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Review

Emerging Evidence on the Effects of Dietary Factors on the Gut Microbiome in Colorectal Cancer

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Abstract: Dietary factors play an important role in shaping the gut microbiome which, in turn, regulates the molecular events in colonic mucosa. The composition and resulting metabolism of the gut microbiome have been implicated in the development of colorectal cancer (CRC). Diets low in dietary fibers and phytomolecules as well as other lifestyle-related factors may predispose to CRC. Emerging evidence demonstrates that the predominance of microbes, such as Fusobacterium nucleatum, can predispose the colonic mucosa to malignant transformation. Dietary and lifestyle modifications have been demonstrated to restrict the growth of potentially harmful opportunistic organisms. In this study, we aim to present evidence regarding the relationship of dietary factors to the gut microbiome and development of CRC.

Keywords: dietary fibers; short chain fatty acid; gut microbiota; colorectal cancer prevention; epigenetics

1. Introduction

The 'gut microbiome' includes the collective genome and products of all the microorganisms residing in the gastrointestinal tract (GIT) [1]. In fact, there are over 100 trillion microbes residing in the GIT, the majority of which, reside in the colon [2]. Metagenomic studies demonstrate that there are approximately 1,952 uncultured bacterial species, many of which remain unclassified to date, contributing to substantial diversity within the microbial ecosystem [3]. The host-microbe relationship can be symbiotic or pathogenic. Several external factors, such as diet, medications, and lifestyle changes heavily influence the microbial ecosystem [4]. Symbiotic relationships with the microbes have a plethora of effects on human physiology and overall health. Microorganisms provide essential micronutrients, regulate the immune response, modulate enterocyte function, influence metabolism, and most importantly prevent colonization by pathogenic microorganisms [5]. The gut ecosystem is highly dependent on the human diet as well as its composition as the microbes thrive on and metabolize 'what we consume'. Dietary fibers or 'microbiota accessible carbohydrates' and certain plant-based proteins are metabolized to short chain fatty acids (SCFAs) which exhibit anti-inflammatory properties, maintain mucosal integrity, and retain microbial diversity [6,7]. Imbalances in ratios of vital nutrients to dangerous toxins are implicated in a wide variety of diseases, including cancer. Transformed microbial diversity, impaired immune response, and release of carcinogenic or genotoxic substances are the major microbiome-induced mechanisms implicated for cancer pathogenesis [8]. In this study, we aim to present emerging evidence on the dietary factors related to the development of CRC and how heathy dietary modifications can restore functional colonic epithelium and prevent CRC.

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2. Gut Microbiome and Colorectal Cancer

The microbiome can influence the development of CRC in several ways. Microbial dysbiosis exposes the GIT to the toxins and superimposes the effects of lifestyle factors such as smoking, alcohol and obesity, thus increasing oncogenic transformations [1]. Figure 1 shows a diet-benefit model that incorporates the host-microbe relationship and factors influencing their harmony. Primarily, the colon is the site which harbors most of the microbial flora (70%) and constitutes the frontline defense against the invading pathogenic strains [9]. Apart from the natural gut defenses, our own symbionts have an important role in fighting pathogenic strains by stimulating the immune system. In turn, the immune system responds by producing a host of inflammatory mediators such as anti-microbial peptides, inflammasomes, and cytokines, such as IL (interleukin)-22, IL-17, and IL-10 [10]. Importantly, persistent activation of the immune system has its own adverse effects. Chronic inflammation can induce oxidative stress by producing reactive oxygen species (ROS), which have both cytotoxic and genotoxic effects, resulting in detrimental effects on intestinal mucosal cells [11]. Inflammasomes produced by the innate immune system secondary to inflammation induce colitis which increases the risk for CRC development [12]. Moreover, inflammation-mediated persistent release of growth factors, apoptosis suppression, and angiogenesis are additional factors which promote tumorigenesis [13] Carcinogenic metabolites or oncotoxins resulting from the microbial metabolism of altered nutritional constituents due to lifestyle factors work as key external factors for promoting CRC [1]. Integrated metagenomic and metabolomic analysis show that CRC-associated microbes are highly associated with production of polyamines (i.e. cadaverine and putrescine) [14]. Diets lacking microbiota-accessible carbohydrates (MAC) are responsible for the increasing incidence of CRC [15]. Healthy diet nurtures microbial diversity by providing essential substrates such as dietary fibers which are metabolized by the microbiome into metabolites like butyrate, which protects the colonic mucosa by impeding inflammatory damage. The various mechanisms by which faulty microbiome mediate CRC includes increased microbial adherence to colon cells, down-regulation of tumor suppressor genes, activation of oncogenes, induction of genotoxic effects on colonic enterocyte, and activation of angiogenesis [16]. Thus, external factors can modulate the gut microbiome resulting in either stimulatory or regulatory roles in priming the intestinal microenvironment towards or against tumorigenesis.

Poor microbial diversity is associated with increased risk for CRC [14,17]. Abundance of Fusobacteria is observed in carcinomas of left colon, while colonization by Helicobacter spp. Is observed in right-sided CRC [18]. In colonic adenomatous polyposis (CAP), a precursor to CRC, there is an abundance of Bacteroides and Citrobacter taxa, as compared to Weissella and Lactobacillus, which are disproportionally low. The chief metabolite observed in the fecal samples of CAP patients is butyric acid while qPCR analysis shows lower butyrate producing bacteria [19]. Even though butyrate has pro-apoptotic and anti-proliferative role in CRC, it has paradoxically been shown to enhance polyp formation in APCmin/+MSH2−/−(adenomatous polyposis colimin/+MutS homolog 2−/−) mice having defective mismatch repair [20,21]. Certain pharmacological agents have also been shown to modulate the colonic microbial diversity and alter the course of CRC. For example, Ternák et al. demonstrated that antibiotic therapy may have positive and negative correlation with development of different malignancies [22]. However, in certain European regions, overconsumption of antibiotics such as penicillin and tetracyclines are associated with higher incidences of CRC, especially among females. Lee et al. reported that antibiotic therapy, either solitary or in cocktail combinations, administered to murine colitis-associated cancer models decreased the bacterial load, suppressed inflammation, and impeded tumorigenesis in a drug-specific manner [23]. This suggests that abnormal bacterial colonies can increase tumorigenesis and may be regulated by antibiotics.

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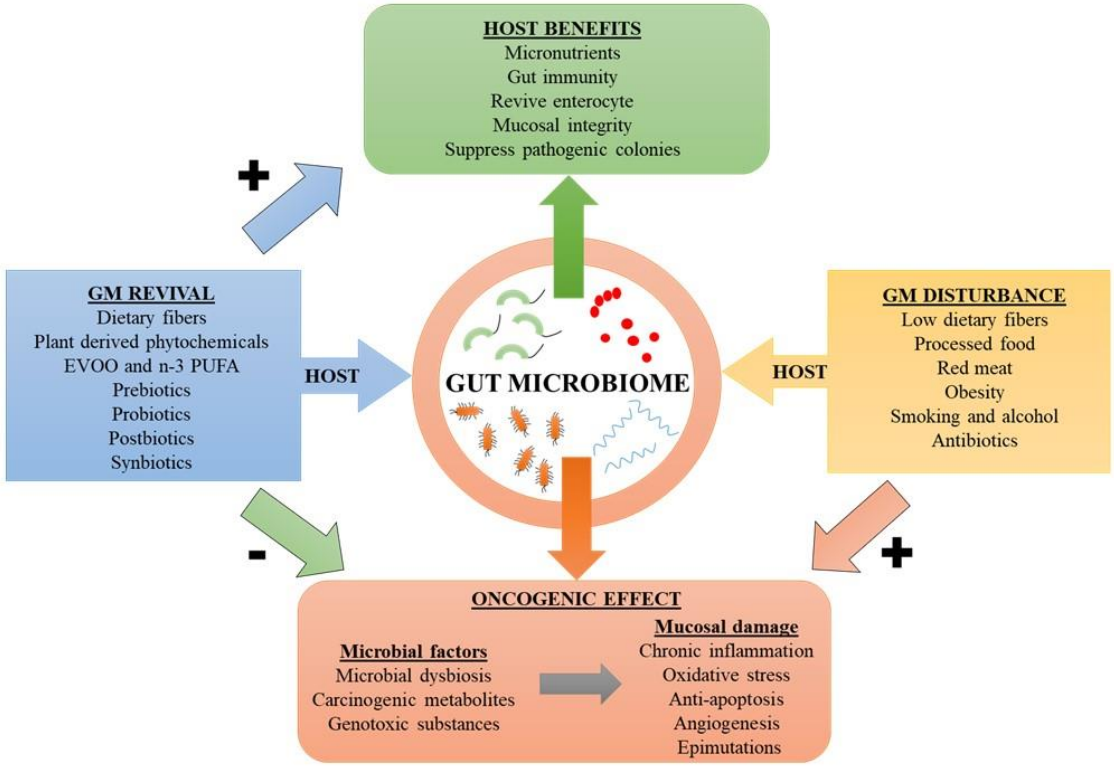

Figure 1. A host diet-benefit model showing the relation between host factors affecting the gut microbiome which can induce oncogenic changes.
Abbreviations: EVOO: extra virgin olive oil; n-3 PUFA: omega-3 polyunsaturated fatty acid. Symbols: Positively associated (+); Negatively associated (-)

Streptococcus gallolyticus subspecies gallolyticus is one such bacteria that is highly implicated in CRC. This bacterial species produces a special protein coded within the type VII secretion system and is noted for its attachment to HT29 colon cancer cells and subsequently inducing proliferative changes. Deletion of the secretion system suppresses the protein expression related to bacterial attachment to the HT29 cells in vitro and decreases Streptococcus gallolyticus subspecies gallolyticus colonization in murine in vivo colon cancer models [24]. This suggests that bacterial proteins produced by selective species can potentially exhibit pro-tumorigenic effects. Similar effects could be responsible for decreased CRC development among type 2 diabetics consuming metformin. This observation is suggested by changes in the gut microbiome consisting of increased number of colonies of Bacteroides, Prevotella, and Bifidobacterium, whereas decreased number of colonies of Firmicutes and Lactobacillus, after starting metformin treatment [25].

Five species of microbes are typically associated with CRC: Bacteroides, Streptococcus, Achromobacter, Alistipes and Fusobacterium [26]. Fusobacterium, a passenger strain from the oral cavity, has been identified with advanced and serrated forms of CRC, mainly localized to the right colon [27]. The abundance of Fusobacterium nucleatum is affected by a number of environmental factors including smoking, chronic periodontitis, and uncontrolled type 2 diabetes [28]. Metformin treatment is associated with reduction in Fusobacterium growth. In APCmin/+ colon cancer mice models, metformin suppressed the tumor growth induced by F. nucleatum colonization [26]. Yu et al. reported that Fusobacterium nucleatum directly targeted the TLR4-MYD88 (toll-like receptor 4-myeloid differentiation primary response 88) axis of the innate immune system to activate autophagy. Autophagic activity mediated by enhanced ULK1 (unc-51 like autophagy activating kinase 1) and ATG7 (autophagy related 7) expression supported cell

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survival and alleviated chemotherapy-induced cytotoxicity [29]. This suggests that Fusobacterium is intricately involved in propagating and sustaining the growth of CRC. Wang et al. in 75 CRC samples identified characteristic taxonomical variation in the tumor niche which showed greater abundance of Eubacterium rectale as a potential 'driver' organism. Eubacterium rectale initiated chronic inflammation by activating downstream NF-κB signaling, which imitates chemokine and cytokine production [30]. Upregulation of NF-κB signaling pathway in CRC has shown to promote cancer growth by inducing cell proliferation, angiogenesis, inflammation, metastasis, and drug resistance [13]. Collectively, these mechanisms demonstrate that pathogenic microbe-induced inflammation can trigger potential oncogenic pathways.

Certain pathogenic microbes present in the proximal colon of CRC patients can also induce biofilm formation which is associated with pro-malignant potential [27]. In familial adenomatous polyposis (FAP), which is a precursor lesion to CRC, colonization and invasion of the intestinal mucosa by carcinogenic toxin producing Escherichia coli and Bacteroides fragilis were associated with the formation of biofilms. Cocolonization of toxigenic Escherichia coli and Bacteroides fragilis into FAP model mice resulted in enhanced colonic inflammation and tumorigenesis. This suggests that toxigenic bacterial strains can enhance the progression of benign colonic lesions to malignant CRC. Therefore, this evolving evidence promotes the notions that gut microbial crosstalk with the colon mucosa, restoration of a healthy microbiome, and maintenance of microbial richness are essential for CRC prevention.

3. The Influence of Diet on Gut Microbiome and Colorectal Cancer Development

The necessary ingredients in the diet fuel the bacterial metabolism which not only aids in digestion, but also synthesizes the byproducts that have immense functional significance to the host. However, when this balance is impaired, nutrition-mediated toxic metabolites are generated by the gut microbes which have cytotoxic and genotoxic effects with oncogenic potential. Moreover, a diet along with prebiotics and probiotics can influence the richness of microbiome by enhancing microbial diversity and nurturing the exiting flora. Thus, the quality of diet delivered to the gut microbiota may be crucial for optimum health benefits. In the current era of highly processed food consumption, adulteration, and food contamination, the gut biodiversity and chemical composition is profoundly affected, leading to chronic colonic inflammation which increases the risk for CRC [31,32].

Dietary factors such as higher levels of red meat, processed meat, refined sugar, alcohol, and harmful fatty acids as well as lower levels of dietary fibers have been suggested as contributing factors to unhealthy microbiome and their metabolites induce mutagenic changes [33]. Both red meat and processed meat have been suggested as potential risk factors for CRC because they alter the composition of gut microbiome [16]. Under the influence of fecal inoculum, in vitro studies demonstrated that pork cooked at higher temperatures leads to carcinogenic O6-carboxy-methylguanine DNA adduct formation which increase the risk of CRC.[34]. In experimental rats, the heme iron of red meat reduces the number of operational taxonomical units (OTUs) in the colonic lumen indicating a reduction in the flora. Firmicutes and Deferribacteres were specifically lowered, whereas Bacteroidetes and Proteobacteria counts were increased. Heme iron increases luminal lipid peroxidation, aldehydes, and ROS, leading to cytotoxic and genotoxic effects on colonic epithelium [35]. Similarly, in a colitis mouse model fed with heme iron Constante et al. reported Firmicutes depletion and Proteobacteria overgrowth. These mice had exacerbation of dextran sodium sulfate (DSS)-induced colitis and subsequently formed adenomas [36]. However, clinical epidemiological support for this pathway. A cohort study of over 48,000 women in Canada showed no association between iron, heme iron, or iron from meat and colorectal cancer [37].

Red meat contains higher levels of N-glycolylneuraminic acid (Neu5Gc) which gets incorporated into cancer cell surface glycans and triggers immunologically mediated in-

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flammation. Neu5Gc rich diet modifies the microbial composition of the gut with higher levels of Clostridium and Bacteroides species which efficiently express sialidases that release the mucopolysaccharide from the glycans [38]. Although it is unclear if this association is causation, bacterial sialidases may prove protective for red meat consumers by potentially reducing Neu5Gc triggered inflammation. Firmicutes, Bacteroidetes, and Proteobacteria phyla also produces enzymes, such as beta-glucuronidase and glycerol/diol dehydratase, which can metabolize heterocyclic amines from red meat into less toxic products, thus proving useful in CRC [39].

Despite the data suggesting that red meat may contribute to carcinogenesis via microbiota alterations, the overall balance of the literature shows that this association is weak at best. A rigorous review of 35 prospective studies showed minimal association between red meat and colorectal cancer with most relative relatively risks below 1.50 and not statistically significant [40]. An alternative hypothesis is that specific combinations of foods may have a detrimental impact on the microbiome. Rats concomitantly fed both red meat and high amylose-resistant starch shift the gut metabolism from protein to carbohydrate fermentation with modulation of gut microbial composition involving mainly Ruminococcus bromii, Bifidobacteriales, Turicibacteraceae and Lactobacillaceae. This change in taxonomical traits were associated with reduced expression of pro-oncogenic miR17-92, protective against CRC [41]. Functional foods such as processed meat fortified with polysaccharide inulin increases the abundance of anti-inflammatory and fiber fermentative Blautia genus which increase SCFAs like propionate and butyrate [42]. This has shown to reduce colonic polyps in experimental rats possibly due to the anti-inflammatory effects. Alternatively, shifting to a fish-inclusive vegetarian diet might have potential benefit over a standard western diet [43]. Collectively it can be postulated that consumption of certain food combinations may be more toxic than others for the colonic epithelium and increases the risk for CRC development.

Dietary constituents significantly modulate chronic inflammation by regulating the immune response. Liu et al. reported that CRC subjects who consumed food with inflammatory potential were positive for Fusobacterium nucleatum in their cancer biopsies. suggesting that healthy diet is a valuable key to a healthy colon [44]. Moreover, consumption of whole grains and dietary fiber rich prudent diets decrease the risk of developing F nucleatum-positive CRC [45]. Fermented foods such as yogurts are protective to the colonic mucosa and maintain microbial diversity, which reduces the risk for CRC, especially in the proximal colon [46]. Moreover, yogurts supplemented with lyophilized jabuticaba (Myrciaria jaboticaba) seed extract have strong prebiotic, antioxidant and anti-cancer properties. When these supplements were fed to CRC rat models, the gut microbiota was modulated and increased the cytotoxic effects on colon cancer cells [47]. This suggests that consumption of yogurt or other probiotic rich foods may be a healthy supplement for the gut and its microbial ecosystem.

Antioxidant consumption is very essential for the survival of certain bacterial strains in the GIT. Absence of ascorbic acid, glutathione and uric acids turned out to be lethal for the anaerobic gut bacterial species (Clostridium sporogenes, Clostridium subterminale and Romboutsia lituseburensis) whereas supplementation of these anti-oxidants in controlled aerobic condition resulted in production of protective SCFA such as propanoic, butanoic, isobutanoic and isopentanoic acids [48]. SCFA were excessively synthesized in aerobic conditions as compared to anerobic environment. It can be hypothesized that aerobic conditions in colon will favor higher SCFA production with dietary supplementation of antioxidants. SCFA such as butyrate produced by the anerobic species has a protective effect in CRC [49]. In CRC survivors, consumption of legumes such as navy beans improved the production of useful metabolites in the stool. Gut microbes metabolize the indigestible substrates present in navy beans to synthesize useful metabolites with antioxidants and anti-inflammatory properties [50]. Individuals with diets deficient in dietary fibers, high in processed meat and high in sugary beverages show colonization with sulfur-digesting bacteria which have been associated with an increased risk for distal colon and rectal malignancies [51]. However, the relative risk for CRC in this pop-

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ulation is only 1.43, and those consuming sulfur-metabolizing diet were also more likely to smoke and have a higher BMI.

Glycyrrhiza uralensis polysaccharide (GCP) extracted from licorice impedes tumor growth and metastasis in mice inoculated with murine colon cancer (CT-26) cells. This is achieved by modifying the composition of gut microbiome such as increased level of Enterorhabdus, Odoribacter, Ruminococcaceae_UCG_014, Ruminococcaceae_UCG_010, Enterococcus, and Ruminiclostridium_5 [52]. Similarly, polysaccharides extracted from jujube have been associated with reductions in inflammation in mouse colon cancer models, due most likely to an associated decrease in Firmicutes and Bacteroidetes taxa in the gut flora [53]. Similarly, combinations of Ganoderma lucidum polysaccharides and Gynostemma pentaphyllum saponins decreased colonic inflammatory and precancerous changes in APCmin/+ mice. Together they altered the microbial richness by increasing SCFA-producing microbes and decreasing sulfate-reducing microbes [54]. This suggests that certain plant and fungi-based products may be effective prebiotics and exert protective effects on the colonic epithelium.

Alcohol consumption is associated with alteration of the gut flora that potentially accelerates CRC carcinogenesis. Alcohol is metabolized by the gut microbiota to toxic intermediates leading to colonic carcinogenesis via formation of DNA-adducts, oxidative stress, epimutations, loss of epithelial barrier function, and immunomodulation [55]. This effect can be potentiated and aggravated by poor nutrition and chronic smoking status; covariates commonly associated with alcohol consumption. The microbiota in alcoholics have decreased dominant obligate anaerobes such as Bacteroides and Ruminococcus and increased Streptococcus taxa [56]. Integrated analysis using 16S rRNA data and epidemiological characteristics by Kim et al. revealed that alcohol consumption increased Fusobacterium OTU levels in gut [57]. Among alcoholics, deficiency of obligate anaerobe OTUs was demonstrated through decreased production of acetaldehyde in formed stool when treated with specific quantities of ethanol under experimental conditions. This suggested that restriction of alcohol can potentially prevent colonic mucosa from genotoxic insults.

4. The Effects of Dietary Interventions on Colorectal Cancer

Dietary fibers provided by plant-based diet are not digested by the human intestinal enzymes and reach the lower GIT unchanged. Figure 2 illustrates the effects of dietary factors on the gut microbiome and their impact on CRC development. Colonic bacteria express the enzymes which metabolize and ferment dietary fibers into useful metabolites such as SCFAs which have roles in decreasing colonic mucosal inflammation and lowering the risk for CRC [58,59]. Butyrate has an inhibitory effect over the histone deacetylases (HDAC) enzymes which results in enhanced expression of genes which arrest the cell cycle [60]. Butyrate also serves as an energy source for normal enterocytes; however rapidly dividing CRC cells are dependent on glycolysis-based metabolism rather than butyrate utilization for energy needs [61]. Co-culturing certain bacterial strains results in enhanced production of butyrate and has extended SCFA-mediated protection in animal models. Faecalibacterium prausnitzii co-cultured with Bifidobacterium catenulatum and supplemented with fructooligosaccharides in anerobic conditions significantly enhanced butyrate production and decreased the release of proinflammatory cytokines such as IL-8 from the HT29 colon cancer cells in vitro. The supernatant from the co-cultured bacteria decreases IL-8 production in DSS-induced colitis mice models as well [62]. More recently, butyrate has also been shown to increase the extracellular tight junction protein complexes in APCmin/+ mice model [63]. This underscores the potential role of butyrate in preventing formation and dissemination of CRC.

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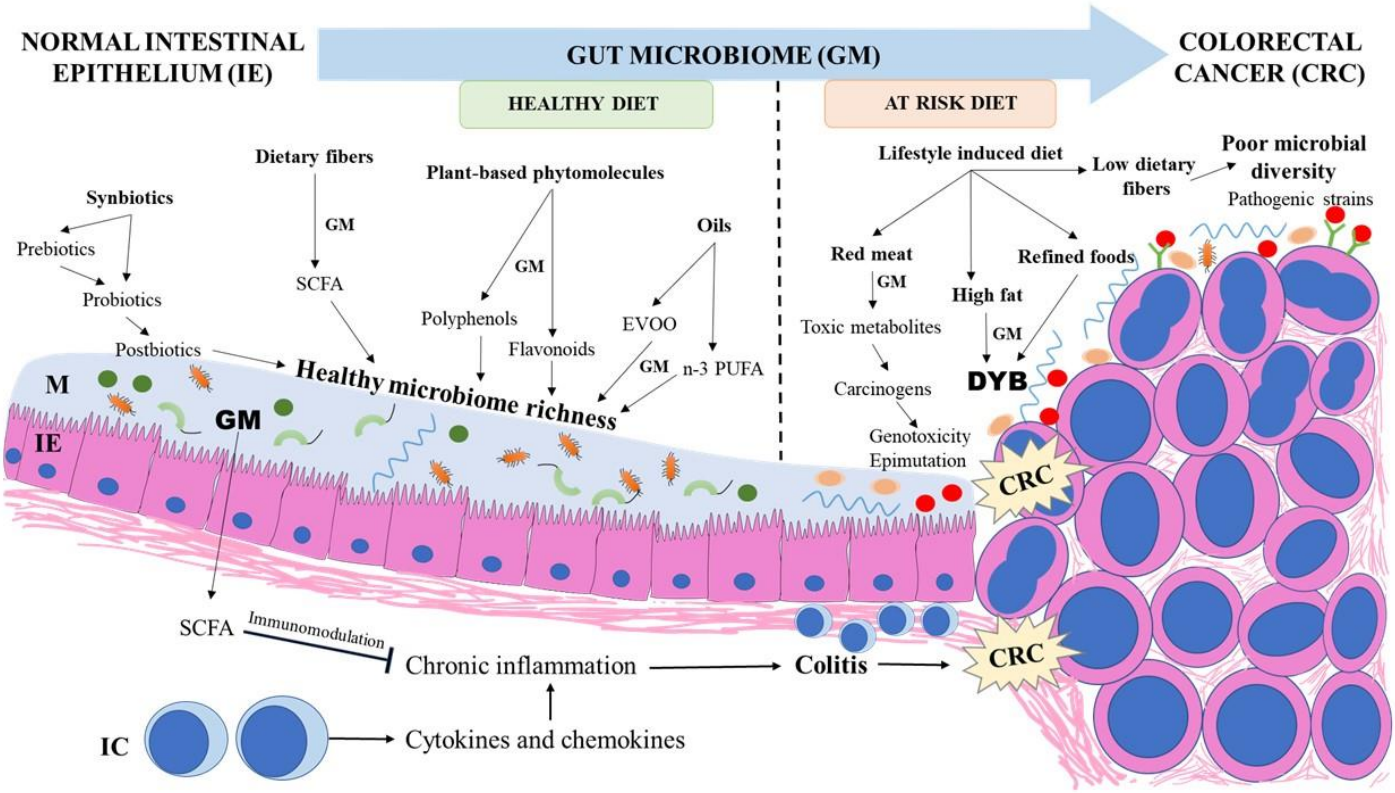

Figure 2. Influence of dietary factors on gut microbiome and its impact on CRC development.
Note: The suspended particles in the intestinal mucus (M) and particles bound to the luminal aspect of CRC tumor represents the gut microbiome (GM) in healthy state or dysbiosis (DYB) respectively.
Abbreviations: CRC: colorectal cancer; DYB: dysbiosis; EVOO: extra virgin olive oil; GM: gut microbiome; IC: immune cells; IE: intestinal epithelium; M: mucus; n-3 PUFA: omega-3 polyunsaturated fatty acids; SCFA: short chain fatty acids

Diet-derived phytochemicals such as polyphenols and flavonoids have protective effects on the colonic mucosa [64,65]. Most of the ingested polyphenols present in plant-based diets and their derivatives reach the colon unaltered and are metabolized by intestinal bacteria to active substances which decrease oxidative stress, inflammation, and tumorigenesis [64]. Polyphenols also act on the gut microbiota to enhance the proliferation of beneficial strains and inhibit pathogenic strains. Polyphenols increases the growth of beneficial butyrate-producing microbiota which inhibit inflammation, while decreasing strains like Lactobacillus and Bifidobacterium which induce colitis and CRC [66]. Polyphenols such epigallocatechin-3-O-gallate and theaflavins present in tea extracts exert anti-inflammatory effects on Fusobacterium nucleatum-induced inflammatory bowel disorders, which are risk factors for CRC [67]. These anti-inflammatory effects are due to the reduction of NF-κB activation that triggers the production of pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α (tumor necrosis factor-α), and CXCL8 (C-X-C motif chemokine ligand 8) in macrophages. Polyphenols present in berries function as prebiotics and improve microbial richness in the form of Bifidobacterium, Lactobacillus and Akkermansia. Berry polyphenols also modulate the production of cytokines which alleviate inflammation and decrease the viability and proliferation of CRC cells [68]. Polyphenols present in mango pulp such as gallotannins and gallic acid exhibit anti-inflammatory effects on the intestinal mucosa. In human subjects, consumption of mango pulp has been shown to decrease pro-inflammatory cytokines such as IL-8, growth-regulated oncogene (GRO), and granulocyte macrophage colony-stimulating factor (GM-CSF). Mango polyphenols increase the abundance of Lactobacillus plantarum, Lactobacillus reuteri, and Lactobacillus lactis as well as increase butyrate levels in

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feces [69]. Date palms, another source of polyphenols and fibers, do not significantly alter gut microbiota or raise SCFAs in healthy volunteers, but decrease genotoxicity, fecal ammonia levels, and increase bowel movements, thereby decreasing the risk for CRC [70]. Similarly, administration of polyphenol-rich green tea extracts to human volunteers results in enhanced Firmicutes to Bacteroidetes ratio and SCFAs producing gut microbes, while decreasing the colonization of oral cavity organisms like Fusobacterium [71]. Thus collectively, polyphenols are natural plant products which may have a potent role in re-shaping the gut microbiome and their regular consumption can stimulate the microbiota to produce active metabolites which prevent CRC tumorigenesis.

Curcumin, a natural product from Curcuma longa plant, is a polyphenol with a significant role in decreasing inflammation, oxidative stress, and gut dysbiosis [72]. Similar to other polyphenols, curcumin is also subjected to bacterial metabolism resulting in production of useful metabolites which have protective effects in CRC. IL-10-deficient CRC mice models fed a curcumin-based diet demonstrated improved gut flora taxonomic profiles such as an abundance of Lactobacillales and deceased levels Coriobacterales. This was also associated with a reduction in tumor size and complete elimination of macroscopic lesions. In addition, there was restoration of β-catenin on plasma membranes. However, there were limited effects on mucosal inflammatory responses [73]. Farhana et al. reported that a combination of essential turmeric oil-curcumin and tocotrienol-rich fraction of vitamin E isomers effectively reduced the proliferation of colon cells (HCT-116 and HT-29 cells) in vitro and suppressed the growth of mice xenograft formed of HCT-116 cells in vivo [74]. The anti-tumor effect was observed with a shift in microbial diversity with a concomitant increase in Lactobacillaceae and Bifidobacteriaceae and Clostridium XIVa which have probiotic and anti-inflammatory actions, and decrease in Bacteroides, Parabacteroides, Lachnospirace, Ruminococcaceae and Firmicutes families. Collectively, this evidence supports the potential role of curcumin in combination with other natural substances in diet in contributing to restricting tumor growth.

Flavonoids are polyphenols abundant in fruits and vegetables and naturally impart their colors due to pigments [75]. The diverse gut microbial flora and associated enzymes convert the flavonoids into bioactive metabolites which result in anti-inflammatory, antioxidant, and anti-tumor effects [75]. Neohesperidin, a flavonoid which is abundant in citrus fruits, imparts tumoricidal activity in APCmin/+ CRC mice models by inhibiting angiogenesis and promoting apoptosis. This effect is achieved through restructuring of the gut microbiota composition as shown by fecal transplantation from neohesperidin-treated mice. Neohesperidin treatment increased Firmicutes and Proteobacteria and decreased Bacteroidetes species [76]. Black raspberry anthocyanins are another group of protective flavonoids that decreased tumorigenesis in colitis-associated CRC model mices by inducing epigenetic changes [77]. Pan et al. reported that consumption of raspberry anthocyanin resulted in a significant increase in anti-inflammatory taxa of Akkermansia and Desulfovibrio as well as butyrate producing Anaerostipes. However, alteration in the microbial composition was achieved only on consumption of whole raspberries [78]. Thus, consumption of flavonoids abundant in a plant-based diet improved microbial richness and effectively decreased CRC growth.

Olive oil, an essential component of the Mediterranean diet, is rich in monounsaturated fatty acids, squalene, phytosterols, and phenols [79]. Phenolic derivatives of some of these nutrients are further metabolized by gut microbiota into active substances that achieve chemoprevention in CRC. Consumption of extra virgin olive oil (EVOO) has a superior effect on the mucosal health when compared to other oils such as coconut and sunflower. In experimental mice models, high-fat diets based on sunflower and coconut oil led to gut microbial dysbiosis with inflammatory changes [80]. Interestingly, EVOO helped in recuperating the gut dysbiosis by increasing the Firmicutes/Bacteroidetes ratio and promoting beneficial microbiota such as Akkermansia growth, while decreasing harmful microbiota such as Enterococcus, Staphylococcus, Neisseria and Pseudomonas. This suggests that diets based on extra virgin olive oil may be beneficial for CRC prevention, compared to other oils. Other lipids such as n-3 polyunaturated fatty acid

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(PUFA) in combination with fermentable dietary fibers have been shown to regulate critical pathways related to programmed cell death and epigenetic dysregulation observed in CRC [81]. In a randomized control trial, administration of n-3 PUFA lead to an increase in butyrate-producing bacteria such as Bifidobacterium, Roseburia, and Lactobacillus suggesting that it has role in reducing inflammation and CRC risk [82]. However, it is noteworthy that several lipid signature molecules including PUFA and sphingolipids are altered in the fecal metabolomic profile of the adenoma-carcinoma sequence, which correlated to many species of Firmicutes and Bacteroidetes in the gut microbiome [83]. This suggests that careful selection of lipids in diet, especially EVOO and n-3 PUFA, is necessary for optimizing healthy colonic mucosa.

The combination of prebiotics and probiotics, also known as synbiotics, and their consumption is presumably an active intervention to modulate the gut microbiome in preventing CRC. This works by enriching the gut microbiome and the microbial strains which protect the intestinal mucosa by decreasing inflammation, uncontrolled proliferation, immune responses, production of toxic metabolites, and oxidative stress [84]. In an experimental in vitro chip-based model (HuMiX gut-on-a-chip), synbionts (consisting of Lactobacillus rhamnosus Gorbach-Goldin strain) have been shown to selectively capacitate the microbes that downregulate oncogenic signaling pathways (in Caco-2 cells). They also enhanced lactate production and drug resistance in colon cancer-derived cells, while increasing acetate and formate levels [85]. A new symbiotic combination of Lactobacillus gasseri 505 and Cudrania tricuspidata leaf extract in fermented milk has been shown to decrease Staphylococcus and increase Lactobacillus, Bifidobacterium, and Akkermansia in the gut microbiota, thus increasing protective effects in DSS/azoxymethane (AOM) induced colitis-CRC model mice. This in vivo intervention decreased tumor proliferation and inflammation (marked by decreased levels of TNF-α, interferon (IFN)-γ, IL-1β, IL-6, inducible nitric oxide synthase and cyclooxygenase-2) and lead to upregulation of anti-inflammatory cytokines IL-4 and IL-10 [86]. Praveen et al. developed raindrop candy consisting of polysaccharides extracted from Indian seaweed (S. wightii, E. compressa, and A. spicifera) and probiotic species L. plantarum NCIM 2083. These seaweed polysaccharides demonstrated anti-cancer effects on RAW 264.7 macrophage and HT-29 human colon cancer cell line in vitro [87]. Thus, synbiotics could be novel therapeutic measures to strengthen the gut microbiome and potentially mitigate CRC by alleviating inflammation and preventing tumorigenesis. Consumption of dietary fibers and diet-derived factors such as phytochemicals and essential fatty acids, as well as inclusion of prebiotics, probiotics, and postbiotics may lead to a multi-pronged protective effect against CRC. Therefore, adopting a healthy fiber-based diet consisting of fruits and vegetables could be effective in promoting gut health. Table 1 presents the studies on dietary factors influencing the gut microbiome and its effect on the colonic mucosa and CRC progression.

Table 1. Studies showing the effect of dietary factors influencing the gut microbiome and its impact on the colonic mucosa and CRC progression.

Author	Human/*in vivo*/*in vitro*	Dietary factors or intervention	Influence on gut microbiome	Impact on colon/CRC
Constante et al., 2017	*in vivo*	Heme iron (red meat)	↓ *Firmicutes* ↑ *Proteobacteria*	↑ DSS induced Colitis ↑ Colitis induced adenoma
Fernández et al., 2019	*in vivo*	Processed meat mixed with polysaccharide inulin (Functional food)	↑ *Blautia*	CRC prevention ↑ SCFA production ↑ Anti-inflammatory

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Lagha et al., 2016	*in vitro*	Epigallocatechin-3-O-gallate and Theaflavins (Tea polyphenols)	↓ *Fusobacterium nucleatum*	↓ Inflammation ↓ NF-κB activation
Kim et al., 2020	Human	Mango pulp polyphenols	↑ *Lactobacillus*	↓ Intestinal inflammation ↓ IL-8, GRO and GM-CSF
Gong et al., 2019	*in vivo*	Neohesperidin (Flavonoid)	↑ *Firmicutes* ↑ *Proteobacteria* ↓ *Bacteroidetes*	↑ Apoptosis ↓ Angiogenesis
Chen et al., 2018	*in vivo*	Black raspberry anthocyanin (Flavonoid)	↑ *Eubacterium rectale* ↑ *Faecalibacterium prausnitzii* ↑ *Lactobacillus*	↓ Tumorigenesis ↓ *SFRP2* promoter methylation
Pan et al., 2017	*in vivo*	Black raspberry anthocyanin (Flavonoid)	↑ *Akkermansia* ↑ *Anaerostipes* ↑ *Desulfovibrio*	CRC prevention
Rodríguez-García et al. 2020	*in vivo*	Extra virgin olive oil	↑ Firmicutes:Bacteroidetes ↑ *Akkermansia* ↓ *Enterococcus* ↓ *Staphylococcus* ↓ *Neisseria* ↓ *Pseudomonas*	↓ Gut dysbiosis ↑ Anti-inflammatory effect
Watson et al., 2018	Human	n-3 PUFA	↑ *Bifidobacterium* ↑ *Roseburia* ↑ *Lactobacillus*	CRC prevention (Increase butyrate producers)
Kim et al., 2020	*in vitro* *in vivo*	Fructooligosaccharides	*Faecalibacterium prausnitzii* *Bifidobacterium catenulatum* (Co-culture)	↓ Pro-inflammatory cytokines
Yuan et al., 2018	Human	Green tea extracts (Polyphenols)	↑ Firmicutes:Bacteroidetes ↑ SCFA producers ↓ *Fusobacterium*	CRC prevention
Pluta et al., 2020	*in vivo*	Curcumin (polyphenol)	↑ *Lactobacillales* ↓ *Coriobacterales*	↓ CRC tumor size
Farhana et al., 2020	*in vivo*	Essential turmeric oil-curcumin and vitamin E isomers	↑ *Lactobacillaceae* ↑ *Bifidobacteriaceae* ↑ *Clostridium XIVa*	↓ CRC proliferation ↑ Probiotic action ↑ Anti-inflammatory effect
Greenhalgh et al., 2019	*in vitro*	Dietary fiber	*Lactobacillus rhamnosus* Gorbach-Goldin (Probiotic)	CRC prevention ↓ Oncogenic pathways

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				↓ Lactate production ↓ Chemoresistance
Mehta et al., 2017	Human	Prudent diet (Whole grain and dietary fiber)	↓ *Fusobacterium nucleatum*	↓ CRC risk
Oh et al., 2020	*in vivo*	Cudrania tricuspidate extracts in fermented milk (Prebiotic)	*Lactobacillus gasseri* 505 (Probiotic)	↑ *Lactobacillus* ↑ *Bifidobacterium* ↑ *Akkermansia* ↓ Inflammatory cytokines ↑ Anti-inflammatory cytokines
Li et al., 2019	Human	Childhood calorie restriction	↓ *Fusobacterium nucleatum*	↓ CIMP and ↓MSI which influences prognosis of CRC
Sobhani et al., 2019	*in vivo*	Fecal microbiota transplantation (of CRC subjects to germ free mice)	↓ Coprococcus ↑ Bacteroides	Enhanced DNA mutation and hypomethylation involving genes of pro-oncogenic W*nt* and N*otch* pathway in mice
Alrafas et al., 2020	*in vivo*	Resveratrol (plant stilbenoid)	↑ SCFA (butyrate and iso-butyrate) producers	CRC prevention ↓ HDAC ↑ Foxp3 ↑ Treg cells and IL-10 ↓ Th1 and Th17 cells

Abbreviations: CIMP: CpG island methylator phenotype; CRC: colorectal cancer; DSS: dextran sulfate sodium; Foxp3: forkhead box P3; GM-CSF: granulocyte-macrophage colony-stimulating factor; GRO: growth-regulated oncogene; HDAC: histone deacetylase; IL-8: interleukin-8; MSI: microsatellite instability; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; PUFA: polyunsaturated fatty acids; SCFA: short chain fatty acid; *SFRP2*: secreted frizzled related protein 2; Symbols: Enhanced (↑); Reduced (↓)

5. The Diet-Gut Microbiome-Epigenetics Axis

Cancer is triggered by a multitude of factors that destabilize the genetic regulatory mechanisms controlling the cell proliferation events. Apart from mutations occurring in the tumor suppressor genes or protooncogenes leading to either loss or gain of resulting protein function, epigenetic changes also transform the transcriptomic profile and the genomic landscape resulting in CRC oncogenic traits (Figure 3). Epigenetic dysregulation, otherwise known as 'epimutations', commonly occur by promoter methylation/demethylation of CpG islands, histone acetylation/deacetylation or by non-coding RNA such as miRNA which alter the expression of genes involved in cellular growth, differentiation, and metabolism [88]. The gut microbiome is unique in the sense that it carries millions of genes which execute functions exotic to the human genome and their metabolic activities depend on the substrate present to them by the host diet, thus estab-

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lishing a symbiotic relationship. However, this symbiosis comes at a cost as impaired nutrition can result in the synthesis of harmful metabolites which potentiate the host genomic architecture's susceptibility to genotoxicity [88].

Figure 3. The host-gut microbiome influencing the CRC associated epigenetics. The resulting gut microbial metabolites can induce pro-oncogenic or onco-suppressive effects on CRC by modulating epigenomics.
Abbreviations: CRC: colorectal cancer; *FFAR2*: free fatty acid receptor 2; FMT: fecal microbiota transplantation; GM: gut microbiome; ncRNA: non-coding RNA; SCFA: short chain fatty acids; SFPR2: secreted frizzled related protein 2; *TET3*: ten eleven translocation 3
Symbols: Enhanced (↑); Reduced (↓); Activation (-)

The SCFAs, bacterial metabolites produced by digestion of dietary fibers by gut microbes, regulate certain epigenetic alterations in enterocytes associated with CRC carcinogenesis [88]. SCFAs such as butyrate protect the genetic and epigenetic architecture of enterocytes by multiple mechanisms [58]. The foremost includes its anti-inflammatory action, whereby it alleviates colonic mucosal inflammation and directly decreases the risk for CRC. Butyrate upregulates the activity of T-regulatory (T-reg) cells which exert an inhibitory effect on pro-inflammatory cytokine production and thereby blocking pro-oncogenic pathways [89]. Butyrate has an inhibitory effect over the HDAC enzymes which results in enhanced expression of genes which arrest the cell cycle [60]. Free fatty acid receptor 2 (FFAR2), which is activated by SCFAs such as butyrate, is known to suppress inflammation and prevent epigenetic dysregulation in CRC. Loss of FFAR2 in DSS/AOM treated APCmin/+ colitis-CRC mice models led to overexpression of HDAC mediated by overactivation of CREB (cAMP-response element binding protein). This resulted in an epigenetic under-regulation of immunomodulating genes such as SFRP1, Dickkopf-related protein 3 (DKK3), and suppressor of cytokine signaling 1 (SOCS1) which were collectively associated with enhanced infiltration of the colonic mucosa and tumor tissue by the neutrophils. The study demonstrated that the epigenetic dysregulation induced by loss of FFAR2 resulted in enhanced colonic inflammation, progressing into adenoma and adenocarcinoma formation [78]. The loss of FFAR2 subjugates the protective immunomodulatory effect of BRB in CRC prevention [78]. This suggests that

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enterocytic expression of functional FFAR2 is important for the beneficial effects of gut microbial metabolites. One carbon metabolism mediated by S-adenosyl methionine (SAM) transfers a methyl group to the CpG islands in the DNA promoter region which affects the gene expression and is of significance in CRC [90]. Thus, bacterial metabolites in the gut also serve as co-factors and epigenetic regulators within the host cell.

The absence of calorie restriction during childhood may negatively impact microbiota composition which may contribute to epigenetic dysregulation and development of CRC later in adulthood [77]. Subjects who were energy restricted during their childhood had decreased abundance of pathogenic species such as Fusobacterium nucleatum, Bacteroides fragilis, and Escherichia coli in later life, compared to non-restricted subjects [77]. Fusobacterium nucleatum is specifically associated with development of genetic and epigenetic defects such as microsatellite instability (MSI) and CpG island methylator phenotype (CIMP), respectively [75]. Similarly, consumption of high caloric foods could lead to histone modifications such as methylation and acetylation at the active enhancers which augments the gene expression pertaining to CRC. Transplantation of colonic microbiota adapted to a high-fat diet into germ-free mice fed on high-calorie diet initiated the reoccurrence of these epigenetic changes [91]. In another experiment, human fecal microbiota transplantation (from CRC subjects) to germ-free mice (treated with azoxymethane, CRC model) resulted in increased rate of DNA mutation and decreased DNA methylation involving the gene families of oncogenic Wnt and Notch pathway, in conjunction with lower abundance of Coprococcus and higher Bacteroides in stools [92]. Sobhani et al. used fecal micriobata transfer techniques to examine differences in mice who receiving micriobiota from human subjects with or without colorectal cancer. With this approach they developed a blood-based cumulative methylation index (CMI) for assessing methylation status from three selected genes WIF1, NPY, and PENK respectively in CRC [92]. It was observed that a CMI>2 had significant correlation with CRC and the associated microbiota significantly composed of microbes from Parvimonas genus [92]. Thus, CMI could be a useful non-invasive tool in analyzing epigenetic derangements associated with increased risk of developing CRC.

Plant-based derivatives and microbiomes together can modulate epigenomic changes associated with CRC. Anthocyanins present in freeze-dried BRB extracts have been shown to induce demethylation of secreted frizzled related protein 2 (SFRP2) promoters, revived by probiotics such as Eubacterium rectale, Faecalibacterium prausnitzii, and Lactobacillus in DSS/AOM colitis-CRC model mice [77]. SFRP2 hypermethylation and subsequent downregulation are highly associated with development of hepatocellular carcinoma and CRC [93]. Gut bacterial dysbiosis activates ten-eleven-translocation 3 (TET3) expression in colonocytes which induces demethylation of lamina-associated domains (LADs) leading to epigenetically programmed tumorigenesis associated with impaired chemotherapeutic response in CRC [94,95]. Resveratrol, a plant based stilbenoid induces changes in the gut microbiome and is associated with an increased production of butyrate and isobutyrate producing taxa, causing release of anti-inflammatory cytokines. This is achieved through resveratrol-induced inactivation of HDAC, which correlated with upregulation of transcription factor forkhead box P3 (Foxp3). This has several immunomodulatory functions, such as concomitant activation of T-regulatory (T-reg) cells, IL-10 synthesis, and reduction in pro-inflammatory Th1 and Th17m cells. This resulted in inhibition of inflammation in association with restoration of gut microbiome thereby reducing the risk of colitis-associated CRC [96]. Lactobacillus reuteri 6475, a commensal and probiotic producing 2-carbon folate metabolite, 5,10-ethenyl-tetrahydrofolyl polyglutamate, biochemically takes part in transfer of 2 carbon atoms from acetate to homocysteine, leading to formation of an exclusive amino acid ethionine, instead of conventional methionine. Incorporation of ethionine instead of methionine in proteins leads to reduced methylation as well as enhanced ethylation of lysine residues in histones [97]. Dietary ethionine can result in immunomodulatory effects by suppressing cell mediated immunity and plausibly by NF-κB inhibition [97,98]. However, ethionine also carries carcinogenic potential, which can be reduced by sup-

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plementing sufficient methionine [99]. Nicotinamide adenine dinucleotide (NAD+) dependent deacetylases such as sirtuin-3 have profound anti-inflammatory and anti-cancer effects. Sirtuin-3 knockout mice showed pro-tumorigenic effects marked by depressed levels of pro-apoptotic caspase 3, together with upregulated p38, and chloride voltage-gated channel 4 (CLCN4), which is possibly caused by abundance of infective gut microbes, Escherichia and Shigella dysenteriae [100]. This suggests that consumption of certain plant-based extracts and probiotics may help to prevent epigenetic alterations associated with CRC.

Finally, the non-coding RNA are also products of the genetic machinery which regulate gene expression in CRC [88]. Yuan et al. reported 76 differentially expressed microRNAs (miRNAs) in tumor samples of which 55 were upregulated and 21 downregulated. miR-182, miR-183, miR-503, and the miR-17~92 clusters were among the most consistently overexpressed miRNA in CRC [101]. Genus Blautia reciprocally correlated with miR-20a, miR-21, miR-96, miR-182, miR-183, and miR-7974, while positively correlated to miR-139, which is significantly expressed in normal tissues [101]. However, enrichment analysis has shown that Akkermansia is the only genus associated with miRNA, which is linked to CRC pathway [101]. This suggest that CRC dysbiosis often changes expression profiles of miRNA linked to cancer pathway. In the case of Fusobacterium nucleatum, selective downregulation of miRNA such as miR-18a and miR-4802 has shown to activate autophagy, inhibit apoptosis, and induce chemoresistance in HCT116 and HT29 CRC cells [29]. miR-18a and miR-4802 post-transcriptionally regulate the expression of pro-autophagic proteins ULK1 and ATG7. However, Fusobacterium nucleatum did not correlate significantly with miR-31 expression which was previously shown to be upregulated in CRC with BRAF mutation [102,103]. Therefore, Fusobacterium nucleatum-associated CRC plausibly has a key miRNA profile related to its pathogenesis.

The gut microbiome is also an enormous source of lipopolysaccharides (LPS) which are immense activators of inflammation and associated with CRC progression. Exosomal miR-200c-3p notably impedes LPS-induced CRC invasion and migration by targeting zinc finger E-box-binding homeobox-1 (ZEB-1) as well as induces apoptosis in HCT116 cells in vitro [104]. Tarallo et al. reported altered bacterial small RNA (elevated in E. coli and low in Bacteroides ovatus) profile in stools of CRC subjects showing bacterial dysbiosis [105]. Stool samples from CRC patients also showed dysbiosis, characterized by abundance of Alistipes putredinis species and Firmicutes phyla. Across human ncRNA, miR-378a-3p and piR-11481 were the most differentially expressed miRNA and small ncRNA, respectively. Thus, it is suggestive that non-coding RNA expression affecting CRC pathogenesis correlates with the composition of the gut microbiome. The microbial dysbiosis pertaining to epigenetic landscape of CRC is highly dependent as well as regulated by our dietary pattern [58]. Therefore, diet has an important role in repopulating the gut microbiome and thereby modulating the epigenetic events.

6. Conclusion

Emerging evidence suggests a significant association between the gut microbiome and colorectal cancer. As a result, dietary constituents such as phytochemicals, essential fatty acids prebiotics, probiotics, and postbiotics may offer benefits in the prevention of CRC through favorable alterations in the gut microbiome. More specifically, dietary and lifestyle factors may enrich the growth of healthy microbes and suppressing the non-beneficial strains. Beneficial strains of gut microbiome produce enterocyte-friendly metabolites such as SCFAs which may protect the mucosa against inflammation and induction of oncogenic pathways. At this time prospective data examining this anti-cancer approach is lacking. Future studies should examine the microbiome impact of dietary risk factor modification in patients at high-risk for CRC.

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