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Carcinogenesis and therapeutics: the microbiota perspective

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Abstract

Cancer arises from the acquisition of multiple genetic and epigenetic changes in host cells over the span of many years, promoting oncogenic traits and carcinogenesis. Most cancers develop following random somatic alterations of key oncogenic genes, which are favoured by a number of risk factors, including lifestyle, diet and inflammation. Importantly, the environment where tumours evolve provides a unique source of signalling cues that affects cancer cell growth, survival, movement and metastasis. Recently, there has been increased interest in how the microbiota, the collection of microorganisms inhabiting the host body surface and cavities, shapes a micro-environment for host cells that can either promote or prevent cancer formation. The microbiota, particularly the intestinal biota, plays a central role in host physiology, and the composition and activity of this consortium of microorganisms is directly influenced by known cancer risk factors such as lifestyle, diet and inflammation. In this Review, we discuss the pro- and anticarcinogenic role of the microbiota, as well as highlighting the therapeutic potential of microorganisms in tumourigenesis. The broad impacts, and, at times, opposing roles of the microbiota in carcinogenesis serve to illustrate the complex and sometimes conflicted relationship between microorganisms and the host—a relationship that could potentially be harnessed for therapeutic benefits.

Microorganisms are present in all terrestrial and aquatic habitats and this ubiquitous environmental presence has produced symbiotic, mutualistic and parasitic coevolution between hosts and microorganisms across kingdoms. In humans, this evolutionary process has led to the acquisition of a rich and diverse set of microorganisms, comprising Bacteria, viruses, fungi, protozoa and Archaea inhabiting every surface and cavity of the human body.

Although essential for life, a narrow segment of the microbial world is categorized as pathogenic and exposure to these microorganisms causes various infectious diseases, some

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leading to the development of cancer[1,2]. For example, subjects infected with microorganisms that are classified as class 1 carcinogens, such as *Helicobacter pylori*, hepatitis B or C viruses, Epstein-Barr virus or Kaposi-sarcoma-associated herpesvirus infection, were found to develop various cancers, including lymphoma, leukaemia, gastric cancer and hepatocellular carcinoma (HCC)[3]. In addition to the presence of specific pathogens, host environmental conditions such as smoking[4], inflammation[5-7], antibiotics[8] and diet[9] can promote changes in microbial community composition or metabolic activity, which may lead to conditions favouring neoplastic changes (Fig. 1). To add another layer of complexity, these host–microbiota interactions also modulate host susceptibility to infectious bacteria (*Helicobacter*)[10] and viruses (such as norovirus, rotavirus, poliovirus)[11-13], suggesting that cancer susceptibility depends on the interactions between the normal microbiota and infectious entities.

Efforts to better understand the microbial communities of humans have become a major focus of research over the last decade. Initial characterization studies were mostly directed at compositional changes within bacterial communities living at different body sites using high-throughput nucleic acid sequencing techniques, but more recent studies have included more comprehensive sampling and analysis strategies, such as the generation of RNA sequences from metagenomes (such as metatranscriptomes)[14,15]. Among the various body locations subjected to these studies, the digestive tract, mouth to anus, was found to harbour the most abundant and rich repertoire of microorganisms, with the lower intestine (colon) showing the highest density of microorganisms[16]—although the ratio of bacteria to human cells is probably lower than the 10:1 ratio often cited[17]. These microorganisms have been studied in the context of health and disease and compared to a state of normalcy or eubiosis, and intestinal bacterial communities have been characterized as dysbiotic in many diseases, including allergy, asthma, rheumatoid arthritis, cardiovascular diseases, metabolic syndrome, obesity, inflammatory bowel diseases (IBDs) and colorectal cancer (CRC)[18–23]. Therefore, the intestinal bacterial community appears to influence local as well as extraintestinal function. This review will focus on recent discoveries on how the intestinal microbiota exerts its modulatory role on carcinogenesis, particularly CRC, and whether and how microorganisms could be integrated into the therapeutic landscape.

Bacteria as a modulating agent of carcinogenesis

The role of bacteria in carcinogenesis is complex, as both pro- and anticarcinogenic functions have been attributed to microorganisms[24]. For example, the pro-neoplastic activity of bacteria in cancer has been known for decades as exemplified in the promotion of gastritis and cancer by pathogenic *Helicobacter*—the first bacteria classified as a group 1 carcinogen[25]. Studies using *Helicobacter* have demonstrated that toxin cytotoxin-associated gene A (CagA)-induced DNA damage and the promotion of host-derived inflammatory mediators and growth factors are direct risk factors for carcinogenesis[26]. *Helicobacter* toxin CagA is an oncoprotein that enhances DNA damage through host-mediated overproduction of reactive oxygen species (ROS)[27–29]. Vaculolating cytotoxin A (VacA) alters membrane permeability and can lead to increased rates of apoptosis, and a recent meta-analysis has confirmed its association with increased gastric cancer risk[30,31]. Remarkably, the link between gastric cancer and *Helicobacter* appears specific, as a recent study suggests that no

other single bacteria has a major influence on the development of some gastric cancers[32]. *Helicobacter* colonization has been shown to increase cancer rates by an estimated six times, possibly through induction of high levels of nitric oxide release from immune cells[33]. However, recent literature suggests that *Helicobacter* can also play protective roles against cancers[34–37]. For example, a meta-analysis of *H. pylori* and oesophageal cancer risk demonstrated a statistically significant decrease in risk associated with infection, although the mechanism for such a protective effect remains unknown[38]. This duality of cancer promotion and protection at the single-species level indicates that host health outcomes that are associated with microorganisms are highly context dependent. *Salmonella typhi* has also been associated with increasing gallbladder cancer risk[39], and recent evidence has shown that effector proteins delivered through the type III secretion system of this bacterium induce MAPK (mitogen-activated protein kinase) and Akt signalling—critical host responses that lead to cellular transformation[40].

While pathogens such as *Helicobacter* can directly promote cancer risk, an indirect mechanistic link between the microbiome and cancer is inflammation[41]. This major environmental stress, derived from either chronic or para-inflammation, is strongly implicated with increased cancer risk[41–45]. At steady state (eubiosis), the microbiota participates in homeostasis by generating metabolites such as short-chain fatty acids (SCFAs) and by engaging protective innate and adaptive immune responses[16,46] (Fig. 2). However, inflammation is associated with the expansion of disease-promoting bacteria and depletion of protective bacteria, leading to a state of microbial dysbiosis[47] with the potential for positive-feedback loops promoting further inflammation. The mechanisms by which inflammation contributes to change in microbial composition and activities are still unclear, but it is likely that they depend on the ability of bacteria to adapt to an inflammatory environment and utilize unique resources present in this environment (see next paragraph). For example, patients with chronic intestinal inflammation displayed reduced overall microbial diversity with increased representation of specific families such as Enterobacteriaceae and Fusobacteriaceae, whose species, including adherent invasive *Escherichia coli* and *Fusobacterium nucleatum*, are well-documented pro-inflammatory bacteria[48]. Patients with IBDs also showed reduced abundance of Lachnospiraceae, a family containing SCFA-producing members (for example, group IV and XIVa Clostridia). These microbial metabolites modulate immune responses and play a critical function in epithelial cell energy balance, a key element in host homeostasis[49,50]. This depletion of SCFA-producing bacteria may not only alter host-dependent immune signalling but may also create favourable conditions for the emergence of potential carcinogenic strains. For example, antibiotic-mediated depletion of butyrate-producing Clostridia leads to expansion of *Salmonella enterica* serovar Typhimurium due to decreased hypoxic conditions at the epithelium level[51]. SCFAs, however, do not always promote beneficial effects on the host. For example, microbiota-derived acetate production leads to metabolic syndrome in TLR5 (toll-like receptor 5) gene-deficient mice, a mechanism that is associated with increased liver glucogenesis[52]. More specific to cancer, a recent study showed that microbial-derived butyrate promotes carcinogenesis by enhancing intestinal epithelial cell proliferation in a *MutS homologue 2* gene-deficient mouse crossed to a multiple intestinal neoplasia (Min or $Apc^{min/+}$) model (ref. 53). It is possible that the impact of SCFAs on the host is beneficial at

steady state but promotes deleterious effects under specific genetic or inflammatory conditions.

Facultative anaerobic pathogen and pathobiont strains thrive in an inflammatory environment due, in part, to their ability to utilize inflammation-derived molecules such as nitrites and oxides as electron acceptors—a feature not shared by many symbionts[54]. In addition, microbial metabolism is altered under dysbiotic conditions, conferring new microbial phenotypes such as enhanced cellular adherence and invasion, mucus utilization, and production of metabolites and toxins (such as H_2S, bile acids and genotoxins)[1,2,55]. Furthermore, it has recently been recognized that the induction of inflammation allows bacteria and tumour cells to communicate via peptides associated with quorum sensing, which could contribute to metastasis[56]. Taken together, these studies demonstrate that inflammation creates host-derived (cytokines, growth factors, radical oxygen and nitrogen species) and bacteria-derived (genotoxins, H_2S, bile acids, radical species, and so on) pro-carcinogenic conditions that provide an ideal landscape for cancer development (Fig. 2).

Establishing an ‘ecosystem’ profile of cancer dysbiosis appears to be an important first step toward the identification of the microbial community implicated in the promotion or protection of carcinogenesis. Microbial dysbiosis has been observed in oral, lung, breast and liver cancers (Box 1)[57]. However, functional experiments causally linking the state of dysbiosis to carcinogenesis have not been extensively performed in these organs. In contrast, microbial dysbiosis has been extensively studied in patients with CRC, and preclinical models have shown clear functional consequences. For example, an altered microbial community is observed between tumour and normal flanked tissue of CRC patients[58,59], distal versus proximal tumours, and across the neoplastic progression from adenoma to adenocarcinoma[60,61]. Across this spectrum, specific changes within the intestinal microbial community are observed in CRC patients, such as increased abundance of *Fusobacteria*, *Alistipes*, Porphyromonadaceae, Coriobacteridae, Staphylococcaceae, *Akkermansia* and Methanobacteriales, while representation of *Bifidobacterium, Lactobacillus, Ruminococcus, Faecalibacterium, Roseburia* and *Treponema* decreased[23,62,63]. *Bifidobacterium* and *Lactobacillus* spp. have been shown to possess antitumourigenic effects in preclinical models[64]. Changes in the microbial community in CRC patients may be robust enough to serve as potential non-invasive biomarkers to predict carcinogenic stages[65–67]. Reproducibility across studies remains a serious challenge, however, although the field is beginning to address these concerns with new tools that evaluate the generalizability of models across studies[68].

Numerous factors can influence intestinal microbiota composition, including diet, medication, disease and health parameters[69]. Host genetics has also been shown to influence human intestinal microbiota composition[70]. It is still unclear whether microbial dysbiosis observed in human CRC patients is a consequence of the pathology or a leading factor promoting the disease. Animal models suggest microbial change occurs before development of neoplasis. For example, at pre-neoplastic stage, the colonic mucosa of *Apc*$^{min/+}$ mice showed increased relative abundance of Bacteroidetes spp. compared to wild-type mice. In addition, microbial composition changed during inflammation onset in *IL10*$^{-/-}$ (interleukin 10 knockout) mice, which preceded development of invasive CRC[71]. Moreover, deletion of

the antimicrobial peptide lipocalin-2 (*Lcn2*) gene in *Il10*$^{-/-}$ mice (*Il10*$^{-/-}$ *Lcn2*$^{-/-}$) leads to intestinal inflammation, microbial dysbiosis and increased tumourigenesis compared to *Il10*$^{-/-}$ mice[72]. Cross-fostering experiments between *Il10*$^{-/-}$ *Lcn2*$^{-/-}$ mother and *Il10*$^{-/-}$ newborn mice showed that tumourigenesis is communicable[72]. In a separate study, faecal transfer of a dysbiotic community from tumourbearing mice into germ-free mice lead to higher tumour numbers compared to healthy community transfer[73]. Whether human cancer-associated dysbiotic microbiota is functionally implicated in carcinogenesis remains unclear. Recent studies showed that colitis developed in germ-free *Il10*$^{-/-}$ mice transplanted with stools from dysbiotic IBD patients but not from eubiotic healthy controls[74]. Surprisingly, germ-free mice colonized with stools from CRC patients displayed lower tumour burdens than mice colonized with stools from healthy subjects after exposure to azoxymethane (AOM) and the inflammatory agent dextran sodium sulfate (DSS)[75]. As the AOM–DSS chronic wound-healing model was used for these experiments, it is possible that dysbiotic bacteria from CRC patients enhanced tissue repair, thereby attenuating DSS-induced wound healing—a critical component of carcinogenesis in this model[76].

To add further complexity to the relationship between the microbial community and development of CRC, bacterial biofilms were identified in 50% of tumour and paired adjacent normal tissue samples from human CRC patients[77]. In addition, biofilm microbial organization is a feature of patients with both ulcerative colitis and Crohn's disease. Bacteria with the biofilm feature are detected in 95% of IBD patients, compared to 35% for healthy subjects[78]. Microorganisms growing in biofilms frequently express phenotypes that are different from their non-adherent planktonic counterparts[79]. Interestingly, higher levels of acetylated polyamines, essential metabolites for cellular growth and proliferation[80], were reported in biofilm-positive cancer tissue when compared to biofilm-negative cancer tissue[81]. Moreover, functional experiments using germ-free *Il10*$^{-/-}$ *Apc*$^{min/+}$ mice showed that mice colonized with biofilm-positive but not biofilm-negative bacteria obtained from CRC patients developed tumours[82]. These findings highlight the key roles played by the microbiota in influencing neoplastic changes in the intestine, which may also play a role in different forms of cancer (Fig. 2; Box 1).

Bacteria as therapeutic tools for cancer

Despite substantial recent progress in understanding the molecular mechanisms of tumourigenesis, cancer remains stubbornly difficult to treat. Even with some encouraging successes in rationally derived drugs, rates of clinical trial failures for new cancer drugs remain high[83]. There is an urgent need to develop new anticancer therapies or to improve current ones, and the potential roles of bacteria in this mission has been the focus of considerable research. An emerging frontier in cancer therapeutics focuses on how the interaction between bacteria, individual metabolism and immune response and established antitumour drugs could shape cancer management. This integration of bacteria to the therapeutic landscape forms the essence of 'pharmacomicrobiomics'[84], where microbial bioactivities and direct interaction with the host become important variables influencing drug toxicity and efficacy (Fig. 3).

Interaction between immunotherapy and bacteria

Targeting co-inhibitory or co-stimulatory receptor–ligand systems, such as programmed death 1 (PD-1)-programmed death-ligand 1 (PD-L1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), on T lymphocytes has emerged as a powerful means to recruit the host immune system in the fight against tumour cells[85,86]. Antibodies against CTLA-4 and PD-1 have been approved for treatment of advanced melanomas and have shown strong therapeutic response, and various clinical trials are ongoing to test their efficacy in other forms of cancer, including ovarian, prostate, bladder, renal and lung cancer[87]. Unsurprisingly new antibody-based immune targets have also been developed and are currently being tested for their antitumour efficacy[87,88]. With the recognized interaction between bacteria and host immune responses, a logical step was to investigate the impact of microorganisms on immunotherapy As with many cancer treatments, utilization of immunotherapy agents requires balancing their effectiveness in eliminating cancer with the burden the drugs place on the patient[89]. In the following section, we discuss in detail how consideration of microbial function may impact the toxicity and effectiveness of this class of drugs.

Bacteria and toxicity.

The inhibitory antibody ipilimumab binds the immune checkpoint protein CTLA-4, impeding regulatory T (Treg)-cell function and consequently promoting T-cell activation. This antibody is highly effective in the treatment of metastatic melanoma but its dampening effect on Treg function causes numerous adverse effects, including intestinal inflammation[90]. In the case of colitis, the deleterious effect of inhibiting CTLA-4 signalling was attenuated by reconstituting microbiota-depleted mice with *Bacteroides fragilis* and *Burkholderia cepacia*[91], which is consistent with the observation that patients who are resistant to ipilimumab-induced colitis show a high abundance of bacteria belonging to the Bacteroidetes phylum[92]. The mechanism by which these microorganisms alleviate the deleterious effect of ipilimumab treatment is unclear, but increased Treg function is probably not implicated as *Bacteroides fragilis* is critical for increased drug efficacy (as discussed in the following section).

Bacteria and efficacy.

Recent literature suggests that bacteria may not only alleviate drug toxicity but could also enhance the efficacy of immune checkpoint inhibitors. The potential role of bacteria in drug efficacy has been highlighted in experiments where microbial content was manipulated through antibiotics, faecal transfer and gnotobiotics. For example, the presence of bacteria appears to be essential for ipilimumab (anti-CTLA-4) therapy as this treatment was less effective in preventing subcutaneous growth of MCA205 sarcoma, MC38 colon carcinoma and Ret melanoma in mice maintained in germ-free conditions or exposed to broad-spectrum antibiotics to eliminate the intestinal biota[91]. When administered by oral feeding, specific strains of bacteria (*Bacteroides thetaiotaomicron, B. fragilis* and *B. cepacia*) were found to stimulate CTLA-4-induced antitumour immune response, thereby increasing therapeutic efficacy[91]. In humans, the intestinal microbiota of metastatic melanoma patients was sampled before and after treatment with ipilimumab. The microbiome of these patients

was found to form three major clusters: one dominated by *Alloprevotella* or *Prevotella*, while the other two were characterized by distinct *Bacteroides* spp. Interestingly, there was some evidence that ipilimumab treatment caused patients to shift between the two *Bacteroides*-dominated clusters. Faecal transfer of these different clusters into germ-free animals appeared to have different consequences for tumour formation in the recipient animals, with increased drug efficacy correlating with the cluster that also produced increased colonization of the immunogenic bacteria *B. fragilis* and *B. thetaiotaomicron* in the animal host. Data from these experiments are consistent with the hypothesis that ipilimumab treatment increases the abundance of immunogenic *Bacteroides* spp., which in turn improves the efficacy of the drug. Although less efficient than live *B. fragilis*, oral administration of polysaccharides isolated from *B. fragilis* caused an immunostimulatory effect and antitumour activity suggesting that the enhanced antitumour efficacy is not dependent on a microbial metabolite. The immunostimulatory effect of *B. fragilis* on dendritic cells and the resulting $CD8^+$ cell response in this system is somewhat surprising as previous observations showed an immunosuppressive effect of a *B. fragilis* bacterial strain and associated polysaccharides[93,94]. These results again highlight the complex nature of host immune system interactions with bacteria, where the final outcomes, immunostimulation or immunosuppression, are dictated by complex combinations of cellular signal processing.

The efficacy of another immune checkpoint blocker, PD-L1, was also shown to depend on bacteria. Microbial genomic analysis and faecal transplantation in mice identified *Bifidobacterium* spp. (*Bifidobacterium breve* and *Bifidobacterium longum*) as important contributors of a PD-L1 blockade-mediated antitumour effect on subcutaneous B16.SIY melanoma growth[95]. *Bifidobacterium* enhanced the antigen-presentation function of dendritic cells, which resulted in augmented activation of $CD8^+$ T cells' antitumour activity. Remarkably, even in the absence of PD-L1 treatment, melanoma tumour growth was impaired in mice colonized with *B. breve* and *B. longum*, suggesting a direct engagement of the host immune antitumour response. Although both immune checkpoint inhibitors (CTLA-4 and PD-L1) synergize with bacteria to achieve antitumour effects, their mechanisms of action appear somewhat different as CTLA-4 may rely on bacteria–tumour cross-reactivity, whereas bacteria-induced antigen presentation is probably a key component of PD-L1 efficacy[96].

Over a century ago, William Coley administered heat-killed bacterial extracts to patients with different forms of inoperable cancer, as part of what is now considered the first immunotherapy experiment[97]. It is not clear which components of this original microbial cocktail activated the immune system, but cell wall components and nucleic acids, such as DNA, all have immunostimulatory properties. For example, unmethylated CpG oligodeoxynucleotides (CpG ODN) have potent immunostimulatory effects that could be utilized for the treatment of various pathologies including cancer[98]. Interestingly, the gut microbiota modulates the antitumour effect of a combined anti-IL10 receptor (anti-IL10R)-antibody–CpG-ODN immunotherapy[99]. Similar to CTLA-4 and PD-L1, the antitumour efficacy of an anti-IL10R-antibody–CpG-ODN immunotherapy against subcutaneous tumour growth (EL4 lymphoma, MC38 colon carcinoma or B16 melanoma) was diminished in germ-free mice or broad-spectrum antibiotic-treated mice, an effect that correlated with the host's ability to generate tumour necrosis factor-α (TNFα). Specific bacteria were

positively (*Alistipes*, *Ruminococcus*) or negatively (*Lactobacillus*) correlated with TNF production and activation of cytotoxic $CD8^+$ T-cell response in the tumour environment. Microbiota-depleted mice colonized with *Alistipes shahii* increased TNF production by tumour-associated myeloid cells following anti-IL10R-antibody–CpG-ODN exposure. It is remarkable that although microorganisms work in concert with immunotherapeutic drugs (CTLA-4, PD-L1, CpG ODN) to enhance their efficacy, a 'pairing' system between each drug and bacteria seems to take place. Therefore, the presence of specific microbial species may determine patient response to a given drug treatment (Fig. 3).

Interaction between chemotherapeutics and bacteria

Chemotherapeutic drugs essentially induce cytotoxicity of rapidly dividing cells, which is a common feature of cancer cells but also of specific healthy cells present in bone marrow and the gastrointestinal (GI) tract. Consequently, these drugs cause significant collateral damage to the patient, generally leading to a state of immunosuppression and severe diarrhoea. Therefore, it has been a challenge to reach maximum antitumour effects with such side effects.

Bacteria and toxicity.

Camptothecin-11 (CPT-11) is an analogue of the natural alkaloid CPT, an inhibitor of topoisomerase I, which is required for DNA replication[100]. CPT-11 (irinotecan) is mainly used in patients with CRC and is biotransformed into the active topoisomerase I inhibitor SN-38 to exert its antitumour effect[101]. The compound is subjected to further metabolism in the liver, where a glucuronide group is added to generate the inactive derivative SN-38G (ref. 101). This derivative is excreted via biliary ducts into the GI tract, where the bacterially derived β-glucuronidase enzyme reactivates the compound to its cytotoxic SN-38 form, thereby causing damage to intestinal epithelial cells and leading to severe diarrhoea. In order to prevent microbially derived reactivation of SN-38 in the intestine, *E. coli*-derived β-glucuronidase inhibitors were screened and successfully tested in mice to alleviate CPT-11-induced GI toxicity[102,103]. Targeting microbially derived β-glucuronidase did not alter irinotecan pharmacokinetics, suggesting that drug toxicity and efficacy could be uncoupled by targeting bacterial enzymes[104].

Similar to CPT-11, methotrexate (MTX) is another chemotherapeutic compound that causes significant GI toxicity in patients[105]. Antibiotic-mediated microbiota depletion increased MTX-induced mucosal injury in mice, an effect linked to TLR2 signalling[106]. The microbiota has also been shown to modulate radiation-induced GI toxicity[107,108] and one could envision the use of selective bacteria to alleviate toxicity due to cancer treatment[109].

Bacteria and efficacy.

As mentioned previously, bacteria modulate antitumour drug efficacy through their influence on host immune responses. Cyclophosphamide (CTX) is a prodrug that, once activated, acts as an alkylating cytotoxic compound, effective in numerous forms of solid tumours[110]. The CTX-mediated antitumour effect in a subcutaneous tumour growth model (P815 mastocytoma and MCA205 sarcoma) was reduced in germ-free, antibiotic-treated or

vancomycin (specific for Gram-positive bacteria)-treated mice[111]. Importantly, CTX failed to induce an antitumour response in a vancomycin-treated transgenic mouse model of lung adenocarcinoma, suggesting that the interplay between bacteria and host immune response is not restricted to xenograft models. Interestingly, administration of CTX led to the disruption of small intestinal barrier function, enabling the translocation of intestinal bacteria, particularly Gram-positive and vancomycin-sensitive *Lactobacillus johnsonii, Lactobacillus murinus* and *Enterococcus hirae*, into mesenteric lymph nodes and spleen[111]. This extra-intestinal interaction between bacteria and immune cells was critical for the induction of pathogenic T helper 17 (pTh17) cells expressing interferon-γ in the spleen, which were responsible for the drug's therapeutic effect[111]. Oral administration of *L. johnsonii* and *E. hirae* restored the pTh17 response in the spleen of antibiotic-treated mice. Hence, although CTX causes damage to the intestinal barrier as part of its toxic effect, it allows for translocation of specific Gram-positive intestinal bacteria, a necessary process for the activation of peripheral immune cells and anticancer efficacy.

Oxaliplatin and cisplatin are both alkylating agents that are widely used in cancer therapy[112]. As observed with CTX, microbiota depletion by broad-spectrum antibiotic exposure reduced the antitumour efficacy of these compounds in subcutaneous tumour growth (EL4 lymphoma and MC38 colon carcinoma)[99]. The reduced antitumour efficacy was linked to reduced production of ROS by myeloid cells, a critical step for the platinum-induced antitumour effect.

The microbiota possesses a large collection of genes with an impressive metabolic potential that not only participates in energy harvest, but also metabolizes numerous xenobiotics[113]. For example, after the preincubation of 30 chemotherapeutic drugs with non-pathogenic *E. coli* Nissle 1917 or *Listeria welshimeri* serovar 6B SLCC533410, 10 out of 30 chemotherapeutic drugs (for example, gemcitabine, cladribine, daunorubicin) showed reduced cytotoxic activity against the Lewis lung carcinoma cell line, while 6 compounds showed increased efficacy (for example, fludarabine phosphate, CB1954)[114]. Bacterial heat inactivation abolished these differential drug modification responses, suggesting that enzymatic activities, and not structural components, are implicated in this phenomenon. These findings suggest that bacteria often directly metabolize chemotherapeutic drugs and change their efficacy (Fig. 3).

Cancer management through bacteriotherapy

While much recent research has focused on how bacteria can contribute to disease in the gut, especially cancer, these microorganisms could also potentially be enlisted for cancer prevention, detection or drug delivery. For example, probiotics or microorganisms associated with health benefits have long been used to attempt to resolve gut dysbiosis with 'good' bacteria, although often with limited clinical evidence of their effectiveness[64]. Lactic acid bacteria, with demonstrated beneficial effects as probiotics[115,116], are beginning to be explored in the context of CRC[117,118]. As mentioned previously, abundances of these microorganisms are often reduced in the biota of CRC patients. Capsule delivery of probiotics (*B. longum, Lactobacillus acidophilus* and *Enterococcus faecalis*) in CRC patients produced increased microbial diversity and lower abundance of *Fusobacterium* with

increased representation of *Enterococcus* on the intestinal mucosal tissue of patients compared to placebo-treated patients[119]. Studies in colitis and enteric infectious diseases have yielded limited evidence suggesting that such multi-species probiotics have greater efficacy relative to that of single-strain probiotics[120,121]. However, the vast majority of studies involving probiotics as cancer therapies have focused on single-species probiotics[117,122–124] and a rigorous comparison of the efficacy of single- versus multispecies probiotics is needed. Such comparisons would allow for the evaluation of tradeoffs between multiple species with multiple cancer targets and potential intra-probiotic antagonism between species. Although probiotics are mainly viewed as preventive agents, some *Lactobacillus* spp. are used to mitigate the GI toxicity associated with anticancer drugs[109]. The proposed mechanisms of action are wide-ranging from providing antioxidants to improving the function of the immune response[64]. However, even when they alter microbial community composition, probiotics do not always offer protection against cancer, and in some models many even enhance tumourigenesis[125]. This again emphasizes the ultimate importance of understanding each individual's unique microenvironments when designing interventions.

Microbial replacement therapy or faecal microbial transplantation (FMT) consists of repopulating the lower GI tract of patients with GI pathologies using stools from healthy subjects. This intervention has emerged as a 'natural' and promising therapy for patients with relapsing *C. difficile* infection, irritable bowel syndrome and IBD[126]. In the case of recurrent *C. difficile* infection, FMT has shown a 90% clinical success rate[127]. Following FMT, donor and recipient bacteria strains coexist for more than 90 days, although optimal ecosystem transfer and duration may be dependent on donor-recipient compatibility[128]. With the growing popularity of FMT, access to donor stools are made easier by nonprofit companies such as OpenBiome, which provides stools from healthy subjects packaged into pills for researchers implicated in clinical trials, as the oral mode of delivery is easier than faecal infusion using colonoscopy, endoscopy or enema. However, as opposed to *C. difficile* infection, where the disease-causing agent has been identified and is trackable, IBD and CRC are complex diseases resulting from polymicrobial interactions between microorganisms, the host and the environment that evolve over a long period of time. In addition, the presence of a microbial biofilm in these patients may limit FMT efficacy[77,78] as these communities are typically resilient to intervention. Indeed, studies on FMT as a remission-inducing approach for IBD has resulted in conflicting reports on its efficacy and safety, and further investigation is needed[129,130]. The effectiveness of FMT in treating CRC is likewise not yet clear. While the idea of utilizing FMT as a preventive measure[65–67], following surgery for remission maintenance or during treatment is intriguing, there is little data to support such incorporation of FMT for cancer management and more studies are urgently needed to evaluate the value of ecosystem transfer in cancer.

Bacteria could also be directly enlisted in the fight against cancer, either by acting as detecting agents or in the shuttle delivery of specific therapeutics. The fact that bacteria such as *Salmonella, Escherichia, Clostridium* and *Listeria* can migrate and penetrate tumour tissues makes them potentially valuable tools for cancer management. The ability of bacteria to detect tumours is probably due to their response to microorganism-specific signals that come from the tumour microenvironment[131]. Administration of attenuated *Salmonella*

typhimurium VNP20009 showed antitumour efficacy in preclinical models[132]. In addition, using a 'tumour-on-a-chip' system, Panteli *et al.* showed that attenuated *Salmonella* engineered to express and release the fluorescent protein ZsGreen was able to detect microscopic solid tumours[133]. Moreover, *E. coli* engineered to sense a glucose gradient were able to penetrate deep into microfluidic tumours[134], suggesting that these engineered bacteria could be 'armed' to deliver therapeutics deep into neoplastic tissues. The armed bacteria could deliver a battery of compounds, ranging from toxins and host signalling molecules (such as TRAIL (TNF-related apoptosis-inducing ligand), Fas, cytokines) to enzymes that selectively activate prodrugs in the tumour tissues, thereby avoiding systemic toxicity[135]. However, although the phase I trial demonstrated a safety profile for *S. typhimurium* VNP20009 in patients with metastatic melanoma, no tumour regression was observed in these patients, possibly due to limited accumulation of the bacterium in the tumours[136]. Nevertheless, similar to oncolytic viruses[137], exploiting the unique properties of bacteria may yet prove to be a powerful tool in combatting carcinogenesis (Fig. 4).

Conclusions

The exciting conceptual models reviewed suggest that the traditional screening and treatment of cancer could be greatly enhanced by monitoring microbial composition and function and manipulating the microbiome. For example, future diagnostics could screen for microbial genes as biomarkers of increased cancer risk. For cancer prevention, the manipulation of the microbiome through microbial replacement, probiotics and/or diet could promote a microbiome that minimized inflammation and carcinogenic activities, thereby reducing cancer risk. The remarkable advances in our understanding of how bacteria interact with host immune response suggest that treatment could be personalized through monitoring the microbiome to determine how individual patients are likely to metabolize individual drugs or 'pair well' with given anticancer drugs to enhance efficacy while reducing toxicity. This holistic concept is supported by the recent demonstration of microbiome–cytokine interaction in humans[138].

Although there exists a great deal of optimism in the potential for mining the microbiome for cancer management drugging this ecosystem for therapeutic purposes will likely be very challenging. There are probably more genes within the human microbiome than human genes, and while it is enticing to think about new targets and biology within this microbiome, the vast increase in complexity when considering both human and microbial targets makes this a daunting mission. In addition, the literature reports time and time again that the taxonomic composition of the gut microbiome of an individual is highly distinct and, at least in some studies, stable in the face of differences in diet[139]. For example, a recent study swapped the diet of a rural cohort in Africa and African Americans and found minor changes in microbial community composition, but large changes in mucosal markers of cancer risk, some of which are probably microbial in origin[140]. This intriguing study suggests that how microorganisms contribute to cancer risk may be determined more by the environments an individual creates for their microbial communities, rather than simple individual differences in community composition. Consistent with this idea, a recent study comparing vegans to non-vegans found robust differences in the metabolome but very few differences in microbial community composition[141].

If what we feed our microorganisms and the resulting metabolites they produce is of greater importance to cancer risk than community composition, then studies that examine microbial differences between cancer and healthy subjects using metatranscriptomics or metabolic profiles may generate a deeper understanding of bacterial roles in tumourigenesis than 16S rRNA or metagenomics studies. Moreover, if the environment of the microbial community, rather than just community composition, needs to be changed to impact risk, this may limit the potential of interventions that rely on probiotics to engineer a healthier gut. It may be that features of the microenvironment beyond just the bacteria themselves are transferred in a faecal transfer procedure, and that removing bacteria from their environmental context also strips them of their beneficial behaviours. In addition, interactions between viruses and bacteria are important components of host homeostasis[142,143] and efforts in engineering defined bacterial cocktails to promote health may prove to be of limited efficacy. These important questions of what the requirements of engineering a protective gut microflora are will unquestionably receive much attention over the coming years.

In summary, attempts to utilize the microbiome to improve cancer detection, progression and therapy have tremendous potential, but substantial efforts in basic and clinical research that will allow us to better understand the complexity of host–environment interactions will probably be needed before the translational potential of the microbiome is fully realized.

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Box 1 |

Microbiota and extra-intestinal cancer.

The microbiome has attracted attention as an environmental factor that is implicated in carcinogenesis[144]. The first microbiome encountered in the digestive tract, that of the oral cavity has long been indicated as an intermediary between diet and cancer risk[145,146]. Oral squamous cell carcinomas have shown changes in microbiota diversity between tumour and healthy tissue, and taxa that are associated with disease status have been identified, though cohort sizes in such studies have been small[147–149]. The relative diagnostic ease of sampling the oral microbiota has the potential to yield biomarkers for colorectal, pancreatic, GI and other cancers[150–153]. Similarly, the lung is a nexus between environmental factors[154], microbial dysbiosis[155] and cancer with its own potential biomarkers[156,157]. In contrast to these 'open environments in human physiology Hieken *et al.* recently confirmed differences in microbiota abundance profiles between aseptically collected cancerous and healthy breast tissue that reduce the concerns of contamination driving profile differences in similar studies[158–160]. The far-reaching influence of gut bacteria such as *Helicobacter* can also be seen through the promotion of cancers distant from the gut, such as breast cancer, through *Helicobacter*'s interactions with neutrophils[161]. *Helicobacter* has also been indicated, with some contention, in the development of HCC, warranting further investigations of microbiota–HCC associations[162–164]. The relationship between the microbiome and HCC is also one of inflammation and dysbiosis, and there has been some evidence of probiotics restoring eubiosis and reducing tumour growth[165,166].

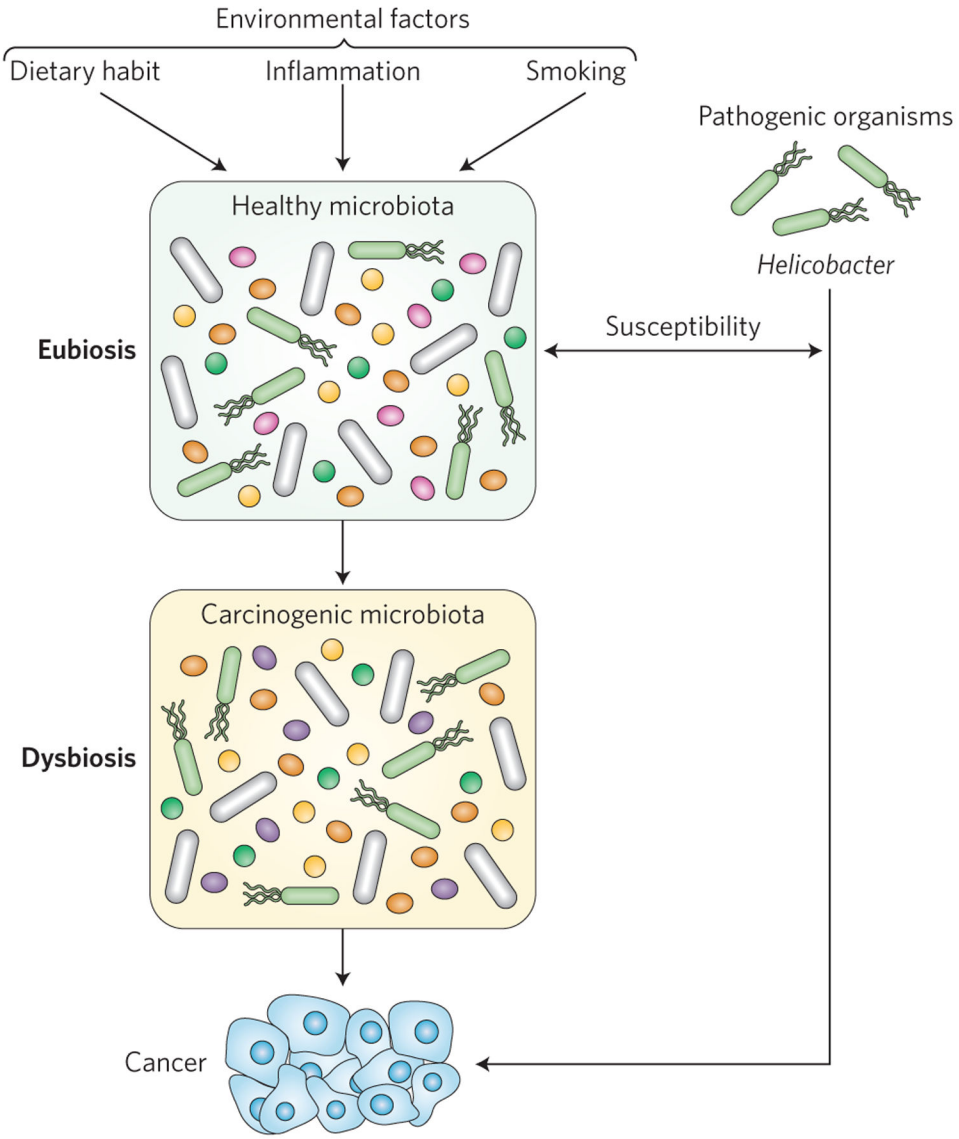

Figure 1 |. Environmental changes can promote dysbiosis and pathogen-derived susceptibility to cancer.

Disruption of the healthy microbiota by environmental factors, such as inflammation, can both increase host susceptibility to carcinogenic pathogens (such as *Helicobacter*) and expose the host to the cancer risks associated with dysbiosis.

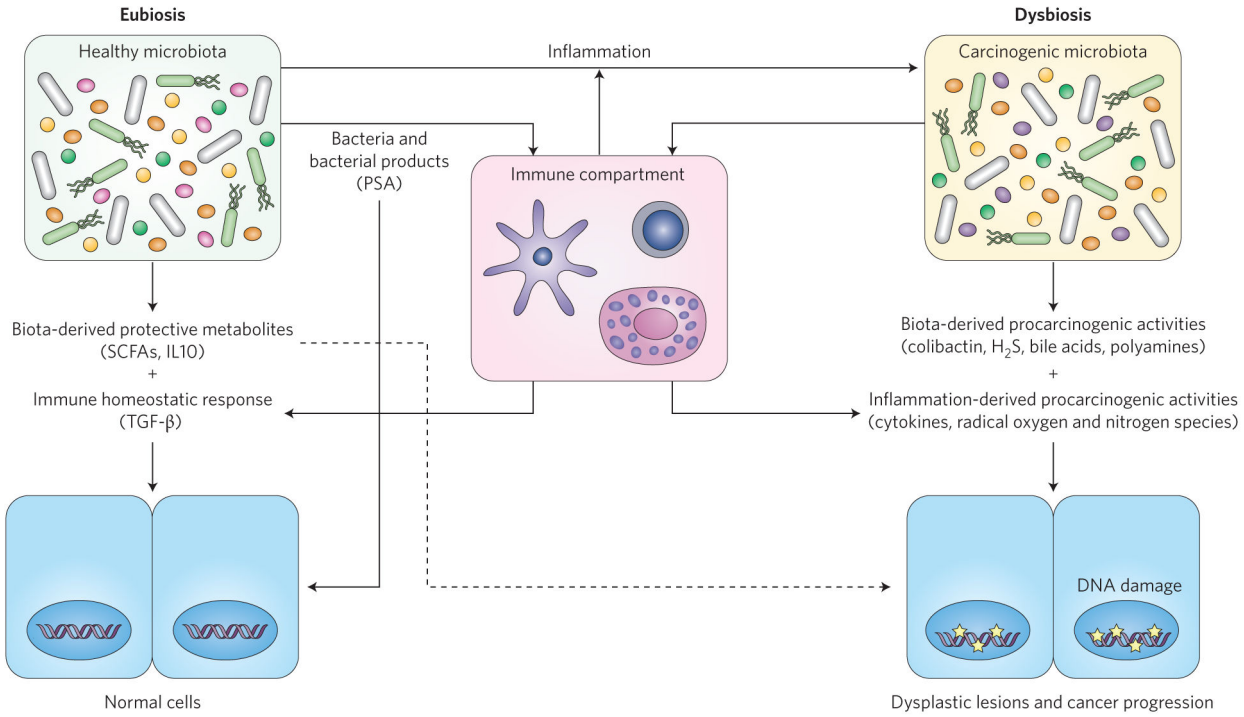

Figure 2 | Microbial interactions with the immune system modulate cancer risk.

Microbiota promote homeostasis directly through metabolites and bacterial products, which influence both the epithelial and immune cell response. In addition, dysregulated immune-host interaction favours the development of dysbiosis, which contributes to carcinogenesis through metabolic activities and activation of immune responses. Some protective microbiota (SCFAs) may promote cellular proliferation of cancer-initiated cells (dashed arrow). Brackets contain example compounds. PSA, polysaccharide A; TGF-β, transforming growth factor-β. Stars indicate DNA damage.

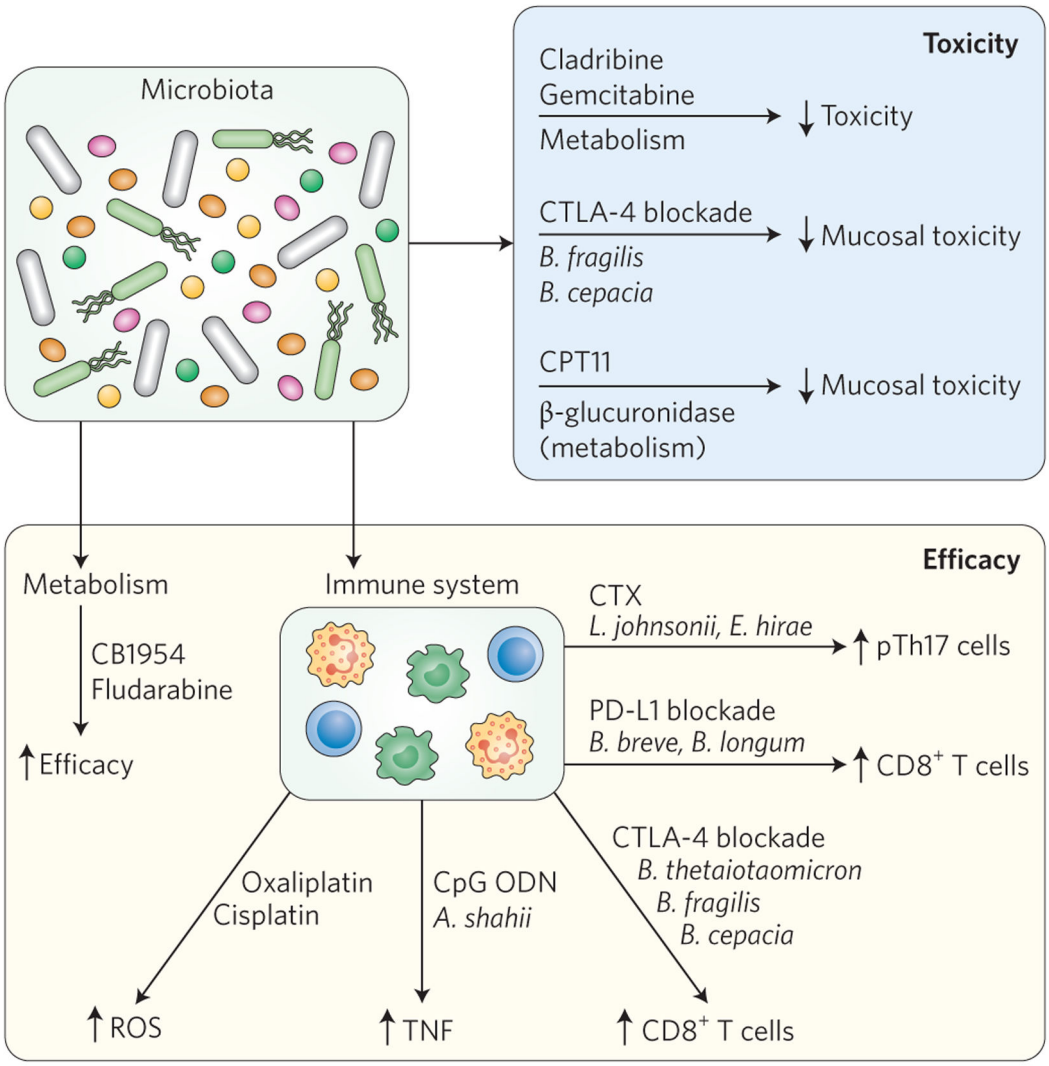

Figure 3 |. The microbiota influences drug toxicity and efficacy.

Efficacy and toxicity of anticancer drugs can be modulated by the microbiota through numerous mechanisms, including microbial metabolism and host-mediated immune responses. Small arrows indicate the direction of the response (increased or decreased).

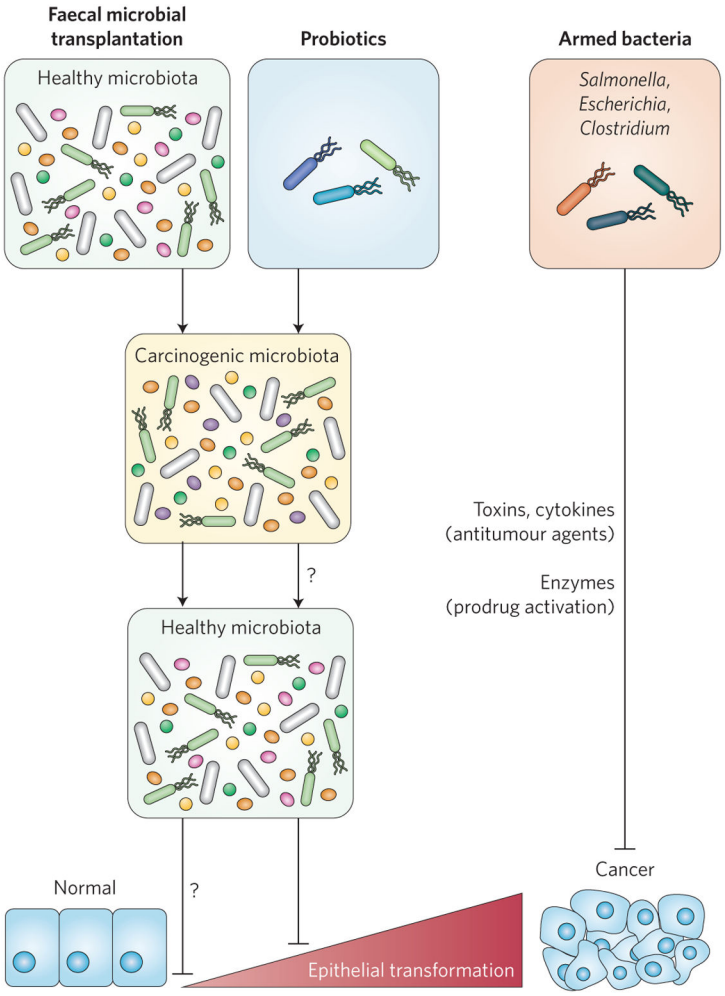

Figure 4 |. Cancer management through the use of bacteriotherapy.

Potential strategies to enlist bacteria to prevent or treat carcinogenesis include FMT, probiotics and armed bacteria. In FMT, the carcinogenic microbiota from patients is ‘replaced’ by a new, healthy microbiota to eliminate carcinogenic activities. The introductions of probiotics may result in a ‘rebalanced’ microbiota with less potential to cause cancer. Bacteria could also be engineered to deliver specific cargo, such as cell death signalling molecules, toxins or enzymes, to selectively activate antitumour prodrugs in the tumour tissues. Whether probiotic intake results in a healthy microbiota or whether healthy microbiota prevents development of carcinogenesis (question marks) is unclear.