Review

Pancreatic Cancer, Gut Microbiota, and Therapeutic Efficacy

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Abstract

Pancreatic cancer remains one of the leading causes of cancer-related death worldwide and has a poor prognosis. Current treatment relies on surgical resection and adjuvant therapies. The gut microbiota plays important roles in metabolism and immunomodulation. Accumulating evidence has implied that the gut microbiota is involved in the metabolism of chemotherapeutic drugs and the tumor microenvironment (TME), which could affect the efficacy of both conventional chemotherapy and immunotherapy for pancreatic cancer. Herein, we comprehensively reviewed the history and highlights of the interactions among pancreatic cancer, the gut microbiota and therapeutic efficacy and showed the promising future of manipulating the gut microbiota to improve clinical outcomes of pancreatic cancer.

Key words: gut microbiota, pancreatic cancer, chemotherapy, immunotherapy, tumor microenvironment

Introduction

Pancreatic ductal adenocarcinoma (PDAC) accounts for more than 85% of pancreatic cancer cases. PDAC is still one of the most devastating malignancies, with a 5-year overall survival of less than 10%. Since less than 20% of PDAC patients have the opportunity for surgical resection, chemotherapy remains the main treatment option. Since its approval by the Food and Drug Administration (FDA) in 1996, gemcitabine has been actively used to treat PDAC that has progressed in the extensive desmoplastic microenvironment surrounding the few remaining cancer cells, but chemoresistance and reduced sensitivity are often acquired during multiple weeks of chemotherapy cycles [1]. This hypovascular and highly desmoplastic tumor tissue leads to poor drug delivery and ineffectiveness of cytotoxic agents [2]. However, morphological characteristics are only partly responsible for resistance. Great efforts have been made to solve this difficult problem. Gemcitabine-based combined therapies, tumor microenvironment (TME)-targeting strategies and immunotherapy are being developed to overcome drug resistance and ineffectiveness in PDAC [3].

The gut microbiota has been recognized as a considerable ecosystem, comprising over $10^{14}$ microorganisms, and it encodes far more genes than the human body [4,5]. Increasing evidence links the microbiota, cancer progression and therapeutic responses [5]. Several population-based studies indicate that oral pathogenic microorganisms are associated with an increased risk of PDAC [6-9]. This may be due to systemic inflammatory and immune responses induced by some specific bacteria and bacterial metabolites, which could regulate cancer-related immunomodulation. Recent data have suggested that the gut microbiota play critical roles in human pancreatic diseases, including pancreatitis and PDAC [10]. Large numbers of bacterial metabolites may participate in the regulation of pancreatic carcinogenesis, the immune system and therapeutic resistance. Intratumoral bacteria have also been found in the TME of PDAC. The interactions between the
host microbiota and therapeutic efficacy may be an important breakthrough in understanding the altered efficacy of chemotherapeutic agents and immunotherapies towards PDAC.

To date, common techniques used to assess microbial communities, e.g., 16S ribosomal RNA (rRNA) sequencing, metagenomic sequencing, quantitative PCR and culturomics, have greatly expanded our knowledge of the diversity and multifunctionality of the microbiota. Herein, we summarize the history and progression of the reciprocal interactions between the gut microbiota and therapeutic efficacy in PDAC and discuss how these developments have paved the way to improve patient survival.

**Therapeutic Dilemma of Pancreatic Cancer**

Difficult issues regarding chemotherapy and immunotherapy of advanced-stage PDAC mainly concentrated on the heterogeneous efficacy of individuals who had the same histopathologic tumor characteristics. Essential factors determining drug sensitivity include pancreatic cancer cells and their surrounding components, such as the extracellular matrix, immune cells (e.g., tumor-associated macrophages [TAMs], myeloid-derived suppressor cells [MDSCs], tumor-associated neutrophils), cancer-associated fibroblasts, pancreatic stellate cells and cancer stem cells, all of which impair the normoxic microenvironment of the pancreas [11,12]. The desmoplastic stroma-rich microenvironment restricts intratumor blood supply and drug delivery. Hypoxia and cancer somatic mutations also contribute to therapeutic resistance [13]. Recent achievements have also promoted clinical research on the TME to increase pharmaceutic penetration into tumor tissue. However, clinical trials of stroma-targeting compounds plus gemcitabine have failed to improve patient survival in metastatic PDAC [14]. PDAC responds poorly to a single application of immune checkpoint inhibitors (ICIs), e.g., anti-programmed cell death protein 1 (PD)-1/anti-PD-L1, anti-CTLA-4, and anti-LAG-3 [15,16]. In most cases, the agents do not work, and their limited effects are offset or compensated under the coordination and crosstalk of multiple suppressive mechanisms of the host immune system.

Recent studies have emphasized the difference in the gut microbiota between cancer patients and healthy individuals. The microbiota of the duodenal mucosa in PDAC patients and healthy controls shared similar species in one study. However, duodenal samples of PDAC were characterized by enrichment with, for example, *Acinetobacter*, *Aquabacterium*, *Oceanobacillus* and *Rahnella* [17]. Limitations exist in determining whether microbiota alterations contribute to tumor progression, and whether the altered host microbiota is merely a concomitant manifestation remains elusive. A considerable proportion of nonantibiotics also influence the growth of bacterial species [18]. A resistant starch diet promoted a decrease in tumor progression in PDAC xenograft mice, which was associated with a reduction in proinflammatory fecal microbiota [19].

The mechanisms of the TME in PDAC that affect the efficacy of therapies remain largely unknown and require further exploration. Recently, accumulating evidence has shown that the gut microbiota has a potential role in regulating cancer-related immunomodulation and treatment, presenting new targets to improve therapeutic efficacy.

**The Role of the Gut Microbiota in Chemotherapy and Immunotherapy**

Based on previous publications in the past decade, the gut microbiota may lead to altered efficacy of pharmacotherapeutics in cancer treatment (Table 1). Although the explicit role of the microbiota in host immunity, especially in the tumor-specific TME, remains unclear, the interactions between tumor control and gut microbiota have become more intertwined than ever before [20,21]. In drug-free conditions, interactions between the host and the gut microbiota involve the mucus layer, epithelial cells, dendritic cells (DCs) and immune cells [22-25]. In cancer patients, summarized evidence implies a bidirectional relationship between the host microbiota and various types of cancer therapies.

**Conventional Chemotherapy**

Previous evidence has demonstrated that both cancer cells and the related TME components participate in cancer development and treatment adaptation. Recent studies revealed that the gut microbiota may contribute to the efficacy of conventional chemotherapeutic agents through drug metabolism, biotransformation and immune regulation [50].

The gut microbiota has favorable effects on chemotherapy *in vitro* and *in vivo*. Previously, the clinical potential for microbial therapeutic use was indicated by the synergism of *Salmonella typhimurium* in mouse models of PDAC treated with gemcitabine and bevacizumab [51]. The culture supernatant of *Lactobacillus plantarum* was shown to have positive effects on improving the chemosensitivity of 5-fluorouracil (5-FU) in colorectal cancer (CRC) cells by inhibiting cancer stem-like cell formation [32]. An intact commensal microbiota, as a modulator in the TME, is required for optimal anticancer drug responses via the functional maintenance of myeloid-
derived cells through Toll-like receptors (TLRs) [35]. Attenuated cytotoxic effects of oxaliplatin were observed in germ-free and antibiotic-treated subcutaneous tumor-bearing animals. An intact microbiota was required for priming tumor-associated myeloid cells that produce reactive oxygen species, which are important for oxaliplatin cytotoxicity [35]. In tumor-bearing mouse models treated with cyclophosphamide (CTX), the gut microbiota promoted an adaptive immune response to restore antitumor efficacy [36]. CD8+ T cells perform important duties in the adaptive antitumor immune response. The commensal bacterial species Enterococcus hirae (E. hirae) and Barnesiella intestinihominis were identified in CTX-induced immunomodulation, with altered TME and enhanced anticaner CTL responses. These bacteria were capable of partially restoring host T cell responses and improving the therapeutic efficacy of CTX or other alkylating agents [31]. Interestingly, translocation of some intestinal bacterial species (gram-positive) into secondary lymphoid organs was observed in response to CTX [36]. Translocated bacteria enhanced the bioactivity of adoptively transferred CD8+ T cells and innate immunity [52]. In addition, chemotherapeutic platinum agents were also found to induce bacterial translocation across the intestinal barrier and activate T helper 1 (Th1) memory responses [53].

However, microbiota, e.g. Fusobacterium nucleatum in CRC, were found to promote chemoresistant status [26,30,54]. A cocktail of antibiotics increased Proteobacteria and reduced 5-FU efficacy in CRC mice [27]. Furthermore, bacterial metabolism was reported to affect the efficacy of CPT, 5-FU and 5-fluoro-2-deoxyuridine (FUDR) against Caenorhabditis elegans [55]. Prior studies investigating chemotherapy-related microbiota alterations revealed a severe imbalance in microbial composition and function, leading to intestinal dysbiosis [56]. Gemicitabine-treated xenografted mouse models of PDAC revealed proinflammatory alterations in the fecal microbiota, with an increase in Proteobacteria and Verrucomicrobia and a decrease in Firmicutes and Bacteroidetes, as well as activation of the NF-kB inflammatory pathway in tumor tissues [57]. The latest multicenter clinical trial revealed that FOLFIRINOX chemotherapy for PDAC led to longer survival than standard gemcitabine adjuvant therapy at the expense of a higher incidence of toxic events, e.g., diarrhea and nausea [58]. The side effect of CPT-11 (irinotecan) chemotherapy, diarrhea, occurs in many cancer cases. CPT-11’s inactive metabolite SN-38G (transferred by carboxylesterase) was restored to its active form by β-glucuronidase–expressing bacteria in the gut, leading to enteral release of the active SN-38 metabolite and severe diarrhea [59].

Using streptomycin reduced enteral epithelium absorption of SN-38 and decreased carboxylesterase activity [60]. Thus, chemotherapeutics induce efficacy, alter the microbiota and cause toxicity, which presents a challenge in achieving optimal anticancer effects and reducing side effects by manipulating the gut microbiota. Collectively, current findings have elucidated the complex influences of the gut microbiota on exogenous drugs and endogenous responses.

**Immunotherapy**

Unlike conventional chemotherapy, immunotherapy targets the immune microenvironment beyond the tumor cells. One of the crucial mediators linking the microbiota to the immune response is TLRs, which are categorized as cytoplasmic pattern recognition receptors. TLR4 binding to bacterial lipopolysaccharides triggers in situ and systemic inflammation. A recent study reported that microbial stimulation of cancer cells overexpressed cathepsin K, which promoted immunosuppressive M2 TAM polarization through the TLR4-mTOR pathway [61]. Attenuated immunocyte-targeting bacterium Listeria monocytogenes modified the suppressive cancer microenvironment by reducing peripheral and intratumor MDSCs and repolarizing the TAM subpopulation from the M2 phenotype to the antitumor M1 phenotype [62,63]. Bacteroides species activate Th1 immune responses and promote the maturation of DCs within tumors [49]. Faecalibacterium and butyrate-producing bacteria were associated with Foxp3+ regulatory T cell (Treg) accumulation in the gut, whereas Bifidobacterium adolescentis, Parabacteroides merdae, Collinsella aerofaciens (C. aerofaciens), and Enterococcus faecium (E. faecium) inhibited Tregs in humans [40,45]. Tregs expressing TLR2 could suppress immune responses in cancer treatment [45,64]. A genetically engineered mouse model (GEMM) has provided us with a more accurate imitation of human cancer progression and natural TME components. Sethi and coworkers found that gut microbial depletion via oral antibiotics caused a significant decrease in pancreatic cancer burden and an activated anticancer immune response in GEMM [65]. C57BL/6 wild-type mice, Rag1 knockout mice lacking mature T and B lymphocytes and Kras^{G12D/+}, Trp53R172H/+, Pdx-1cre (KPC) mice were comparatively analyzed. Microbial ablation led to significant changes in critical components of the TME, presenting as increased populations of IFNγ+CD4+CD3+ T helper 1 cells and IFNγ+CD8+CD3+ cytotoxic T cell 1 (Tc1) population, with a simultaneous decrease in pro-tumor immune cells [65]. These inflammatory cells are usually cancer-associated and infiltrate into the TME, which may influence therapeutic efficacy.
Table 1. Preclinical and clinical studies on the microbiota and therapeutic efficacy against solid tumors in the past decade.

| Studies          | Therapeutic drugs or targets | Microbiota or microbial intervention                                      | Efficacy            | Mechanisms                                                                 |
|------------------|------------------------------|-----------------------------------------------------------------------------|---------------------|-----------------------------------------------------------------------------|
| **Chemotherapy** |                              |                                                                             |                     |                                                                            |
| Zhang et al. [26] | 5-Fluorouracil               | *Fusobacterium nucleatum*                                                   | Nonbeneficial       | Induce BIRC3 expression via the TLR4/NF-κB pathway                          |
| Yuan et al. [27]  | 5-Fluorouracil               | Antibiotics increase Proteobacteria                                          | Nonbeneficial       | -                                                                           |
| Deng et al. [28]  | Tegafur plus oxaliplatin     | *Fusobacterium nucleatum*                                                   | Nonbeneficial       | -                                                                           |
| Geller et al. [29] | Gemcitabine                 | Gammaproteobacteria                                                         | Nonbeneficial       | Bacterial CDD inactivates gemcitabine and autophagy                         |
| Yu et al. [30]    | 5-Fluorouracil/oxaliplatin  | *Fusobacterium nucleatum*                                                   | Nonbeneficial       | Activate TLR4/MyD88 signaling and autophagy                                 |
| Daillère et al. [31] | Cyclophosphamide           | *Enteroctococcus hirae*                                                     | Beneficial          | Translocation increases CD8/Treg ratio within tumor                         |
| An and Ha [32]   | 5-Fluorouracil               | *Barnesiella intestinohominis*                                               | Beneficial          | Increase IFN-γγ+γδ T cells within tumor                                      |
| Lehouritis et al. [33] | Gemcitabine     | *Lactobacillus plantarum*                                                   | Beneficial          | Decrease cancer stem-like cells                                             |
| Vande et al. [34] | Gemcitabine                 | *Mycoplasma hypothesis*                                                     | Nonbeneficial       | Drug modification                                                            |
| lida et al. [35]  | Oxaliplatin/cisplatin        | Antibiotic treatment                                                        | Nonbeneficial       | Reduce myeloid-cell ROS                                                     |
| Vian et al. [36]  | Cyclophosphamide             | *Lactobacillus johnsonii, Lactobacillus murinus, Enterococcus hirae*         | Beneficial          | Induce bacterial translocation, which stimulates pathogenic Th17 and memory Th1 immune responses |

**Immunotherapy (Underlined microbiota were involved in the mechanisms)**

| Studies          | Therapeutic drugs or targets | Microbiota or microbial intervention                                      | Efficacy            | Mechanisms                                                                 |
|------------------|------------------------------|-----------------------------------------------------------------------------|---------------------|-----------------------------------------------------------------------------|
| Zheng et al. [37] | PD-1                        | Akkermansia muciniphila, Ruminococcaceae spp.                               | Beneficial          | -                                                                           |
| Peters et al. [38] | PD-1/CTLA-4                 | Facalibacterium prausnitzii, Coprococcus eutactus, Prevotella stercorarum, Streptococcus sanguinis, Streptococcus anginosus, Lachnocipiraeceum bacteria 3 146AA | Beneficial          | -                                                                           |
| Zhao et al. [39]  | PD-1                        | Bacteroides ovatus, Bacteroides dorei, Bacteroides massiliensis, Ruminococcus gaurus, Blautia producta | Nonbeneficial       | -                                                                           |
| Matson et al. [40] | PD-1                        | Enterococcus lacu, Clostridial aerogenes, Bifidobacterium aditinscens, Klebsiella pneumoniae, Veillonella parouda, Parabacteroides merdah, Lactobacillus sp., Bifidobacterium longum | Nonbeneficial       | Decrease Tregs                                                              |
| Gopalakrishnan et al. [41] | PD-1 | Ruminococcaceae/Faecalibacterium | Nonbeneficial       | Increase peripheral and infiltrating effector T cells                       |
| Pushalkar et al. [42] | PD-1 | Bacteriodales | Nonbeneficial       | -                                                                           |
| Routy et al. [43]  | PD-1                        | Akkermansia muciniphila, Enteroctococcus hirae, Alistipes indistinctus       | Beneficial          | Increase CD4+ central memory T cells, IL-12 secretion of DC, and intratumor CD4/Foxp3 ratios and elicit Th1 immune responses |
| Derosa et al. [44]  | PD-1/CTLA-4                 | Antibiotic treatment                                                        | Nonbeneficial       | -                                                                           |
| Chaput et al. [45]  | CTLA-4                      | Facalibacterium prausnitzii, butyrate-producing bacterium, Gemmiger formitisc | Beneficial          | Induce Tregs in the gut                                                     |
| Frankel et al. [46] | PD-1/CTLA-4                 | Bacteroides cacae, Streptococcus parاضoxangiunis, aceticolae prausnitzii, Bacteroides theiaotamicron, Holdemania filiformis, Dorea formicogenaruns | Nonbeneficial       | -                                                                           |
| Kaderbhai et al. [47] | PD-1 | Antibiotic treatment                                                        | Nonbeneficial       | -                                                                           |
| Sivan et al. [48]  | PD-1                        | Bifidobacterium                                                            | Beneficial          | Induce DC maturation and intratumor CD8+ T cell accumulation               |
| Vetizou et al. [49] | CTLA-4                      | Bacteroides theiaotamicron, Bacteroides fragilis                         | Beneficial          | Synergize with TLR2/TLR4                                                   |
| lida et al. [50]    | IL-10R Plus Cpg oligonucleotide | Alistipes, Ruminococcus                                                   | Beneficial          | Activate tumor-infiltrating myeloid cells via TLR4 and increase TNF response |

The mechanisms refer to the underlined components when only a portion of the microbiota have been clarified. TLR, Toll-like receptor; CDD, cytidine deaminase; Tregs, regulatory T cells; Th, T helper; DC, dendritic cells; TNF, tumor necrosis factor.

Attention has been paid to ICIs in the clinic. Immunotherapies targeting PD-1 has emerged as an effective strategy for the treatment of several cancers. Accumulating data revealed that T cell infiltration and variable immune regulators, including the gut microbiota, were associated with PD-1/PD-L1 blockade in patients, with beneficial outcomes for some cancers [66-69]. Among melanoma patients, fecal microbiota analysis identified a favorable abundance of the Ruminococcaceae family and Clostridiales in anti-PD-L1 responders with enhanced antitumor immune responses, whereas nonresponders were enriched with Bacteroidales [41]. Ruminococcaceae bacteria in the gut were associated with a higher

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density of peripheral and infiltrating effector T cells. A higher relative abundance of gut bacterial species, including Bifidobacterium longum, C. aerobacteriens, E. faecium and Bacteroides caccae, was detected in immunotherapeutic responders versus nonresponders among metastatic melanoma patients [40,46]. Intertumor heterogeneity may be derived from differences in individual microbial composition. A favorable composition of commensal microbiota in a melanoma mouse model resulted in enhanced antitumor immunity and improved therapeutic activity of anti-PD-L1 treatment [48]. Hereinto, Bifidobacterium in mice directly stimulated DCs and induced the maturation of DCs. These novel findings indicated the potential of gut microbiota for regulating host responses towards immunotherapies. Routy et al. [43] observed that antibiotic treatment suppressed the clinical benefit of ICIs (overall survival and progression-free survival) when treating epithelial tumor (non–small cell lung cancer, renal cell carcinoma and urothelial carcinoma) patients. Metagenomic analysis of patient fecal samples revealed the correlation between ICI responses and Akkermansia muciniphila (A. muciniphila) dysbiosis, which showed a restoration of PD-1 blockade resistance in mouse tumor models after oral administration. Intestinal A. muciniphila increased the recruitment of CCR9+CXCR3+CD4+ T cells in the tumor bed, suggesting that future immunotherapeutic targets could manipulate the gut microbiota in individuals with cancer. Furthermore, in vitro, A. muciniphila and E. hirae stimulated DCs to secrete interleukin-12 (IL-12), which is the crucial cytokine for Th1 cell differentiation and function [43,70]. However, other clinical observations in non–small-cell lung cancer showed no beneficial impact of antibiotics on anti-PD-1 therapy [39,44,47].

Briefly, host immunity and TME always play crucial roles in microbiota-modified therapeutic responses. Specific gut microbiota have the potential to predict the efficacy of certain kinds of immunotherapies, and colonization of tumor-specific bacteria has been found to play regulatory roles in the antitumor effects of immune-targeting treatment. The presence of microbiota-derived mediating factors and host variability will create a heterogeneous local TME and relevant alterations in systemic communication.

Intratumor Microbiota of Pancreatic Cancer

Recent advances have begun to elucidate the potential roles of intratumoral microorganisms in anticancer therapeutics, e.g., pancreatic cancer [71]. Based on conventional speculation, the pancreas tissue has no direct contact with the gut microbiota from both a clinical and anatomical perspective. Many clinicians believe that pancreatic tissue is germ free; otherwise, the patient or individual may be infected and will have a fever of pancreatic origin. Notably, recent studies in mice and humans found that bacteria exist not only in pancreatic tumor tissues but also in normal pancreatic tissues. Nevertheless, cancerous tissue harbors an increased abundance of microorganisms [42]. Geller et al. [29] reported that 15% (3/20) of normal pancreatic tissues contain bacterial DNA via qPCR detection. In both PDAC and noncancer patients, similar bacterial profiles were detected at different sites of the pancreas and duodenum tissues within the same individual, suggesting that intrapancreatic bacteria may migrate from the surrounding gut tract across the intestinal wall [72].

The association of gut bacteria and tumor tissue has been previously reported, such as Helicobacter pylori and gastric cancer, Salmonella typhi and gallbladder cancer, and altered bacterial species and CRC [73-75]. These microorganisms within tumors may stimulate host immune responses and generate beneficial or disruptive impacts on anticancer therapy, as determined by pharmacological mechanisms, as well as the major response pathways [53]. Some human solid tumors were found to be infected with Mycoplasma hyorhinis (M. hyorhinis), which was shown to have a relationship with gemcitabine drug resistance [76]. M. hyorhinis infection led to weakened therapeutic efficacy of gemcitabine treatment via the microbial enzyme cytidine deaminase (CDD). Gemcitabine (2’,2’-difluorodeoxycytidine) was metabolized into its inactive deaminated form, 2’,2’-difluorodeoxyuridine (dFdU), in M. hyorhinis-infected conditioned cultures [29,34]. In human PDAC, 76% (86/113) of the tissue samples exhibited the presence of bacteria from the intratumoral Gammaproteobacteria class, which contain the enzyme CDD; this enzyme was indicated to be responsible for the ineffectiveness of gemcitabine in PDAC [29,77]. Bacterial CDD exhibits two different forms: long CDD (CDDL) and short CDD (CDDS). The expression of the resistance-related isoform CDDL led to the metabolism of gemcitabine [34]. Moreover, high-performance liquid chromatography and mass spectrometry identified Escherichia coli (E. coli)-induced chemical structure modification of gemcitabine, fludarabine, cladribine and CB1954 [33]. Nonpathogenic E. coli lowered the cytotoxicity of gemcitabine in vitro and in subcutaneous colorectal carcinoma models containing intratumoral bacteria [33].

The Fusobacterium species, a group of oral bacteria, were initially detected in PDAC tissues by
Mitsuhashi et al. [78] and were found to be independently associated with a worse patient survival probability. The intrapancreatic abundance of *Fusobacterium* species was found to be relatively higher in PDAC subjects than in noncancer controls [72]. *Fusobacterium nucleatum* elicited chemoresistance to 5-FU and oxaliplatin in CRC, targeting TLR4 and MyD88 immune signaling and activating the cancer autophagy pathway via downregulation of miR-18a* and miR-4802 [30]. TLR4/MyD88 signaling was also previously associated with chemoresistance to paclitaxel in ovarian cancer [79]. With respect to PDAC, the desmoplastic response induced by cancer cells was dependent on MyD88 signaling to create an immunosuppressive TME, suggesting the potential impact of *Fusobacterium* species on the chemoresistance of PDAC [80].

Bacteria are capable of translocating from the gut to the pancreas in mice and influencing the PDAC microenvironment [42]. Moreover, local bacteria in the human pancreas that migrate from the gut play a specific role in cancer progression and treatment and, to some extent, may be a neglected component of the traditionally defined TME. In the complex TME of PDAC, TAMs and MDSCs constituted the leading population of infiltrated immunosuppressive components [81]. Antibiotic-mediated removal of intrapancreatic bacteria was associated with immune remodeling of the TME in PDAC-bearing KPC mice. The observed cellular events mainly involve (1) reduced MDSCs; (2) increased M1-polarized TAMs (repolarized from the M2 phenotype); and (3) immune-promoting activation of CD4+ and CD8+ T cells with elevated expression of T-BET, TNF-α, CD38, PD-1 and CD44 [42]. The study revealed that antibiotics plus PD-1 inhibition generated synergistic anticancer efficacy, with enhanced intratumoral T cell activation. However, these mouse-based preclinical results for PDAC are in contrast to the abovementioned research by Routy et al. [43] on multiple solid tumors of nondigestive systems. This indicates that distinct microbiota alterations may cause bacteria-specific effects on the corresponding organs [82]. In addition, enteral and parenteral translocation, local and systemic responses, and the predominant microflora will determine the final direction of therapeutic efficacy.

Recently, intratumor microbiota were further confirmed to be highly predictive of long-term survivorship in PDAC patients, and experimental evidence suggested that intratumor microbiota were modified by the gut microbiota [83]. Transplantation of fecal microbiota from PDAC long-term survivors restored a treatment-friendly immune microenvironment in tumor mice. These findings highlight the role of microbiota as a promising therapeutic intermediate for PDAC.

To date, continued attempts to overcome cancer chemoresistance and immune tolerance have not attained beneficial overall survival for PDAC patients, even after R0 resection with pathologically tumor-free surgical margins. The gut microbiota has been recognized as a crucial mediator in the TME of PDAC; thus, the crosstalk among these regulators becomes more complex and pluralistic (Figure 1). Considering that microorganisms outnumber human somatic cells, further use of antibiotics to shape the gut microbiome is likely to overcome the hurdles.

**Microbiota Transplantation**

The concept of fecal microbiota transplantation (FMT), which originates from the fourth century in ancient China, has overcome challenges and instigated wide discussion both technologically and theoretically [84]. With the increasing use of FMT, fecal therapy has become a promising strategy in diverse human diseases, as shown in novel clinical reports on epilepsy, hepatic encephalopathy and metabolic syndrome [85-87]. Le Bastard et al. [88] reported that ampicillin- and/or 5-FU-pretreated C57BL/6J mice exhibited a critical alteration of bacterial species distribution and presented functional disruption, which was corrected by receiving FMT. This may be helpful to prevent and treat several
gastrointestinal side effects caused by chemotherapy, immunotherapy and antibiotics. Moreover, the first successful attempt at treating refractory ICI-associated colitis with FMT was recently reported [89]. The two enrolled patients achieved improvement in clinical symptoms during the follow-up. Recently, Tanoue et al. [90] separated 11 bacterial strains from the fecal microbiota of healthy volunteers with the ability to induce interferon-gamma (IFN-γ)-expressing CD8+ T cell accumulation in the intestine. The 11 isolated strains showed effectiveness in spontaneous ICI treatment and dependent tumor inhibition via enhanced CD8+ T cell antitumor immunity. The reported mixture of 11 strains, which were mostly rare and low-abundance species among normal human microbiota, showed great therapeutic potential for resistance to chemotherapy and immunotherapy for widespread cancer types.

Given its success with metastatic melanoma, FMT could allow PDAC patients to seek improved prognosis safely [91,92]. Although it remains unknown whether FMT could restrict oncogenesis in humans, microbiota transplantation seems to be a promising approach to further manipulate microbial composition and function to enhance host anticancer immunity and to improve resistance and ineffectiveness in cancer patients with relatively short survival. These gut and intratumoral microflora will become future targets to overcome pancreatic oncogenesis and immunosuppression.

**Microbial Markers for Therapeutics**

Distinct responses to chemotherapy and immunotherapy in cancer patients have provoked robust interest in identifying useful biomarkers to optimize patient selection and management. Fecal sample analysis using 16S rRNA sequencing has provided evidence that *Fusobacterium nucleatum* is related to the chemoresistance of CRC, as well as other specifically chemotherapy-associated bacterial strains [28]. This may be an optimal microbial marker candidate during anticancer treatment. Chemotherapy-induced gastrointestinal mucositis, which is clinically manifested as diarrhea, abdominal pain, malnutrition and bacteremia, remains an unpleasant side effect of chemotherapeutic agents [93]. A higher abundance of baseline gut microbiota (*Faecalibacterium* genus and other Firmicutes) predicted favorable clinical responses among ipilimumab-treated individuals with metastatic melanoma but increased onset of ipilimumab-related colitis [45]. Inflammation can be either a cause or a consequence of the gut microbiota. A hypothesis concerning commensal intestinal bacteria, chemotherapy and mucositis has been proposed with five potential aspects: inflammatory process and oxidative stress, intestinal permeability, mucus layer constituents, epithelial repair and immune effector molecules [94]. From the perspective of mechanism and etiology, microbial shifts may also reflect the development of intestinal mucositis.

Use of the gut microbiota as a marker for drug efficacy and side effects should be qualified with significantly altered abundance and differential function between postoperative adaptation and drug response. Some issues should be urgently addressed, such as the specificity of biomarker bacteria, the accuracy of comparative studies and how the remote prediction of bacteria works [95]. Future studies will discuss potential biomarkers for monitoring treatment responses, explore possible mechanisms underlying resistance and guide clinical dosage adjustment.

**Perspectives**

Altered therapeutic efficacy resulting from the host microbiota typically changes in terms of multiple parameters. The quantitative approach used to evaluate the contributions of the microbiota, as previously described in the study of brivudine metabolism, will be appreciated for its role in the effort against drug tolerance in PDAC [96]. Pharmacomicrobiomics has been frequently used in recent years to investigate the interactions between the microbiota and drugs [97,98]. Current microbe-based anticancer therapy has attracted increasing attention from clinicians and oncologists and has inspired them to increase their efforts to control immunoregulation, therapeutic efficacy and drug safety [99].

**Conclusions**

It is almost impossible for the human body to be absolutely sterile or germ free. All therapeutic strategies and host responses will be directly or indirectly influenced by the microbiota. Future studies should focus on not only the tumor itself but also the treatment dilemma, which might have a long-term influence on the ecosystem in the gastrointestinal tract [4]. We reviewed the potential association between the gut microbiota and therapy regimens and highlighted the microbiota-therapeutic interactions in PDAC, which is of significance for patients lacking effective anticancer drugs.

The reciprocal interactions between the gut microbiota and cancer therapies are complicated and are cancer-dependent, therapy-dependent, and even tumor stage-dependent, leading to the paradox that in some cancers, the gut microbiota is a prerequisite to maintain therapeutic efficacy; however, in other cancers, depletion of the gut microbiota significantly
improves efficacy. A large amount of exploratory work is needed to comprehensively understand the role of the microbiota in influencing therapeutic responses in PDAC. We suggested that modified dietary supplements, FMT and the application of certain antibiotics would have an impact on augmented drug efficacy, reduced toxicity and restricted PDAC recurrence and metastasis [100,101]. Using 16S rRNA identification, metagenomics analyses and other high-throughput techniques enables clinicians to monitor therapeutic efficacy before the development of invasiveness and to provide a possible salvaged target to make paradigm shifts to the current first-line regimens.

Abbreviations

5-FU: 5-Fluorouracil; A. muciniphila: Akkermansia muciniphila; C. aerofaciens: Collinsella aerofaciens; CDD: Cytidine deaminase; CRC: Colorectal cancer; CTX: Cyclophosphamide; DCs: Dendritic cells; dFdU: 2’,2’-Difluorodeoxouridine; E. coli: Escherichia coli; E. faecium: Enterococcus faecium; E. hirae: Enterococcus hirae; FDA: Food and Drug Administration; FMT: Fecal microbiota transplantation; FUDR: 5-Fluoro-2’-deoxyuridine; GEMM: Genetically engineered mouse model; ICIs: Immune checkpoint inhibitors; IL-12: Interleukin-12; IFN-γ: Interferon-gamma; M. hyorhinis: Mycoplasma hyorhinis; MDSCs: Myeloid-derived suppressor cells; PD-1: Programmed cell death protein 1; PDAC: Pancreatic ductal adenocarcinoma; rRNA: Ribosomal RNA; TAMs: Tumor-associated macrophages; Tc1: Cytotoxic T cell 1; Th1: T helper 1; TLRs: Toll-like receptors; TME: Tumor microenvironment; TNF: tumor necrosis factor; Treg: Regulatory T cell.

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Competing Interests

The authors have declared that no competing interest exists.

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