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Microbiota as a mediator of cancer progression and therapy

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Abstract

Complex and intricate circuitries regulate cellular proliferation, survival, and growth, and alterations of this network through genetic and epigenetic events result in aberrant cellular behaviors, often leading to carcinogenesis. Although specific germline mutations have been recognized as cancer inducers, the vast majority of neoplastic changes in humans occur through environmental exposure, lifestyle, and diet. An emerging concept in cancer biology implicates the microbiota as a powerful environmental factor modulating the carcinogenic process. For example, the intestinal microbiota influences cancer development or therapeutic responses through specific activities (immune responses, metabolites, microbial structures, and toxins). The numerous effects of microbiota on carcinogenesis, ranging from promoting, preventing, or even influencing therapeutic outcomes, highlight the complex relationship between the biota and the host. In this review, we discuss the latest findings on this complex microbial interaction with the host and highlight potential mechanisms by which the microbiota mediates such a wide impact on carcinogenesis.

Introduction

Cancer is a multifactorial disease involving genetic and epigenetic alterations, environmental factors, and lifestyle components. Cancer genetic studies have offered a spectacular view of the complexity and intricacy of events at play during carcinogenic evolution.[1-3] Similarly, significant progress has been made on the identification and functional effect of environmental elements and lifestyles on tumorigenesis.[4] As a whole, these studies have contributed important knowledge regarding mechanisms implicated in cancer initiation, progression, metastasis, and therapeutic responses. Beside the previously mentioned factors, a relatively novel component named the microbiota has recently been recognized as a potent modulator of the carcinogenic process. The microbiota is a consortium of microorganisms composed of bacteria, viruses, fungi, and protozoa living in various body sites, including oral,[5] urogenital,[6] and gastrointestinal (GI) cavities,[7] forming a community living in a eubiotic state. Noteworthy, genetic, environmental, and lifestyle components all influence microbial composition and one should not view these as independent factors but rather as

integrated components of carcinogenesis[8] (Fig 1). The vast majority of microorganisms reside within the intestine, and influence not only the local gut function but also exert long-distant effects on host homeostasis and disease states such as allergy, asthma,[9] rheumatoid arthritis,[10] cardiovascular diseases,[11] metabolic syndrome,[12] and obesity.[12,13] This review will focus on recent advances about the local and wide range effects the intestinal microbiota exerts as it mediates numerous phases of cancer, particularly colorectal cancer (CRC), spanning initiation, progression, and treatment.

The mechanisms by which the microbial community exerts such a profound and wide impact on the host are still unclear but likely originate from microbial metabolism and microbial-derived structures interacting with the host cellular compartment through receptors or receptor-independent fashion. Moreover, the identities of specific microorganisms responsible for health maintenance or disease development are still unclear and probably result from an ensemble of organisms rather than any particular one. A general consensus in the field is that alterations in the microbiome, a phenomenon termed dysbiosis, are often linked to disease development, including CRC.[14] In addition, preclinical models suggest that microbial dysbiosis has a causative impact on cancer development, at least for CRC. As such, some forms of cancer may be influenced by the action of a microbial community as opposed to a single organism paradigm as seen with *Helicobacter pylori* (gastric cancer), hepatitis B or C virus (liver cancer), or Epstein–Barr virus (lymphomas) infection.[15]

Microbial Dysbiosis and Tumorigenesis

Although numerous body sites have been shown to harbor a microbiota, the intestine has the most compelling evidence that microbial composition is linked to carcinogenesis. In this pathology, phylogenic differences were reported between bacteria present in the intestine of healthy subjects compared with CRC patients.[16] Microbial dysbiosis is also observed between tumor and healthy adjacent tissue of the same patient,[17] distal vs proximal tumors,[18] and between tumor staging from adenoma to adenocarcinoma.[19] A systematic review of reports documented microbial dysbiosis in CRC patients highlighting specific changes within the intestinal microbial community such as increased representation of fusobacteria, *Alistipes*, porphyromonadaceae, coriobacteridae, staphylococcaceae, *Akkermansia*, and methanobacteriales and decreased abundance of *Bifidobacterium*, *Lactobacillus*, *Ruminococcus*, *Faecalibacterium*, *Roseburia*, and *Treponema*.[14] In line with microbial dysbiosis, novel prognostic approaches have made use of the unique microbial signature present in patients to predict the carcinogenic stage, a step toward the generation of noninvasive biomarkers to test stools.[20-22] In addition, microbial dysbiosis has also been observed in other forms of cancers including breast, lung, urogenital, and liver, which was the subject of a recent review.[23]

Independent of dysbiosis, the intestinal microbiota may play an important role in the progression of extra-intestinal cancer. A study analyzing public data to assess bacterial DNA integration into the somatic genome identified leukemia as having the highest number of reads, with a high frequency of *Acinetobacter* gene transfer into leukemic cells.[24] In addition, mice genetically predisposed for B-cell lymphoma, another form of blood cancer,

exhibit delayed disease and a distinct separation in bacterial diversity when exposed to stringent husbandry conditions or associated with a restricted microbiota in contrast to being housed or raised in specificpathogen-free (SPF) conditions.[25] These data suggested a causative role for the microbiota in disease progression. For some time, studies have linked *H. pylori* to mucosa-associated lymphoid tissue lymphoma.[26] Accordingly, treatment with antibiotics to deplete *H. pylori* resulted in regression of disease.[27] Strikingly, low-grade mucosa-associated lymphoid tissue patients with undetectable levels of *H. pylori* that were subjected to the standard *H. pylori* eradication treatment, containing a mixture of antibiotics, showed complete remission of disease,[28] suggesting an even broader mechanistic role for intestinal microbiota in lymphoma beyond *H. pylori* infection.

Studies in preclinical models support a causal role for dysbiosis in CRC. For example, the 2 most commonly used models of colon tumorigenesis, the adenomatous polyposis coli ($Apc^{Min/+}$) spontaneous model and the colitis-associated cancer (CAC) model involving the procarcinogenic compound azoxymethane (AOM) and the inflammatory agent dextran sodium sulfate (DSS), showed differences in the microbiota of mice with tumors vs controls.[29,30] Son et al compared the gut microbial composition between $Apc^{Min/+}$ and wild-type (WT) mice at the age of 6 weeks, a stage preceding intestinal neoplasia, and showed the presence of dysbiosis.[30] Germ-free (GF) studies have greatly expanded the depth of research in the microbiota field allowing the ability to study specific contributions of a single bacterial species or communities. These studies, along with many additional cancer models, including those of the gut, lung, breast, and immune system, underline a role for the microbiota in tumor development.[31] It is important to note that the role of bacteria in CRC development may be model specific. For example, GF AOM/DSS mice develop more tumors compared with SPF mice, which was attributed to delayed inflammatory and proliferative responses.[32] However, for most CRC murine models including $AOM/IL10^{-/-}$ and $Apc^{Min/+}$ mice, GF mice typically develop fewer tumors.[31] Altogether, these data highlight a direct link between dysbiosis and tumor development. It is important to note that the cross talk between microbiota and cancer can be bidirectional as cancer may provide an environment fostering changes in microbial composition, which could then further influence carcinogenesis.

In addition to microbiota composition, the organization and location of the microbiota in CRC patients also impact tumorigenesis. For example, bacterial bio-films were recently identified in 50% of tumor and paired adjacent normal tissue samples from human CRC patients using fluorescence in situ hybridization.[33] In addition, stratifying CRC patients by tumor location revealed biofilms associated with 89% of right-sided (proximal) CRCs vs 13% of left-sided (distal) CRCs.[33] The presence of a biofilm on CRC patient normal tissue was associated with increased epithelial interleukin (IL)-6 expression, Stat3 phosphorylation, and epithelial cell proliferation and decreased E-cad-herin expression, suggesting a possible mechanism by which biofilms drive tumorigenesis.[33] On the microbial side, metabolites may partly explain how biofilms contribute to tumorigenesis.[34] Indeed, higher levels of acetylated polyamines were detected in biofilm-positive cancer tissue compared with biofilm-negative cancer tissue.[34] In addition, antibiotic treatment of CRC patients eliminated the presence of a biofilm and was associated with decreased acetylated polyamines in tumor and paired normal tissue substantiating the association between

bacterial biofilms and acetylated polyamines.[34] Further studies are needed to address other mechanisms by which biofilms promote cancer as acetylated polyamines are significantly higher in CRC patient tumor tissue compared with normal tissue regardless of biofilm status.[34]

Diet, Microbiota, and Host Mechanisms

Components of the diet that cannot be digested in the GI tract are biotransformed by the microbiota to generate critical nutrients and metabolites for the host. The type of diet consumed by the host has a profound impact on the spectrum of bacterial-derived metabolites, a phenomenon that could have important repercussions for homeostasis. For example, the microbial-derived metabolomic signature of vegans is significantly different than omnivore subjects,[35] although the functional impact of these metabolites on health status is unclear. Nevertheless, knowing the strong link between diet and cancer development, the biotransformation function of the microbiota has drawn intense attention. One of the earliest and most widely known associations between diet and cancer was that involving red meat. Consumption of red meat leads to the production of dietary heme, which has been shown to have host cytotoxic effects.[36] The microbiota was recently identified as a mediator of heme-induced preneoplastic events, which include hyperplasia and hyper-proliferation.[37] Administration of heme-supplemented diet in mice was sufficient to augment bacterial load with a significant increase in *Bacteroides* abundance and host cellular proliferation. These data support a direct connection among diet, the microbiota composition, and the host function.

Of interest are recent studies identifying the micro-biota as a mediator of diet-induced modulation of host cellular processes, likely through the generation of selective metabolites. We direct readers to a series of comprehensive reviews highlighting the production and role of microbial metabolites including short-chain fatty acids (SCFAs) in cancer and immune homeostasis.[38-40] One of the most recent human studies analyzing the tunable role of diet in cancer susceptibility was that of African Americans, influenced by a high-fat western diet that is strongly implicated in the etiology of many diseases including cancer, and the genetically similar rural African population having a more restricted diet that has a high fiber content. African Americans carry a greater risk for cancer development, specifically CRC, than White Americans.[41] Moreover, cancer risk is more than 10-fold higher in African Americans than in rural Africans.[42] The role of diet in this high-risk group was examined by studying fecal samples from African Americans and rural Africans.[42,43] Rural Africans contained higher amounts of *Prevotella*, whereas African Americans were colonized more abundantly by *Bacteroides*.[43] Further analysis of African American samples revealed enrichment of bile acids, known for their protumorigenic effects.[44] Conversely, rural Africans were enriched for genes involved in the production of metabolites such as the SCFA butyrate, known for its protective, antitumorigenic effects in mice.[45] The cytoprotective effects of butyrate have been shown to depend on the receptor Gpr109a, as genetic deletion results in a significant increase in colonic tumors.[46] Interestingly, diet intervention was able to alter the production of these microbial metabolites. When African Americans were switched to a high-fiber, low-fat diet, an increase in butyrate-producing microbes was observed.[42] In contrast, when rural Africans were switched to a high-fat, low-

fiber diet, an increase in bacterial gene expression responsible for bile acid production was noted. The diet switch also reversed cellular proliferation and immune cell infiltration in colonic biopsies of the subjects, suggesting that dietary changes modulate microbiota activities, leading to functional consequences on the host.

As stated in the previous section, bacteria and their associated metabolites could impact carcinogenesis at extraintestinal locations. Many of these long-range effects are because of metabolite dissemination throughout the body, with or without further transformation by the liver.[47,48] For example, high-fat diet can promote hepatocarcinogenesis through microbial metabolic activities. In a carcinogen-driven model of tumorigenesis using the chemical dimethylbenz(a) anthracene, administration of high-fat diet resulted in a significant increase in liver tumor burden. This was shown to be a result of diet-induced intestinal dysbiosis responsible for the increased production of the secondary bile acid, deoxycholic acid.[49]

Knowledge of the influence diet intervention plays in microbial output can also be used for the benefit of the host. Leukemia is commonly associated with cachexia, a frequent adverse effect to most cancers that is characterized by decreased muscle mass and energy loss. Exploitation of the microbiome's metabolic function using nondigestible carbohydrates has been shown to reverse these effects.[50,51] Nondigestible carbohydrates serve as precursors for microbial production of SCFAs and their effect resulted in increased muscle mass and reduced inflammation.[51] In addition, when combined with the bacterial administration of *Lactobacillus reuteri*, known as synbiotic approach, intestinal homeosta-sis was restored and cytokine production and immune cell recruitment that was observed during carcinogen-esis were reversed in mice.[50]

Altogether, these studies highlight the pronounced effect of diet on the microbiota and its functional consequences on the host.

Interplay Among Host Immunity, Microbiota, and Crc

As mentioned in the previous section, the microbiota has been implicated in multiple types of cancer, but the mechanisms by which bacteria influence carcinogenesis are still unclear. It is clear that numerous processes are at play during bacteria-induced carcinogenesis, one of which is the ability of microorganisms to induce inflammation on recognition by the host innate immune system.[52] There is considerably more information on the immune mechanisms involved in CRC driven by the microbiota, and this section will highlight the recent developments in this field. Pattern recognition receptors (PRRs) are part of the host innate immune response and include C-type lectin receptors, helicase receptors, toll-like receptors (TLRs), and the NOD-like receptor (NLR) family.[53] PRRs sense microbes (microbe-associated molecular patterns) and tissue damage (damage-associated molecular patterns) and play crucial roles in maintaining host and microbiota homeostasis (eubiosis).[53] Dysregulation of PRRs has been linked to multiple inflammatory disorders including obesity and inflammatory bowel disease (IBD), both of which are risk factors of CRC.[54-56] IBD patients have an estimated 2%–40% risk of developing CAC depending on IBD severity, duration, and location.[57]

Single-nucleotide polymorphisms in NOD2, an NLR member, have previously been implicated in IBD, and meta-analysis of 30 human cancer studies shows an association between several polymorphisms in the leucine-rich region of *NOD2* and cancer risk, particularly for GI cancer.[58] Furthermore, review of 10 CRC microarray expression data sets found reduced *NLRC3* expression and increased *NOD1* and *NOD2* correlated with CRC.[59] Interestingly, Nod2-derived intestinal stem cell signaling promotes cellular proliferation after an injury,[60] suggesting that bacteria could feed prolifer-ative signaling to stem cells.[61] In contrast, genetic deletion of *Nod1* or *Nod2* exacerbates CAC development, suggesting that these sensors exert protective functions against carcinogenesis.[62,63] Overall, the data on *NOD2* appear contradictory with *NOD2* polymorphisms and increased *NOD2* expression associated with human CRC, whereas in contrast CAC is worse in *Nod2*$^{-/-}$ mice.

Nod2 deficiency in mice resulted in microbiota dysbiosis that promoted inflammation and tumorigenesis in AOM/DSS-induced CAC through an IL-6–dependent mechanism.[63] Another study found that *Nod2* control of microbiota composition is cell compartment specific as *Nod2* deficiency in nonhematopoietic cells resulted in an altered microbiota, associated with altered mucin and antimicrobial peptide expression in the ileum and colon.[64] However, *Nod2*-mediated dysbiosis is controversial because a study using littermate controls (co-housed WT and *Nod2*$^{-/-}$ mice) failed to observe intestinal dysbiosis.[65] It is unknown whether the *NOD2* polymorphisms or increased *NOD1*/2 expression in human CRC patients are associated with an altered microbiota composition. Clearly, more studies are needed to address how *NOD1*/2 signaling modulates carcinogenesis and which bacteria contribute.

Another critical innate immune response implicated in gut homeostasis is inflammasome activation.[53] This response is triggered by the sensing of various microbe-associated molecular patterns or damage-associated molecular patterns by specific sensors, leading to caspase-1 activation, which then cleaves pro–IL-1β and IL-18 proteins to generate functional cytokines.[66] Gene expression patterns of inflammasome components in CRC microarray data sets revealed reduced *NLRP1, NLRP3, NLRC4*, and *AIM2* in CRC patients compared with healthy controls.[59] In the context of a CAC mouse model, *Nlrp6* and the adaptor protein apoptosis-associated speck-like protein containing CARD (*Asc*) knockout mice are more susceptible to AOM/DSS-induced tumorigenesis than WT mice.[67] Cohousing and antibiotic experiments revealed the involvement of the microbiota in tumorigenesis via induction of the inflammatory cytokines IL-18 and che-mokine (C-C motif) ligand 5, which promoted epithelial cell proliferation through IL-6.[67] A metabolomic screen performed on cecal contents from WT and *Asc*$^{-/-}$ mice suggests that microbial metabolites modulate inflammasome signaling and consequently host response.[68] For example, taurine, a bile acid component, was found to activate the Nlrp6-mediated inflam-masome, inducing antimicrobial angiogenin 4, promoting the epithelial barrier function, and restoring homeostasis.[68] In contrast, histamine and the polyamine spermine were identified as microbial metabolites that inhibit Nlrp6 inflammasome activation and promote dysbiosis.[68] Although these studies suggest that micro-bial metabolites are able to modulate inflammasome signaling, the functional impact of these metabolites in human inflammasome activation remains to be defined. The Nlrp1, Nlrp3, and Nlrc4 inflammasomes have also been implicated in regulating susceptibility to AOM/DSS-induced CAC, but the contribution of microbiota in these findings was not examined.[69-71]

Migration of cancer cells from the organ of origin to other body sites, a process called metastasis, is a hallmark of late-stage cancer and poor prognosis.[72] A model of CRC metastasis where mouse CRC cell lines are intrasplenically injected into mice showed that *Nlrp3* suppresses metastatic growth to the liver.[73] *Nlrp3*-mediated suppression of metastatic growth appeared to be microbially independent because antibiotic treatment did not alter liver metastasis.[73] However, residual bacteria, bacterial products, or microbial-derived metabolites may still be present in antibiotic-treated mice. Stringent experiments involving GF mice are needed to define the relationship among NLRP3, bacteria, and metastasis.

Although, the cytosolic double-stranded DNA sensor, AIM2, was previously implicated in host defense against infection via inflammasome activation,[74,75] 2 groups have demonstrated a protective role for AIM2 in CAC and CRC mouse models that operates through an inflammasome-independent mechanism.[76,77] In one study, *Aim2* deficiency led to increased AOM/DSS-induced tumorigenesis by promoting stem cell proliferation.[76] Microbiota dysbiosis accompanied tumorigenesis in $Aim2^{-/-}$ mice with an increased abundance of *Akkermansia muciniphila* and decreased *Anaerostipes, Bifidobacterium, Flexispira*, and *Prevotella*.[76] Cohousing experiments reduced tumors in $Aim2^{-/-}$ mice and increased tumors in WT mice, suggesting Aim2 protection against CAC is dependent on microbiota.[76] In the other study, *Aim2* deficiency increased tumorigenesis in $Apc^{Min/+}$ and AOM/DSS mice.[77] Additional experiments revealed that Aim2 associates with the PI3K-related family member, DNA-dependent protein kinase (DNA-PK) to limit Akt activation, controlling epithelial cell proliferation and apoptosis, although the role of the microbiota was not addressed in this study.[77]

Multiple proinflammatory cytokines are implicated in CRC pathogenesis, many of which are directly or indirectly affected by the microbiota.[78] Increased IL-23 and IL-17A expressions are found in tumors from human CRC patients and in a spontaneous mouse model of CRC (CPC-APC mice) compared with normal tissue.[79] Additional experiments with antibiotics or $Myd88^{-/-}$ and $Tlr2,4,9^{-/-}$ mice suggest the microbiota promotes IL-23 and IL-17A expressions. Decreased barrier protein expression and function in mouse and human CRC tissues suggests that infiltration of bacteria and microbial components (together or individually) promote tumor inflammation.[79] Another IL-17 family cytokine, IL-17C, is also upregulated in tumors from human CRC patients and mouse CRC models ($Apc^{Min/+}$, AOM/DSS) in a TLR-MyD88–dependent signaling manner.[80] IL-17C induces the prosurvival genes, Bcl-Xl and Bcl-2, in intestinal epithelial cells to promote tumor formation.[80] The microbiota is implicated in IL-17C production because antibiotic-treated mice and GF mice exhibit reduced IL-17C messenger RNA expression.[80] Importantly, *Enterobacteriaceae* abundance, in particular, was shown to be increased during DSS treatment and *Escherichia coli* monocolonized GF mice–induced IL-17C expression after DSS treatment.[80] However, it is unknown whether increased *Enterobacteriaceae* in human CRC stool or tumor tissue samples is associated with increased IL-17C expression.

Mucosa-associated invariant T (MAIT) cells are innate-like T cells that can produce both interferon gamma (IFN-γ) and IL-17, which have been shown to promote either antitumor immunity or tumorigenesis, respectively.[81] MAIT cells are activated by riboflavin (vitamin B2) metabolites, which are produced by bacteria and yeast, suggesting interaction with the

microbiota.[82] To examine the role of MAIT cells in CRC, Sundström et al[81] compared MAIT cell numbers and functional activities in colon tumor and unaffected tissue (>10 cm from tumor) from CRC patient resections. A higher accumulation of MAIT cells in tumor tissues was observed compared with unaffected tissues.[81] Functionally, there was a lower frequency of IFN-γ producing MAIT cells in tumor tissue, which was attributed to factors present in the tumor microenvironment, as tumor tissue–conditioned medium decreased IFN-γ production in vitro. A similarly high number of MAIT cells in CRC tumors were observed in a separate study.[83] However, these studies have not addressed the relationship between the microbiota and MAIT cell frequency and function in CRC, and further studies will be needed to define this possible interplay.

Autophagy can affect multiple aspects of the immune system: PRR signaling, proinflammatory signaling, adaptive immunity, and secretion of immune mediators.[84] Assessment of LC3 vesicular staining in human CRC samples and an *Apc* mouse model revealed autophagy genes are active during CRC.[85] Conditional inactivation of the autophagy gene *Atg7* in intestinal epithelial cells inhibits tumorigenesis in tamoxifen-treated *VilCreER^{T2}Apc$^{flox/+}$* mice (*Apc$^{+/-}$Atg7$^{-/-}$*) by suppressing proliferation and enhancing antitumor CD8+ T cells.[85] This phenotype is dependent on micro-biota because antibiotic-treated Apc$^{1/2}$Atg7$^{-/-}$ mice have a diminished antitumor response.[85] In addition, *Apc$^{+/-}$Atg7$^{-/-}$* mice have disrupted gut mucosal integrity resulting in altered microbiota localization and composition with a higher abundance of firmicutes and a lower abundance of proteobacteria compared with *Apc$^{+/-}$* mice.[85] Thus, the microbiota influences a range of host immune responses including PRRs, inflammasomes, cytokines, MAIT cells, and immune responses affected by autophagy, all of which contribute to cancer susceptibility (Fig 2).

Immune Mechanisms Involved in Specific Bacteria-Driven CRC

Although the nature of microbial interactions with the host is polymicrobial, it is important to dissect the individual contributions of these microorganisms to carcinogenesis. Specific bacterial candidates implicated in human CRC include enterotoxigenic *Bacteroides fragilis* (ETBF), *E. coli*, and *Fusobacterium nucleatum* and have been shown to influence the carcinogenic process through various strategies including production of toxins, genotoxins, and specific microbial genes.[31,86]

Interestingly, some microbial activities impact host immune responses or are modulated by host-derived inflammation. Mucosal T regulatory lymphocytes (Tregs) have been shown to promote cancer initiation via the enhancement of IL-17A production in ETBF-colonized *Apc* [716] mice.[87] Genetic depletion of Tregs for the first 2 weeks after ETBF colonization reduced microadenoma numbers but not inflammation (increased IFN-γ, decreased IL-17A) in *Apc* [716] mice, unexpectedly suggesting that Tregs enhance cancer initiation by promoting T helper 17 (Th17) differentiation through an IL-2–dependent mechanism.[87] The cellular source of IL-17A in ETBF-colonized *Apc* [716] mice was examined by ablating IL-17 production via Stat3 inactivation in CD4+T cells (*CD4$^{Stat3-/-}$*).[88] Tumorigenesis was delayed in *CD4$^{Stat3-/-}$* mice and their tumors still had increased IL-17A expression, suggesting the involvement of additional IL-17A–producing cells.[88] $\gamma\delta$T cells were

identified as the other IL-17A producers by flow cytometry of $CD4^{Stat3-/-}$ tumor tissue, which was then confirmed with bone marrow chimera experiments.[88] In addition, the presence of both Th17 and $\gamma\delta$T17 cells in human CRC tumor tissues suggests that both cell types contribute to IL-17A production in CRC, although the role of ETBF in this observation was not examined.[88] Strikingly, ETBF seems important for both tumor initiation and progression in Apc^{716} mice, as ETBF clearance with the antibiotic cefoxitin 5 or 14 days after colonization differentially impacts IL-17A cytokine expression and adenoma numbers, with both time points reducing microadenoma formation.[89]

Colibactin is a microbial-derived genotoxin encoded on the pathogenicity island *pks* found predominantly in phylogroup B2 *E. coli.*[90,91] This genotoxin induces double-strand DNA breaks and is essential for *E. coli*–induced CRC.[91,92] Interestingly, microbial RNA-sequencing suggests that host inflammation altered 3% of cancer-promoting genes in *pks*+ *E. coli*, including 5 *pks* island genes that were increased in *E. coli* monoassociated *AOM/IL10^{-/-}* compared with AOM *IL10^{-/-}; Rag2^{-/-}* mice.[93] Among these *pks* genes was *clbM*, which was recently identified as a multi-antimicrobial extrusion protein transporter of precolibactin.[94] High abundance of *Enterobacteriaceae* is observed in CRC patients and in mouse models of CRC,[95] but the fact that *pks*+ *E. coli* failed to promote CRC in *AOM/IL10^{-/-}; Rag2^{-/-}* mice (inflammation deficient) suggests that inducible microbial activities in addition to abundance are critical for *E. coli*–induced CRC.[93] The inflammatory-derived factors influencing bacterial carcinogenic potential are unknown but could be secondary to inflammation-induced tissue damage (cell-derived nucleotides, amino acids, minerals, and so forth).

As opposed to *E. coli* and ETBF, *F. nucleatum* is not considered a proinflammatory bacterium in mice.[96] Nevertheless, this microorganism has a profound impact on immune responses, an effect important for carcinogenesis. Flow cytometry on tumors from *F. nucleatum*–colonized mice and RNA-sequencing data of human CRC patients suggest a positive association between the bacteria and the presence of tumor-infiltrating myeloid cells.[96] In vitro assays with primary human cells suggest that *F. nucleatum* is capable of interfering with host immunity by binding the human inhibitory receptor T cell immunoglobulin and ITIM domain (TIGIT) via its Fap2 surface protein, leading to inhibition of natural killer cell cytotoxicity and other T cell activities.[97] A correlation has also been observed between *F. nucleatum* levels in human colorectal carcinoma tissue and reduced CD3+ T cells.[98] Thus, *F. nucleatum* may promote immune evasion, one of the new hallmarks of cancer.[72]

Although *Helicobacter hepaticus* has not been associated with human CRC, infection of AOM/129SvEv.*Rag2^{-/-}* with *H. hepaticus* is used to promote CAC in mice.[99] IL-17+IL-22+ group 3 innate lymphoid cells (ILC3s) accumulate in the colon of *H. hepaticus*–infected AOM/129SvEv.*Rag2^{-/-}* mice. Depletion of ILCs with anti-Thy1 or anti–IL-22 treatment reduced inflammation and tumors, suggesting a driving role for IL-22 producing ILC3s in *H. hepaticus*–induced CAC.[99] Additional work suggests that ILC3-produced IL-22 promotes CAC by inducing epithelial cell proliferation and antimicrobial peptide production in a Stat3-dependent manner.[99] Adaptive immune cells also produce IL-22, which cannot be studied in *Rag2^{-/-}* mice, and therefore more investigations will be needed to address the role

of these cells. IL-22 may also play a role in human CRC, as there was higher IL-22 expression in tumor tissue compared with normal tissue in 7 of 12 matched CRC patient samples; unfortunately, the role of bacteria in this observation is unknown.[99] In summary, the microbiota affects and is affected by a range of host immune responses, all of which may contribute to CRC pathogenesis. Moreover, the impact of the intestinal microbiota on host immune responses extends beyond CRC, influencing both extraintestinal cancers and cancer therapeutics.

Microbiota, Drug Toxicity, and Cancer Therapy

The implication of the microbiota in cancer pathogen-esis has opened new opportunities for preventive or therapeutic intervention through microbiota manipulation, and various modalities (eg, antibiotics, probiotics, prebiotics, postbiotics, and so forth) have been proposed and tested in preclinical models.[100-103] However, the modulatory impact of the microbiota on cancer extends beyond pathogenesis as recent evidence highlighted an interaction between bacteria and established cancer therapeutics. These bacteria–drug interactions originate from the extensive metabolic capacity and profound immunomodulatory effect of the microbiota (Fig 3).

Microbiota and drug toxicity. Chemotherapeutics

Chemotherapeutic drugs designed to target rapidly growing cancer cells are commonly used in cancer treatments but are frequently associated with severe cytotoxicity for the host.[104] For example, the prodrug irinotecan (CPT-11) is a topoisomerase I inhibitor typically used in patients with metastatic carcinoma of the colon.[105] The prodrug is transformed into the active topoisomerase I inhibitor SN-38, which is further processed in the liver to form inactive SN-38G derivative. SN-38G is excreted via biliary ducts into the GI tract, where it is converted back to cyto-toxic SN-38 by bacterial β-glucuronidases. The presence of active SN-38 in the intestine causes severe diarrhea in a significant subset of patients, leading to dose reductions or treatment termination. Early studies showed that Kampo medicine (Hangeshashinto) and D-saccharic acid 1,4-lactone, both possessing inhibitory activities against β-glucuronidases, could alleviate CPT-11–induced diarrhea in patients and mucosal damage in rats, respectively.[106,107] Subsequently, inhibitors of *E. coli*–derived β-glucuronidase were shown to protect the host against CPT-11–induced GI toxicity in mice.[108,109] These studies provide direct evidence that reactivation of SN-38G by the commensal gut micro-biota plays an essential role in CPT-11's toxic effect and suggests that targeting bacterial β-glucuronidases has great translational potential. More recently, representative β-glucuronidases were characterized from other commensal bacteria including the firmicutes *Streptococcus agalactiae* and *Clostridium perfringens* and the bacteroidetes *B. fragilis*.[110] The β-glucuronidases produced by different bacteria display distinct catalytic properties and inhibition propensities.[110] Future studies need to identify high potent inhibitors of these β-glucuronidases for clinical tests. Interestingly, while inducing CPT-11 toxicity in the intestine via β-glucuronidase activities, the microbiota could also promote resistance to the same drug by generating mucosal protective metabolites. Studies have shown that dietary fiber supplementation ameliorates CPT-11 toxicity without affecting microbial β-glucuronidase activity, a phenomenon attributed to high levels of

butyrate generated from microbial metabolism.[111] This highlights the complexity of microbiota–drug interactions in the intestine.

Methotrexate (MTX) is another chemotherapeutic agent widely used in cancer treatments.[112] Similar to CPT-11, MTX-induced GI toxicity is the major dose-limiting aspect for patient management.[112] Although the precise pathophysiology underlying MTX-associated GI toxic effects remains elusive, a recent study suggests that the gut microbiota is involved.[113] Frank et al[113] reported exacerbation of MTX-induced mucositis in mice lacking the innate receptor TLR2 compared with WT mice. Further investigation revealed that stimulation of TLR2 by the receptor agonist Pam_3-CysSK4 protects against MTX-induced GI toxicity through activation of the multidrug efflux system ABCB1/(MDR)1 p-glycoprotein.[113] Importantly, microbiota depletion by antibiotics led to increased susceptibility to MTX-induced mucosal injury in WT mice.[113] Together, the results suggest that microbial activation of TLR2 signaling attenuates MTX-induced GI toxicity. Whether microbial-driven TLR2 signaling plays a role in detoxifying other cancer drugs remains to be investigated.

Immune checkpoint blockade

Ipilimumab, an antibody against the immune checkpoint protein cytotoxic T-lymphocyte–associated protein 4 (CTLA-4), is used mainly to treat metastatic melanoma and often leads to colitis development in patients because of poor function of Tregs.[114] Recent studies showed that intestinal reconstitution of microbiota-depleted mice with the combination of *B. fragilis* and *Burkholderia cepacia* attenuated CTLA-4-blockade–induced histopathologic signs of colitis, likely via enhancing Treg response.[115] Consistent with this observation, high abundance of gut bacteria belonging to the bacteroidetes phylum correlated with resistance to CTLA-4-blockade–induced colitis in patients.[116] Thus, supplementation of *B. fragilis* and *B. cepacia* could be beneficial for patients undergoing ipilimumab treatment.

Microbiota and Cancer Therapy Efficacy

Chemotherapy

As mentioned previously, cancer drug efficacy could be influenced by the microbiota. A recent survey of in situ bacterial effects on frequently used chemotherapeutics suggests profound influence of distinct bacteria speciesonthe antitumor effect of these drugs.[117] Lehouritis et al found that after preincubation with nonpathogenic gram-negative *E. coli* Nissle 1917 or gram-positive *Listeria welshimeri* Serovar 6B SLCC5334, 10 of 30 chemotherapeutic drugs (eg, gemcitabine, cladribine, daunorubicin, and so forth) showed reduced cancer cell killing efficacy, whereas 6 drugs (eg, fludarabine phosphate, CB1954, and so forth) showed increased cancer cell killing efficacy in vitro.[117] High-performance liquid chromatography–mass spectrometry studies showed that these effects are often bacteria specific, likely because of the unique biotransforming activities associated with each bacterial species/strain.[117] The in vitro bacteria-specific effect on drug cytotoxicity can be replicated in vivo using a xenograft mouse tumor (CT26 colon carcinoma) model.[117] Thus, bacteria could directly metabolize chemotherapeutic drugs to affect their efficacy.

Understanding the microbial pathways involved could help to improve cancer therapy outcomes.

Another means by which bacteria impact anticancer drug efficacy is through modulation of host inflammatory and immune responses. Viaud et al reported that nonmyeloablative doses of cyclophosphamide (CTX), a potent alkylating cytotoxic drug used to treat lymphomas and certain solid tumors,[118] induced IL-17 and IFN-γ–expressing "pathogenic" Th17 (pTh17) cells in the spleen, which mediate the therapeutic effect of the drug in xenograft (P815 mastocytoma and MCA205 sarcoma) and genetic (lung adenocarcinoma) mouse tumor models.[119] Importantly, this antitumor immune response was diminished in GF, antibiotics-treated mice, or vancomycin (specific for gram-positive bacteria)-treated mice, indicating that gram-positive commensal bacteria are required for CTX efficacy. CTX treatment disrupted the small intestine barrier and facilitated translocation of commensal bacteria, particularly the gram-positive *Lactobacillus johnsonii, Lactobacillus murinus,* and *Enterococcus hirae,* into mesenteric lymph nodes and spleen. Oral administration of *L. johnsonii* and *E. hirae,* but not *Lactobacillus plantarum,* which did not translocate after CTX treatment, restored the pTh17 response in the spleen of antibiotics-treated mice.[119] This study clearly demonstrates that specific gram-positive commensal bacteria contribute to the anticancer efficacy of CTX through engagement of host-derived immune response.

In support of this concept, a study led by Iida et al[120] showed that the commensal microbiota promotes the antitumor effects of the platinum compounds oxaliplatin and cisplatin, as microbiota depletion by antibiotics reduced the efficacy of these drugs against subcutaneous tumors (EL4 lymphoma and MC38 colon carcinoma). Mechanistically, the commensal-dependent reactive oxygen species production by myeloid cells is responsible, at least partially, for the efficacy of these platinum compounds. In line with the observation, Gui et al reported that antibiotic cotreatment reduced the efficacy of cisplatin in the Lewis lung cancer mouse model.

Immunotherapy

The work by Iida et al[120] also demonstrated that the gut microbiota modulates the antitumor effect of CpG-oligonucleotide immunotherapy. The researchers found that combined anti–IL-10R treatment and CpG-oligonucleotide immunotherapy slowed xenograft tumor growth (EL4 lymphoma, MC38 colon carcinoma, and B16 melanoma) and prolonged mouse survival by inducing tumor necrosis factor (TNF)-dependent cytotoxic CD8+ T cell response in the tumor environment, which is significantly impaired in microbiota-depleted or GF mice.[120] Further investigation identified specific bacteria positively (eg, *Alistipes, Ruminococcus*) or negatively (eg, *Lactobacillus*) correlated with TNF production from tumor-associated myeloid cells.[120] Oral administration of Alistipes shahii reconstituted TNF production by tumor-associated myeloid cells in microbiota-depleted mice, whereas gavage of *Lactobacillus fermentum* attenuated the response in SPF mice.[120]

More recently, specific commensals have been reported to mediate the antitumor effects of immune checkpoint blockers. Vetizou et al[115] showed that anti–CTLA-4 antibody failed to inhibit tumor growth (MCA205 sarcoma, MC38 colon carcinoma, and Ret melanoma) in GF or antibiotics-treated mice because of defective antitumor Th1 response. Microbial profiling

identified bacterial species (eg, *Bacteroides* genus and species) associated with anti–CTLA-4 treatment, and functional studies revealed that *Bacteroides thetaiotaomicron*, *B. fragilis*, and *B. cepacia* could stimulate the CTLA-4–induced antitumor immune response and thereby therapeutic efficacy.[115] Clinically, the intestinal microbiota of patients with metastatic melanoma can be distinguished into 3 clusters: cluster A driven by the *Alloprevotella* or *Prevotella*, and clusters B and C by distinct *Bacteroides* spp.[115] After ipilimumab (anti–CTLA-4) therapy, many cluster B patients switch to cluster C.[115] Only mice colonized by cluster C, but not A or B fecal microbiota, showed increased abundance of *B. fragilis*, which negatively correlated with the antitumor effect of CTLA-4 blockade.[115] Thus, CTLA-4 blockade can modify the abundance of immunogenic *Bacteroides* spp., which in turn affect the efficacy of treatment.[115]

Programmed death–ligand 1 (PD-L1) is another immune checkpoint molecule targeted for cancer therapy and often used in combination with CTLA-4 blockade agents. Sivan et al[122] compared subcutaneous B16.SIY melanoma growth and response with PD-L1 blockade in C57BL/6 mice obtained from Jackson Laboratory (JAX) and Taconic Farms (TAC). These mice have previously been shown to display different intestinal microbiota and immune responses.[123] Interestingly, tumors grew more aggressively in TAC mice, which are paralleled by dampened tumor-specific T cell responses compared with JAX mice.[122] Moreover, the TAC phenotype can be reversed by cohousing or transplanting JAX fecal materials, suggesting that the mi-crobiota drives the differential phenotype.[122] PD-L1 blockade showed better outcomes in JAX mice than TAC, as assessed by tumor growth inhibition and anti-tumor CD8+ T cell response.[122] Microbiota analyses revealed that *Bifidobacterium* was strongly associated with the antitumor T cell response.[122] Strikingly, oral gavage of a *Bifidobacterium* species cocktail, which included *Bifidobacterium breve* and *Bifidobacterium longum*, significantly decreased tumor growth in TAC mice and when combined with PD-L1 blockade abolished tumor growth.[122] Mechanistically, the researchers showed that dendritic cells play a role in bridging *Bifidobacterium*-derived signals and the antitumor immune response.[122] These findings support the concept that bacteria strongly influence the antitumor efficacy of drugs targeting immune checkpoint molecules. Because these studies were mostly conducted with xenograft models, the clinical relevance of the findings will need to be extended in model with primary tumors. Nevertheless, it is clear that the microbiota has tremendous impact on cancer therapeutics (efficacy–toxicity) and this "drug–bug" interaction deserves further mechanistic investigation and translational validation.

Conclusion and Future Directions

The microbiome is deeply embedded in human metabolic function and represents an integral part of host homeostasis. The impact of this microbiota on homeostasis starts early at birth and is sustained throughout life, with modifying pressure coming from environmental cues such as lifestyle and diet. Therefore, it is not surprising that disruption of the microbial ecosystem has repercussions on host homeostasis, which could lead to pathologies such as cancer (Fig 4). The wide impact of the microbiota on the carcinogenic process is remarkable and spans initiation, progression, tumor evasion, and therapeutic responses. The long-distance impact of the intestinal microbiota on carcinogenesis suggests that cancer in a given

organ is not strictly linked to a local biota, again showing the wide networking activities of microorganisms.

Despite all the connections made between the microbiota and carcinogenesis, numerous questions remain unanswered. For example, it is unclear how microbial dysbiosis influences tumorigenesis (local or long distance) and whether ensembles of microbial activities (protective and deleterious) are at play in the process. Are these activities originating from tissue-associated or planktonic microorganisms? The biotransformation capacity of the intestinal microbiota is certainly an important element in the selection and maintenance of microbial consortia throughout evolution. Linking functional anticarcinogenic nutrients to biotransformative ability of specific microorganisms would have a tremendous impact in cancer prevention. An exciting and emerging field of research in microbiome cancer is undeniably the interaction between bacteria and anticancer drugs. However, it is imperative to strengthen this field of research and document the interaction between anti-cancer therapies and bacteria using preclinical primary tumor models to ascertain the physiological impact of bacteria in cancer therapeutics. Although challenging to set up, studies linking treatment outcomes using specific drugs with that of intestinal microbial composition would help define the physiological impact of drug–bug interactions. In addition, identification of microbes and microbial genes responsible for drug biotransformation as well as the specific immune cells engaged by microbes and implicated in the therapeutic response should be investigated. As more mechanistic understandings emerge from this new drug–bug field of research, one could envision pairing bacteria (or bacterial-derived molecules) with a given compound to obtain maximum efficacy and lower toxicity.

In summary, microbiota unifies numerous processes including nutrition, metabolism, and immunity, which represent key biological activities for carcinogenesis, and it is clear that the microbiota-cancer is not a flash in the pan but rather a transformative new field of research that would likely impact the way cancer is detected, treated, and managed in the future.

Acknowledgments

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Abbreviations

AOM	azoxymethane
Apc	adenomatous polyposis coli
CAC	colitis associated cancer
CRC	colorectal cancer
DNA-PK	DNA-dependent protein kinase
GF	germ free
GI	gastrointestinal
IBD	inflammatory bowel diseases
IL	interleukin
MAIT	mu-cosa-associated invariantT
MTX	methotrexate
NLR	NOD-like receptor
NLRP1	NLR family, pyrin domain containing 1
NOD	nucleotide-binding oligomerization domain-containing protein
PDL-1	programmed cell death protein1ligand1

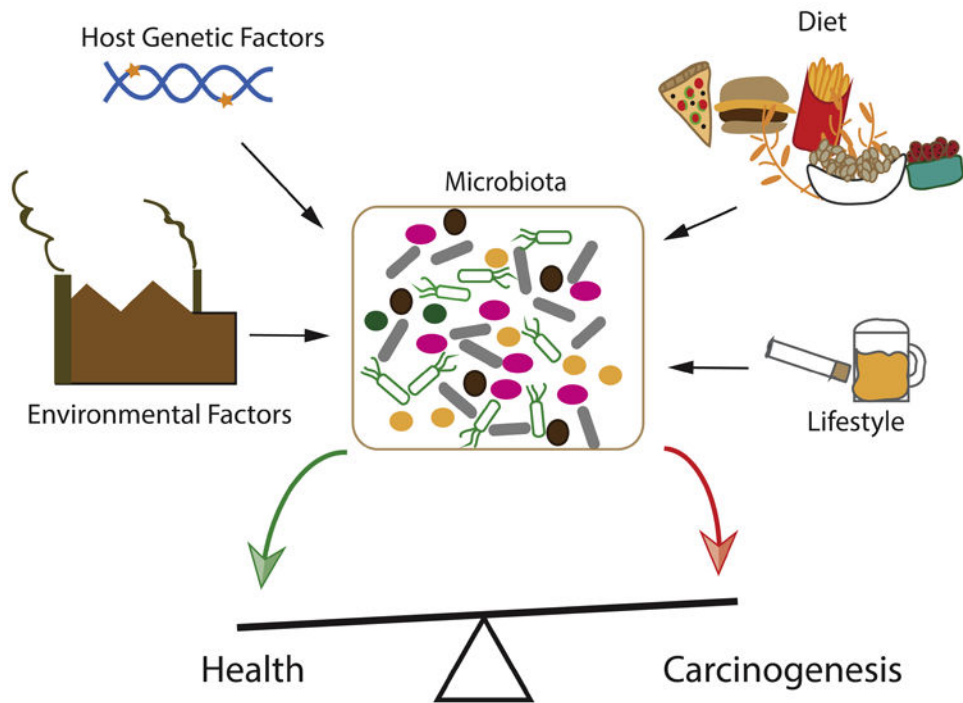|	
PI3K	phosphoinositide 3-kinase
PRR	pattern recognition receptors
SCFA	short chain fatty acids
TIGIT	T cell immunoglobulin and ITIM domain
TLR	toll-like receptors
Tregs	regulatory T cells
WT	wild type

Fig 1.
The microbiota regulates the balance between health and disease. A combination of external factors can influence microbial composition, including host genetics, diet, lifestyle, and environmental factors. These perturbations in the microbiota shift the balance between healthy and carcinogenesis.
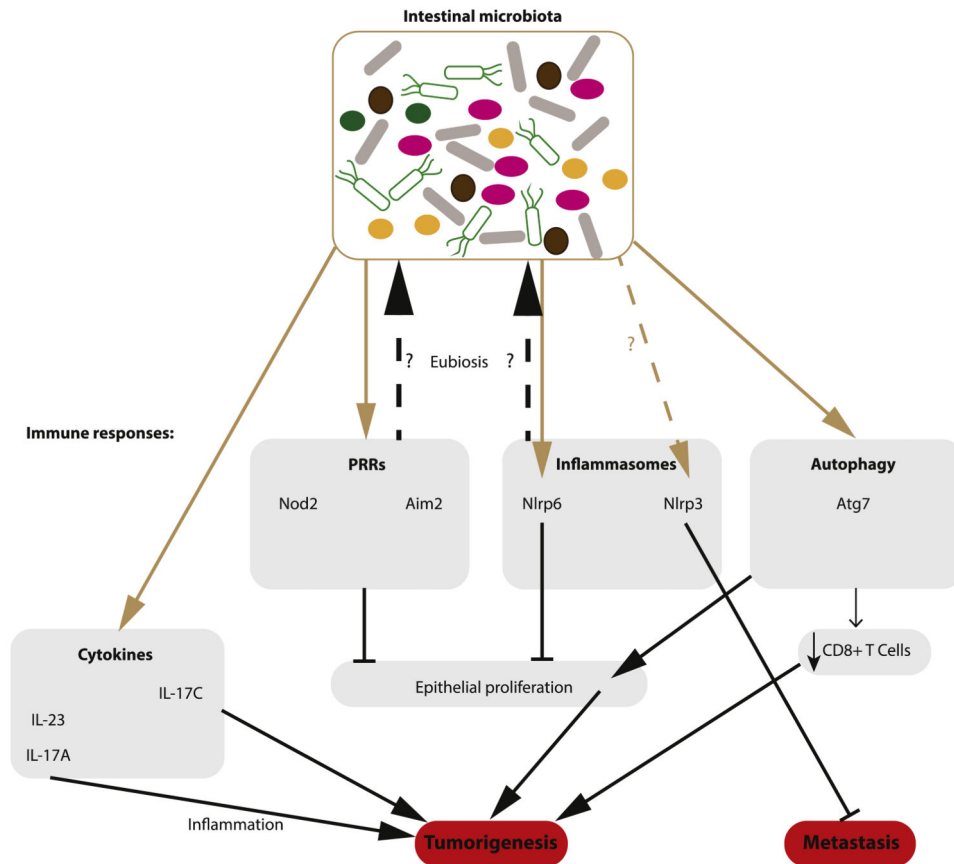
Fig 2.
Interplay among the microbiota, host immunity, and CRC. The intestinal microbiota induces a variety of host immune responses (brown arrows) including cytokine production, PRRs, inflammasomes, and autophagy, all of which contribute to cancer development. The microbiota induces the proinflammatory cytokines IL-23, IL-17A, and IL-17C, which promote CRC. The PRRs Nod2 and Aim2 and the Nlrp6 inflammasome protect against tumor-igenesis by regulating epithelial proliferation and may contribute to eubiosis. Nlrp3 suppresses metastatic growth to the liver, a phenotype which is unaffected by antibiotics. The autophagy gene Atg7 promotes tumorigenesis in an *Apc* model by promoting proliferation and decreasing the antitumor CD8+ T cell response. CRC, colorectal cancer; IL, interleukin; PRR, pattern recognition receptor. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
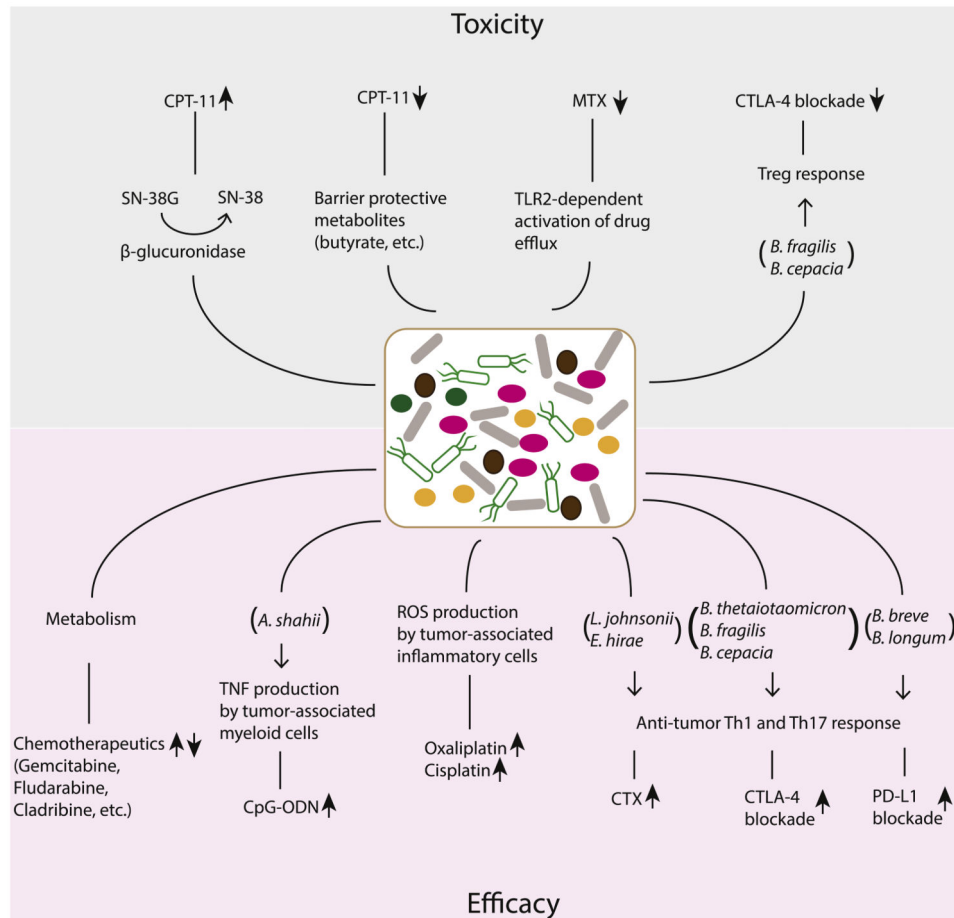
Fig 3.
Interaction between bacteria and anticancer drugs. The microbiota influences anticancer drug efficacy and toxicity through direct or indirect mechanisms. Bacterial β-glucuronidases convert SN-38G to active SN-38, leading to the toxic effect of CPT-11. Microbiota can generate barrier-protective metabolites such as butyrate and activate TLR2/drug efflux response to attenuate CPT-11 and MTX toxicity, respectively. *B. fragilis* (via Treg response) and *B. cepacia* can ameliorate CTLA-4-blockade–induced intestinal inflammation. On the other hand, bacteria profoundly influence the efficacy of chemotherapeutics via a metabolic route. Microbial-driven ROS production by tumor-associated inflammatory cells promotes the antitumor effect of oxaliplatin and cisplatin. *A. shahii* induces TNF production by tumor-associated myeloid cells, which contributes to the antitumor effect of CpG-ODN. Efficacies of CTX, CTLA-4 blockade, and PD-L1 blockade can be enhanced by specific and distinct bacteria. CpG-ODN, CpG-oligonucleotide; CPT, irinotecan; CTLA, cytotoxic T-lymphocyte–associated protein 4; MTX, methotrexate; ROS, reactive oxygen species; TLR, toll-like receptor; TNF, tumor necrosis factor.
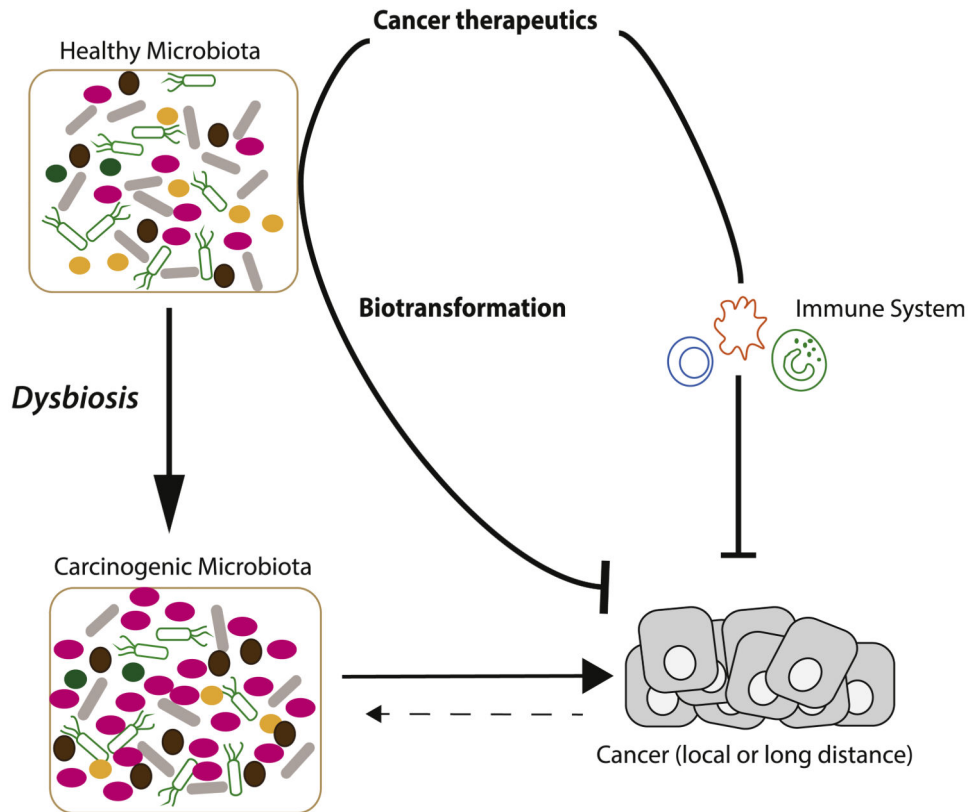
Fig 4.
Gut microbiota regulates carcinogenesis at various levels. Perturbations in the healthy microbiota lead to dysbiosis, increasing the number of procarcinogenic bacteria that can have local or long-distance effects. Healthy microbiota can biotransform anticancer therapeutic drugs, impacting their toxicity and efficacy. In addition, cancer therapeutics can function synergistically with the immune system to inhibit cancer.