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REVIEW ARTICLE

Can we change our microbiome to prevent colorectal cancer development?

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ABSTRACT

Background. Colorectal cancer represents an important disease as one of the major causes of death worldwide. Although a lot of genetic and epigenetic research has been conducted, all the pieces of the puzzle of colorectal cancer carcinogenesis have not yet been identified. New recent data has highlighted that gut microbiota could have an influence on colorectal carcinogenesis. Gut microbiota represents the microbe population living in the human intestine and contains tens of trillions of microorganisms.

Material and methods. A systematic search in Medline and PubMed for studies reporting the influence of gut microbiota and inflammation on patients with colorectal cancer was made.

Results. In this review we discuss many of the specific bacteria, as well as their metabolites which may have an important role in development of colorectal cancer. Furthermore, we emphasize the molecular mechanisms modulated by gut microbiota, which promote inflammation, toxic metabolites, DNA damaging and pro-carcinogenic compounds, as support for colorectal carcinogenesis. The interrelation between microbiota and inflammation is complex because bacteria and inflammation could mutually impact upon each other. In this context, both endogenous and exogenous miRNAs may have an important role to modulate tumor-related inflammation in colorectal cancer.

Conclusions. Better understanding of the role of gut microbiota in colorectal carcinogenesis could provide promising new directions to improve both prevention and treatment of colorectal cancer. Moreover, the discovery of novel biomarkers in the gut microbiome in order to detect colorectal cancer in an early stage or even in a precancerous stage is of outmost importance.

Colorectal cancer (CRC) represents third common malignancy and one of the considerable causes of death [1–3]. The etiology of CRC is multifactorial and has been correlated with diet, genetic alterations, inflammatory processes, and recently with gut microbiota. Epidemiological data indicate that inflammation plays a significant role in the development and progression of CRC. Maintenance of healthy of intestinal epithelia is critical to provide optimal nutrient absorption, as well as an efficient immune barrier. The balance between intestinal microbiota, intestinal epithelium and host immune system is decisive for normal functionality of the intestinal cells. Therefore, changes in any of these three factors may influence the functionality of the intestinal epithelium [4]. Structural alterations of gut microbiota have been shown to follow the gradual development of CRC. Different events, such as infection, diet, stress, inflammation may influence microbial composition, determining the formation of a dysbiotic microbiota, with direct influence on colon health state. Increasing evidence [5–7] indicates that the collective activities of the resident gut microbiota, particularly their metabolic products, strongly influence

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protection against, and predisposition to, the development of CRC. In this review we discuss recent studies that illustrate the role of gut microbiota, recent assumptions on the mechanisms involved in the bacteria-mediated carcinogenesis and the role of inflammation in CRC development.

Human intestinal microbiota and its influence for colorectal cancer development

Gut microbiota represents the name given today to the trillions of microorganisms living in human intestine, covering at least 1000 different species of known bacteria with more than 3 million genes [8]. The formation of gut microbiota begins after birth when the newborn's digestive system is populated by microorganisms from the mother, the location in which the birth takes place and other factors. From the third day, the composition of the microbiota is b ased on how the infant is fed: gut microbiota of breastfed babies' is mainly dominated by *Bifidobacterium*, whereas *Enterococci* prevail in formula-fed infants [9]. It has been suggested that Firmicutes and Bacteroidetes account for more than 90% of the known phylotypes of the human gut, while Proteobacteria, Verrucomicrobia, Fusobacteria, Actinobacteria, Spirochaetes and Lentisphaerae are present in a proportion of less than 1% and up to 15% [10–12]. Moreover, it seems that gut microbiota in children from rural Africa and Europe has considerable variation in microbial structure probably a result of different diet habits. The principal difference was that rural Africa children had a significant enrichment in Bacteroidetes and reduction in Firmicutes compared to European children [11,13].

The benefits of the body in relation to gut microbiota are related to extraction of the energy from the fermentation of undigested carbohydrates and from the absorption of short-chain fatty acids. Butyrate is the most important of these fatty acids being metabolized by the colonic epithelium and is the favorite energy source of colonocytes [14]. The most important bacteria producing this fatty acid are *Faecalibacterium prausnitzii*, which belongs to the Clostridium leptum cluster, and *Eubacterium rectale*/*Roseburia* spp., which belong to the Clostridium coccoides [15]. In healthy colonocytes, butyrate hampers apoptosis and further mucosal atrophy [16]. In contradiction, in CRC cells, butyrate has been proved to stimulate differentiation, impede cell proliferation, lead to apoptosis and inhibit angiogenesis [17,18]. Furthermore, butyrate protects human colon cells from DNA damage [19]. Besides butyrate, gut microbiota is also implicated in the constitution of another category of beneficial fatty acids, such as conjugated linoleic acids, having anti-inflammatory and cancer protective properties [20]. Intestinal bacteria also play a role in synthesizing vitamin B and vitamin K, as well as metabolizing bile acids, sterols and xenobiotics [14]. Other recognized functions of gut microbiota include the assistance for colonization resistance versus incoming enteropathogens; the mechanisms involved in this process include: prevention the growth of pathogenic species by competing for nutrition and attachment sites to the epithelium of the colon [21,22], inhibition of pathogen growth through acetate production [23] and promotion of the early development of the gut's mucosal immune system [24,25].

The composition of gut microbiota evolves throughout human life, from birth to old age, and is modulated, temporarily or permanently, by many factors such as dietary components, environment, age, stress, treatment (medical or surgical) and disease [26]. Antibiotic-based therapy represents one of the most important factors with the effect on the composition of the microbiota. This therapy can cause diarrhea which generally is associated with altered intestinal microbiota resulting in enteropathogens overgrowth, loss of mucosal integrity and altered metabolism of vitamins and minerals [27,28]. Gut microbiota's balance can be influenced over the ageing process and, hence, the elderly have significantly different microbiota than younger adults. Aging can influence the gut microbiota structure through age-related physiological processes implicating systemic and local inflammation, and inducing changes in dietary habits and lifestyle [29]. The age-related growing drug intake and the interaction between various medications can also be listed among the potential factors that run changes in the gut microbiota [30]. Enck et al. [31] pointed out that some individual bacterial species (*Escherichia coli, Enterococci* spp.) significantly increased with age, others declined with aging (*Bacteroides* spp.), or were stable along the life duration (*Lactobacilli*).

In one of the previous studies exploring gut microbiota was highlighted that people can be classified into one of three prevalent variants or "enterotypes" according to the abundance of predominant genera which are *Bacteroides*, *Prevotella* and *Ruminococcus* [32]. However, there is a lack of consensus on the analytical basis for enterotypes in the literature [33]. The generality of enterotypes across populations, and the existence of similar cluster types for other body sites, remains to be evaluated. Wu et al. [34] showed that there is a connection between the proportion of each bacterial type and dietary constituents; it seems that *Bacteroides* enterotypes are related to amino acids, animal proteins and saturated fats, constituents typical to Western diet, while *Prevotella* is connected to carbohydrates and simple sugars, suggesting an interconnection with a carbohydrate-based diet more

common of rural societies. Nevertheless, people whose microbiota are mainly *Bacteroides* and commute their dietary patterns to a diet based on high proportions of carbohydrates, will acquire a *Prevotella* enterotype in the long term [34]. Substantial changes in the composition of fecal microbiota are also detectable in a few days after the carbohydrate intake, such as the increasing of Firmicutes and Actinobacteria [35,36]. Numerous studies indicate that fruit, vegetable and a high-fiber intake, particularly of cereals and whole grains, is associated with a decreased risk of CRC [37–39]. However, diets that are rich in red and processed meat, fat and alcohol are associated with an increased risk of CRC [40,41]. The animal-based diet causes higher numbers of Bacteroidetes and *Bilophila wadsworthia*, whereas the number of several members of the Firmicutes (*Roseburia*, *E. rectale* and *Ruminococcus bromii*) are decreasing [35]. These findings yield evidence that higher dietary intakes of animal products may modify gut microbiota and consequently play an important role in carcinogenesis.

Overweight/obesity and other metabolic disorders (hyperglycemia, hyperinsulinemia, dyslipidemia, and type 2 diabetes) were found to be important risk factors for CRC [42,43]. A lot of studies have showed the association between an alteration of the dominant phylotypes of bacteria in the gut and body weight [44–46]. Turnbaugh et al. [47] used genetic sequencing to recognize the various strains of bacteria in the gut of obese persons and compared them with lean volunteers; they observed that obese people had more Firmicutes and almost 90% less Bacteroidetes than the lean people.

Specific strains of bacteria, such as *Streptococcus bovis*, *Clostridia*, *Bacteriodes* and *Helicobacter pylori* have been involved in the pathogenesis of cancer [48–50]. The best known connection is the one between *S. bovis* bacteremia and CRC, recognized by McCoy and Mason in 1951 when they first reported a case of endocarditis, probable from *S. bovis*, associated with a cecum carcinoma. Ever since, the connection between *S. bovis* septicemia and CRC has been certified by various other case reports and case-control studies [51–53]. Furthermore, an importantly higher fecal portage of *S. bovis* has been reported in patients with CRC as compared with control subjects [48]. Likewise, CRC microbiota is enriched in pro-inflammatory opportunistic pathogens, such as *Fusobacterium*, *Enterococcaceae* and *Campylobacter* [54–56], and depleted in microbial strains strategic to preserve the intestinal homeostasis, such as *Bifidobacterium longum* [23,57]. Interestingly, probiotic bacteria like *Lactobacillus* and *Bifidobacterium* have anticarcinogenic effects by inactivating microbial enzymes which are important for pro-carcinogen activation [58]. For example, *L. casei* and *L. acidophilus* can decrease the activity of β-glucuronidase, azoreductase, and nitroreductase [59]. In addition, other *Lactobacillus* and *Bifidobacterium* can inhibit DNA damage and tumorigenesis induced by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), 1,2-dimethylhydrazine (DMH), azoxymethane (AOM) and heterocyclic amines [60–62]. This balance of activation and detoxification suggests that microbial community structure play a significant role in the initiating step of carcinogenesis.

The relationship between the gut microbiota and CRC opens new approaches about cancer prevention. Therefore, a favorable modulation of the gut microbiota structure might represent an important strategy to reduce the risk of CRC development. At the present time, there are high quality experimental studies that yield the scientific evidences for the clinical use of probiotic in the prevention of CRC. Apás et al. [63] reported that the probiotic supplement was able to modify microbiota structure by reducing enterobacteria like *Salmonella*/*Shigella* and increasing lactic acid bacteria and *Bifidobacteria*, which are the most common types of microbes used as probiotics. In the same study, the probiotics consumption was associated with a 10-fold reduction of fecal putrescine and a diminution of 60% mutagen fecal concentration, indicating the protective role of the probiotics. Furthermore, Matsumoto et al. [64] showed that the consumption of *Bifidobacterium lactis* LKM512 yogurt reduces gut mutagenicity by increasing gut polyamine contents in healthy adult subjects.

Mechanisms of colorectal cancer modulated by gut microbiota

Gut microbiota may promote CRC genesis and progression by different mechanisms, such as the induction of a chronic inflammatory state, the production of toxic metabolites, DNA damaging and the transformation of dietary heterocyclic amines to pro-carcinogenic compounds.

The role of chronic inflammation in CRC progression

Inflammatory bowel diseases (IBD) are a major risk factor for the development of colon cancer, by a mechanism called in literature colitis-associated cancer (CAC). The increase prevalence of CAC in IBD patients is influenced by disease severity and duration, and by efficacy of anti-inflammatory therapies [65–67]. It seems that IBD are induced and preserved by divers' microorganisms and frequently involves signs of global dysbiosis, according to changes in the number, diversity and stability of microbiota. Increasing evidence shows that dysbiosis induces the production of geno-

toxins and metabolites associated with tumorigenesis and produces disorder of the immune response which promotes and maintains inflammation in IBD leading to CRC [68]. Microorganisms frequently found in IBD patients include different species of *E. coli*, species of *Chlamydia*, *Mycobacterium*, *Clostridia*, *Candida*, as well as *Proteus mirabilis*, *Klebsiella pneumonia* and diverse *Proteobateria*, including *Helicobacter* [69,70]. However, normal colonic components, such as Firmicutes and Bacteroidetes decrease in IBD [71]. In addition, Machiels et al. [72] reported a reduction in *Roseburia hominis* and *F. prausnitzii*, both well known butyrate producing bacteria, and this has been linked to disease severity. All these findings suggest that different bacterial species contribute to the pathogenesis of IBD. IBD, the fundamental condition of CAC development, is associated with enhanced activation of transcription factor NF-κB, which is an important regulator of inflammatory processes [73]. Whereas NF-κB in myeloid cells controls the expression of different inflammatory cytokines and its suppression can improve IBD development [74], it has been hinted that NF-κB dependent cytokines are key agents which signal from inflammatory cells to tumor cells in microenvironment [75,76]. Some of these tumor promoting cytokines, such as tumor necrosis factor alpha (TNF-α) and IL-1, have the capacity to activate NF-κB in malignant or epithelial cells, consequently completing the connection between immune and epithelial NF-κB activation (Figure 1). Furthermore, it has been demonstrated that elevated NF-kB signaling can stimulate mutations in the Wnt pathway, determining the transformation of epithelial non-stem cells into malignant initiating cells [77]. In CAC, mutations that affect the Wnt/b-catenin pathway occur in later stages of the disease, following mutations in the *TP53* and *K-Ras* genes [78]. Another mediator of CAC tumorigenesis is represented by transcriptional factor STAT3 [75]. Grivennikov et al. [75] demonstrated that specific STAT3 ablation in intestinal epithelial cells interferes with tumor formation and tumor growth in a mouse model of CAC. Activation of STAT3 increases tumor cell proliferation, survival and invasion and has dual role in tumor inflammation and immunity by promoting pro-oncogenic inflammatory pathways, including NFκB, IL-6-gp130-Janus kinases (JAK) pathways (Figure 1) [79]. Numerous

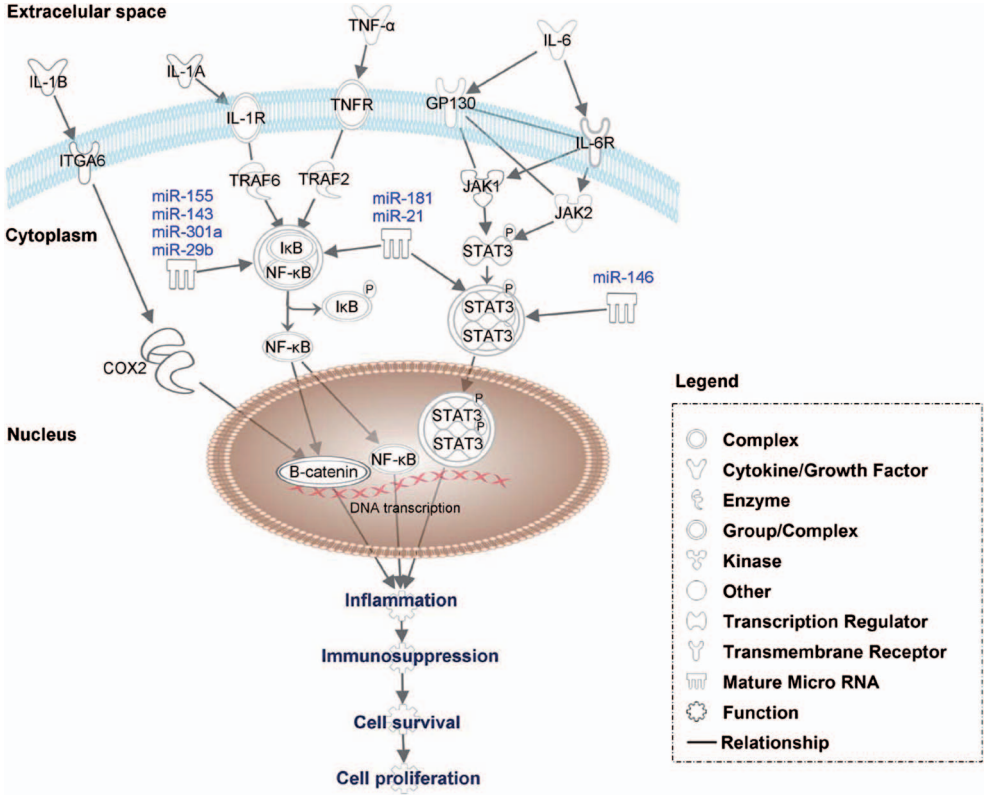

Figure 1. Constitutive activation of pro-oncogenic inflammatory pathways including STAT3, NF-kB and B-catenin by proinflamatory factors such as IL-6, TNF-α, IL-1A and IL1-B. IL-6 activates via tyrosine kinases and cytokine receptors as JAK1 and JAK2 the STAT3, which after dimerization translocate to the nucleus, to directly activate genes involved in inflammation, immunosupression or cell proliferation. TNF-α and IL-1A modulate via TRAF6 and TRAF2 the IkB –NF-kB complex. IKB is released then from IkB –NF-kB complex by phosphorylation and NF-kB translocate to the nucleus where activate specific set of genes involved in carcinogenesis. B-catenin can be activated by IL-1B proinflammatory factor via COX2. Moreover, the expression of STAT 3 and NF-kB can be together or separately modulated by specific miRNAs.

Table I. Tumor-promoting cytokines implicated in colorectal carcinogenesis.

Tumor-promoting cytokines	Mechanism involved in carcinogenesis	References
IL-6	Activation of STAT3	Grivennikov et al. (2009) [67]
	Changing the expression of miRNAs	Ma et al. (2011) [119]
IL-1	Activation of NF-κB	Wang et al. (2009) [70]
IL-1B	COX-2 activation	Kaler et al. (2009) [75]
	Activation of Wnt signaling	Schwitalla et al. (2013) [69]
	Activation of NF-κB	Wang et al. (2009) [68]
	Changing the expression of miRNAs	Ma et al. (2011) [119]
TNF-α	Activation of NF-κB	Wang et al. (2009) [68]
	Inducing DNA damage	Schwitalla et al. (2013) [69]
	Changing the expression of miRNAs	Murata et al. (2012) [74] Ma et al. (2011) [119]
IL-23	Regulate expression of tumor-promoting cytokines, such as IL-6	Grivennikov et al. (2012) [73]

inflammatory cytokines can stimulate STAT3 in epithelial cells, and one of them is IL-6 (Table I). IL-6 is a complex cytokine which plays a major role in IBD development, both in mouse models and in human patients, and its inhibition has been shown efficient to ameliorate IBD [80].

Some of other cancer promoting cytokines include IL-23 and IL-1B. For instance, Grivennikov et al. [81] showed that IL-23 signaling promotes tumor growth and progression. IL-23 is mainly generated by tumor-associated myeloid cells that are probably stimulated by microbial products, which infiltrate the tumors but not the adjacent tissue. In chronic inflammation, proinflammatory cytokines, such as TNF-α, can induce DNA damage through reactive oxygen species (ROS) and nitrogen species, which leads to tumor initiation [82]. IL-1B is involved in COX-2 activation and activates the Wnt cell cycle pathway, the primary pathway of colon cell proliferation [83]. In contradiction, IL-10 and transforming growth factor beta (TGF-β) inhibit carcinogenesis. IL-10 inhibits NF-κB signaling and thus, it can down regulate proinflammatory cytokine expression and act as an antitumor cytokine [84]. TGF-β is a powerful pleiotropic cytokine with immune suppressing and anti-inflammatory properties, inhibiting cell cycle progression and promoting apoptosis [85].

The biosynthesis of bacterial toxins and the production of toxic metabolites

Bacterial toxins have a high capacity to produce CRC. *B. fragilis* toxin is an enterotoxin produced by members of the genus *Bacteroides* which induces proliferation of the colon epithelial cells by activation of the oncogene *c-MYC* [86] and production of IL-8 in colon epithelial cells [87,88]. IL-8 is a member of the neutrophil-specific CXC subfamily of chemokines who can act not only on leukocyte chemotaxis, inflammatory responses and infectious diseases, but also on endothelial cells via their receptors to induce migration, invasion, proliferation and in vivo angiogenesis [89]. Toprak et al. [90], by investigating the prevalence of *B. fragilis* toxin in stool samples from 73 CRC patients and 59 controls reported the enterotoxin gene in 38% of the samples from CRC patients compared with 12% of the samples from the control group. The capacity of the *B. fragilis* toxin producing strains to contribute to colon carcinogenesis is moderated by the enhanced expression of transcriptional factor STAT3 [91]. *B. fragilis* toxin is a metalloprotease that attaches to colonic epithelial cells and stimulates the cleavage of E-cadherin, augmenting intestinal barrier permeability and stimulating cell signaling via the β-catenin/Wnt pathway, which is activated in CRC [86,91].

Recent studies demonstrated that active toxins found in tumor tissue from CRC patients besides *B. Fragilis* toxin are those derived from *E. coli* which is the main aero-anaerobic Gram-negative species of the normal intestinal flora and takes part in supporting the stability of the luminal microbial flora and in maintaining normal intestinal homeostasis. Various studies of CRC patients in the UK and Germany pointed out that mucosa-associated *E. coli* are more often detected in colon tissue from patients with adenocarcinomas than in that of controls [92,93]. Furthermore, Maddocks et al. [94] showed that the colonic mucosa of patients with adenomas and carcinomas has revealed an increased intracellular mucosal portage of *E. coli* compared to healthy controls. Colibactin is a potent bacterial genotoxin produced by particular pathogenic and commensal strains of *E. coli* who determine the DNA damage of gut epithelial cells both in vitro and in vivo, probably influencing the development and progression of CRC [95,96]. The *E. coli* cytotoxic necrotizing factor 1 (CNF1) activates the Rho GTPases, causing dysfunctions in already

changed epithelial cells, as apoptosis, the liberation of proinflammatory cytokines, COX2 expression and NF-κB activation [97]. Furthermore, CNF1 determines inactive cells to gain access to the cell cycle and induce DNA synthesis, interferes with normal cytokines, with the result in the generation of multinucleated cells and in the initiation of aneuploidia [98]. All these arguments are admissible that CNF1 has a role in tumorigenesis [99]. Lately, Buc et al. [100] outlined a high preponderance of genotoxin and cyclomodulin producing mucosa-related *E. coli* strains in CRC patients. Through *E. coli* virulence factors, various toxins, such as cyclomodulins, are attracting attention because they are genotoxic and/or modulate cellular differentiation, apoptosis and proliferation [101].

The role of heterocyclic amines and of N-nitroso compounds in CRC

The human diet is abundant in a multitude of heterocyclic amines (HCA_S). A major source of these compounds occurs in fish and red meat, which can generate HCA_S during cooking [102]. Epidemiological studies have linked consumption of well done meats with an enhanced risk of certain cancers, including CRC [103]. The most important classes of HCA_S consist of amino-imidazo-quinolines known as IQ-type compounds and amino-imidazo-pyridines as PhIP. Cooking methods that contribute to the constitution of HCAs are believed to play a significant role in CRC risk [104]. Secondary bile acids (SBAs), recognized as cancer promoting components could be a part of the problem; they are more hydrophobic and thus more potent at disrupting cell membranes, which is likely to determine the production of ROS via the activation of membrane-associated proteins, such as NAD(P)H oxidases and phospholipase A2 [105]. In high concentrations, SBAs are cytotoxic, leading to oxidative DNA damage and cell death [106]. In addition, SBAs could up-regulate proinflammatory cytokines including NFκβ and could also induce changes of the gut microbiota composition by endotoxin producing species [107].

High protein intake causes an increase in the fermentation of diet-derived protein in the colon, as suggested by the increase level of amino acid-derived products [108,109]. A branch of bacteria, such as several *Bacteroides* spp. and some Firmicutes, ferment aromatic amino acids to produce potentially bioactive products, including phenylacetic acid, phenols, indoles and p-cresol [110]. Some nitrogenous products, especially N-nitroso compounds (NOC), have the potential to promote cancer [111]. NOC are constituted by N-nitrosation of amines and amides, generated primarily in the presence of a nitrosating component by bacterial decarboxylation of amino acids [112]. Joosen et al. [113] found 60 times higher concentration of NOC in the fecal of volunteers receiving processed red meat than in volunteers that followed a vegetarian diet. Therefore, several arguments suggest that NOC may be significant genotoxins. First of all, most NOC, such as nitrosamines, nitrosamides, and nitrosoguanidines, can produce alkylating agents during metabolism, and cause DNA damage. Jacoby et al. [114] showed that N-methyl-N-nitrosurea perfused intrarectally caused G-A transitions in K-ras in 30% of rat with CRC. Likewise, nitrosated glycine compounds reacted with DNA to yield promutagenic adducts including O^6-methylguanine and O^6-carboxymethylguanine [115]. Furthermore, potassium diazoacetate, a stable form of nitrosated glycine, was found to initiate mutations in the p53 gene [116]. This supports the hypotheses that NOC linked to glycine subscribes to p53 mutations in humans, and that O^6-carboxymethylguanine adducts in exfoliated colorectal cells are connected to CRC.

Polyamines are other molecules that are implicated in CRC development. Polyamines are small polycationic molecules found in almost all cells and associated with a wide variety of essential physiological functions, such as the maintenance of the structural integrity of membranes and nucleic acids, gene regulation and translation [117]. The major polyamines putrescine, spermidine and spermine are produced from arginine in host tissues, but polyamine synthesis also appears in gut bacteria [118]. High levels of polyamines are toxic and are associated with several diseases, including cancer, and oxidative stress that results from polyamine catabolism is the underlying mechanism of toxicity [119]. Several pathogens, including *Shigella flexneri*, *Streptococcus pneumoniae*, *Salmonella enterica* and *H. pylori*, utilize polyamines to increase their virulence [117]. Ignatenko et al. [120] reported that polyamine export is down-regulated by loss of wildtype APC and can contribute to elevate colonic polyamines in APC-dependent colon carcinogenesis.

Chronic inflammation can deeply alter local immune response and cause the liberation of nitric oxide (NO) and ROS that may induce DNA damage and therefore alter tissue homeostasis [121]. ROS can be produced by the gut microbiota or generated by immune cells during inflammation. Huycke et al. [122,123] demonstrated that *Enterococcus faecalis* generates hydroxyl radicals in both in vitro and in vivo studies. It seems that *Lactobacilli* and *Bifidobacteria* are able of producing high amounts of NO from nitrite (NO_2), and dietary addition of NO_3^- could expand NO production [124]. ROS are potent mutagens that lead to DNA breaks, point mutations, and protein-DNA crosslinking and could influence chromosomal instability and the risk of CRC [125,126].

The role of miRNAs as modulators of inflammation in CRC

MicroRNA (miRNAs) are small (21-25 nucleotide) non-coding RNAs (ncRNAs) that regulate the translation and stability of their specific mRNA targets [127]. During the last decade, it has become clear that aberrant expression of microRNAs is related to the initiation and progression of CRC. MiRNAs can act as tumor suppressors or oncogenes, depending on the cellular environment in which they are expressed. The expression of miRNAs is reproducibly altered in CRC, their expression profiles being associated with diagnosis, prognosis and therapeutic outcome. Inflammation determines changes in expression of miRNAs, primarily through the actions of proinflammatory cytokines such as IL-1B, IL-6 and TNF-α [128]. Numerous reports have endorsed the role of miRNAs in the initiation and progression of human cancer, as well as their role in immune responses, inflammation, cell proliferation and cell death which are known to be regulated by NFκB [129]. Numerous miRNAs, such as miRNA-155, miRNA-146a, miRNA-143, mi-RNA-301a, miRNAs-17-92, mi-RNA-29b, mi-RNA-21 and miRNA-181 regulate signaling pathways like JAK/STAT, TLR/MyD88, NF-κB, and AkT, which are important for the immune responses (Figure 1) [130–133]. For instance, STAT3 and miRNA-181b-1 expression levels are positively correlated with colon adenocarcinomas [133]. Recent data have shown that in breast cancer the overexpression of miRNA-181b-1 will lead to repression of tumor suppressor gene promoting tumor survival and cell migration through NF-κB activation [134].

As we mentioned above, the gut microbiota interacts directly with the host through the production of metabolites, peptides and other molecules. However, how microbiota regulates miRNA expression and therefore contributes to the maintenance of intestinal homeostasis and to IBD pathogenesis is still largely unknown. Endogenous miRNA may play a significant role in ascertaining how microbiota-produced signals are received by the host, and balance the line between maintaining an effective barrier and preventing inadequate inflammation response to microbiota. There are some studies that have been highlighted the distinctive mechanisms by which miRNAs are produced by components of the microbiota. In one of these studies, Hu et al. [135] identified an important growth regulatory role for colonic epithelial miRNAs in mediating the effects of the microbe-derived short chain fatty acid butyrate on host gene expression. These data could suggest that these miRNAs play a role in colonic carcinogenesis and that their reduction by butyrate is an important mechanism of its anti-cancer effects. Another study concerning the microbiota regulation by miRNAs expression has been outlined by Xue et al. [136] who demonstrated a link between the expression of miR-10a and IL-12/IL-23p40, a significant molecule for innate immune responses to commensal bacteria. The better understanding of relationship between microbiota and miRNAs relating to inflammation in CRC still represent a great challenge.

The interrelation between microbiota and inflammation, as notified, is complex because bacteria and inflammation could mutually impact upon each other. All of the examples illustrated above reveal the variety of mechanisms through which microbial-derived products interact with host and induce CRC. Probably, the main mechanism linking intestinal microbiota and CRC is represented by the appearance of dysbiosis which induces the production of various metabolites and determines disruptions of the immune response which promotes inflammation and lead to CRC.

Conclusions

There is growing evidence that microbiota contributes to colon tumorigenesis. Various bacteria have been correlated with carcinogenesis in animal models or linked with CRC in human studies. The identification of bacteria-derived metabolites that can induce and/or promote CRC development will be important future discoveries that will tremendously influence the prevention and the treatment of this disease. For many years all efforts to treat cancer have focused on the inhibition/destruction of tumor cells. Strategies to modulate the host microbiota and the miRNAs-induced inflammation could offer a complementary perspective. Therefore, more longitudinal microbiota surveys need to be performed, in order to resume the gut microbiota changes over time and its impact on the development of CRC. In the same context, the anti-inflammatory therapy focused on the main regulators of inflammatory responses, STAT3 and NFκB, could be an additional tool in CRC therapy. In addition, because antibiotic treatment causes disturbance of the microbiota, new therapeutic tools, including probiotics, prebiotics and fecal microbiome transplantation, could be safer and natural treatment options to restore the dysbiosis.

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References

[1] Tenesa A, Dunlop MG. New insights into the aetiology of colorectal cancer from genome-wide association studies. Nat Rev Genet 2009;10:353–8.

[2] Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010;127:2893–917.

[3] Boyle P, Ferlay J. Cancer incidence and mortality in Europe, 2004. Ann Oncol 2005;16:481–8.

[4] Kau AL, Ahern PP, Griffin NW, Goodman AL, Gordon JI. Human nutrition, the gut microbiome and the immune system. Nature 2011;474:327–36.

[5] Compare D, Nardone G. The bacteria-hypothesis of colorectal cancer: Pathogenetic and therapeutic implications. Transl Gastrointest Cancer 2014;3:44–53.

[6] Louis P, Hold GL, Flint HJ. The gut microbiota, bacterial metabolites and colorectal cancer. Nat Rev Microbiol 2014;12:661–72.

[7] Arthur JC, Jobin C. The struggle within: Microbial influences on colorectal cancer. Inflamm Bowel Dis 2011; 17:396–409.

[8] Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature 2010; 464:59–65.

[9] Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography. Nature 2012; 486:222–7.

[10] Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature 2012;486:207–14.

[11] Rajilić-Stojanović M, Heilig HG, Molenaar D, Kajander K, Surakka A, Smidt H, et al. Development and application of the human intestinal tract chip, a phylogenetic microarray: Analysis of universally conserved phylotypes in the abundant microbiota of young and elderly adults. Environ Microbiol 2009;11:1736–51.

[12] Tap J, Mondot S, Levenez F, Pelletier E, Caron C, Furet JP, et al. Towards the human intestinal microbiota phylogenetic core. Environ Microbiol 2009;11:2574–84.

[13] De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci USA 2010;107:14691–6.

[14] Koropatkin NM, Cameron EA, Martens EC. How glycan metabolism shapes the human gut microbiota. Nat Rev Microbiol 2012;10:323–35.

[15] Louis P, Flint HJ. Diversity, metabolism and microbial ecology of butyrate producing bacteria from the human large intestine. FEMS Microbiol Lett 2009;294:1–8.

[16] Klampfer L, Huang J, Sasazuki T, Shirasawa S, Augenlicht L. Inhibition of interferon gamma signaling by the short chain fatty acid butyrate. Mol Cancer Res 2003;1: 855–62.

[17] Pryde SE, Duncan SH, Hold GL, Stewart CS, Flint HJ. The microbiology of butyrate formation in the human colon. FEMS Microbiol Lett 2002;17:133–9.

[18] Basson MD, Liu YW, Hanly AM, Emenaker NJ, Shoney SG, Gould Rothberg BE. Identification and comparative analysis of human colonocyte short-chain fatty acid response genes. J Gastrintest Surg 2000;4:501–12.

[19] Ebert MN, Klinder A, Peters WH, Schaferhenrich A, Sendt W, Scheele J, et al. Expression of glutathione S-transferases (GSTs) in human colon cells and inducibility of GSTMs by butyrate. Carcinogenesis 2003;10:1637–44.

[20] Wong CS, Sengupta S, Tjandra JJ, Gibson PR. The influence of specific luminal factors on the colonic epithelium: high-dose butyrate and physical changes suppress early carcinogenesis events in rats. Dis Colon Rectum 2005; 48:549–59.

[21] Neish AS. Microbes in gastrointestinal health and disease. Gastroenterology 2009;136:65–80.

[22] Guarner, F; Malagelada, J. Gut flora in health and disease. Lancet 2003;361:512–9.

[23] Fukuda S, Toh H, Hase K, Oshima K, Nakanishi Y, Yoshimura K, et al. Bifidobacteria can protect from enteropathogenic infection through production of acetate. Nature 2011;469:543–7.

[24] Stecher B, Hardt WD. Mechanisms controlling pathogen colonization of the gut. Curr Opin Microbiol 2011;14: 82–91.

[25] Maynard CL, Elson CO, Hatton RD, Weaver CT. Reciprocal interactions of the intestinal microbiota and immune system. Nature 2012;489:231–41.

[26] Tennyson CA, Friedman G. Microecology, obesity, and probiotics. Curr Opin Endocrinol Diabetes Obes 2008;15:422–7.

[27] de La Cochetière MF, Montassier E, Hardouin JB, Carton T, Le Vacon F, et al. Human intestinal microbiota gene risk factors for antibiotic-associated diarrhea: Perspectives for prevention. Risk factors for antibiotic-associated diarrhea. Microb Ecol 2010;59:830–7.

[28] Iapichino G, Callegari ML, Marzorati S, Cigada M, Corbella D, Ferrari S, Morelli L. Impact of antibiotics on the gut microbiota of critically ill patients. J Med Microbiol 2008;57:1007–14.

[29] Biagi E, Candela M, Fairweather-Tait S, Franceschi C, Brigidi P. Aging of the human metaorganism: The microbial counterpart. Age (Dordr) 2012;34:247–67.

[30] Genser D. Food and drug interaction: Consequences for the nutrition/health status. Ann Nutr Metab 2008;52(Suppl 1):29–32.

[31] Enck P, Zimmermann K, Rusch K, Schwiertz A, Klosterhalfen S, Frick JS. The effects of ageing on the colonic bacterial microflora in adults. Z Gastroenterol 2009;47:653–8.

[32] Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, et al. Enterotypes of the human gut microbiome. Nature 2011;473:174–80.

[33] Koren O, Knights D, Gonzalez A, Waldron L, Segata N, et al. A guide to enterotypes across the human body: Meta-analysis of microbial community structures in human microbiome datasets. PLoS Comput Biol 2013;9: e1002863.

[34] Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science 2011;334:105–8.

[35] David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature 2014;505: 559–63.

[36] Walker AW, Ince J, Duncan SH, Webster LM, Holtrop G, Ze X, et al. Dominant and diet-responsive groups of bacteria within the human colonic microbiota. ISME J 2011;5:220–30.

[37] Aune D, Chan DS, Lau R, Vieira R, Greenwood DC, Kampman E, et al. Dietary fibre, whole grains, and risk of

colorectal cancer: Systematic review and dose-response meta-analysis of prospective studies. Br Med J 2011;343:d6617.

[38] Beresford SA, Johnson KC, Ritenbaugh C, Lasser NL, Snetselaar LG, Black HR, et al. Low-fat dietary pattern and risk of colorectal cancer: The Women's Health Initiative Randomized Controlled Dietary Modification Trial. JAMA 2006;295:643–54.

[39] Terry P, Giovannucci E, Michels KB, Bergkvist L, Hansen H, Holmberg L, et al. Fruit, vegetables, dietary fiber, and risk of colorectal cancer. J Natl Cancer Inst 2001;93:525–33.

[40] Sandhu MS, White IR, McPherson K. Systematic review of the prospective cohort studies on meat consumption and colorectal cancer risk: A meta-analytical approach. Cancer Epidemiol Biomarkers Prev 2001;10:439–46.

[41] Norat T, Lukanova A, Ferrari P, Riboli E. Meat consumption and colorectal cancer risk: Dose-response meta-analysis of epidemiological studies. Int J Cancer 2002; 98:241–56.

[42] Esposito K, Chiodini P, Capuano A, Bellastella G, Maiorino MI, Rafaniello C, et al. Colorectal cancer association with metabolic syndrome and its components: A systematic review with meta-analysis. Endocrine 2013;44:634–47.

[43] Aleksandrova K, Nimptsch K, Pischon T. Influence of obesity and related metabolic alterations on colorectal cancer risk. Curr Nutr Rep 2013;2:1–9.

[44] Collado MC, Isolauri E, Laitinen K, Salminen S. Effect of mother's weight on infant's microbiota acquisition, composition, and activity during early infancy: A prospective follow-up study initiated in early pregnancy. Am J Clin Nutr 2010;92:1023–30.

[45] Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: Human gut microbes associated with obesity. Nature 2006;444:1022–3.

[46] Kalliomäki M, Collado MC, Salminen S, Isolauri E. Early differences in fecal microbiota composition in children may predict overweight. Am J Clin Nutr 2008;87:534–8.

[47] Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006;444:1027–31.

[48] Gold JS, Bayar S, Salem RR. Association of Steptoccocus bovis bacterium with colonic neoplasia and extracolonic malignancy. Arch Surg 2004;139:760–5.

[49] Nakamura J, Kubota Y, Miyaoka M, Saitoh T, Mizuno F, Benno Y. Comparison of four microbial enzymes in Clostridia and Bacteroides isolated from human feces. Microbiol Immunol 2002;46:487–90.

[50] Peek RM, Blaser MJ. Helicobacter pylori and gastrointestinal tract adenocarcinomas. Nat Rev Cancer 2002; 2:28–37.

[51] Klein R, Catalano M, Edberg S, Casey JI, Steigbigel NH. Streptococcus bovis septicemia and carcinoma of the colon. Ann Intern Med 1979;91:560–2.

[52] Goumas P, Naxakis S, Rentzis G, Tsiotos PD, Papadas TA. Lateral neck abscess caused by Streptococcus bovis in a patient with undiagnosed colon cancer. J Laryngol Otol 1997;111:666–8.

[53] Gupta A, Madani R, Mukhtar H. Streptococcus bovis endocarditis; a silent sign for colonic tumor. Literature review. Colorectal Dis 2010;12:164–71.

[54] Zheng J, Meng J, Zhao S, Singh R, Song W. Campylobacter-induced interleukin-8 secretion in polarized human intestinal epithelial cells requires Campylobacter-secreted cytolethal distending toxin- and Toll-like receptor-mediated activation of NF-kappaB. Infect Immun 2008;76:4498–508.

[55] Castellarin M, Warren RL, Freeman JD, Dreolini L, Krzywinski M, Strauss J, et al. Fusobacterium nucleatum infection is prevalent in human colorectal carcinoma. Genome Res 2012;22:299–306.

[56] Kostic AD, Chun E, Robertson L, Glickman JN, Gallini CA, Michaud M, et al. Fusobacterium nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. Cell Host Microbe 2013;14:207–15.

[57] Fanning S, Hall LJ, Cronin M, Zomer A, MacSharry J, Goulding D, et al. Bifidobacterial surface-exopolysaccharide facilitates commensal-host interaction through immune modulation and pathogen protection. Proc Natl Acad Sci USA 2012;109:2108–13.

[58] Geier MS, Butler RN, Howarth GS. Probiotics, prebiotics and synbiotics: A role in chemoprevention for colorectal cancer? Cancer Biol Ther 2006;5:1265–9.

[59] Goldin BR, Gorbach SL. Alterations of the intestinal microflora by diet, oral antibiotics, and Lactobacillus: Decreased production of free amines from aromatic nitro compounds, azo dyes, and glucuronides. J Natl Cancer Inst 1984;73:689–95.

[60] Uronis JM, Mühlbauer M, Herfarth HH, Rubinas TC, Jones GS, Jobin C. Modulation of the intestinal microbiota alters colitis-associated colorectal cancer susceptibility. PLoS One 2009;4:e6026.

[61] Nowak A, Libudzisz Z. Ability of probiotic Lactobacillus casei DN 114001 to bind or/and metabolise heterocyclic aromatic amines in vitro. Eur J Nutr 2009;48:419–27.

[62] Zsivkovits M, Fekadu K, Sontag G, Nabinger U, Huber WW, Kundi M, et al. Prevention of heterocyclic amine-induced DNA damage in colon and liver of rats by different lactobacillus strains. Carcinogenesis 2003;24:1913–8.

[63] Apás AL, Dupraz J, Ross R, González SN, Arena ME. Probiotic administration effect on fecal mutagenicity and microflora in the goat's gut. J Biosci Bioeng 2010;110:537–40.

[64] Matsumoto M, Benno Y. Consumption of Bifidobacterium lactis LKM512 yogurt reduces gut mutagenicity by increasing gut polyamine contents in healthy adult subjects. Mutat Res 2004;568:147–53.

[65] Neumann H, Vieth M, Langner C, Neurath MF, Mudter J. Cancer risk in IBD: How to diagnose and how to manage DALM and ALM. World J Gastroenterol 2011;17:3184–91.

[66] Rubin DT, Cruz-Correa MR, Gasche C, Jass JR, Lichtenstein GR, Montgomery EA, et al. Colorectal cancer prevention in inflammatory bowel disease and the role of 5-aminosalicylic acid: A clinical review and update. Inflamm Bowel Dis 2008;14:265–74.

[67] Grivennikov SI. Inflammation and colorectal cancer: Colitis-associated neoplasia. Semin Immunopathol 2013; 35:229–44.

[68] Tomasello G, Tralongo P, Damiani P, Sinagra E, Di Trapani B, et al. Dismicrobism in inflammatory bowel disease and colorectal cancer: Changes in response of colocytes. World J Gastroenterol 2014;20:18121–30.

[69] Nanau RM, Neuman MG. Metabolome and inflammasome in inflammatory bowel disease. Transl Res 2012;160: 1–28.

[70] Neuman MG, Nanau RM. Inflammatory bowel disease: Role of diet, microbiota, life style. Transl Res 2012;160:29–44.

[71] Blumberg R, Powrie F. Microbiota, disease, and back to health: A metastable journey. Sci Transl Med 2012; 4:137rv137.

[72] Machiels K, Joossens M, Sabino J, De Preter V, Arijs I, et al. A decrease of the butyrate-producing species Roseburia hominis and Faecalibacterium prausnitzii defines dysbiosis in patients with ulcerative colitis. Gut 2014;63:1275–83.

[73] Grivennikov SI, Karin M. Dangerous liaisons: STAT3 and NF-kappaB collaboration and crosstalk in cancer. Cytokine Growth Factor Rev 2010;21:11–9.
[74] Greten FR, Eckmann L, Greten TF, Park JM, Li ZW, Egan LJ, et al. IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. Cell 2004;118:285–96.
[75] Grivennikov S, Karin E, Terzic J, Mucida D, Yu GY, Vallabhapurapu S, et al. IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. Cancer Cell 2009;15:103–13.
[76] Wang S, Liu Z, Wang L, Zhang X. NF-kappaB signaling pathway, inflammation and colorectal cancer. Cell Mol Immunol 2009;6:327–34.
[77] Schwitalla S, Fingerle AA, Cammareri P, Nebelsiek T, Göktuna SI, Ziegler PK, et al. Intestinal tumorigenesis initiated by dedifferentiation and acquisition of stemcell-like properties. Cell 2013;152:25–38.
[78] Lakatos, PL, Lakatos L. Risk for colorectal cancer in ulcerative colitis: Changes, causes and management strategies. World J Gastroenterol 2008;14:3937–47.
[79] Yu H, Kortylewski M, Pardoll D. Crosstalk between cancer and immune cells: Role of STAT3 in the tumour microenvironment. Nature Rev Immunol 2007;7:41–51.
[80] Ito H, Takazoe M, Fukuda Y, Hibi T, Kusugami K, Andoh A, et al. A pilot randomized trial of a human anti-interleukin-6 receptor monoclonal antibody in active Crohn's disease. Gastroenterology 2004;126:989–96.
[81] Grivennikov SI, Wang K, Mucida D, Stewart CA, Schnabl B, Jauch D, et al. Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. Nature 2012;491:254–8.
[82] Murata M, Thanan R, Ma N, Kawanishi S. Role of nitrative and oxidative DNA damage in inflammation-related carcinogenesis. J Biomed Biotechnol 2012;2012:623019.
[83] Kaler P, Godasi BN, Augenlicht L, Klampfer L. The NF-kappaB/AKT-dependent induction of Wnt signaling in colon cancer cells by macrophages and IL-1beta. Cancer Microenviron 2009;2:69–80.
[84] Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 2001;19:683–765.
[85] Massague J, Blain SW, Lo RS. TGFβ signaling in growth control, cancer, and heritable disorders. Cell 2000;103: 295–309.
[86] Wu S, Morin PJ, Maouyo D, O'Garra A. Bacteroides fragilis enterotoxin induces c-Myc expression and cellular proliferation. Gastroenterology 2003;124:392–400.
[87] Sanfilippo L, Li CK, Seth R, Balwin TJ, Menozzi MG, Mahida YR. Bacteroides fragilis enterotoxin induces the expression of IL-8 and transforming growth factor-beta (TGF-beta) by human colonic epithelial cells. Clin Exp Immunol 2000;119:456–63.
[88] Wu S, Powell J, Mathioudakis N, Kane S, Fernandez E, Sears CL. Bacteroides fragilis enterotoxin induces intestinal epithelial cell secretion of interleukin-8 through mitogen-activated protein kinases and a tyrosine kinase-regulated nuclear factor-kappaB pathway. Infect Immun 2004; 72:5832–9.
[89] Raman D, Baugher PJ, Thu YM, Richmond A. Role of chemokines in tumor growth. Cancer Lett 2007; 256:137–65.
[90] Toprak NU, Yagci A, Gulluoglu BM, Akin ML, Demirkalem P, Celenk T, et al. A possible role of Bacteroides fragilis enterotoxin in the aetiology of colorectal cancer. Clin Microbiol Infect 2006;12:782–6.
[91] Wu S, Rhee KJ, Albesiano E, Rabizadeh S, Wu X, Yen HR, et al. A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. Nat Med 2009;15:1016–22.
[92] Swidsinski A, Khilkin M, Kerjaschki D, Schreiber S, Ortner M, Weber J, et al. Association between intraepithelial Escherichia coli and colorectal cancer. Gastroenterology 1998; 115:281–6.
[93] Martin HM, Campbell BJ, Hart CA, Mpofu C, Nayar M, Singh R, et al. Enhanced Escherichia coli adherence and invasion in Crohn's disease and colon cancer. Gastroenterology 2004;127:80–93.
[94] Maddocks OD, Short AJ, Donnenberg MS, Bader S, Harrison DJ. Attaching and effacing Escherichia coli downregulate DNA mismatch repair protein in vitro and are associated with colorectal adenocarcinomas in humans. PLoS One 2009;4:e5517.
[95] Cuevas-Ramos G, Petit CR, Marcq I, Boury M, Oswald E, Nougayrède JP. Escherichia coli induces DNA damage in vivo and triggers genomic instability in mammalian cells. Proc Natl Acad Sci U S A 2010;107:11537–42.
[96] Arthur JC, Perez-Chanona E, Mühlbauer M, Tomkovich S, Uronis JM, Fan TJ, et al. Intestinal inflammation targets cancer-inducing activity of the microbiota. Science 2012;338:120–3.
[97] Fiorentini C, Fabbri A, Flatau G, Donelli G, Matarrese P, Lemichez E, et al. Escherichia coli cytotoxic necrotizing factor 1 (CNF1), a toxin that activates the Rho GTPase. J Biol Chem 1997;272:19532–7.
[98] Falzano L, Filippini P, Travaglione S, Miraglia AG, Fabbri A, Fiorentini C. Escherichia coli cytotoxic necrotizing factor 1 blocks cell cycle G2/M transition in uroepithelial cells. Infect Immun 2006;74:3765–72.
[99] Fabbri A, Travaglione S, Ballan G, Loizzo S, Fiorentini C. The cytotoxic necrotizing factor 1 from E. coli: A janus toxin playing with cancer regulators. Toxins (Basel) 2013 ;5:1462–74.
[100] Buc E, Dubois D, Sauvanet P, Raisch J, Delmas J, Darfeuille-Michaud A, et al. High prevalence of mucosa associated E. coli producing cyclomodulin and genotoxin in colon cancer. PLoS One 2013;8:e56964.
[101] Collins D, Hogan AM, Winter DC. Microbial and viral pathogens in colorectal cancer. Lancet Oncol 2011; 12:504–12.
[102] Hatch FT, Knize MG, Colvin ME. Extended quantitative structure-activity relationships for 80 aromatic and heterocyclic amines: structural, electronic, and hydropathic factors affecting mutagenic potency. Environ Mol Mutagen 2001;38:268–91.
[103] Weisburger JH. Comments on the history and importance of aromatic and heterocyclic amines in public health. Mut Res 2002;506–7:9–20.
[104] Butler LM, Sinha R, Millikan RC, Martin CF, Newman B, Gammon MD, et al. Heterocyclic amines, meat intake, and association with colon cancer in a population-based study. Am J Epidemiol 2003;157:434–45.
[105] Májer F, Sharma R, Mullins C, Keogh L, Phipps S, Duggan S, et al. New highly toxic bile acids derived from deoxycholic acid, chenodeoxycholic acid and lithocholic acid. Bioorgan Med Chem 2014;22:256–68.
[106] Baptissart M, Vega A, Maqdasy S, Caira F, Baron S, et al. Bile acids: From digestion to cancers. Biochimie 2013; 95:504–17.
[107] Tsuei J, Chau T, Mills D, Wan YJ. Bile acid dysregulation, gut dysbiosis, and gastrointestinal cancer. Exp Biol Med (Maywood) 2014;239:1489–504.

[108] Ou J, Carbonero F, Zoetendal EG, DeLany JP, Wang M, Newton K, et al. Diet, microbiota, and microbial metabolites in colon cancer risk in rural Africans and African Americans. Am J Clin Nutr 2013;98:111–20.

[109] Russell WR, Gratz SW, Duncan SH, Holtrop G, Ince J, Scobbie L, et al. High-protein, reduced carbohydrate weight-loss diets promote metabolite profiles likely to be detrimental to colonic health. Am J Clin Nutr 2011;93:1062–72.

[110] Russell WR, Duncan SH, Scobbie L, Duncan G, Cantlay L, Calder AG, et al. Major phenylpropanoid-derived metabolites in the human gut can arise from microbial fermentation of protein. Mol Nutr Food Res 2013;57:523–35.

[111] Gill CI, Rowland IR. Diet and cancer: Assessing the risk. Br J Nutr 2002;88:S73–87.

[112] Hughes R, Cross AJ, Pollock JRA, Bingham S. Dose-dependent effect of dietary meat on endogenous colonic N-nitrosation. Carcinogenesis 2001;22:199–202.

[113] Joosen AM, Kuhnle GG, Aspinall SM, Barrow TM, Lecommandeur E, Azqueta A, et al. Effect of processed and red meat on endogenous nitrosation and DNA damage. Carcinogenesis 2009;30:1402–7.

[114] Jacoby RF, Alexander RJ, Raicht RF, Brasitus TA. K-ras oncogene mutations in rat colon tumors induced by N-methyl-N-nitrosourea. Carcinogenesis 1992;13:45–9.

[115] Shuker DE, Margison GP. Nitrosated glycine derivatives as a potential source of O6-methylguanine in DNA. Cancer Res 1997;57:366–9.

[116] Gottschalg E, Scott GB, Burns PA, Shuker DE. Potassium diazoacetate-induced p53 mutations in vitro in relation to formation of O^6-carboxymethyl- and O^6-methyl-2`-deoxyguanosine DNA adducts: Relevance for gastrointestinal cancer. Carcinogenesis 2007;28:356–62.

[117] Di Martino ML, Campilongo R, Casalino M, Micheli G, Colonna B, Prosseda G. Polyamines: Emerging players in bacteria-host interactions. Int J Med Microbiol 2013; 303:484–91.

[118] Hanfrey CC, Pearson BM, Hazeldine S, Lee J, Gaskin DJ, Woster PM, et al. Alternative spermidine biosynthetic route is critical for growth of Campylobacter jejuni and is the dominant polyamine pathway in human gut microbiota. J Biol Chem 2011;286:43301–12.

[119] Pegg AE. Toxicity of polyamines and their metabolic products. Chem Res Toxicol 2013;26:1782–800.

[120] Ignatenko NA, Gerner EW, Besselsen DG. Defining the role of polyamines in colon carcinogenesis using mouse models. J Carcinog 2011;10:10.

[121] Terzić J, Grivennikov S, Karin E, Karin M. Inflammation and colon cancer. Gastroenterology 2010;138:2101–14.e5.

[122] Huycke MM, Moore D, Joyce W, Wise P, Shepard L, Kotake Y, et al. Extracellular superoxide production by Enterococcus faecalis requires demethylmenaquinone and is attenuated by functional terminal quinol oxidases. Mol Microbiol 2001;42:729–40.

[123] Huycke MM, Moore DR. In vivo production of hydroxyl radical by Enterococcus faecalis colonizing the intestinal tract using aromatic hydroxylation. Free Radic Biol Med 2002;33:818–26.

[124] Sobko T, Reinders CI, Jansson E, Norin E, Midtvedt T, Lundberg JO. Gastrointestinal bacteria generate nitric oxide from nitrate and nitrite. Nitric Oxide 2005;13:272–8.

[125] Cooke MS, Evans MD, Dizdaroglu M, Lunec, J. Oxidative DNA damage: Mechanisms, mutation, and disease. FASEB J 2003;17:1195–214.

[126] Evans MD, Dizdaroglu M, Cooke MS. Oxidative DNA damage and disease: Induction, repair and significance. Mutat Res 2004;567:1–61.

[127] Croce CM. Causes and consequences of microRNA dysregulation in cancer. Nat Rev Genet 2009;10:704–14.

[128] Ma X, Becker Buscaglia LE, Barker JR, Li Y. MicroRNAs in NFkappaB signaling. J Mol Cell Biol 2011;3:159–66.

[129] Baud V, Karin M. Is NF-kB a good target for cancer therapy? Hopes and pitfalls. Nat Rev Drug Discov 2009; 8:33–40.

[130] Baltimore D, Boldin MP, O'Connell RM, Rao DS, Taganov KD. MicroRNAs: New regulators of immune cell development and function. Nat Immunol 2008;9: 839–45.

[131] Xiang M, Birkbak NJ, Vafaizadeh V, Walker SR, Yeh JE, Liu S, et al. STAT3 induction of miR-146b forms a feedback loop to inhibit the NF-κB to IL-6 signaling axis and STAT3-driven cancer phenotypes. Sci Signal 2014;7:ra11.

[132] O'Connell RM, Rao DS, Baltimore D. MicroRNA regulation of inflammatory responses. Annu Rev Immunol 2012; 30:295–312.

[133] Iliopoulos D, Jaeger SA, Hirsch HA, Bulyk ML, Struhl K. STAT3 activation of miR-21 and miR-181b-1 via PTEN and CYLD are part of the epigenetic switch linking inflammation to cancer. Mol Cell 2010;39:493–506.

[134] Hayashi M, Jono H, Shinriki S, Nakamura T, Guo J, Sueta A, et al. Clinical significance of CYLD downregulation in breast cancer. Breast Cancer Res Treat 2014;143: 447–57.

[135] Hu S, Dong TS, Dalal SR, Wu F, Bissonnette M, Kwon JH, et al. The microbe derived short chain fatty acid butyrate targets miRNA-dependent p21 gene expression in human colon cancer. PLoS One 2011;6:e16221.

[136] Xue X, Feng T, Yao S, Wolf KJ, Liu CG, Liu X, et al. Microbiota downregulates dendritic cell expression of miR-10a, which targets IL-12/IL-23p40. J Immunol 2011; 187:5879–86.