The Gastrointestinal Microbiota and Colorectal Cancer

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Abstract

The human gut is home to a complex and diverse microbiota that contributes to the overall homeostasis of the host. Increasingly, the intestinal microbiota is recognized as an important player in human illness such as colorectal cancer (CRC), inflammatory bowel diseases, and obesity. CRC in itself is one of the major causes of cancer mortality in the Western World. The mechanisms by which bacteria contribute to CRC are complex and not fully understood, but increasing evidence suggests a link between the intestinal microbiota and CRC as well as diet and inflammation which are believed to play a role in carcinogenesis. It is thought that the gut microbiota interact with dietary factors to promote chronic inflammation and CRC through direct influence on host cell physiology, cellular homeostasis, energy regulation, and/or metabolism of xenobiotics. This review provides an overview on the role of commensal gut microbiota in the development of human colorectal cancer and explores its association with diet and inflammation.

Keywords: Gastrointestinal microbiota, Colorectal adenoma, Colorectal cancer, Diet, Inflammation, Bacterial Metabolites

Abbreviations:

CRC: Colorectal cancer
IBD: Inflammatory bowel diseases
IBS: Irritable bowel syndrome
GIT: Gastrointestinal tract
TGF-β: Transforming growth factor-β
ETBF: Enterotoxigenic Bacteroides fragilis
BFT: B. fragilis toxin
PRRs: Pattern recognition receptors

PAMPs: Pathogen-associated molecular patterns

TLRs: Toll-like receptors

NLRs: Nod-like receptors

NF-κB: Nuclear factor κB

DMH: Dimethylhydrazine

STAT3: Signal transducer and activator of transcription

DCA: Deoxycholic acid

LCA: Lithocholic acid

PGE2: Prostaglandin E2

NOC: Nitroso compounds

IFN-γ: Interferon-γ

TNF-α: Tumor necrosis factor-α

CFU: Colony-forming units

SCFAs: Short chain fatty acids

BCFAs: Branched chain fatty acids

ROS: Reactive oxygen species

RNOS: Reactive nitric oxide species

H2S: Hydrogen sulfide

TNF: Tumor necrosis factor
Colorectal cancer

Colorectal cancer (CRC) has the third highest cancer incidence in the world. In 2012 CRC accounted for approximately 1,361,000 new cases (9.7% of total cancer incidence) and approximately 694,000 deaths (8.5% of total cancer deaths) globally (45). In the United States, CRC is the second leading cause of cancer mortality and the third most commonly diagnosed cancer. It is estimated to account for roughly 50,310 deaths and about 136,830 new cases in 2014 (142).

Despite extensive research, the precise etiology for colorectal cancer is still unknown, but genetic, epigenetic, and environmental factors such as diet have been implicated. CRC arises as part of a multi-step process associated with the accumulation of a series of genetic and epigenetic alterations (49) that influence fitness and clonal expansion of altered cells in the transformation of normal colonic epithelium to adenomas and cancer.

The first model depicting key genetic alterations in CRC included the activation of K-ras oncogene, (44, 84, 163) and the inactivation of tumor suppressor genes such as adenomatous polyposis coli (APC) (104, 133) and p53 (encoded by TP53 gene in human) (164, 169).

Following this initial model, a number of acquired genetic mutations including PIK3CA, FBXW7, SMAD4, TCF7L2, NRAS, FAM123B, CTNNB1, SMAD2, alterations in pathways of chromosomal and microsatellite instability (MSI), mismatch repair (MMR) (23, 25), and epigenetic CpG island methylation (CIMP) (2, 128), have also been observed to contribute to colorectal carcinogenesis.

In addition to genetic factors, an environmental component to CRC is also strongly implicated. Studies of immigrants show that cancer rates for migrants quickly match those of their current country of residence, even when they originated from a country with a lower baseline cancer rate (46, 102). Since genes do not change over such a short period, diet, a
component that changes with migration, is thought to be an important contributor to colorectal cancer development. However, studies of dietary elements such as fiber and colorectal cancer to date are largely inconsistent with some studies reporting protective effects of fiber and others showing no effects (6, 118). This is likely due an overlooked component of the large bowel, the gut microbiome. There is mounting evidence that the intestinal gut bacteria (microbiota) play an important role in colorectal carcinogenesis (37, 69, 126, 157, 174).

**Intestinal microbiota**

The human large bowel is home to complex and diverse communities of microbiota, which play important roles in health and disease. Several studies suggest that the depending on the route of delivery, the human gastrointestinal tract (GIT) is colonized at birth, at least with limited microbes (73, 134) which gradually become diverse to reach more than $10^{14}$ microbes comprising 1000 or more heterogeneous species of bacteria, viruses, archaea, and fungi (135). It is estimated that the number of bacterial cells of the human gut exceeds the number of human cells by 10-fold (48). The collective bacterial genome referred to as the gut microbiota, harbors approximately 150-fold more genes than the human genome (120, 171).

Within the large bowel, bacteria are present in two compartments: a luminal compartment and a mucosal adherent compartment. The microbiota in the luminal compartment, while reflective of the bacterial communities in the colonic lumen, may not reflect the composition and localization of epithelial and cryptal communities (135). The colonic mucosa harbors dense cohesive communities of bacteria that adhere to surface-associated polysaccharide matrices and resist hydrodynamic shear forces (146). Some of these bacteria establish themselves as entrenched residents of the mucosa (146) despite the rapid turnover and propulsion of debris and water through the gut. These adherent resident bacteria interact
with the mucosal immune system and as such may be more relevant to diseases such as inflammatory bowel diseases and colorectal cancer.

Although the gut bacteria have long been considered commensal residents, it is now recognized that they serve diverse and important functions (18) some of which may contribute to colorectal cancer etiology. The intestinal bacteria are essential in digestion and absorption of indigestible carbohydrates (fibers), production of vitamins B and K, metabolism of endogenous and exogenous compounds, immune potentiation, are actively involved in innate and cell mediated immunity, help to maintain intestinal barrier function and assist with an appropriate immune response against pathogenic microbes (9, 74). Their symbiotic relationship (“normobiosis”) with the host is critical to maintaining a balance (homeostasis) in the gut. A shift in this balance (“dysbiosis”) under abnormal conditions can lead to detrimental consequences for the host. For instance, dysbiosis of the normal microbiota is associated with overgrowth of opportunistic pathogens that are normally inhibited by commensal bacteria (12). Moreover, microbial dysbiosis has been observed in Inflammatory Bowel Diseases (IBD) (32, 61, 80, 152), obesity (11, 20, 140, 154, 159), colorectal adenomas and cancer (26, 37, 92, 99, 109, 116, 130, 138, 144, 145, 171). One cause of dysbiosis may be antibiotic treatment, which has been linked to development of Clostridium difficile colitis (59, 64, 139, 170).

The composition of intestinal microbiota is determined by various factors including host genetics (159), environment (16, 21), diet (26, 43, 103, 160, 176) and hygiene (7). Recent findings demonstrated that paneth cell defensins, innate antimicrobial peptides that contribute to mucosal host defense, can regulate the composition of the intestinal bacteria (129). Although host genetics may have a significant impact on the microbiota composition, discovery of specific genetic factors that interact with the gut microbiota is in the beginning stages. α1,2-Fucosyltransferases (FUT2) secretor genotype is correlated with the abundance of
bifidobacteria in the colon. The α1,2-fucosyltransferases (FUT2) gene is responsible for the histo-blood group antigens and polymorphisms in this gene have been associated with IBD and CRC (56, 76).

**Intestinal microbiota and colorectal cancer**

Several human studies have demonstrated a link between the gut bacteria and CRC. One of the earliest studies to correlate gut bacteria with colorectal neoplasm was conducted by Moore *et al.* (101) who assessed fecal samples from polyp patients using culture methods. They observed that the abundance of *Bacteroides* and bifidobacteria were associated with increased risk of colon polyps while *Lactobacillus* and *Eubacterium aerofaciens* were protective. Early studies by Swidsinski *et al.* (150) also reported an association between the abundance of *E. coli* and colorectal adenomas and cancer. O'Keefe *et al.* (113) observed that high abundance of hydrogen sulfide and bile salt producing bacteria were associated with increased risk of colon cancer. The majority of bacteria are not culturable, therefore these early studies evaluated only bacteria that are culturable. However, the advances in molecular biology and sequencing technology, has revolutionized the microbiome field such that it is now possible to characterize bacteria without culturing. Human studies using high throughput molecular sequencing methods reveal changes in fecal microbiota composition in colorectal cancer subjects compared to healthy controls (145, 166). Although the ease of collection facilitates the evaluation of fecal samples, the luminal contents may not accurately represent the adherent microbiome as they contain many transient organisms. Several studies have used high throughput molecular 16S based methods to examine either the fecal or mucosal microbiome in relation to colorectal adenomas and cancer. Compared to control subjects without adenomas case subjects with, adenomas had significantly increased diversity and richness of bacterial species (130, 141). Chen *et al.* (26) observed reduced abundance of *Clostridium, Roseburia, Eubacteria* spp. and other butyrate-producing bacteria in fecal samples of adenoma subjects.
compared to healthy controls. Marchesi et al. (92) profiled the microbiota in colon tumors and matching normal colon tissues and observed very different microbial patterns and signatures between the two sites. Specifically, they observed an overabundance of *Fusobacterium* on the tumor compared to matching normal tissue. Sobhani et al. (145) observed that altered fecal bacterial profile was linked with elevated IL-17 in CRC patients compared to healthy controls. A summary of the findings from human studies on gut bacteria and colorectal cancer is presented in Table 1. Together, these studies suggest that alterations that favor increased abundance of potentially pathogenic bacteria and reduction of beneficial bacteria are associated with colorectal adenomas and cancer. Although bacterial dysbiosis is associated with CRC, there is limited information on the contribution of specific bacteria. This is currently an area of intense investigation.

In order to fully understand the role of gut bacteria in CRC, mechanistic studies in animal models are critical. Vital studies of genetically engineered rodents raised in germ free environments support the role of bacteria in CRC. For example, IL-10 or TGFB /Rag2 knockout mice maintained under germ-free conditions do not develop tumors and have significantly lower levels of inflammation (29, 41, 137, 172). Findings from studies in murine models of chemically induced of colon cancer indicate that intestinal bacteria promote colon carcinogenesis by increasing proliferation and formation of aberrant crypt foci (40, 115). Horie et al. (65) found that the incidence of tumors in germ free mice monoassociated with bacteria ranged from 30-68% depending on the colonizing bacteria and thus supports the notion that the intestinal microflora has potent effects on cancer development. Another study showed that infection with *Helicobacter* spp. promoted colon cancer in *SMAD3* deficient mice, suggesting that gut bacteria in combination with genetic alterations in the TGF-β pathway may contribute to colon carcinogenesis (90). More recent studies in animal models suggest that the bacterial dysbiosis phenotype is transferable. Transplantation of feces from tumor bearing mice to conventionalized
germ free mice resulted in an increased colon inflammation and tumorigenesis (175). Similarly, fecal transplants from human CRC patients into germ free mice resulted in increased tumor burden in mice (14). A summary of the findings from animal studies on gut bacteria and colorectal cancer is presented in Table 2. While these studies in animal models provide compelling evidence to support the contribution of intestinal microbiota to CRC, the relationship is likely to be more complex and may involve interactions between the gut bacteria, chronic inflammation, mutations in oncogenic pathways and diet to promote colorectal carcinogenesis (63, 69, 108).

Although the exact role of the microbiota and the mechanisms by which it promotes CRC is yet to be fully elucidated, potential mechanisms have been described:

a. Cell wall antigens and bacterial colicins — Klein et al., and Huycke et al. (67, 78) showed that *Streptococcus gallolyticus* (formerly *S. bovis*) antigens promote premalignant lesions through aberrant crypt formation. Colibactin produced by *E. coli* strains have been shown to induce DNA double strand breaks in intestinal cells and trigger chromosomal instability, gene mutations and cell transformation (3, 14, 26, 108). Cougnoux et al. (33) reported that colibactin-producing *E. coli* enhanced colon tumor growth in both xenograft and AOM/DSS IL-10−/− mouse models by inducing a senescence-associated secretory phenotype (3).

b. Inflammation—the long standing presence of infection with potential pathogenic bacteria can induce chronic inflammation. Chronic inflammation in the colorectal mucosa has been linked to CRC development (77, 79, 95, 161).

c. Production of toxic metabolic byproducts from dietary carbohydrates, protein, bile and mutagenic precursors - H₂S produced by bacteria in the gut are related to CRC etiology. H₂S and reactive oxygen radicals are toxic to the epithelium (5, 35, 50, 68, 112, 122).
Phenolic compounds such as amines, N-nitroso compounds from meat consumption can also be toxic to the host (91, 143).

This review will focus on inflammation and diet as potential mechanisms as these risk factors for colorectal cancer are well established in the literature.

Inflammation, intestinal microbiota, and colorectal cancer

The normal colon maintains a continuous state of low-grade inflammation in response to the presence of endogenous bacteria and dietary antigens. Under normal physiologic conditions, this low-grade inflammation is maintained by components of the innate and adaptive immune systems which contribute to the homeostasis between pro-inflammatory mediators (e.g. IL-1B, IFN-γ, IL-8, TNF-α, IL-23, IL-12 IL-17 and IL-6) (24, 100, 111), and anti-inflammatory mediators (e.g. IL-10 and TGF-β). The specific components of the innate immune system that regulate this state of homeostasis include monocytes, macrophages, dendritic cells, and natural killer cells, while the adaptive immune system lymphocytes (B cells, T cells, and helper cells) and regulatory cells serve a similar role. Disruption of such inflammatory mediator homeostasis may contribute to chronic inflammation and increased risk of colorectal adenomas and cancer (28, 97, 168).

There is evidence to suggest that chronic inflammation increases colon cancer risk (52, 106, 155). Evidence comes from studies in Inflammatory Bowel Disease (IBD) (34, 86) and colitis in animal models (17, 123). Studies in animal models support the importance of IL-10 in the suppression of chronic inflammation and CRC. In addition, anti-inflammatory agents such as aspirin and non-steroidal anti-inflammatory drugs have been repeatedly shown to decrease the risk of colorectal adenomas and cancer (10, 13, 123).

An important aspect to consider is the resident gut microbiota and their role in inflammation and cancer. Under normal conditions, commensal gut bacteria maintain a
symbiotic relationship with the mucosal immune system and are thought to be in a state of
tolerance. The presence of commensal bacteria is vital to the development of the gut immune
system. For instance, the immune systems of conventionalized animals are highly developed
compared to GF animals (17, 30, 57, 72). Disruption of the symbiotic relationship between the
intestinal microbiota and the host is thought to trigger chronic inflammation. For example, the
gut microbiota is a source of antigens for the inflammatory processes associated with IBD (18,
151) and the pathogenesis of enterocolitis and colon cancer in \textit{IL-10} knockout mice (114).
Recent studies have shown reduced inflammation in inflammatory bowel diseases (IBD) through
manipulation of the gut microbiota with probiotics (1, 15, 114).

Alterations in the ratio of ‘harmful’ and ‘beneficial’ commensal bacteria contributes to
increased mucosal permeability, bacterial translocation and increased activation of components
of the innate and adaptive immune system to promote chronic inflammation (71, 94, 132).
Activation of the innate immune system by commensal bacteria (Figure 1) leads to increased
production of proinflammatory cytokines such as IL-12, IL-23, TNF-\(\alpha\) and interferon gamma
(IFN\(\gamma\)) by macrophages, dendritic cells, and natural killer cells, with subsequent induction of the
acquired immune system that includes lymphocytes such as T cells, B cells, and various
inflammatory mediators. Bacteria may also be responsible in regulating Th17 cells and IL-17 in
the lamina propria (71). For example, the presence of segmented filamentous bacteria was
shown to promote the accumulation of Th17 cells and increase expression of inflammatory
genes in the intestinal mucosa (4, 70). A major consequence of the inflammatory response to
commensal bacteria is the activation of key pro-survival and pro-proliferative signaling pathways
by transcription factors such as NF-\(\kappa\)B and STAT3 in epithelial cells (51, 53, 62, 75, 87, 156) as
well as generation of reactive oxygen or nitrogen species that leads to oxidative stress, DNA
damage, aberrant proliferation and, ultimately development of colorectal adenomas and cancer
(Fig. 1). Colonization of germ free animals with \textit{Enterococcus faecalis} and \textit{Bacteroides vulgatus}

has been observed to induce NF-κB signaling (75, 127) in epithelial cells. Thus, studies to date suggest that disruption of the normal homeostasis between the host and its commensal bacteria community is crucial to inducing inflammation and further downstream changes that promote colon carcinogenesis.

**Intestinal microbiota and diet**

Diet has been extensively studied in relation to colorectal adenomas and cancer. Diet has a major impact on the composition and activity of intestinal bacteria (38, 119). As such, the link between diet and CRC may be explained, in part, by the activities of the intestinal microbiota. The principal components of dietary intake, carbohydrates, protein, and fat undergo bacterial digestion to generate byproducts, some of which have potential carcinogenic activity (Figure 2). Humans lack the enzymes to digest fiber but bacteria have glycoside hydrolases and polysaccharide lysases that can ferment plant cell wall polysaccharides (147). One of the major functions of bacteria in the colon is fermentation of non-digestible dietary fiber residues and carbohydrates such as resistant starches, pectins, gums, and cellulose. Fermentation leads to generation of short chain fatty acids (SCFA) such as butyrate, which serves as a source of energy for colonocytes (121) and demonstrate a protective effect against the development of CRC (22, 36, 124, 125, 148). Butyrate promotes large bowel functions such as modulation of colonic motility, enhanced visceral blood flow, and prevention of potential pathogen overgrowth (19, 54, 85, 89, 158). Butyrate has also been shown to reduce colonic inflammation, induce apoptosis, inhibit tumor cell progression, and protect against development of CRC (19, 54, 60, 85, 117, 136, 177). Increased production of SCFA significantly lowers the intestinal pH which promotes colonic fermentation and reduced absorption of carcinogens (89), all of which have a potential to reduce CRC risk (88, 153). Diets rich in fiber and complex carbohydrates are thought to have preventive effects against CRC due to the effect of polyphenol metabolites produced by colonic microbiota on COX-2 and GSTT2 expression in colon cells (98), promotion
of colonocyte homeostasis, and DNA repair. The chemopreventive effects of butyrate is mediated through induction of p21 (66) as illustrated in Figure 2a.

With increased protein ingestion, the colonic residue contains more sulfur, nitrates, ammonia, amines, branched chain amino acids, and hydrogen sulfide (H$_2$S). Residues from protein digestion can stimulate growth of sulfur-reducing bacteria such as Desulfovibrio and Desulfomonas spp (110). Hydrogen sulfide, an end product of protein metabolism is proinflammatory and genotoxic (50). Many of the end products of protein catabolism are mutagens and genotoxic and are associated with development of CRC via genetic mutations summarized in Fig. 2b. Increased intake of red or processed meats has generally been shown to be a risk factor for development of colorectal adenomas and cancer (81, 107, 131). Bacteria metabolize meat proteins to produce nitrosamines, known promoters of colon tumors in animal models (31).

Bile acids are byproducts of fat metabolism. Secondary bile acids such as deoxycholic acid contribute to increased reactive oxygen species (ROS), DNA damage, genomic instability, and tumor growth (39, 47) (Figure 2). Bacteria such as Clostridium spp convert bile acids into secondary bile acids such as deoxycholic acid (DCA) to generate free radicals and induce chronic inflammation, ROS and CRC (8). The high fat, low fiber (low intake of fruits and vegetables) diet in Western countries is thought to increase the risk of colon cancer possibly through increased secondary bile acids and ROS, which induces DNA damage and genomic instability. Consumers of a Western diet rich in fat and protein have a higher proportion of inflammatory 7-alpha-dehydroxylating bacteria and sulfur-reducing bacteria producing H$_2$S and secondary bile acids respectively (50).

Together, studies suggest that byproducts of carbohydrates protein and fat metabolism may contribute to colorectal carcinogenesis through a variety of mechanisms. Additional studies in humans and animal models are needed to fully understand the mechanisms underlying the
interactions of diet, microbiota, and colorectal adenomas and cancer. In particular, studies are needed to characterize the functional aspects of the microbiome. In a recent study Faith et al. (42), employed this approach to evaluate the interrelationship between diet and human gut bacteria. They evaluated germ-free mice colonized with a model bacterial community (10 defined human bacteria phylotypes) and four different diets. They found that certain factors in the diet influenced bacterial community membership. This type of approach will not only help to define underlying mechanisms but also identify metabolic functions, host-microbe and microbe-microbe interactions as well as develop ways to manipulate the gut microbiota to prevent or treat colorectal cancer.

Summary and Future Directions

Until recently, much of our understanding of the complexity and diversity of the microorganisms in the GI tract relied on observations from microbiological culture. However, the development of culture independent molecular methods based on the highly conserved bacterial 16S ribosomal RNA (rRNA) gene and advances in high throughput sequencing technology have allowed more in depth probing of the human gut microbiota (58, 82, 93, 149).

Several studies have demonstrated an association of bacterial dysbiosis with development of colorectal adenomas and cancer. However, specific causative bacteria have yet to be identified. Microbial signatures of colorectal adenomas and cancer identified in human studies will need to be verified in animal studies using humanized and gnotobiotic mice. Similarly, mouse models can be employed to investigate the potential protective role of specific microbial groups on CRC onset.

A better understanding of the complex interactions between the microbiome and development of colorectal cancer will require system-based approaches that incorporate metagenomics, metabolomics, proteomics, and transcriptomics studies. Such an approach will
facilitate the identification of possible interactions between the intestinal microbiota, pro-
carcinogenic factors and colorectal cancer.

Challenges in CRC include identifying at-risk individuals and early detection. While no
specific bacteria have been identified as a causative agent for colorectal cancer, ascertaining a
bacterial signature for development of adenomas and cancer holds great promise for providing
novel preventive and therapeutic strategies. It is conceivable that an intestinal bacterial profile
might (a) predict an individual’s risk of developing adenomas or CRC; and (b) allow
manipulation of the microbiota for prevention or treatment. Modulation of the human gut
microbiota will likely be accomplished with pro- and pre-biotics, although other agents may also
be beneficial. Large scale clinical trials will be required to introduce such therapeutic or
preventive interventions.

In order to understand the relationship between intestinal microbiota and development of
CRC in humans, epidemiological studies are needed to verify the findings from animal models in
humans. Ideally, these studies should be conducted in various populations, with a sufficient
sample size that would allow meaningful examination of the contribution of the microbiota to
disease after taking into account confounders (e.g. age and gender) and interaction effects with
diet. The studies should be prospective to provide information on temporal sequence. Moreover,
these studies will require interdisciplinary collaborations with experts in biostatistics,
bioinformatics, microbiology, molecular biology epidemiology and others to analyze and
interpret the massive microbial data generated by the high throughput sequencing technologies.

**Conclusions**

The human gut microbiome and its role in CRC and other diseases is an active field of
research. While initial correlative studies have informed us on the question of: “Who is there?”
they also raise questions concerning the functional aspects of the gut microbiota and the exact
means by which they influence human disease. These initial studies are likely to give rise to
mechanistic studies that will explore host-microbe interactions, microbe-microbe interactions as well as studies on modulation of the gut microbiome to prevent disease. Continued funding in microbiome research could potentially increase the number of significant discoveries in the microbiome field and provided much needed mechanistic insights that could contribute to disease prevention and treatment.
ACKNOWLEDGEMENTS:

This work was supported by funding from the National Institutes of Health: NCI P50CA106991
NCI R01 CA136887, NCI R01CA44684, and NIDDK P30 DK 034987.

The authors thank Drs. Robert Sandler, Gary Asher, and Andrea Azcarate-Peril for their helpful suggestions. The authors also thank Ms. Amber McCoy for editorial assistance.


Figure Captions

Figure 1. Mechanisms of gut microbiome to chronic inflammation, adenoma and CRC.

The gut microbiome involves in several mechanisms to modulate colorectal carcinogenesis. Bacterial dysbiosis weakens host defense mechanism of barrier function disrupting of epithelial layer tight junctions and the mucus film covering causes increased permeability of the intestinal epithelium favors bacterial translocation through mucosal epithelium (173). Macrophages on recognition of microorganism-associated molecular patterns (MAMPs) expressed by commensal microbiota, through molecular pattern-recognition receptors (TLR), triggers various signal pathways to produce inflammatory responses (55, 96). Activation of NF-kB pathways stimulate the transcription of proinflammatory genes, resulting in increased production of pro-inflammatory cytokines (TNF, IL-1, IL-23). Pro-inflammatory cytokines induce
STAT3 and NF-κB signalling which leads to the suppression of apoptosis and the promotion of cell cycle progression which progress to chronic inflammation and carcinogenic activity (52, 83, 105, 155). During chronic inflammation, imbalances between the production of ROS/RNS released from inflammatory cells such as macrophages, as well as bacterial genotoxins (colibactin) and hydrogen sulphide (H₂S) from the bacteria results in oxidative stress with in the target tissue result in DNA damage and further reduce DNA repair (46, 167).

TLR=Toll-like receptor. IL=interleukin. TNF=tumor necrosis factor. NF-κB=nuclear factor-κB. T_H17, T_helper 17Th=T-helper. STAT3, signal transducer and activator of transcription 3.

Figure 2. Roles of various components of diet and bacteria in CRC.

The principal components of diet intake are carbohydrates, protein, and fat which undergo colonic bacterial digestion to generate byproducts that are shown to involve in CRC via various mechanism. Some gut bacteria such as Clostridium spp. convert primary bile acids (intermediate product of fat) into a secondary bile acid such as deoxycholic acid (DCA). DCA is widely considered as a carcinogen that is associated with DNA damage via the production of free radicals or ROS and further induce chronic inflammation and CRC (138).

Fig. 2a. Metabolism of complex carbohydrates and fibers by colonic bacteria produces the metabolites for the involvement of CRC inhibition.

A wide variety of fermentable fibers in the diet include non-starch polysaccharides (cellulose, pectins, gums, arabinogalactans, mucilages, insulin, galacto-oligosaccharides), carbohydrate fibers (resistant starches, dextrins), and lignans (waxes, tannins). Fermentation of these complex carbohydrates and fibers by gut bacteria results in the production of short-chain fatty acids (SCFAs) such as formic acid, acetic acid, propionic acid, butyric acid, and valeric acid. SCFAs promote large bowel functions modulating colonic motility, promoting visceral blood flow, and preventing potential pathogens overgrowth (153, 158). Butyric acid or butyrate promotes colonic fermentation, lowers the absorption of procarcinogens, protects against inflammation and reduces CRC risk (162). Butyrate can also act as a
histone deactylase (HDAC), inhibiting the function of HDAC thereby favoring an acetylated state of histone in the cell (60). HDAC inhibition via SCFAs results in upregulation of p21, a key regulatory molecule of cell cycle arrest, which is also involved in cell proliferation, differentiation, and apoptosis (27, 66). SCFAs enhance the barrier functions promoting epithelial cells attachment to the basement while suppressing type IV collagen activity. All these mechanisms involve maintaining gut homeostasis and CRC inhibition.

**Fig. 2b. Breakdown of protein by colonic bacteria to potential carcinogenic metabolites and CRC.** (Modified from (89)).

The breakdown of proteins and peptides by colonic microorganisms also generate end-products that are considered as pro-carcinogenic, mutagenic and genotoxic besides health benefiting SCFAs and energy. A variety of branched chain fatty acids (BCFAs), are usually one carbon shorter than their amino acid precursors, such as isobutyrate, 2-methylbutyrate, and isovalerate are produced by many gut bacteria (Clostridia) (89). BCFAs are not considered to be risk factors for CRC. Ammonia, phenol, and H₂S produced during amino acid fermentation are putrefactive toxic substances. Amines (end-product of aminoacids via deamination) act as mutagen-precursors, phenols and indoles act as pro-carcinogens (89). Thioles and H₂S are also numerous reported as genetoxic can release ROS and damage host DNA and induce CRC.
Gut Microbiome

Adenoma
Colon Cancer

Chronic Inflammation

Genetic Mutations

DNA Damage

ROS, RNS

Genotoxin

TLR

MAMP

Macrophage

Cytokines
IL-23, IL-1, TNF

TH17

STAT3

NF-κB

Epithelial Cell Hyperproliferation

Adenoma

Colon Cancer
Host Diet Intake

Carbohydrate
- Complex/Fibers (Fig. 2a)
- Simple Sugars

Protein (Fig. 2b)

Fat

Mucosal Bacteria

Bacterial Enzymes:
- β-Gluconidase
- β-Glucosidase
- Nitroreductase

Pro-carcinogen

Heterocyclic Amines

Reactive Oxygen Species (ROS)

Primary Bile Acids

Secondary Bile Acids - (DCA)

Pro-carcinogen

Carcinogen

DNA Damage

Genetic Mutations

Inflammation

CRC
Complex Carbohydrates & Fibers

- Mucosal Bacteria
- Short Chain Fatty Acids (SCFAs)
- P21 Stimulator
- Cell Cycle Arrest at G1
- Induction of Apoptosis
- Fibronectin (Type IV Collagen) Activity
- Inhibits Metalloproteases & Histone Deactylase
- Lows pH / Acidic Environment
- Absorption of Carcinogens
- Epithelial Cell-Basement Attachment
- Homeostasis/ DNA Repair
- CRC Inhibition
- HDAC
- Histone Hyperacetylation
SCFAs
Homeostasis
CH4
CO2
H2
Mucosal Bacteria
Protein
Peptides/Amino acids
SCFAs
H2S/HS-
BCFAs
Phenols
Indoles
Ammonia
Amines
Thiols
H2S/HS-
Unknown Function
Pro-carcinogens
Mutagens
Mutagen Precursors
Genotoxic
DNA Damage & Genetic Mutations
CRC
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<td>Feces</td>
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<td>Bacterial dysbiosis, altered diversity, higher relative abundance of <em>Proteobacteria</em> and lower relative abundance of <em>Bacteroides</em> in adenoma cases to non-adenoma controls.</td>
</tr>
<tr>
<td>Geng et al. 2014 [171]</td>
<td>Biopsy samples</td>
<td>Adenoma and CRC</td>
<td>Members of <em>Enterobacteriaceae</em> (7 genera such as <em>Enterobacter</em>, <em>Pseudomonadaceae</em>, <em>Neisseriaceae</em>) as potential bacterial drivers and 12 genera such as <em>Streptococcaceae</em>, <em>Streptophyta</em>, <em>Microbacter</em>, <em>Methylbacter</em>, <em>Staphylococcus</em> as possible pro-inflammatory passenger bacteria in adenoma and CRC suggesting bacterial driver-passenger model for CRC.</td>
</tr>
<tr>
<td>Mira-Pascual et al. 2014 [47]</td>
<td>Mucosa and Feces</td>
<td>Adenoma and CRC</td>
<td>Bacterial dysbiosys, higher abundance of <em>F. nucleatum</em> and <em>Enterobacteriaceae</em> in CRC, altered microbial composition in adenoma</td>
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<tr>
<td>Zackular et al. 2014 [78]</td>
<td>Feces</td>
<td>Adenoma and CRC</td>
<td>Microbial dysbiosis, enrichment of pathogenic bacteria in adenoma and CRC, higher relative abundances of <em>Fusobacterium</em>, <em>Porphyromonas</em>, <em>Lachnospiraceae</em>, <em>Enterobacteriaceae</em>, and lower relative abundances of <em>Bacteroides</em>, <em>Lachnospiraceae</em>, <em>Clostridiales</em>, and <em>Clostridium</em> in adenoma and CRC groups compared to healthy groups.</td>
</tr>
<tr>
<td>Ohigashi et al. 2013 [172]</td>
<td>Fecal samples from CRC/adenoma/non-adenoma</td>
<td>Adenoma and CRC</td>
<td>Drastic alterations in intestinal environment; altered microbiota (decreased particularly obligate anaerobes), decreased SCFAs, and elevated pH in CRC.</td>
</tr>
<tr>
<td>Scanian et al. 2008 [173]</td>
<td>Feces</td>
<td>Adenoma (Polyps) and</td>
<td>Lower temporal stability and altered intestinal microbial diversity and metabolites in polyps/CRC compared to control. Higher</td>
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<td>Study</td>
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<td>Findings</td>
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<td>Swidsinski et al. 1998 [66]</td>
<td>Biopsy specimens</td>
<td>Adenoma and CRC</td>
<td>Marked abundance of <em>E. coli</em> and <em>coli-like</em> bacteria in CRC tissue</td>
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<td>Kubota et al. 1990 [174]</td>
<td>Feces</td>
<td>Adenoma and CRC</td>
<td>A decreased tendency of <em>Bifidobacterium</em> and <em>Clostridium</em> in CRC tissue</td>
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<td>Tahara et al. 2014 [175]</td>
<td>CRC tissue and normal mucosae</td>
<td>CRC</td>
<td>Significant enrichment (250 fold) of <em>F. nucleatum</em> in CRC tissue</td>
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<td>Ahn et al. 2013 [176]</td>
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<td>Reduced bacterial diversity in CRC cases</td>
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<tr>
<td>Chen et al. 2013 [43]</td>
<td>Feces</td>
<td>CRC</td>
<td>Distinct differences in fecal microbiota communities, <em>Clostridium</em>, <em>Roseburia</em>, and <em>Eubacterium</em> significantly less prevalent, whereas Enterococcus and Streptococcus more prevalent in the CRA group compared to healthy control.</td>
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<tr>
<td>Geng et al. 2013 [177]</td>
<td>Tumor/matching normal tissue</td>
<td>CRC</td>
<td>Overabundance of <em>Fusobacterium</em> spp., <em>Roseburia</em> in tumor tissue</td>
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<td>Ohigashi et al. 2013 [178]</td>
<td>Fecal samples before/after surgery</td>
<td>CRC</td>
<td>Marked decreased of obligate anaerobes, increased pathogenic bacteria, and reduction of short chain fatty acids detected after surgery for CRC</td>
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<td>Warren et al. 2013 [179]</td>
<td>CRC/matching normal tissues</td>
<td>CRC</td>
<td>Enriched <em>Fusobacterium</em>, <em>Leptotrichia</em> and <em>Campylobacter</em> in tumor tissues compared to normal tissues. Bacteria detected in tumors are mostly Gram (-)ve anaerobes. Host proinflammatory genes such as IL-8 overexpressed in Tumor tissues having enriched polymicrobial signature.</td>
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<td>Weir et al. 2013 [180]</td>
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<td>Decreased butyrate producing bacteria in CRC.</td>
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<td>Wu et al. 2013 [49]</td>
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<td>Bacterial dysbiosis, altered diversity, enriched Bacteroides, overabundance of <em>Fusobacterium</em> and <em>Campylobacter</em> and decreased butyrate producing bacteria in CRC cases compared to healthy controls.</td>
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<td>Kostic et al. 2012 [182]</td>
<td>Tumor/matching normal tissues</td>
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<td>Altered microbiota, higher relative abundance of <em>Fusobacterium</em> sequences and lower <em>Bacteroides</em> and <em>Firmicutes</em> sequences in tumors compared to matching normal tissues.</td>
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<td>Marchesi et al. 2011 [48]</td>
<td>Tumor/matching normal tissues</td>
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<td>Bacterial dysbiosis, high relative abundance of <em>Fusobacterium</em> in tumors to normal matching tissues.</td>
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<td>Sobhani et al. 2011 [45]</td>
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<td>Bacterial dysbiosis linked with elevated IL-17 in CRC patients</td>
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<td>Baxter et al. 2014 [79]</td>
<td>GF C57BL/6 mice, AOM/DSS</td>
<td>Fecal microbiota from CRC patients/control healthy human transplanted into GF mice</td>
<td>Gram negative Bacteroides, Parabacteroides, Alistipes, Akkermansia and mucin-degrading bacteria increase tumor burden however butyrate producing bacteria (Clostridium Group XIVa) protects against tumor</td>
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<td>Lu et al. 2014 [183]</td>
<td>SPF C57BL/6 mice, AOM/DSS induced CRC</td>
<td>Salmonella typhimurium AvrA-inflammation-associated CRC</td>
<td>Salmonella expressing AvrA activates β-catenin, significantly increased CRC</td>
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<td>Zhu et al. 2014 [184]</td>
<td>Wister rats, 1,2-dimethylhydrazine (DMH)-induced CRC</td>
<td>Microbial composition in lumen</td>
<td>Altered microbial composition of intestinal lumen in tumor groups, significant difference in intestinal bacterial flora in CRC rats vs healthy control, reduction of butyrate-producing bacteria (Roseburia and Eubacterium), significant increase in Desulfovibrio, Erysipelotrichaceae and Fusobacterium and decrease in probiotic species (Ruminococcus and Lactobacillus) in tumor groups</td>
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<tr>
<td>Kostic et al. 2013 [185]</td>
<td>C57BL/6-Apc&lt;sup&gt;Min&lt;/sup&gt;+, BALB/c Il-10&lt;sup&gt;−/−&lt;/sup&gt;, and BALB/c T-bet&lt;sup&gt;−/−&lt;/sup&gt; × Rag2&lt;sup&gt;−/−&lt;/sup&gt; mice</td>
<td>Fusobacterium nucleatum, Streptococcus anginosus, S. parasanguinis, and S. sanguinis</td>
<td>Apc&lt;sup&gt;Min&lt;/sup&gt; mice colonized with &lt;i&gt;F. nucleatum&lt;/i&gt; developed a significantly higher number of colonic tumors (but no colitis) as compared to Apc&lt;sup&gt;Min&lt;/sup&gt; mice colonized with Streptococcus spp. Higher expression of proinflammatory genes detected in mouse/human tumors with higher abundance of &lt;i&gt;F. nucleatum&lt;/i&gt;.</td>
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<td>Zackular et al. 2013 [19]</td>
<td>SPF/GF C57BL/6-AOM/DSS induced colon tumorigenesis</td>
<td>Microbiome roles in the development of CRC, using fecal transplants from tumor bearing mice to conventionalized GF mice</td>
<td>Abnormal microbial community structure in colonic tumorigenesis in mice. Enriched Bacteroides, Odoribacter, and Akkermansia genera and decreased Prevotellaceae and Porphyromonadaceae families in the colon of tumor bearing mice. Microbiota transplant from tumor-bearing mice to germfree mice significantly increased colon tumorigenesis compared to mice colonized with a healthy gut microbiome. Changes in the gut microbiome associated with inflammation directly contribute to tumorigenesis in mice.</td>
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<td>Arthur et al. 2012 [84]</td>
<td>GF IL10&lt;sup&gt;−/−&lt;/sup&gt; and WT129/SvEv mice, AOM-induced CRC</td>
<td>Genotoxic island polyketide synthase (pks) containing &lt;i&gt;Escherichia coli&lt;/i&gt; and pks deleterion &lt;i&gt;E. coli&lt;/i&gt; mutant, Enterococcus faecalis</td>
<td>No tumor detected in pks + &lt;i&gt;E. coli&lt;/i&gt;-associated WT mice, &lt;i&gt;E. faecalis&lt;/i&gt; mono-associated, AOM-treated IL10&lt;sup&gt;−/−&lt;/sup&gt; mice developed severe colitis but rarely adenocarcinoma, pks /Colibactin containing &lt;i&gt;E. coli&lt;/i&gt; NC101 induced severe inflammation associated colitis and invasive mucinous adenocarcinoma in AOM-treated IL10&lt;sup&gt;−/−&lt;/sup&gt; mice.</td>
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<td>Uronis et al. 2009 [186]</td>
<td>SPF and conventionalized IL10&lt;sup&gt;−/−&lt;/sup&gt;; Myd88&lt;sup&gt;−/−&lt;/sup&gt; and control IL10&lt;sup&gt;−/−&lt;/sup&gt; C57BL/6, 129ScEv mice, AOM</td>
<td>Bacteroides vulgatus</td>
<td>&lt;i&gt;B. vulgatus&lt;/i&gt; (a weak inflammation inducer) colonized with IL10&lt;sup&gt;−/−&lt;/sup&gt;GF WT mice displayed a significantly lower tumor multiplicity compared to their conventionalized counterparts. Bacterial-induced inflammation is essential for the development of colitis-associated colorectal cancer dependent on TLR/MyD88 pathway signaling.</td>
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<td>Wu et al. 2009 [187]</td>
<td>Multiple intestinal neoplasia (Min) mice</td>
<td>Enterotoxigenic Bacteroides fragilis (ETBF) and Nontoxigenic B. fragilis (NTBF)</td>
<td>Only ETBF triggers inflammatory colitis via BFT and promotes infection induced colon carcinogenesis</td>
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<td>Nagamine et al. 2008 [188]</td>
<td>BALB/c-Rag2&lt;sup&gt;−/−&lt;/sup&gt; Apc&lt;sup&gt;Min&lt;/sup&gt; mice</td>
<td>Helicobacter hepaticus infection</td>
<td>Significant increase in colon tumor incidence in &lt;i&gt;H. hepaticus&lt;/i&gt;-infected BALB-RagMin mice compare to uninfected BALB-RagMin mice.</td>
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<td>Nagamine et al. 2008</td>
<td>BALB/c-IL10, AOM-induced colon tumorigensis</td>
<td><em>Helicobacter hepaticus</em></td>
<td>Significant increase in AOM-induced, exophytic adenomas and colon tumors in IL10KO mice</td>
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<td>Newman et al. 2001</td>
<td>Apc&lt;sup&gt;Min&lt;/sup&gt; (Min) C57BL/6J mice, DMH</td>
<td><em>Citrobacter rodentium</em> infection</td>
<td><em>C. rodentium</em> infection promotes adenoma formation and enhances carcinogenesis in the colon of genetically susceptible Min mice.</td>
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<td>Ellmerich et al. 2000</td>
<td>Wister rats, AOM-induced CRC</td>
<td><em>Streptococcus bovis</em> and <em>S. bovis</em> cell wall-extracted antigens</td>
<td><em>S. bovis</em> and its cell wall proteins act as promotores for the progression of preneoplastic lesions in colonic mucosa.</td>
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