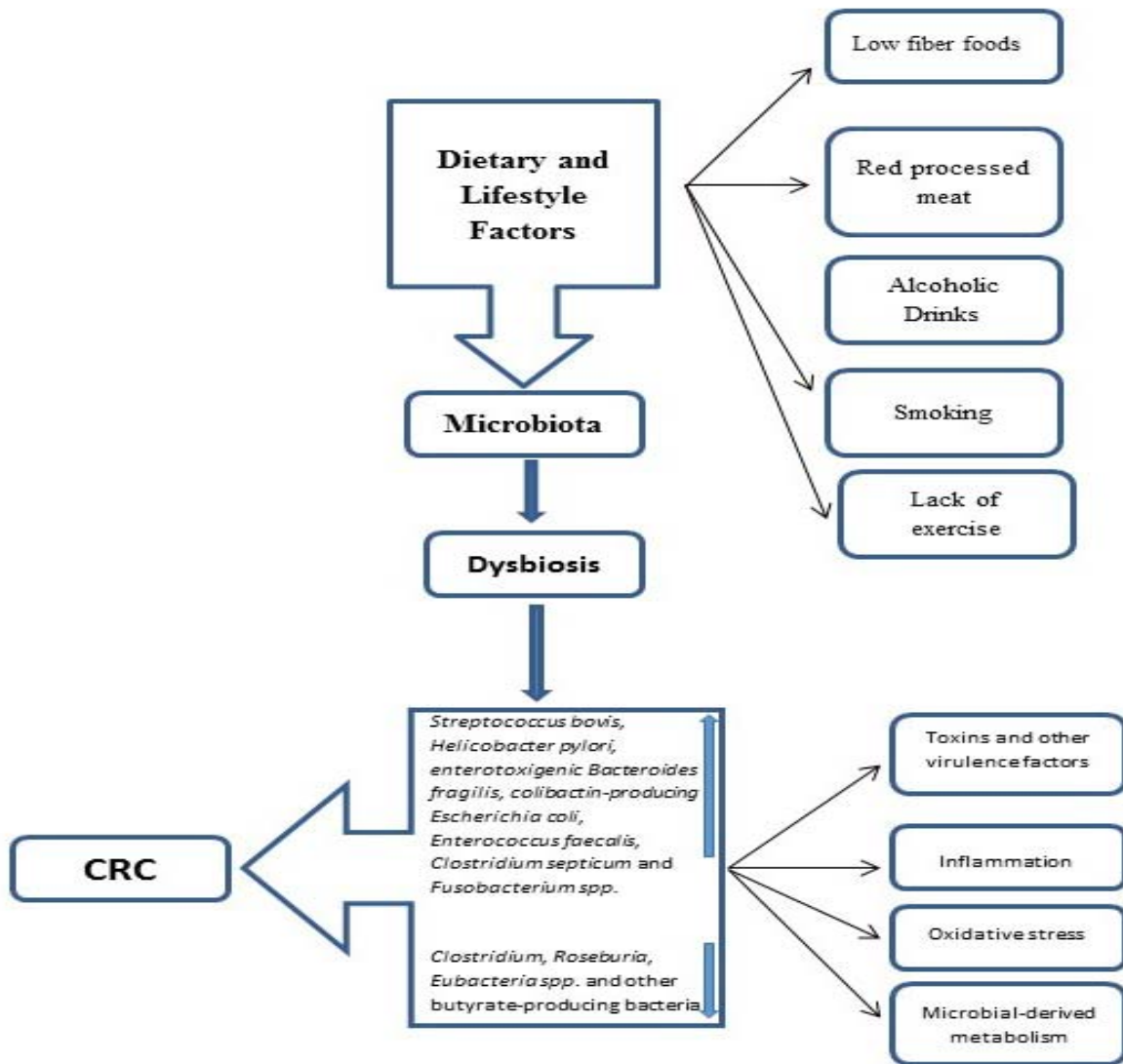


Figure 1. The linked of environment factors influenced gastrointestinal microbiota and promote colorectal cancer via various mechanisms

the normal population but significantly increased in CRC patients (36). *S. bovis/gallolyticus* could colonize and grow in colorectum tissues via collagen-binding and histone-like protein A to collagen I, IV, fibronectin, fibrinogen in colon tissues (35). The activity of present microbiota causes severe inflammatory response by inducing inflammatory and angiogenic cytokines in colorectum tissues and leading to the development or proliferation of colorectal cancer (3).

In a study by Sobhani et al, 179 subjects including 60 colorectal cancer and 119 healthy individuals underwent colonoscopy and the results showed higher levels of *Bacteroides/Prevotella* in patients with colorectal cancer (23). *Entero toxigenic, Bacteroides fragilis* increased in fecal samples of CRC patients. *B. fragilis* degraded the E-cadherin protein and activated nuclear beta-catenin signaling and induces c-Myc expression and cellular proliferation (30, 60).

In a study by Gao *et al.* no significant difference was observed between proximal and distal colon microbiota in 30 healthy compare to 31 cancer patients; nevertheless, in colorectal cancer patients, *Firmicutes* and *Fusobacteria*, *Lactococcus* and *Fusobacterium* were more prevalent and *Proteobacteria*, *Pseudomonas* and *Escherichia-Shigella* were less frequent in tissues samples compared to control group (47).

Several studies showed higher prevalence of *F. nucleatum* in CRC tissue compare to a matched normal tissue (14, 19, and 24). *F. nucleatum* is showed as a probable candidate for CRC predisposition (61-66). *F. nucleatum* adheres to colonic epithelial cells through its FadA adhesion. FadA binds to E-cadherin, activates β -catenin signaling, and differentially regulates the inflammatory and oncogenic responses (41). Fap2 protein of *F. nucleatum* can stimulates CRC expansion by inhibition of the antitumor immune cell activity via TIGIT (67).

Enterococcus faecalis (*E. faecalis*), a commensal microorganism in the intestinal tract, has been repeatedly found in colorectal cancer patients (34, 68). *E. faecalis* has recently been considered as a human pathogen (68). Balamurugan et al. had reported statistically significant higher levels of *E. faecalis* from the feces of patients with CRC compared to healthy volunteers (34). These bacteria can produce reactive oxygen and nitrogen species (RONS) that directly lead to DNA break, point mutation and chromosomal

instability. These functions demonstrated this common colonic commensal has rendered an organism with the potential to contribute to oncogenic transformation in the colon (68).

Controversial result have been reported regarding the role of *H. pylori* in CRC. Zumkeller et al., in their meta-analysis study, reported a 1.4 time increased risk of CRC in patients with a *H. pylori* infection around the world (39). Guo *et al.*, in a meta-analysis study of 7679 Asian patients, recommended a carcinogenic role of *H. pylori* at a primary phase of carcinogenesis (69). Bacterial cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA) are encoded in some *H. pylori* strains and induce the activation of inflammation pathways (70). There is also another hypothesis that direct and indirect production of RONS by some strains could participate in tumorigenesis in the colon (71).

While *E. coli* is a commensal microbe of the human gut, several surveys have verified a strong association between mucosa-adherent *E. coli* and CRC (42-44). In 2004 Martin et al. reported that more than 70% of mucosa samples of CRC patients were inhabited by *E. coli* (42). Majority of *E. coli* isolated from CRC patients harbors the *pks* genomic island that is responsible for the synthesis of colibactin. Colibactin is another bacterial-derived genotoxin that can interference with the cell cycle and promote proliferation of epithelial cells via DNA damage, mutation and genomic instability, subsequently and, tumor growth (44).

Colon cancer and inflammatory pathways

As mentioned, increased gut microbiota release inflammatory agents via the inflammatory pathway and therefore promote the change of normal cells to cancerous cells. On the other hand, intestinal inflammation as observed in inflammatory bowel disease (IBD) is a risk factor for the development of CRC (72). Increasing evidence suggests that inflammation-associated pathways also contribute to CRC development in the absence of clinically overt intestinal inflammation. Thus, signaling pathways with central roles in myeloid and lymphoid cells, such as those associated with signal transducer and activator of transcription 3 (STAT3) and nuclear factor (NF)- κ B, are also active in the transformed intestinal epithelium and promote tumor development (73, 74).

Table 1. Summary of increased gut microbiota variations in fecal sample of colorectal cancer patients

Reference	Sample type	Increased bacteria	Method
Sinha et al. 2016(51)	lyophilized feces	<i>Fusobacterium and Porphyromonas</i>	16S rRNA gene pyrosequencing
Flemer et al. 2016(52)	Fecal and mucosal samples	<i>Bacteroidetes and Prevotella</i>	16S rRNA amplicon sequencing
Wong et al. 2016(53)	Fecal samples	<i>F. nucleatum</i>	qPCR
Liang et al. 2016(54)	Fecal samples	<i>F. nucleatum</i>	qPCR
Suehiro et al. 2016(55)	Fecal samples	<i>F. nucleatum</i>	droplet digital PCR
Kasai et al. 2016(58)	Fecal samples	<i>Actinomyces odontolyticus, Bacteroides fragiles, Clostridium nexile, Fusobacterium varium, Haemophilus parainfluenzae, Prevotella stercorea, Streptococcus gordonii, and Veillonella dispar</i>	T-RFLP and NGS
Fukugaiti et al. 2015(45)	Fecal samples	<i>F. nucleatum and C. difficile</i>	qRT-PCR
Chen et al. 2013(68)	feces	<i>Enterococcus and Streptococcus</i>	Pyrosequencing based on 16S ribosomal RNA.
Wu et al. 2013 (69)	Feces	<i>enriched Bacteroides, overabundance of Fusobacterium and Campylobacter</i>	pyrosequencing of the 16S rRNA gene V3 region
Sobhani et al. 2011 (23)	Fecal samples	<i>Bacteroides/Prevotella</i>	Pyrosequencing and qRT-PCR
Ahn et al. 2013 (70)	Feces	<i>Atopobium/Porphyromonas and Fusobacterium</i>	Pyrosequencing and Real-time PCR
Balamurugan et al. 2008 (34)	Feces	<i>Enterococcus Faecalis</i>	Real-time PCR

F. nucleatum: *Fusobacterium nucleatum*; *E.faecalis*: *Enterococcus faecalis*; ETBF: *Enterotoxigenic Bacteroides fragilis*; qPCR: quantitative polymerase chain reaction; qRT-PCR: quantitative Real-time PCR; FQ-PCR: Fluorescent quantitative polymerase chain reaction; T-RFLP: terminal restriction fragment length polymorphism; NGS: next-generation sequencing

A study by Wang et al. has recently shown a critical role of the microbiota, and its TLR-dependent recognition in intestinal tumor progress in human and rodents (75). Intestinal microbiota are also known to be involved in the initiation and development of colorectal cancer, which is a risk factor for inflammatory bowel disease.

The investigations confirmed profound modifications in the gut microbiota before or during the progression of colorectal cancer (9). Intestinal microbiota-dependent nutritional or lifestyle intermediation beside colorectal carcinoma deserve additional research. The result of different studies advocate that fecal microbiome-based approaches might be valuable for prompt diagnosis and treatment of colorectal cancer.

Conclusion

According to the presented studies, more prevalent gut microbiota variations in the fecal and biopsy samples of CRC patients were *Fusobacterium*, *Porphyromonas*, *Bacteroidetes* and *Prevotella*. (Table

1, 2). However, it seems that there is no difference between bacteria variation in developed and developing countries. Therefore, the strong association between the gut microbiota and CRC is evident, but several questions remain unanswered. As previously declared, the gut microbiota acts as a key role in the development of CRC through numerous mechanisms, comprising genotoxin, metabolism and inflammation. Thus, studies have provided supportive data that modifications in gut microbiota structure could induce a host immune response and plays a critical part in intestinal epigenic mechanisms of the host.

The studies that are discussed in this review did not highlight the classification of tumors according to their molecular phenotype and it is not clear why some adenomas growth to malignancy, while others are stable or even regress. According to investigations, a greater abundance of *Fusobacterium* was detected in cancer tissues than in normal tissues. Thus, the increased abundance of *Fusobacterium* could be linked with high risk of CRC.

Therefore, we recommended future studies should consider the heterogeneity of CRC tumors by focusing

Table 2. Summary of increased gut microbiota variations in biopsy sample of colorectal cancer patients

Reference	Sample type	Increased bacteria	Method
Mima et al. 2016(49)	Tumor tissue samples	<i>F. nucleatum</i>	quantitative PCR assay
Wei et al. 2016(50)	Tumor tissue samples	<i>F. nucleatum</i> and <i>Bacteroides fragilis</i>	16S rRNA gene pyrosequencing
Li et al. 2016(56)	Tumor tissue samples	<i>F. nucleatum</i>	FQ-PCR
Zhou et al. 2016(57)	Tumor tissue samples	<i>Fusobacterium</i> spp., <i>E.faecalis</i> , <i>ETBF</i>	Real-time PCR
Burns et al. 2015(46)	Tumor tissue samples	<i>Fusobacterium</i> and <i>Providencia</i>	qPCR and 16S rRNA gene pyrosequencing
Gao et al. 2015(47)	Tumor tissue samples	<i>Firmicutes</i> and <i>Fusobacteria</i>	16S rRNA gene pyrosequencing
Mira-Pascual et al. 2015(48)	mucosal and fecal samples	<i>F. nucleatum</i> and <i>Enterobacteriaceae</i>	qPCR and 16S ribosomal RNA gene pyrosequencing
Viljoen et al. 2015(75)	CRC tissues	<i>fusobacterium</i> spp., <i>enterotoxigenic Bacteroides fragilis (ETBF)</i>	Real-time PCR
Tahara et al. 2014 (76)	CRC tissues	<i>F. nucleatum</i> and <i>Pan-fusobacterium</i>	Real-time PCR
Geng et al. 2013(77)	Tumor/matching normal tissue of Chinese CRC patients	<i>Fusobacterium</i> spp., <i>Roseburia</i>	pyrosequencing-based molecular monitoring of bacterial 16S rRNA gene
Warren et al. 2013(78)	CRC/matching normal tissues	<i>Fusobacterium</i> , <i>Leptotrichia</i> and <i>Campylobacter</i>	16S rRNA gene pyrosequencing
Castellarin et al. 2012(14)	Tumor/matching normal tissues	<i>Fusobacterium nucleatum</i>	RNA sequencing
Kostic et al. 2012 (65)	Tumor/matching normal tissues	<i>Fusobacterium</i>	whole genome sequences and confirmed by quantitative PCR and 16S rDNA sequence
Marchesi et al. 2011 (20)	Tumor/matching normal tissues	<i>Fusobacterium</i>	rRNA sequencing

on microbiota imbalances in relation to molecular pathways involved in colorectal carcinogenesis. Also performing such studies may be useful to explore links between pathological features of adenomas and type of cytotoxic microbiota. On the other hand, development of research techniques are expected to provide important evidence concerning healthy and dysbiotic microbiota conformation. In conclusion, the role of the gut microbiota in the pathogenesis of CRC is clear and perhaps represents new techniques for better therapeutic management of patients with CRC.

Conflict of interests

The authors declare that they have no conflict of interest.

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