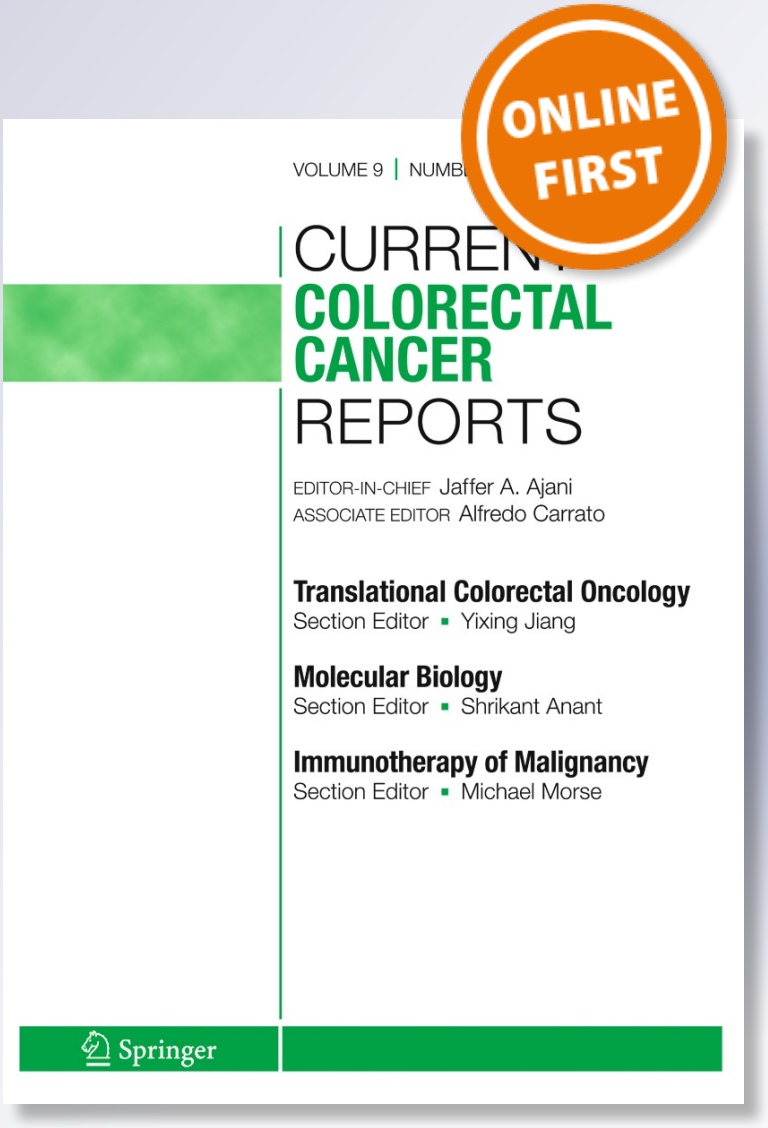
The Microbiota: A New Player in the Etiology of Colorectal Cancer

Maayan Levy, Christoph A. Thaiss & Eran Elinav

Current Colorectal Cancer Reports

ISSN 1556-3790

Curr Colorectal Cancer Rep
DOI 10.1007/s11888-013-0196-z



Springer

Curr Colorectal Cancer Rep
DOI 10.1007/s11888-013-0196-z

PREVENTION AND EARLY DETECTION (N ARBER, SECTION EDITOR)

The Microbiota: A New Player in the Etiology of Colorectal Cancer

Maayan Levy · Christoph A. Thaiss · Eran Elinav

Abstract Colorectal cancer is one of the commonest forms of cancer worldwide. Although the molecular pathogenesis of colorectal cancer shares many characteristics with that of other cancers, the tissue environment is unique in that the intestinal mucosal surface is continuously exposed to a vast community of microorganisms. It is increasingly recognized that the intestinal microbiota is a critical component of the tumor environment that contributes to the development of colorectal cancer, and certain members of the commensal microbiota have been identified as critical elements in intestinal carcinogenesis. As sensors of the presence of microbes at mucosal surfaces, pattern-recognition receptors of the innate immune system are equally involved in this process. This review summarizes our current knowledge of the role of the microbiota in colorectal cancer development and provides an overview of the mechanisms involved in the cross talk between intestinal microbial colonization and tumorigenesis.

Keywords Colon cancer · Microbiota · Dysbiosis · Inflammation · Therapy

Introduction

Almost one in five cases of cancer are associated with microbial infection [1]. The most prominent examples of cancer-associated microorganisms are human papillomavirus, hepatitis B virus, hepatitis C virus, human herpes virus 8, Epstein-Barr virus, and *Helicobacter pylori.* In the case of viruses, a connection between infection and neoplastic cell growth was first documented more than 100 years ago, when Oluf Bang and Vilhelm Ellerman demonstrated the transmissibility of chicken leukemia [2]. For bacteria, the concept of microbe-induced tumor growth has remained much more controversial. About 50 years ago, following numerous reports of the appearance of bacteria in cancerous tissue [3, 4], the American physician Virginia Livingston postulated the direct causal relationship between the presence of a bacterial species in a tissue and neoplastic transformation [5]. However, both the existence of this cancer-causing bacterium and the efficiency of the derived Livingston–Wheeler cancer therapy were later refuted [6]. To date, experimental and clinical evidence exists for the causal involvement of pathogenic bacterial species in cancer development. This is well exemplified in *Helicobacter pylori* in gastric adenocarcinoma [7] and *Salmonella enterica* ssp. I ser. Typhi. in gall bladder cancer [8].

The organ system most widely exposed to microbial influence and pathogenic infection is the gastrointestinal tract. Remarkably, however, the microbial influence on colorectal cancer (CRC) has remained elusive. Recently, the notion has emerged that in addition to pathogenic microorganisms, even members of the commensal microbiota, which densely colonizes the lumen and mucosal surface of the gastrointestinal tract, may play a role in the pathogenesis of CRC. Members of the commensal microbiota that perform homeostatic functions under normal conditions but become pathogenic in the case of aberrations in host–microbiota mutualism are called pathobionts. In this review, we provide an overview of the recent findings on the role of pathobionts in the pathophysiology of CRC. We summarize the knowledge of the involvement of intestinal microbe-sensing pattern-recognition receptors (PRR) of the innate immune system in CRC. We conclude with a perspective on the conclusions that should be drawn from these recent findings for the development of novel probiotic and antimicrobial strategies for potential future treatment options in CRC.

Maayan Levy and Christoph A. Thaiss are equal contributors.

M. Levy · C. A. Thaiss · E. Elinav (✉)
Department of Immunology, Weizmann Institute of Science, 100 Herzl Street, 76100 Rehovot, Israel
e-mail: eran.elinav@weizmann.ac.il

Published online: 17 November 2013

The Intestinal Microbiota

The human gastrointestinal tract harbors a highly complex and abundant microbial community, encompassing more than 1000 bacterial species that can reach levels as high as 10^{13}–10^{14} microorganisms in the large intestine [9]. The members of this microbial ecosystem, termed the microbiota, outnumber the host cells by a factor of 10–100 and collectively encompass 1000-fold more genes than the host [10]. The host and its resident microbiota have a mutually beneficial relationship. The commensal microbiota intimately interacts with the host and provides it with metabolites and enhances host digestive efficiency, thus allowing efficient uptake of nutrients that contribute to the fitness of the host [11]. Moreover, the gut microbiota protects against enteropathogens, contributes to development and maintenance of the intestinal epithelial barrier, and has diverse immunological functions [12–14]. In turn, the intestinal lumen of the host provides a safe and nourishing environment and is an efficient natural incubator for bacteria. Despite the mutualistic nature of the intestinal host–microbiota relationship, the dense microbial population poses a threat of translocation into the sterile intestinal milieu with ensuing diseases and immense health challenges.

Interactions between the gut microbiota and the host immune system begin at birth. The microbiota shapes the development of the immune system, and the immune system in turn shapes the composition and localization of the microbiota [15]. The establishment of the intestinal microbiota occurs progressively, beginning after birth and being stabilized to its adult configuration by the age of 3 years [16]. The initial gut microbiota composition is relatively simple, and is determined by the maternal microbiota [17], with variation in microbial communities and functional gene repertoires between individuals [16]. Early in life, however, the microbiota markedly changes [16], and obligate anaerobes become prominent, with much lower numbers of facultative anaerobes. The healthy adult gut microbiota is highly variable, although the microbial community is mostly dominated by bacteria belonging to just a few phyla, *Bacteroidetes* (including *Bacteroides*) and *Firmicutes* (including *Bacillus*, *Clostridium*, and *Lactobacillus*). Lower-abundance phyla are mainly composed of *Proteobacteria* (including *Escherichia*) and *Actinobacteria* (including *Bifidobacterium*) [18].

The microbiota is sensitive to host genetics and external factors, such as diet, lifestyle, hygiene, and use of antibiotics, which drive normal variation between individuals [19]. Diet is one of the most important factors shaping microbial diversity in the gut owing to different substrate preference and intense competition for resources that changes the community composition and function of the microbiota. Inflammation can also lead to alterations in community membership (reduction in both the total number of resident intestinal bacteria and bacterial diversity as host-mediated inflammation in response to an infecting agent, a chemical trigger), or genetic predisposition markedly alters the colonic microbial community [20].

Bacterial Influence on Cancer Development

CRC is one of the most prevalent cancer types worldwide, with over one million cases occurring every year, with steadily increasing incidence and mortality rates over the last five decades. It is the third most common malignancy and the fourth biggest cause of cancer mortality. CRC is a complex trait influenced by genetic and environmental factors and their interactions. Genome-wide association studies identified gene variants contributing to CRC risk, such as genes of the transforming growth factor β superfamily signaling pathway, which has been previously implicated in tumor biology, cadherin 1 (*CDH1*), and eukaryotic translation initiation factor 3 (*EIF3H*) [21]. The malignant tumor is composed of transformed cells with aberrant genomes and immune and stromal cells. In tissues that have a high level of exposure to microbes, the tumors can include microbes such as bacteria and viruses.

The overall microbial structures of cancerous tissue and noncancerous tissue are similar; however, the tumor microbiota exhibits lower diversity, and specific strains of bacteria have been implicated in the pathogenesis of cancer, including *Streptococcus bovis*, *Bacteroides* spp., *Clostridia* spp., *H. pylori*, and *Fusobacterium* spp. [22–26]. CRC patients have been shown to harbor an increased abundance of anaerobic bacteria belonging to the *Bacteroides–Prevotella* group compared with healthy individuals [27••, 28]. *Helicobacter pylori* infection is associated with development of gastric cancer [29]. Scanlan et al. [30] demonstrated that the *Clostridium leptum* and *Clostridium coccoides* subgroups were specific to CRC and polyposis. Using culture-independent pyrosequencing, Wu et al. [31] observed significant elevation of the levels of several bacterial groups, such as *Bacteroides* and *Fusobacterium* spp., in CRC patients.

Altered intestinal microbial ecology plays a causal role in mammalian physiological and pathological processes. However, it remains unclear how aberrant microbiota composition, termed dysbiosis, is established and sufficient to drive the development of CRC. Although many observations regarding microbial dysbiosis in CRC patients have been made, no clear picture has emerged from these studies regarding a common group of microorganisms associated with the disease. Several lines of evidence were found regarding the potential mechanisms by which the microbiota poses a risk for the development of CRC. The intestinal microbiota interacts with the host through metabolic exchange and co-metabolism of substrates to maintain the normal homeostasis. One feature of the microbiota composition in CRC patients is the depletion of butyrate-producing bacteria [32], indicating the potential

benefit of bacterial metabolites. Butyrate arising from microbial fermentation is important for the energy metabolism and normal development of colonic epithelial cells, and has a mainly protective role in relation to colonic disease. Butyrate has been implicated in the protection against colitis and CRC by reducing oxidative damage to DNA, inducing apoptosis in DNA-damaged cells, inhibiting tumor cell growth, and decreasing the activity of co-carcinogenic enzymes [33, 34]. Martin et al. [35] found an increased proportion of hemagglutinin-expressing *Escherichia coli* in Crohn's disease and colon cancer patients compared with healthy controls. Enteropathogenic *E. coli* possesses the ability to downregulate DNA mismatch repair proteins and, therefore, promotes colonic tumorigenesis [36]. In a rat model, *Streptococcus bovis* promotes the progression of preneoplastic lesions by increasing cell proliferation and IL-8 production [35].

Reddy et al. [37] linked the microbiota to CRC when a strong association has been established between microbially modified bile acids and cholesterol metabolites, and the risk of colon cancer among different populations of patients with high concentrations of fecal bile acids and cholesterol metabolites compared with the controls. Later, it was shown that under germ-free conditions, colitis and subsequent tumor formation are suppressed compared with their occurrence in either monoassociated or colonized mice [38].

The normal microbiota present in the gut is known to produce and release toxins that can bind specific cell surface receptors and affect intracellular signal transduction [39]. One example of such a toxin is produced by enterotoxigenic *Bacteroides fragilis*, which can secrete a virulence factor, *B. fragilis* toxin, that can bind to colonic epithelial cells and induce E-cadherin cleavage, IL-8 secretion, and epithelial cell proliferation [40]. Cleavage of E-cadherin then increases the permeability of the intestinal barrier and augments cell signaling via the β-catenin/Wnt pathway, which is constitutively active in most CRCs. Wu et al. [41] demonstrated that enterotoxigenic *B. fragilis* triggers colitis and strongly induces colonic tumors by activation of signal transducer and activator of transcription 3 and inducing the infiltration of the lamina propria by IL-17-producing $CD4^+$ T cells (T_H17) and γδ T cells (Fig. 1).

Inflammation and Cancer-Associated Pathobionts

Patients with ulcerative colitis and Crohn's disease have an increased risk of developing CRC, and prolonged inflammatory bowel disease (IBD) is a major risk factor for the development of CRC, with prevalence ranging from 2 % of patients after 10 years of IBD to up to 18 % after 30 years of IBD [42, 43].

Chronic inflammation is believed to promote cancer development through various inflammatory mediators and genotoxic substances, including proinflammatory cytokines and reactive oxygen and nitrogen species, which introduce genetic and epigenetic modifications [44]. The current research paradigm for IBD-associated CRC is that the gut microbiota promotes the development of colitis, with intestinal inflammation leading to tumorigenesis. For colon carcinogenesis, it is becoming increasingly evident that the large, complex bacterial population of the large intestine plays an important role. We have recently demonstrated in a mouse model of inflammation-induced CRC that microbiota-induced secretion of IL-6 and consequent signaling directly on intestinal epithelial cells promotes CRC development [45••]. Importantly, individuals with chronic inflammation have a lower bacterial diversity of the microbiota than healthy individuals, and maintaining the diversity of the gut microbial community is likely a prerequisite for a stable and healthy gastrointestinal tract [46].

The evidence for the involvement of microbiota in the induction of chronic colonic inflammation is growing, and it is now clear that chronic inflammation plays important roles in the development of various cancers. The inflammatory microenvironment is a risk factor for cancer development and chronic inflammation, regardless of infectious agents, and plays important roles in the development of various cancers [29]. Inflammatory cells and cytokines found in tumors are more likely to contribute to tumor growth, angiogenesis, progression, and immunosuppression than they are to mount an effective host antitumor response by enhancing DNA damage [47]. Altered mucosal glycosylation in IBD and colon cancer could affect mucosal bacterial adherence. It has been found that ileal and colonic mucosal surfaces of IBD and CRC patients harbor increased numbers of adherent–invasive *E. coli* bacteria relative to healthy individuals [35, 48, 49]. It is not clear whether the increase is a result or a cause of the intestinal inflammation.

A link between specific bacteria, inflammation, and cancer was revealed in recent work [27••], in which high-throughput sequencing demonstrated that the altered microbial composition of IL-10-deficient (*Il10*$^{-/-}$) mice promotes tumorigenesis. Colitis-susceptible *Il10*$^{-/-}$ mice exhibited a reduction in luminal microbial richness, relative to healthy wild-type mice, with increased abundance of *Proteobacteria* and adherent–invasive *E. coli* in the *Il10*$^{-/-}$ microbiota. *E. coli* was able to induce cancer in azoxymethane (AOM)-treated *Il10*$^{-/-}$ mice, and wild-type mice monoassociated with *E. coli* NC101 developed neither inflammation nor tumors. These results suggest that inflammation alone is insufficient to induce CRC and that microbial entities play a key role in the disease.

Cuevas-Ramos et al. [50] also showed that adherent–invasive *E. coli* strains were highly abundant in the colonic mucosa of patients with colorectal carcinoma and adenoma, yet not in normal colonic mucosa. *E. coli* bacteria harbor a 54-kb polyketide synthase (pks) pathogenicity island that encodes the multienzymatic machinery for a putative peptide–polyketide genotoxin called colibactin [50–52]. Pks^+ bacteria were

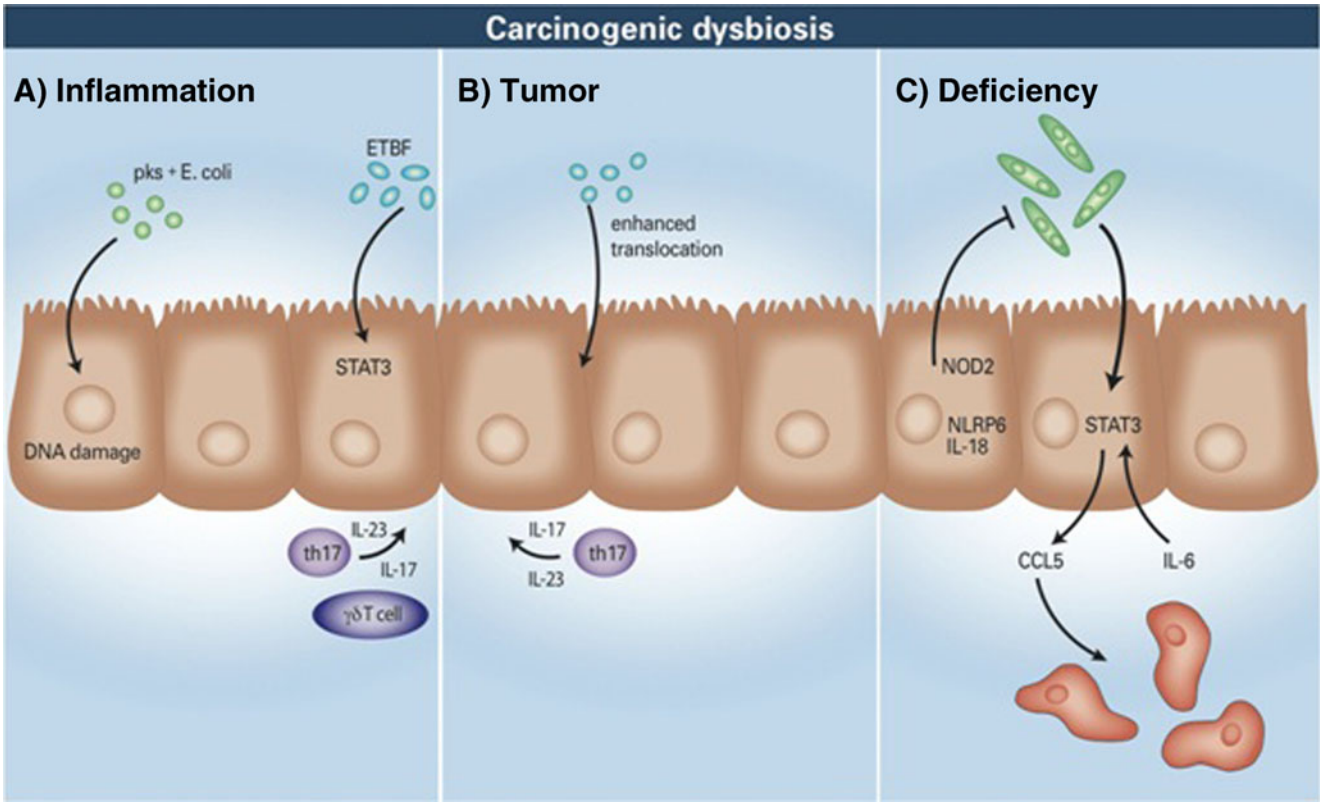


Fig. 1 Examples of dysbiosis associated with colorectal cancer development. *A* inflammation causes the blooming of certain bacteria associated with carcinogenesis, such as polyketide synthase (*pks*)-positive *Escherichia coli* and enterotoxigenic *Bacteroides fragilis* (*ETBF*). These bacteria induce direct genotoxic events or trigger proinflammatory signaling cascades, respectively. *B* tumor-induced inflammation also promotes loss of epithelial barrier integrity, resulting in enhanced bacterial translocation and ensuing inflammation. This process drives T_H17 cell infiltration to the site of tumor growth and elevated T_H17 signature cytokine production. *C* deficiency in host innate immune pathways (such as NOD2 or the NLRP6 inflammasome) results in aberrant bacterial growth and community representation. In the case of NLRP6 deficiency, dysbiosis is characterized by the overrepresentation of *Prevotellaceae* and TM7. This aberrant bacterial community induces proinflammatory signaling in the host, predominantly CCL5 secretion by epithelial cells, which in turn leads to the recruitment of inflammatory cells. Aberrantly high IL-6 production and signal transducer and activator of transcription 3 (*STAT3*) signaling induces neoplastic events and carcinogenesis

associated with chronic intestinal inflammation and CRC, indicating that pks may play an active role in promoting tumorigenesis and that pks^+ bacteria alone can induce DNA damage in the absence of a carcinogen such as AOM [27] (Fig. 1).

Taken together, during inflammation there is an increase in the abundance of bacteria with procarcinogenic activities that influence the development of CRC. As inflammation changes the microbial composition and induces the increase in abundance of microbes with cancer-promoting activities, the host is faced with a double-hit system in which both endogenous inflammatory mediators and bacterial-derived mediators influence CRC development.

The Role of Innate Sensors in the Development of Colorectal Cancer

The enormous impact of intestinal microorganisms on host physiology necessitates sensing platforms which allow communication between the eukaryotic and the prokaryotic parts of the intestinal ecosystem. The innate immune system serves as a sophisticated system for sensing signals of loss of tissue homeostasis, such as the presence of pathogenic microbes or host-derived signals of cellular stress. The mucosal immune system coevolves with the microbiota beginning at birth, acquiring the capacity to tolerate components of the microbial community while remaining unresponsive to nondangerous motifs, such as normal host molecules, dietary antigens, and commensal gut flora. This sensing system engages an array of germline-encoded PRRs to detect invariant microbial motifs. PRRs are expressed in both hematopoietic and nonhematopoietic cell types and include the membrane-bound Toll-like receptors (TLRs) and C-type lectins, which scan the extracellular milieu and endosomal compartments for pathogen-associated molecular patterns. The RNA-sensing RIG-like helicases RIG-I and MDA5 and the DNA sensors DAI and AIM2 provide cytoplasmic sensing of nucleic acid. NOD-like receptors (NLRs) are cytosolic PRRs that recognize pathogen-associated molecular patterns as well as host-

derived damage signals [53–55]. Some NLRs initiate the formation of a cytoplasmic multiprotein complex termed the inflammasome, consisting of an upstream sensor NLR protein, the adaptor protein apoptosis-associated speck-like protein containing a CARD (also known as PYD and CARD domain containing protein, PYCARD), and the effector protein caspase 1 [56]. Inflammasome activation leads to catalytic processing of IL-1β and IL-18, and can be triggered by both microbial ligands and endogenous signals of tissue damage. As such, inflammasome signaling provides an integrative assessment of both the presence of microbes and the consequences of their penetration into host tissue [56, 57].

Consistent with its role in sensing and shaping the microbial community in the intestine, the innate immune system has also been shown to play a role in regulating colon carcinogenesis. In the gut, the activation of PRRs initiates regulatory pathways, including mitogen-activated protein kinase and nuclear factor κB/Rel pathways, as well as caspase-dependent signaling cascades involving the inflammasome. Nod1 deficiency results in the increased development of both dextran sodium sulfate (DSS)-colitis-associated and adenomatous polyposis coli (APC)-tumor-suppressor-related colon tumors [58]. In the absence of Nod1 signaling, there is a greater disruption of the intestinal epithelial cell barrier due to chemically induced injury as manifested by increased surface epithelial apoptosis early on during chemically induced colitis and increased intestinal permeability. The increased intestinal permeability is associated with enhanced inflammatory cytokine production and epithelial cell proliferation in Nod1-deficient mice as compared with wild-type mice. Depletion of the gut microbiota suppressed tumor development in Nod1-deficient mice [58].

Further evidence for microbiota-induced inflammatory tumorigenesis came from a study showing that the TLR signaling adaptor protein MyD88 contributes to cancer progression in the $Apc^{Min/+}$ mouse model of spontaneous intestinal tumorigenesis and in mice treated with multiple injections of AOM [59], suggesting that innate sensing of microorganisms in the intestine is necessary for regulation of inflammation and cancer development. In the DSS-colitis-associated cancer model, $Myd88^{-/-}$ mice showed enhanced susceptibility to CRC development [60]. Upstream of MyD88, TLR signaling might be involved in the microbiota-mediated development of CRC. $Tlr4^{-/-}$ mice are protected from DSS–AOM-elicited intestinal tumor development [61], whereas $Tlr2^{-/-}$ mice are more prone to develop CRC in this model [62].

Besides TLRs, the inflammasome-derived cytokines IL-1β and IL-18 signal through MyD88 on target cells. In addition to orchestrating multiple innate and adaptive immune responses, inflammasomes were demonstrated to mediate gut homeostasis and tumorigenesis [63, 64•, 65–71]. Mice lacking the inflammasome adaptor protein PYCARD and caspase-1 demonstrate enhanced autoinflammation, morbidity, histopathological changes, and polyp formation. The increased tumor burden is correlated with attenuated levels of IL-1β and IL-18 at the tumor site. $Nlrp3^{-/-}$ mice show an increase in acute and recurring colitis and colitis-associated cancer, although the disease outcome is less severe in $Nlrp3^{-/-}$ mice than in $Pycard^{-/-}$ or $Casp1^{-/-}$ mice. *Nlrp3* expression and function in hematopoietic cells, rather than intestinal epithelial cells or stromal cells, is responsible for protection against increased tumorigenesis. Moreover, the NLRC4 inflammasome is central to colonic inflammation-induced tumor formation through regulation of epithelial cell response to injury [68]. $Nlrp12^{-/-}$ mice are highly susceptible to colitis and colitis-associated colon cancer. Polyps isolated from $Nlrp12^{-/-}$ mice showed elevated noncanonical nuclear factor κB activation and increased expression of target genes that were associated with cancer [70]. $Nlrp6^{-/-}$ and $Pycard^{-/-}$ mice harbor a colitogenic gut microflora that is transmissible to cohoused wild-type mice, resulting in an exacerbated colitis phenotype [64•]. With use of the AOM–DSS-induced colitis-associated CRC model, it was demonstrated that wild-type mice cohoused with $Nlrp6^{-/-}$ and $Pycard^{-/-}$ mice develop a dramatically enhanced tendency for inflammation-induced CRC formation, which is mediated through IL-18-induced alterations in the microflora and resultant induction of CCL5-dependent colonic inflammation and activation of the IL-6 pathway, resulting in enhanced epithelial cell proliferation culminating in tumor formation.

Probiotics in Colorectal Cancer

The recognition that the intestinal microbiota significantly contributes to CRC development warrants the exploitation of microbial-community-modifying probiotics for CRC prevention or treatment of CRC patients. By definition, prebiotics are live bacteria, which confer a health benefit when administered in adequate amounts [72]. Currently, about ten bacterial strains are used as probiotics. Prospectively, such bacteria can be used as part of a patient's diet to strategically alter the gut microbiota to ameliorate CRC symptoms, delay CRC onset, or even stop CRC development. The general use of probiotics aims at stabilizing a healthy gut microbial community, thereby preventing the outgrowth of pathobionts and inhibiting colonization with intestinal pathogens. Furthermore, secreted factors may positively influence the mucosal barrier through effects on intestinal epithelial cells and components of the mucosal immune system.

Although very little is known about the mechanisms that promote the beneficial effect of probiotics on the host, studies have shown that various molecular pathways are influenced by probiotic colonization, including the cyclooxygenase 2 pathway, nitric oxide synthesis, fatty acid production, regulation of intestinal pH, and the secretion of cytokines [73–75].

The kinetics of probiotic colonization of the gut is complex and likely not stable, meaning that probiotic strains do not persist as stable members of the microbial community [76]. In an in vitro system, *Lactobacillus rhamnosus* GG was able to decrease proliferation of cancer cell lines, with the cytoplasmic fraction of the bacteria being responsible for the effect exerted [77]. In another in vitro study, *Bacillus polyfermenticus* SCD showed strong adherent properties toward a colonic cell line. The same study also demonstrated an anticarcinogenic effect in rats [78]. Similarly, *Bifidobacterium adolescentis* SPM0212 was shown to inhibit the proliferation of cell lines derived from human colon [79]. In vivo studies in rats have assessed the effect of administration of *Lactobacillus acidophilus* and *Bifidobacterium lactis* on carcinogen-induced apoptosis and damage in the colon. They documented a lower grade of dysplasia in rats given the probiotics and antigenotoxic effects [80, 81].

Conclusions and Perspective

The notion that dysbiosis is involved in the molecular pathogenesis of CRC prospectively opens new avenues for our understanding of this devastating disease. The use of probiotics or targeted antimicrobial therapy in CRC patients is the logical consequence of the studies summarized herein. Other potential microbiota-altering interventions that are likely to be added to the antineoplastic arsenal include modifiers for the microbiota composition and function (prebiotics), and microbiota ecosystem replacement, also known as fecal transplantation. The latter approach has recently been suggested to be efficacious in a number of infectious and autoinflammatory disorders [82, 83••]. Nonetheless, our understanding of the bacterial strains involved in CRC pathogenesis, the mechanisms leading to pathobiont outgrowth, and the processes connecting pathobiont activity to tumor initiation and progression are still poorly understood. Before an effective new therapy based on probiotics or targeted antimicrobial therapy can be exploited, several central questions need to be addressed: What contributes to microbial community stability in the intestine? How can this stability be maintained? Which characteristics are unique to tumor-associated pathobionts? Are pathobionts causally involved in CRC pathogenesis or merely a consequence of tumor growth? How can tumor-associated dysbiosis be eradicated? These intriguing questions warrant further research, both at the basic level in animal models and through the initiation of the first human studies on the effect of interventions targeting the microbiota as a new player in CRC.

Acknowledgments We thank the members of the Elinav laboratory for fruitful discussions. We apologize to authors whose relevant work was not included in this review owing to space constraints. Eran Elinav is supported by grants provided by the Abisch-Frenkel Foundation, the Kenneth Rainin Foundation, the Leona M. and Harry B. Helmsley Charitable Trust, the Israel Science Foundation, and the German-Israel Foundation, and a Marie Curie Career Integration Grant Christoph Thaiss is funded by a Boehringer Ingelheim Fonds PhD Fellowship. We thank A. Levy and Z. Levy for excellent support.

Compliance with Ethics Guidelines

Conflict of Interest Maayan Levy, Christoph A. Thaiss, and Eran Elinav declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Kuper H, Adami HO, Trichopoulos D. Infections as a major preventable cause of human cancer. J Intern Med. 2000;248:171–83.
2. Hu WY, Bushman FD, Siva AC. RNA interference against retroviruses. Virus Res. 2004;102:59–64.
3. Russell W. An address on a characteristic organism of cancer. Br Med J. 1890;2:1356–60.
4. Wuerthele-Caspe V et al. Cultural properties and pathogenicity of certain microorganisms obtained from various proliferative and neoplastic diseases. Am J Med Sci. 1950;220:638–46.
5. Livingston VW, Alexander-Jackson E. An experimental biologic approach to the treatment of neoplastic disease; determination of actinomycin in urine and cultures as an aid to diagnosis and prognosis. J Am Med Womens Assoc. 1965;20:858–66.
6. Unproven methods of cancer management. Livingston-Wheeler therapy. CA Cancer J Clin. 1991;41:A7–12.
7. Polk DB, Peek Jr RM. Helicobacter pylori: gastric cancer and beyond. Nat Rev Cancer. 2010;10:403–14.
8. Samaras V, Rafailidis PI, Mourtzoukou EG, Peppas G, Falagas ME. Chronic bacterial and parasitic infections and cancer: a review. J Infect Dev Ctries. 2010;4:267–81.
9. Berg RD. The indigenous gastrointestinal microflora. Trends Microbiol. 1996;4:430–5.
10. Gordon JI. Honor thy gut symbionts redux. Science. 2012;336:1251–3.
11. Sonnenburg JL et al. Glycan foraging in vivo by an intestine-adapted bacterial symbiont. Science. 2005;307:1955–9.
12. Candela M et al. Interaction of probiotic Lactobacillus and Bifidobacterium strains with human intestinal epithelial cells: adhesion properties, competition against enteropathogens and modulation of IL-8 production. Int J Food Microbiol. 2008;125:286–92.
13. Fukuda S et al. Bifidobacteria can protect from enteropathogenic infection through production of acetate. Nature. 2011;469:543–7.
14. Hosokawa T, Kikuchi Y, Nikoh N, Shimada M, Fukatsu T. Strict host-symbiont cospeciation and reductive genome evolution in insect gut bacteria. PLoS Biol. 2006;4:e337.
15. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. Science. 2012;336:1268–73.

16. Yatsunenko T et al. Human gut microbiome viewed across age and geography. Nature. 2012;486:222–7.
17. Dominguez-Bello MG et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A. 2010;107:11971–5.
18. Eckburg PB et al. Diversity of the human intestinal microbial flora. Science. 2005;308:1635–8.
19. Spor A, Koren O, Ley R. Unravelling the effects of the environment and host genotype on the gut microbiome. Nat Rev Microbiol. 2011;9:279–90.
20. Lupp C et al. Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of Enterobacteriaceae. Cell Host Microbe. 2007;2:204.
21. Tenesa A, Dunlop MG. New insights into the aetiology of colorectal cancer from genome-wide association studies. Nat Rev Genet. 2009;10:353–8.
22. Peek Jr RM, Blaser MJ. Helicobacter pylori and gastrointestinal tract adenocarcinomas. Nat Rev Cancer. 2002;2:28–37.
23. Gold JS, Bayar S, Salem RR. Association of Streptococcus bovis bacteremia with colonic neoplasia and extracolonic malignancy. Arch Surg. 2004;139:760–5.
24. Nakamura J et al. Comparison of four microbial enzymes in Clostridia and Bacteroides isolated from human feces. Microbiol Immunol. 2002;46:487–90.
25. Ellmerich S, Djouder N, Scholler M, Klein JP. Production of cytokines by monocytes, epithelial and endothelial cells activated by Streptococcus bovis. Cytokine. 2000;12:26–31.
26. Castellarin M et al. Fusobacterium nucleatum infection is prevalent in human colorectal carcinoma. Genome Res. 2012;22:299–306.
27. •• Arthur JC et al. Intestinal inflammation targets cancer-inducing activity of the microbiota. Science. 2012;338:120–3. *This article demonstrates the impact of intestinal inflammation on the outgrowth of a bacterial strain that exerts genotoxic effects on the host*.
28. Sobhani I et al. Microbial dysbiosis in colorectal cancer (CRC) patients. PLoS One. 2011;6:e16393.
29. Chiba T, Marusawa H, Ushijima T. Inflammation-associated cancer development in digestive organs: mechanisms and roles for genetic and epigenetic modulation. Gastroenterology. 2012;143:550–63.
30. Scanlan PD et al. Culture-independent analysis of the gut microbiota in colorectal cancer and polyposis. Environ Microbiol. 2008;10:789–98.
31. Wu N et al. Dysbiosis signature of fecal microbiota in colorectal cancer patients. Microb Ecol. 2013;66:462–70.
32. Wang T et al. Structural segregation of gut microbiota between colorectal cancer patients and healthy volunteers. ISME J. 2012;6:320–9.
33. Pryde SE, Duncan SH, Hold GL, Stewart CS, Flint HJ. The microbiology of butyrate formation in the human colon. FEMS Microbiol Lett. 2002;217:133–9.
34. Rose DJ, DeMeo MT, Keshavarzian A, Hamaker BR. Influence of dietary fiber on inflammatory bowel disease and colon cancer: importance of fermentation pattern. Nutr Rev. 2007;65:51–62.
35. Martin HM et al. Enhanced Escherichia coli adherence and invasion in Crohn's disease and colon cancer. Gastroenterology. 2004;127:80–93.
36. 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.
37. Reddy BS, Mastromarino A, Wynder EL. Further leads on metabolic epidemiology of large bowel cancer. Cancer Res. 1975;35:3403–6.
38. Kim SC et al. Variable phenotypes of enterocolitis in interleukin 10-deficient mice monoassociated with two different commensal bacteria. Gastroenterology. 2005;128:891–906.
39. Fasano A. Cellular microbiology: can we learn cell physiology from microorganisms? Am J Physiol. 1999;276:C765–76.
40. Rhee KJ et al. Induction of persistent colitis by a human commensal, enterotoxigenic Bacteroides fragilis, in wild-type C57BL/6 mice. Infect Immun. 2009;77:1708–18.
41. Wu S 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.
42. Jess T, Rungoe C, Peyrin-Biroulet L. Risk of colorectal cancer in patients with ulcerative colitis: a meta-analysis of population-based cohort studies. Clin Gastroenterol Hepatol. 2012;10:639–45.
43. Eaden JA, Abrams KR, Mayberry JF. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. Gut. 2001;48:526–35.
44. Ullman TA, Itzkowitz SH. Intestinal inflammation and cancer. Gastroenterology. 2011;140:1807–16.
45. •• Hu B et al. Microbiota-induced activation of epithelial IL-6 signaling links inflammasome-driven inflammation with transmissible cancer. Proc Natl Acad Sci U S A. 2013;110:9862–7. *This report demonstrates that susceptibility to CRC development is transmissible through the transfer of dysbiotic microbiota*.
46. Qin J et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010;464:59–65.
47. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet. 2001;357:539–45.
48. Darfeuille-Michaud A et al. Presence of adherent Escherichia coli strains in ileal mucosa of patients with Crohn's disease. Gastroenterology. 1998;115:1405–13.
49. Swidsinski A et al. Association between intraepithelial Escherichia coli and colorectal cancer. Gastroenterology. 1998;115:281–6.
50. Cuevas-Ramos G et al. 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.
51. Nougayrede JP et al. Escherichia coli induces DNA double-strand breaks in eukaryotic cells. Science. 2006;313:848–51.
52. Putze J et al. Genetic structure and distribution of the colibactin genomic island among members of the family Enterobacteriaceae. Infect Immun. 2009;77:4696–703.
53. Geijtenbeek TB, van Vliet SJ, Engering A, 't Hart BA, van Kooyk Y. Self- and nonself-recognition by C-type lectins on dendritic cells. Annu Rev Immunol. 2004;22:33–54.
54. Medzhitov R, Janeway Jr CA. Decoding the patterns of self and nonself by the innate immune system. Science. 2002;296:298–300.
55. Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Mol Cell. 2002;10:417–26.
56. Martinon F, Mayor A, Tschopp J. The inflammasomes: guardians of the body. Annu Rev Immunol. 2009;27:229–65.
57. Elinav E, Strowig T, Henao-Mejia J, Flavell RA. Regulation of the antimicrobial response by NLR proteins. Immunity. 2011;34:665–79.
58. Chen GY, Shaw MH, Redondo G, Nunez G. The innate immune receptor Nod1 protects the intestine from inflammation-induced tumorigenesis. Cancer Res. 2008;68:10060–7.
59. Rakoff-Nahoum S, Medzhitov R. Regulation of spontaneous intestinal tumorigenesis through the adaptor protein MyD88. Science. 2007;317:124–7.
60. Salcedo R et al. MyD88-mediated signaling prevents development of adenocarcinomas of the colon: role of interleukin 18. J Exp Med. 2010;207:1625–36.
61. Fukata M et al. Toll-like receptor-4 promotes the development of colitis-associated colorectal tumors. Gastroenterology. 2007;133:1869–81.
62. Lowe EL et al. Toll-like receptor 2 signaling protects mice from tumor development in a mouse model of colitis-induced cancer. PLoS One. 2010;5:e13027.
63. Dupaul-Chicoine J et al. Control of intestinal homeostasis, colitis, and colitis-associated colorectal cancer by the inflammatory caspases. Immunity. 2010;32:367–78.
64. • Elinav E et al. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. Cell. 2011;145:745–57. *This article shows the involvement of the NLRP6 inflammasome in the prevention of dysbiosis development. Absence of NLRP6 leads to the outgrowth of colitogenic microbiota*.

65. Allen IC et al. The NLRP3 inflammasome functions as a negative regulator of tumorigenesis during colitis-associated cancer. J Exp Med. 2010;207:1045–56.
66. Zaki MH et al. The NLRP3 inflammasome protects against loss of epithelial integrity and mortality during experimental colitis. Immunity. 2010;32:379–91.
67. Bauer C et al. Colitis induced in mice with dextran sulfate sodium (DSS) is mediated by the NLRP3 inflammasome. Gut. 2010;59:1192–9.
68. Hu B et al. Inflammation-induced tumorigenesis in the colon is regulated by caspase-1 and NLRC4. Proc Natl Acad Sci U S A. 2010;107:21635–40.
69. Chen GY, Liu M, Wang F, Bertin J, Nunez G. A functional role for Nlrp6 in intestinal inflammation and tumorigenesis. J Immunol. 2011;186:7187–94.
70. Allen IC et al. NLRP12 suppresses colon inflammation and tumorigenesis through the negative regulation of noncanonical NF-κB signaling. Immunity. 2012;36:742–54.
71. Zaki MH et al. The NOD-like receptor NLRP12 attenuates colon inflammation and tumorigenesis. Cancer Cell. 2011;20:649–60.
72. Zhu Y, Michelle Luo T, Jobin C, Young HA. Gut microbiota and probiotics in colon tumorigenesis. Cancer Lett. 2011;309:119–27.
73. Pala V et al. Yogurt consumption and risk of colorectal cancer in the Italian European prospective investigation into cancer and nutrition cohort. Int J Cancer. 2011;129:2712–9.
74. Goncharova GI, Dorofeichuk VG, Smolianskaia AZ, Sokolova K. [Microbial ecology of the intestines in health and in pathology]. Antibiot Khimioter. 1989;34:462–6.
75. Arumugam M et al. Enterotypes of the human gut microbiome. Nature. 2011;473:174–80.
76. Corthesy B, Gaskins HR, Mercenier A. Cross-talk between probiotic bacteria and the host immune system. J Nutr. 2007;137:781S–90.
77. Orlando A, Messa C, Linsalata M, Cavallini A, Russo F. Effects of Lactobacillus rhamnosus GG on proliferation and polyamine metabolism in HGC-27 human gastric and DLD-1 colonic cancer cell lines. Immunopharmacol Immunotoxicol. 2009;31:108–16.
78. Lee NK, Park JS, Park E, Paik HD. Adherence and anticarcinogenic effects of Bacillus polyfermenticus SCD in the large intestine. Lett Appl Microbiol. 2007;44:274–8.
79. Kim Y et al. Inhibition of proliferation in colon cancer cell lines and harmful enzyme activity of colon bacteria by Bifidobacterium adolescentis SPM0212. Arch Pharm Res. 2008;31:468–73.
80. Le Leu RK et al. A synbiotic combination of resistant starch and Bifidobacterium lactis facilitates apoptotic deletion of carcinogen-damaged cells in rat colon. J Nutr. 2005;135:996–1001.
81. Pool-Zobel BL et al. Lactobacillus- and bifidobacterium-mediated antigenotoxicity in the colon of rats. Nutr Cancer. 1996;26:365–80.
82. Borody TJ, Paramsothy S, Agrawal G. Fecal microbiota transplantation: indications, methods, evidence, and future directions. Curr Gastroenterol Rep. 2013;15:337.
83. •• van Nood E et al. Duodenal infusion of donor feces for recurrent Clostridium difficile. N Engl J Med. 2013;368:407–15. *This study presents the successful use of fecal transplantation to cure disease*.