nature reviews clinical oncology

https://doi.org/10.1038/s41571-023-00766-x

Review article

Check for updates

Gut microbiota in colorectal cancer development and therapy

Chi Chun Wong & Jun Yu

Abstract

Colorectal cancer (CRC) is one of the commonest cancers globally. A unique aspect of CRC is its intimate association with the gut microbiota, which forms an essential part of the tumour microenvironment. Research over the past decade has established that dysbiosis of gut bacteria, fungi, viruses and Archaea accompanies colorectal tumorigenesis, and these changes might be causative. Data from mechanistic studies demonstrate the ability of the gut microbiota to interact with the colonic epithelia and immune cells of the host via the release of a diverse range of metabolites, proteins and macromolecules that regulate CRC development. Preclinical and some clinical evidence also underscores the role of the gut microbiota in modifying the therapeutic responses of patients with CRC to chemotherapy and immunotherapy. Herein, we summarize our current understanding of the role of gut microbiota in CRC and outline the potential translational and clinical implications for CRC diagnosis, prevention and treatment. Emphasis is placed on how the gut microbiota could now be better harnessed by developing targeted microbial therapeutics as chemopreventive agents against colorectal tumorigenesis, as adjuvants for chemotherapy and immunotherapy to boost drug efficacy and safety, and as non-invasive biomarkers for CRC screening and patient stratification. Finally, we highlight the hurdles and potential solutions to translating our knowledge of the gut microbiota into clinical practice.

Sections

Institute of Digestive Disease and Department of Medicine and Therapeutics, State Key Laboratory of Digestive Disease, Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Hong Kong SAR, China.
e-mail: junyu@cuhk.edu.hk

Introduction

The human gut microbiota is an ecosystem populated by bacteria, fungi, viruses, Archaea and parasites whose cells in total typically outnumber those of the host[1]. The number of microbial species residing in the human gut is usually in the order of thousands at any given time[2], representing a much greater level of genetic diversity than that of the human genome. Colonization of the newborn gastrointestinal (GI) tract begins in early life and co-evolves concomitantly with the host throughout development and adult life. The content of the gut microbiota varies considerably from person to person, and is readily modified by changes in lifestyle, such as diet, smoking and exercise; yet collectively these diverse ecosystems have similar roles in maintaining homeostasis. Gut microorganisms are indispensable for the fermentation of non-digestible carbohydrates, nutrient absorption and vitamin biosynthesis, as well as modulation of host immunity[3].

A dynamic but largely stable gut microbiota is believed to be beneficial for health. The disruption of this ecosystem, termed dysbiosis, can therefore become a threat to the host's health, as it is associated with a multitude of disease states potentially including gastrointestinal disorders, certain neurological, respiratory, metabolic, hepatic and cardiovascular conditions, and stomach and colon cancers[4]. Dysbiosis is disease-specific and is defined as any abnormalities in function and/or composition of the microbiota that are associated with disease pathogenesis[5], including in colorectal cancer (CRC). Despite the existence of large interindividual variations in the microbiota that often overwhelm disease-specific changes, large-scale multicohort analysis of the CRC metagenome (defined as all genetic material present within a sample) has unravelled consistent enrichment of a 'core microbiome' with pro-tumorigenic functions[6], consistent with the definition of dysbiosis. Gut dysbiosis is of particular importance in colorectal tumorigenesis, not least owing to the existence of close interactions between the colonic epithelium and the gut microbiota. Over the past decade, research has demonstrated that the gut microbiota becomes dysregulated during the development of CRC and is an active participant in tumorigenesis. Mechanistic dissection has underscored the roles of gut microorganisms in all aspects of CRC, including the roles of *Fusobacterium nucleatum* and *Peptostreptococcus anaerobius* in tumour initiation, disease progression and metastasis. Besides contributing to CRC pathogenesis, gut microorganisms can modify responses to treatment including chemotherapy and immune checkpoint inhibitors (ICIs).

Despite knowledge of these associations, precise and personalized management of the gut microbiota to yield clinical benefits with a minimal risk of adverse events remains challenging. We believe that the stage is now set to harness our knowledge to move from largely untargeted interventions (such as faecal microbiota transplantation (FMT)) to more targeted microbial modulation strategies involving the ablation of specific subsets of CRC-inducing pathogens and/or the administration of formulated probiotic consortia, microbial products and/or metabolites. Owing to the ability of gut microorganisms to modulate responses to chemotherapy and/or immunotherapy, intense interest exists in using microbial-based adjuvants to promote more robust therapeutic responses and/or to alleviate adverse events. Herein, we provide an overview of current knowledge of the role of the gut microbiota in colorectal tumorigenesis and discuss the potential clinical implications for patients with CRC, with a focus on the mechanisms of action of disease-causing or protective microorganisms and how these could be targeted using interventions. We also highlight the many unknown aspects of the gut microbiota and suggest potential paths forward in this exciting area of research.

The microbiota and CRC

Microbial dysbiosis

Bacterial dysbiosis. As the most abundant microorganisms residing in the GI tract, dysbiosis of gut bacteria is an established phenomenon that contributes to several aspects of CRC development and progression[7–24]. Owing to the existence of substantial geographical and ethnicity-specific heterogeneity of the gut microbiota, several meta-analyses have been conducted to identify universal associations between the presence of specific bacteria and CRC[6,16,17,25]. Large-scale meta-analysis of sequencing data from 768 (ref. 17) and 969 (ref. 16) faecal metagenomes from geographically distinct cohorts of patients with CRC has unravelled several core pathogenic species that are enriched in these patients. Most notable is the enrichment of oral pathogens, including *F. nucleatum*, *Parvimonas micra*, *Peptostreptococcus stomatis*, *P. anaerobius*, *Porphyromonas asaccharolytica*, *Solobacterium moorei* and *Prevotella intermedia*. Besides CRC, elevated levels of oral pathogens can be found in the gut microbiota of patients with colorectal adenomas, including *F. nucleatum*[18,26], *S. moorei*[18] and *Lachnoclostridium* spp.[27], suggesting that gut dysbiosis is an early event in colorectal tumorigenesis. Specific colon-resident bacterial strains have also been shown to be enriched in patients with CRC in other studies, including enterotoxigenic *Bacteroides fragilis*[28], pks$^+$ *Escherichia coli*[29], *Streptococcus gallolyticus*[30] and *Morganella morganii*[31]. Individually, these bacterial species have all been linked with colorectal tumorigenesis in mechanistic studies, although whether they function alone or synergize with other microorganisms to accelerate CRC development remains unclear. In support of the latter hypothesis, pathogenic bacteria seem to form strong mutualistic networks in both the faecal[6,8] and colon mucosal[23] microbiota of patients with CRC. Conversely, the role of depletion of beneficial gut bacteria in patients with CRC has received less attention but could be equally critical in skewing the microbiota towards tumorigenesis. Bacteria that are often depleted in CRC include several probiotics with putative beneficial effects such as *Streptococcus thermophilus*[32], *Streptococcus salivarius*[33], *Lactobacillus gallinarum*[34], *Clostridium butyricum*[35] and *Carnobacterium maltaromaticum*[36], which can form a co-exclusive network with pathobionts, implying the existence of competitive or antagonistic interactions between pathobionts and probiotics. Hence, bacterial dysbiosis deregulates the balance between pro-tumorigenic pathobionts and anti-tumorigenic probiotics, with potential implications for pathogenesis, as well as opportunities for active interventions and biomarker development.

In addition to gut dysbiosis, both clinical and preclinical evidence indicates that bacteria can colonize tumours[21,24,37–42]. For example, bacteria of the *Fusobacterium* genus, in particular the CRC pathobiont *F. nucleatum*, are consistently enriched in the intratumoural CRC microbiome[21,37–40,42] and are able to persist in metastatic lesions[37]. Spatial mapping of the intratumoural CRC microbiome[43] indicates *Fusobacterium* and *Bacteroides* as the dominant genera, and that *F. nucleatum*-occupied niches are associated with induction of metastasis-promoting signalling pathways, such as epithelial-to-mesenchymal transition, implying that intratumoural *F. nucleatum* has a role in CRC progression and metastasis.

Non-bacterial dysbiosis. Thus far, most studies of microbial dysbiosis in the context of CRC have focused on bacteria owing to their relatively high abundance in the GI tract (comprising 93% of gut microbial sequences)[44]. However, improvements in sequencing technologies have enabled the detection of non-bacterial components of the gut

microbiota such as viruses, fungi and Archaea. Several oncogenic viruses capable of infecting eukaryotic cells, such as Epstein–Barr virus[45], human papillomavirus[46] and human polyomavirus 2 (ref. 47), have been detected in patients with CRC, although no conclusive evidence exists supporting a causative role. Nonetheless, enteric viruses chiefly comprising bacteriophages can control the gut bacteriome through phage predation[48]. In a multicohort study, the investigators used shotgun metagenomics to demonstrate that the enteric virome is altered in patients with CRC compared to that in patients without cancer[49], with enrichment of members of several genera including *Orthobunyavirus*, *Inovirus* and *Tunalikevirus*. The latter two genera are bacteriophages participating in trans-kingdom interactions that suppress pathogenic bacteria[50]. Data from several studies demonstrate the existence of trans-kingdom interactions between oral bacterial and viral communities in patients with CRC[49,51,52], implying that synergy between viruses and bacteria might be involved in the development of CRC.

The role of the mycobiome is under-studied in patients with CRC owing to the often-limited abundance of fungi and the high level of interindividual and intraindividual variability[53]. Data from several studies suggest the existence of distinct mycobiome profiles in patients with CRC, characterized by an elevated ratio of Basidiomycota to Ascomycota[54,55]. At the species level, several *Aspergillus* species are highly enriched in patients with CRC, an observation that has since been validated in cohorts containing patients from both Asia and Europe[55]. In a meta-analysis of data from >1,000 patients with CRC from eight cohorts, investigators identified several fungal species that are consistently enriched (*Aspergillus rambellii*, *Cordyceps* sp. RAO-2017, *Erysiphe pulchra*, *Moniliophthora perniciosa*, *Sphaerulina musiva* and *Phytophthora capsici*) and one that is consistently depleted (*Aspergillus kawachii*) in faecal samples from patients with CRC[53]. Dysbiosis of the mycobiome has also been detected, albeit to a lesser extent, in patients with colorectal polyps[56] and adenomas[57], implying the involvement of fungi in early stage CRC. A multi-kingdom analysis of the presence of Archaea, bacteria, fungi and viruses in faecal samples from patients with CRC demonstrated the strongest inter-kingdom interaction between bacteria and fungi, with distinct patterns of co-occurrence[58] and co-exclusion[55] observed in bacterial–fungal networks in patients with CRC compared with the patterns in patients without CRC. These observations imply potential interplay between bacteria and fungi in CRC. Notably, faecal samples enriched with *A. rambellii* also harbour high levels of the pathogenic bacteria *F. nucleatum* and *P. micra*, and enrichment with both fungi and bacteria is more predictive of CRC than enrichment with bacteria alone[53]. The underlying reasons for this apparent link between certain fungi and bacteria and CRC are unknown, although this might reflect diet, nutrient competition, the presence of secondary metabolites and/or toxins as well as extracellular enzymes[59].

Archaea, one of the most ancient forms of life, have long ben considered to inhabit extreme environments such as hot springs, and are able to use a diverse range of substrates for energy metabolism[60,61]. Research published in 2022 suggests that Archaea inhabit the GI tract[62], where they are able to derive energy from the fermentation products of gut bacteria[63]. Thus far, only one study has investigated the Archaea profile of patients with CRC[64]. Compared with those without cancer, faecal samples from patients with CRC have a greater abundance of halophilic Archaea, but fewer methanogenic Archaea. Halophilic *Haloplanus* sp. CBA1113 and *Natrinema* sp. J7-2 are the most-enriched archaeal species, with levels of the latter increasing progressively during the course of colorectal carcinogenesis[64]. CRC-enriched archaeal species positively correlate with the presence of oncogenic *B. fragilis*, whilst being antagonistic with probiotic *Clostridium* species[64]. Hence, Archaea constitute a part of the dysbiotic microbial network in patients with CRC. Together, mounting evidence suggests that non-bacterial microorganisms are altered in the pathogenesis of CRC, although major gaps remain in our understanding of both the direct and indirect roles of these microorganisms.

Dark matter. Microbial 'dark matter' refers to the substantial fraction of microbial taxa that researchers are unable to culture or for which a high-quality reference genome is currently not available[65], which is often the case for non-bacterial microorganisms such as viruses, fungi and Archaea[44,66]. Advances in sequencing and computational techniques have enabled cultivation-independent investigations of de novo genome assembly[67], which have the potential to unearth the identity of certain uncultured microorganisms, as evidenced by reports on the metagenomic-assembled genomes of microorganisms present in the human gut[68], skin[69] and oral cavity[70]. In light of the limited availability of reference viral genomes, investigators profiled the gut virome using third-generation PacBio sequencing in conjunction with metagenome sequencing. This method utilizes less accurate (>90%) PacBio long reads with lower accuracy to guide the assembly of highly accurate (>99.9%), shotgun metagenome sequencing short reads to reconstruct complete viral genomes, leading to the identification of 1,058 novel viruses. Some of these novel viruses were found to be potential biomarkers of CRC. Single-cell techniques, which are already widely utilized to characterize human cells, are also being developed for the characterization of single microbial genomes and transcriptomes[65]. Microfluidics technologies, such as droplet-based[71–73] or compartmentation-based[74] approaches, have been proposed as promising tools for the large-scale isolation of single microorganisms, and these have already enabled the publication of single-cell atlases of the human[75] and mouse[76] gut microbiome. The application of single-cell approaches is expected to offer a glimpse into the role of microbial dark matter in colorectal tumorigenesis.

Causality and molecular mechanisms

The existence of a causal relationship between microbial dysbiosis and colorectal tumorigenesis is still a matter of debate[5], although experimental evidence suggests that the gut microbiota could, at least in part, contribute to tumorigenesis. Reconstitution of the faecal microbiota of patients with CRC in germ-free or gnotobiotic mouse models enhances colonic cell proliferation and accelerates carcinogen-induced colon tumorigenesis, as compared with transplantation of the faecal microbiota from individuals without cancer[77]. This observation has been recapitulated in $Apc^{Min/+}$ mice (a mouse model of human familial adenomatous polyposis)[78], and provides valuable evidence supporting a causal role of the gut microbiota in the development of CRC. Further evidence is provided by mono-colonization studies involving pathogenic bacteria such as *F. nucleatum*[23,79–84], pks^{+} *E. coli*[29,85–90], *P. anaerobius*[91,92] and enterotoxigenic *B. fragilis*[28,93–97], all of which have demonstrated tumour-promoting potential in experimental models of CRC. Evidence supporting a role of non-bacterial components of the microbiota in CRC remains limited, although the fungus *A. rambellii* has been shown to promote the development of CRC in experimental models[53]. Aside from evidence supporting a direct role in causality, the gut microbiota can also interact with certain environmental factors such as a high-fat diet[98] and cigarette smoking[99] to accelerate the development of CRC. The molecular mechanisms through which microorganisms might promote the development of CRC are highly diverse. A single

pathogen often has multifaceted effects on the colonic epithelium that are typically non-exclusive and culminate in the promotion of tumorigenesis. These mechanisms can be broadly classified into several categories including genotoxicity, signal transduction, inflammation, immunity and metabolism (Fig. 1).

Several mouse models have been used to investigate potentially causal roles of gut microorganisms in CRC. The most popular models include transgenic (*Apc*-mutant mouse strains, such as $Apc^{Min/+}$) and carcinogen-induced (azoxymethane-exposed) models that recapitulate the sporadic development of adenomas followed by carcinomas seen in patients with CRC. *Apc*-mutant models form tumours preferentially in the small intestine, although this limitation can be overcome using CDX2-Cre-mediated[100] or CAC-Cre-mediated[101], colon-specific *Apc* deletion (*Apc* floxed mice). Alternatively, the combination of azoxymethane plus dextran sodium sulfate (DSS) or azoxymethane alone in $Il10^{-/-}$ knockout mice can elicit colonic inflammation, and is widely used as an experimental model of colitis-associated CRC. These models rarely develop tumour invasion and metastasis, and are therefore more suitable for the evaluation of tumour initiation and/or growth, but not for metastatic progression.

Genotoxin-mediated promotion of mutagenesis. Genetic alterations leading to oncogene activation and/or inactivation of tumour suppressor genes are a fundamental aspect of CRC tumorigenesis[102]. Pathogenic microorganisms can promote the emergence of genetic alterations through the production of genotoxins. Examples of such toxins include cytolethal distending toxin (CDT)[103], a potent DNase that induces double-strand breaks and is produced by pathogenic *E. coli*[104] and *Campylobacter jejuni*[105], both of which are enriched in patients with CRC. The introduction of *C. jejuni* harbouring a defective form of *CdtB*, which encodes a subunit of CDT, abolished the tumorigenic effects of this bacterium in mouse models[105], implying a causal role of CDT in tumorigenesis. Colibactin is another genotoxin produced by *E. coli* harbouring a gene cluster known as the pks pathogenicity island, which contains genes encoding the enzymes necessary for colibactin synthesis[85]. Colibactin forms DNA adducts[106] and crosslinks[107], leading to double-strand breaks that result in AT-rich hexameric sequence motifs[108,109]. Monocolonization of $Apc^{Min/+}$ mouse models with pks^+ *E. coli* leads to the development of colitis-associated CRC[29,110], and this effect can be attenuated by deletion of the pks island[29]. Importantly, the locations of colibactin-induced double-strand breaks have been shown to correspond with those of mutational hotspots in the CRC genome, thus emphasizing the likely role of this genotoxin in CRC tumorigenesis[108]. Small-molecule inhibitors of colibactin biosynthesis[111,112] could potentially reverse these genotoxic and tumour-promoting effects of pks^+ *E. coli* and possibly provide a prophylactic treatment approach. Similar to colibactin, indolimines, a class of microbial metabolites containing a functional imine group that are secreted by the commensal *M. morganii*, which is enriched in faecal samples obtained from patients with inflammatory bowel disease (IBD) and from those with CRC, are also able to mediate DNA damage and promote the development of CRC[31]. As well as producing genotoxins, pathogenic bacteria are also able to induce DNA damage through less-direct mechanisms. *B. fragilis* toxin from enterotoxigenic *B. fragilis* causes DNA damage in colonic cells in vivo by inducing the accumulation of reactive oxygen species (ROS)[113]. *Enterococcus faecalis* also promotes genomic instability in the colon through a similar mechanism[114]. Other mechanisms of DNA damage include the secretion of EspF, an effector protein that suppresses host cell DNA mismatch repair (MMR) proteins by enteropathogenic *E. coli*[115]. Despite these promising results, more data from patients with CRC are required to verify these indirect effects. A survey of 122 bacterial isolates obtained from patients with IBD revealed the presence of bacteria capable of inducing DNA damage in 24 patients (20%), highlighting a tremendous capacity of gut microorganisms to induce DNA damage in cells of the colonic epithelium[31]. The genotoxic potential of pathogenic bacteria could be further exacerbated by their co-enrichment in the colonic mucosa. For example, bacterial biofilms from the colonic mucosa of individuals with familial adenomatous polyposis have been shown to harbour concomitant elevations of colibactin-producing *E. coli* and enterotoxigenic *B. fragilis*, leading to accelerated DNA damage and tumorigenesis[110].

Modulation of oncogenic signalling cascades. Oncogenic signalling pathways, such as the WNT–β-catenin, MAPK and PI3K–AKT cascades, are frequently activated in patients with CRC, and these signalling cascades can be augmented by pathogenic bacteria. A number of pathobionts transduce their signals by directly interacting with receptors expressed on the surface of colonic epithelial cells. *F. nucleatum* expresses a cell-surface adhesin, FadA, which binds to E-cadherin on colonic epithelial cells and activates β-catenin signalling, leading to increased expression of cyclin D1, annexin A1 and Chk2 and tumorigenesis[79–81]. Fap2 is another *F. nucleatum* adhesin that interacts with Gal–GalNAc on the surface of CRC cells, enabling this bacterium to selectively migrate towards such cells[82]. Deletion of *FadA* impairs the ability of *F. nucleatum* to attach to and subsequently invade mammalian cells, including those derived from patients with CRC[79,116,117], thereby potentially abrogating the tumour-promoting effects of *F. nucleatum*[79]. Patients with adenomas or CRC have consistently increased FadA expression in colon tissue samples that is correlated with activation of WNT signalling and expression of several pro-inflammatory genes[79]. *P. anaerobius*, another oral bacterium that is often enriched in patients with CRC, uses its putative cell wall binding repeat 2 (PCWBR2) protein to interact with integrin α2β1 on CRC cells, which in turn activates the PI3K–AKT signalling pathway via FAK kinase[92]. Similarly, *Porphyromonas gingivalis* is able to selectively invade CRC cells and activate MAPK–ERK signalling owing to the activity of gingipain, a cell-surface protease[118]. The disruption of bacterial surface protein–host cell receptor interactions might be a viable therapeutic approach, given that pharmacological blockade of integrin α2β1 using RDGS peptides has been shown to abolish the pro-tumorigenic effects of *P. anaerobius* on both CRC cells and in $Apc^{Min/+}$ mice[92].

Induction of inflammation. Inflammation is a hallmark of cancer[119] and an established risk factor for CRC[120]. Gut dysbiosis is closely associated with inflammation of the GI tract, and has an especially important role in the development of colitis-associated CRC. Studies involving the transplantation of stools from patients with CRC into germ-free mice elicit inflammation of the colon with upregulation of pro-inflammatory cytokines[77,78]. Colonization with individual cancer-promoting pathobionts, including *F. nucleatum*[20], *P. anaerobius*[92], *E. faecalis*[121], enterotoxigenic *B. fragilis*[28], *P. micra*[122] and pks^+ *E. coli*[29], is associated with colonic inflammation. For certain microorganisms, such as enterotoxigenic *B. fragilis*, inflammation is the principal mechanism in promoting colorectal tumorigenesis[28,93–95,113,123], whereas the pro-tumorigenic effect of pks^+ *E. coli* appears to be independent of its inflammatory potential[29], although inflammation might still have a role by maintaining the expression of pks-associated genes[124]. Inflammation induced by gut pathobionts frequently involves activation of IL-17, NF-κB, and

pattern recognition receptor signalling, as well as gut barrier dysfunction, which are highly interconnected cascades that collectively confer an inflammatory phenotype. Enterotoxigenic *B. fragilis*-elicited inflammation, for example, begins with loss of gut barrier function[93], leading to rapid activation of the T helper 17 (T_H17) cell-dependent inflammatory cascade and activation of STAT3 and NF-κB signalling in

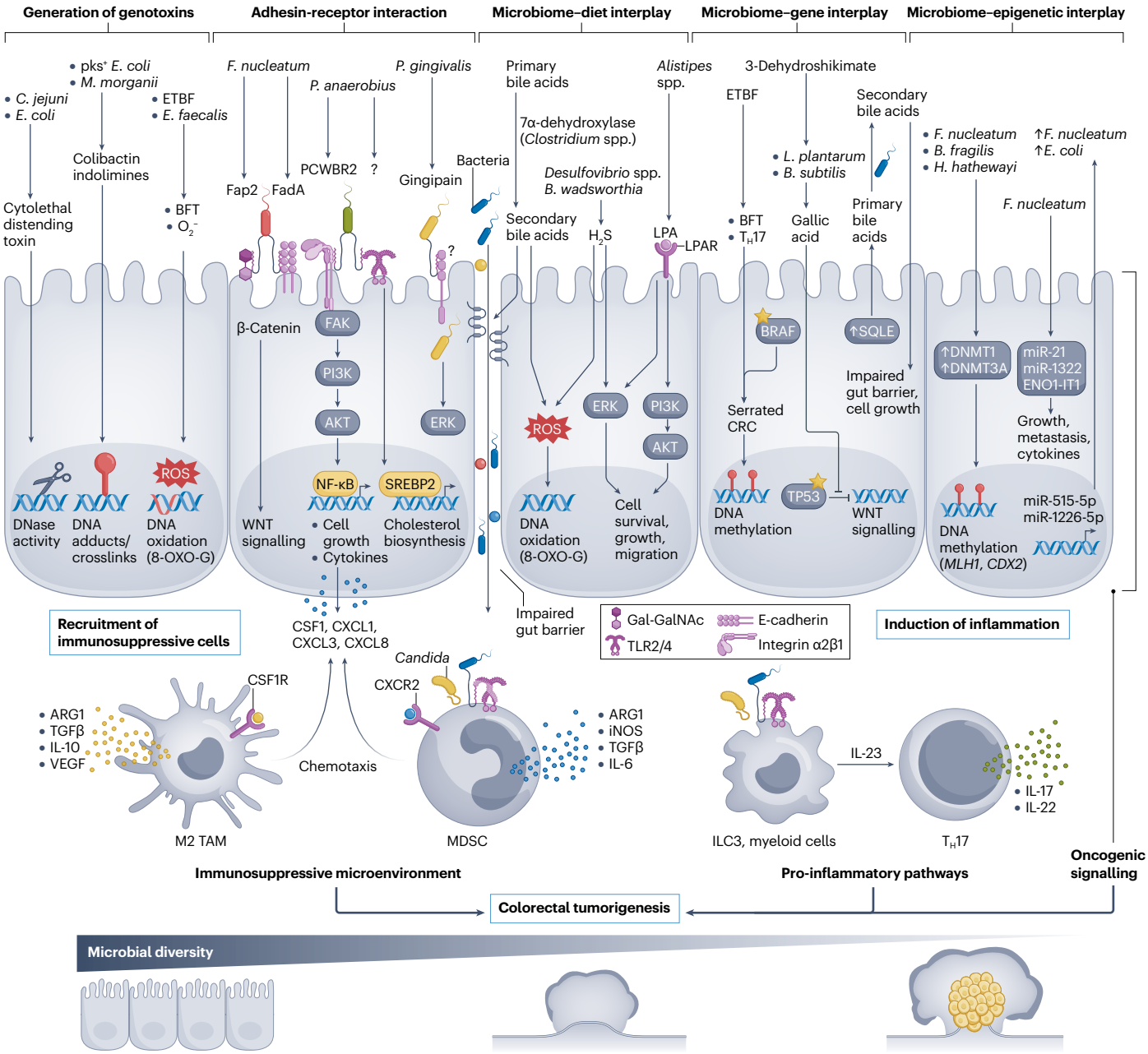

Fig. 1 | Gut microbial dysbiosis contributes to the development of CRC through a diverse range of molecular mechanisms. Gut microbial dysbiosis is a co-evolving hallmark of colorectal tumorigenesis, with decreasing microbial diversity and enrichment of cancer-inducing pathobionts observed as precancerous lesions such as adenomas that progress to colorectal carcinoma (CRC). Pathobionts present in the gut are involved in both tumour initiation and progression by generating carcinogenic genotoxins, participating in bacterial adhesin–host-cell receptor interactions, metabolism of dietary components to produce tumorigenic metabolites, interaction with genetic or epigenetic alterations, induction of inflammation, and the recruitment of immunosuppressive cells. These interactions culminate in an increased incidence of tumour-promoting genetic alterations and tumour cell proliferation and foster a repressed immune microenvironment that enables immune evasion, leading to the establishment of CRC. *B. subtilis*, *Bacillus subtilis*; BFT, *B. fragilis* toxin; DNMT, DNA methyltransferase 1; ETBF, enterotoxigenic *B. fragilis*; ILC3, type 3 innate lymphoid cells; iNOS, inducible nitric oxide synthase; LPA, lysophosphatidic acid; LPAR, LPA receptor; MDSC, myeloid-derived suppressor cell; 8-OXO-G, 8-oxoguanine; PCWBR2, putative cell wall binding repeat 2; ROS, reactive oxygen species; TAM, tumour-associated macrophage; T_H17, T helper 17; TLR, Toll-like receptors.

IL-17R-expressing colonic epithelial cells[28,93]. Reciprocally, enterotoxigenic *B. fragilis* inhibits exosomal miR-149-3p release from CRC cells to facilitate T_H17 cell differentiation[95], thereby forming a positive feedforward cycle. Blockade of IL-17 signalling thus abolishes enterotoxigenic *B. fragilis*-driven inflammation and inhibits the development of CRC in mouse models[28]. Conversely, *P. micra* induces a T_H17 cell response and secretion of pro-inflammatory cytokines. Pattern recognition receptors can also transduce their signals via NF-κB or STAT3. Toll-like receptors (TLRs) and nucleotide binding and oligomerization domain (NOD)-like receptors are primary sensors of bacterial products. TLR2 and TLR4 (TLR2/4) sense extracellular microbial products, lipopolysaccharide or lipoteichoic acids from Gram-negative and Gram-positive bacteria, respectively. *F. nucleatum*[20], *P. anaerobius*[92] and *E. faecalis*[125] activate TLR2/4 signalling through its adaptor MYD88, which activates NF-κB and downstream expression of pro-inflammatory cytokines. NF-κB activation also promotes tumour growth and metastatic dissemination[126], an effect reversed by TLR2/4 knockdown[91]. NOD-like receptors sense intracellular microbial products. Contrary to TLRs, they protect against CRC by suppressing DSS-induced colonic inflammation and its consequential gut dysbiosis[127], and antagonizing TLR-mediated activation of NF-κB and MAPK signalling pathways[128,129]. Taken together, gut dysbiosis-elicited inflammation, at least in part, can contribute to both the initiation and progression of CRC.

Promoting immune evasion. Immunosurveillance imparts potent selection pressures on tumour cells[130]. Pathobionts have also been shown to foster an immunosuppressive tumour microenvironment that supports CRC growth. Myeloid-derived suppressor cells (MDSCs) are immunosuppressive cells that antagonize the activities of T cells by depleting amino acids[131], and expressing TGFβ and PD-L1 (ref. 132). Gut dysbiosis or the presence of certain pathogenic bacteria (such as *F. nucleatum* and *P. anaerobius*) promotes intratumoural MDSC infiltration[92,133,134] by inducing tumour-derived CXCL1, an MDSC chemoattractant[92,133]. Microbial activation of the TLR–calcineurin–NFAT–IL-6 signalling cascade on polymorphonuclear MDSCs further promotes crosstalk with CRC cells to induce STAT3-dependent expression of the co-inhibitory proteins B7H3/4 (ref. 135), leading to suppression of cytotoxic T cells. The commensal fungus *Candida tropicalis*, when allowed to accumulate in *Card9*$^{-/-}$ mice, which have impaired fungicidal activity, also participates in CRC by inducing the differentiation and activation of MDSCs[136]. Multiple pathobionts including *F. nucleatum*[137], *P. anaerobius*[92] and *Klebsiella pneumoniae*[138] are also able to regulate the activity of tumour-derived cytokines that induce the infiltration of tumour-associated macrophages of the M2 subtype, thus promoting the development and dissemination of CRC. In line with these observations, intratumoural enrichment of *F. nucleatum* in patients with CRC is associated with enrichment of immunosuppressive myeloid cells[43].

Co-metabolism of host and dietary components. Gut microorganisms produce a rich repertoire of compounds that have an essential role in supporting their dietary needs, and are also able to metabolize a multitude of diet-derived and host-derived components. In particular, diet has a major influence on the metabolic output of the gut microbiota. Here, we focus on microbial metabolites that promote tumorigenesis.

Secondary bile acids, such as deoxycholic and lithocholic acids, are generated from primary bile acids by the sequential actions of bacterial bile acid hydrolases[139] and bile acid 7α-dehydroxylases[140]. Elevated levels of secondary bile acids are typically found in the colon of individuals consuming high-fat diets, and are associated with an increased risk of CRC[141]. Secondary bile acids promote tumorigenesis by inflicting direct damage on the colonic epithelial barrier[142], induction of oxidative DNA damage resulting in genomic instability[143] and NF-kB activation[144]. Lithocholic and deoxycholic acids accelerate the colon tumorigenesis induced by carcinogens[145] or loss-of-function mutations in a single copy of *Apc*[146]. Secondary bile acids are also able to promote gut dysbiosis[147], which in turn supports the development of CRC. In line with this notion, antibiotics have been demonstrated to inhibit the tumour-promoting effects of deoxycholic acid in *Apc*$^{Min/+}$ mice, whereas FMT from deoxycholic acid-treated mice to *Apc*$^{Min/+}$ mice is sufficient to provoke inflammation and CRC[146]. Nevertheless, ursodeoxycholic acid, a secondary bile acid produced by *Ruminococcus gnavus*[148], can protect against the development of CRC[149–151]. Thus, the effects of secondary bile acids are variable and can either promote or inhibit tumorigenesis depending on the metabolic activity of certain gut microorganisms.

Hydrogen sulfide is generated by sulfur-metabolizing bacteria, such as *Desulfovibrio* spp.[152], and other bacteria that metabolize taurine (*Bilophila wadsworthia*) or cysteine (*F. nucleatum*) to form hydrogen sulfide[153,154]. The abundance of sulfur-metabolizing bacteria is increased by an animal-based, high-fat Western-style diet[155], and is associated with both a higher risk of CRC[15,156] and early onset of CRC (diagnosed at ≤50 years of age)[157]. Hydrogen sulfide is genotoxic at doses attainable in the colon[158] owing to the generation of oxidative stress[159]. Exogenous hydrogen sulfide is also capable of directly promoting the phosphorylation of AKT and ERK, and the proliferation of CRC cells in vitro[160].

Integrated analyses of the gut microbiome and metabolome have enabled the identification of novel gut microbiome–metabolite associations that contribute to the development of CRC. One such study deciphered the interaction between a high-fat diet and the gut microbiota in mouse models of CRC[98]. Dysbiosis is essential for the tumorigenic effects of a high-fat diet, and FMT from donor mice receiving a high-fat diet modulates the microbiota of azoxymethane-exposed germ-free mice and can accelerate the development of CRC. Data from correlation analyses indicate a robust correlation between pathogenic *Alistipes* species and lysophosphatidic acid (LPA), and data from in vitro assays involving CRC cells confirm that LPA is a pro-proliferative metabolite[100]. Multiple datasets integrating analyses of the gut microbiome and metabolome have been published[18,161–164]. Although largely descriptive in nature, these studies provide rich resources for attempts to experimentally investigate the role of gut microbiome–metabolome crosstalk in CRC. For example, CRC-associated faecal metabolomes are typically enriched with branched-chain amino acids and aromatic amino acids[18,164], whereas several amino acids and related metabolites are also upregulated in the serum metabolome of patients with CRC[161,162]. Further investigations of crosstalk between the gut microbiome and amino acid metabolites might provide novel insights into the role of gut microbiome–metabolome interactions in the development of CRC.

Interactions with host genetics and/or epigenetics

The gut microbiota is clearly able to modulate colorectal tumorigenesis, although few studies have explored the role of the microbiota in the context of genetic and/or epigenetic factors that promote tumorigenesis. Data from the past 5 years offer clues on the bidirectional interaction between host genetic and epigenetic characteristics and the gut microbiota. Environmental factors are considered the main influence on the gut microbiota in healthy individuals[155,165], association studies have linked cancer-related genetic and/or epigenetic alterations

with the enrichment or depletion of specific gut microorganisms. In one study, investigators mapped the microbiota of 436 gastric mucosal biopsy samples obtained from individuals with adenomas and those with CRC and investigated correlations with *KRAS* and microsatellite instability (MSI) status[38]. Bacteria of several genera were found to be strongly enriched in patients with *KRAS*-mutant CRC (such as *Peptostreptococcus* and *Parvimonas*) and in those with adenomas (*Peptostreptococcus* and *Clostridium*), while *Gallionella* and *Dechloromonas* are enriched in patients with MSI-high (MSI-H) CRC. Data from other small-scale studies indicate an association between the gut microbiota and the CRC consensus molecular subtypes[166] and *KRAS* mutations[167,168] and enrichment with specific gut microorganisms.

Despite the microbiota having an important role in the development of CRC, interplay between gut microorganisms and genetic and/or epigenetic alterations is also crucial in this regard. p53 is a tumour-suppressive transcription factor that is mutated, leading to a loss of wild-type activity potentially with gains of certain oncogenic or even de novo acquisition of tumour-suppressive functions, in approximately half of all CRCs. Specifically, the depletion of the gut microbiota using a cocktail of antibiotics supports the tumour-suppressive potential of mutant p53 in the distal colon in conditional *Trp53*^{R270H}-knockin mice, leading to the attenuation of colorectal tumorigenesis[169]. Mechanistically, gut microbiota-derived gallic acid abolishes the tumour-suppressing function of mutant p53 and promotes the development of CRC in mice harbouring *Trp53*^{R270H} via hyperactivation of WNT signalling.

Somatic oncogenic alterations in *BRAF* (predominantly *BRAF*^{V600E}) are found in around 8–10% of CRCs, and are associated with a serrated histology and DNA CpG island hypermethylation[123]. Curiously, the midproximal, serrated-like CRCs associated with this alteration in patients only emerge following colonization by enterotoxigenic *B. fragilis* in *Apc*$^{\Delta 716}$/*BRAF*^{V600E}-mutant mice[123], suggesting that both elements must be present to induce tumorigenesis. Moreover, these tumours often have high levels of IFNγ^{+}CD8^{+} T cell infiltration and are sensitive to anti-PD-L1 antibodies, suggesting that the presence of this microorganism–oncogene interplay could guide therapeutic decision-making in patients. Apart from driver mutations, gut microorganisms are also able to participate in colorectal tumorigenesis driven by SQLE, the rate-limiting enzyme in cholesterol biosynthesis. Colon-specific SQLE overexpression induces gut dysbiosis in mice with enrichment of secondary bile acid-producing pathogenic bacteria, leading to gut barrier impairment and increased colon cell proliferation[170]. Data from these studies demonstrate that gut microorganisms are able to amplify the downstream effects of oncogenic factors to promote the development of CRC. Further validation of the clinical relevance of this interplay between genetic alterations and the gut microbiota in patients with CRC is required to support mechanistic data from mouse models.

Accumulating evidence indicates that gut microorganisms might also drive CRC-promoting epigenetic alterations. Hypermethylation of multiple tumour suppressor genes is often detected in CRC DNA samples and is associated with transcriptional silencing. Transplantation of stool samples from patients with CRC into germ-free mice also induces the hypermethylation of multiple promoters, in contrast to the lower rate of methylation following transplantation of samples from individuals without CRC, and the resulting methylation signature was successfully validated in patients[171]. The level of certain pathogenic bacteria, such as *F. nucleatum* and *Hungatella hathewayi*, showed strong correlations with CpG methylation in clinical specimens. Consistently, monocolonization by single strains of pathogenic bacteria such as *B. fragilis*[123,172], *F. nucleatum*[173,174] and *H. hathewayi*[174] is associated with promoter hypermethylation of CpG islands of tumour suppressor genes, such as *MLH1* and *CDX2*. Inoculation with *F. nucleatum* or *H. hathewayi* has been found to upregulate expression of DNA methyltransferase 1 (DNMT1) and DNMT3A in CRC cells and in conventional or germ-free mouse models[174], indicating a direct role in de novo DNA methylation.

Gut microorganisms have also been associated with certain non-coding RNAs in CRC samples[175]. *F. nucleatum* infection in mouse models of CRC modulates the expression of miR-21 (ref. 176) and miR-1322 (ref. 137), which mediate tumorigenesis and metastasis, respectively, and can also activate the long non-coding RNA ENO1-IT1 to promote glycolysis[177]. Reciprocally, host cell-secreted miR-515-5p and miR-1226-5p are able to enter gut bacteria such as *F. nucleatum* and *E. coli*, where they upregulate bacterial gene transcripts and promote growth[178], highlighting a role for microRNAs in enabling host–bacteria crosstalk, including in CRC[179]. *F. nucleatum* has also been shown to disrupt m^{6}A-modification in CRC cells and to facilitate metastatic dissemination[180]. Understanding the complex interplay between gut microorganisms and host genetics/epigenetics is expected to provide potential therapeutic targets for intervention.

Microbiota and cancer prevention

Probiotics as anticancer agents

As with all diseases, prevention of CRC is better than cure. Probiotics are defined as living microorganisms that, when administered in adequate amounts, confer health benefits on the host[181]. Probiotics such as those containing lactobacilli or bifidobacteria have a long history of use and are generally recognized as safe. Probiotic strains such as *L. acidophilus*[182,183], *L. rhamnosus* GG[184] and *Bifidobacterium longum*[185–187] have been recognized as being able to inhibit carcinogen-induced colorectal tumorigenesis in rodents since the early 1980s. Data from early clinical trials testing probiotic preparations in patients with CRC[188,189] indicated potentially beneficial effects such as a reduced risk of disease recurrence. Increasingly, the definition of probiotics has expanded to include other gut commensals with beneficial effects on health, referred to as 'next-generation' probiotics[190]. Owing to their excellent safety profiles and potential for broad health benefits, probiotics are viewed as a highly attractive CRC prophylactic. Generally, and despite containing live microorganisms, probiotics do not directly colonize the gut mucosa. The effects of these bacteria are instead primarily mediated via microbial metabolites and products, and their interplay with pathogens, resulting in protection of gut barrier function (Fig. 2; Table 1).

Growth inhibitory effects of probiotic metabolites. Probiotics or their products can have direct inhibitory effects on CRC cell viability in preclinical models[191–194]. Heat-killed probiotics have been shown to maintain these anticancer properties both in vitro and in vivo, suggesting that probiotic structural molecules and metabolites have a key role in suppressing CRC[195–197]. Such formulations can include several classes of molecules, such as low-molecular-weight metabolites (<5 kDa), peptides, and high-molecular-weight proteins and polysaccharides.

The microorganisms included in probiotic formulations can produce a diverse range of probiotics. Short-chain fatty acids (SCFAs) such as butyrate, propanoate and acetate are all abundant metabolites produced by gut bacteria following the fermentation of soluble fibre. Major sources of butyrate include bacteria of the phylum Firmicutes, in particular *Faecalibacterium prausnitzii*, and *Clostridium leptum* of

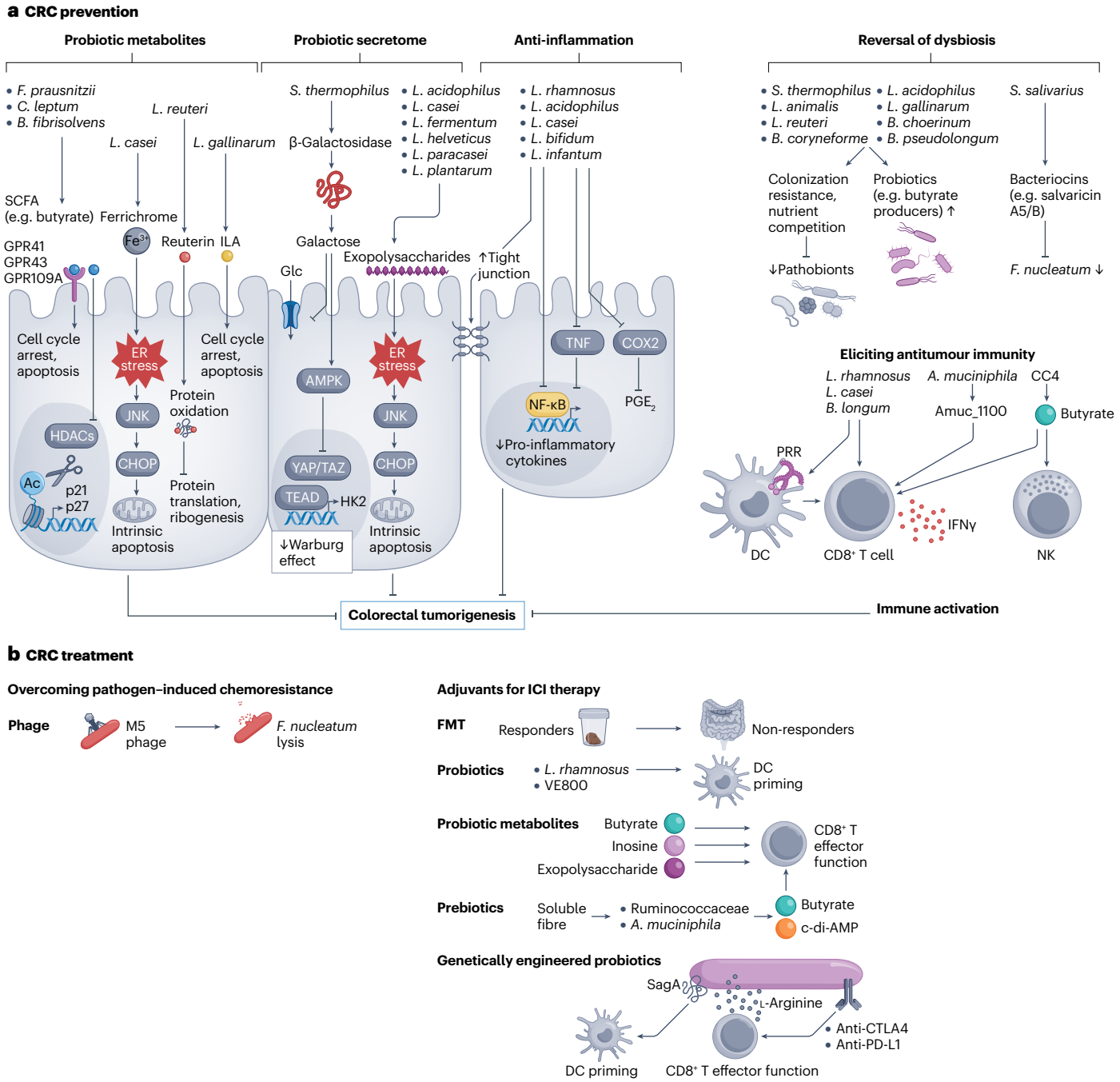

Fig. 2 | Clinical implications of gut microorganisms in the prevention and treatment of colorectal cancer. Probiotics can potentially prevent the development of colorectal tumorigenesis by generating antiproliferative or pro-apoptotic low-molecular-weight metabolites and large molecules, including proteins and exopolysaccharides, inhibition of inflammation, reversal of gut dysbiosis by impairing pathobionts and boosting beneficial commensals, and reactivation of antitumour immunity, which leads to the clearance of premalignant cells. Data from emerging studies also underscore the potential of targeting the gut microbiota to improve the efficacy of chemotherapy[258,262] and immune-checkpoint inhibitors (ICIs)[316,342]. For example, phage-mediated ablation of specific pathobionts is able to reverse *Fusobacterium nucleatum*-induced chemoresistance in mouse models of colorectal cancer (CRC)[258]. Using the gut microbiota to improve responsiveness to ICIs is currently an area of considerable research interest. Current approaches largely involve faecal microbiota transplantation (FMT), although more selective strategies such as probiotics, probiotic metabolites, prebiotics and genetically engineered probiotics hold great promise in improving responses to ICIs in patients with CRC. c-di-AMP, cyclic di-AMP; CC4, mix of four probiotic Clostridiales species (*Roseburia intestinalis*, *Eubacterium hallii*, *Faecalibacterium prausnitzii*, *Anaerostipes caccae*); DC, dendritic cell; ER, endoplasmic reticulum; GPR, G protein-coupled receptor; HDAC, histone deacetylase inhibitor; HK2, hexokinase 2; ILA, indole-3-lactic acid; NK, natural killer cell; PRR, pattern recognition receptor; SagA, secreted antigen A; SCFA, short-chain fatty acids.

Table 1 | Probiotic-derived metabolites and macromolecules in CRC prevention

Probiotic	Associated metabolites or macromolecules	Mechanism of action	Level of evidence
Low-molecular-weight probiotic metabolites			
Butyrate producers, such as Ruminococcaceae and Lachnospiraceae	Fatty acids such as butyrate, propionate, acetate	Histone deacetylase inhibition	Cell lines and mouse models[199,200]
		Induction of cell-cycle arrest	Cell lines[200]
		Activation IFNγ$^+$CD8$^+$ T cells and NK cells	Primary cells and mouse models[246,259]
		Activation of FFAR2 and/or FFAR3	Cell lines and mouse models[201,203,206]
Lactobacillus casei	Ferrichrome	ER stress and CHOP-induced apoptosis	Cell lines and mouse models[207]
Lactobacillus reuteri	Reuterin	Protein oxidation and inhibition of translation	Cell lines and mouse models[209]
Lactobacillus gallinarum	Indole-3-lactic acid	Inhibition of cell growth	Cell lines and mouse models[208]
Lactobacillus brevis	Polyphosphate	Induction of apoptosis	Cell lines[210,211]
Lactococcus lactis	Nisin	Induction of apoptosis	Cell lines[212]
Non-pathogenic *Escherichia coli*	tRNAs	Inhibition of cell growth	Cell lines and mouse models[213]
High-molecular-weight probiotic macromolecules			
Akkermansia muciniphila	Amuc_1100	Expansion and activation CD8$^+$ T cells	Primary cells and mouse models[247]
Streptococcus thermophilus	β-Galactosidase	Catalyse galactose formation and activation of AMPK signalling	Cell lines and mouse models[214]
Genetically engineered *Streptococcus thermophilus*	Catalase and superoxide dismutase	Antioxidant and anti-inflammatory effect	Mouse models[234]
Streptococcus salivarius	Salivaricin A5/B	Antimicrobial effect against *Fusobacterium nucleatum*	In vitro and ex vivo[243]
Lactobacillus acidophilus	Exopolysaccharides	Induction of apoptosis and autophagy	Cell line and mouse models[216,217]
Lactobacillus casei	Exopolysaccharides	Induction of apoptosis	Cell lines[218]
Lactobacillus fermentum	Exopolysaccharides (YL-11)	Induction of apoptosis, cell-cycle arrest and inhibition of PI3K–AKT signalling	Cell line and mouse models[219]
Lactobacillus helveticus	Exopolysaccharides (LHEPS-1)	Induction of apoptosis	Cell lines[220,221]
Lactobacillus paracasei	Exopolysaccharides	ER stress and CHOP-induced apoptosis	Cell lines[222]
Lactobacillus plantarum	Exopolysaccharides	Induction of apoptosis	Cell line[223]
Cell wall components			
Lactobacillus paracasei	Peptidoglycan	ER stress and immunogenic cell death	Cell line[226]

CHOP, C/EBP homologous protein; ER, endoplasmic reticulum; NK, natural killer.

the Ruminococcaceae family[198]. Experimental in vivo data demonstrate that the anti-CRC effects of the butyrate producer *Butyrivibrio fibrisolvens* plus a high-fibre diet is dependent on a functioning butyryl-CoA operon in gnotobiotic mice (colonized with four commensal bacteria, with or without *B. fibrisolvens*)[199]. Moreover, dietary fortification with tributyrin, a butyrate glycerol ester, has been shown to arrest the development of CRC in mice[199], implying that butyrate is an effective anticancer metabolite. Butyrate, by acting as a histone deacetylase inhibitor (HDAC), promotes histone acetylation and the expression of tumour suppressor proteins such as FAS, p21 and p27 (ref. 200). SCFAs are also able to bind with several tumour-suppressive G protein-coupled receptors (GPRs) including free fatty acid receptor 3 (FFAR3), FFAR2 (refs. 201,202) and hydroxycarboxylic acid receptor 2 (ref. 203), which are expressed on the surface of non-malignant colonic epithelial cells with loss of expression often observed in CRC cells[204]. Restoring GPR43 expression has been shown to sensitize CRC cells to the antitumour effects of butyrate[204], whereas *Gpr43* silencing attenuates these antitumour effects in mouse models[205]. *Ffar2* knockout (*Ffar2*$^{-/-}$) consistently exacerbates the severity of CRC in azoxymethane/DSS-induced and *Apc*$^{Min/+}$ mouse models[206]. Together, these data imply that butyrate is a microbiota-derived chemopreventive agent that is active in the colon.

Beyond SCFAs, data from several studies have revealed a plethora of other low-molecular-weight metabolites with antitumour effects. Multiple lactobacillus strains have been demonstrated to release other antitumour metabolites. Ferrichrome is a metabolite recovered from the <3 kDa fraction of *Lactobacillus casei* ATCC334 supernatant[207]. This metal-chelating siderophore induces ER stress responses, leading to selective JNK–CHOP-mediated apoptosis in CRC cells, and suppresses the growth of CRC xenografts in mouse models. *L. gallinarum* is another probiotic with chemopreventive effects in *Apc*$^{Min/+}$ mice with chemically induced CRC[208] that acts via catabolism of L-tryptophan to produce indole-3-lactic acid, which reduces both the number and size of colorectal tumours. Likewise[209], *Lactobacillus reuteri* has been shown to generate a unique and highly electrophilic metabolite, reuterin, which induces oxidative stress through glutathione depletion and causes selective oxidation of cysteine residues in ribosomal proteins, leading

to suppression of protein translation and arrested CRC growth in vitro and in vivo. Other examples of microbiota-derived antitumour small molecules include polyphosphates[210,211], the antimicrobial peptide nisin[212] and tRNAs[213].

S. thermophilus is a probiotic that suppresses the development of CRC in azoxymethane/DSS-exposed or $Apc^{Min/+}$ mice. Contrary to other probiotics, we localized the active component of this bacterium to the >100 kDa fraction and identified β-galactosidase as a major secreted protein[214]. Mechanistically, *S. thermophilus*-derived β-galactosidase catalyses the formation of galactose both in vitro and in vivo, which impairs glucose utilization and activates oxidative phosphorylation, resulting in a 'reverse Warburg' effect, whereby tumour cells become dependent on the same energy-generating processes as non-malignant cells, resulting in tumour suppression. Exopolysaccharides are another class of microbial-derived extracellular macromolecules with tumour-suppressive properties. Exopolysaccharides derived from various lactobacilli, including *L. acidophilus*[215–217], *L. casei*[218], *L. fermentum*[219], *L. helveticus*[220,221], *L. paracasei*[222] and *L. plantarum*[223], can suppress the growth of CRC cells in vitro[215,224] and in mouse models[219,225] by selectively promoting tumour cell apoptosis. Besides secreted molecules, bacterial cell wall components can also elicit antitumour effects. Peptidoglycans, an essential cell wall component of the *L. paracasei* cell wall, can induce the death of CRC cells by provoking ER stress[226]. Collectively, probiotic metabolites and cell wall components provide a rich and highly diverse repertoire of tumour-suppressive molecules.

Suppression of inflammation. Chronic inflammation is an important initiating factor in colorectal tumorigenesis[227] by causing DNA damage, gut barrier dysfunction and immunosuppression. Administration of probiotics has been associated with reduced inflammation of the colonic epithelium, and such a strategy is highly effective in preclinical models of colitis-associated CRC. *L. rhamnosus* GG, for example, suppresses the expression of several inflammatory mediators including NF-κB, p65, COX2 and TNF in a rat dimethyl hydrazine model of CRC[228]. A probiotic mixture of *L. acidophilus*, *Bifidobacterium bifidum* and *B. infantum* was found to suppress inflammation and restore gut barrier function in a similar model[229]. Likewise, orally administered probiotic cocktails[230,231] or yogurts[232] have been shown to suppress inflammation and delay tumour development in mouse models of CRC. In other investigations, *L. casei* BL23 suppressed the development of colitis-induced CRC in mice by downregulating IL-22 expression[233]. The antitumour activity of probiotic formulations can further be enhanced by genetic engineering[234]. For example, genetically modified *S. thermophilus* carrying antioxidant enzymes and/or IL-10 are able promote the release of anti-inflammatory cytokines, thus preventing CRC in mouse models. The engineering of *L. acidophilus* to abrogate the production of lipoteichoic acid, a pro-inflammatory cell wall polymer, also renders this bacterium more effective at ameliorating colonic inflammation and CRC in similar models[235,236]. These data highlight the potential of probiotics to suppress inflammation, which has a key role in the pathogenesis of CRC and particularly in the context of chronic inflammation such as in IBD.

Reversal of gut dysbiosis. Reversal of gut dysbiosis can be defined as the disruption of the previously described tumour-promoting network of CRC-associated pathobionts and/or the enrichment of probiotic strains with tumour suppressive potential that negate the potentially carcinogenic effects of gut pathogens. The regular consumption of probiotics might also reverse such dysbiosis. For example, administration of *S. thermophilus* reduces the size of the Firmicutes population and increases that of Actinobacteria in $Apc^{Min/+}$ mice[214]. This approach also leads to enrichment of several potentially tumour-suppressive strains of *Bifidobacterium* and *Lactobacillus* (*B. choerinum*, *B. pseudolongum*, *B. coryneforme*, *L. reuteri*, *L. animalis* and *L. acidophilus*)[214]. Administration of *L. gallinarum* to $Apc^{Min/+}$ mice also leads to the development of a distinct microbial profile, characterized by an increased abundance of the probiotic *L. helveticus* and *L reuteri*, and a reduced abundance of the pathogenic genera *Alistipes*, *Allobacullum*, *Dorea*, *Odoribacter*, *Parabacteroides* and *Ruminococcus*[208]. Similarly, administration of *L. rhamnosus* GG and *L. rhamnosus* LS8 can enrich for butyrate producers such as species of the genera *Faecalibaculum*, *Akkermansia*, *Roseburia* and *Coprococcus* and suppress CRC development in mouse models[237,238]. Even short-term, early-life exposure of $Apc^{Min/+}$ mice to *L. rhamnosus* GG promotes the enrichment with the probiotic genera *Bifidobacterium* and *Anaeroplasma*, whilst depleting *Peptostreptococcus*[239]. Data from intervention studies in humans validate the possibility of modifying the gut microbiota with probiotics. For example, administration of a probiotic mixture of *B. lactis* and *L. acidophilus* to patients with CRC increases the abundance of butyrate producers of the genera *Faecalibacterium* and *Clostridium*, with simultaneous reductions in the *Fusobacterium* and *Peptostreptococcus* populations[240]. Nonetheless, more research is required to assess whether reversal of dysbiosis correlates with suppression of tumorigenesis in humans. Mechanistically, probiotics contribute to resistance to colonization and expansion of pathogenic bacteria. This resistance can involve passive mechanisms such as nutrient competition and release of certain metabolites (such as SCFAs and bile acids)[241,242]. Microbiota-derived butyrate activates PPARγ to maintain hypoxia and limit the expansion of pathogenic bacteria, such as those of the genera *Escherichia* and *Salmonella*[242]. Colonization resistance also involves active antagonism; for example, gut commensals can produce bacteriocins and antimicrobial proteins that are known to suppress specific pathogenic bacteria. In a study screening 16,000 bacterial clones from human stool samples, *S. salivarius* was found to have selective antimicrobial activity against pro-tumorigenic *F. nucleatum* strains via its bacteriocins salivaricin A5 and salivaricin B[243]. Hence, the identification of bacteriocins and other antimicrobial proteins might lead to biotherapeutics capable of targeting CRC-inducing pathogens.

Eliciting antitumour surveillance. The host's immune system is an important mechanism of resistance to tumorigenesis. In this regard, probiotics can promote enhanced innate and adaptive immune responses. Bacterial extracts, such as Coley toxins, have long been known to elicit antitumour immune responses. For example, gavage with *L. casei* and *Bifidobacterium longum* extracts induces the expansion of natural killer (NK) and $CD8^+$ T cells in mice[244]. Similarly, administration of live *L. rhamnosus* GG elicited $CD8^+$ T cell-dependent antitumour effects in a mouse model of CRC[245], suggesting that immunomodulation has a role in the activity of this probiotic. In a separate study[246] investigators selected a mix of four probiotic Clostridiales species (CC4) (*Roseburia intestinalis*, *Eubacterium hallii*, *F. prausnitzii* and *Anaerostipes caccae*) that are all capable of producing butyrate from distinct substrates. This formulation inhibits the development of, and in some cases can even be used to mediate a reduction in, CRC tumour diameter by eliciting an $IFNγ^+CD8^+$ T cell and NK cell-mediated antitumour response, with concomitant reductions in regulatory T cell (T_{reg}) activity. The antitumour activity of CC4 was found to be superior to that

of anti-PD-1 antibodies and chemotherapy in a mouse MC38 allograft model. Thus, butyrate has a two-pronged effect including suppression of CRC cell growth and promotion of antitumour immunity. *Akkermansia muciniphila*, another bacterium capable of producing butyrate, additionally expresses an outer membrane protein, Amuc_1100, that suppresses colitis-associated CRC by inducing the expansion and activation of $CD8^+$ T cells in colonic and mesenteric lymph nodes in a mouse model of colitis[247]. *A. muciniphila* has also been demonstrated to induce innate immune responses that delay the development of CRC by enriching M1-like macrophages via TLR2–NLRP3 signalling in mouse models[248].

Modulating the efficacy of chemopreventive interventions. A number of chemopreventive drugs have been proposed for the prevention of CRC, with the strongest clinical evidence available supporting the use of aspirin and other non-steroidal anti-inflammatory drugs[249]. Regular aspirin use is associated with a CRC-suppressive microbiota. In volunteers without cancer, regular aspirin use has been shown to influence several microbial taxa, with enrichment of bacteria of the probiotic genera *Akkermansia* and *Prevotella* together with depletion of *Parabacteroides*[250]. Data from mouse models also indicate that aspirin leads to a greater abundance of the probiotics *Bifidobacterium* and *Lactobacillus*, while reducing that of the pathogenic bacteria *B. fragilis* and *F. nucleatum*[251,252], which might explain the protective effects of aspirin use against CRC. Conversely, the gut microbiota can also degrade aspirin, which reduces bioavailability and the associated antitumour activity in azoxymethane or DSS-exposed mice compared with their antibiotic-treated or germ-free counterparts[252]. In this context, arylesterase-expressing *Lysinibacillus sphaericus* was found to lower the bioavailability of aspirin and salicylic acid, thus impairing the efficacy of aspirin in chemoprevention of CRC. The levels of *L. sphaericus* and potentially other aspirin-degrading bacteria might thus be predictive of clinical benefit from aspirin.

Role of the microbiota in drug response and resistance

Mounting evidence indicates that the gut microbiota can modulate the responses of patients with CRC to chemopreventive and/or chemotherapeutic drugs, such as aspirin[252], 5-fluorouracil (5-FU)[253–257], irinotecan[258] and oxaliplatin[259–262]. Several mechanisms might explain these effects, including microbial biotransformation, modulation of chemoresistance pathways and therapy-elicited immunity. Studies involving antibiotic-treated gnotobiotic mice or germ-free mice have demonstrated both increased[252,263] and diminished[254,262,264] activity of chemotherapy in the absence of gut microorganisms, thus highlighting the complexity of host microbiota–drug interplay. Improved characterization of the roles of pivotal bacteria might enable selective targeting of the gut microbiota to improve the efficacy of chemotherapy and/or limit the incidence of adverse events.

The gut microbiota modulates drug bioavailability. Intratumoural bacteria[38,265] can modulate the bioavailability of chemotherapies in tumours. For example, intratumoural Gammaproteobacteria express a long isoform of cytidine deaminase (CDD_L) that degrades gemcitabine to an inactive metabolite[263]. Co-culture of CDD_L-expressing bacteria with CRC cells in vitro and the colonization of mouse colon allograft models in vivo contributes to gemcitabine resistance, an effect that can be reversed by antibiotics. Intratumoural *E. coli* isolates obtained from patients with CRC also have the ability to degrade 5-FU, which might lead to diminished therapeutic efficacy[266]. These studies highlight a possible role of host–microbial co-metabolism in modulating the efficacy of chemotherapy in patients with CRC.

Role of the gut microbiota in chemoresistance. The role of the gut microbiota in chemoresistance is less well understood than its role in tumorigenesis. *F. nucleatum* is abundant in CRC samples obtained from patients[20,79,267] and is associated with inferior outcomes in patients with recurrent disease[256]. Data from functional studies indicate that *F. nucleatum* confers resistance to both oxaliplatin and 5-FU by eliciting protective autophagy to prevent apoptosis[256,268]. Mechanistically, *F. nucleatum* promotes TLR4–MyD88 signalling and reverses miR-18a*/4802-mediated repression of the transcription of several genes involved in autophagy (such as *ULK1* and *ATG7*). *F. nucleatum*-mediated TLR4 activation drives NF-κB signalling and expression of the transcriptional target BIRC3 (refs. 255,269), which inhibits caspase-dependent apoptosis[270]. Cancer stem cells are thought to be a source of chemoresistance and metastasis in CRC[271]. *F. nucleatum* has been shown to promote self-renewal of colon cancer stem cells by promoting fatty acid oxidation[272] and activation of Notch signalling[272]. *F. nucleatum*-derived formate has also been shown to promote AhR signalling, leading to tumour invasion and stemness in mouse models[273]. Thus *F. nucleatum* is a potential target of interventions designed to overcome chemoresistance in patients with CRC.

Modulation of therapy-elicited antitumour immunity. Accumulating evidence indicates that effective tumour control with chemotherapy requires a functioning immune system[274]. Chemotherapies can induce immunogenic cell death, a type of cell death that releases damage-associated molecular patterns into the tumour microenvironment[275], and thus prime antitumour immunity[276], and potentially have direct or indirect effects on immunosuppressive cells[277] and/or effector cells[264]. The gut microbiota has an important role in eliciting the immunological effects of chemotherapy[264]. Data from multiple studies indicate that the gut microbiota is able to potentiate the antitumour activity of oxaliplatin through immunomodulation, and that this activity is diminished in antibiotic-treated or germ-free mouse models[259,261,262]. Several diverse mechanisms have been suggested in an attempt to explain these positive effects of the gut microbiota. In one study[262] gut commensals were found to promote the acute cytotoxic effects of oxaliplatin by promoting intratumoural ROS production by myeloid cells. Gut bacteria can also participate in oxaliplatin-induced immunogenic cell death[278,279]. Roberti et al.[261] showed that oxaliplatin-induced immunogenic cell death involves a T follicular helper cell (T_{FH})-mediated immune response that is abolished in both germ-free and TLR2/4 knockout mice. Elsewhere, several bacterial species, such as non-enterotoxigenic *B. fragilis* and Erysipelotrichaceae species, have been identified as immunogenic and able to stimulate the release of IL-1β and IL-12 from dendritic cells (DCs), which is required for optimal priming of T_{FH} cells and in turn contributes to an enhanced effector T cell response and tumour suppression. Although the exact microbial structures involved are unclear, bacterial macromolecules (such as proteins and RNA) could potentially elicit a T_{FH} response[280].

SCFAs, such as butyrate, from gut microbial species are able to regulate T cells through GPRs[281,282] and epigenetic mechanisms[283,284]. Effects of such microbial metabolites can include restoration of the antitumour activity of oxaliplatin in antibiotic-treated mice by activating $CD8^+$ T cells[259]. Gut bacteria-derived butyrate can also evoke cytotoxic T cell activity through epigenetic regulation of ID2-dependent IL-12 signalling. Accordingly, the combination of oxaliplatin and

butyrate has been shown to synergize in inducing tumour regression in mouse models of CRC[259]. These data underscore the potential of gut microorganisms or their products as adjuvants that might improve the responses of patients with CRC to chemotherapy.

Improving the effectiveness of chemotherapy

Modulating efficacy. Currently, only limited evidence exists on how manipulation of the gut microbiota can modulate the anticancer activity of chemotherapy. Butyrate-producing bacteria might potentiate the efficacy of chemotherapy by activating adaptive immunity[259]. In vitro evidence suggests that *Lactobacillus* spp. either improve the activity of, or reverse resistance to, 5-FU[285,286] and irinotecan[287] in CRC by secreting the metabolites butyrate[287], γ-aminobutyric acid[288] and extracellular vesicles[289]. Additional in vivo evidence is required to validate the potential of probiotics to improve the activity of chemotherapy.

Targeting chemoresistance-inducing pathogens is another plausible method of improving chemotherapy response. However, antibiotics typically have broad-spectrum effects, often leading to simultaneous depletion of gut commensals. In this regard, bacteriophages could potentially provide species-specific or even strain-specific, selective depletion of bacteria. For example, investigators[290] used an M13 phage display library to identify an M5 phage clone that selectively targets *F. nucleatum*. Silver nanoparticles coated with the M5 phage were able to selectively kill *F. nucleatum* and potentiate the activity of folinic acid–5-FU–irinotecan chemotherapy in mouse models of CRC, thus underscoring the potential of phages to target pathogenic bacteria (Fig. 2).

Reducing toxicity. Beyond improving antitumour activity, the gut microbiota can also affect toxicity. Intestinal mucositis and diarrhoea are both frequently observed toxicities in patients with cancer receiving chemotherapy[291–293]. SN-38, the active metabolite of irinotecan, is detoxified in the liver by glucuronidation and secreted via bile into the gut[294]. Gut microorganisms release active SN-38 via bacterial β-glucuronidase, which can cause enteric toxicities and diarrhoea[295,296]. Consistent with this notion, irinotecan-induced enteric toxicities are attenuated in antibiotic-treated[297] or germ-free mice[298,299]. β-Glucuronidase, the enzyme responsible for glucuronidation of irinotecan, is broadly represented in the human gut microbiome[300], and this agent might augment β-glucuronidase activity by promoting enrichment in Enterobacteriaceae and Clostridia, forming a vicious cycle that exacerbates such adverse effects. Thus, selective inhibition of bacterial β-glucuronidase has shown the potential to alleviate adverse effects and improve the therapeutic index of irinotecan in CRC[296,301,302].

Role of the microbiota in responsiveness to ICIs

Targeted inhibition of T cell inhibitory checkpoints, such as with anti-PD-1 or anti-CTLA4 antibodies, has revolutionized the treatment of many cancers[303,304]. However, ICIs are largely ineffective in patients with CRC, with the notable exception of the minority (~5%) with MMR-deficient or MSI-H disease[305–308]. Preclinical and clinical studies have identified an indispensable role of the gut microbiota in responsiveness to ICIs. For example, antibiotic-treated and germ-free mouse models of several solid tumours have impaired responses to anti-CTLA4 and/or anti-PD-1 antibodies[309–312]. Transplantation of stool samples from patients with metastatic melanoma who respond to anti-PD-1 antibodies enables responsiveness to ICIs to be recapitulated in these models[310,313,314]. Antibiotic use is correlated with inferior outcomes in patients with cancer receiving ICIs[311,313]. In light of the role of gut microorganisms in responsiveness to ICIs, this is an exciting area with translational potential in CRC.

Innate immune cell crosstalk

The gut microbiota harbours a rich repertoire of antigens with immunostimulatory potential that are recognized by pattern recognition receptors expressed on innate immune cells, particularly antigen-presenting cells. Potentiation of the antitumour activity of ICIs in mouse models by individual microorganisms[309,313,315] or cocktails of commensal bacteria[316,317] has been attributed to the ability to activate DCs. A diverse range of bacterial components can prime DCs. For example, *B. fragilis* capsular polysaccharides are able to stimulate $CD11b^+$ DCs in the lamina propria and induce IL-12-dependent T_H1 antitumour activity[309]. A cocktail of 11 commensals derived from human faeces was also able to prime $CD103^+$ DCs in the lamina propria, thus promoting $IFN\gamma^+CD8^+$ T cell accumulation in mouse models[316]. Oral administration of live *L. rhamnosus* GG induced the production of type I interferons in DCs by activating cyclic GMP–AMP synthase (cGAS)/stimulator of IFN genes (STING) signalling pathways in mouse models of CRC and melanoma[315]. Thus, the gut microbiota is able to stimulate DCs and promote an enhanced $CD8^+$ T cell response that improves the antitumour activity of ICIs.

Enterococcus are enriched in stool samples from patients with ICI-responsive solid tumours[310,313]. Specific strains of this genus have been found to express a peptidoglycan hydrolase secreted antigen A (SagA), which hydrolyses the bacterial cell wall and generates muropeptide fragments. These muropeptides are able to stimulate pro-inflammatory gene signatures in intratumoural, NOD2-expressing myeloid cell clusters that potentiate an antitumour T cell response following administration of anti-PD-L1 antibodies in mouse models[318]. In addition to bacteria-derived products, translocation of bacteria such as *Bifidobacterium* into tumours can occur under certain conditions and can trigger NK cell and $CD8^+$ T cell responses capable of killing tumour cells as a bystander effect in mouse models[319]. Thus, intratumoural injections of *Bifidobacterium* might provide a useful adjuvant for potentiating the efficacy of immunotherapies in patients.

Gut microbiota–effector cell crosstalk. Gut microorganisms can directly modulate the antitumour activity of effector cells in a number of ways. For example, the bacterial metabolite inosine can be produced by both *B. pseudolongum* and *A. muciniphila*, and binds to the adenosine A2A receptor on T cells leading to the activation of T_H1 cells in combination with anti-CTLA4 antibodies[320]. Inosine can also provide a fuel to support T cell activity in a glucose-depleted tumour microenvironment[321]. SCFAs are another class of microbial metabolites capable of potentiating the activity of ICIs through inhibition of HDACs[259,322]. Butyrate epigenetically activates the ID2-mediated upregulation of IL-12 receptor expression in $CD8^+$ T cells, thus priming them for activation by DC-derived IL-12 (ref. 259). Similarly, pentanoate induces the production of effector molecules in $CD8^+$ T cells and is able to augment the activity of chimeric antigen receptor T cells in mouse models of melanoma or pancreatic cancer[322]. Macromolecules derived from the gut microbiota are also able to stimulate immune effector cells. For example, the *Lactobacillus delbrueckii* subsp. *bulgaricus* OLL1073R-1 secretes the exopolysaccharide EPS-R1 (ref. 323), which binds to LPA receptor 2 on $CD8^+$ T cells and is able to stimulate the activity of $CCR6^+CD8^+$ T cells by competing with endogenous lipids. EPS-R1 is also able to augment the activity of ICIs by inducing the infiltration of $IFN\gamma^+CCR6^+CD8^+$ T cells into CCL20-expressing mouse CT26 colon

carcinoma xenografts. Secreted bacterial products are thus promising adjuvants for use in combination with ICIs.

Molecular mimicry by gut microbial antigens is implicated in autoimmunity, although this effect can also be exploited to mimic cancer antigens and elicit T cell responses[324,325]. Examples from melanoma include cross-reactivity between the tail-length tape measure protein epitope from an *Enterococcus hirae* prophage and PSMB4 (ref. 325), and the SVY epitope from *Bifidobacterium breve* and SIY[324]. Microbial epitopes are highly diverse[68] and these examples are possibly only a very limited snapshot of the full repertoire of tumour-mimicking antigens.

Gut microbiota–CRC cell crosstalk. Curiously, pathobionts that promote colorectal tumorigenesis could have contrasting effects on the antitumour activity of ICIs. In contrast to its tumour-promoting and chemoresistance-inducing effects, data from 2021 indicate that *F. nucleatum* is able to enhance the efficacy of anti-PD-L1 antibodies and prolong the survival durations of mouse CRC allografts[326]. *F. nucleatum* colonization activates STING–NF-κB signalling and PD-L1 expression in CRC cells, but paradoxically also promotes the activation of $CD8^+$ T cell activation when PD-L1 is inhibited[326]. Similarly, colonization of $Apc^{\Delta 716/+}BRAF^{V600E}$ mice with enterotoxigenic *B. fragilis* promotes the development of tumours with serrated-like morphology, CpG island hypermethylation, infiltration of IFNγ-expressing $CD8^+$ T cells and increased sensitivity to anti-PD-L1 antibodies[123]. Apparently pathogenic bacteria might thus have discordant effects in the context of treatment with ICIs.

Harnessing the microbiota to improve ICI therapy

Faecal microbiota transplantation. FMT is one of the early gut microbiota modulation strategies and has been tested in combination with ICIs in clinical trials, on the basis that administering FMT to germ-free animals recapitulates the responses to ICIs of human donors[310,313,314,327,328]. Conversely, FMT involving donors with IBD, who have a dysbiotic microbiota, causes resistance and implies that a healthy microbiome is critical for supporting the efficacy of ICIs[327]. FMT involving transfer of the entire faecal microbiota from responders to patients with ICI-refractory melanomas, has been shown to overcome resistance to anti-PD-1 antibodies in trials involving small cohorts[329,330]. Trials testing similar approaches in patients with CRC are currently ongoing (NCT04729322 and NCT04130763).

Probiotics and their products. Individual gut bacteria, including *A. muciniphila*[310], *B. fragilis*[309], *Bifidobacterium*[313], *L. rhamnosus* GG[315], *Lacticaseibacillus paracasei*[331] or probiotic cocktails[316,317], are able to potentiate the activity of ICIs in mouse models. In a phase I trial, administration of the live bacterial formulation CBM588 (containing *C. butyricum*) was found to improve the response rate to nivolumab plus ipilimumab (58% versus 20%, $P = 0.06$) in a cohort of 30 patients with metastatic renal cell carcinoma[332]. Most studies of such interventions in patients with CRC have thus far focused on those with MMR-deficient or MSI-H disease, and whether probiotics might overcome resistance to ICIs in patients with microsatellite-stable (MSS) CRCs remains understudied. Nonetheless, Probio-M9 (containing *L. rhamnosus*) was found to improve the activity of anti-PD-1 antibodies in mouse CT26 allograft models[333]. VB800 (a probiotic cocktail comprising a total of 11 strains)[316] is currently being tested in combination with nivolumab in a phase I/II study involving patients with MSS CRC (NCT04208958), thus highlighting the potential of probiotics. Nonetheless, contrasting results have been reported in experimental models, in which gavage with *B. longum* or *L. rhamnosus* GG attenuated the activity of anti-PD-1 antibodies in melanoma allografts, and that the use of over-the-counter probiotics containing these bacteria within 1 month of treatment reduces the effectiveness of ICIs in patients with advanced-stage melanomas[334]. Indiscriminate use of probiotics should thus be discouraged in patients receiving ICIs until more data become available on the potential of rationally designed probiotics or probiotic cocktails.

Several probiotic-derived therapeutics supported by knowledge of the effects of the active components are being assessed as adjuvants for use alongside ICIs. These include bacterial lysates[335], extracellular vesicles[336] or more-defined components such as exopolysaccharides[337], muropeptides[318] and single bacterial metabolites[259,320], which can either fully or partly recapitulate the effects of live probiotics on the efficacy of ICIs.

Prebiotics. A positive association exists between dietary fibre intake and improved responses to ICIs, as well as a lower incidence of immune-related adverse events in patients with cancer across several globally distributed cohorts[334,338]. The administration of prebiotics, soluble fibre including inulin[339] and pectin[340,341], but not polyphenols or oligosaccharides[339], improves the activity of anti-PD-1 antibodies in various mouse models. Mechanistically, inulin and pectin enrich for certain bacterial genera (such as those of the Ruminococcaceae family), individual bacteria (such as *A. muciniphila*), and their metabolites (such as SCFAs and cyclic diadenosine monophosphate (cyclic di-AMP)) to induce antitumour immunity. SCFAs potentiate the activity of cytotoxic $CD8^+$ T cells[334,339], whereas cyclic di-AMP activates STING-dependent type I IFN production, leading to activation of innate immunity (including monocytes, NK cells and DCs) and promotion of responsiveness to ICIs[340]. In an attempt improve the functionality of dietary fibre as an immune adjuvant, Han et al.[339] devised an inulin hydrogel formulation with improved colonic residence time that demonstrated robust synergy with anti-PD-1 antibodies in eliciting tumour regression in mouse CT26 allografts, a model of MSS CRC. A phase II trial examining the effects of a high-fibre dietary intervention in patients with stage III–IV melanoma who are also receiving ICIs is currently ongoing (NCT04645680).

Genetically engineered probiotics. Engineered probiotics with enhanced functionality might provide a safe and effective method of delivering adjuvant therapy. For example, investigators engineered a *Lactobacillus lactis* strain expressing SagA that is capable of augmenting the activity of anti-PD-L1 antibodies to a similar extent to that achieved with natural SagA-expressing *Enterococcus* strains in MC38 CRC allograft mice[318]. Genetically engineered bacteria could also be used as a vehicle to provide metabolic support for intratumoural T cells. A tumour-colonizing probiotic form of *E. coli* has been engineered to synthesize arginine from ammonia, thus boosting intratumoural L-arginine availability[342], which is essential for the proper functioning of cytotoxic T cells[343]. Engineered probiotics have also been developed in an attempt to overcome the immunologically 'cold' immune microenvironment of most MSS CRCs. Investigators designed a probiotic Nissle 1917 strain of *E. coli* that expresses single-domain antibody fragments targeting PD-L1 and CTLA4, with a controlled mechanism of release designed for tumour-specific delivery of the payload[344]. In a mouse CT26 model of MSS CRC, triple-engineered *E. coli* expressing anti-PD-L1 and anti-CTLA4 antibodies, and granulocyte–macrophage colony-stimulating factor, reduced the rate of tumour growth and

improved survival[344]. These proof-of-concept studies highlight the potential of genetically engineered probiotics as versatile tools for promoting the efficacy of ICIs.

Reducing toxicity. ICIs have transformed the treatment of patients with cancer, although these antibodies can elicit immune-related adverse events, such as ICI-associated colitis[345]. As well as being life threatening in a small subset of patients, this and other adverse events are commonly treated with immunosuppressants, which might have undesirable adverse effects of their own. In this context, microbial-based therapies have shown beneficial effects. For example, FMT from donors without cancer improves treatment-refractory ICI-associated colitis by promoting T_{reg} activation in the colonic mucosa[346]. Probiotics containing bifidobacteria[347] and *L. reuteri*[348] have also been shown to ameliorate ICI-associated colitis in experimental models. Thus, modulation of gut dysbiosis not only promotes the efficacy of ICIs but could also reduce the incidence of adverse events.

The faecal microbiota as a source of biomarkers

Screening for CRC and/or colorectal adenomas

Early diagnosis of CRC (as stages I/II) is associated with excellent clinical outcomes, providing a clear rationale for the development of non-invasive biomarkers for CRC screening. The current approach to CRC screening primarily relies on faecal immunochemical testing (FIT), which is relatively specific (typically 75–90%), but lacks sensitivity especially for adenomas (<30%)[349]. A positive FIT test requires follow-up colonoscopy procedures that are both invasive and costly at the population level. Newer multitarget stool tests provide a more sensitive method of CRC detection, but still lack sensitivity for detecting adenomas (<50%)[350]. Given that gut dysbiosis is typically an early event in colorectal tumorigenesis, a number of studies have mined the faecal microbiome for possible diagnostic markers[16,18,27,49,55,64,351–367]. Large-scale assessments of gut microbiome–disease associations[17,368,369] have revealed the existence of disease-specific and shared gut microbial signatures. Consistent patterns unique to CRC are dominated by the enrichment of a small number of pathogenic bacteria[17,368] that are largely undetectable in individuals without cancer and in those with other chronic conditions (such as type 2 diabetes, Parkinson disease and IBD)[17]. These signatures are able to discriminate between patients with CRC and those without cancer with a high level of accuracy (area under the curve (AUC) > 0.9) in both single-cohort and multicohort studies[7,16,18]. For translational application in clinical settings, a reductionist approach involving only the optimal bacterial marker(s) would be preferable. As a single bacterial marker, *F. nucleatum* is the most frequently reported to be diagnostic for CRC[352,353,370], and can be combined with three or four other species to achieve a good level of accuracy. A signature combining *F. nucleatum, C. hathewayi*, *Bacteroides clarus* and m7 resulted in a superior AUC of 0.89 and outperformed *F. nucleatum* alone[352], while quantification of that ratio of *F. nucleatum* to *F. prausnitzii* or *Bifidobacterium* yielded an AUC of 0.94 (ref. 351), both of which are superior to the AUCs typically achieved with conventional FIT (AUC 0.69–0.86)[371]. *F. nucleatum* is enriched in colorectal adenomas[26], although the diagnostic performance of this bacterium decreases considerably in precancerous lesions[372]. An alternative microbial marker, *Lachnoclostridium* sp. 'm3', has demonstrated improved sensitivity (~50%) for adenoma detection compared with that of *F. nucleatum* (34%) and is superior to FIT testing in this regard[27]. Faecal bacterial markers (*F. nucleatum*, *C. hathewayi*, *B. clarus* and m3) are also effective for the diagnosis of CRC (AUC 0.88) and detection of adenomas (AUC 0.67) in asymptomatic individuals[356], verifying the potential utility of this approach in screening programmes. Gut dysbiosis is partially normalized following surgery or chemotherapy[373], and therefore quantification of CRC-associated bacterial markers has been proposed as a method of non-invasive post-treatment surveillance[374] including monitoring for adenoma recurrence[375]. The presence of CRC-associated pathobionts, including *B. fragilis*, *S. gallolyticus*, and *F. nucleatum*, in blood can also predict a subsequent diagnosis of CRC[376], suggesting that the bacterial composition of the gut or circulation could be used as an early biomarker of CRC.

Emerging metagenomics datasets suggest that the non-bacterial microbiome is also a source of diagnostic biomarkers for CRC. Taxa-level (22 taxa, AUCs 0.72–0.80) or strain-level viral gene expression signatures (14 viruses, AUCs 0.73–0.85) are able to discriminate between patients with CRC and individuals without in independent cohorts[49]. Chen et al.[52] constructed a CRC-associated viral operational taxonomic unit comprising 405 viruses based on a meta-analysis of nine cohorts of patients with CRC comprising 1,282 metagenomes with a similar level of predictive performance compared with that of bacterial markers (average AUC 0.83). Impressively, these investigators also demonstrated the ability of an 88-virus marker panel to differentiate individuals with adenomas from those without with an AUC of 0.77 (ref. 52). Signatures based on the mycobiome (14 markers) and Archaea (six markers) were also able to effectively differentiate between patients with CRC and individuals without cancer with AUCs of 0.74–0.93 (ref. 55) and 0.82–0.83 (ref. 64), respectively. Multi-kingdom analysis of stool samples from patients with CRC has yielded a novel microbial panel comprising 11 bacterial, four fungal and one archaeal marker with an average AUC of 0.83, which is superior to that of any biomarker panel focused on single kingdoms when tested under identical analysis conditions[375]. The key distinguishing features in multi-kingdom analysis include known pathobionts such as *F. nucleatum*, *P. micra* and *A. rambellii*. Besides quantification of CRC-specific strains, predictive models have been built that incorporate other microbial features, including single-nucleotide variants[377] and microbial gene functions[375]. Despite the not inconsiderable population-level variability of the gut microbiota, which reflects several variables including geographical, lifestyle-related and dietary factors[11], the process of CRC tumorigenesis invariably involves the modification of specific subsets of microbial species, thus providing a sound rationale for the further development of microbial markers enabling the early diagnosis of CRC.

Integrated biomarkers

Microbial markers have been used in combination with other established CRC biomarkers to further improve the accuracy of CRC or adenoma detection. Most notably, the use of such markers in conjunction with FIT has been shown to improve the sensitivity and specificity of CRC detection. A simple combination of *F. nucleatum* plus FIT results in a dramatic improvement in sensitivity from 73.1% to 92.3%[353]. Similar levels of improvement have been reported when bacterial marker panels plus FIT are applied to CRC detection[27,352] and m3 plus FIT for colorectal adenomas[27]. Leveraging faecal microbial markers is thus a promising approach for improving the accuracy of FIT.

Given the close interplay between the gut microbiota and metabolites in the pathogenesis of CRC, a number of studies have explored the diagnostic capabilities of biomarker panels that combine microbial and metabolic features[161,164,378–380]. For example, combining 11 metabolites and six bacterial species resulted in an AUC for CRC detection of 0.92,

Box 1

Clinical applications of microbiota in CRC: current evidence

FMT

- Faecal microbiota transplantation (FMT) to germ-free colorectal cancer (CRC) allograft mouse models recapitulates the activity of immune checkpoint inhibitors (ICIs) seen in the donor. Clinical trials (NCT04729322 and NCT04130763) are ongoing.
- FMT improves refractory ICI-induced colitis in patients. Probiotics such as *Bifidobacterium* and *Lactobacillus reuteri* alleviate ICI-induced colitis in experimental models.

Probiotics

- Individual probiotics (*Lactobacillus rhamnosus* Probio M9) or cocktails (VE800) improve the activity of ICIs in mouse models of microsatellite instability-high and microsatellite-stable CRC. Clinical trials (NCT04208958) are ongoing.
- In vitro studies indicate that probiotics such as *Lactobacillus* species and their metabolites sensitize drug-resistant CRC cells to chemotherapy.

Prebiotics

- Dietary fibre and the related metabolites (such as butyrate) potentiate the activity of anti-PD-1 antibodies in allograft models of CRC, although clinical association studies are currently lacking.

Genetically engineered probiotics

- Genetically engineered probiotics with improved function (such as expression of SagA, PD-L1, CTLA4, and/or arginine-producing enzymes) that are able to enhance the activity of ICIs in animal models of CRC. Data are mainly limited to proof-of-concept preclinical studies.

Phage-targeted depletion of pathogenic bacteria

- Selective bacteriophages targeting *Fusobacterium nucleatum* have been identified, and one clone was able to selectively kill *F. nucleatum* in mouse models and potentiate the activity of chemotherapy in allografts.

Pharmacological inhibition

- Inhibition of bacterial β-glucuronidase alleviates irinotecan-induced diarrhoea in animal models, with an improved therapeutic index. Specific bacterial β-glucuronidase inhibitors have been identified.

Faecal biomarkers

- Studies testing combinations of faecal bacterial markers typically achieve areas under the curve (AUCs) in the range 0.8–0.9 for the detection of CRC, and 0.6–0.7 for colorectal adenoma. Such tests are generally more effective in conjunction with faecal immunochemical testing.
- Bacterial, viral and fungal markers predict inferior survival in patients with CRC. However, no single microbial marker consistently predicts response in the context of specific chemotherapy and/or immunotherapy regimens.

Plasma biomarkers

- Quantification of plasma microbial metabolites alone or in combination with faecal bacteria markers enables the detection of CRC and colorectal adenomas with AUCs > 0.9 in single-cohort studies.

whereas a panel of two metabolites plus 14 bacterial markers was able to discriminate between individuals with adenoma and those without with an AUC of 0.88. Similarly, a test involving the quantification of eight gut microbiota-associated serum metabolites also had a high level of accuracy for the diagnosis of CRC (AUC 0.98) and adenoma (AUC 0.92)[161]. Theoretically, an assay integrating complementary risk factors from the host (FIT, genetics and epigenetics) and environment (the microbiota) will provide the most accurate method of CRC detection. This principle was demonstrated by a study that integrated FIT, genetic analysis (for oncogenic *KRAS*, *BRAF* and *PI3KCA* mutations), epigenetics (Septin9, NDRG4 and BMP3 methylation) and the presence of specific bacteria in the microbiota (*F. nucleatum* and *P. micra*); this study found improved detection of CRC (sensitivity 81.5%) and adenoma (27.8%) compared with FIT alone (sensitivity of 69.4% and 11.1%)[381]. Robust data from multicohort, independent validation studies are imperative for confirming the feasibility and performance of such multitarget approaches.

Predictive biomarkers

Data indicating associations between the presence of specific gut bacterial[382], viral[49] and fungal[383] species or groups of species and/or their metabolites and CRC prognosis suggest that microbial markers might be predictive of a response to systemic therapy in patients with CRC. However, data from studies specifically examining the gut microbiota longitudinally over the course of chemotherapy are sparse. Given that the gut microbiota has a critical role in an effective response to ICIs, several studies have attempted to identify associations between gut microbial signatures, ICI responses and survival outcomes. Several associations exist between specific bacterial species and a response to ICIs, including *A. muciniphila*[310,384], *Bifidobacterium* spp.[313,385,386] and *Roseburia* spp.[246]. Nonetheless, few studies have focused on microbial signatures that predict a response to ICIs in unselected patients with CRC, probably owing to the low response rates in most patients who have MSS disease.

Comparisons of responders and non-responders in a cohort of 14 patients with CRC receiving cetuximab plus the anti-PD-L1 antibody avelumab indicated that butyrate-producing bacteria, *Agathobacter* M104/1 and *Blautia* SR1/5 are enriched in faecal samples from responders and associated with improved survival outcomes[387]. Another study involving patients with CRC receiving anti-PD-1 antibodies demonstrated that those with tumours harbouring *F. nucleatum* or with high levels of *F. nucleatum* in faecal samples have improved responses and progression-free survival (PFS) durations on anti-PD-1 antibodies[326]. However, neither gut microbiota diversity nor the presence of specific

Box 2

Clinical applications of the microbiota in CRC: future directions

FMT

- Explore the use of faecal microbiota transplantation (FMT) to improve the activity of immune-checkpoint inhibitors (ICIs) in patients with microsatellite instability-high colorectal cancers (CRCs) as well as to overcome resistance to these agents in microsatellite-stable CRCs.
- Identify the critical components of FMT material that elicit immunotherapeutic responses in patients with CRC.
- Evaluate whether FMT that alleviates ICI-induced colitis has adverse or beneficial effects on the efficacy of ICIs in patients with CRC.
- Identify the key microorganisms that contribute to the alleviation of ICI-induced colitis.

Probiotics

- Determine the potentiating effects of probiotics or their cocktails on the efficacy of ICIs in the context of the complex human microbiota by comparing their effects in germ-free mice transplanted with stool samples obtained from non-responders and from responders.
- Design clinical trials to evaluate the safety and efficacy of probiotic cocktails in patients with CRC receiving ICIs.
- Define the components of probiotics and/or their cocktails that elicit immunotherapeutic responses in patients with CRC, including metabolites and peptides.
- Determine the effects of probiotic strains in animal models of chemoresistant CRC.
- Elucidate the molecular mechanisms of probiotics in overcoming chemoresistance in CRC.
- Testing of candidate probiotics for the alleviation of ICI-induced colitis in patients.

Prebiotics

- Analyse associations between prebiotics and outcomes in clinical trials involving patients with CRC receiving ICIs.
- Further validation of the effects of dietary fibre and metabolites in physiologically relevant mouse models of CRC, such as orthotopic implantation or models that spontaneously develop CRC.
- Elucidate the molecular basis of prebiotics in potentiating the efficacy of ICIs in patients with CRC.

Genetically engineered probiotics

- Selection of appropriate probiotics that are able to colonize the human gut as a carrier for molecules designed to sensitize patients to ICIs.
- Comprehensive assessments of the safety of engineered probiotics, including comprehensively addressing any regulatory concerns.

Phage-targeted depletion of pathogenic bacteria

- Define the core network of pathobionts that contribute to CRC chemoresistance.
- Devise an optimized cocktail of bacteriophages that eliminate pathobionts in experimental models and validate their effects on chemoresistance.
- Validate the safety and efficacy of phage cocktails in depleting pathobionts in the human gut.

Pharmacological inhibition

- Determine whether pharmacological β-glucuronidase inhibition influences the efficacy of irinotecan in patients with CRC.
- Evaluate the safety and efficacy of bacterial β-glucuronidase inhibitors in patients.

Faecal biomarkers

- Identify additional microbial markers enabling the high-specificity and high-sensitivity detection of CRCs, especially adenomas.
- Validate these markers in screening studies enrolling asymptomatic individuals and in geographically and ethnically diverse cohorts.
- Combine bacterial markers with faecal immunohistochemical testing and other genetic and/or epigenetic markers to establish a multitarget stool panel.
- Design prospective studies with longitudinal follow-up of gut microbiota content at baseline and at intervals during and after treatment.
- Combine microbial markers with their genetic and/or epigenetic counterparts (such as tumour mutational burden or PD-L1 for ICIs) and metabolic biomarkers to improve the predictive power of microbial markers.
- Consider the role of the intratumoural microbiota in predicting therapy responses.

Plasma biomarkers

- Standardize sample preparation procedures, instrumentation and data analysis methods to reduce the variability typically seen in the results of metabolomics analyses.
- Validate these markers in independent cohorts of patients with diverse geographical and ethnic backgrounds.

individual microbial species was shown to predict PFS at 3 months after treatment in a cohort of 30 patients with CRC receiving anti-PD-1 antibodies[388]. Data from cross-cohort studies involving patients with melanoma also demonstrated no consistent associations between single microorganisms and responsiveness to ICIs[389]. Given that microbial metabolites and structures associated with the effectiveness of ICIs could be produced by different members of the gut microbiota, functional analysis of the gut microbiota is a plausible approach to the identification of predictive markers of ICI responsiveness. In line with this notion, data from a meta-analysis indicate a more consistent association between microbial gene content and responsiveness to ICIs as opposed to microbiome composition[390].

Future directions

As summarized in this Review, major inroads have been made in our knowledge of the gut microbiome, its role in the pathogenesis of CRC and its clinical significance in CRC diagnosis, prevention and treatment (Boxes 1,2). Yet, these discoveries are merely the very tip of an iceberg of unknown information on the gut microbiota. The advent of metagenome sequencing technologies has widened our horizons considerably in providing an improved understanding of the diversity of the gut microbiome, although a reliance on this method alone creates major limitations and might be biased towards the best-characterized portions of the microbiome. Gaining a more in-depth view of the CRC microbiota, will require the development of readily accessible tools that enable us to perform metagenome-wide strain level analyses that enable the genetic variability of gut microorganisms to be discerned at the strain or even individual level, and to probe the previously unannotated parts of the gut microbiota, especially for fungi, viruses and Archaea. Only then would we be able to fully grasp the landscape of the gut microbiota in the context of colorectal tumorigenesis. To this end, emerging third-generation sequencing[391,392] and single-cell microbial sequencing[75,393] technologies are expected to offer the ability to analyse microbial genomes at strain level resolution, and have the ability to decipher uncharted microbial genomes via de novo genomic assembly. With a more comprehensive picture of the diversity of gut microorganisms potentially involved in CRC, investigators could begin to dissect the various multi-kingdom interactions that contribute to dysbiosis. Identifying interactions such as co-occurrence or co-exclusive interplay could help to identify potential agonistic and antagonistic interactions that could be harnessed to target specific tumour-promoting pathogens and/or to enrich the populations of beneficial commensals.

Another consistently under-studied theme emerging from the majority of the studies, which mostly analysed bulk tissue samples, is a lack of information on spatial heterogeneity, thus obscuring any analysis of the differential distribution of intratumoural microorganisms, and their potential interactions with distinct cell types within tumours and/or their microenvironments, including tumour cells, immune cells and fibroblasts. Multisite sampling[38], multiplexed spatial imaging[394] and single cancer cell-associated microbial profiling[43] are emerging technologies that might enable the organization of microbial niches within the tumour microenvironment to be uncovered. Indeed, data published in 2022 indicate that single cancer cells infected with bacteria behave differently from those without such infections[43]. Our own observations suggest that one bacterium can interact with a divergent range of receptors on several different cell types including tumour cells, MDSCs and T cells to mediate phenotypic effects on colorectal tumorigenesis. Hence, the elucidation of the underlying host cell–microbial niches that support cancer progression might reveal novel molecular targets for CRC prevention or treatment.

A perennial question from studies of the CRC-associated microbiota is whether changes in the microbiota cause cancer, or whether cancer causes changes to the microbiota. Even with the identification of pathobionts and commensals that might skew the balance to and from tumorigenesis and/or disease progression, whether the gut microbiota has an essential role in CRC tumorigenesis or merely enables it to happen is currently uncertain. A more likely scenario is that genetic and epigenetic changes in the colonic epithelium work hand-in-hand with gut dysbiosis to initiate cellular transformation, tumour progression and eventually invasion and metastasis. Whole-genome sequencing of non-malignant colonic epithelial cells has demonstrated that the presence of 'driver' mutations in epithelial cells does not necessarily contribute to the emergence of precancerous lesions[395] owing to intrinsic[396] as well as immune surveillance[397] programmes. A further need exists to understand the interplay between genetic or epigenetic driver events occurring in non-malignant colonic epithelial cells and a dysfunctional microbiota. In this regard, studying longitudinal changes in the gut microbiota of mouse models harbouring reversible tumour-promoting genetic and/or epigenetic disruptions[398] could be used to track the co-evolution of the gut microbiota in order to better understand its contribution to tumorigenesis.

Conclusions

Despite our limited knowledge of the role of the gut microbiota in CRC, much scope exits for clinical translation. Precise and/or personalized treatments of CRC that are focused on the gut microbiota are likely to target either the key cancer-promoting pathobionts or involve the administration of functional probiotics or their bioactive components or metabolites. At present, the vast majority of studies propose the use of functional probiotics for CRC prevention and treatment. These methods are often highly promising in experimental models of CRC, although such probiotics might not achieve the desired effect in the context of a more complex human microbiota owing to difficulties relating to colonization and unpredictable interactions with the host microbiota. More clinical studies with long-term follow-up are required to establish the benefits versus risks associated with the use of probiotics in the settings of CRC prevention and treatment. The use of probiotics to boost the efficacy of ICIs is currently an exciting area of development. Given that most patients with MSS CRCs are not responsive to anti-PD-1 antibodies, the discovery of probiotics or their products that are able to sensitize these tumours to ICIs would be a major achievement. Conversely, interventions targeting pathobionts have been largely ignored by researchers, owing to the inherent difficulties in selectively targeting specific bacterial strains. Most antibiotics have a broad spectrum of antibacterial activity and are unlikely to be useful for selective depletion of specific bacteria, whereas bacteriophages are species-specific, or even strain-specific, and are a safe and potentially effective method of targeted depletion of gut microorganisms, with some initial successes in ameliorating colitis in early clinical trials[399]. Ultimately, armed with the proper tools for selective modulation, the future is bright for harnessing the power of the gut microbiota to either prevent or slow the onset of CRC, to potentiate the efficacy of chemotherapy or immunotherapy, and to devise biomarkers enabling more accurate early diagnosis and to inform treatment decisions.

Published online: 11 May 2023

References

1. Sender, R., Fuchs, S. & Milo, R. Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. *Cell* **164**, 337–340 (2016).
2. Turnbaugh, P. J. et al. The human microbiome project. *Nature* **449**, 804–810 (2007).
3. Wu, H. J. & Wu, E. The role of gut microbiota in immune homeostasis and autoimmunity. *Gut Microbes* **3**, 4–14 (2012).
4. Carding, S., Verbeke, K., Vipond, D. T., Corfe, B. M. & Owen, L. J. Dysbiosis of the gut microbiota in disease. *Microb. Ecol. Health Dis.* **26**, 26191 (2015).
5. Scott, A. J. et al. International Cancer Microbiome Consortium consensus statement on the role of the human microbiome in carcinogenesis. *Gut* **68**, 1624–1632 (2019).
6. Dai, Z. et al. Multi-cohort analysis of colorectal cancer metagenome identified altered bacteria across populations and universal bacterial markers. *Microbiome* **6**, 70 (2018).
7. Feng, Q. et al. Gut microbiome development along the colorectal adenoma–carcinoma sequence. *Nat. Commun.* **6**, 6528 (2015).
8. Yu, J. et al. Metagenomic analysis of faecal microbiome as a tool towards targeted non-invasive biomarkers for colorectal cancer. *Gut* **66**, 70–78 (2017).
9. Sobhani, I. et al. Microbial dysbiosis in colorectal cancer (CRC) patients. *PLoS ONE* **6**, e16393 (2011).

10. Wang, T. et al. Structural segregation of gut microbiota between colorectal cancer patients and healthy volunteers. *ISME J.* **6**, 320–329 (2012).
11. Ahn, J. et al. Human gut microbiome and risk for colorectal cancer. *J. Natl Cancer Inst.* **105**, 1907–1911 (2013).
12. Zeller, G. et al. Potential of fecal microbiota for early-stage detection of colorectal cancer. *Mol. Syst. Biol.* **10**, 766 (2014).
13. Zackular, J. P., Rogers, M. A. M., Ruffin, M. T. & Schloss, P. D. The human gut microbiome as a screening tool for colorectal cancer. *Cancer Prev. Res.* **7**, 1112–1121 (2014).
14. Baxter, N. T., Ruffin, M. T., Rogers, M. A. M. & Schloss, P. D. Microbiota-based model improves the sensitivity of fecal immunochemical test for detecting colonic lesions. *Genome Med.* https://doi.org/10.1186/s13073-016-0290-3 (2016).
15. Yazici, C. et al. Race-dependent association of sulfidogenic bacteria with colorectal cancer. *Gut* **66**, 1983–1994 (2017).
16. Thomas, A. M. et al. Metagenomic analysis of colorectal cancer datasets identifies cross-cohort microbial diagnostic signatures and a link with choline degradation. *Nat. Med.* **25**, 667–678 (2019).
17. Wirbel, J. et al. Meta-analysis of fecal metagenomes reveals global microbial signatures that are specific for colorectal cancer. *Nat. Med.* **25**, 679 (2019).
18. Yachida, S. et al. Metagenomic and metabolomic analyses reveal distinct stage-specific phenotypes of the gut microbiota in colorectal cancer. *Nat. Med.* **25**, 968 (2019).
19. Chen, W., Liu, F., Ling, Z., Tong, X. & Xiang, C. Human intestinal lumen and mucosa-associated microbiota in patients with colorectal cancer. *PLoS ONE* **7**, e39743 (2012).
20. Kostic, A. D. et al. *Fusobacterium nucleatum* potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. *Cell Host Microbe* **14**, 207–215 (2013).
21. Warren, R. L. et al. Co-occurrence of anaerobic bacteria in colorectal carcinomas. *Microbiome* **1**, 16 (2013).
22. Zhang, Z. et al. Spatial heterogeneity and co-occurrence patterns of human mucosal-associated intestinal microbiota. *ISME J.* **8**, 881–893 (2014).
23. Nakatsu, G. et al. Gut mucosal microbiome across stages of colorectal carcinogenesis. *Nat. Commun.* **6**, 8727 (2015).
24. Flemer, B. et al. Tumour-associated and non-tumour-associated microbiota in colorectal cancer. *Gut* **66**, 633–643 (2017).
25. Drewes, J. L. et al. High-resolution bacterial 16S rRNA gene profile meta-analysis and biofilm status reveal common colorectal cancer consortia. *NPJ Biofilms Microbiomes* https://doi.org/10.1038/s41522-017-0040-3 (2017).
26. McCoy, A. N. et al. *Fusobacterium* is associated with colorectal adenomas. *PLoS ONE* https://doi.org/10.1371/journal.pone.0053653 (2013).
27. Liang, J. Q. et al. A novel faecal *Lachnoclostridium* marker for the non-invasive diagnosis of colorectal adenoma and cancer. *Gut* **69**, 1248–1257 (2020).
28. Wu, S. et al. A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. *Nat. Med.* **15**, 1016–1022 (2009).
29. Arthur, J. C. et al. Intestinal inflammation targets cancer-inducing activity of the microbiota. *Science* **338**, 120–123 (2012).
30. Boleij, A. & Tjalsma, H. The itinerary of *Streptococcus gallolyticus* infection in patients with colonic malignant disease. *Lancet Infect. Dis.* **13**, 719–724 (2013).
31. Cao, Y. et al. Commensal microbiota from patients with inflammatory bowel disease produce genotoxic metabolites. *Science* **378**, eabm3233 (2022).
32. Correa, N. B., Peret Filho, L. A., Penna, F. J., Lima, F. M. & Nicoli, J. R. A randomized formula controlled trial of *Bifidobacterium lactis* and *Streptococcus thermophilus* for prevention of antibiotic-associated diarrhea in infants. *J. Clin. Gastroenterol.* **39**, 385–389 (2005).
33. Horz, H. P., Meinelt, A., Houben, B. & Conrads, G. Distribution and persistence of probiotic *Streptococcus salivarius* K12 in the human oral cavity as determined by real-time quantitative polymerase chain reaction. *Oral. Microbiol. Immunol.* **22**, 126–130 (2007).
34. Roy, D., Ward, P., Vincent, D. & Mondou, F. Molecular identification of potentially probiotic lactobacilli. *Curr. Microbiol.* **40**, 40–46 (2000).
35. Szajewska, H., Ruszczynski, M. & Radzikowski, A. Probiotics in the prevention of antibiotic-associated diarrhea in children: a meta-analysis of randomized controlled trials. *J. Pediatr.* **149**, 367–372 (2006).
36. Pilchova, T., Pilet, M.-F., Cappelier, J.-M., Pazlarová, J. & Tresse, O. Protective effect of *Carnobacterium* spp. against *Listeria monocytogenes* during host cell invasion using in vitro HT29 model. *Front. Cell. Infect. Microbiol.* **6**, 88 (2016).
37. Bullman, S. et al. Analysis of *Fusobacterium* persistence and antibiotic response in colorectal cancer. *Science* **358**, 1443–1448 (2017).
38. Liu, W. et al. Microbial community heterogeneity within colorectal neoplasia and its correlation with colorectal carcinogenesis. *Gastroenterology* **160**, 2395–2408 (2021).
39. Okuda, S. et al. Profiling of host genetic alterations and intra-tumor microbiomes in colorectal cancer. *Comput. Struct. Biotechnol. J.* **19**, 3330–3338 (2021).
40. Burns, M. B., Lynch, J., Starr, T. K., Knights, D. & Blekhman, R. Virulence genes are a signature of the microbiome in the colorectal tumor microenvironment. *Genome Med.* **7**, 55 (2015).
41. Kosumi, K. et al. The amount of *Bifidobacterium* genus in colorectal carcinoma tissue in relation to tumor characteristics and clinical outcome. *Am. J. Pathol.* **188**, 2839–2852 (2018).
42. Yamamoto, S. et al. Heterogeneous distribution of *Fusobacterium nucleatum* in the progression of colorectal cancer. *J. Gastroenterol. Hepatol.* **36**, 1869–1876 (2021).
43. Galeano Nino, J. L. et al. Effect of the intratumoral microbiota on spatial and cellular heterogeneity in cancer. *Nature* **611**, 810–817 (2022).
44. Shkoporov, A. N. & Hill, C. Bacteriophages of the human gut: the "known unknown" of the microbiome. *Cell Host Microbe* **25**, 195–209 (2019).
45. Salyakina, D. & Tsinoremas, N. F. Viral expression associated with gastrointestinal adenocarcinomas in TCGA high-throughput sequencing data. *Hum. Genomics* **7**, 23 (2013).
46. Bodaghi, S. et al. Colorectal papillomavirus infection in patients with colorectal cancer. *Clin. Cancer Res.* **11**, 2862–2867 (2005).
47. Goel, A. et al. Association of JC virus T-antigen expression with the methylator phenotype in sporadic colorectal cancers. *Gastroenterology* **130**, 1950–1961 (2006).
48. Shkoporov, A. N., Turkington, C. J. & Hill, C. Mutualistic interplay between bacteriophages and bacteria in the human gut. *Nat. Rev. Microbiol.* https://doi.org/10.1038/s41579-022-00755-4 (2022).
49. Nakatsu, G. et al. Alterations in enteric virome are associated with colorectal cancer and survival outcomes. *Gastroenterology* **155**, 529–541.e5 (2018).
50. Niu, Y. D., McAllister, T. A., Nash, J. H., Kropinski, A. M. & Stanford, K. Four *Escherichia coli* O157:H7 phages: a new bacteriophage genus and taxonomic classification of T1-like phages. *PLoS ONE* **9**, e100426 (2014).
51. Hannigan, G. D., Duhaime, M. B., Ruffin, M. T. T., Koumpouras, C. C. & Schloss, P. D. Diagnostic potential and interactive dynamics of the colorectal cancer virome. *mBio* https://doi.org/10.1128/mBio.02248-18 (2018).
52. Chen, F. et al. Meta-analysis of fecal viromes demonstrates high diagnostic potential of the gut viral signatures for colorectal cancer and adenoma risk assessment. *J. Adv. Res.* https://doi.org/10.1016/j.jare.2022.09.012 (2022).
53. Lin, Y. et al. Altered mycobiota signatures and enriched pathogenic *Aspergillus rambellii* are associated with colorectal cancer based on multicohort fecal metagenomic analyses. *Gastroenterology* **163**, 908–921 (2022).
54. Anandakumar, A., Pellino, G., Tekkis, P. & Kontovounisios, C. Fungal microbiome in colorectal cancer: a systematic review. *Updates Surg.* **71**, 625–630 (2019).
55. Coker, O. O. et al. Enteric fungal microbiota dysbiosis and ecological alterations in colorectal cancer. *Gut* **68**, 654–662 (2019).
56. Gao, R. et al. Dysbiosis signature of mycobiota in colon polyp and colorectal cancer. *Eur. J. Clin. Microbiol. Infect. Dis.* **36**, 2457–2468 (2017).
57. Luan, C. et al. Dysbiosis of fungal microbiota in the intestinal mucosa of patients with colorectal adenomas. *Sci. Rep.* **5**, 7980 (2015).
58. Liu, N. N. et al. Multi-kingdom microbiota analyses identify bacterial–fungal interactions and biomarkers of colorectal cancer across cohorts. *Nat. Microbiol.* **7**, 238–250 (2022).
59. Deveau, A. et al. Bacterial–fungal interactions: ecology, mechanisms and challenges. *FEMS Microbiol. Rev.* **42**, 335–352 (2018).
60. Eme, L., Spang, A., Lombard, J., Stairs, C. W. & Ettema, T. J. G. Archaea and the origin of eukaryotes. *Nat. Rev. Microbiol.* **15**, 711–723 (2017).
61. Evans, P. N. et al. An evolving view of methane metabolism in the Archaea. *Nat. Rev. Microbiol.* **17**, 219–232 (2019).
62. Hoegenauer, C., Hammer, H. F., Mahnert, A. & Moissl-Eichinger, C. Methanogenic Archaea in the human gastrointestinal tract. *Nat. Rev. Gastroenterol. Hepatol.* https://doi.org/10.1038/s41575-022-00673-z (2022).
63. Nakamura, N., Lin, H. C., McSweeney, C. S., Mackie, R. I. & Gaskins, H. R. Mechanisms of microbial hydrogen disposal in the human colon and implications for health and disease. *Annu. Rev. Food Sci. Technol.* **1**, 363–395 (2010).
64. Coker, O. O., Wu, W. K. K., Wong, S. H., Sung, J. J. Y. & Yu, J. Altered gut Archaea composition and interaction with bacteria are associated with colorectal cancer. *Gastroenterology* **159**, 1459–1470.e5 (2020).
65. Llorens-Rico, V., Simcock, J. A., Huys, G. R. B. & Raes, J. Single-cell approaches in human microbiome research. *Cell* **185**, 2725–2738 (2022).
66. Epstein, S. S. The phenomenon of microbial uncultivability. *Curr. Opin. Microbiol.* **16**, 636–642 (2013).
67. Parks, D. H. et al. Recovery of nearly 8,000 metagenome-assembled genomes substantially expands the tree of life. *Nat. Microbiol.* **2**, 1533–1542 (2017).
68. Almeida, A. et al. A unified catalog of 204,938 reference genomes from the human gut microbiome. *Nat. Biotechnol.* **39**, 105–114 (2021).
69. Saheb Kashaf, S. et al. Integrating cultivation and metagenomics for a multi-kingdom view of skin microbiome diversity and functions. *Nat. Microbiol.* **7**, 169–179 (2022).
70. Zhu, J. et al. Over 50,000 metagenomically assembled draft genomes for the human oral microbiome reveal new taxa. *Genomics Proteom. Bioinforma.* https://doi.org/10.1016/j.gpb.2021.05.001 (2021).
71. Villa, M. M. et al. Interindividual variation in dietary carbohydrate metabolism by gut bacteria revealed with droplet microfluidic culture. *mSystems* https://doi.org/10.1128/mSystems.00864-19 (2020).
72. Watterson, W. J. et al. Droplet-based high-throughput cultivation for accurate screening of antibiotic resistant gut microbes. *Elife* https://doi.org/10.7554/eLife.56998 (2020).
73. Lan, F., Demaree, B., Ahmed, N. & Abate, A. R. Single-cell genome sequencing at ultra-high-throughput with microfluidic droplet barcoding. *Nat. Biotechnol.* **35**, 640–646 (2017).
74. Ma, L. et al. Gene-targeted microfluidic cultivation validated by isolation of a gut bacterium listed in Human Microbiome Project's Most Wanted taxa. *Proc. Natl Acad. Sci. USA* **111**, 9768–9773 (2014).
75. Zheng, W. et al. High-throughput, single-microbe genomics with strain resolution, applied to a human gut microbiome. *Science* **376**, eabm1483 (2022).
76. Chijiiwa, R. et al. Single-cell genomics of uncultured bacteria reveals dietary fiber responders in the mouse gut microbiota. *Microbiome* **8**, 5 (2020).

77. Wong, S. H. et al. Gavage of fecal samples from patients with colorectal cancer promotes intestinal carcinogenesis in germ-free and conventional mice. *Gastroenterology* **153**, 1621–1633.e6 (2017).
78. Li, L. et al. Gut microbiota from colorectal cancer patients enhances the progression of intestinal adenoma in Apc$^{min/+}$ mice. *EBioMedicine* **48**, 301–315 (2019).
79. Rubinstein, M. R. et al. *Fusobacterium nucleatum* promotes colorectal carcinogenesis by modulating E-cadherin/β-catenin signaling via its FadA adhesin. *Cell Host Microbe* **14**, 195–206 (2013).
80. Rubinstein, M. R. et al. *Fusobacterium nucleatum* promotes colorectal cancer by inducing Wnt/β-catenin modulator Annexin A1. *EMBO Rep.* https://doi.org/10.15252/embr.201847638 (2019).
81. Guo, P. et al. FadA promotes DNA damage and progression of *Fusobacterium nucleatum*-induced colorectal cancer through up-regulation of chk2. *J. Exp. Clin. Cancer Res.* **39**, 202 (2020).
82. Abed, J. et al. Fap2 mediates *Fusobacterium nucleatum* colorectal adenocarcinoma enrichment by binding to tumor-expressed Gal-GalNAc. *Cell Host Microbe* **20**, 215–225 (2016).
83. Casasanta, M. A. et al. *Fusobacterium nucleatum* host-cell binding and invasion induces IL-8 and CXCL1 secretion that drives colorectal cancer cell migration. *Sci. Signal* https://doi.org/10.1126/scisignal.aba9157 (2020).
84. Gur, C. et al. Binding of the Fap2 protein of *Fusobacterium nucleatum* to human inhibitory receptor TIGIT protects tumors from immune cell attack. *Immunity* **42**, 344–355 (2015).
85. Nougayrede, J. P. et al. *Escherichia coli* induces DNA double-strand breaks in eukaryotic cells. *Science* **313**, 848–851 (2006).
86. Cuevas-Ramos, G. et al. *Escherichia coli* induces DNA damage in vivo and triggers genomic instability in mammalian cells. *Proc. Natl Acad. Sci. USA* **107**, 11537–11542 (2010).
87. Iftekhar, A. et al. Genomic aberrations after short-term exposure to colibactin-producing *E. coli* transform primary colon epithelial cells. *Nat. Commun.* **12**, 1003 (2021).
88. Cougnoux, A. et al. Bacterial genotoxin colibactin promotes colon tumour growth by inducing a senescence-associated secretory phenotype. *Gut* **63**, 1932–1942 (2014).
89. Lucas, C. et al. Autophagy of intestinal epithelial cells inhibits colorectal carcinogenesis induced by colibactin-producing *Escherichia coli* in Apc$^{Min/+}$ mice. *Gastroenterology* **158**, 1373–1388 (2020).
90. Lopes, A. et al. Colibactin-positive *Escherichia coli* induce a procarcinogenic immune environment leading to immunotherapy resistance in colorectal cancer. *Int. J. Cancer* **146**, 3147–3159 (2020).
91. Tsoi, H. et al. *Peptostreptococcus anaerobius* induces intracellular cholesterol biosynthesis in colon cells to induce proliferation and causes dysplasia in mice. *Gastroenterology* **152**, 1419–1433.e5 (2017).
92. Long, X. et al. *Peptostreptococcus anaerobius* promotes colorectal carcinogenesis and modulates tumour immunity. *Nat. Microbiol.* **4**, 2319–2330 (2019).
93. Chung, L. et al. *Bacteroides fragilis* toxin coordinates a pro-carcinogenic inflammatory cascade via targeting of colonic epithelial cells. *Cell Host Microbe* **23**, 203–214.e5 (2018).
94. Geis, A. L. et al. Regulatory T-cell response to enterotoxigenic *Bacteroides fragilis* colonization triggers IL17-dependent colon carcinogenesis. *Cancer Discov.* **5**, 1098–1109 (2015).
95. Cao, Y. et al. Enterotoxigenic *Bacteroides fragilis* promotes intestinal inflammation and malignancy by inhibiting exosome-packaged miR-149-3p. *Gastroenterology* https://doi.org/10.1053/j.gastro.2021.08.003 (2021).
96. Hwang, S. et al. Enterotoxigenic *Bacteroides fragilis* infection exacerbates tumorigenesis in AOM/DSS mouse model. *Int. J. Med. Sci.* **17**, 145–152 (2020).
97. Thiele Orberg, E. et al. The myeloid immune signature of enterotoxigenic *Bacteroides fragilis*-induced murine colon tumorigenesis. *Mucosal Immunol.* **10**, 421–433 (2017).
98. Yang, J. et al. High-fat diet promotes colorectal tumorigenesis through modulating gut microbiota and metabolites. *Gastroenterology* **162**, 135 (2022).
99. Bai, X. W. et al. Cigarette smoke promotes colorectal cancer through modulation of gut microbiota and related metabolites. *Gut* https://doi.org/10.1136/gutjnl-2021-325021 (2022).
100. Akyol, A. et al. Generating somatic mosaicism with a Cre recombinase-microsatellite sequence transgene. *Nat. Methods* **5**, 231–233 (2008).
101. Xue, Y., Johnson, R., Desmet, M., Snyder, P. W. & Fleet, J. C. Generation of a transgenic mouse for colorectal cancer research with intestinal Cre expression limited to the large intestine. *Mol. Cancer Res.* **8**, 1095–1104 (2010).
102. Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* **487**, 330–337 (2012).
103. Nesic, D., Hsu, Y. & Stebbins, C. E. Assembly and function of a bacterial genotoxin. *Nature* **429**, 429–433 (2004).
104. Johnson, W. M. & Lior, H. A new heat-labile cytolethal distending toxin (CLDT) produced by *Escherichia coli* isolates from clinical material. *Microb. Pathog.* **4**, 103–113 (1988).
105. He, Z. et al. *Campylobacter jejuni* promotes colorectal tumorigenesis through the action of cytolethal distending toxin. *Gut* **68**, 289–300 (2019).
106. Wilson, M. R. et al. The human gut bacterial genotoxin colibactin alkylates DNA. *Science* https://doi.org/10.1126/science.aar7785 (2019).
107. Xue, M. et al. Structure elucidation of colibactin and its DNA cross-links. *Science* https://doi.org/10.1126/science.aax2685 (2019).
108. Dziubanska-Kusibab, P. J. et al. Colibactin DNA-damage signature indicates mutational impact in colorectal cancer. *Nat. Med.* **26**, 1063–1069 (2020).
109. Pleguezuelos-Manzano, C. et al. Mutational signature in colorectal cancer caused by genotoxic *pks*$^{+}$ *E. coli*. *Nature* **580**, 269–273 (2020).
110. Dejea, C. M. et al. Patients with familial adenomatous polyposis harbor colonic biofilms containing tumorigenic bacteria. *Science* **359**, 592–597 (2018).
111. Cougnoux, A. et al. Small-molecule inhibitors prevent the genotoxic and protumoural effects induced by colibactin-producing bacteria. *Gut* **65**, 278–285 (2016).
112. Volpe, M. R. et al. A small molecule inhibitor prevents gut bacterial genotoxin production. *Nat. Chem. Biol.* https://doi.org/10.1038/s41589-022-01147-8 (2022).
113. Goodwin, A. C. et al. Polyamine catabolism contributes to enterotoxigenic *Bacteroides fragilis*-induced colon tumorigenesis. *Proc. Natl Acad. Sci. USA* **108**, 15354–15359 (2011).
114. Wang, X. & Huycke, M. M. Extracellular superoxide production by *Enterococcus faecalis* promotes chromosomal instability in mammalian cells. *Gastroenterology* **132**, 551–561 (2007).
115. Maddocks, O. D., Scanlon, K. M. & Donnenberg, M. S. An *Escherichia coli* effector protein promotes host mutation via depletion of DNA mismatch repair proteins. *mBio* **4**, e00152-13 (2013).
116. Ikegami, A., Chung, P. & Han, Y. P. W. Complementation of the fadA mutation in *Fusobacterium nucleatum* demonstrates that the surface-exposed adhesin promotes cellular invasion and placental colonization. *Infect. Immun.* **77**, 3075–3079 (2009).
117. Xu, M. H. et al. FadA from *Fusobacterium nucleatum* utilizes both secreted and nonsecreted forms for functional oligomerization for attachment and invasion of host cells. *J. Biol. Chem.* **282**, 25000–25009 (2007).
118. Mu, W. et al. Intracellular *Porphyromonas gingivalis* promotes the proliferation of colorectal cancer cells via the MAPK/ERK signaling pathway. *Front. Cell Infect. Microbiol.* **10**, 584798 (2020).
119. Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell* **144**, 646–674 (2011).
120. Lasry, A., Zinger, A. & Ben-Neriah, Y. Inflammatory networks underlying colorectal cancer. *Nat. Immunol.* **17**, 230–240 (2016).
121. Ruiz, P. A., Anna, S., Kim, S. C., Sartor, R. B. & Haller, D. IL-10 gene-deficient mice lack TGF-β/Smad signaling and fail to inhibit proinflammatory gene expression in intestinal epithelial cells after the colonization with colitogenic *Enterococcus faecalis*. *J. Immunol.* **174**, 2990–2999 (2005).
122. Zhao, L. et al. *Parvimonas micra* promotes colorectal tumorigenesis and is associated with prognosis of colorectal cancer patients. *Oncogene* **41**, 4200–4210 (2022).
123. DeStefano Shields, C. E. et al. Bacterial-driven inflammation and mutant BRAF expression combine to promote murine colon tumorigenesis that is sensitive to immune checkpoint therapy. *Cancer Discov.* **11**, 1792–1807 (2021).
124. Arthur, J. C. et al. Microbial genomic analysis reveals the essential role of inflammation in bacteria-induced colorectal cancer. *Nat. Commun.* **5**, 4724 (2014).
125. Zou, J. & Shankar, N. Roles of TLR/MyD88/MAPK/NF-κB signaling pathways in the regulation of phagocytosis and proinflammatory cytokine expression in response to *E. faecalis* infection. *PLoS ONE* **10**, e0136947 (2015).
126. Xia, Y. F., Shen, S. & Verma, I. M. NF-κB, an active player in human cancers. *Cancer Immunol. Res.* **2**, 823–830 (2014).
127. Couturier-Maillard, A. et al. NOD2-mediated dysbiosis predisposes mice to transmissible colitis and colorectal cancer. *J. Clin. Invest.* **123**, 700–711 (2013).
128. Udden, S. M. N. et al. NOD2 suppresses colorectal tumorigenesis via downregulation of the TLR pathways. *Cell Rep.* **19**, 2756–2770 (2017).
129. Chen, G. Y., Shaw, M. H., Redondo, G. & Nunez, G. The innate immune receptor Nod1 protects the intestine from inflammation-induced tumorigenesis. *Cancer Res.* **68**, 10060–10067 (2008).
130. Martin, T. D. et al. The adaptive immune system is a major driver of selection for tumor suppressor gene inactivation. *Science* **373**, 1327 (2021).
131. Veglia, F., Sanseviero, E. & Gabrilovich, D. I. Myeloid-derived suppressor cells in the era of increasing myeloid cell diversity. *Nat. Rev. Immunol.* **21**, 485–498 (2021).
132. Bronte, V. et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. *Nat. Commun.* **7**, 12150 (2016).
133. Zhang, Q. et al. Gut microbiome directs hepatocytes to recruit MDSCs and promote cholangiocarcinoma. *Cancer Discov.* **11**, 1248–1267 (2021).
134. Schneider, K. M. et al. Imbalanced gut microbiota fuels hepatocellular carcinoma development by shaping the hepatic inflammatory microenvironment. *Nat. Commun.* **13**, 3964 (2022).
135. Peuker, K. et al. Microbiota-dependent activation of the myeloid calcineurin-NFAT pathway inhibits B7H3- and B7H4-dependent anti-tumor immunity in colorectal cancer. *Immunity* **55**, 701–717.e7 (2022).
136. Wang, T. T. et al. The adaptor protein CARD9 protects against colon cancer by restricting mycobiota-mediated expansion of myeloid-derived suppressor cells. *Immunity* **49**, 504–514.e4 (2018).
137. Xu, C. C. et al. *Fusobacterium nucleatum* promotes colorectal cancer metastasis through miR-1322/CCL20 axis and M2 polarization. *Gut Microbes* https://doi.org/10.1080/19490976.2021.1980347 (2021).
138. Chiang, M. K. et al. Two ST11 *Klebsiella pneumoniae* strains exacerbate colorectal tumorigenesis in a colitis-associated mouse model. *Gut Microbes* **13**, 1980348 (2021).
139. Jones, B. V., Begley, M., Hill, C., Gahan, C. G. & Marchesi, J. R. Functional and comparative metagenomic analysis of bile salt hydrolase activity in the human gut microbiome. *Proc. Natl Acad. Sci. USA* **105**, 13580–13585 (2008).

140. Ridlon, J. M. & Hylemon, P. B. Identification and characterization of two bile acid coenzyme A transferases from *Clostridium scindens*, a bile acid 7α-dehydroxylating intestinal bacterium. *J. Lipid Res.* **53**, 66–76 (2012).
141. Ou, J. H. et al. Diet, microbiota, and microbial metabolites in colon cancer risk in rural Africans and African Americans. *Am. J. Clin. Nutr.* **98**, 111–120 (2013).
142. Payne, C. M., Bernstein, C., Dvorak, K. & Bernstein, H. Hydrophobic bile acids, genomic instability, Darwinian selection, and colon carcinogenesis. *Clin. Exp. Gastroenterol.* **1**, 19–47 (2008).
143. Booth, L. A., Gilmore, I. T. & Bilton, R. F. Secondary bile acid induced DNA damage in HT29 cells: are free radicals involved? *Free Radic. Res.* **26**, 135–144 (1997).
144. Turner, D. J. et al. Bile salts induce resistance to apoptosis through NF-κB-mediated XIAP expression. *Ann. Surg.* **245**, 415–425 (2007).
145. Bernstein, C. et al. Carcinogenicity of deoxycholate, a secondary bile acid. *Arch. Toxicol.* **85**, 863–871 (2011).
146. Cao, H. et al. Secondary bile acid-induced dysbiosis promotes intestinal carcinogenesis. *Int. J. Cancer* **140**, 2545–2556 (2017).
147. Islam, K. B. M. S. et al. Bile acid is a host factor that regulates the composition of the cecal microbiota in rats. *Gastroenterology* **141**, 1773–1781 (2011).
148. Lee, J. Y. et al. Contribution of the 7β-hydroxysteroid dehydrogenase from *Ruminococcus gnavus* N53 to ursodeoxycholic acid formation in the human colon. *J. Lipid Res.* **54**, 3062–3069 (2013).
149. Wali, R. K. et al. Ursodeoxycholic acid and F_6-D_3 inhibit aberrant crypt proliferation in the rat azoxymethane model of colon cancer: roles of cyclin D1 and E-cadherin. *Cancer Epidemiol. Biomark. Prev.* **11**, 1653–1662 (2002).
150. Peiro-Jordan, R. et al. The chemopreventive agent ursodeoxycholic acid inhibits proliferation of colon carcinoma cells by suppressing c-Myc expression. *Eur. J. Cancer Prev.* **21**, 413–422 (2012).
151. Kim, E. K., Cho, J. H., Kim, E. & Kim, Y. J. Ursodeoxycholic acid inhibits the proliferation of colon cancer cells by regulating oxidative stress and cancer stem-like cell growth. *PLoS ONE* https://doi.org/10.1371/journal.pone.0181183 (2017).
152. Marquet, P., Duncan, S. H., Chassard, C., Bernalier-Donadille, A. & Flint, H. J. Lactate has the potential to promote hydrogen sulphide formation in the human colon. *FEMS Microbiol. Lett.* **299**, 128–134 (2009).
153. Braccia, D. J., Jiang, X. F., Pop, M. & Hall, A. B. The capacity to produce hydrogen sulfide (H_2S) via cysteine degradation is ubiquitous in the human gut microbiome. *Front. Microbiol.* https://doi.org/10.3389/fmicb.2021.705583 (2021).
154. Laue, H., Denger, K. & Cook, A. M. Taurine reduction in anaerobic respiration of *Bilophila wadsworthia* RZATAU. *Appl. Environ. Microbiol.* **63**, 2016–2021 (1997).
155. David, L. A. et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* **505**, 559–563 (2014).
156. Nguyen, L. H. et al. Association between sulfur-metabolizing bacterial communities in stool and risk of distal colorectal cancer in men. *Gastroenterology* **158**, 1313–1325 (2020).
157. Nguyen, L. H. et al. The sulfur microbial diet is associated with increased risk of early-onset colorectal cancer precursors. *Gastroenterology* **161**, 1423–1432.e4 (2021).
158. Attene-Ramos, M. S. et al. DNA damage and toxicogenomic analyses of hydrogen sulfide in human intestinal epithelial FHs 74 Int cells. *Environ. Mol. Mutagen.* **51**, 304–314 (2010).
159. Attene-Ramos, M. S., Wagner, E. D., Gaskins, H. R. & Plewa, M. J. Hydrogen sulfide induces direct radical-associated DNA damage. *Mol. Cancer Res.* **5**, 455–459 (2007).
160. Cai, W. J., Wang, M. J., Ju, L. H., Wang, C. & Zhu, Y. C. Hydrogen sulfide induces human colon cancer cell proliferation: role of Akt, ERK and p21. *Cell Biol. Int.* **34**, 565–572 (2010).
161. Chen, F. et al. Integrated analysis of the faecal metagenome and serum metabolome reveals the role of gut microbiome-associated metabolites in the detection of colorectal cancer and adenoma. *Gut* **71**, 1315–1325 (2022).
162. Kong, C. et al. Integrated metagenomic and metabolomic analysis reveals distinct gut-microbiome-derived phenotypes in early-onset colorectal cancer. *Gut* https://doi.org/10.1136/gutjnl-2022-327156 (2022).
163. Gao, R. et al. Integrated analysis of colorectal cancer reveals cross-cohort gut microbial signatures and associated serum metabolites. *Gastroenterology* **163**, 1024–1037.e9 (2022).
164. Coker, O. O. et al. Altered gut metabolites and microbiota interactions are implicated in colorectal carcinogenesis and can be non-invasive diagnostic biomarkers. *Microbiome* https://doi.org/10.1186/s40168-021-01208-5 (2022).
165. Rothschild, D. et al. Environment dominates over host genetics in shaping human gut microbiota. *Nature* **555**, 210–215 (2018).
166. Purcell, R. V., Visnovska, M., Biggs, P. J., Schmeier, S. & Frizelle, F. A. Distinct gut microbiome patterns associate with consensus molecular subtypes of colorectal cancer. *Sci. Rep.* **7**, 11590 (2017).
167. Gao, R. et al. Mucosa-associated microbiota signature in colorectal cancer. *Eur. J. Clin. Microbiol. Infect. Dis.* **36**, 2073–2083 (2017).
168. Yuan, D. et al. A comprehensive analysis of the microbiota composition and host driver gene mutations in colorectal cancer. *Invest. N. Drugs* **40**, 884–894 (2022).
169. Kadosh, E. et al. The gut microbiome switches mutant p53 from tumour-suppressive to oncogenic. *Nature* **586**, 133–138 (2020).
170. Li, C. et al. Squalene epoxidase drives cancer cell proliferation and promotes gut dysbiosis to accelerate colorectal carcinogenesis. *Gut* **71**, 2253–2265 (2022).
171. Sobhani, I. et al. Colorectal cancer-associated microbiota contributes to oncogenic epigenetic signatures. *Proc. Natl Acad. Sci. USA* **116**, 24285–24295 (2019).
172. Maiuri, A. R. et al. Mismatch repair proteins initiate epigenetic alterations during inflammation-driven tumorigenesis. *Cancer Res.* **77**, 3467–3478 (2017).
173. Park, H. E., Kim, J. H., Cho, N. Y., Lee, H. S. & Kang, G. H. Intratumoral *Fusobacterium nucleatum* abundance correlates with macrophage infiltration and CDKN2A methylation in microsatellite-unstable colorectal carcinoma. *Virchows Arch.* **471**, 329–336 (2017).
174. Xia, X. X. et al. Bacteria pathogens drive host colonic epithelial cell promoter hypermethylation of tumor suppressor genes in colorectal cancer. *Microbiome* https://doi.org/10.1186/s40168-020-00847-4 (2020).
175. Yuan, C., Burns, M. B., Subramanian, S. & Blekhman, R. Interaction between host microRNAs and the gut microbiota in colorectal cancer. *Msystems* **3**, e00205-17 (2018).
176. Yang, Y. Z. et al. *Fusobacterium nucleatum* increases proliferation of colorectal cancer cells and tumor development in mice by activating toll-like receptor 4 signaling to nuclear factor-κB, and up-regulating expression of microRNA-21. *Gastroenterology* **152**, 851–866.e24 (2017).
177. Hong, J. et al. *F. nucleatum* targets lncRNA ENO1-IT1 to promote glycolysis and oncogenesis in colorectal cancer. *Gut* **70**, 2123–2137 (2021).
178. Liu, S. et al. The host shapes the gut microbiota via fecal microRNA. *Cell Host Microbe* **19**, 32–43 (2016).
179. Tomkovich, S. et al. Human colon mucosal biofilms and murine host communicate via altered mRNA and microRNA expression during cancer. *Msystems* https://doi.org/10.1128/mSystems.00451-19 (2020).
180. Chen, S. et al. *Fusobacterium nucleatum* reduces METTL3-mediated m^6A modification and contributes to colorectal cancer metastasis. *Nat. Commun.* **13**, 1248 (2022).
181. Hill, C. et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat. Rev. Gastroenterol. Hepatol.* **11**, 506–514 (2014).
182. Goldin, B. R. & Gorbach, S. L. Effect of *Lactobacillus acidophilus* dietary supplements on 1,2-dimethylhydrazine dihydrochloride-induced intestinal cancer in rats. *J. Natl Cancer Inst.* **64**, 263–265 (1980).
183. Rao, C. V., Sanders, M. E., Indranie, C., Simi, B. & Reddy, B. S. Prevention of colonic preneoplastic lesions by the probiotic *Lactobacillus acidophilus* NCFMTM in F344 rats. *Int. J. Oncol.* **14**, 939–944 (1999).
184. Goldin, B. R., Gualtieri, L. J. & Moore, R. P. The effect of *Lactobacillus* GG on the initiation and promotion of DMH-induced intestinal tumors in the rat. *Nutr. Cancer* **25**, 197–204 (1996).
185. Koo, M. & Rao, A. V. Long-term effect of Bifidobacteria and Neosugar on precursor lesions of colonic cancer in CF1 mice. *Nutr. Cancer* **16**, 249–257 (1991).
186. Singh, J. et al. *Bifidobacterium longum*, a lactic acid-producing intestinal bacterium inhibits colon cancer and modulates the intermediate biomarkers of colon carcinogenesis. *Carcinogenesis* **18**, 833–841 (1997).
187. Rowland, I. R., Rumney, C. J., Coutts, J. T. & Lievense, L. C. Effect of *Bifidobacterium longum* and inulin on gut bacterial metabolism and carcinogen-induced aberrant crypt foci in rats. *Carcinogenesis* **19**, 281–285 (1998).
188. Ishikawa, H. et al. Randomized trial of dietary fiber and *Lactobacillus casei* administration for prevention of colorectal tumors. *Int. J. Cancer* **116**, 762–767 (2005).
189. Rafter, J. et al. Dietary synbiotics reduce cancer risk factors in polypectomized and colon cancer patients. *Am. J. Clin. Nutr.* **85**, 488–496 (2007).
190. O'Toole, P. W., Marchesi, J. R. & Hill, C. Next-generation probiotics: the spectrum from probiotics to live biotherapeutics. *Nat. Microbiol.* **2**, 17057 (2017).
191. Wan, Y. et al. Fermentation supernatants of *Lactobacillus delbrueckii* inhibit growth of human colon cancer cells and induce apoptosis through a caspase 3-dependent pathway. *Oncol. Lett.* **7**, 1738–1742 (2014).
192. Wang, S. M. et al. Induction of HT-29 cells apoptosis by lactobacilli isolated from fermented products. *Res. Microbiol.* **165**, 202–214 (2014).
193. Nami, Y. et al. Assessment of probiotic potential and anticancer activity of newly isolated vaginal bacterium *Lactobacillus plantarum* 5BL. *Microbiol. Immunol.* **58**, 492–502 (2014).
194. Tiptiri-Kourpeti, A. et al. *Lactobacillus casei* exerts anti-proliferative effects accompanied by apoptotic cell death and up-regulation of TRAIL in colon carcinoma cells. *PLoS ONE* **11**, e0147960 (2016).
195. Orlando, A. et al. Antiproliferative and proapoptotic effects of viable or heat-killed *Lactobacillus paracasei* IMPC2.1 and *Lactobacillus rhamnosus* GG in HGC-27 gastric and DLD-1 colon cell lines. *Nutr. Cancer* **64**, 1103–1111 (2012).
196. Karimi Ardestani, S., Tafvizi, F. & Tajabadi Ebrahimi, M. Heat-killed probiotic bacteria induce apoptosis of HT-29 human colon adenocarcinoma cell line via the regulation of Bax/Bcl2 and caspases pathway. *Hum. Exp. Toxicol.* **38**, 1069–1081 (2019).
197. Chung, I. C. et al. Pretreatment with a heat-killed probiotic modulates the NLRP3 inflammasome and attenuates colitis-associated colorectal cancer in mice. *Nutrients* https://doi.org/10.3390/nu11030516 (2019).
198. Vital, M., Karch, A. & Pieper, D. H. Colonic butyrate-producing communities in humans: an overview using omics data. *mSystems* https://doi.org/10.1128/mSystems.00130-17 (2017).
199. Donohoe, D. R. et al. A gnotobiotic mouse model demonstrates that dietary fiber protects against colorectal tumorigenesis in a microbiota- and butyrate-dependent manner. *Cancer Discov.* **4**, 1387–1397 (2014).
200. Archer, S. Y., Meng, S., Shei, A. & Hodin, R. A. p21(WAF1) is required for butyrate-mediated growth inhibition of human colon cancer cells. *Proc. Natl Acad. Sci. USA* **95**, 6791–6796 (1998).
201. Kim, M. H., Kang, S. G., Park, J. H., Yanagisawa, M. & Kim, C. H. Short-chain fatty acids activate GPR41 and GPR43 on intestinal epithelial cells to promote inflammatory responses in mice. *Gastroenterology* **145**, 396–406.e10 (2013).

202. Zhao, Y. et al. GPR43 mediates microbiota metabolite SCFA regulation of antimicrobial peptide expression in intestinal epithelial cells via activation of mTOR and STAT3. *Mucosal Immunol.* **11**, 752–762 (2018).
203. Singh, N. et al. Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. *Immunity* **40**, 128–139 (2014).
204. Tang, Y., Chen, Y., Jiang, H., Robbins, G. T. & Nie, D. G-protein-coupled receptor for short-chain fatty acids suppresses colon cancer. *Int. J. Cancer* **128**, 847–856 (2011).
205. Chen, D. et al. *Clostridium butyricum*, a butyrate-producing probiotic, inhibits intestinal tumor development through modulating Wnt signaling and gut microbiota. *Cancer Lett.* **469**, 456–467 (2020).
206. Sivaprakasam, S. et al. An essential role of Ffar2 (Gpr43) in dietary fibre-mediated promotion of healthy composition of gut microbiota and suppression of intestinal carcinogenesis. *Oncogenesis* **5**, e238 (2016).
207. Konishi, H. et al. Probiotic-derived ferrichrome inhibits colon cancer progression via JNK-mediated apoptosis. *Nat. Commun.* **7**, 12365 (2016).
208. Sugimura, N. et al. *Lactobacillus gallinarum* modulates the gut microbiota and produces anti-cancer metabolites to protect against colorectal tumourigenesis. *Gut* https://doi.org/10.1136/gutjnl-2020-323951 (2021).
209. Bell, H. N. et al. Reuterin in the healthy gut microbiome suppresses colorectal cancer growth through altering redox balance. *Cancer Cell* **40**, 185–200.e6 (2022).
210. Sakatani, A. et al. Polyphosphate derived from *Lactobacillus brevis* inhibits colon cancer progression through induction of cell apoptosis. *Anticancer. Res.* **36**, 591–598 (2016).
211. Kashima, S. et al. Polyphosphate, an active molecule derived from probiotic *Lactobacillus brevis*, improves the fibrosis in murine colitis. *Transl. Res.* **166**, 163–175 (2015).
212. Ahmadi, S., Ghollasi, M. & Hosseini, H. M. The apoptotic impact of nisin as a potent bacteriocin on the colon cancer cells. *Microb. Pathog.* **111**, 193–197 (2017).
213. Cao, K. Y. et al. Antitumor activities of tRNA-derived fragments and tRNA halves from non-pathogenic *Escherichia coli* strains on colorectal cancer and their structure–activity relationship. *mSystems* **7**, e0016422 (2022).
214. Li, Q. et al. *Streptococcus thermophilus* inhibits colorectal tumorigenesis through secreting β-galactosidase. *Gastroenterology* **160**, 1179–1193.e14 (2021).
215. Di, W. et al. Exopolysaccharides produced by *Lactobacillus* strains suppress HT-29 cell growth via induction of G0/G1 cell cycle arrest and apoptosis. *Oncol. Lett.* **16**, 3577–3586 (2018).
216. Kim, Y., Oh, S., Yun, H. S., Oh, S. & Kim, S. H. Cell-bound exopolysaccharide from probiotic bacteria induces autophagic cell death of tumour cells. *Lett. Appl. Microbiol.* **51**, 123–130 (2010).
217. El-Deeb, N. M., Yassin, A. M., Al-Madboly, L. A. & El-Hawiet, A. A novel purified *Lactobacillus acidophilus* 20079 exopolysaccharide, LA-EPS-20079, molecularly regulates both apoptotic and NF-κB inflammatory pathways in human colon cancer. *Microb. Cell Fact.* **17**, 29 (2018).
218. Di, W. et al. Physicochemical characterization and antitumour activity of exopolysaccharides produced by *Lactobacillus casei* SB27 from yak milk. *Carbohydr. Polym.* **171**, 307–315 (2017).
219. Li, F. et al. Antitumor mechanisms of an exopolysaccharide from *Lactobacillus fermentum* on HT-29 cells and HT-29 tumor-bearing mice. *Int. J. Biol. Macromol.* **209**, 552–562 (2022).
220. Li, W. et al. Characterization of a novel polysaccharide with anti-colon cancer activity from *Lactobacillus helveticus* MB2-1. *Carbohydr. Res.* **411**, 6–14 (2015).
221. Xiao, L. et al. Anticancer potential of an exopolysaccharide from *Lactobacillus helveticus* MB2-1 on human colon cancer HT-29 cells via apoptosis induction. *Food Funct.* **11**, 10170–10181 (2020).
222. Song, W. et al. Oxidative stress and endoplasmic reticulum stress contribute to *L. paracasei* subsp. *paracasei* M5L exopolysaccharide-induced apoptosis in HT-29 cells. *Food Sci. Nutr.* **9**, 1676–1687 (2021).
223. Sun, M. et al. The effects of *Lactobacillus plantarum*-12 crude exopolysaccharides on the cell proliferation and apoptosis of human colon cancer (HT-29) cells. *Probiotics Antimicrob. Proteins* **13**, 413–421 (2021).
224. Tukenmez, U., Aktas, B., Aslim, B. & Yavuz, S. The relationship between the structural characteristics of lactobacilli-EPS and its ability to induce apoptosis in colon cancer cells in vitro. *Sci. Rep.* **9**, 8268 (2019).
225. Deepak, V. et al. Exopolysaccharides from *Lactobacillus acidophilus* modulates the antioxidant status of 1,2-dimethyl hydrazine-induced colon cancer rat model. *3 Biotech* **11**, 225 (2021).
226. Tian, P. J. et al. Extraction of peptidoglycan from *L. paracasei* subp. *paracasei* X12 and its preliminary mechanisms of inducing immunogenic cell death in HT-29 cells. *Int. J. Mol. Sci.* **16**, 20033–20049 (2015).
227. Schmitt, M. & Greten, F. R. The inflammatory pathogenesis of colorectal cancer. *Nat. Rev. Immunol.* **21**, 653–667 (2021).
228. Gamallat, Y. et al. *Lactobacillus rhamnosus* induced epithelial cell apoptosis, ameliorates inflammation and prevents colon cancer development in an animal model. *Biomed. Pharmacother.* **83**, 536–541 (2016).
229. Kuugbee, E. D. et al. Structural change in microbiota by a probiotic cocktail enhances the gut barrier and reduces cancer via TLR2 signaling in a rat model of colon cancer. *Dig. Dis. Sci.* **61**, 2908–2920 (2016).
230. Do, E. J. et al. Suppression of colitis-associated carcinogenesis through modulation of IL-6/STAT3 pathway by balsalazide and VSL#3. *J. Gastroenterol. Hepatol.* **31**, 1453–1461 (2016).
231. Chung, E. J. et al. Combination of metformin and VSL#3 additively suppresses western-style diet induced colon cancer in mice. *Eur. J. Pharmacol.* **794**, 1–7 (2017).
232. Del Carmen, S., de Moreno de LeBlanc, A. & LeBlanc, J. G. Development of a potential probiotic yoghurt using selected anti-inflammatory lactic acid bacteria for prevention of colitis and carcinogenesis in mice. *J. Appl. Microbiol.* **121**, 821–830 (2016).
233. Jacouton, E., Chain, F., Sokol, H., Langella, P. & Bermudez-Humaran, L. G. Probiotic strain *Lactobacillus casei* BL23 prevents colitis-associated colorectal cancer. *Front. Immunol.* **8**, 1553 (2017).
234. Del Carmen, S. et al. Anti-cancer effect of lactic acid bacteria expressing antioxidant enzymes or IL-10 in a colorectal cancer mouse model. *Int. Immunopharmacol.* **42**, 122–129 (2017).
235. Mohamadzadeh, M. et al. Regulation of induced colonic inflammation by *Lactobacillus acidophilus* deficient in lipoteichoic acid. *Proc. Natl Acad. Sci. USA* **108**, 4623–4630 (2011).
236. Khazaie, K. et al. Abating colon cancer polyposis by *Lactobacillus acidophilus* deficient in lipoteichoic acid. *Proc. Natl Acad. Sci. USA* **109**, 10462–10467 (2012).
237. Ni, Y. et al. A metagenomic study of the preventive effect of *Lactobacillus rhamnosus* GG on intestinal polyp formation in $Apc^{Min/+}$ mice. *J. Appl. Microbiol.* **122**, 770–784 (2017).
238. Wang, T. et al. *Lacticaseibacillus rhamnosus* LS8 ameliorates azoxymethane/dextran sulfate sodium-induced colitis-associated tumorigenesis in mice via regulating gut microbiota and inhibiting inflammation. *Probiotics Antimicrob. Proteins* **14**, 947–959 (2022).
239. Liu, X. et al. Early life *Lactobacillus rhamnosus* GG colonisation inhibits intestinal tumour formation. *Br. J. Cancer* **126**, 1421–1431 (2022).
240. Hibberd, A. A. et al. Intestinal microbiota is altered in patients with colon cancer and modified by probiotic intervention. *BMJ Open. Gastroenterol.* **4**, e000145 (2017).
241. Sorbara, M. T. & Pamer, E. G. Interbacterial mechanisms of colonization resistance and the strategies pathogens use to overcome them. *Mucosal Immunol.* **12**, 1–9 (2019).
242. Byndloss, M. X. et al. Microbiota-activated PPAR-γ signaling inhibits dysbiotic Enterobacteriaceae expansion. *Science* **357**, 570–575 (2017).
243. Lawrence, G. W. et al. Effect of a bacteriocin-producing *Streptococcus salivarius* on the pathogen *Fusobacterium nucleatum* in a model of the human distal colon. *Gut Microbes* **14**, 2100203 (2022).
244. Lee, J. W. et al. Immunomodulatory and antitumor effects in vivo by the cytoplasmic fraction of *Lactobacillus casei* and *Bifidobacterium longum*. *J. Vet. Sci.* **5**, 41–48 (2004).
245. Owens, J. A. et al. *Lactobacillus rhamnosus* GG orchestrates an antitumor immune response. *Cell Mol. Gastroenterol. Hepatol.* **12**, 1311–1327 (2021).
246. Montalban-Arques, A. et al. Commensal *Clostridiales* strains mediate effective anti-cancer immune response against solid tumors. *Cell Host Microbe* **29**, 1573–1588.e7 (2021).
247. Wang, L. et al. A purified membrane protein from *Akkermansia muciniphila* or the pasteurised bacterium blunts colitis associated tumourigenesis by modulation of $CD8^+$ T cells in mice. *Gut* **69**, 1988–1997 (2020).
248. Fan, L. et al. *A. muciniphila* suppresses colorectal tumorigenesis by inducing TLR2/NLRP3-mediated M1-like TAMs. *Cancer Immunol. Res.* **9**, 1111–1124 (2021).
249. Katona, B. W. & Weiss, J. M. Chemoprevention of colorectal cancer. *Gastroenterology* **158**, 368–388 (2020).
250. Prizment, A. E. et al. Randomised clinical study: oral aspirin 325 mg daily vs placebo alters gut microbial composition and bacterial taxa associated with colorectal cancer risk. *Aliment. Pharmacol. Ther.* **52**, 976–987 (2020).
251. Brennan, C. A. et al. Aspirin modulation of the colorectal cancer-associated microbe *Fusobacterium nucleatum*. *mBio* https://doi.org/10.1128/mBio.00547-21 (2021).
252. Zhao, R. et al. Aspirin reduces colorectal tumor development in mice and gut microbes reduce its bioavailability and chemopreventive effects. *Gastroenterology* **159**, 969–983.e4 (2020).
253. Garcia-Gonzalez, A. P. et al. Bacterial metabolism affects the *C. elegans* response to cancer chemotherapeutics. *Cell* **169**, 431–441.e8 (2017).
254. Hou, X. Y. et al. *Prevotella* contributes to individual response of FOLFOX in colon cancer. *Clin. Transl. Med.* **11**, e512 (2021).
255. Zhang, S. et al. *Fusobacterium nucleatum* promotes chemoresistance to 5-fluorouracil by upregulation of BIRC3 expression in colorectal cancer. *J. Exp. Clin. Cancer Res.* **38**, 14 (2019).
256. Yu, T. et al. *Fusobacterium nucleatum* promotes chemoresistance to colorectal cancer by modulating autophagy. *Cell* **170**, 548–563.e16 (2017).
257. Scott, T. A. et al. Host–microbe co-metabolism dictates cancer drug efficacy in *C. elegans*. *Cell* **169**, 442–456.e18 (2017).
258. Zheng, D. W. et al. Phage-guided modulation of the gut microbiota of mouse models of colorectal cancer augments their responses to chemotherapy. *Nat. Biomed. Eng.* **3**, 717–728 (2019).
259. He, Y. et al. Gut microbial metabolites facilitate anticancer therapy efficacy by modulating cytotoxic $CD8^+$ T cell immunity. *Cell Metab.* **33**, 988–1000.e7 (2021).
260. Yoon, Y., Kim, G., Jeon, B. N., Fang, S. & Park, H. *Bifidobacterium* strain-specific enhances the efficacy of cancer therapeutics in tumor-bearing mice. *Cancers* https://doi.org/10.3390/cancers13050957 (2021).

261. Roberti, M. P. et al. Chemotherapy-induced ileal crypt apoptosis and the ileal microbiome shape immunosurveillance and prognosis of proximal colon cancer. *Nat. Med.* **26**, 919–931 (2020).
262. Iida, N. et al. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. *Science* **342**, 967–970 (2013).
263. Geller, L. T. et al. Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. *Science* **357**, 1156–1160 (2017).
264. Viaud, S. et al. The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. *Science* **342**, 971–976 (2013).
265. Nejman, D. et al. The human tumor microbiome is composed of tumor type-specific intracellular bacteria. *Science* **368**, 973–980 (2020).
266. LaCourse, K. D. et al. The cancer chemotherapeutic 5-fluorouracil is a potent *Fusobacterium nucleatum* inhibitor and its activity is modified by intratumoral microbiota. *Cell Rep.* **41**, 111625 (2022).
267. Castellarin, M. et al. *Fusobacterium nucleatum* infection is prevalent in human colorectal carcinoma. *Genome Res.* **22**, 299–306 (2012).
268. Ramos, A. & Hemann, M. T. Drugs, bugs, and cancer: *Fusobacterium nucleatum* promotes chemoresistance in colorectal cancer. *Cell* **170**, 411–413 (2017).
269. Chu, Z. L. et al. Suppression of tumor necrosis factor-induced cell death by inhibitor of apoptosis c-IAP2 is under NF-κB control. *Proc. Natl Acad. Sci. USA* **94**, 10057–10062 (1997).
270. Wang, C. Y., Mayo, M. W., Korneluk, R. G., Goeddel, D. V. & Baldwin, A. S. Jr NF-κB antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. *Science* **281**, 1680–1683 (1998).
271. Pang, R. et al. A subpopulation of $CD26^+$ cancer stem cells with metastatic capacity in human colorectal cancer. *Cell Stem Cell* **6**, 603–615 (2010).
272. Liu, H. et al. *Fusobacterium nucleatum* promotes colorectal cancer cell to acquire stem cell-like features by manipulating lipid droplet-mediated numb degradation. *Adv. Sci.* **9**, e2105222 (2022).
273. Ternes, D. et al. The gut microbial metabolite formate exacerbates colorectal cancer progression. *Nat. Metab.* **4**, 458–475 (2022).
274. Bracci, L., Schiavoni, G., Sistigu, A. & Belardelli, F. Immune-based mechanisms of cytotoxic chemotherapy: implications for the design of novel and rationale-based combined treatments against cancer. *Cell Death Differ.* **21**, 15–25 (2014).
275. Nagata, S. & Tanaka, M. Programmed cell death and the immune system. *Nat. Rev. Immunol.* **17**, 333–340 (2017).
276. Wang, Y. J., Fletcher, R., Yu, J. & Zhang, L. Immunogenic effects of chemotherapy-induced tumor cell death. *Genes. Dis.* **5**, 194–203 (2018).
277. Wang, Z., Till, B. & Gao, Q. Chemotherapeutic agent-mediated elimination of myeloid-derived suppressor cells. *Oncoimmunology* **6**, e1331807 (2017).
278. Apetoh, L. et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nat. Med.* **13**, 1050–1059 (2007).
279. Ghiringhelli, F. et al. Activation of the NLRP3 inflammasome in dendritic cells induces IL-1β-dependent adaptive immunity against tumors. *Nat. Med.* **15**, 1170–1178 (2009).
280. Barbet, G. et al. Sensing microbial viability through bacterial RNA augments T follicular helper cell and antibody responses. *Immunity* **48**, 584–598.e5 (2018).
281. Marino, E. et al. Gut microbial metabolites limit the frequency of autoimmune T cells and protect against type 1 diabetes. *Nat. Immunol.* **18**, 552–562 (2017).
282. Trompette, A. et al. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat. Med.* **20**, 159–166 (2014).
283. Luu, M. et al. Regulation of the effector function of $CD8^+$ T cells by gut microbiota-derived metabolite butyrate. *Sci. Rep.* **8**, 14430 (2018).
284. Furusawa, Y. et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* **504**, 446–450 (2013).
285. An, J. & Ha, E. M. Combination therapy of *Lactobacillus plantarum* supernatant and 5-fluouracil increases chemosensitivity in colorectal cancer cells. *J. Microbiol. Biotechnol.* **26**, 1490–1503 (2016).
286. An, J. & Ha, E. M. Lactobacillus-derived metabolites enhance the antitumor activity of 5-FU and inhibit metastatic behavior in 5-FU-resistant colorectal cancer cells by regulating claudin-1 expression. *J. Microbiol.* **58**, 967–977 (2020).
287. Encarnacao, J. C. et al. Butyrate, a dietary fiber derivative that improves irinotecan effect in colon cancer cells. *J. Nutr. Biochem.* **56**, 183–192 (2018).
288. An, J., Seok, H. & Ha, E. M. GABA-producing *Lactobacillus plantarum* inhibits metastatic properties and induces apoptosis of 5-FU-resistant colorectal cancer cells via GABAB receptor signaling. *J. Microbiol.* **59**, 202–216 (2021).
289. An, J. & Ha, E. M. Extracellular vesicles derived from *Lactobacillus plantarum* restore chemosensitivity through the PDK2-mediated glucose metabolic pathway in 5-FU-resistant colorectal cancer cells. *J. Microbiol.* **60**, 735–745 (2022).
290. Dong, X. et al. Bioinorganic hybrid bacteriophage for modulation of intestinal microbiota to remodel tumor-immune microenvironment against colorectal cancer. *Sci. Adv.* **6**, eaba1590 (2020).
291. Keefe, D. M. Intestinal mucositis: mechanisms and management. *Curr. Opin. Oncol.* **19**, 323–327 (2007).
292. van Vliet, M. J., Harmsen, H. J., de Bont, E. S. & Tissing, W. J. The role of intestinal microbiota in the development and severity of chemotherapy-induced mucositis. *PLoS Pathog.* **6**, e1000879 (2010).
293. Stein, A., Voigt, W. & Jordan, K. Chemotherapy-induced diarrhea: pathophysiology, frequency and guideline-based management. *Ther. Adv. Med. Oncol.* **2**, 51–63 (2010).
294. Haaz, M. C., Rivory, L., Jantet, S., Ratanasavanh, D. & Robert, J. Glucuronidation of SN-38, the active metabolite of irinotecan, by human hepatic microsomes. *Pharmacol. Toxicol.* **80**, 91–96 (1997).
295. Yamamoto, M. et al. Metabolism of irinotecan and its active metabolite SN-38 by intestinal microflora in rats. *Oncol. Rep.* **20**, 727–730 (2008).
296. Cheng, K. W. et al. Pharmacological inhibition of bacterial β-glucuronidase prevents irinotecan-induced diarrhea without impairing its antitumor efficacy in vivo. *Pharmacol. Res.* **139**, 41–49 (2019).
297. Takasuna, K. et al. Involvement of β-glucuronidase in intestinal microflora in the intestinal toxicity of the antitumor camptothecin derivative irinotecan hydrochloride (CPT-11) in rats. *Cancer Res.* **56**, 3752–3757 (1996).
298. Pedroso, S. et al. Evaluation of mucositis induced by irinotecan after microbial colonization in germ-free mice. *Microbiology* **161**, 1950–1960 (2015).
299. Brandi, G. et al. Intestinal microflora and digestive toxicity of irinotecan in mice. *Clin. Cancer Res.* **12**, 1299–1307 (2006).
300. Pollet, R. M. et al. An atlas of β-glucuronidases in the human intestinal microbiome. *Structure* **25**, 967–977.e5 (2017).
301. Wallace, B. D. et al. Alleviating cancer drug toxicity by inhibiting a bacterial enzyme. *Science* **330**, 831–835 (2010).
302. Bhatt, A. P. et al. Targeted inhibition of gut bacterial β-glucuronidase activity enhances anticancer drug efficacy. *Proc. Natl Acad. Sci. USA* **117**, 7374–7381 (2020).
303. Rousseau, B. et al. The spectrum of benefit from checkpoint blockade in hypermutated tumors. *N. Engl. J. Med.* **384**, 1168–1170 (2021).
304. Robert, C. A decade of immune-checkpoint inhibitors in cancer therapy. *Nat. Commun.* **11**, 3801 (2020).
305. Le, D. T. et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science* **357**, 409–413 (2017).
306. Le, D. T. et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N. Engl. J. Med.* **372**, 2509–2520 (2015).
307. Overman, M. J. et al. Durable clinical benefit with nivolumab plus ipilimumab in DNA mismatch repair-deficient/microsatellite instability-high metastatic colorectal cancer. *J. Clin. Oncol.* **36**, 773–779 (2018).
308. Overman, M. J. et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. *Lancet Oncol.* **18**, 1182–1191 (2017).
309. Vetizou, M. et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science* **350**, 1079–1084 (2015).
310. Routy, B. et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science* **359**, 91–97 (2018).
311. Gopalakrishnan, V. et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science* **359**, 97–103 (2018).
312. Xu, X. et al. Gut microbiome influences the efficacy of PD-1 antibody immunotherapy on MSS-type colorectal cancer via metabolic pathway. *Front. Microbiol.* **11**, 814 (2020).
313. Matson, V. et al. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. *Science* **359**, 104–108 (2018).
314. Shaikh, F. Y. et al. Murine fecal microbiota transfer models selectively colonize human microbes and reveal transcriptional programs associated with response to neoadjuvant checkpoint inhibitors. *Cancer Immunol. Immunother.* https://doi.org/10.1007/s00262-022-03169-6 (2022).
315. Si, W. et al. *Lactobacillus rhamnosus* GG induces cGAS/STING-dependent type I interferon and improves response to immune checkpoint blockade. *Gut* **71**, 521–533 (2022).
316. Tanoue, T. et al. A defined commensal consortium elicits CD8 T cells and anti-cancer immunity. *Nature* **565**, 600–605 (2019).
317. Li, Y. et al. Gut microbiota dependent anti-tumor immunity restricts melanoma growth in $Rnf5^{-/-}$ mice. *Nat. Commun.* **10**, 1492 (2019).
318. Griffin, M. E. et al. Enterococcus peptidoglycan remodeling promotes checkpoint inhibitor cancer immunotherapy. *Science* **373**, 1040–1046 (2021).
319. Rizvi, Z. A. et al. High-salt diet mediates interplay between NK cells and gut microbiota to induce potent tumor immunity. *Sci. Adv.* **7**, eabg5016 (2021).
320. Mager, L. F. et al. Microbiome-derived inosine modulates response to checkpoint inhibitor immunotherapy. *Science* **369**, 1481–1489 (2020).
321. Wang, T. et al. Inosine is an alternative carbon source for $CD8^+$-T-cell function under glucose restriction. *Nat. Metab.* **2**, 635–647 (2020).
322. Luu, M. et al. Microbial short-chain fatty acids modulate $CD8^+$ T cell responses and improve adoptive immunotherapy for cancer. *Nat. Commun.* **12**, 4077 (2021).
323. Makino, S. et al. Enhanced natural killer cell activation by exopolysaccharides derived from yogurt fermented with *Lactobacillus delbrueckii* ssp. *bulgaricus* OLL1073R-1. *J. Dairy. Sci.* **99**, 915–923 (2016).
324. Bessell, C. A. et al. Commensal bacteria stimulate antitumor responses via T cell cross-reactivity. *JCI Insight* https://doi.org/10.1172/jci.insight.135597 (2020).
325. Fluckiger, A. et al. Cross-reactivity between tumor MHC class I-restricted antigens and an enterococcal bacteriophage. *Science* **369**, 936–942 (2020).
326. Gao, Y. et al. *Fusobacterium nucleatum* enhances the efficacy of PD-L1 blockade in colorectal cancer. *Signal. Transduct. Target. Ther.* **6**, 398 (2021).
327. Goc, J. et al. Dysregulation of ILC3s unleashes progression and immunotherapy resistance in colon cancer. *Cell* **184**, 5015–5030.e6 (2021).
328. Huang, J. et al. Metagenomic and metabolomic analyses reveal synergistic effects of fecal microbiota transplantation and anti-PD-1 therapy on treating colorectal cancer. *Front. Immunol.* **13**, 874922 (2022).

329. Baruch, E. N. et al. Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. *Science* **371**, 602–609 (2021).
330. Davar, D. et al. Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. *Science* **371**, 595–602 (2021).
331. Zhang, S. L. et al. *Lacticaseibacillus paracasei* sh2020 induced antitumor immunity and synergized with anti-programmed cell death 1 to reduce tumor burden in mice. *Gut Microbes* **14**, 2046246 (2022).
332. Dizman, N. et al. Nivolumab plus ipilimumab with or without live bacterial supplementation in metastatic renal cell carcinoma: a randomized phase 1 trial. *Nat. Med.* **28**, 704–712 (2022).
333. Gao, G. et al. Adjunctive probiotic *Lactobacillus rhamnosus* Probio-M9 administration enhances the effect of anti-PD-1 antitumor therapy via restoring antibiotic-disrupted gut microbiota. *Front. Immunol.* **12**, 772532 (2021).
334. Spencer, C. N. et al. Dietary fiber and probiotics influence the gut microbiome and melanoma immunotherapy response. *Science* **374**, 1632–1640 (2021).
335. Zhuo, Q. et al. Lysates of *Lactobacillus acidophilus* combined with CTLA-4-blocking antibodies enhance antitumor immunity in a mouse colon cancer model. *Sci. Rep.* **9**, 20128 (2019).
336. Morishita, M. et al. Characterizing different probiotic-derived extracellular vesicles as a novel adjuvant for immunotherapy. *Mol. Pharm.* **18**, 1080–1092 (2021).
337. Kawanabe-Matsuda, H. et al. Dietary lactobacillus-derived exopolysaccharide enhances immune-checkpoint blockade therapy. *Cancer Discov.* **12**, 1336–1355 (2022).
338. Simpson, R. C. Diet-driven microbial ecology underpins associations between cancer immunotherapy outcomes and the gut microbiome. *Nat. Med* **28**, 2344–2352 (2022).
339. Han, K. et al. Generation of systemic antitumour immunity via the in situ modulation of the gut microbiome by an orally administered inulin gel. *Nat. Biomed. Eng.* **5**, 1377–1388 (2021).
340. Lam, K. C. et al. Microbiota triggers STING-type I IFN-dependent monocyte reprogramming of the tumor microenvironment. *Cell* **184**, 5338–5356.e21 (2021).
341. Zhang, S. L. et al. Pectin supplement significantly enhanced the anti-PD-1 efficacy in tumor-bearing mice humanized with gut microbiota from patients with colorectal cancer. *Theranostics* **11**, 4155–4170 (2021).
342. Canale, F. P. et al. Metabolic modulation of tumours with engineered bacteria for immunotherapy. *Nature* **598**, 662–666 (2021).
343. Geiger, R. et al. L-Arginine modulates T cell metabolism and enhances survival and anti-tumor activity. *Cell* **167**, 829–842.e13 (2016).
344. Gurbatri, C. R. et al. Engineered probiotics for local tumor delivery of checkpoint blockade nanobodies. *Sci. Transl. Med.* https://doi.org/10.1126/scitranslmed.aax0876 (2020).
345. Chen, J. H., Pezhouh, M. K., Lauwers, G. Y. & Masia, R. Histopathologic features of colitis due to immunotherapy with anti-PD-1 antibodies. *Am. J. Surg. Pathol.* **41**, 643–654 (2017).
346. Wang, Y. et al. Fecal microbiota transplantation for refractory immune checkpoint inhibitor-associated colitis. *Nat. Med.* **24**, 1804–1808 (2018).
347. Sun, S. et al. *Bifidobacterium* alters the gut microbiota and modulates the functional metabolism of T regulatory cells in the context of immune checkpoint blockade. *Proc. Natl Acad. Sci. USA* **117**, 27509–27515 (2020).
348. Wang, T. et al. Probiotics *Lactobacillus reuteri* abrogates immune checkpoint blockade-associated colitis by inhibiting group 3 innate lymphoid cells. *Front. Immunol.* **10**, 1235 (2019).
349. Hundt, S., Haug, U. & Brenner, H. Comparative evaluation of immunochemical fecal occult blood tests for colorectal adenoma detection. *Ann. Intern. Med.* **150**, 162–169 (2009).
350. Imperiale, T. F. et al. Multitarget stool DNA testing for colorectal-cancer screening. *N. Engl. J. Med.* **370**, 1287–1297 (2014).
351. Guo, S. H. et al. A simple and novel fecal biomarker for colorectal cancer: ratio of *Fusobacterium nucleatum* to probiotics populations, based on their antagonistic effect. *Clin. Chem.* **64**, 1327–1337 (2018).
352. Liang, Q. Y. et al. Fecal bacteria act as novel biomarkers for noninvasive diagnosis of colorectal cancer. *Clin. Cancer Res.* **23**, 2061–2070 (2017).
353. Wong, S. H. et al. Quantitation of faecal *Fusobacterium* improves faecal immunochemical test in detecting advanced colorectal neoplasia. *Gut* **66**, 1441–1448 (2017).
354. Xie, Y. H. et al. Fecal *Clostridium symbiosum* for noninvasive detection of early and advanced colorectal cancer: test and validation studies. *Ebiomedicine* **25**, 32–40 (2017).
355. Suehiro, Y. et al. Highly sensitive stool DNA testing of *Fusobacterium nucleatum* as a marker for detection of colorectal tumours in a Japanese population. *Ann. Clin. Biochem.* **54**, 86–91 (2017).
356. Liang, J. Q. et al. Fecal microbial DNA markers serve for screening colorectal neoplasm in asymptomatic subjects. *J. Gastroenterol. Hepatol.* **36**, 1035–1043 (2021).
357. Yang, J. et al. Establishing high-accuracy biomarkers for colorectal cancer by comparing fecal microbiomes in patients with healthy families. *Gut Microbes* **11**, 918–929 (2020).
358. Lowenmark, T. et al. *Parvimonas micra* as a putative non-invasive faecal biomarker for colorectal cancer. *Sci. Rep.* https://doi.org/10.1038/s41598-020-72132-1 (2020).
359. Gao, R. Y. et al. Gut microbiota dysbiosis signature is associated with the colorectal carcinogenesis sequence and improves the diagnosis of colorectal lesions. *J. Gastroenterol. Hepatol.* **35**, 2109–2121 (2020).
360. Clos-Garcia, M. et al. Integrative analysis of fecal metagenomics and metabolomics in colorectal cancer. *Cancers* https://doi.org/10.3390/cancers12051142 (2020).
361. Shen, X. N. et al. Fecal enterotoxigenic *Bacteroides fragilis–Peptostreptococcus stomatis–Parvimonas micra* biomarker for noninvasive diagnosis and prognosis of colorectal laterally spreading tumor. *Front. Oncol.* https://doi.org/10.3389/fonc.2021.661048 (2021).
362. Yuan, B. A. et al. Fecal bacteria as non-invasive biomarkers for colorectal adenocarcinoma. *Front. Oncol.* https://doi.org/10.3389/fonc.2021.664321 (2021).
363. Liu, K. et al. The role of fecal *Fusobacterium nucleatum* and pks$^+$ *Escherichia coli* as early diagnostic markers of colorectal cancer. *Dis. Markers* **2021**, 1171239 (2021).
364. Yao, Y. Z. et al. A new biomarker of fecal bacteria for non-invasive diagnosis of colorectal cancer. *Front. Cell. Infect. Microbiol.* https://doi.org/10.3389/fcimb.2021.744049 (2021).
365. Osman, M. A. et al. *Parvimonas micra*, *Peptostreptococcus stomatis*, *Fusobacterium nucleatum* and *Akkermansia muciniphila* as a four-bacteria biomarker panel of colorectal cancer. *Sci. Rep.* https://doi.org/10.1038/s41598-021-82465-0 (2021).
366. Wu, Y. et al. Identification of microbial markers across populations in early detection of colorectal cancer. *Nat. Commun.* **12**, 3063 (2021).
367. Gao, R. Y. et al. Alterations of gut mycobiota profiles in adenoma and colorectal cancer. *Front. Cell. Infect. Microbiol.* https://doi.org/10.3389/fcimb.2022.839435 (2022).
368. Duvallet, C., Gibbons, S. M., Gurry, T., Irizarry, R. A. & Alm, E. J. Meta-analysis of gut microbiome studies identifies disease-specific and shared responses. *Nat. Commun.* **8**, 1784 (2017).
369. Priya, S. et al. Identification of shared and disease-specific host gene–microbiome associations across human diseases using multi-omic integration. *Nat. Microbiol.* **7**, 780–795 (2022).
370. Eklof, V. et al. Cancer-associated fecal microbial markers in colorectal cancer detection. *Int. J. Cancer* **141**, 2528–2536 (2017).
371. Lee, J. K., Liles, E. G., Bent, S., Levin, T. R. & Corley, D. A. Accuracy of fecal immunochemical tests for colorectal cancer: systematic review and meta-analysis. *Ann. Intern. Med.* **160**, 171 (2014).
372. Flanagan, L. et al. *Fusobacterium nucleatum* associates with stages of colorectal neoplasia development, colorectal cancer and disease outcome. *Eur. J. Clin. Microbiol. Infect. Dis.* **33**, 1381–1390 (2014).
373. Sze, M. A., Baxter, N. T., Ruffin, M. T., Rogers, M. A. M. & Schloss, P. D. Normalization of the microbiota in patients after treatment for colonic lesions. *Microbiome* https://doi.org/10.1186/s40168-017-0366-3 (2017).
374. Yao, Q. L. et al. Potential of fecal microbiota for detection and postoperative surveillance of colorectal cancer. *BMC Microbiol.* https://doi.org/10.1186/s12866-021-02182-6 (2021).
375. Liang, J. Q. et al. Novel microbiome signatures for non-invasive diagnosis of adenoma recurrence after colonoscopic polypectomy. *Aliment. Pharm. Ther.* **55**, 847–855 (2022).
376. Kwong, T. N. Y. et al. Association between bacteremia from specific microbes and subsequent diagnosis of colorectal cancer. *Gastroenterology* **155**, 383–390.e8 (2018).
377. Ma, C. et al. Establishing a novel colorectal cancer predictive model based on unique gut microbial single nucleotide variant markers. *Gut Microbes* **13**, 1–6 (2021).
378. Bosch, S. et al. The potential of fecal microbiota and amino acids to detect and monitor patients with adenoma. *Gut Microbes* **14**, 2038863 (2022).
379. Du, X. H. et al. Alterations of the gut microbiome and fecal metabolome in colorectal cancer: implication of intestinal metabolism for tumorigenesis. *Front. Physiol.* https://doi.org/10.3389/fphys.2022.854545 (2022).
380. Bosch, S. et al. Integration of stool microbiota, proteome and amino acid profiles to discriminate patients with adenomas and colorectal cancer. *Gut Microbes* **14**, 2139979 (2022).
381. Mo, S. B. et al. Fecal multidimensional assay for non-invasive detection of colorectal cancer: fecal immunochemical test, stool DNA mutation, methylation, and intestinal bacteria analysis. *Front. Oncol.* https://doi.org/10.3389/fonc.2021.643136 (2021).
382. Mima, K. et al. *Fusobacterium nucleatum* in colorectal carcinoma tissue and patient prognosis. *Gut* **65**, 1973–1980 (2016).
383. Li, X. & Saxena, D. The tumor mycobiome: a paradigm shift in cancer pathogenesis. *Cell* **185**, 3648–3651 (2022).
384. Derosa, L. et al. Intestinal *Akkermansia muciniphila* predicts clinical response to PD-1 blockade in patients with advanced non-small-cell lung cancer. *Nat. Med.* **28**, 315–324 (2022).
385. Lee, S. H. et al. *Bifidobacterium bifidum* strains synergize with immune checkpoint inhibitors to reduce tumour burden in mice. *Nat. Microbiol.* **6**, 277–288 (2021).
386. Sivan, A. et al. Commensal *Bifidobacterium* promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science* **350**, 1084–1089 (2015).
387. Martini, G. et al. Gut microbiota correlates with antitumor activity in patients with mCRC and NSCLC treated with cetuximab plus avelumab. *Int. J. Cancer* **151**, 473–480 (2022).
388. Pi, H., Huang, L., Liu, H., Liang, S. & Mei, J. Effects of PD-1/PD-L1 signaling pathway on intestinal flora in patients with colorectal cancer. *Cancer Biomark.* **28**, 529–535 (2020).
389. Lee, K. A. et al. Cross-cohort gut microbiome associations with immune checkpoint inhibitor response in advanced melanoma. *Nat. Med.* **28**, 535–544 (2022).
390. Gharaibeh, R. Z. & Jobin, C. Microbiota and cancer immunotherapy: in search of microbial signals. *Gut* **68**, 385–388 (2019).
391. Zhao, L. et al. Uncovering 1058 novel human enteric DNA viruses through deep long-read third-generation sequencing and their clinical impact. *Gastroenterology* **163**, 699–711 (2022).
392. Li, Y. et al. Recovery of human gut microbiota genomes with third-generation sequencing. *Cell Death Dis.* **12**, 569 (2021).

393. Kuchina, A. et al. Microbial single-cell RNA sequencing by split-pool barcoding. *Science* https://doi.org/10.1126/science.aba5257 (2021).
394. Merritt, C. R. et al. Multiplex digital spatial profiling of proteins and RNA in fixed tissue. *Nat. Biotechnol.* **38**, 586–599 (2020).
395. Lee-Six, H. et al. The landscape of somatic mutation in normal colorectal epithelial cells. *Nature* **574**, 532–537 (2019).
396. Ayukawa, S. et al. Epithelial cells remove precancerous cells by cell competition via MHC class I-LILRB3 interaction. *Nat. Immunol.* **22**, 1391–1402 (2021).
397. Geyer, R. J., Tobet, R., Berlin, R. D. & Srivastava, P. K. Immune response to mutant neo-antigens: cancer's lessons for aging. *Oncoimmunology* **2**, e26382 (2013).
398. Dow, L. E. et al. Apc restoration promotes cellular differentiation and reestablishes crypt homeostasis in colorectal cancer. *Cell* **161**, 1539–1552 (2015).
399. Federici, S. et al. Targeted suppression of human IBD-associated gut microbiota commensals by phage consortia for treatment of intestinal inflammation. *Cell* **185**, 2879–2898.e24 (2022).

Acknowledgements
This project was supported by funds from Research Grants Council-General Research Fund (14101321; 24100520), RGC-Collaborative Research Fund (C4039-19G); Heath and Medical Research Fund (06170686; 08190706); and the Science and Technology Program Grant Shenzhen (JCYJ20170413161534162).

Author contributions
C.C.W. researched data for this manuscript. Both authors discussed the content of the manuscript, and wrote, reviewed and/or edited the manuscript prior to submission.

Competing interests
The authors declare no competing interests.

Additional information
Peer review information *Nature Reviews Clinical Oncology* thanks A. Chan who co-reviewed with C. Geraghty and W. Jia, who co-reviewed with H. Li and Y. Ni; and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.