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Gut Microbiome and Colorectal Adenomas

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Abstract

The trillions of bacteria that naturally reside in the human gut collectively constitute the complex system known the gut microbiome, a vital player for the host's homeostasis and health. However, there is mounting evidence that dysbiosis, a state of pathological imbalance in the gut microbiome is present in many disease states. In this review, we present recent insights concerning the gut microbiome's contribution to the development of colorectal adenomas and the subsequent progression to colorectal cancer (CRC). In the United States alone, CRC is the second leading cause of cancer deaths. As a result, there is a high interest in identifying risk factors for adenomas, which are intermediate precursors to CRC. Recent research on CRC and the microbiome suggest that modulation of the gut bacterial composition and structure may be useful in preventing adenomas and CRC. We highlight the known risk factors for colorectal adenomas and the potential mechanisms by which microbial dysbiosis may contribute to the etiology of CRC. We also underscore novel findings from recent studies on the gut microbiota and colorectal adenomas along with current knowledge gaps. Understanding the microbiome may provide promising new directions towards novel diagnostic tools, biomarkers, and therapeutic interventions for CRC.

Keywords

colorectal adenoma; colorectal cancer (CRC); gut microbiota; bacterial dysbiosis; inflammation; microbiome

Introduction

Globally in 2012 alone, colorectal cancer (CRC) accounted for approximately 694,000 deaths (approx. 8.5% of total cancer deaths) and 1.36 million new cases [1]. In the United States, CRC is the third most commonly diagnosed cancer and the second leading cause of cancer deaths and will account for about 136,830 new cases and 50,310 deaths in 2014 [2].

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The annual economic burden of CRC in 2010 was approx. 14.1 billion dollars and this is expected to increase to about 17.4 billion dollars in 2020 [3]. Mortality from CRC is more broadly associated with metastatic disease; therefore, early detection and screening are vital.

CRC occurs in a stepwise fashion beginning with abnormal cell proliferation, and aberrant crypt foci leading to the development of adenomatous polyps, which are widely considered to be CRC precursors [4]. Colonic polyps are mostly classified on the basis of their properties to progress to malignancy (hyperplastic or adenomatous) as well as their structure including types (sessile, pedunculated, and flat), shape (tubular, villous, serrated) and size (small 1–5 mm, medium 5–10 mm, and large ≥ 10mm) [5]. Hyperplastic polyps are usually small, located in the rectum and sigmoid colon, and are generally thought to have no malignant potential. However, subsets of serrated hyperplastic polyps are associated with a risk of CRC [6]. Adenomatous polyps or adenomas account for approximately 70% of colon polyps and have the potential to progress to CRC over time if not screened and removed by colonoscopy or sigmoidoscopy [7].

In clinical settings, the number, and structure (shape, size, and type) of adenomatous polyps are vital indicators when predicting which patients are more prone to develop CRC based on polyp morphology. Thus, adenomas are important intermediates in colorectal carcinogenesis and identifying adenoma risk factors is important in preventing CRC. Although the specific etiologic agents responsible for adenomas and CRC are unknown, several genetic and environmental risk factors have been implicated.

Risk factors for colorectal adenomas and CRC

The role of genetic alterations in the progression of adenomas to CRC was initially described by Fearon and Vogelstein [2]. Genetic mutations in oncogenes (*KRAS*), tumor suppressor genes such as adenomatous polyposis coli (*APC*), *CTNNB1* and *p53*, [2, 8–13], and alterations in pathways that revolve around chromosomal and microsatellite instability (MSI), mismatch repair (MMR) [14, 15], and CpG island methylation (CIMP) [16, 17] are key players in colorectal adenomas and CRC [18].

In addition, findings from genome-wide association studies (GWAS) support a polygenic model of CRC in which several common low penetrance susceptibility genes such genetic variants in vitamin D [19], cyclin D1, and Smad7 [20] contribute to increased risk of adenoma and CRC [21, 22]. Family history and age are also considered to be important CRC predictors as they have been associated with higher risk of adenomas and CRC. Studies suggest that genetic predisposition and somatic alterations in combination with environmental factors are responsible for CRC as a complex disease [20].

The most common environmental factors implicated in association with colorectal adenomas and CRC are lifestyle and diet. Several studies demonstrate that unhealthy diets such as those high in fat, alcohol, red meat, and low in fiber are associated with increased risk of adenomas and CRC [23]. Moreover, smoking, obesity, low physical activity [24, 25], sex (increased risk for males), ethnicity (predominantly in Non-Hispanic Black population [19, 26], and lifestyle (lack of physical exercise) all contribute to the development of CRC. Adopting a healthy lifestyle, incorporating regular exercise and a diet high in fruits,

vegetables, and high-fiber foods could potentially reduce the risk of CRC. However, not all the results from dietary studies are consistent. A pooled study of fiber and CRC reported inconsistent findings in which about half of the studies showed a protective effect of fiber while the others did not [27]. These discrepancies could relate to the influence of the gut microbiota on fiber. The gut microbiota was not assessed in these studies.

Gut microbiota

The human colon hosts a very diverse and complex microbial community comprising an estimated 100 trillion bacteria of more than 1,000 heterogeneous species (harboring approx. 4 million genes) along with viruses, archaea, and fungi. The collective bacterial genome referred to as the gut microbiome, harbors 150-fold more genes than the human genome [28, 29]. Bacterial cells of the gut exceed the total number of host cells in the human body by 10-fold [30]. These bacteria play key roles in modulating host metabolism such as absorption of indigestible carbohydrates, production of vitamins B and K, and promotion, maturation and development of innate and cell-mediated immunity and also help to maintain intestinal barrier function and appropriate immune response against pathogens [31, 32]. Under normal physiological conditions, the gut bacteria and the host co-exist in a state of homeostasis. However, the gut microbiota is increasingly associated with a variety of diseases including obesity, inflammatory bowel diseases, adenomas, and CRC [12, 33, 34].

Gut microbiota, adenomas, and CRC

Several studies implicate microbial dysbiosis, a pathological imbalance in the microbial community, in the etiology of colorectal adenomas and CRC. This is summarized in Fig. 1A. Shen *et al.* used molecular fingerprinting and clone sequencing methods to characterize the adherent bacterial composition in normal rectal mucosal biopsies and observed that the gut bacterial composition of subjects with adenomas differed significantly from that of control subjects without adenomas [35]. They reported a higher proportion of *Proteobacteria* and lower abundance of *Bacteroidetes* in cases than in controls. These initial findings were confirmed in a follow-up study that used 16S rRNA gene amplicon 454 pyrosequencing methods to characterize the gut bacteria. Sanapareddy *et al.* [36] found an overabundance of potential pathogens, *Pseudomonas*, *Helicobacter*, *Acinetobacter* and other genera belonging to the phylum *Proteobacteria* in rectal mucosal biopsies of adenoma cases compared to non-adenoma controls [36]. Brim *et al.* compared the fecal microbiota from a small sample group of African American patients with or without colorectal adenomas and noted a trend of altered microbial changes between adenoma patients and healthy controls [37]. In experimental models of CRC, Wei *et al.* observed dysbiosis associated with an increased abundance of *Ruminococcus obeum*, and *Allobaculum* spp. in precancerous lesions [38]. These findings suggest that changes in the gut adherent microbial community composition may play a role in the development of adenomas.

Other studies have also examined the microbiota in relation to CRC (Table 1). Marchesi *et al.* assessed the microbiota in colon tumors and matching normal tissue and observed bacterial dysbiosis in the tumors [39]. In particular, they noted an overabundance of *Fusobacterium* in tumors compared to matching normal tissue. Their initial findings for

Fusobacterium and CRC have been confirmed by others [40–45]. Furthermore, some studies characterized the microbiota in fecal samples from CRC subjects and healthy controls. Sobhani *et al.* examined fecal samples from CRC patients and controls and found that bacterial dysbiosis was associated with CRC and was characterized by an increased abundance of *Prevotella* [46]. Bacterial dysbiosis associated with CRC has been reported to have relative decreased abundance of obligate anaerobes, increased potential pathogenic bacteria, and reduction in proportions of beneficial butyrate-producing bacteria [45, 47–49]. Zackular *et al.* demonstrated that changes in the gut microbiota associated with inflammation and tumorigenesis directly contribute to colorectal cancer [50]. In experimental models, they transferred the fecal microbiota of tumor bearing mice to germ free mice and showed that the microbiota from the tumor bearing mice (donor) promoted tumorigenesis in recipient animals with twice as many colon tumors than mice given healthy microbiota. Similar to the donor microbiota, the microbiota of recipient mice was characterized by elevated abundance of *Akkermansia*, *Odoribacter*, and *Bacteroides*. Their observations suggest that the gut microbiota may be amenable to manipulation with antibiotics or probiotics to prevent the development of adenomas and CRC.

The overall consensus from these studies is that a combination of the expansion of procarcinogenic bacteria concomitant with the reduction of tolerogenic commensals such as *Faecalibacterium prausnitzii* [51] or spore-forming *Clostridium* clusters IV and XIV [52] may link bacterial dysbiosis to the risk of adenomas and CRC. However, it is difficult to discern from human studies whether gut bacterial dysbiosis is a cause or consequence of adenomas and CRC.

Specific gut bacteria, adenoma, and CRC

Overall, the mechanisms by which the gut microbiota influences adenoma and CRC development remain to be fully established. Moreover, the contribution of specific bacterial signatures and potential mechanisms are not yet elucidated. Potential mechanisms include promotion of chronic inflammation, DNA damage, and production of bioactive carcinogenic metabolites. We describe current reports on some specific bacteria.

Fusobacterium nucleatum: Various studies suggest that overabundance of *Fusobacterium* spp. is a common feature of CRC that may contribute to disease progression from adenoma to cancer. However, it is not clear whether *Fusobacterium* spp. is a cause or consequence of adenomas and CRC [53]. Two recent experimental studies provide further mechanistic insights into the relationship between *F. nucleatum* and colorectal neoplasia. Rubinstein *et al.* [54] observed that binding of *F. nucleatum* via its FadA adhesion molecule to E-cadherin leads to activation of β-catenin signaling to induce pro-oncogenic and inflammatory pathways (Fig. 1B. I). The second study by Kostic *et al.* showed that *Fusobacterium* modulates the tumor immune microenvironment to promote inflammation and tumorigenesis [43]. In the APC min mouse model of CRC, they showed that *Fusobacterium* increases infiltration of myeloid cells such as CD11b positive T cells, macrophages, and dendritic cells to induce an NF-κB-driven pro-inflammatory response to promote CRC. In a companion human study, increased FadA expression (> 10–100 times) correlated with elevated expression of oncogenic and inflammatory genes in CRC subjects. While these

findings support a role for *Fusobacterium* spp. and FadA in colorectal carcinogenesis, it is too early to determine their potential as a CRC biomarker or their utility as potential diagnostic and therapeutic targets. Thus, additional studies are needed.

Streptococcus gallolyticus (formerly *S. bovis*): DNA from *S. gallolyticus* is present in about 20–50% of colon tumors compared to less than 5% in the normal colon [55]. It has also been associated with increased colonization of collagen-rich surfaces of colorectal adenomas and tumors [56]. It is thought that *S. gallolyticus* may contribute to neoplastic transformation in the colon via invasion through a breach in the epithelial barrier or virulence factors, which ultimately enhance inflammation and tumorigenesis [55, 56].

Enterotoxigenic Bacteroides fragilis (ETBF): Other bacteria possessing virulence traits such as ETBF are pro-oncogenic and may remodel the microbiota as a whole to promote mucosal immune responses and epithelial changes, which promote colorectal adenomas and cancer. ETBF produces a toxin known as fragilysin (*B. fragilis* toxin; BFT) which activates the Wnt/β-catenin signaling pathway to increase cell proliferation [57]. BFT also activates NF-kB to induce production of inflammatory mediators. This leads to mucosal inflammation and, ultimately, colorectal carcinogenesis [58, 59]. ETBF was shown to promote tumorigenesis in a study by Wu *et al.* in which they colonized the APC min model of intestinal neoplasia with a pig isolate of ETBF. They observed a marked increase in colon adenoma and tumor formation in mice colonized with ETBF compared to control mice [60]. The enhanced tumorigenesis by ETBF could occur via activation of Stat3, induction of IL-17 [61] and DNA damage [62]. These observations support a link between bacterial antigens, virulence factors and colon adenomas and CRC.

Enterococcus faecalis: In experimental models, certain strains of *E. faecalis* have been associated with CRC and colitis- associated CRC. Some strains promote release of extracellular superoxide in host cells. The superoxide is converted by hydrogen peroxide could induce DNA damage [63], chromosome instability [64], and cancer in germfree Interleukin-10 (*IL-10*$^{-/-}$) mice (Fig. 1B. III) [65, 66].

Escherichia coli: DNA damage induced by genotoxic *E. coli* strains could result in CRC-initiating lesions. *E. coli* possessing the polyketide synthase (*pks*) Genotoxic Island, which encodes the enzymatic machinery to make Colibactin may also promote CRC via induction of DNA double strand breaks (Fig. 1B. I) [67]. Arthur *et al.* recently showed that deletion of *pks* from a strain of *E. coli* results in reduced DNA damage, tumor numbers, and tumor invasion, but not inflammation in mono-associated *IL10*$^{-/-}$ mice treated with azoxymethane (AOM) [68]. A few human studies suggest that *E. coli* harboring the *pks* is more common in CRC and inflammatory bowel disease patients [68, 69]. Thus, these findings lend strong support to the contribution of genotoxic *E. coli* in colorectal cancer.

Acidovorax: *Acidovorax* spp., an acid degrading member of the phylum *Proteobacteria* is also associated with increased risk of adenomas [36]. *Acidovorax* may promote colon neoplasia through increased metabolism of nitro-aromatic compounds [70] in the gut as well as induction of local inflammation by its flagellar proteins [71, 72].

In addition to DNA damage and superoxide release, activation of inflammation is a common theme across these studies. Further research is needed to identify additional mechanisms by which bacteria and their virulence factors promote colorectal carcinogenesis. While monoassociation studies involving individual bacteria provide useful mechanistic insights, they may not fully represent the complex interactions between gut bacterial communities and adenomas and CRC.

Bacteria metabolites, adenomas, and CRC

The colonic microbiota influences a wide range of metabolic processes and functions that may lead to beneficial or detrimental effects within the human colon. Metabolites produced by colonic microbiota might play a critical role in the progression of adenomas to CRC, though limited information about the function of most of the gut bacteria and their metabolites is known to date. Certain gut bacteria produce short chain fatty acids (SCFAs) such as butyrate, which can serve as an energy source for colonic epithelial cells. Wang and colleagues observed a reduction in butyrate-producing bacteria in feces of CRC patients suggesting that microbial metabolites may contribute to the etiology of CRC [73]. A few members of the *Clostridium* cluster IX, XI, and XVIa are capable of metabolizing primary bile acids into secondary bile acids [74]. Secondary bile acids such as deoxycholic acid (DCA) might contribute to CRC progression (Fig. 1B. IV) by interacting with host metabolism and immunity [75–78].

Few human studies have evaluated the metabolome and microbiota in relation to adenomas or CRC. Findings from a recent study suggests that there is a correlation between bacterial dysbiosis, the metabolome, and colorectal adenomas [79]. More studies are needed to fully explore the relationship between the microbiota, metabolome, adenomas, and CRC.

Summary and conclusions

Although gut bacterial dysbiosis is increasingly recognized as a phenomenon in colorectal carcinogenesis, host-bacterial interactions still remain to be fully elucidated. In studying the gut microbiota and adenomas or CRC, it is unclear whether sampling the mucosa or the luminal content is the most appropriate. Bacteria in the lumen are transient and may be more influenced by diet while the adherent mucosal bacteria are considered residents and may be more relevant to CRC because of their close contact with the host mucosa and immune cells. To date, there is no clear consensus. Studies suggest that bacteria communities in the feces differ from the mucosa [80, 81]. Findings from two studies that compared the microbiota in mucosa, rectal swabs, and feces of the same patients were inconclusive [82, 83]. Additional studies are needed to further define the best sampling location so as to enhance uniformity and reproducibility among studies.

The role of the gut microbiota in the progression from adenomas to CRC is undoubtedly multifactorial and can affect the various stages of the neoplastic process. Microbial dysbiosis, induction of mucosal inflammation, and production of reactive metabolites are all processes that might act in concert to set the colonic mucosa on the initial stage of the adenoma-carcinoma process. Further research in experimental animal models is necessary to better understand the mechanisms that underlay the association between the gut

microorganisms and CRC. The intestinal microbiota represents an enormous reservoir for the discovery of novel signatures that could be potentially useful as biomarkers and predictors for adenomas and CRC. Manipulation of the gut microbiota to restore normal physiologic balance might be beneficial in preventing colon adenomas and CRC. Furthermore, beneficial or "friendly" bacteria that have been specifically engineered to provide desired inflammatory responses and epigenetic expression could have the potential to be useful therapeutically in CRC.

In conclusion, the advances in microbiome research provides an opportunity to elucidate the exact connections between the host gut microbiome and the onset of CRC, which will hopefully lead to safer and more efficacious treatments in the near future.

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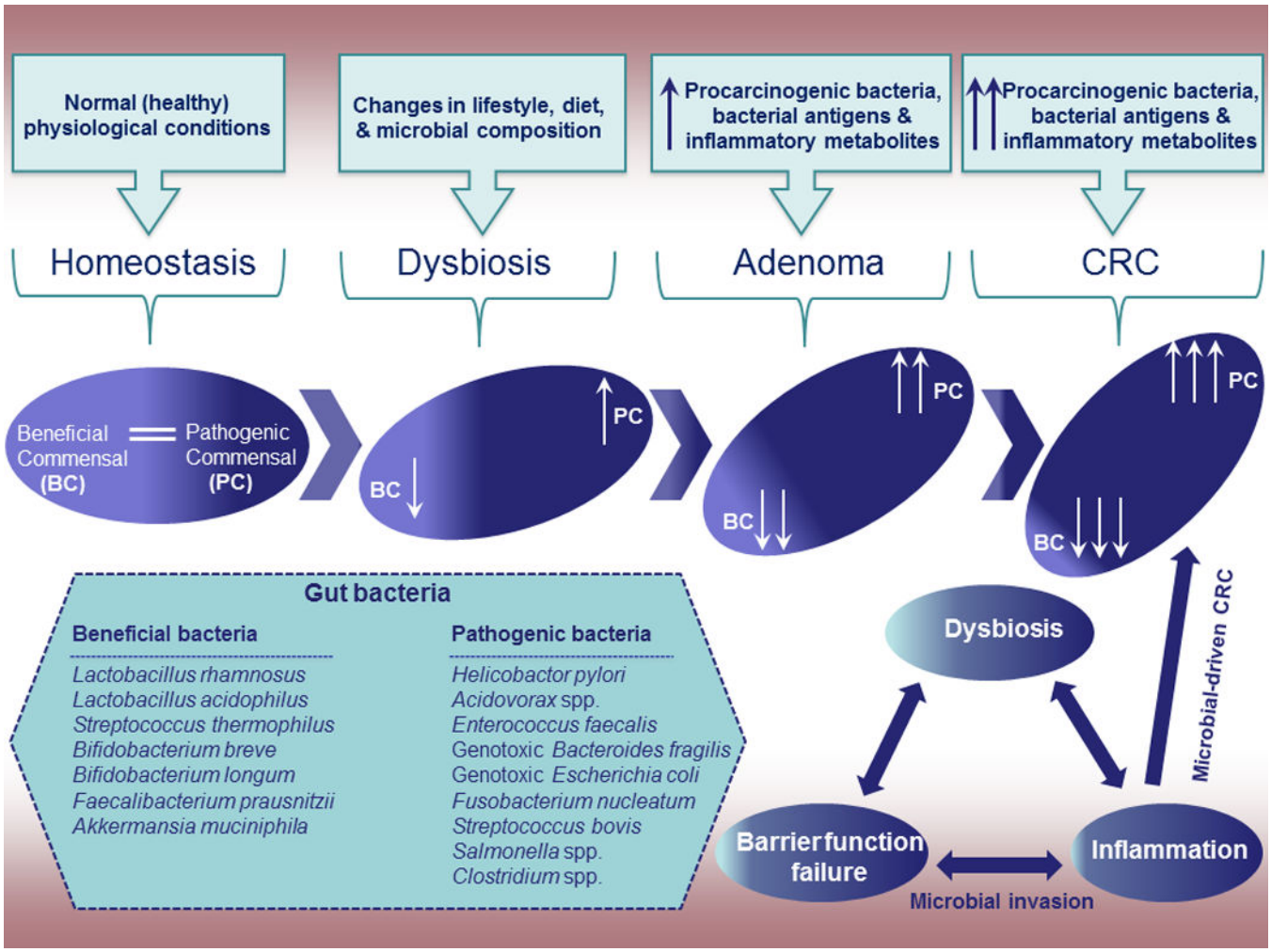

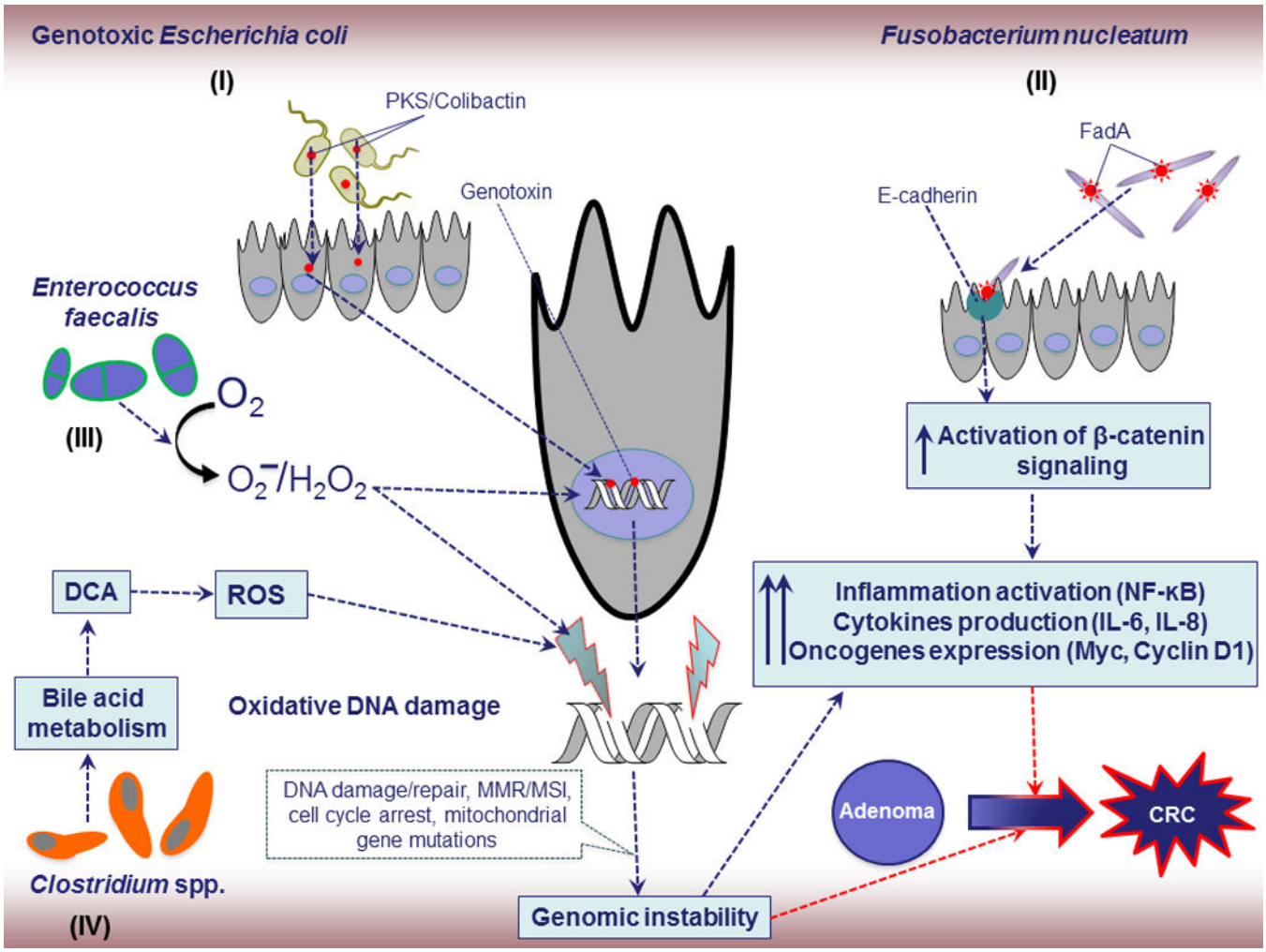

Figure 1.

A. Schematic diagram of colonic microbiota and adenomas progression to CRC.

Shifts in the balance of host-microbial symbiotic relationships derail the state of homeostasis (normal physiology) in the human gut. Dysbiosis, an imbalance of microbial population dynamics, is characterized by decreased beneficial commensals/symbionts, overexpression of pathogenic microbiota such as genotoxic bacteria, invasive and inflammation triggering microbiota, procarcinogenic bacteria and cancer enhancing bacterial antigens and metabolites. Consequences of the microbial dysbiosis lead to the chronic inflammation after damaging the host defenses (natural barrier) can further drive to the enhancement of small adenomas to adenocarcinoma by multistep processes.

B. Proposed mechanisms of specific bacteria and CRC.

The human gut microbiome drives CRC via several mechanisms. Some of the reported mechanisms of specific bacteria for the development of CRC are highlighted here.

A. *E. coli*, Gram-negative facultative anaerobic bacterium, considered as one of the potential etiological agents of CRC due to its genotoxins such as Colibactin, and cytolethal distending toxin (CDT). These products could induce DNA damage and

influence the progression of CRC due to genomic instability from MSI, MMR, and mutations.

B. *F. nucleatum*, Gram-negative anaerobic bacterium, has been linked to CRC progression but the exact underlying mechanisms are still unknown. A potential *F. nucleatum*-driven CRC mechanism is its invasion into epithelial cells and activation of oncogenic and inflammatory responses through its unique FadA adhesin. Active FadA binds to E-cadherin, mediating *Fusobacterium* attachment and invasion into the epithelial cells. This activates β-catenin signaling, leading to increased activation of inflammatory genes (NF-κB) and secretion of cytokines interleukin-6 (IL-6), IL-8, and IL-18, and oncogenes and drives to adenoma to adenocarcinoma.

C. *E. faecalis*, has been shown to produce extracellular superoxide and hydrogen peroxide, which damage DNA and also further enhances chromosomal instability in colonic epithelial cells. Chromosomal instability, a common cause of genomic instability in tumors, is characterized by nucleotide additions or deletions, inversions, translocations, and complex rearrangements, and ultimately contributes to the dramatic and unstable alteration in genomic state critical for tumor initiation in the colorectum.

D. Gram-positive, spore forming bacteria in cluster IX of the genus *Clostridium spp.* convert primary bile acids 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 reactive oxygen species (ROS) and implicated to adenoma-inflammation-CRC through enhancing genomic instability and inflammation.

Table 1

Human studies of gut bacteria and colorectal cancer

Study	Sampling site	Disease	Findings	Reference
Geng *et al.* 2013	Tumor/matching normal tissue of Chinese CRC patients	CRC	Overabundance of *Fusobacterium* spp., *Roseburia* in tumor tissues and over-representation of *Microbacterium, Anoxybacillus* bacteria away from tumor site	[45]
McCoy *et al.* 2013	Rectal mucosa	Adenoma	*Fusobacterium* spp., higher abundance in adenoma subjects.	[43]
Castellarin *et al.* 2012	Tumor/matching normal tissue	CRC	Overabundance of *Fusobacterium nucleatum* sequences	[41]
Chen *et al.* 2012	Intestinal lumen, mucosa (rectal swabs), fecal samples, tumor/ matching normal tissue	CRC	Lower bacterial diversity in tumor, altered microbial structures in CRC lumen compared to mucosa. CRC might be due to cometabolism by lumen microflora and direct interaction between host and mucosa-associated microbiota.	[81]
Kostic *et al*. 2012	Tumor/matching normal tissue	CRC	Altered microbiota, high abundance of *Fusobacterium* sequences and low *Bacteroides* and *Firmicutes* sequences in tumors	[42]
Sanapareddy *et al.* 2012	Rectal mucosa	Adenoma	Bacterial dysbiosis, altered diversity and increased richness	[37]
Marchesi *et al.* 2011	Tumor/matching normal tissue	CRC	Bacterial dysbiosis, high abundance of *Fusobacterium* in tumors	[40]
Shen *et al.* 2010	Colonic mucosa of adenoma/non-adenoma	Adenoma	Bacterial dysbiosis, altered diversity, higher abundance of *Proteobacteria* and lower abundance of *Bacteroides* in adenoma cases	[36]
Ahn *et al*. 2013	Fecal sample	CRC	Reduced bacterial diversity in CRC cases	[83]
Brim *et al*. 2013	Fecal sample	Adenoma	Microbiota changes at the sub-genus level but not genome/functions level in colon polyps.	[38]
Ohigashi *et al*. 2013	Fecal samples from CRC/adenoma/ non-adenoma	CRC& Adenoma	Significant differences in the intestinal environment; altered microbiota (decreased particularly obligate anaerobes), decreased SCFAs, and elevated pH in CRC.	[48]
Ohigashi *et al*. 2013	Fecal sample before/after surgery	CRC	Marked decreased of obligate anaerobes, increased pathogenic bacteria, and reduction of short chain fatty acids detected after surgery for CRC	[49]
Weir *et al.* 2013	Fecal sample	CRC	Decrease butyrate producing bacteria	[50]
Wu *et al*. 2013	Fecal sample	CRC	Bacterial dysbiosis, altered diversity, enriched *Bacteroides*, more abundant of *Fusobacteriumand Campylobacter sps*. Decreased butyrate producing bacteria	[46]
Sobhani *et al*. 2011	Fecal sample	CRC	Bacterial dysbiosis linked with elevated IL-17 in CRC patients	[47]