Bugs, drugs, and cancer: can the microbiome be a potential therapeutic target for cancer management?

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Outnumbering our own cells over ten times, gut microbes can even be considered an additional organ. Several studies have explored the association between microbiomes and antitumor drug response. It has been reported that the presence of specific bacteria might modulate cancer progression and the efficacy of anticancer therapeutics. Bacteria-targeting intervention can provide crucial guidance for the design of next-generation antitumor drugs. Here, we review previous findings elucidating the impact of gut microbiomes on cancer treatment and the possible underlying mechanisms. In addition, we examine the role of microbiome manipulation in controlling tumor growth. Finally, we discuss concerns regarding the alteration of the microbiome composition, and the potential approaches to surpass existing limitations.

Introduction

The microbiota is defined as all microorganisms that are associated with a specific host cellular environment [1]. These microorganisms are identified using 16S ribosomal RNA (rRNA) sequencing. Whereas the term 'microbiome' generally refers to the combination of genes of the microbiota and its products, as well as the host environment [1]. The number of human gut microbiota could exceed 1000 species, with a total of 10^{14} microorganisms [2,3]. Outnumbering our own cells more than ten times, the diverse microbial communities in the human body vary in composition and function based on their location, and the age, sex, race, and diet of their host [3,4]. Generally, gastrointestinal (GI) microbiota can be described in terms of global parameters (richness and diversity), compositional features (bacterial phyla and taxa), and functional features (metabolic modules and pathways) [3]. Sequence-based metagenomic analyses from stool and intestinal biopsy specimens that are based on 16S rRNA gene sequencing and/or whole-genome sequencing can be used to derive various indices of richness and diversity for the comparison of bacterial communities [3]. In addition, the distribution of gut microbiota differs from the oral to rectal cavities and can change significantly during life [5] (Fig. 1). Gut microbial density increases from the proximal to the distal GI tract and along the tissue–lumen axis. Similarly, diversity further increases in the same pattern [6]. Moreover, the microbial inhabitants of the human gut can even be considered an additional organ because their metabolic capacity is approximately equal to that of the liver [6]. These microbiomes have a vital role in the maintenance of GI homeostasis, which includes metabolism, protection against pathogens, and the development and differentiation of host epithelial cells [2,6]. The establishment of animal models mimicking human disease and advances in organoid culture have provided opportunities for scientists to explore the specific health benefits or harm caused by various gut microbiomes [3]. Accumulating evidence suggests that dysbiosis of the microbial community can contribute to disease pathogenesis, such as obesity, inflammatory bowel disease (IBD), and even cancer [5]. Although still at the early discovery stage, current research in this field has largely transformed our understanding of the relationships among microorganisms, drugs, and cancer. As reported earlier, the host genetics, immune system, age, diet, and antibiotic use can alter the gut microbiome [7]. These alterations are counteractive to the development and progression of the GI neoplasms [7]. For example, a diet rich in soluble fiber, which can be fermented by gut microbiota into short-chain fatty acids (SCFA), can induce the development of

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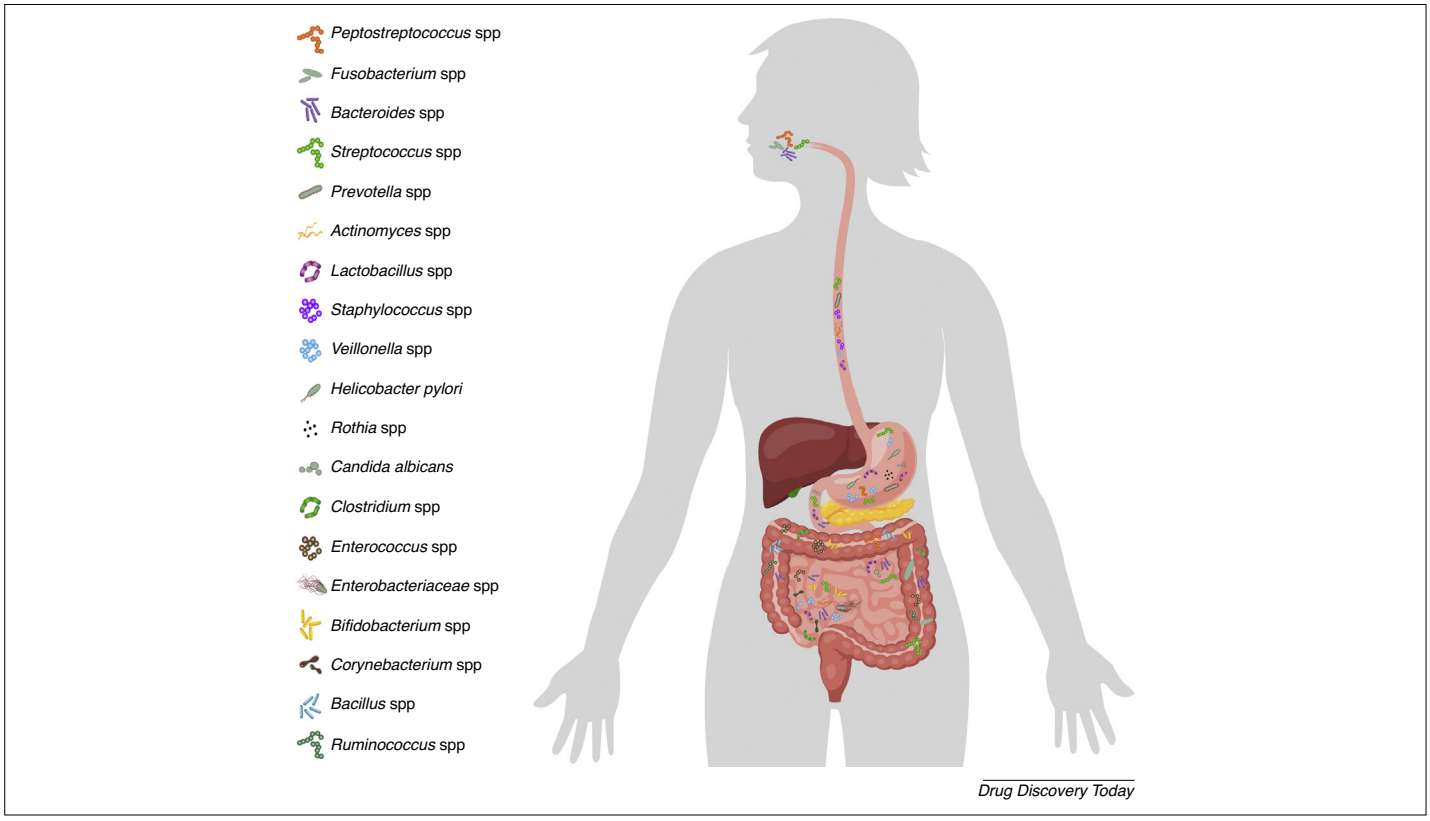

FIGURE 1

Predominant microbial communities within different sites of the oral cavity, oropharyngeal region and gastrointestinal (GI) tract. *Peptostreptococcus*, *Fusobacterium*, *Bacteroides*, and *Streptococcus* are the predominant genera in the oropharyngeal region. The microbial ecosystem in the esophageal cavity is relatively limited in diversity and dominated by *Streptococcus* spp., with other genera, such as *Prevotella*, *Actinomyces*, and *Lactobacillus*. The microbial diversity of the human stomach is limited because of the low pH of the gastric lumen. *Streptococcus* is the most dominant genus in the distal esophagus and the stomach in the absence of *Helicobacter pylori* infection. In the duodenum and jejunum, *Streptococcus* again appears to be a dominant genus. The microbial composition in the ileum and colon are similar, including bacteria such as *Bacteroides*, *Streptococcus*, and *Bifidobacterium*.

hepatocellular carcinoma (HCC) [8]. In addition to its role in carcinogenesis, the effects of the gut microbiome on modulating the response to cancer therapy have gradually been revealed [9]. Here, we summarize research progress regarding the relationship between gut microbiomes and treatment outcomes of human cancer. We further discuss the potential of bacteria-targeting interventions with regard to cancer management.

The gut microbiome influences immunotherapy and chemotherapy

Accumulating evidence indicates that the dysregulation of microbiome–host interactions is involved in various diseases, such as IBDs [10], diabetes [11], and liver diseases [12]. Despite advances in our understanding of the contribution of the microbiome to human health, its influence on the response of the host to drug treatment remains obscure. Recently, several research reports have generated a paradigm shift in concepts regarding the interactions between bacteria and cancer therapeutic drugs [13,14] (Table 1).

Immunotherapy

Immunotherapy is a pillar of cancer treatment [15]. Harnessing the host immune system is a promising cancer control strategy because of its potential for the specific targeting of tumor cells and reduced adverse effects on normal tissues. Given that the microbiome strongly modulates inflammation and immunity, it is plausible that alterations in the microbial composition can affect the response to immunotherapy [16].

CpG oligodeoxynucleotides

Small, oligodeoxynucleotides rich in unmethylated cytidyl guanosyl dinucleotides (CpG ODNs) have been used as immune adjuvants in tumor immunotherapy. Serving as synthetic ligands, CpG ODNs can mimic bacterial infection and activate the pathogen-associated molecular pattern (PAMP) receptor Toll-like receptor 9 (TLR9) [16,17]. In addition to potent immune-stimulatory properties, the intratumoral administration of CpG ODNs can induce effective antitumor activity [17]. In mouse models bearing lymphoma, colon carcinoma, and melanoma, the intratumor administration of CpG ODNs in combination with antibody treatment against the interleukin (IL)-10 receptor delayed tumor growth and prolonged animal survival [18]. This antitumor effect was associated with the increased production of tumor necrosis factor (TNF) by tumor-associated myeloid cells and the activation of cytotoxic $CD8^+$ T cells [18]. However, the efficacy of this approach was reduced in germ-free mice or mice treated with antibiotics [18]. *Alistipes shaii* was identified as a bacterial species positively associated with TNF production by

TABLE 1

Influence of gut microbiome on anticancer therapy[a]

Phylum	Microbiota	Cancer	Model	Impact on treatment	Effect	Mechanism	Refs
Actinobacteria	*Bifidobacterium*	Melanoma	Mouse	αPD-L1	Beneficial	Immunomodulation: increase $CD4^+$ and $CD8^+$ T cells	[22,61]
	Collinsella aerofaciens	Melanoma	Mouse	αPD-L1	Beneficial	Immunomodulation: increase $CD4^+$ and $CD8^+$ T cells	[22]
Bacteroidetes	*Bacteroidales*	Melanoma	Mouse	αPD-1	Harmful	Immunomodulation: increase Tregs and MDSCs	[21]
	Bacteroides fragilis *Bacteroides thetaiotaomicron*	Melanoma	Mouse	αCTLA-4	Beneficial	Immunomodulation: affect IL-12-dependent TH1 immune responses	[26]
	Barnesiella intestinihominis	Lung cancer, ovarian cancer	Mouse	CTX	Beneficial	Immunomodulation: promote infiltration of IFN-γ-producing γδT cells in cancer lesions	[44]
	Alistipes	Lymphoma, colon cancer, melanoma	Mouse	CpG ODN	Beneficial	Immunomodulation: increase $CD4^+$ and $CD8^+$ T cells	[18]
Firmicutes	*Ruminococcaceae*	Melanoma	Mouse	αPD-1	Beneficial	Immunomodulation: increase $CD4^+$ and $CD8^+$ T cells	[21]
	Faecalibacterium	Melanoma	Mouse	αPD-1	Beneficial	Immunomodulation: increase $CD4^+$ and $CD8^+$ T cells	[21]
	Enterococcus faecium	Melanoma	Mouse	αPD-L1	Beneficial	Immunomodulation: increase $CD4^+$ and $CD8^+$ T cells	[22]
	Enterococcus hirae	Lung cancer, ovarian cancer, fibrosarcoma	Mouse	CTX, ADM	Beneficial	Translocate from small intestine to secondary lymphoid organs; immunomodulation: stimulate generation of pTh17 cells and increase intratumoral CD8:Treg ratio	[44]
	Lactobacillus	Lung cancer	Mouse	DDP	Beneficial	Modulate VEGFA, BAX, and CDKN1B expression and enhance T cell immunity	[48]
	Lactobacillus casei	Bladder cancer	Human	EPI	Beneficial	Not known	[55]
Fusobacteria	*Fusobacterium nucleatum*	CRC	Mouse	OXA, 5-FU	Harmful	Immunomodulation: Fap2 protein of *Fn* interacts with TIGIT, leading to inhibition of NK cell cytotoxicity; orchestrate Toll-like receptor, miRNAs, and autophagy network	[25]
Proteobacteria	*Gammaproteobacteria*	Colon cancer	Mouse	GEM	Harmful	Metabolism: metabolize GCM into its inactive form	[46]
		Pancreatic cancer	Mouse	αPD-1	Harmful	Immunomodulation: are associated with MDSCs and M1 macrophages	[24]
	Comamonas aquatica DA1877		*C. elegans*	CPT	Harmful	Not known	[27,28]
				FUDR		Metabolism: inhibit bacterial ribonucleotide metabolism	
	Escherichia coli OP50		*C. elegans*	CPT	Harmful	Not known	[27,28]
				FUDR		Metabolism: inhibit bacterial ribonucleotide metabolism	
Verrucomicrobia	*Akkermansia muciniphila*	Lung cancer, kidney cancer	Mouse	αPD-1	Beneficial	Immunomodulation: increase $CD4^+$ and $CD11^+$ T cells	[23]

[a] Abbreviation: CTX: Cyclophosphamide; CpG ODN: CpG oligodeoxynucleotides; *C. elegans*: *Caenorhabditis elegans*; CPT: Camptothecin; FUDR: 5-fluoro-20-deoxyuridine; CRC: colorectal cancer; Tregs: regulatory T cells; MDSCs: myeloid-derived suppressor cells; pTh17: pathogenic T helper 17, ADM: Doxorubicin; GEM: Gemcitabine; OXA: Oxaliplatin; DDP: Cisplatin; EPI: Epirubicin; α: anti.

tumor-associated myeloid cells [18]. Oral administration of this microorganism to antibiotic-treated mice restored TNF production [18]. A previous study further underlined the importance of an intact commensal microbiome to the efficacy of cancer treatment [16].

Immune checkpoint therapy

Another treatment strategy in anticancer immunotherapy is the blockade of immune checkpoints, specifically CTLA-4 and the axis between programmed cell death protein 1 (PD-1) and its ligand (PD-L1). The US Food and Drug Administration (FDA) has ap-

proved the use of immune therapies that involve checkpoint blockade for the treatment advanced melanoma and lung cancers [19]. Effector T lymphocytes represent a crucial branch of the adaptive immune response to antigens. The control of the length and strength of T lymphocyte activation is necessary to preserve healthy tissues from the destructive potential of these cells [13]. A series of co-inhibitory molecules, termed 'immune checkpoints', are expressed by antigen-presenting cells (APCs) and are responsible for switching off T cell activation and terminating the immune response. The expression of immune checkpoint molecules by tumor cells leads to inactivation of cytotoxic $CD8^+$ T cells (a type of effector T lymphocyte) and, as a result, evasion of the antitumor immune response [13]. Thus, unleashing the power of adaptive immune responses by targeting immune checkpoints, such as the PD-1–PD-L1 axis has emerged as a promising cancer therapeutic approach for solid tumors [13]. Checkpoint inhibitors have shown consistent efficacy in the chronic treatment of certain types of cancer. However, only 25% of all patients respond to PD-1 blockers [20]. The mechanisms underlying variable immune responses between individuals are not well understood. Interestingly, the composition of the gut microbiome is associated with the patient response to anti-PD-1 therapy, as demonstrated by numerous studies.

Previous studies have demonstrated that patients who responded to checkpoint inhibitors exhibited a more abundant diversity of microbiota notably with regard to the accumulation of specific bacteria [21]. As reported, *Ruminococcaceae* [21], *Faecalibacterium* [21], *Clostridales* [21], *Bifidobacterium longum* [22], *Collinsella aerofaciens* [22], *Enterococcus faecium* [22], and *Akkermansia muciniphila* [23] were enriched in patients who responded to treatment, denoted as responders (R). These species were regarded as 'favorable bugs', whereas *Bacteroidales* [21] was found to be enriched in nonresponders (NR) and, hence, were regarded as 'unfavorable bugs'. The comparisons of pathway enrichment between R and NR patients also indicated differences in metabolic effects [21]. Anabolic functions predominated in the former, including amino acid biosynthesis, which might promote host immunity, whereas catabolic functions predominated in the latter [21]. Furthermore, patients with a high abundance of 'favorable bugs' exhibited higher frequencies of effector $CD4^+$ and $CD8^+$ T cells in the systemic circulation with a preserved cytokine response to anti-PD-1 therapy, whereas patients with a higher abundance of 'unfavorable bugs' presented with higher frequencies of regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) in the systemic circulation and with a blunted cytokine response [21]. These findings are in line with fecal microbiota transplantation (FMT) experiments performed in germ-free recipient mice [21]. These data suggested that patients with a favorable gut microbiome had enhanced antitumor immune responses mediated by increased antigen presentation and improved effector T cell function in the periphery and the tumor microenvironment [21]. In contrast to these patients, patients with an unfavorable gut microbiome exhibited impaired antitumor immune responses, possibly resulting from reduced intratumoral lymphoid and myeloid infiltration and weakened antigen presentation capacity [21]. In addition to melanoma, patients with non-small cell lung cancer (NSCLC), renal cell carcinoma, and urothelial carcinoma exhibited a negative correlation of the response to anti-PD-1 treatment with antibiotic exposure during the course of cancer therapy used to treat various infections [13,23]. However, these findings contradict those of a recent study focused on pancreatic ductal adenocarcinoma (PDA). In PDA, bacterial depletion by antibiotic increased responses to immunotherapy by upregulating PD-1 expression and reprogramming the tumor microenvironment, such as decreasing MDSCs, increasing M1 macrophage differentiation, and promoting TH1 differentiation of $CD4^+$ T and $CD8^+$ T cells [24]. These conflicting findings suggest that different cancer types induce diverse alterations of microbiomes and that the varied composition exerts either a positive or negative effect on the immune checkpoint therapy [25].

Anti-PD-1 mainly induces the expansion of specific tumor-infiltrating exhausted-like CD8 T cell subsets. In contrast to this type of treatment, anti-CTLA-4 induces the expansion of an $ICOS^+$ Th1-like CD4 effector population in addition to engaging specific subsets of exhausted-like CD8 T cells [15]. The optimal immune-mediated response to anti-CTLA-4 is also limited to a few patients. For example, only 22% of patients with advanced melanoma treated with anti-CTLA-4 exhibited durable responses extending beyond 10 years [15]. The antitumor effects of this drug have been reported to rely on distinct *Bacteroides* spp. [21,26]. Melanomas in germ-free or antibiotic-treated mice did not respond efficiently to CTLA-4 inhibitor treatment [26]. However, these results are partly in conflict with work demonstrating that *Bacteroidales* were abundant in NRs to anti-PD-1 therapy and, thus, were deemed 'bad bugs' [21]. Therefore, despite recent clinical progress, further investigations are required for a detailed understanding of the fundamental mechanisms underlying the interplay between the anticheckpoint therapies and gut microbiome. Generally, valuable insights have been derived from the improvement of current therapies and the rational design of combination therapy approaches that aim to modulate the gut microbiome in patients with cancer.

Chemotherapy

The gut microbiome can affect the response to anticancer immunotherapy, and several studies have investigated simultaneously the involvement of the microbiome in the patient response to chemotherapeutics and their underlined mechanisms [27].

Fluoropyrimidines

Fluoropyrimidines are antimetabolite drugs primarily used to treat cancer. The archetype fluoropyrimidine, 5-fluorouracil (5-FU), is the principal therapeutic option used for colorectal cancer (CRC). 5-FU and its prodrugs, such as capecitabine, are uracil analogs that impede nucleotide biosynthesis and, hence, cell division, by inhibiting the activity of the enzyme thymidylate synthase (TS) [28]. Despite the widespread use of 5-FU-based chemotherapy, no universally accepted dose has been classified, and significant pharmacokinetic variations exist between patients [28]. Recently, fluoropyrimidines have been reported to be associated with microbiota metabolism and two distinct mechanisms were proposed by which bacteria modulate their efficacy [28]. Initially, it was shown that these drugs are activated or inactivated by bacteria ribonucleotide metabolism [28]. Inhibition of bacterial ribonucleotide metabolism can drastically antagonize drug efficacy, whereas inhibition of deoxyribonucleotide metabolism can improve it [28]. For example, *Escherichia*

coli and *Comamonas* can affect the response of *Caenorhabditis elegans* to 5-fluoro-2′-deoxyuridine (FUDR) and camptothecin (CPT) in opposite directions, by regulating bacterial nucleotide metabolic networks. 5-FU and FUDR can produce FdUMP, which directly inhibits thymidylate synthase and causes thymineless cell death. In addition, the products of 5-FU and FUDR can be converted to FUTP or FdUTP, leading to RNA or DNA damage, respectively. However, mutations in *E. coli* or *Comamonas upp* can generate 5-fluorouridine monophosphate (FUMP) but not FdUMP from 5-FU and, thus, can reduce the efficacy of 5-FU and FUDR [27]. The second mechanism involves the influence of the bacterial deoxynucleotide pools on the host response to drugs therapy [28]. The alterations in bacterial deoxynucleotide pools amplify 5-FU-induced autophagy and cell death in host cells and, thus, increase the efficacy of treatment, and this process is specifically regulated by nucleoside diphosphate kinase [28].

Recently, a specific bacterium, *Fusobacterium nucleatum* (*Fn*), was shown to directly promote CRC chemoresistance to oxaliplatin (OXA) and 5-FU [29]. *Fn* has attracted considerable attention as a potential cancer-promoting microbe since 2012, when two laboratories simultaneously found that the levels of this bacterium were enriched in human CRCs compared with those in healthy subjects [30,31]. Subsequent studies further indicated that a higher abundance of *Fn* in CRC tissue was associated with metastasis [32], worse prognostic outcomes [33–35], and specific tumor molecular features, including CpG island methylator phenotype (CIMP)-high, microsatellite instability (MSI) and genetic mutations (such as *BRFA, KRAS*, and *TP53*), possibly contributing to both the adenoma-carcinoma sequence (e.g., *APC*-mutated adenoma [36,37]) and the serrated polyp-carcinoma sequence [38–40]. The mechanisms underlying these phenomena are complex. Current studies demonstrated that *Fn* can adhere to and invade endothelial and epithelial cells via its virulence factors, such as adhesion A (FadA) [2], *Fusobacterium* autotransporter protein 2 (Fap2) [41,42], and fusobacterial outer membrane protein A (FomA), which can activate inflammatory responses, induce tumor-immune evasion, and promote tumor development [43]. Increased expression of miRNA-21 was also observed following *Fn* infection, which is considered another novel angle used to clarify the carcinogenic role of *Fn* [35]. However, the effect of this bacterium on the chemotherapeutic response was only reported recently, suggesting that the tumor microenvironment promotes chemoresistance. CRC cells resident in the gut can enhance this colonization property by interacting with *Fn*. The activation of the TLR4/MYD88 pathway by this microbe downregulated miRNA-18a* and miRNA-4082, inducing a switch from apoptosis to autophagy in CRC cells that, in turn, allowed them to resist the drug therapeutic action [37].

Cyclophosphamide

Cyclophosphamide (CTX), a prominent alkylating anticancer agent, is used in the treatment of breast cancers, lymphomas, and some brain cancers [44]. This compound induces immunogenic cancer cell death, subverting immunosuppressive T cells, and promoting T_H1 and T_H17 cells to control cancer outgrowth [44]. According to the literature, CTX can alter the composition of microbiota in the small intestine and induce the translocation of selected species of Gram-positive bacteria into secondary lymphoid organs [44]. Recently, two commensals, namely *Enterococcus hirae* and *Barnesiella intestinihomini*, were identified to orchestrate the therapeutic efficacy of CTX [45]. The Gram-positive *E. hirae* occurs in the small intestine, whereas the Gram-negative *B. intestinihomini* occurs in the colon [45]. Following CTX treatment, *E. hirae* was able to translocate from the small intestine to secondary lymphoid organs, such as the lymph nodes and spleen, inducing pathogenic (p)Th17 and Th1 responses and increasing the cytotoxic $CD8^+$ T:Treg ratio [45]. In contrast to *E. hirae*, *B. intestinihomini* does not translocate and accumulates in the colon, where it induces systemic polyfunctional Th1 and Tc1 responses and causes an increase in intratumoral interferon (IFN)-γ-producing γδT cells [45]. In addition, the pattern recognition receptor nucleotide-binding oligomerization domain-containing protein 2 (NOD2) can control these two immunogenic bacteria and limit their adjuvant effects [45]. In tumor-bearing animals after CTX treatment, when the NOD2 receptor was deficient in intestinal epithelial cells, *E. hirae* and *B. intestinihomini* induced epithelial cell death [45]. This barrier dysfunction resulted in increased translocation of *E. hirae* from the small intestine and higher accumulation of *B. intestinihomini* in the colon [45]. Conclusively, the efficacy of CTX was modulated by these two bacteria and could be improved in a NOD2-deficient environment [45].

Gemcitabine

In addition to CTX, gemcitabine (GCM) has attracted considerable attention in the field of microbiology. GCM is a nucleoside analog (2′,2′-difluorodeoxycytidine) used to treat patients with pancreatic, lung, breast, or bladder cancers [46]. In mouse models bearing CRC, *Gammaproteobacteria* within tumors are able to metabolize GCM into its inactive form, 2′,2′-difluorodeoxyuridine, thereby rendering it ineffective [46]. Expression of the long isoform of the bacterial cytidine deaminase enzyme, which exists as long and short versions, might be responsible for this metabolic process. Such GCM resistance could be abolished by antibiotics or deletion of the long version of cytidine deaminase from the bacteria genome [46,47].

Platinum

Platinum is one of the most widely used regimens for the treatment of lung cancer. Despite over 80% of patients responding to chemotherapy, the cancer is rarely cured because of rapidly developing drug resistance [48]. In a Lewis lung cancer xenograft model, tumor size following combined treatment of cisplatin (DDP) and ABX (vancomycin, ampicillin and neomycin) was larger than that noted with DDP alone and the survival rate was significantly reduced [48]. Furthermore, gene expression analysis demonstrated that antibiotics can partially impair the function of DDP via upregulating VEGFA (oncogenes) and downregulating BAX, CDKN1B (suppressor genes), IFN-γ, GZMB (granzyme B), and PRF1 (perforin 1) in $CD8^+$ T cell subsets [48].

Gut microbiomes modulation improves cancer management

Following careful appraisal of the aforementioned studies, a crucial question results: can the manipulation of the microbiome be applied to prevent cancer or stop its recurrence in the clinic? Based on recent studies, the effects of dietary modulation, FMT, or other ways to manipulate microbial communities can achieve desirable effects with regard to the treatment modulation of the gut microbiome [3]. The modulation of the microbiome can be divided into

the supplement or activation of the favorable gut microbiome and the inhibition of the unfavorable gut microbiome.

Inhibition of unfavorable microbiome

The taking of antibiotics is a vital factor contributing to the alteration of the microbiome composition and, thus, can cause a series of cascade effects. As reported in the aforementioned studies, antibiotics could be applied as anticancer treatments [16]. Bacteria from the tumor microenvironment can induce GCM resistance by producing a form of the enzyme cytidine deaminase, which inactivates this drug [46]. Mice with colon cancer that were treated with GCM plus the antibiotic ciprofloxacin exhibited enhanced drug response compared with mice receiving GCM alone [46]. In addition to ciprofloxacin, *Clostridium* spp. are responsible for the decrease in the immune response of both primary liver tumors and liver metastases [49]. The mechanism underlying this phenomenon involves modification of host bile acid production and not the modulation of the concentration of GCM metabolites [49]. In this study, primary liver tumors in transgenic mice were analyzed and liver metastases from lymphoid or melanoma tumor cell lines were engrafted outside the liver. All mice received ABX treatment (vancomycin, neomycin and primaxin) by drinking water to minimize gut commensal bacteria colonization [50]. As a result, antibiotic treatment induced a liver-selective antitumor effect, along with an increase in the number of hepatic $CXCR6^+$ natural killer T (NKT) cells and the levels of IFN-γ production following antigen stimulation [49]. Further investigations revealed that the gut microbiome was involved in mediating primary-to secondary bile acid conversion, controlling CXCL16 expression levels of the liver sinusoidal endothelial cells (LSECs) [49]. Following ABX treatment, LSECs produced higher CXCL16 levels and recruited NKT cells to the liver, effectively combating tumor cell growth [49].

The antibiotic metronidazole might also have a role in cancer management. The treatment of mice bearing a colon cancer xenograft with metronidazole reduced *Fusobacterium* load, cancer cell proliferation, and overall tumor growth [51]. To identify whether treatment of *Fusobacterium*-positive colon cancer xenografts with either a *Fusobacterium*-resistant or a *Fusobacterium*-sensitive antibiotic could affect tumor growth, erythromycin and metronidazole were used in two separate mouse models respectively [51]. Following oral administration of metronidazole, the mice containing *Fusobacterium*-positive patient-derived xenografts (PDXs) resulted in a statistically significant decrease in the trajectory of tumor growth [51]. Significant reductions were also noted with regard to the *Fusobacterium* load in the tumor tissue and the tumor cell proliferation. However, the erythromycin group showed no significant differences in these parameters [51]. These results were also supported by a recent study showing that colorectal tumors with a high *Fusobacterium* load were more likely to develop recurrence [29]. This finding suggests that *Fusobacterium*-positive tumors would benefit from antifusobacterial therapy [51].

Supplement or activation of favorable microbiome

Probiotics

The notion that administration of viable bacteria to humans can lead to health benefits was initially originated by Élie Metchnikov, who was awarded a Nobel prize in 1908 [52]. The latest definition of 'probiotics' elaborated by FAO and WHO is as follows: viable microorganisms which when administered in adequate amounts confer a health benefit on the host [53]. Most probiotics are lactic acid-producing bacteria, notably those belonging to the genera *Lactobacillus* and *Bifidobacterium*; other genera, such as *Streptococcus*, *Bacillus*, and *Enterococcus*, are also used [16]. In addition to the role of probiotics in alleviating the adverse effects of chemotherapy, probiotic administration has also been reported to improve chemotherapeutic and immunotherapeutic outcomes [16].

Lactobacillus

The genus *Lactobacillus* has been reported to be involved in various cancer types, such as bladder cancer, lung cancer, CRC, and breast cancer. In mice with bladder cancer, the intravesical instillation of *Lactobacillus casei* Shirota strain (LC9018) displayed an antitumor effect [54]. The supplement of this bacterium significantly reduced the rate of tumor progression with locally increased IFN-γ and TNF-α levels, and induced the infiltration of neutrophils surrounded by macrophages [54]. In a prospective, randomized, control trial, patients treated with *L. casei* (sold commercially as Yakult) and intravesical instillation of epirubicin (EPI) exhibited recurrence rates that were 15% lower than those of patients who received chemotherapy alone [55]. In addition, the use of antibiotics attenuated the efficacy of DDP in mouse models bearing lung cancer. In contrast to a single treatment of DDP, mice treated with DDP and *Lactobacillus acidophilus* displayed a decreased tumor size along with an improved survival rate, suggesting that probiotic cotreatment could promote the cytostatic and proapoptotic effects of DDP [48]. Moreover, according to previous studies, several *Lactobacillus* spp. have great potential in CRC treatment. Oral treatment of mice with *L. casei* BL23 and *Lactobacillus salivarius* Ren (Ren) significantly protected the animals against 1,2-dimethylhydrazine (DMH)-induced CRC formation [56,57]. Injection of DMH affected the composition of gut microbial flora that was characterized by increased number of *Ruminococcus* spp. and *Clostridiales* bacteria, as well as a decreased number of *Prevotella* spp. [57]. Supplement with Ren reversed this dysbiosis back to the healthy state, along with a declined cancer incidence from 87.5% to 25% compared with that in the group administered with DMH alone [57]. With regard to *L. casei* BL23, further studies demonstrated that its antitumor effect might be associated with the regulation of Treg and Th17 cell populations [56]. Other bacteria belonging to the *Lactobacillus* genus, such as *Lactobacillus acidophilus* NCFM, have been reported to have a role in attenuating colon carcinogenesis, with a 50.3% decrease in tumor size [58]. The antitumor efficacy of selenium nanoparticle (SeNP)-enriched *Lactobacillus plantarum* and *Lactobacillus brevis* in mouse breast cancer models was also examined. Oral supplementation of these two bacteria exhibited similar results with regard to the increase of IFN-γ levels, NK cell activity, and animal survival rate. Manipulation of *L. brevis* can also further decrease liver metastasis [59,60].

Bifidobacterium

Oral administration of Bifidobacterium alone improved tumor treatment to the same degree as anti-PD-L1 therapy, whereas the combination treatment nearly abolished tumor outgrowth [61].

FMT experiments have been used frequently in germ-free recipient mice. Mice administered with fecal material from patients who responded to anti-PD-1 therapy (R-FMT) exhibited significantly reduced tumor size, augmented T cell responses, and higher

antitumor efficacy of the anti-PD-L1 therapy [21–23]. In NR-FMT mouse models, oral supplementation of a specific bacterium, *Akkermansia muciniphila*, restored the efficacy of PD-1 blockade in an IL-12-dependent manner by increasing the recruitment of $CCR9^+CXCR3^+CD4^+$ T lymphocytes to mouse tumor beds [23]. Furthermore, other approaches, including gavage with *Bacteroides fragilis*, immunization with *B. fragilis* polysaccharides, or adoptive transfer of *B. fragilis*-specific T cells, were able to reverse the poor response of tumor-bearing mice to the anti-CTLA-4 therapy caused by antibiotic treatment [26].

Prebiotics

Prebiotics are nondigestible food ingredients that can be selectively fermented by gut microbiota; they mainly comprise soluble fibers, resistant starch (RS), and oligosaccharides. These macromolecules can boost the health of the host by selectively stimulating the growth and/or activity of one or a few microorganisms in the colon [16,62]. Dietary fibers can be categorized into soluble or insoluble forms. Soluble forms can be readily metabolized by gut bacteria to SCFA, whereas insoluble forms resist fermentation [8]. It has been shown that pathogenesis induced in the colonic mucus layer by low fiber consumption and high meat intake can be prevented by fiber supplementation with enriched *Bifidobacteria* [63,64]. In addition, it has been proved that prudent diets rich in fiber are related to lower-risk CRC, especially *Butyrivibrio fibrosolvens*- or *Fn*-enriched CRC [65,66]. However, a diet rich in soluble fiber, but not insoluble fiber, such as inulin, can induce HCC in a mouse model [8]. The development of HCC could be prevented by reducing the levels of SCFA via the inhibition of fermenting bacteria or their fermentation process [8]. RS is one of the most studied prebiotics and is able to promote the growth of bacteria involved in the production of butyrate. The latter is a well-known postbiotic with anti-inflammatory and anticancer activities [16]. Pancreatic cancer xenografts of mice on an engineered resistant-starch (ERS) diet displayed significant retardation in tumor growth. This suggested the use of ERS dietary intervention as a potential alternative adjuvant treatment for patients with pancreatic cancer [67]. In addition to RS, in mice bearing transplantable liver tumors, inulin and fructooligosaccharides (FOS) dietary treatment potentiated the effect of six cytotoxic drugs [5-FU, doxorubicin (ADM), vincristine, cyclophosphamide, methotrexate, and cytarabine], and was further capable of increasing the lifespan of the animals [68]. FOS, β (1-4) galacto-oligosaccharides (GOS), and lactulose have been shown to be involved in the increased levels of lactate and SCFA in the GI tract and the decreased level of secondary bile acids in feces, suggesting a significant role in CRC prevention [69].

Other food ingredients in diets also exert a cancer-preventive effect, which might be linked to their capacity to influence the gut microbiome. For example, higher marine omega-3 fatty acid (MO3FA) intake is associated with alleviated CRC pathological progress resulting from the increased abundance of SCFA-producing bacteria (mostly *Lactobacillus* and *Bifidobacteria*), and the decreased richness of *Fn*, *Akkermansia* and lipopolysaccharide-producing bacteria (e.g., *E. coli*) [63,70,71]. Furthermore, exercise can also affect the composition and function of the gut microbiome. Professional athletes have a higher variety of their microbiota as determined by their fecal samples and higher production of immune-friendly secondary metabolites, such as SCFA. This suggests the potential role of exercise in enhancing the survival of patients with CRC by restraining the immune system [63,72].

Difficulties and possibilities associated with gut microbiome manipulation in cancer management

A variety of approaches to manipulate the gut microbiome have been discussed to improve the therapeutic outcomes of patients with cancer. However, the adoption of these strategies includes several concerns, as discussed below.

Concerns regarding antibiotic treatment

Since the middle of the previous century, antibiotics have revolutionized the treatment of infections and exerted considerable impacts on extending life expectancy of humans. However, it is well known that antimicrobial agents affect the pathogens to which they are directed and further impact other members of the gut microbiome [73]. For example, streptomycin treatment can deplete butyrate-producing *Clostridia* from the mouse intestinal lumen, leading to decreased butyrate levels, increased epithelial oxygenation, and aerobic expansion of *Salmonella enterica* serovar Typhimurium [74]. In addition, three broad-spectrum antimicrobials (vanomycin, metronidazole, and lacticin 3147) can cause dramatic shifts in the relative proportions of the dominant phyla of the human microbiota, as determined by in a distal colon model. These changes include population shifts from *Firmicutes* to *Proteobacteria* in the presence of these antimicrobials [75]. In addition, a study demonstrated that healthy volunteers treated for 1 week or less with antibiotics reported effects on their bacterial flora that persisted 6 months to 2 years after treatment, including a dramatic loss in diversity, such as the predominance of a specific taxa, an insurgence of antibiotic-resistant strains, and an upregulation of antibiotic-resistance genes (ARGs) [73]. Numerous examples have been reported that are similar to these and strongly support the notion that antibiotics have a tremendous impact on the composition and functionality of the human microbiome. Therefore, the discovery of complementary strategies is imperative and various approaches have been proposed to address this issue.

Novel antimicrobial agents can bypass common modes of multidrug resistance, while retaining their selectivity for individual strains [76]. In a fecal-culture system, which mimics the human colon, thuricin-CD, a bacteriocin produced by *Bacillus thuringiensis*, was reported to target *Clostridium difficile*. Thuricin-CD was detected as an effective agent with equivalent potency to that of vancomycin or metronidazole but had no impact on microbial composition [75,77]. In addition, previous reports revealed the potential of creating narrow-spectrum therapies via antibacterial drug combinations [78]. Over 20% of the drug–drug interactions displayed strain specificity and 70% were species specific among six strains from three Gram-negative pathogens [78]. The use of synergies that are specific to pathogens and of antagonisms that are specific to abundant commensals can enable drug combinations to impart species specificity to drug action [78]. Furthermore, nonantibiotic drugs, such as food additives, might also offer a new path for narrowing spectrum therapies; for example, vanillin was reported to potentiate the activity of spectinomycin in *E. coli* multidrug-resistant isolates [78]. An additional strategy that can be used includes the combined use of the CRISPR/Cas9 system and phagemids to eliminate selected bacterial targets. CRISPR/Cas9 is a

bacterial immune system that can be used to edit genes of specific organisms. Phagemids carrying CRISPR/Cas9 components can be constructed to cleave ARGs on chromosomes or plasmids of specific pathogens. The advantage of this approach lies in the ability of the CRISPR/Cas9 phagemids to selectively eliminate the target strains, while sparing nontarget strains in a consortium of two to three bacterial isolates of the same strain [76,77,79,80].

Concerns regarding probiotic agents

Unlike chemical drugs, the composition and purity of which can be determined with precision, viable bacteria must be cultured, often in complex media. Bacteria can mutate to either gain or lose functions upon extended culturing despite their maintenance in a pure form [52]. Although empirical probiotics are commonly used by healthy individuals to improve their life quality and prevent diseases, the efficacy of probiotic gut mucosal colonization remains debatable. In fact, both host and indigenous microbiome factors contribute to differential colonization susceptibility to probiotics, possibly via competitive exclusion of related species and site-specific immune responses [81]. Therefore, the efficient design of novel probiotics requires estimation of interindividual variability [81]. In addition to the efficacy, the safety of viable microorganisms use is another question requiring attention. Extensive analyses have uncovered the impact of distinct commensal bacterial compositions on the development of obesity [82], autoimmunity [83], intestinal inflammation [84], and metabolic syndrome [85]. The broad influence of commensal bacterial species on a variety of health issues also requires consideration for microbiome reconstitution. Therefore, studies that involve next-generation probiotics require long-term follow-up to evaluate the development of such diseases in study participants.

Concerns regarding current study methods

Over the past decade, most studies that examined the role of microbiome manipulation in cancer treatment used animal models. Despite similarities in phylum composition, substantial differences in lower taxa exist between the human and murine microbiomes. Mice have smaller and more homogeneous repertoires of commensal bacteria compared with humans [44]. For example, antibiotics specific for Gram-positive bacteria were reported to decrease the anticancer activity of CTX in animal models [45]. However, follow-up clinical studies in patients with chronic lymphocytic leukemia (CLL) treated with CTX and DDP confirmed that antibiotic treatment targeting Gram-positive bacteria was independently associated with shorter progression-free survival and overall survival of the patients [86]. Thus, it remains debatable whether findings obtained in mice could be replicated in human studies and whether they can alter the future options for cancer treatment. Ultimately, the assessment of microbiome manipulations will have to rely on interventional clinical trials. At present, the establishment of PDXs or FMT could help translate results gained from animal models to the clinical setting. Furthermore, the systematic tracking and recording of the effects of antibiotic use in patients, in the context of immunotherapy administration, will be important for understanding the pertinent clinical impact. Specific interactions need to be clearly determined between the composition of the microbiome and the germline polymorphisms of the host. The effects of diet and exercise require further clarification. As such, an integrated analysis of multiple dimensions of data in patients could reveal additional pathways regarding the biology of commensal bacteria and their impact on treatment outcomes.

It has been suggested that the gut microbiome should be included in precision medicine strategies as a potential treatment modifier based on studies that have examined its interactions with antitumor therapies [13]. Molecular pathological epidemiology (MPE) is an approach that successfully demonstrates the interaction of pathology and data science, and is recommended for extensive application in this field [87]. MPE is an integrative transdisciplinary science that integrates the methodology of molecular pathology to population-based epidemiological research [5]. Unlike conventional epidemiology, MPE aims to clarify associations between exposed factors and a specific disease subtype classified by molecular pathology instead of a single disease entity [5]. Currently, MPE has been successfully applied in the treatment of lung, breast, prostate, and colorectal cancers, while further integration of several disciplines into MPE has also been achieved, such as pharmaco-MPE, immune-MPE, and microbial MPE [88,89]. Microbial MPE addresses etiological heterogeneity according to carcinoma subgroups classified by tumor tissue microbial profiling [5]. A previous study that utilized two large prospective cohorts reported that prudent diets (rich in whole grains and dietary fiber) correlated with a lower risk for developing *Fn*-positive but not *Fn*-negative CRC [65]. These findings suggested that diet altered the gut microbiome to promote or inhibit colorectal carcinogenesis and that dietary interventions could be useful in cancer prevention and precision medicine. Therefore, by utilizing quantitative PCR and 16S rRNA next-generation sequencing (NGS), several cancer types can be classified into subtypes based on the heterogeneity of bacteria, such as *Fn*-positive CRC. This molecular subtyping can be used to gather similar pathogenic cases to enhance statistical inference, even with a relatively small number of cases [65,90].

Concluding remarks

In conclusion, the efficacy of immunotherapy and chemotherapy is affected by the microbial composition under various types of mechanism, including microbial translocation to other sites, bacterial metabolism, modulation of the immune system, and influence on ecological network function. Importantly, supplementation with specific probiotics or prebiotics and the activation of the favorable gut microbiome or the inhibition of the unfavorable microbiome via narrow-spectrum antibiotics can offer improved tumor control or enhanced drug efficacy, as demonstrated by various animal models. Moreover, alterations in diet and lifestyle could also prevent carcinogenesis and cancer progression. However, the proper use of antibiotics or probiotics, the feasibility of clinical trials, and the substantial interpatient variability in the microbiome pose challenges to all scientists. Despite these difficulties, the microbial-MPE approach has offered valuable insights for the development of precision medicine. Through further investigations into the interaction between drugs and the microbiome, and the combined application of molecular-editing techniques, such as the CRISPR/Cas9 system, it is likely that microbiome manipulation for cancer management will become a reality.

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