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The role of the microbiome in drug resistance in gastrointestinal cancers

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Abstract

Introduction: The microbiota is recognized for its impact on both human health and disease. The human microbiota is made up of trillions of cells, including bacteria, viruses, and fungi. The largest population of microbes reside in the gut, prompting research for better understanding of the impact of gastrointestinal microbiota in different diseases. Evidence from numerous studies has pointed out the role of commensal microbes as key determinants of cancer pathogenesis. Moreover, gut microbiota may play an important role in chemoresistance; consequently, this knowledge might be important for novel strategies to improve anticancer treatment efficacy.

Areas covered: We describe the role of microbiota in different gastrointestinal cancer types (esophageal, gastric, colorectal, hepatocellular and pancreatic-biliary tract cancers). Moreover, we analyzed the impact of the microbiota on resistance to anticancer therapies, and, lastly, we focused on possibilities of microbiota modulation to enhance anticancer therapy efficacy.

Expert opinion: Increasing evidence shows that gut microbiota might influence resistance to anticancer treatment, including conventional chemotherapy, immunotherapy, radiotherapy and surgery. Therefore, a better knowledge of gut microbiota and its interactions with anticancer drugs will enable us to develop novel anticancer treatment strategies and subsequently improve the cancer patients' outcome.

Keywords: gut microbiota; tumor microbiota; radiotherapy; chemotherapy resistance; immunotherapy; anticancer drug efficacy

Article highlights:

- Gut microbiota plays an important role in the pathogenesis of cancer
- Different gastrointestinal cancer types are linked to gut microbiota dysbiosis
- Gut microbiota influences efficacy of anticancer treatments, such as conventional chemotherapy, immunotherapy, radiotherapy and surgery
- Gut microbiota modulation could maximize the response to anticancer treatments

1. Introduction

The human microbiota homes approximately 100 trillion communities of microorganisms (bacteria, fungi and viruses) within the epithelial surfaces of the human body [1-3]. The microbiota plays an important role in different physiological functions in humans, such as metabolism, neurological and cardiovascular functions, inflammation and immunity [4,5]. Remarkably, these functions are pathologically influenced by changes in composition of commensal microbes [5,6]. It is estimated that microorganisms could be associated with 15% to 20% of cancers [7]. The largest population of microbes resides in the gut. Therefore, there is an increasing interest in better understanding thea gastrointestinal microbiota or gut microbiota. Some clinical and many preclinical studies support the important role of the gut bacteria in the modulation of host response to anti-tumor drugs, especially conventional chemotherapy and immunotherapy [8].

In this review, we describe the role of microbiota in various cancer types, as summarized in Table 1, and how its modification can influence the response to anticancer treatment, including conventional chemotherapy, immunotherapy, radiotherapy, as well as oncological surgery.

2. Gut microbiota and cancer

2.1 Esophageal cancer

The composition of the microbiota differs from esophagus to rectum throughout life. Given the impact of the gut microbiota on cancer, many efforts have been made to study its correlation with esophageal cancer. In healthy individuals, esophageal harbors a diverse microbial community containing *Streptococcus, Prevotella,* and *Veillonella* [9]. Gagliardi *et al*. [10] showed that *Streptococcus viridans* is the most frequent microorganism residing in the healthy esophagus. Esophageal adenocarcinoma (EAC) is the predominant type of esophageal cancer in North America and Europe, whereas esophageal squamous cell carcinoma (ESCC) is the major esophageal cancer in Asia, Africa, and South America. Barret's esophagus (BE) is the precursor lesion and evaluated for early detection of dysplasia and EAC. The composition of microbiota is different among normal esophagus, BE, EAC and ESCC [11-15]. As compared to the normal microbiota, BE contained reduced abundance of *Streptococcus* and higher proportion of Gram-negative anaerobes, such as *Campylobacter* or *Escherichia*, and microaerophiles [12-14]. The microbiome of EAC and ESCC are poorly described [9]. Generally the microbial diversities of both types cancer decreased; as compared to the normal tissues [11,15], the microbiota of EAC was also featured with increased relative abundance of *Lactobacillu fermentum* [16] and that of ESCC was associated with increased quantity of *Fusobacterium nucleatum* [15]. Moreover, a link between toll-like receptors (TLR) signaling pathway and microbiota has been found[17]. TLRs are highly conserved in evolution and widely expressed on immune cells, where they have an important role in the innate immune system by evoking inflammatory responses. Individual TLRs

play important roles in recognizing specific microbial components derived from pathogens including bacteria, fungi, protozoa and viruses, therefore creating an important link between microbiota and immune system.

2.2 Gastric cancer

The main bacterial inhabitants of the stomach include *Streptococcus*, *Staphylococcus*, *Lactobacillus*, *Peptostreptococcus*, and some types of yeast[18]. It is well known that the strongest risk factor for the onset of gastric cancer is the progression of *Helicobacter pylori* infection. In fact, *H. pylori* presents VacA and CagA, two virulence factors involved in cell migration and carcinogenesis[19]. In particular, VacA, is present in every strain of *H. pylori*, leading to gastric epithelial cells apoptosis[20]. It is also responsible for the immunosuppressive activity that enhance the survival of the cancer cells [21]. CagA is the component of type Ⅳ bacterial secretion system which can undergo phosphorylation by entering gastric epithelial cells, thereby leading to the proliferation of gastric epithelial cells and the onset of carcinomas [22,23]. The *H. pylori* infection is not only a recognized direct risk factor for gastric cancer but it might be also involved indirectly in carcinogenesis, because it alters gastric microbiome by reducing gastric acidity. *H. pylori* has been shown to cause a reduction in relative abundance of *Actinobacteria, Bacteroides* and *Firmicutes* and elevation in abundance of non-*Helicobacter* bacteria from *Proteobacteria*, *Spirochetes* and *Acidobacteria* [24]. Several studies have indicated, using the transgenic insulin-gastrin (INS-GAS) mouse model, that non-*H. pylori* bacteria also contributed to stomach cancer [25]. Lertpiriyapong *et al.* [26] showed that *H. pylori* can act synergistically with bacterial species such as *Clostridium* species, *Lactobacillus murinus* and *Bacterioides* species to promote gastric cancer. Although *H. pylori* infection has the strongest association with the development of gastric cancer, increasing evidence has suggested that non-*H. pylori* bacteria may also play a role in the onset of gastric cancer [27,28]. The relative abundance of genera *Prevotella, Streptococcus* were frequently found higher in the tumor tissues than the non-tumor tissues. Interestingly, mycoplasma infections are more abundant in gastric cancer tissues than in other gastric diseases, and in particularly *M. hyorhinis* seems to be found in highly differentiated tissues[3].

2.3 Colorectal cancer

Generally, the colon comes into contact with a large number of microorganisms. Most colon microbiota are composed by *Bacteroides* and *Firmicute*s, but also *Proteobacteria* and *Fusobacteria* [29]. Microbiota might increase colorectal cancer (CRC) risk [30]. The way in which these microorganisms can contribute to the pathogenesis of cancer is the production of toxic metabolites or direct effects. Considering various microorganisms studies, some specific bacteria such as *Escherichia*, *Bacteroides*, *Enterococcus faecalis*, *Streptococcus gallolyticus* and *Clostridium septium* seem to be most commonly correlated with the onset of colon cancer [31,32]. In particular, *Escherichia*, *Bacteroides*, *Enterococcus* and *Clostridium* could directly promote colorectal carcinogenesis in mice [33]. Human studies showed that the gut microbiota related to

CRC was different from healthy individuals, the former has a higher species diversity as well as increased abundance of procarcinogenic taxa [32,34,35]. For example, increased abundance (20-50%) of *S. gallolyticus* was found in the microbiota related to colon cancer compared to in normal colon (5%) tissues [36]. Patients with colorectal adenomas showed a lower abundance of *Bacteroides* and a higher of *Proteobacteria* as compared with the subjects without adenomas [33]. In addition, *Fusobacteria nucleatum* is also considered to play an important role in colorectal cancer development and possibly affects patient survival outcome. Higher abundance of *F. nucleatum* was detected in the stool samples from colorectal adenoma and carcinoma patients than those from healthy subjects [31, 37]. The CRC patients with high levels of *F. nucleatum* had a significantly shorter survival time than those with low levels of *F. nucleatum* [37]. Considering that the abundance of some key microorganisms seems to be important in increasing the risk for colorectal cancer, it has been suggested that specific microbial abundance could represent an early diagnostic tool. Of note, diet could also represent an indirect factor involved because microorganisms use bile acids primarily for their metabolism and these are involved in some cancer-related events, such as apoptosis and cell proliferation[38].

2.4 Hepatobiliary and pancreatic cancers

Hepatobiliary and pancreatic cancers are aggressive diseases with a poor prognosis [39-42]. Though pancreas, gallbladder and liver are not part of the alimentary canal, these organs are essential to digestion and pancreatic cancer, biliary tract and liver cancer are exposed to the gut microbiome via blood flow through the portal vein [43]. It has been demonstrated that the composition of intestinal microbiota is associated with the progression of non-alcoholic steatohepatitis [44] and liver cirrhosis [45] which are correlated with liver tumor development. Furthermore, a possible role of *H. pylori* and other *Helicobacter species* has been found in hepatocellular cancer. In particular, *Helicobacter hepaticus* may colonize the bile tract and the large intestine and promote liver tumor development in a mouse model [46]. Similarly, biliary tract cancers have been associated with *Helicobacter species*, in particular *H. pylori*, *Helicobacter bilis* and *H. hepatics* [47,48]. In addition, *Salmonella typhi* infection is associated with an increased risk of gallbladder cancer [49].

Interestingly, epidemiologic studies have demonstrated associations of periodontitis with an increased risk of pancreatic cancer development [50]. *Neisseria elongata* and *Porphyromonas gingivalis* in the saliva are associated with an increased risk of pancreatic cancer development [51]. High levels of plasma antibodies against *P. gingivalis* have also been associated with an increased risk of pancreatic cancer [52]. In addition, Mitsuhashi *et al*. [53] have shown that a high amount of *Fusobacterium* species in tumor tissue is independently associated with worse prognosis in patients with pancreatic cancer.

3. Intestinal microbiota and its impact on resistance of anticancer therapies

Microbiota plays an important role in the cell response to anticancer therapy in several ways: by modulating drug efficacy, abolishing the anticancer effect or mediating drug toxicity [8]. Moreover, emerging data support the interaction between diet, microbiota and conventional chemotherapy or immunotherapy. Modification of gut microbiota can be obtained through fecal microbiota transplantation, changes in diet or lifestyle or probiotics administration.

3.1 Conventional chemotherapy

An increasing number of studies has shown that gut microbiota might influence chemoresistance to some frequently used anticancer drugs such as irinotecan, oxaliplatin, cyclophosphamide, 5-fluorouracil, gemcitabine or anthracyclines (Table 2; Figure 1). Gut microbiota can indeed promote or reduce the efficacy of these anticancer drugs. The main findings in this field of research are summarized in the following sections.

3.1.1 Cyclophosphamide

Cyclophosphamide (CTX) is an alkylating agent used both in hematological malignancies and solid tumors. CTX treatment alters the gut microbial composition via the disruption of gut epithelial barrier. Its tumoricidal activity depends on the translocation of selective Gram-positive bacteria, such as *Lactobacillus johnsonii* and *Enterococcus hirae*, from the small intestine into secondary lymphoid organs, where T-helper cells may be activated [54]. The broad spectrum antibiotics vancomycin and colistin diminished the anticancer activity of CTX in mastocytoma- and sarcoma-bearing mice, indicating that the efficacy of CTX is microbiota dependent [55]. Moreover, Al *et al*. [56] showed that nucleotide-binding oligomerization domain 2 (NOD2), an intracellular pattern recognition receptor that senses bacterial peptidoglycan and stimulates host immune response, can function as a gut immune checkpoint regulating the efficacy of CTX. The anticancer activity of CTX was found to be superior in mice presenting a genetic defect in the intestinal NOD2 expression. The analysis of the gut microbiota in these NOD2-deficient animals, identified an overrepresented Gram-negative bacterium, *Barnesiella intestinehominis,* after chemotherapy with CTX. The abundance of *B. intestinihominis* in the colon seemed to be correlated with the superior anticancer efficacy of CTX in NOD2-deficient mice. When CTX was combined with *B. intestinihominis* in mice which contained antibiotics-induced dysbiotic microflora, the reduced tumoricidal activity of CTX was restored. In agreement with these findings, cancer patients who possessed *E. hirae* and *B. intestinihominis* exhibited longer survival after the treatment of CTX [55].

3.1.2 Oxaliplatin

Oxaliplatin is a platinum derivative that is used in conventional chemotherapy of gastrointestinal tumors such as in the combination regimens FOLFOX (colorectal cancer) and FOLFIRINOX (pancreatic cancer). Similar to other platinum-based compounds, oxaliplatin exerts its major therapeutic effect through formation of DNA adducts resulting in DNA damage, which leads to apoptosis. Apoptosis of cancer cells can be caused by formation of these DNA lesions, arrest of DNA synthesis, inhibition of RNA synthesis, and triggering of immunologic reactions [57]. Ida *et al.* [58] investigated how gut microbiota influenced the efficacy of anticancer agents oxaliplatin and cisplatin in MC38 and B16 tumor-bearing mice. The researchers discovered that a healthy microbiota could enhance the efficacy of these two agents by inducing reactive oxygen species (ROS) release from myeloid cells, thereby enhancing inflammatory cytokine production and tumor regression. However, when the microbiota was eliminated by antibiotics, the efficacy of oxaliplatin was largely impaired. A similar reduction in efficacy due to the absence of resident flora was also observed in germ-free mice [58]. These results underline that cancer treatment could be improved by the modulation of human gut microbiota.

3.1.3 Anthracyclines

Anthracyclines intercalate between the base pairs of DNA (or RNA), thus inhibiting the DNA replication and transcription of RNA, resulting in a decrease in replication of fast growing cancer cells. Anthracyclines are synthesized by *Streptomyces* strains. As an antibiotic, it can modulate the composition of the gut microbiota through bacteriostatic effect. In contrast, a few bacterial species can metabolize (or detoxify) anthracyclines[59]. *Streptomyces* WAC04685 can inactivate doxorubicin via a deglycosylation mechanism [60]. Using the same mechanism, another gut bacteria *Raoultella planticola* is able to deglycosylates doxorubicin to the metabolites 7-deoxydoxorubicinol and 7-deoxydoxorubicinolone[61].

Notably, the use of anthracyclines is limited due to cumulative toxicity in nontumor tissues, due to their mechanism of action. For instance, the drug can damage gut mucosal tissue by inducing apoptosis of epithelial cells in the jejunum. Therefore, detoxification of doxorubicin has been an important topic for the clinical use of this drug. The findings mentioned above indicate the potential of gut microbiome modulation (by increasing the amount of *R. planticola*) for the detoxification of anthracyclines and extend their clinical anticancer application.

3.1.4 Irinotecan

Irinotecan hydrochloride (CPT-11) is a topoisomerase-1 inhibitor frequently used in combination with other anticancer drugs in the treatment of different gastrointestinal cancers (FOLFIRI for colon cancer and FOLFIRINOX for pancreatic cancer). CPT-11 is converted by liver carboxylesterases into the active metabolite SN-38. SN-38 can be further metabolized by uridine diphosphate glucuronosyl- transferase 1As

(UGT1As) to a non-toxic glucuronide SN-38G and being excreted via the biliary ducts [62,63]. SN-38 causes toxicity by damaging crypt cells of the cecum and by inducing submucosal inflammation [64]. Hence high SN38 levels can cause side effects such as diarrhea. Consequently, the patients have to adjust the doses frequently. Because resident microbial β-glucuronidases (GUS) in the intestines can convert the inactive SN-38G back to the active and toxic SN-38 [62,65], microbial GUS are believed to be responsible for the side effects of CPT-11. Using a rat model, Lin *et al.* [66] demonstrated that CPT-11-based chemotherapy induced microbial dysbiosis in the gut by favoring the potentially pathogenic bacteria, such as *Enterobacteriaceae* and *Clostridium spp.* while reducing the beneficial bacteria such as *Lactobacillus spp.* and *Bifidobacterium spp.* Interestingly, although GUS activity has been identified in all major bacterial phyla of the gut microbiota, such as *Bacterioidetes*, *Firmicutes*, *Proteobacteria* and *Actinobacteria*[67,68], the specific GUS from different bacterial phyla was recently shown to have different substrate preferences[67]. Dashnyam *et al.*[67] showed that the GUS of enterobacteria and opportunistic bacteria, including *Escherichia coli* and *Clostridium spp.* can be the key players mediating the CPT-11-induced toxicity in the gut; whereas the GUS of commensal bacteria, such as *Bifidobacterium spp.* are less active in the SN-38 conversion. Hence the side-effects caused by CPT-11 might be reduced by promoting the homeostasis of gut microbiota through the enhancement of beneficial gut bacteria and suppression of the pathogenic or opportunistic gut bacteria. These findings underline the role of gut microbiota in the modulation of different effects of chemotherapy.

3.1.5 5-Fluorouracil

5-Fluorouracil (5-FU) is a pyrimidine analogue, belonging to the family of antimetabolites [69]. It represents one of the most widely used chemotherapeutic drugs in oncology. Several studies have demonstrated that the efficacy of fluoropyrimidines can be reduced by the presence of mycoplasma species or bacteria. In particular, *F. nucleatum* infection has been linked with resistance of colorectal cancer to 5-FU via upregulation of Baculoviral IAP Repeat Containing 3 (BIRC3) expression, an inhibitor apoptotic protein (IAP). The abundant presence of *F. nucleatum* in colorectal cancer patients has indeed been correlated with the resistance to a chemotherapy cocktail containing tegafur and oxaliplatin [70]. Moreover, *F. nucleatum* plays an important role in colon cancer microenvironment and interacts with the immune cells in different ways: it increases tumor-associated neutrophils, dendritic cells and pro-cancer M2 macrophages but also prevents the cytotoxicity of T and NK cells, resulting in reduced ability of immune host system [3,71,72]. Macrophages are key participants in tumor pathogenesis. They can be divided into two general classes (M1 and M2) based on function [73]. M1 possess anti-tumor functions whereas M2 tumor associated macrophages (TAMs) promote tumor growth [73].

Furthermore, mycoplasmas inside the tumor microenvironment might interact with fluoropyrimidine analogs. In particular, Bronckaers *et al*. [74], showed that *Mycoplasma-hyorhinis*-infected cell lines reduced

the activity of pyrimidine nucleoside analogues directly in the tumor cells. The cytostatic activity of 5-fluoro-2'-deoxyuridine and trifluorothymidine was dramatically reduced (20-150-fold) by degradation to the less active base, 5FU or the inactive trifluorothymine, respectively. The efficacy could be restored completely by the thymidine phosphorylase inhibitor, TPI ((5-chloro-6-[1-(2-iminopyrrolidinyl)methyl]uracil hydrochloride), Since TPI is part of the clinically approved formulation TAS-102 [69], mycoplasma contamination would not affect the efficacy of TAS-102. In contrast *Mycoplasma-hyorhinis* infection increased the efficacy of 5-fluoro-5'-deoxyuridine (DFUR) which needs activation to 5FU. DFUR is an intermediate of the 5FU prodrug, capecitabine (Xeloda) [69]. Moreover, *F. nucleatum* is responsible for the chemoresistance to 5-FU and oxaliplatin in patients with colorectal cancer because of the activation of innate immune system, while in cell culture it was also demonstrated that *F. nucleatum* induced autophagy mediated via microRNA (miR-4802 and miR-18a*) downregulation leading to 5FU and oxaliplatin resistance [75]. Chloroquine, an autophagy lysosomal inhibitor, blocked the autophagic flux. Therefore, the combination of fluoropyrimidine-based therapy with antibiotics or anti-mycoplasma agents could improve the efficacy of some anticancer drugs [3,74], while for other drugs the mycoplasma infection might even be beneficial [74].

3.1.6 Gemcitabine

Gemcitabine is a pyrimidine nucleoside antimetabolite frequently used in the treatment of pancreatic or biliary tract cancer [76]. Several studies have demonstrated that microbiota reduces the efficacy of gemcitabine. In particular, different bacterial species within pancreatic cancer tissues and microenvironment has been described to be responsible for gemcitabine resistance [77]. This intratumor microbiota can produce bacterial cytidine deaminase (CDD), an enzyme that metabolizes gemcitabine into its inactive metabolite 2',2;-difluoro-2'-deoxyuridine (dFdU), primarily by the long form of CDD (CDD_L). One of the most common bacterial class found in pancreatic cancer tissue is *Gammaproteobacteria*. *Gammaproteobacteria* which can express CDD_L leading to inefficacy of gemcitabine. The resistance to gemcitabine could be neutralized by some antibiotics such as ciprofloxacin [77, 78]. Moreover, Vande Voorde et al. [3] demonstrated that the *Mycoplasma hyorhinis* -infected tumor cells were considerably less sensitive to gemcitabine than the non-infected cells, as was also found by Geller et al [77], both in *in vitro* (using RKO colorectal cancer cells) and *in vivo* (by using infected MC-26 tumors) systems. These authors also demonstrated an increased degradation of gemcitabine to its inactive metabolite dFdU. The reduced sensitivity is attributed to the breakdown of gemcitabine by mycoplasma CDD [3]. Although gemcitabine is not a substrate for pyrimidine nucleoside phosphorylase, a high mycoplasma pyrimidine nucleoside phosphorylase was also related to a lower sensitivity to gemcitabine, which can be explained by degradation of normal nucleosides so that they cannot compete with gemcitabine for deamination by CDD.

Other gut bacterial species such as *F. nucleatum* or *E. coli* have been reported to induce gemcitabine resistance as well, but the molecular mechanisms underlying these effects are largely unknown [77-79].

3.2 Immunotherapy

The microbiome modulates the immune system, both local and systemic, and it may also affect the efficacy of immunotherapy [80]. The interaction between immune checkpoint (IC) molecules on tumor cells and their receptors on host immune cells is responsible for host immune response to cancer cells. The blockade of IC molecules is used in oncology, by using antibodies against programmed cell death 1 (PD-1), programmed cell death 1 ligand 1 (PD-L1), and cytotoxic T-lymphocyte associated protein 4 (CTLA-4), which influence anti-tumor immunity [81].

Unfortunately, the patients' responses to immune checkpoint inhibitor (ICI) therapy vary and are often transient. So far, ICI therapy is only effective in 10–30% of treated patients [82]. Several studies have shown that abnormal gut microbiota composition can induce resistance to ICI [5,83].

3.2.1 Anti-CTLA-4

Ipilimumab is a monoclonal antibody that neutralizes CTLA-4. It is mainly used for treatment of metastatic melanoma [84,85]. It has been shown that its efficacy relies on the intestinal microbiota, in particular, *Bacteroidales* and *Burkholderiales* [86-88]. During ipilimumab therapy, *Bacteroides fragilis* is overrepresented in the ileum, followed by a splenic Th1 cell memory response against *B. fragilis* polysaccharide. A recent study performed a fecal microbial transplantation (FMT) of feces harvested from patients with metastatic melanoma into germ-free mice with tumor. The mice were subsequently treated with ipilimumab. The results showed that the feces enriched with *Bacteriodes* spp. facilitated the colonization of *Bacteriodes fragilis* and *Bacteriodes thetaiotaomicron* in mice. This group responded the best to ipilimumab treatment, and the size of the tumors was negatively correlated with the outgrowth of *Bacteriodes fragilis*. This example clearly demonstrates that the composition of the gut microbiota can be modulated by an anticancer drug, which in turn affects the anticancer efficacy of the drug.

3.2.2 Anti-PD1 and anti-PD-L1

Similarly, the antitumor sensitivity of monoclonal antibodies against-PD1 or its ligand PD-L1 was shown to be affected by *Bifidobacterium longum* and *Bifidobacterium breve* [89]. Several reports highlighted that antibiotic-induced dysbiosis compromised the anti-PD1 efficacy in lung and renal cancer patients [83]. The presence of *Akkermansia muciniphila* in stools of cancer patients was, however, linked to a favorable clinical outcome during anti-PD1 therapy. Gopalakrishnan *et al.* [87] studied metastatic melanoma patients treated with immunotherapy. Good responders to immunotherapy possessed a characteristic gut microbiome, featured with high diversity and high abundance of *Clostridiales/Ruminococcaceae*. This

"microbiome signature" was associated with an enhanced systemic T cell immune response. In contrast, poor responders to immunotherapy presented microbiome with low diversity and high abundance of *Bacteroidales*, which was associated with a suppressive immune reply.

Interestingly, a direct link between the gut microbiota and efficacy/toxicity of ICI treatment exists [84]. The authors used germ-free mice transplanted with fecal microbiota from anti-PD-1 good or poor responders. The addition of *Clostridiales* species and *Faecalibacterium* in the gut of mice leads to better efficacy/response to anti- PD1/PD-L1 therapy [85].

3.3 Radiotherapy

Ionizing radiation directly induces DNA damage through the production of ROS or reactive nitrogen species (RNS) [90,91]. Furthermore, radiotherapy can induce local immunogenic effects and stimulate the innate immune system [92,93]. Several studies indicate that the intestinal microbiota can play an important role in modulation of systemic immune response to radio sensitivity and radio-induced toxicity [94]. Ferreira *et al*. [95] examined 128 patients who underwent high-dose intensity-modulated radiotherapy and found that radiotherapy considerably reduced the diversity of gut microbiota and induced high abundance of *Clostridium IV*, *Roseburia* and *Phascolarctobacterium*. This shift in microbial composition seemed to coincide with the reduction of homeostatic intestinal mucosa cytokines and could cause radiation-induced side-effects [95].

3.4 Surgery

Anastomotic leak (AL) in the most common life-threatening postoperative complication, reported in 1% to 19% of patients [96,97]. Despite advances in surgical techniques and patient selection, the rate of AL has remained the same [98]. The risk factors and pathogenesis of AL are not yet clear. The importance of gut microbiota in AL occurrence has become increasingly acknowledged. Cohn and Rives [99] were the first to demonstrate that the gut and specific colon microbiome played a role in AL. The intraluminal use of antibiotic provides protection to the devascularized segment of colon. The gut microbiome may function in two ways. On one hand, gut bacteria *Enterococcus faecalis* and *Pseudomonas aeruginosa* contribute to AL via their collagenolytic and matrix metalloproteinase 9-activation functions [98,100]; one the other hand, the gut microbiome may promote anastomotic healing through the short-chain fatty acid producing bacteria, which show beneficial effects on anastomotic integrity and suppress the growth of deleterious bacterial pathogens [101,102].

4. Modulation of the gut microbiota to enhance therapy efficacy

As reported in the above-mentioned studies gut microbiota has important effects through its ability to metabolize drugs, including anticancer drugs. This activity can induce a superior or inferior activity of the drug with decreased or increased toxicity. As an example, *Bacteroides spp.,* resident in the gastrointestinal tract, accelerate the conversion of sorivudine (synthetic analogue of thymidine used as an antiviral agent) into bromovinyluracil (BVU), which is an intermediate product that inhibits 5-FU degradation by the enzyme dihydropyrimidine dehydrogenase. BVU accumulates in the blood, resulting in an increased (sometimes lethal) toxicity [103] in patients taking oral UFT (a combination of ftorafur, a 5FU prodrug, with uracil).

The link between 5-FU activity and dysbiosis was extensively studied in animal models [103]; after the administration of this drug, the abundance of *Staphylococcus* and *Clostridium* was higher, while that of *Bacteroides* and *Lactobacillus* was reduced. This suggests that the microbiome could represent a target for treatment; taking probiotics together with 5-FU could be a useful opportunity. Probiotics have also been studied because of their effect on cachexia in cancer patients. Schieber *et al*. [104] have demonstrated that infection with *E. coli* in mice protects against cancer-induced muscle degradation. Thus, microbiota can directly influence the efficacy of chemotherapy

In addition, antibiotics which are often used to control the side effects of chemotherapy can influence the efficacy of treatment [8]. For example, some gram-positive bacteria species such as *Lactobacillus murinus, Lactobacillus johnsonii* were found to be important for the modulation of the antitumor activity of CTX. Therefore, antibiotics that are specifically directed against gram-positive bacteria can reduce cancer therapy efficacy [54].

These results demonstrate that the microbiome could be exploited as a useful biomarker to evaluate the therapy efficacy: more studies should be directed to investigate this clinically relevant role. Importantly, the possible negative efficacy modulation of anticancer drug if combined with antibiotics should also be taken in consideration.

5. Expert opinion

The gut microbiota has a strong influence on the efficacy of anticancer therapy. On one hand, it may impair the efficacy of one therapy or enhance the side effects; on the other hand, it may promote the efficacy of another type of therapy or reduce the side effects. Conventional chemotherapy and novel anti-signaling targeted therapy may affect the microbiota as is evident by the damage that these anti-cancer drugs may cause to the intestinal barrier, facilitating translocation of the resident flora which change the response to microorganisms [105]..For certain therapies, such as irinotecan, a few bacterial species in the microbiota are detrimental whereas other bacterial species can be beneficial. In this case, antibiotics may also control irinotecan induced toxicity as was found for inhibitors of bacterial β-glucuronidase (amoxapine), which reduce irinotecan mediate mucositis [106]. Therefore, the function of the gut microbiota depends on the specific anticancer agent, as also illustrated in Figure 1. The populations of “favorable” and “unfavorable”

microbiota could be enriched or reduced to obtain a better therapeutic efficacy and less toxicity [109,110]. Thus, modulation of a microbial network through fecal transplantation or probiotics, can be a promising strategy to obtain a superior efficacy of treatment of cancer patients. We should also keep in mind that human microbiota is a diverse and highly heterogenous community. Many microbiome studies revealed that the human microbiota is highly variable both within and between individuals. But individual microbiota is also very stable. Samples obtained from the same individual over time are more similar to one another than those from different individuals [111]. The reported success of microbiota transplantation in treating the recurrent difficile-associated disease [112] gives the hope for the therapy of microbiota modulation.

The use of antibiotics is often suggested to be a method to eliminate the "unwanted" microbes. However, their effects remain insufficient specific. Antibiotics might induce microbiota dysbiosis, which can promote chemoresistance and affect treatment outcome [83,86].. Furthermore, patients with lung or renal cancer who were treated with antibiotics within one month of the initiation of ICI therapy have a worse clinical outcome [110], though, in contrast, antibiotic treatment is associated with improved efficacy of ICI therapy for patients with pancreatic cancer, in which intratumoral microbes generate an immunosuppressive tumor microenvironment 113,114]. Thus, the effects of antibiotics on immunotherapeutic efficacy may depend both on the therapy and on the type of cancer.

Interestingly, specific targeting of tumor microbiota can take advantage of the hypoxic tumor microenvironments, such as anaerobic bacteria which can home the tumors. As an example, the intravenously injected spores of strictly anaerobic *Clostridium* species can germinate in the hypoxic regions present in solid tumors and nowhere else in the body [115]. This unique feature has been used as an anti-tumor strategy since the beginning of the 21st century. The non-toxinogenic strain of *Clostridium, C. novyi-NT,* has been genetically engineered to carry the anticancer drug. The initial application of this strategy in a mouse xenograft model of human colon carcinoma achieved substantial tumor suppression [116]. This encouraging result underlines the great potential of genetically engineered of gut microbes as novel antitumor therapeutic approach.

Another aspect of modulating gut microbiota is the change in metabolism by diet or probiotics [109. 117, 118]; this may not only modulate metabolism of normal metabolites, but also signaling, miRNA expression and cross-talk between microbiota and mammalian cells (normal and tumor), subsequently altering the host response to the drug. It has also been demonstrated that crosstalk may be mediated by extracellular vesicles, which can carry not only proteins but also RNA and DNA , which may modulate genetic expression [119]

6. Conclusions

Despite clinical success obtained with many anticancer therapeutic regimens, heterogeneous response and resistance to chemotherapy and immunotherapy remain the hallmarks of cancer therapy. Emerging

evidence revealed that there is a correlation between the microbiota and chemoresistance as summarized in scheme 1. Therefore, combining anticancer treatment with microbiome-modulating regimens (antibiotics, probiotics, and diet) might provide novel therapeutic strategies to treat cancers that have a correlation with dysbiosis.

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Table 1: Possible link between the human microbiome and cancers in the gastrointestinal tract

Intestinal tract	Normal tissue Microbiota	Tumor tissue Genus or species	Effect/Cancer risk	Ref.
Esophagus	*Streptococcus*	*Lactobacillus fermentum*	Higher abundance EAC	[16,17,
	Prevotella	*Campylobacter*	Lower abundance in EAC	107,108]
	Veillonella	*Escherichia Coli*	Associated with EAC	
Stomach	*Streptococcus*	*Helicobacter pylori*	Carcinogenesis	[3,18,
	Staphylococcus	*Clostridium*		19,28]
	Lactobacillus	*Lactobacillus*		
	Peptostreptococcu	*Bacterioides*		
	Yeast	*Mycoplasma hyorhinis*	Higher abundance in GC	
Colon	*Bacteroides*	*Clostridium septicum*	Carcinogenesis	[31,32,
	Firmicutes	*Enterococcus faecalis*	Carcinogenesis	34,36,37]
	Proteobacteria	*Streptococcus gallolyticus*	Higher abundance in CRC	
	Fusobacteria	*Fusobacterium nucleatum*	Carcinogenesis	
		Bacteroides	Lower abundance in CRA	
		Proteobacteria	Higher abundance in CRA	
Liver	/	*Helicobacter pylori*	Carcinogenesis	[46-49]
		Helicobacter hepaticus	Carcinogenesis	
		Helicobacter bilis	Carcinogenesis	
		Salmonella. tyohi	Carcinogenesis	
Pancreas	/	*Neisseria elongate*	Carcinogenesis	[51-53]
		Porphyromonas gingivalis	Carcinogenesis	
		Fusobacterium/	Higher abundance in PDAC	

EAC, Esophageal adenocarcinoma; GC, gastric cancer; CRC, colorectal cancer; CRA, colorectal adenomas; PDAC, pancreatic ductal adenocarcinoma

Table 2. Potential role of microbiome in gastrointestinal cancer therapy

Therapy	Drugs	Cancer type	microbiome	Effect	Ref.
Conventional chemotherapy	Cyclophosphamide (CTX)	Hematologic malignancies Solid tumors	*Barnesiella intestinehominis* *Lactobacillus johnsonii* *Enterococcus hirae*	Promoted CTX efficacy	[55]
	Oxaliplatin	/	Commensal bacteria	Promoted oxaliplatin efficacy	[58]
	Anthracyclines	/	*Stretomyces* WAC04685	Induced anthracycline resistance and reduced anthracycline efficacy	[60]
	Irinotecan (CPT-11)	Gastrointestinal cancer	*Escherichia Coli* *Staphylococcus* *Clostridium* *Lactobacillus** *Bifidobacterium**	Reduced irinotecan efficacy *Reduce irinotecan side-effect	[67]
	5-fluorouracil (5-FU)	Colorectal cancer	*Fusobacterium nucleatum* *Mycoplasma hyorhinis*	Induced 5-FU resistance and reduced 5-FU efficacy	[70,74]
	Gemcitabine	Pancreatic cancer Biliary tract cancer	*Gammaproteobacteria* *Fusobacterium nucleatum* *Mycoplasma hyorhinis*	Induced gemcitabine resistance and reduced gemcitabine efficacy	[3, 77-79]
Immunotherapy	Anti-CTLA-4	Metastatic melanoma	*Bacteroides fragilis* *Bacteroides thetaiotaomicron* *Burkholderiales*	Promoted ipilimumab efficacy	
	Anti-PD1 & anti-PD-L1	Metastatic melanoma	*Bifidobacterium longum* *B. breve* *Akkermansia muciniphila* *Clostridiales* *Ruminococcaceae* *Faecalibacterium* *Bacteroides thetaiotaomicron* *Bacteroides fragilis**	Promoted anti-PD1 *Reduced anti-PD1 efficacy	[85,87, 89]
Radiotherapy	/	/	*Clostridium* Ⅳ	Induced side-	[95]

			Roseburia Phascolarctobacterium	effects	
Surgery	/	/	*Pseudomonas aeruginosa*	Reduced postoperative complications	[101, 102]

Scheme 1: MAIN MICROBIOTA INVOLVED IN TUMOR TREATMENT AND THEIR EFFECT	
CYCLOPHOSPHAMIDE	
Barnesiella intestinehominis	Enhanced activity of CTX
ANTHRACYCLINES	
Streptomyces Raoultella planticola	Inactivation of Doxorubicin
IRINOTECAN	
Escherichia Coli Clostridium spp	Enhanced toxicity of Irinotecan; reduced efficacy
Bifidobacterium	Reduced side effects
OXALIPLATIN	
Fusobacterium nucleatum	Resistance due to increased autophagy
5-FLUOROURACIL	
Fusobacterium nucleatum	Resistance due to increased autophagy
Mycoplasma-hominis	Reduced activity of 2'-deoxy-5-fluorouridine, but increased effect of 5'-deoxy-5-fluorouridine-(prodrug of Xeloda)
GEMCITABINE	
Gammaproteobacteria	Reduced activity of Gemcitabine by increased degradation
Fusobacterium nucleatum Escherichia Coli	Indirectly increase gemcitabine resistance
Immune Checkpoint Inhibitors	
Bacteroidales	Enhanced activity of Ipilimumab
Bifidobacterium	Increased activity of anti PD1 treatment

Figure 1. Microbiota-drug interactions