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Review Article

Most Frequent Bacteria in Colorectal Cancer Patients and Their Roles in Colorectal Carcinogenesis

Samad Farashi Bonab & Nemat Khansari*

Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

***Correspondence to:** Dr. Nemat Khansari, Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.

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Abstract

Colorectal cancer is the third most common cancer worldwide. The intestinal mucosal surface is continuously exposed to enormous microorganisms. The human intestinal tract harbors approximately 10^{14} bacteria. Most of these bacteria have roles in intestinal homeostasis and may exert anticancer effects. However, bacterial metabolites that are genotoxic can trigger epithelial cell carcinogenesis. Furthermore, a variety of inflammatory mediators produced during bacterial infection can also promote cancer development and progression. Some bacterial species are associated with human colorectal cancer. These bacteria can be involved in colorectal cancer development and progression through induction of chronic inflammation, DNA damage, aberrant cell proliferation, and immunosuppression in the intestinal tissue. In this paper, we discuss most frequent bacteria in colorectal cancer patients and their roles in colorectal carcinogenesis.

Abbreviations

NK: Natural Killer; NKT: Natural Killer T; IL: Interleukin; CD: Cluster of Differentiation; TH: T Helper: TLR4: Toll Like Receptor-4; Cox: Cyclooxygenase; PGE: Prostaglandin E; EGFR: Epidermal Growth Factor Receptor; STAT3: Signal Transducer and Activator of Transcription-3; APC: Adenomatous Poly

posis Coli; TNF-α: Tumor Necrosis Factor-Alpha; CCL20: C-C Motif Chemokine Ligand 20; NF-κB: Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B cells; FOXP3: Forkhead Box P3; IFN-γ: Interferon-Gamma; MAP: Mitogen-Activated Protein.

Introduction

Colorectal cancer is the third most common cancer worldwide [1] and prevention of colorectal cancer is among the most urgent needs regarding public health. Bacteria numbers in the colon are about one million-fold higher than that in the small intestinal, and the frequency of cancers in the colon is higher than that in the small intestinal (approximately 12-fold) [2]. Elucidation of mechanisms by which pro-carcinogenic bacteria initiate and/or promote colorectal cancer could stimulate the development of novel colorectal cancer prevention and therapy approaches.

The human microbiota are communities of commensal, mutualistic and pathogenic microorganisms that reside on or within human tissues and biofluids. These microorganisms consists of 10-100 trillion microorganisms, primarily bacteria in the gut [3]. The gastrointestinal tract of a normal fetus is sterile. During and after the birth, microorganisms from the environment orally enter the mouth and finally colonize the gastrointestinal tract [4]. The intestinal microbiota, composed of bacteria, archaea, viruses, and fungi, plays an important role in intestinal mucosal homeostasis [5]. Approximately 1014 bacteria, which can be classified into at least 1000 species, live in human colon and make up the microbiota [6]. These microorganisms are important to human health, with the exception of defined pathogens as their colonization is associated with disease states [7]. Most of bacterial species in the gastrointestinal tract have beneficial effects on host metabolism as well as the maintenance of intestinal homeostasis [8].

Microbial pathogens are estimated to be the causative agents for about 20% of cancers [9]. In the gastrointestinal tract, the presence of specific bacteria may promote the initiation or progress of gastrointestinal tract cancers. These bacteria can increase cancer susceptibility or encourage its progression through establishing chronic inflammation, induction of DNA damage, and production of metabolites involved in tumorigenesis. In the past decade, several studies on microbiota composition have suggested that intestinal microbiota dysbiosis is associated with colorectal cancer development. Specific bacteria, such as *Fusobacterium nuleatum*, *Bacteroides fagilis,* and *Escherichia coli,* have been correlated to colorectal cancer development or progress, whereas others can be protective.

Evidences Showing Bacteria as Colorectal Cancer Causative Agents

In 1974, Reddy and colleagues for the first time reported that germ-free (without intestinal microbiota) rats display lower incidence of chemically (1,2-dimethylhydrazine) induced duodenal and colonic tumors compared to those reared in conventional conditions [10], suggesting a possible role of intestinal microbiota in development of intestinal cancers. Germ-free rats injected intrarectally with a potent mutagenic substance (N-methyl-N'-nitro-N-nitrosoguanidine) developed doubled adenomas compared to conventional rats. However, germ-free status had no effect on the incidence of adenocarcinomas [11]. Later investigations in murine tumor models also indicated reduced incidence of tumors derived under germ-free conditions [12,13]. Induction of colorectal tumors in rats reared in germ-free conditions resulted in development of

less and smaller intestinal tumors compared to rats reared conventional conditions. Furthermore, germ-free reared rats that did not developed cancer showed an increase in the number NK, NKT, T, and B cells and T cell cytotoxicity in the peripheral blood [14].

Azoxymethane-exposed, conventionalized-IL-10 knock-out ($il10^{-/-}$) mice showed high susceptibility to colon tumor development compared with azoxymethane-exposed, conventionalized-wild-type mice. Spontaneous colitis was developed in azoxymethane-exposed conventionalized-$il10^{-/-}$ mice which was directly correlated with colorectal cancer growth. Importantly, germ-free azoxymethane-exposed $il10^{-/-}$ mice displayed no colitis and were devoid of tumors, indicating that modulation of host intestinal microbiota alerts the susceptibility to colitis-associated colorectal cancer [15]. Aberrant cytokine production and CD4+ TH1 responses was associated with the enterocolitis induced in $il10^{-/-}$ mice [16]. Overexpression of TLR4 has been detected in human and murine inflammation-associated colorectal neoplasia. TLR activation promotes Cox-2 expression, PGE2 production, and EGFR signaling in chronic colitis, indicating a critical role for TLR4 in development of colitis-associated colorectal tumors [17]. Further investigation in a murine model of colorectal cancer showed that lipopolysaccharide, a cell wall component of gram negative bacteria, accelerates tumor growth via c-jun and STAT3 phosphorylation [18].

In a recent study, more proportion of conventional mice fed with stool from patients with colorectal cancer developed carcinogen (azoxymethane) induced high-grade dysplasia and macroscopic polyps than those fed with stool from healthy individuals. An increased epithelial cell proliferation in the colons were observed in germ-free mice fed with stool from patients with colorectal cancer compared with mice fed with stool from healthy individuals. Increased expression of cytokines IL-17A, IL-22, and IL-23A, C-X-C motif chemokine receptor 1 (Cxcr1), and C-X-C motif chemokine receptor 2 (Cxcr2), as well as increased infiltration of TH1 and TH17 cells in the colon were detected in intestines from conventional and germ-free mice fed with stool from patients with colorectal cancer compared with conventional and germ-free mice fed with stool from healthy individuals. Upregulation of genes involved in the cell proliferation, stemness, survival (anti-apoptosis), angiogenesis, invasiveness, and metastases were also detected in mice fed with stool from patients with colorectal cancer [19].

Most Frequent Bacteria in Patients With Colorectal Cancer and Their Associations With Colorectal Cancer

Increased proportions of *Bacteroides fragilis*, and certain *Fusobacterium, Streptococcus*, and *Peptostreptococcus* species have been reported in the intestinal microbiota of colorectal cancer patients [20,21]. Co-occurrence of gram negative anaerobic bacteria *Fusobacterium, Leptotrichia,* and *Campylobacter* species were also detected in colorectal cancer tissues [22]. In a cohort study, bacterial quantitative analysis of the intestinal microbiota of colorectal cancer patients and healthy individuals showed that *Fusobacterium nucleatum* and *Clostridium difficile* in fecal samples were significantly more in colorectal cancer patients compared to healthy controls [23]. *Clostrium septicum* has been rarely isolated from colorectal cancer patients. Isolation of *Clostrium septicum* from two patients with perforated cecal tumor or perforated rectal tumor [24], and other cases of colorectal tumor [25,26] suggests the possibility of sepsis occurrence as a fatal infection in colorectal cancer patients. In another cohort study, quantification of bacteria, including *Fusobacterium* species,

Streptoccus gallolyticus (formerly known as Streptococcus bovis biotype I), *Enterococcus faecalis,* enterotoxigenic *Bacteroides fragilis*, entheropathogenic *Escherichia coli,* and afaC- or *pks*-positve *Escherichia coli*, in paired tumor and normal tissue samples from colorectal cancer patients showed that with the exception of *Streptoccus gallolyticus,* all these bacteria were detected in both tumor and normal samples. The levels of enterotoxigenic Bacteroides fragilis and *Fusobacterium* species were significantly higher in late stage colorectal cancers. *Fusobacterium* was the only bacterium that was significantly detected at higher levels in tumor compared with normal samples. There was also a significant association between high level colonization of *Fusobacterium* [27]. Investigation of possible association between bacteremia and subsequent diagnosis of colorectal cancer in 13,096 adult patients with bacteremia without a previous diagnosis of cancer showed that the risk of colorectal cancer is increased in patients with bacteremia from *Bacteroides fragilis, Streptococcus gallolyticus, Fusobacterium nucleatum, Peptostreptococcus species, Clostridium septicum, Clostridium perfringens*, or *Gemella morbillorum* compared with the unexposed group. There was no increased risk of colorectal cancer in patients with bacteremia caused by bacteria not previously associated with colorectal cancer [28].

Evidences Showing Roles of Colorectal Cancer-Associated Bacteria in Colorectal Tumorigenesis

Fusobacerium nucleatum. *Fusobacerium nucleatum*, an obligate anaerobic gram negative bacterium, is a common resident of the human oral cavity and gut [29,30]. *Fusobacterium* species were found to be enriched in human colonic adenomas relative to surrounding tissues as well as in stool samples from patients with adenomas and colorectal carcinomas compared to healthy subjects [20,31]. The presence of *Fusobacterium* nucleatum in a noticeable proportion of patients with colorectal cancer suggests a potential role of *Fusobacerium* in colorectal cancer. Higher abundance of *Fusobacterium nucleatum* in colorectal cancer tissues, than that in adjacent normal tissues, was associated with lymph node metastasis [32].

In the $Apc^{Min/+}$ mouse model of intestinal tumors, introduction of Fusobacterium nucleatum to mice increased tumor multiplicity and infiltration of myeloid cells into tumors. However, Fusobacterium nucleatum did not induce colitis, enteritis or inflammation-associated intestinal carcinogenesis, in contrast with entrotoxigenic Bacteroides fragilis which induced colitis and accelerated tumorigenesis in $Apc^{Min/+}$ mice. These finding indicates that Fusobacterium nucleatum may accelerate tumorigenesis in the absence of enteritis in $Apc^{Min/+}$ mice. In addition, increased bacterial tumor load was not observed in $Apc^{Min/+}$ mice fed Fusobacterium nucleatum [31]. Expression of several genes, such as IL-1β, IL-6, IL-8, TNF-α, and COX-2, is induced by Fusobacterium nucleatum in human and mouse colonic cancer cell lines *in vitro* [21,33]. Fusobacterium nucleatum can also induce upregulation of CCL20 protein expression in colorectal cancer cells and monocytes, and stimulate monocytes activation and migration *in vitro* [34]. Fusobacterium nucleatum also promoted colorectal carcinogenesis by modulating the E-cadherin and β-catenin signaling pathways and subsequent activation of downstream proinflammatory responses, including activation of expression of NF-κB and cytokines IL-6, IL-8, and IL-18 [35]. Furthermore, a greater amount of Fusobacterium nucleatum in colorectal carcinoma tissues was associated with a lower density of T cells in tumor tissues of colorectal cancer patients. The amount of Fusobacterium nucleatum was not significantly associated with the density of $CD8^+$, $CD45RO^+$, or $FOXP3^+$ T cells [36]. More recently, Fusobacterium nucleatum has been shown to potentiate intestinal tumorigenesis in $Apc^{Min/+}$ mice via a TLR4/phosphorylated p21-activated kinase 1/phosphorylated β-catenin S675 cascade [37].

Bacteroides fragilis. Colonization of Enterotoxigenic *Bacteroides fragilis,* a human colon commensal bacterium, is detected in about 50% of healthy individuals and about 90% of colorectal cancer patients [38]. Both enterotoxigenic *Bacteroides fragilis* and nontoxigenic *Bacteroides fragilis* can chronically colonize mice, but only enterotoxigenic *Bacteroides fragilis* triggered colitis and strongly induced colonic tumors in multiple intestinal neoplasia (Min) mice. Further investigation demonstrated that enterotoxigenic *Bacteroides fragilis* promotes colon tumorigenesis via activation of STAT3 and TH17 response [39].

Bacteroides fragilis toxin is a zinc-dependent metalloprotease that targets epithelial tight junctions via γ-secretase-dependent signal transduction [40]. Recently, it was shown that this toxin coordinates a pro-requiring IL-17 receptor, NF-κB, and Stat3 signaling in colonic epithelial cells of ApcMin mice. Furthermore, IL-17-dependent NF-κB activation in colonic epithelial cells mediated the recruitment of polymorphonuclear immature myeloid cells with parallel initiation of enterotoxigenic Bacteroides fragilis-induced colon tumorigenesis [41].

Escherichia coli. Increased adherence of *Escherichia coli,* a facultative anaerobic gram negative bacterium, to the intestinal mucosa and invasion has been reported to be associated with Crohn's disease and colon cancer [42]. Increased levels of mucosa-associated or internalized *Escherichia coli* were found in tumor tissue of patients with colon cancer compared with normal mucosa tissue. Pathogenic cyclomodulin-positive *Escherichia coli* strains were more prevalent on mucosa of patients with stages III/IV than those with stage I colon cancer [43]. A high prevalence of cycomodulin-producing *Escherichia coli* was detected in biopsies of patients with colorectal cancers than in those of patients with diverticulosis [44]. *Escherichia coli* strains possessing a gene cluster named the *pks* island were reported to be more prevalent in colon tissue specimens derived from colorectal cancer patients compared to those from controls, indicating that they might have a causative role in the development of human colorectal cancer [43-46]. However, in a recent study, no significant differences in the relative concentrations of *pks*-positive bacterial DNA was found in colonic samples from colorectal cancer, adenoma, and control patients [47]. *pks*-positive *Escherichia coli*, but not *pks*-negative *Escherichia coli,* induces DNA double-strand breaks and triggers genomic instability in mammalian cells [48,49]. The *pks* genomic island is responsible for the synthesis of colibactin, a genotoxic compound. Transient contact of malignant cells with colibactin-producing *Escherichia coli* increased tumor growth in a xenograft mouse model. Furthermore, colibactin has been shown to promote colon tumor growth by modifying the tumor microenvironment [50].

Inoculation of a colon cancer-associated Escherichia strain (11G5) to multiple intestinal neoplasia (Min) mice resulted in a marked decrease in the number of visible colonic polyps, when compared to control mice [43]. Escherichia coli strains isolated from colonic mucosa of patients with colon cancer were able to highly persist in the gut, and to induce colon inflammation, epithelial damage, and cell proliferation in transgenic mice expressing carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6) [51]. Both the commensal murine adherent-invasive *Escherichia coli* (NC101) and the human commensal Enterococcus faecalis (OG1RF) caused aggressive colitis in germ-free azoxymethane-treated il10$^{-/-}$ mice. Mono-colonization with Escherichia coli resulted in development of invasive colon carcinoma in 80% of mice, while mono-colonization with Enterococcus faecalis rarely resulted in colon tumor development. The levels of expression of colonic cytokine genes associated with inflammation and carcinogenesis, including IL-

6, TNF-α, IFN-γ, IL-1β, IL-8, IL-17 and IL-23, as well as infiltrating CD3+ T cells, F4/80+ macrophages, and Ly6B.2+ monocytes and neutrophils were similar between Escherichia coli and Enterococcus faecalis infected mice [45].

Streptococcus bovis/Streptococcus gallolyticus. *Streptococcus bovis*, a gram positive bacterium, occasionally present in the human gastrointestinal tract flora [52]. The association of *Streptococcus bovis* bacteremia with colon tumors has been reported in the late 1970s [53]. A large proportion of patients with *Streptococcus bovis* biotype 1 (*Streptococcus gallolyticus* subsp. *gallolyticus*) were found to have colorectal neoplasm [54-56]. A meta-analysis study showed a significant association between *Streptococcus bovis* endocarditis or *Streptococcus bovis* septicemia and the occurrence of colorectal neoplasia in patients. Furthermore, patients with colorectal neoplasia showed a higher incidence of *Streptococcus bovis* in feces upon stool culture [57]. In addition, endocarditis and bacteremia caused by *Streptococcus gallolyticus* infection have been reported to be associated with increased risk of colorectal cancer [58-60].

Streptococcus bovis II/1 (*Streptococcus infantarious*) proteins were able to activate MAP kinases and to release IL-8 and PGE2, correlated with overexpression of COX-2, by human colon cancer cells *in vitro*. These proteins were effective in the promotion of pro-neoplastic lesions in azoxymethane-treated rats [61]. *Streptococcus gallolyticus* subsp. *gallolyticus* colonization was 1,000-fold higher in tumor-bearing mice than that in normal mice and colonization by *Streptococcus gallolyticus* subsp. *gallolyticus* was strongly associated with the occurrence of colorectal cancer [62]. Administration of *Streptococcus gallolyticus* subsp. *gallolyticus* to mice resulted in more tumors, higher tumor burden and dysplasia grade, and increased cell proliferation and β-catenin staining in colonic crypts compared to mice receiving control bacteria [63]. In azoxymethane and dextran sodium sulphate-induced colitis associated colorectal cancer in mice, pretreatment of *Streptococcus gallolyticus* provoked tumor formation and selectively recruits tumor-infiltrating myeloid cells, including marrow-derived suppressor cells, tumor-associated macrophages and dendritic cells [64]. Higher levels of serum *Streptococcus gallolyticus* IgG antibodies, in comparison with control subjects, were observed in colorectal cancer and adenoma patients while no similar association was found with serum IgG antibodies of *Bacteroides fragilis* [65]. A higher expression of NF-κB and increased mRNA levels of inflammatory molecules IL-1, COX-2, and IL-8 were detected in colorectal tissues of *Streptococcus gallolyticus* positive colorectal cancer patients than that in control or *Streptococcus gallolyticus* negative colorectal cancer patients [58,65].

In summary, these findings show that some specific bacteria are associated with colorectal cancer. Bacterial-activated inflammatory and immune responses can be involved in colorectal tumorigenesis and tumor progression. Prevention of chronic infections with colorectal cancer-associated bacteria is required to decrease the development of cancer.

Conclusion

Numerous evidences indicate that some strains of specific bacteria, such as *Fusobacerium nucleatum, Bacteroides fragilis, Escherichia coli*, and S*treptococcus bovis/ gallolyticus*, are involved in colorectal carcinogenesis through induction of inflammatory mediators and immune responses-dependent mechanisms. Immune cells and other cells during inflammation secrete cytokines, chemokines and other mediators that modulate the

growth, differentiation, and migration of many cell types. Some of these mediators can promote colon cancer cell development, proliferation and/or survival. Identification of bacterial-induced molecular mechanisms involved in the tumor cell development, proliferation, survival, and invasiveness is of great importance and may result in development of new therapeutic strategies.

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