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REVIEW

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Roles of microbiota in pancreatic cancer development and treatment

Mariana Santos Cruz[a,b,c], Joseph Tintelnot[a,c*], and Nicola Gagliani[b,c,d*]

[a]II. Department of Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [b]Department of General, Visceral and Thoracic Surgery, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [c]Hamburg Center for Translational Immunology (HCTI), Hamburg, Germany; [d]I. Department of Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive disease with poor prognosis. This is due to the fact that most cases are only diagnosed at an advanced and palliative disease stage, and there is a high incidence of therapy resistance. Despite ongoing efforts, to date, the mechanisms underlying PDAC oncogenesis and its poor responses to treatment are still largely unclear. As the study of the microbiome in cancer progresses, growing evidence suggests that bacteria or fungi might be key players both in PDAC oncogenesis as well as in its resistance to chemo- and immunotherapy, for instance through modulation of the tumor microenvironment and reshaping of the host immune response. Here, we review how the microbiota exerts these effects directly or indirectly via microbial-derived metabolites. Finally, we further discuss the potential of modulating the microbiota composition as a therapy in PDAC.

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) represents the third leading cause of cancer-related deaths worldwide and is the most common form of pancreatic cancer.[1] It is associated with a 5-year survival rate as low as 11% due to its late diagnosis and limited responses to therapy.[1,2] Surgical resection followed by adjuvant chemotherapy represents the best treatment approach in PDAC.[3] However, most patients present with unresectable or metastatic disease at diagnosis or experience disease relapse after successful surgery.[4] Chemotherapy is often administered to locally advanced[4–6] or metastatic[7–9] patients in combinatorial regimens, including gemcitabine combined with nab-paclitaxel or FOLFIRINOX (5-fluorouracil, leucovorin, oxaliplatin and irinotecan). However, most patients either already exhibit chemotherapy resistance when treatment starts, or develop it over time.[10] In addition, novel immune checkpoint blockade therapies that have been revolutionizing the cancer paradigm in several tumor entities do not work in PDAC.[11] Likewise, the therapeutic approaches targeting the tumor stroma, which is recognized as one main factor compromising chemotherapy efficacy, have been equally disappointing.[12]

In the past decades, efforts have been made toward trying to understand how PDAC is initiated and progresses while acquiring such aggressive features. PDAC comprises very heterogeneous tumor phenotypes that differ not only in the tumor cell-intrinsic transcriptional and epigenetic profiles but also in the composition of the stroma and immune cells infiltrating the tumor microenvironment (TME).[13] PDAC may originate from either acinar or ductal cells in the exocrine pancreas, giving rise to these different disease subtypes.[14–16] *In vivo* experiments have suggested that Kirsten rat sarcoma virus (KRAS) activation and Tumor protein 53 (TP53) mutations in pancreatic acinar cells activate a process of metaplasia to ductal-like cells, leading to the onset of pancreatic intraepithelial neoplastic (PanIN) lesions that may progress to malignant disease.[16,17] However, ductal cells bearing both KRAS and TP53 genetic events may also give rise to the more aggressive basal-like PDAC subtype in an alternative and accelerated process of oncogenesis.[15,16] In human disease, it has been reported that more than 90% of PDAC tumors bear KRAS mutations, suggesting that this may represent the initiating event that unleashes malignancy.[18] As the disease progresses, tumor cells may collect other genetic mutations, mainly in *TP53*, mothers against

CONTACT Joseph Tintelnot j.tintelnot@uke.de II. Department of Medicine, University Medical Center Hamburg-Eppendorf, Hamburg 20246, Germany
*These authors jointly supervised this work.

decapentaplegic homolog 4 (*SMAD4*) and cyclin-dependent kinase inhibitor 2A (*CDNK2A*) genes.[18] However, targeted therapies based on the presence of each one of these mutations have so far been unsuccessful in improving patient care.[18]

Currently, there is an urgent need to understand which cues dictate treatment efficacy and to improve clinical outcomes of PDAC patients. While the presence of strong homogeneity in key driver mutations in PDAC has long been acknowledged, it is now being recognized that external factors contribute significantly to the heterogeneity of tumor phenotypes observed during disease development. Among these external factors, recent research has put forward the concept that the gut microbiota – a collection of microorganisms residing in the gut – can play a pivotal role in tumor progression and responses to treatment. Indeed, the concept of the microbiota's central role in maintaining health and homeostasis is not novel, and there has been an increasing association between the microbiota and the development of various diseases throughout the body, extending beyond the gastrointestinal tract.[19] Gut dysbiosis has also emerged as a notable phenotypic feature of human PDAC patients over the past decades.[20] Sequencing and immunofluorescence studies have contributed toward characterizing the microbiota of PDAC patients, and these associations are being increasingly explored in preclinical models of PDAC. Overall, research suggests that microbes play a role in PDAC carcinogenesis via the promotion of inflammation[21–23] and can modulate the response to therapies directly[24] or indirectly via the production of microbiota-derived metabolites.[25]

In this review, we discuss and summarize what is currently known on how PDAC development and treatment are affected by the microbiota. We provide insights on the current limitations of this field and speculate about promising microbiota-based approaches that may arise to improve the future outcomes of PDAC patients.

Main

The microbiota of PDAC patients differs from healthy controls

The first clues that the microbiota could explain PDAC development arose from observational studies comparing its composition between PDAC patients and healthy controls. By using a combination of 16S rRNA sequencing and shotgun metagenomics, several studies reported that the microbiota signatures of treatment-naïve PDAC patients differ from those of healthy controls across different locations within the human body, id est (i.e.), the oral cavity, the gut and the healthy or diseased pancreas, as reviewed in the following sections.

Composition of the oral microbiota in PDAC patients

Several studies have investigated the oral microbiota of treatment-naïve pancreatic cancer patients compared to healthy controls based on analysis of saliva,[26–31] tongue coat[32] or oral wash[33,34] samples. Most studies observed no differences in the alpha diversity of patient oral microbiota compared with controls,[27,28,34] except for one where diversity increased in patients with PDAC.[30] In general, reports across these cohorts are inconsistent regarding the enrichment of certain bacteria taxa. Despite this, oral samples from PDAC patients were shown to exhibit higher relative abundance of the phylum Firmicutes[27,30,32] and *Neisseria* and *Haemophilus* genera were consistently less abundant in the saliva of PDAC patients.[27–29] In addition to Firmicutes, Fusobacteria and Actinobacteria phyla were also found to be enriched in the tongue coat of pancreatic carcinoma patients,[32] although others reported that lower abundance of Fusobacteria in prediagnostic mouthwash samples was associated with increased risk of pancreatic cancer.[33] Contrasting results have also been observed for the *Leptotrichia* genus, which belongs to the Fusobacteria phylum and whose abundance was associated with either higher[29] or lower[33] risk of pancreatic cancer. Notably, there are few other studies that did not find any differential taxa abundance in the oral cavity when comparing PDAC patients and controls[31,34] or between cases of PDAC and intraductal papillary mucinous neoplasms (IPMN), a potential precursor for pancreatic cancer.[27]

Although it has been challenging to systematically connect specific pathogens to PDAC across

different patient cohorts, the bacterium *Porphyromonas gingivalis (P. gingivalis)* seems to be closely linked to pancreatic cancer. *P. gingivalis* is an oral pathogen driving periodontitis and epidemiological studies have indeed suggested a correlation between periodontal disease and pancreatic cancer.[35,36] The relative abundance of the *Porphyromonas* taxa in oral samples of PDAC patients was inconsistently observed to be similar[28] or lower[29,32] compared with non-cancer subjects. Even so, Fan *et al.* identified that the presence of *P. gingivalis* was associated with increased risk of pancreatic cancer in a prospective study with 361 patients.[33] In accordance with this, others have reported that higher levels of circulating antibodies against *P. gingivalis* in blood samples collected up to 10 years before diagnosis represented a two-fold higher risk of developing pancreatic cancer.[37] Besides *P. gingivalis*, *Aggregatibacter actinomycetemcomitans* was also linked to a higher risk of developing PDAC[33] and Farret *et al.* demonstrated that two other species – *Neisseria elongata and Streptococcus mitis* – are less present in the saliva of PDAC patients compared to healthy controls.[26]

Fewer studies have focused on the composition of the fungal community, also referred to as mycobiota, in the oral cavity of PDAC patients. Some of the first observation studies reported that presence of *Candida*-related oral lesions[38] and confirmed *Candida* infection[39] correlated with higher PDAC risk in two independent cohorts. More recently, Wei *et al.* took advantage of Internal Transcribed Spacer (ITS) rRNA sequencing to further characterize the mycobiota in saliva samples from PDAC patients.[40] Decreased alpha diversity was found in PDAC patients compared to healthy controls. The taxa *Aspergillus* and *Cladosporium* were not only less abundant in PDAC but also presented high classification power to discriminate between both groups.[40] However, the study of the mycobiota in PDAC is still in its early stages, and further research is required to solidify this link.

In short, the oral microbiota of PDAC patients is suggested to be distinguishable from healthy patients, but consistent identification and validation of a unique profile of keystone microbes has been difficult. In addition, whether the reported oral dysbiosis precedes or arises after PDAC development remains to be clarified. Notably, it is known that each subregion of the oral cavity hosts a unique microbiota[41] and thus direct comparison between the microbiota signatures of different types of oral samples across PDAC patient cohorts should be avoided. In addition, composition of rare microorganisms varies over time,[41] so time of sampling is another factor that may influence these results. Oral microbiota is also highly dependent on lifestyle-related determinants, such as tobacco use,[42] which itself represents a risk factor for pancreatic cancer and may thereby act as a confounding factor.[1] Considering these challenges, oral-associated microbiota profiles specifically linked to PDAC should be carefully identified. This could be of use in the clinical setting, providing potential biomarkers for noninvasive screening of the disease.

Composition of the intestinal microbiota in PDAC patients

Growing evidence suggests that treatment-naïve PDAC patients also have a unique bacterial signature in the intestinal content. By using a combination of 16S rRNA sequencing and shotgun metagenomics, the fecal microbiota has been characterized in several independent patient cohorts from China,[43,44] Japan,[30] Israel,[45] Spain and Germany.[31] Alpha diversity analysis yielded contrasting findings across cohorts, with microbial diversity within PDAC cases being reported to be lower,[30,43] higher[21] or similar[44,45] in comparison with the control group. Despite this, beta-diversity analysis revealed that the composition of the gut microbiota in PDAC was indeed distinguishable from that of healthy subjects.[30,31,43–45]

When comparing microbial abundance between PDAC patients and healthy controls, different taxa were found to be differentially present in both groups. Overall, lipopolysaccharide-producing bacteria were enriched in the gut of PDAC patients,[43] whereas there was a reduction in butyrate-producing bacteria.[43,44] Specifically, the *Veillonellaceae* family[45] and its genus *Veillonella* ,[21,30,31,43,44] as well as the genera *Klebsiella* ,[21,43] *Streptococcus* [30,31,43] and *Akkermansia* [31,45] were consistently identified to be enriched in fecal samples from PDAC patients. In contrast, *Bifidobacterium* ,[31,43] *Eubacterium* [30,44] and

Faecalibacterium [31,44,45] taxa were more abundant in the gut microbiota of healthy subjects than in PDAC patients. However, contrasting results were reported for other bacteria strains. *Prevotella*, for instance, was reported to be enriched in PDAC patients compared to healthy subjects in two independent studies,[30,43] whereas the opposite was observed in a third cohort.[21] Other genera, including *Odoribacter* ,[45] *Selenomonas*, *Hallella*, *Enterobacter* and *Cronobacter* ,[43] were found to be highly abundant in the gut microbiota of PDAC patients in individual studies, but this was not reproduced in the remaining populations.

When comparing the fecal microbiota of PDAC patients with that of patients with pre-malignant lesions, a clear microbial pattern could not be observed between earlier stages and advanced PDAC.[45] In a similar trend, the gut microbiota of PDAC was also found to be similar to chronic pancreatitis, a risk factor in pancreatic cancer,[30,31] reflecting that microbiota-based early detection of cancer may be challenging. Although most studies carried out so far have not investigated how the intestinal microbiota signature may correlate to responses to treatment, we have recently observed that the pre-treatment microbiota of PDAC patients responding to chemotherapy differs from the microbiota of non-responders.[25] In summary, the gut microbiota of PDAC patients is suggested to be distinguishable from healthy patients and to vary according to treatment responses. Hence, it may serve as a diagnostic tool or prognostic marker in the future.

Composition of the intratumoral microbiota in PDAC

The concept of intratumoral microbiota arose from evidence across several cancers suggesting that bacteria could be detected within the tumor microenvironment (TME).[46] In the specific case of the pancreas, although it was long considered sterile, a considerable number of studies has now established that bacteria may colonize this organ even under steady state conditions.[21–23,31,47]

Possible routes for bacteria translocation into the pancreas

Due to the anatomical proximity between the pancreas and intestine, it has been hypothesized that bacteria detected in the pancreas originate in the gastrointestinal tract and translocate via the pancreatic duct. By orally gavaging mice with fluorescently-labeled bacteria, Pushalkar *et al*. showed that fluorescent bacteria could be detected in the pancreas, possibly through retrograde migration from the duodenum.[21] This oral-pancreas migration was further supported by two other studies upon gavage with the green fluorescent protein-labeled fungus *S. cerevisiae* [48] or with the oral pathogen *P. gingivalis* .[49] Indeed, the pancreatic microbiota profile of PDAC patients resembled that of the duodenum, further supporting this hypothesis.[47] *Proteobacteria*, for instance, was shown to be highly abundant in both the duodenum and the pancreas.[24,47] In addition, tumors from patients undergoing endoscopic retrograde cholangiopancreatography – a procedure that examines bile duct and pancreatic duct abnormalities, exempli gratia (e.g.), an obstruction – before surgery were shown to have significantly more bacteria in the pancreas than those that were not subjected to this procedure.[24] Alternatively, bacteria could also translocate to the pancreas as a result of inflammation-driven increased intestinal permeability.[50]

However, others have described that no bacteria could be detected *in vivo* in pancreatic cancer xenografts regardless of antibiotic treatment.[22] In addition, bacteria infiltration could not be observed in the pancreas from germ-free mice that had been transferred back to specific pathogen-free (SPF) conditions, nor from interleukin (IL) 10 (IL-10) knockout (KO) mice where the barrier function of the intestinal mucosa is compromised.[22] This data suggests that colonization of the pancreas with intestinal bacteria does not happen under physiological conditions, but depends on certain predisposing factors that remain to be identified.

It has also been suggested that microbes may reach the pancreas via other routes, including the lymphatic system. Bacteria may translocate from the intestine to mesenteric lymph nodes independently or via CX_3CR1^{hi} mononuclear phagocytes.[51,52] From there, they can reach the pancreas as lymphatic drainage takes place.[52]

Researchers have started to explore how bacteria translocate to the pancreas, but the triggers that unleash this translocation and the mechanisms

that enable them to remain there under tumorigenic conditions still need to be elucidated. In addition, the reported antimicrobial properties of the pancreatic fluids and their ability to shape the composition of the intestinal microbiota[53] should be taken into account while investigating this intrapancreatic colonization. Whether bacteria or fungi reach the pancreas as a consequence or as a mediator of carcinogenesis is also largely unknown. The relationship between the microbiota and PDAC is still unclear, and further research is needed to establish causality between the presence of bacteria and tumor progression. Indeed, even though microbiota sequencing has also been employed in prospective clinical studies, the presence of certain bacterial strains could solely act as a marker of cancer-associated inflammatory processes.

Composition of the intratumoral microbiota in PDAC

Using a combination of qPCR and fluorescence *in situ* hybridization (FISH) against the bacterial 16S rRNA region, Geller *et al.* first demonstrated that bacterial DNA was present in 76% of a collection of PDAC tumors, as opposed to only 15% of normal pancreas from control donors.[24] Supporting these findings, a study conducted by Nejman *et al.* detected bacterial DNA in 68% of PDAC tumors and showed that the most abundant species in PDAC were members of the Proteobacteria phylum.[46] Interestingly, bacterial DNA was mainly detected in the cytoplasm of both cancer cells and immune cells.[46] Members of the *Gammaproteobacteria* were consistently detected in PDAC tissues across studies.[24,46,49] Furthermore, high relative abundance of Proteobacteria and Firmicutes phyla in PDAC tumors has also been consistently reported.[21,22,46,47,54] *Fusobacterium* species, which are increasingly being implicated in different gastrointestinal cancers, were detected in 8.8% of the tested PDAC samples and its presence correlated with worse survival rates.[55] *Fusobacterium nucleatum* (*F. nucleatum*) has also been found in cyst fluid from high-grade IPMN.[56] Other studies also compared the microbial abundance in PDAC tumor tissue and adjacent healthy pancreatic tissue. *Lactobacillus* species (spp.), *Akkermansia muciniphila* (*A. muciniphila*) and *Bacteroides* spp. were found to be enriched in PDAC tumor tissue compared with healthy tissue.[31] Despite this, the profiles between both tissues shared some similarities at several taxonomic levels.[49]

Recent evidence also highlighted a clear difference in the intratumoral microbiota of short-term and long-term survival PDAC patients.[54,57] Long-term survival patients from two geographically separated cohorts exhibited a higher microbial alpha-diversity and a unique intratumoral microbiota signature characterized by *Saccharopolyspora, Pseudoxanthomonas, Streptomyces and Bacillus clausii* .[57] Huang *et al.*, however, did not observe differences in alpha-diversity between these groups, but beta-diversity analysis unveiled an enrichment of *Sphingomonas, Megasphaera, Bradyrhizobium, Desulfovibrio, Flavobacterium, Enhydrobacter* and *Megamonas* .[54] It should be noted that the threshold to define short-term and long-term survivors and the geographic location of each cohort differed between the two studies, which may be the reason for those inconsistencies. Whereas Riquelme *et al.* considered those surviving more than 5 years after surgery to be long-term survivors,[57] the long-term group in the study by Huang *et al.* included patients with a survival time of longer than 600 days (less than 2 years) after surgery.[54]

The microbiota of different molecular subtypes of PDAC was also suggested to be different in a study by Guo *et al* .[58] Basal-like (BL) PDAC tumors are poorly differentiated and typically exhibit a more aggressive phenotype accompanied by chemoresistance, whereas the classical subtype is characterized by better therapy responses and overall survival. In this study, the authors carried out FISH against 16S rRNA sequences, demonstrating that BL tumors had an increased bacterial DNA load compared to classical or hybrid subtypes. In addition, *Acinetobacter, Pseudomonas* and *Sphingopyxis* genera were highly abundant in the BL subtype and were strongly associated with inflammatory and carcinogenic pathways.[58]

Fungal DNA was detected in the tumors of PDAC patients in two separate studies[48,59] and was significantly increased in PDAC in comparison with healthy donors.[48] The pancreatic mycobiome of patients clustered separately from that of healthy subjects and *Malassezia* spp. were particularly

enriched in the tumor samples.[48] By using 18S rRNA sequencing and FISH for fungal DNA, Alam *et al* further confirmed higher presence of fungal DNA in tumors of $Kras^{G12D}$ $Trp53^{R172H}$ Pdx-Cre (KPC) mice, a mouse model of PDAC, compared with healthy mouse pancreata.[60]

Other studies reported low amounts of microbial biomass (median of 3682 copies of bacterial DNA/ng of DNA, versus a median of 2766 in the negative control) in pancreatic tissue that were relatively conserved across healthy pancreas, IPMN or PDAC tissues, and therefore unlikely to contribute to malignant transformation.[61] Recent reanalysis of previously published microbiota[62] and mycobiota[48] sequencing datasets did not identify the differences reported for PDAC intratumor microbial composition in the original publications.[63,64] This highlights the importance of using complementary techniques, such as fluorescent *in situ* hybridization, in addition to sequencing, to validate the presence of specific bacteria or fungi within PDAC tumors.

Nevertheless, taken together, sequencing data from the different regions throughout the body underlines that patients suffering from PDAC seem to exhibit unique microbial signatures across tissues of the gastrointestinal tract which could serve as diagnostic and prognostic markers. However, there is marked inter-individual variability within each cohort and across different studies, making it more complicated to identify and validate oral, fecal and pancreatic microbiota predictors with potential for PDAC detection. These inconsistencies may be justified by different factors, including: (a) differences in geographic locations and personal lifestyles, which are known to affect the microbiota; (b) small patient sample size and reduced statistical power; (c) challenges in the analysis of microbiota sequencing data from low biomass samples; (d) missing correction for other confounding factors and co-morbidities; (e) differences in sample collection and processing, as well as in methods used for extraction and sequencing of microbial DNA and (f) redundancy of different microbiota taxa. Therefore, larger studies analyzing ethnically and geographically different cohorts of PDAC patients and healthy controls are needed to establish microbiota signatures that might be used as diagnostic markers. Finally, and most importantly, since it is still unclear whether this is the cause or consequence of PDAC development and progression, further mechanistic and functional studies should be performed to evaluate the potential causal role for the microbiota in this disease.

The microbiota promotes pancreatic tumor development in preclinical models

Sequencing studies have contributed greatly to characterizing the unique microbiota signatures of PDAC patients and propose that microbes potentially support PDAC progression. To investigate this putative causative effect, *in vivo* experiments have been carried out in several animal models of PDAC.

One of the first studies was carried out by Pushalkar *et al* .[21] Here, the authors took advantage of two mouse models: $Ptf1a^{Cre}$ LSL-$Kras^{G12D}$ (KC) mice, which spontaneously develop slowly-progressing pancreatic tumors; and the orthotopic model, where tumor cells isolated from the KPC mouse model of PDAC are injected into the pancreas of WT mice. Germ-free KC mice exhibited delayed oncogenesis in the pancreas and, similarly, tumor growth was dramatically reduced in the orthotopic model upon microbial depletion by a cocktail of oral antibiotics composed of vancomycin, neomycin, metronidazole, amphotericin, ampicillin and amphotericin B. Protection from tumor progression in the absence of the microbiota was accompanied by increased tumor infiltration of Th1 $CD4^+$ T cells and cytotoxic $CD8^+$ T cells, reprogramming of tumor-associated macrophages (TAMs) toward higher expression of MHC II, CD86, TNFα, IL-12 and IL-6 and reduced frequencies of myeloid-derived suppressor cells (MDSCs).[21] Complementary experiments either depleting $CD4^+$ and $CD8^+$ T cells with neutralizing antibodies or reactivating their function using PD1 antibodies suggested that the beneficial effect of microbiota depletion depends on the induced T cell-mediated immune responses.[21]

In a similar approach of antibiotic-mediated bacterial ablation in $Kras^{G12D}PTEN^{lox/+}$ mice, Thomas *et al.* also reported a central role for the gut microbiota in PDAC development.[22] Here, the authors used a different antibiotic cocktail

administered *ad libitum* in the drinking water and containing streptomycin, gentamicin, bacitracin and ciprofloxacin. At 3 months of age, antibiotic-treated mice showed lower numbers of malignant lobules than microbiota-intact mice, indicating delayed progression.

In accordance with the data reported by these studies, Sethi *et al*. observed that microbial depletion by antibiotics also reduced tumor growth in a third model of PDAC consisting of subcutaneous injection of KPC tumor cells.[23] Upon ablation of bacteria, there was increased infiltration of interferon-γ (IFN-γ)-producing $CD4^+$ and $CD8^+$ T cells and decreased numbers of IL-17a and IL-10-secreting T cells. Indeed, the protective effect on tumor growth was not present in Rag1 KO mice lacking mature T cells (and B cells) nor after treatment with an IL-17a-neutralizing antibody, underlining that this effect is dependent on a functional adaptive immune system.[23]

Whereas most studies so far have analyzed the role of the microbiota as a whole in PDAC oncogenesis, others are starting to explore whether this process may be dependent on specific microbes. In fact, considering the classical examples of *Fusobacterium nucleatum* [65,66] and *Helicobacter pylori* [67] being implicated in colorectal and gastric cancer, respectively, it appears plausible that specific bacteria may also have drastic outcomes on tumor development in PDAC. A recent preclinical study solidified the association between *P. gingivalis*-induced periodontitis and pancreatic cancer observed in human PDAC. *P. gingivalis* was able to colonize the tumor both in patients and upon oral gavage in an orthotopic model of PDAC.[49] Strikingly, orthotopic tumors grew faster in the presence of this bacterium, highlighting for the first time that *P. gingivalis* can promote PDAC tumorigenesis. This pro-tumor function was mechanistically linked to a CXCR2-mediated infiltration of neutrophils and decreased frequencies of tumor-infiltrating $CD8^+$ T cells and relied on neutrophil-derived elastase.[49] In addition, the potential roles of *F. nucleatum* in PDAC progression have also been addressed.[68] *F. nucleatum* was shown to invade human PDAC cell lines and 3D spheroids, increasing their proliferation and migration capacity. Infection also stimulated secretion of GM-CSF, CXCL1, IL-8 and MIP-3α by both normal and tumor pancreatic cells.[68]

Within the fungal community, Aykut *et al*. showed that mycobiome depletion through oral administration of amphotericin B or fluconazole prevented tumor growth in KC mice and in the orthotopic model.[48] In addition, *Malassezia* species were found to be particularly enriched in murine and human PDAC tumors,[48] similar to what has been observed in colorectal cancer (CRC).[69] Repopulation of antifungal-treated mice with *Malassezia globosa* but not with other commensal fungi restored carcinogenesis, confirming an active role for *Malassezia spp*. in PDAC etiology. According to the findings, this effect was dependent on activation of the mannose-binding lectin signaling pathway and of the C3 complement cascade.[48] Notably, the antifungal amphotericin B was also included in the experiments performed by Pushalkar *et* al along with the remaining antibiotics,[21] indicating that depletion of both bacteria and fungi might potentially be responsible for the observed effect.

Recently, another study suggested a role of fungi in the pathogenicity of PDAC.[60] Alam *et al*. showed that cancer cell-derived IL-33 triggered recruitment and activation of Th2 and innate lymphoid cells 2 (ILC2) cells to stimulate tumor progression in KPC mice. Importantly, IL-33 secretion, infiltration of Th2 and ILC2 cells and overall tumor burden were highly reduced after amphotericin B treatment. In contrast, repopulation with *M. globosa* or *A. alternata* had the opposite effect, suggesting that fungi can promote tumor growth via IL-33-mediated remodeling of the TME.[60]

Altogether, it is becoming increasingly clear that gut microbes may directly control PDAC onset and progression, but the specific molecular mechanisms underlying these effects are still being elucidated. Considering that bacteria and fungi highly depend on each other,[70] it would be relevant to explore whether these pathogenic roles require interactions between both communities. In addition, whether general dysbiosis is the main factor contributing to tumorigenesis or whether specific microbes act as carcinogens in PDAC still needs to be clarified.

Microbiota plays a role in PDAC therapy efficacy and resistance

Besides contributing to pancreatic tumor development, growing evidence suggests that the microbiota may also modulate therapy efficacy and toxicity by affecting drug pharmacokinetics, influencing the host metabolic environment or modulating the composition of the tumor milieu.[71,72] Considering that FOLFIRINOX and gemcitabine/nab-paclitaxel are the main therapy regimens applied in PDAC patients, we will focus on these therapies in the following sections.

Influence of the microbiota on chemotherapy efficacy

Taking advantage of the *Caenorhabditis elegans (C. elegans)* model and the fact that it uses bacteria as a natural food source, it has been shown that distinct bacteria have differential effects on the host response to chemotherapeutic agents.[73,74] In fact, specific gut microbes have been described to orchestrate resistance of cancer cells to different chemotherapeutic agents, including oxaliplatin, 5-fluorouracil (5-FU) and gemcitabine. In the context of CRC, for instance, *F. nucleatum* promotes resistance to oxaliplatin and/or 5-FU regimens, either by activating autophagy in tumor cells[75] or by upregulating BIRC3 expression to prevent apoptosis.[76] Retrospective studies in CRC patients also showed that antibiotic treatment improves the efficacy of oxaliplatin but not irinotecan, although the causes behind this specificity are unclear.[77]

In contrast, others have proposed that the intestinal microbiota mediates treatment efficacy. In a CRC mouse model, the gut microbiota was demonstrated to ensure responses to 5-FU[78] or oxaliplatin via induction of anti-tumor immune responses.[79] Research conducted by Geller *et al.* has also demonstrated that *Gammaproteobacteria* expressing the long isoform of the enzyme cytidine deaminase metabolize gemcitabine to its inactive form, thereby promoting resistance in CRC.[24] In a mouse model of CRC, intratumoral presence of *Gammaproteobacteria* induced resistance to gemcitabine, which was reverted by antibiotic treatment.[24]

Although the roles of the microbiota in treatment outcomes have not been studied extensively in PDAC yet, it can be speculated that some of the aforementioned CRC-related mechanisms may also apply to PDAC, considering that (a) the same chemotherapy regimens are also used for PDAC treatment; and (b) *F. nucleatum* and *Gammaproteobacteria* have also been identified to be present in PDAC patients.[24,46] However, Fulop *et al.* found no association between perichemotherapeutic administration of antibiotics and survival in PDAC patients treated with 5-FU. In contrast, antibiotic treatment correlated with improved survival among patients receiving first-line gemcitabine, suggesting that microbiota-mediated resistance to gemcitabine may also occur in PDAC.[80] Similar observations were also reported in an independent retrospective study highlighting that survival rates during gemcitabine-containing regimens were higher when patients were treated with antibiotics.[81] Besides bacteria, the fungal community was also reported to orchestrate efficacy of gemcitabine-based chemotherapy, since *in vivo* depletion of the mycobiome with antifungals was shown to potentiate responses of orthotopic PDAC tumors to chemotherapy.[48]

Altogether, these findings support that the microbiota can affect the outcomes of patients treated with chemotherapy regimens. However, there is high interindividual variability and redundancy of microbiota taxa and the interplay between the host and the microbiota is remarkably dynamic and complex, involving not only direct microbiota-tumor interactions, but also indirect interactions via the immune system. Due to this, more research is needed to better characterize the mechanisms employed by the microbiota to control chemotherapy responses in PDAC and identify microbiota-based biomarkers that may define better therapy outcomes.

Influence of the microbiota on chemotherapy toxicity

Microbes may also play a role in chemotherapy side effects. Irinotecan is a prodrug that needs to be metabolized by hepatic and intestinal carboxylesterases to form its bioactive metabolite SN-38, responsible for the potent anti-tumor activity.[82] SN-38 is subsequently detoxified in the liver by glucuronyltransferases, resulting in the inactive glucuronide SN-38 metabolite (SN-

38 G). However, SN-38 G is excreted into the gut via the bile, where bacterial β-glucuronidases can hydrolyze SN-38 G back to the toxic form SN-38. β-glucuronidase-producing bacteria essentially belong to the Bacteroidetes, Firmicutes, Verrucomicrobia and Proteobacteria phyla, which were reported to be enriched in PDAC patients, as previously described.[21,22,24,27,30,32,45–47,54] Accumulation of SN-38 in the gut results in delayed severe diarrhea which may even influence continuation of treatment.[82] Different strategies have been tested to prevent reactivation of this compound. The use of β-glucuronidase inhibitors, for instance, has been shown to attenuate irinotecan-induced diarrhea without harming the host cells.[83,84] Lower intestinal toxicity has also been reported upon use of broad-spectrum antibiotics or under germ-free conditions.[85] However, the prophylactic use of antibiotics, for example neomycin[86–88] and clarithromycin,[89] to prevent diarrhea incidence is controversial.

In addition, the microbiota may also mediate irinotecan toxicity via other β-glucuronidase-independent mechanisms. Streptomycin treatment was shown to be efficient in reducing diarrhea in rats by inhibiting intestinal absorption of irinotecan.[90] Importantly, gastrointestinal mucositis and diarrhea are not exclusive to irinotecan and have also been reported upon 5-FU treatment with active participation of the gut microbiota.[91]

Hyperalgesia, defined by extreme sensitivity to pain, is a common adverse reaction in oxaliplatin-treated patients that was shown to be induced by the gut microbiota.[92] It has been shown that germ-free or SPF mice treated with antibiotics did not develop mechanical hyperalgesia in contrast with controls in a mechanism partly dependent on the LPS-TLR4 axis. Conventionalization of GF mice to SPF conditions restored hyperalgesia, further supporting that the gut microbiota contributes to this oxaliplatin-induced effect.[92]

Chemotherapy-induced dysbiosis

Dysbiosis of the gut microbiota is often observed following chemotherapy and may paradoxically affect the anti-tumor properties of the treatment. In a pancreatic cancer xenograft mouse model, gemcitabine was shown to decrease the proportion of Firmicutes and Bacteroidetes while increasing the relative abundance of Proteobacteria and Verrucomicrobia.[93] Other studies have also reported gut dysbiosis after 5-FU treatment. In a CRC model, Yuan *et al*. described decreased alpha diversity and Actinobacteria abundance in the 5-FU-treated group, whereas no differences were observed in the Firmicutes and Bacteroidetes proportion.[78] In contrast, there was decreased Firmicutes and increased Bacteroidetes and Verrucomicrobia abundances in a model of 5-FU-driven intestinal mucositis.[94]

Although most of these studies focused on the gut microbiota, chemotherapy may also affect the composition of the biliary microbiome. A retrospective study has shown that PDAC patients receiving neoadjuvant therapy (NT) including gemcitabine-based or FOLFIRINOX regimes prior to surgical resection had a distinct bile microbiota compared to primary surgery alone.[95,96] However, although marked resistance to cephalosporins was observed in the NT-treated group and in patients undergoing surgery with a biliary stent,[95] the opposite was later described by Nadeem *et al* .[97]

Overall, the current state of the art suggests that chemotherapy modulates the composition of the microbial communities within the gastrointestinal tract. Considering how the microbiota itself may also determine chemotherapy efficacy, as described earlier, this therapy-induced dysbiosis may paradoxically be a factor that explains their limited therapeutic success and the inevitable chemoresistance observed in PDAC.

Influence of the microbiota on immunotherapy

Immunotherapies, including immune checkpoint blockade with anti-PD-1, anti-PD-L1 and anti-CTLA-4, have been revolutionizing cancer therapies. However, these have not shown promising results in PDAC so far.[11] The relationship between the microbiota and the immune system was among the earliest to be investigated. It is now widely acknowledged that the microbiota plays a significant role in modulating immune responses and, as a result, it has been recognized as a potent

influencer of the effectiveness of immunotherapies. Efficacy of CTLA-4 blockade with ipilimumab was shown to be compromised under germ-free conditions or after administration of a cocktail of broad-spectrum antibiotics in mouse models of MCA205 sarcoma, melanoma and colon cancer.[98] Remarkably, oral administration of *Bacteroides* species was sufficient to restore the anti-tumor properties of anti-CTLA-4 treatment in an immune-activating process that involved induction of Th1 responses and maturation of dendritic cells within the tumor. It has also been shown that the microbiota modulates the anti-tumor immune responses of PD-1/PD-L1-based immunotherapy in subcutaneous or spontaneous melanoma and MCA205 sarcoma.[99,100] Specifically, *Bifidobacterium* ,[99] *A. muciniphila* and *Enterococcus hirae* (*E. hirae*) [100] increased the efficacy of anti-PD-1 treatment *in vivo*. These effects were associated with dendritic cell maturation and IL-12 secretion, enhanced priming and intratumoral infiltration of $CD8^+$ T cells and recruitment of $CCR9^+CXCR3^+CD4^+$ T cells to the tumor, underlining that the microbiota affects treatment responses via the immune system. A similar effect was also reported for *Lactobacillus rhamnosus* GG, which improved the responses to anti-PD-1 in melanoma and colon cancer mouse models via $CD8^+$ T cell activation and enhanced IL-6 and IFN-γ production.[101] Colonization of germ-free MC38 tumor-bearing mice with a mixture of IFN-γ-producing bacteria further corroborated a better efficacy of anti-PD-1 treatment induced by the microbiota.[102] Recently, two studies highlighted a causal role for the microbiota in mediating immunotherapy efficacy in melanoma patients.[103,104] Here, transplantation of gut microbiota isolated from responder patients combined with anti-PD-1 treatment induced responses in patients that were previously anti-PD-1--refractory.[103,104] Further corroborating the bidirectional interaction between the microbiota and therapies, immunotherapy with anti-CTLA-4[98] or anti-PD-1[100] were shown to trigger changes in the gut microbiota composition both i*n vivo* and in independent cohorts of cancer patients.

Although all this data suggests that modulation of the gut microbiota may represent a novel immunotherapeutic option in combination with immune checkpoint inhibitors, this area is still understudied in PDAC due to the lack of efficacy of immunotherapies. However, what is known so far also seems to support this notion, since *in vivo* bacterial ablation reprogrammed the microenvironment of PDAC subcutaneous tumors toward a more pro-tumorigenic phenotype.[23] Indeed, as mentioned above, in an orthotopic model of PDAC, tumors did not normally respond to anti-PD-1 treatment but were rendered sensitive to it upon microbiota depletion with oral antibiotics.[21] This effect was accompanied by increased activation of $CD4^+$ and $CD8^+$ T cells within the TME.[21] Accordingly, activation of $CD8^+$ T cells and lower infiltration of $CD4^+$ $Foxp3^+$ cells were observed in another study following human-into-mice transplantation of microbiota from long-term survival PDAC patients, further supporting that the gut microbiota alters the host immune composition.[57] Even so, more studies are needed to confirm these effects and evaluate whether microbiota signatures of PDAC patients may be used to predict clinical responses to future immunotherapy approaches.

Overall, this data reinforces that each individual's gut microbiota composition should be considered as a factor while addressing the therapeutic benefits of immune checkpoint blockade. In the future, an enhanced comprehension of the microbiota in PDAC patients and potential modifications based on the microbiota composition may contribute to increased responsiveness to immunotherapies.

Microbiota-derived metabolites may affect PDAC development and treatment

Other than translocating to the pancreatic tumor microenvironment to directly interact with PDAC cells and thereby affect tumor development locally, it has been proposed that pancreatic carcinogenesis may also be mediated by microbes indirectly and even from a distance via the host immune system or through microbial-derived metabolites. These remote functions are supported by observations of lower tumor burden upon microbial depletion even in subcutaneous PDAC xenograft models lacking intratumoral bacteria.[22] This phenotype was also accompanied by increased infiltration of immune cells in the

tumor xenografts even in the absence of local bacteria.[22] Thus, it is plausible that these extra-pancreatic effects arise through immune pathways. In fact, the *Bifidobacterium*-driven anti-tumor functions described by Sivan *et al.* were also suggested to occur without the need for translocation, since the bacterium was not detected in the mesenteric lymph nodes, spleen or tumors.[99]

Considering this, researchers started to hypothesize that, besides microbes themselves, microbiota-derived metabolites could also play a role in cancer. To date, the microbiota-derived factors that have been most studied in PDAC lie within five main metabolite groups of metabolites widely known for their immunomodulatory properties,[105] comprising short-chain fatty acids (SCFAs), indole derivatives, bile acids (BAs), polyamines and purines. Among SCFAs, *in vitro* studies have reported anti-proliferative, anti-fibrogenic and pro-apoptotic effects of butyrate (a SCFA) against PDAC cells.[106–108] *In vivo* oral administration of sodium butyrate alone or in combination with gemcitabine to mice bearing subcutaneous PDAC xenografts induced stroma remodeling and was suggested to decrease tumor growth, although without statistical significance.[108] In accordance with this, treatment of the colorectal cancer cell line HCT116 with sodium butyrate also inhibited tumor cell growth *in vitro* and promoted epigenetic methylation reprogramming.[109] A recent study demonstrated a synergistic effect of butyrate with oxaliplatin in preventing proliferation, invasion and metastasis of CRC cells *in vitro* and *in vivo* .[109] A protective role for butyrate and the other two main SCFAs (propionate and acetate) was also reported in 5-FU-induced gastrointestinal mucositis by dampening excessive inflammation.[110] In PDAC patients, the relative abundance of butyrate-producing bacteria in the gut was found to be decreased[43,44] and lower concentrations of butyrate in fecal samples of PDAC patients were also reported.[44] Overall, this data suggests that the lower levels of butyrate in PDAC patients may contribute to the high tumor proliferation rates and high rates of therapy resistance, although this has to be thoroughly validated in future studies. If this link can be confirmed, using butyrate or butyrate-producing bacteria as an adjuvant therapy to improve chemotherapy efficacy while also controlling its toxicity might be a future application.

Indoles are also increasingly being linked to PDAC. In the gut, metabolism of dietary tryptophan by the microbiota generates indole derivatives, including indole-3-acetic acid (3-IAA) and indole-3-propionic acid (3-IPA). Several studies have explored how the tryptophan metabolism influences the composition of the gut microbiota and intestinal immunity.[111] Recently, a pro-tumorigenic role for tryptophan-derived metabolites in PDAC was highlighted by Hezaveh *et al* .[112] In this study, indoles produced by *Lactobacillus*-mediated catabolism of tryptophan activated the aryl hydrocarbon receptor (AhR) in macrophages. This promoted reprogramming of tumor-associated macrophages into a tumor-supporting phenotype and inhibited IFN-γ expression in $CD8^+$ T cells. Importantly, deleting Ahr in macrophages or removing tryptophan from the diet prevented tumor growth.[112] More recently, we have shown that 3-IAA increases the efficacy of FIRINOX (5-FU, irinotecan and oxaliplatin) and gemcitabine+nab-paclitaxel in murine models of PDAC, leading to decreased tumor weight.[25] Serum metabolomics unveiled a positive association between 3-IAA and progression-free and overall survival in two human PDAC cohorts. In supplemental murine studies, PDAC tumors were rendered susceptible to chemotherapy upon colonization of germ-free mice with microbiota from responders and oral supplementation of 3-IAA. Mechanistically, this 3-IAA-mediated effect required immune cell-derived myeloperoxidase, which metabolized 3-IAA into toxic products, including 3-methylene-2-oxindole (MOI), that promoted accumulation of reactive oxygen species and autophagy downregulation in the tumor cells, boosting the effect of chemotherapy.[25]

Besides SCFAs and indole derivatives, BAs have also been explored in the context of PDAC. Primary BAs, including glycocholic acid (GCA), glycochenodeoxycholic acid (GCDCA), taurocholic acid (TCA) and taurochenodeoxycholic acid (TCDCA) are metabolized by gut bacteria upon secretion into the duodenum, giving rise to secondary BAs which include deoxycholic acid (DCA), lithocholic acid (LCA) and ursodeoxycholic acid (UDCA).[113] Levels of primary BAs

were reported to be higher in PDAC patients,[114] especially in those with obstructive jaundice.[115] *In vitro* treatment of Capan-1 and BxPC-3 PDAC cell lines with BAs induced their proliferation, adhesion to collagen 1, migration, invasion and colony-forming ability.[115] BAs also upregulated the expression of mucin 4 in a dose-dependent manner and a siRNA-mediated knockdown of mucin 4 prevented these carcinogenic processes.[115] Upregulation of cyclooxygenase-2 expression, which is suggested to be linked to cancer development, was also induced in PDAC cell lines upon exposure to DCA and chenodeoxycholic acid.[116] In the specific case of DCA, it has also been reported to have oncogenic functions in PDAC via activation of epidermal growth factor receptor (EGFR), Signal transducer and activator of transcription 3 (STAT3) and mitogen-activated protein kinase (MAPK) signaling pathways, promoting cell cycle progression.[117] Moreover, the effects of bile itself (which is mainly composed of BAs) in PDAC peritoneal metastasis have also been investigated. Bile samples isolated from PDAC patients reduced peritoneal tumor growth upon co-injection of Panc02 tumor cells and bile into the peritoneum.[118] However, since analyses of the roles of specific BAs in PDAC oncogenesis have mainly relied on *in vitro* studies so far, *in vivo* functional assessment is still required. Besides carcinogenesis, whether BAs modulate treatment responses in PDAC has not been fully addressed, except for a study by Yang *et al*., which demonstrated that extracellular TCA decreases the sensitivity of Panc-1 and CFPAC-1 cells to gemcitabine via the S1PR2-ERK pathway.[114]

The metabolism of polyamines such as putrescine, spermidine and spermine is commonly dysregulated across different tumor entities and has also been implicated in PDAC. Levels of polyamines were found to be enriched in serum of PDAC patients and in KPC mice during disease progression from early stages to PDAC.[119] Moreover, expression of polyamine-related genes was inversely associated with survival in a PDAC patient cohort,[120] further suggesting a role for polyamines in pancreatic cancer. Indeed, a recent study further explored how PDAC cells synthesize polyamines to ensure tumor growth.[121] The authors revealed that glutamine was used in tumor cells as a substrate for ornithine production, which subsequently contributed to polyamine synthesis in a process dependent on ornithine aminotransferase (OAT). Notably, PDAC cells also relied on mutated KRAS to upregulate the expression of proteins required for polyamine synthesis, including OAT.[121] Pharmacological targeting of OAT, thus compromising polyamine synthesis, suppressed PDAC growth both *in vitro* and *in vivo*.[121] Although this study did not specifically investigate bacteria as a source of polyamines, these compounds may also be produced by the gut microbiota.[122] Mendez *et al*., for instance, reported that *Lactobacillus reuteri*, which was detected in later stages of PDAC development, was associated with polyamine metabolism.[119] Overall, this data opens the possibility to target polyamine metabolism in PDAC. However, more research is needed to elucidate to what extent the microbiota contributes to polyamine availability within the TME.

Finally, since purine synthesis is essential for cell proliferation, a dysregulated purine metabolism is often linked to cancer progression.[123] Indeed, in PDAC cells, oncogenic KRAS is able to enhance the synthesis of purines and pyrimidines to support proliferation.[124] In particular, inosine, xanthine and hypoxanthine, which are degradation products of adenine nucleotides during purine synthesis, were found to be decreased in gemcitabine-treated PDAC-xenografted mice.[93] Importantly, the microbiota may also produce these purine-related metabolites[125] and microbial-derived inosine was shown to promote Th1 activation and optimize responses to anti-CTLA-4 treatment in intestinal, bladder cancer and melanoma mouse models.[126] However, further research is needed to provide mechanistic insights into the potential roles of microbial-derived nucleosides in PDAC.

Altogether, the biological activity of microbiota-derived metabolites in PDAC still remains largely unknown. However, this is a growing field of interest, and high-throughput metabolomic screens are currently being used to help identify metabolites participating in PDAC development and determining responses to chemo- and immunotherapy.

Microbiota modulation has therapeutic potential in PDAC

Currently, treatment of advanced PDAC patients relies mainly on chemotherapy-based regimens, although resistance to chemotherapy is often observed in the clinics.[127] Therefore, PDAC treatment remains a challenge, and there is an urgent need to sensitize PDAC tumors to available therapies or develop novel strategies with better efficacy.[128] Considering this scenario and the described roles for microbes in PDAC onset, progression and resolution, modulation of the microbiota may represent a novel therapeutic target to optimize the clinical outcomes in PDAC. So far, there are several approaches to modulate the microbiota which rely on diet, administration of pro- or antibiotics, fecal microbial transplantation, use of bacteriophages and substitution of specific bacteria isolates, among others.

Diet is a key factor influencing the composition of the microbiota in the gut and the availability of bioactive microbial-derived metabolites,[129] making dietary interventions an appealing option in microbiota-associated diseases. The association between diet and pancreatic cancer risk is, however, still poorly explored. High-fat diets negatively imbalance the gut microbiota while favoring pathogenic taxa, impairing gut barrier integrity and even driving colorectal carcinogenesis in a microbiota-dependent manner.[130] High-fiber diets, in contrast, seem to have anti-tumor properties in CRC as a result of the metabolism of fibers into SCFAs such as butyrate.[131] Accordingly, high intake of dietary fibers correlates negatively with pancreatic cancer risk.[132] In line with this, in a xenograft mouse model of PDAC, an engineered resistant-starch (ERS) diet, which is fermented into SCFAs by colon bacteria, was shown to delay tumor growth.[133] These types of high-fiber non-digestible foods that promote the growth and functions of probiotic bacteria in the gut are often referred to as prebiotics.[129]

The diet could also be considered an interesting option to improve treatment outcomes in PDAC. Caloric restriction through fasting was described to improve responses to gemcitabine both *in vitro* and *in vivo* .[134] In addition, we have recently shown that efficacy of chemotherapy *in vivo* was enhanced by 3-IAA resulting from a short-term tryptophan-high diet, as opposed to a more prolonged administration of dietary tryptophan which promotes tumor growth.[25] It seems that dietary changes may even impact therapy toxicity, since dietary fibers were shown to reduce irinotecan-induced toxicity in a rat model of colon carcinoma.[135] Even so, despite the promising effects of diet-based interventions in PDAC treatment, we are still lacking solid results from human-based trials. However, dietary clinical trials and clinical implementation of nutritional interventions involve several challenges that should be carefully considered, including difficulties in ensuring patient compliance, absence of standardized protocols and timing and duration of the interventions.[136]

Probiotics, defined as living microbes associated with a health benefit to the host, are also being considered to ameliorate clinical outcomes in cancer. Research on probiotics in PDAC is still in its early stages, and the available data is scarce. Nevertheless, the most attractive candidates at the moment are butyrate-producing bacteria, such as *Faecalibacterium prausnitzii* (*F. prausnitzii*), *Eubacterium rectale* (*E. rectale*) and *Roseburia intestinalis*, given the reported anti-tumor roles of butyrate.[137] Interestingly, these three species were shown to be less present in PDAC patients,[44] as further confirmed in another study for *F. prausnitzii* and *E. rectale*.[30] It has also been suggested that the administration of a probiotic mixture (composed mainly of *Bifidobacterium* spp. and *Lactobacterium* spp.) to PDAC-xenografted mice may retard epithelial-to-mesenchymal transition in the pancreas.[138] In addition to probiotics, the use of postbiotics, consisting of inanimate microorganisms and/or bioactive compounds secreted by the intestinal microbiota (such as SCFAs), may also represent an interesting therapeutic strategy in PDAC, as

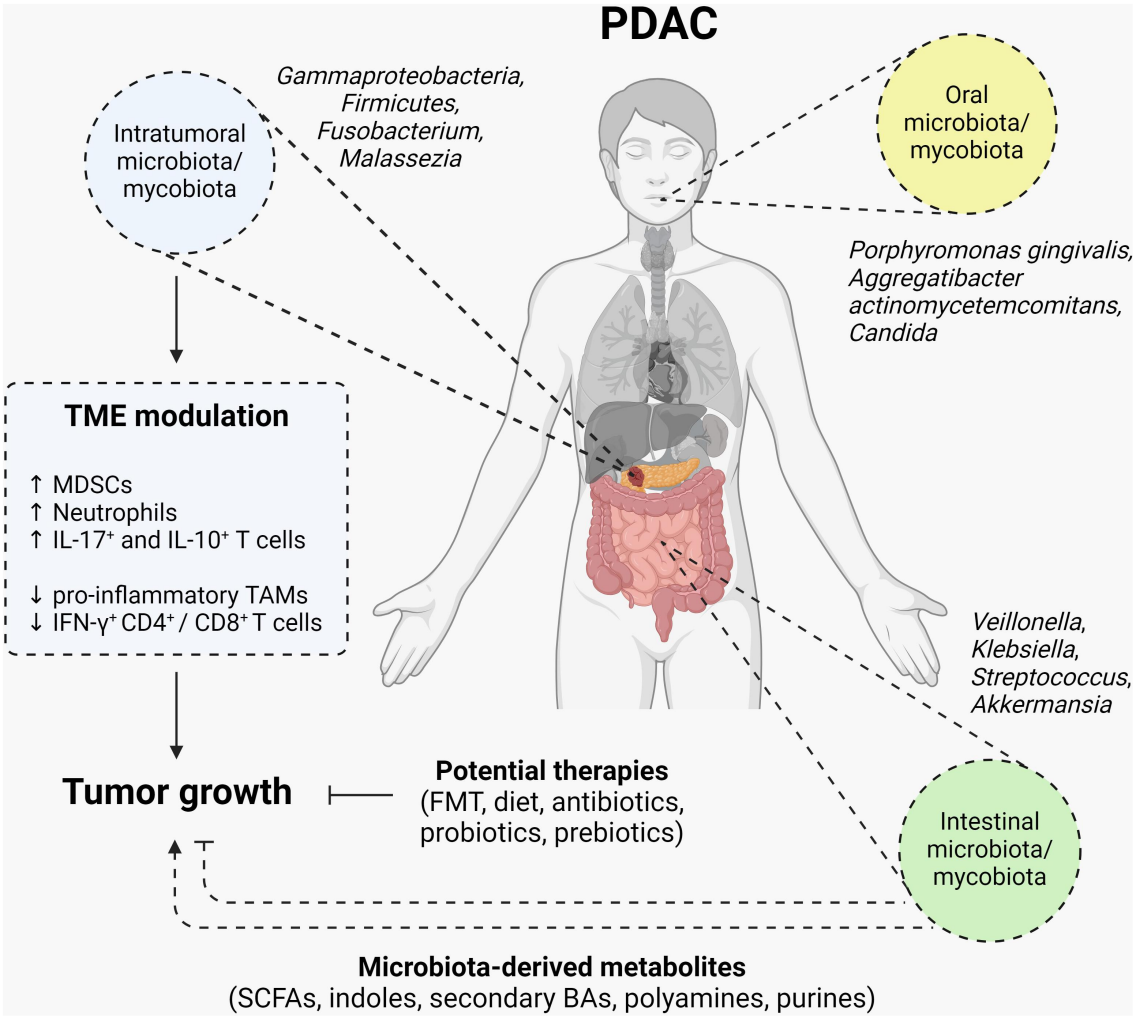

Figure 1. Roles of the microbiota in PDAC. The microbiota plays a role in PDAC carcinogenesis via the modulation of the immune compartment composition in the tumor microenvironment or via the production of microbiota-derived metabolites. PDAC patients have unique oral, intestinal and intratumoral microbiota signatures and its characterization may be useful as clinical biomarkers of PDAC diagnosis and prognosis. Considering this, strategies modulating the composition of the microbiota have therapeutic potential in PDAC. MDSCs: myeloid-derived suppressor cells. TAMs: tumor-associated macrophages. SCFAs: short-chain fatty acids. BAs: bile acids. Created with BioRender.com.

discussed earlier in section 4. However, research on postbiotics in PDAC is still evolving and their clinical use still faces several challenges, including the limited understanding of their mechanisms of action, as well as the need for standardization within the clinical setting.[139]

In the case of antibiotics, antibiotic therapy is often used in cancer patients prior to surgery or during treatment as a measure against opportunistic infections.[140] The beneficial roles of antibiotics and antifungals in PDAC have been thoroughly analyzed in preclinical models[21–23,48] and retrospective cohort studies.[77,80,141] However, broad spectrum antibiotics may not only eliminate pathogenic bacteria which have been shown to mediate carcinogenesis or resistance to chemo- or immunotherapeutic drugs, but also commensal bacteria that may suppress tumor progression. Therefore, its use as an anti-tumor strategy should be carefully considered. Considering this, bacteriophage therapy is emerging as an alternative to precisely target specific bacteria associated with progression and poor prognosis in cancer,[30,142,143] but it has not been formally explored in PDAC yet.

Another possible strategy to modulate the gut microbiota is fecal microbial transplantation

(FMT), in which a mixture of gut microbes isolated from a healthy donor is used to colonize the gut of a recipient. Evidence supporting this approach in PDAC has been provided by Riquelme *et al.*, who showed that FMT from patients with long-term survival into mice elicited anti-tumor immune activation characterized by higher numbers of activated $CD8^+$ T cells and reduced infiltration of $CD4^+Foxp3^+$ cells and MDSCs.[57] To evaluate whether these findings translate into the clinical setting, a phase I trial is ongoing with resectable PDAC patients receiving FMT delivered through colonoscopy or oral capsules, estimated to end in December 2023.[144]

Concluding remarks and future perspectives

In conclusion, research over the last decade has clearly shown that PDAC is associated with unique oral, gut and pancreatic microbiota signatures (Figure 1). However, these signatures were not consistent across different cohorts and high inter-individual variability within each cohort was often reported. This poor reproducibility may be due to differential environmental and lifestyle-related factors, including geographical location, smoking and dietary habits, as well as lack of adjustment for potential existing confounding factors and differences in sampling and sequencing methods used. Recently, increasing concerns in the scientific community[63,64] have also reinforced the importance of appropriate and careful sampling and analysis of microbial sequencing datasets, which should include control for sample contamination and correct separation of bacteria or fungi and human genomic reads to minimize false positive findings. Accordingly, despite the vast availability of literature on this topic, identifying a clearly consistent and transversal gut and intratumoral microbial composition still remains a challenge in PDAC. In addition, although experiments conducted in preclinical models of PDAC have been defining several organisms as causative agents of PDAC, more functional experiments are still needed to prove the role of microbe-mediated pathogenicity and thus provide mechanistic evidence of this interplay, especially in the case of fungi.

In addition to the direct roles of microbes themselves, microbial-derived metabolites are also increasingly being explored in this field. In the future, these metabolites may not only become useful as diagnostic biomarkers but also as mediators of therapy efficacy. Importantly, the profile of microbiota-derived metabolites arises from the diverse activities of various bacterial strains, potentially serving as a comprehensive representation of complex bacterial communities. Consequently, investigating these metabolites could aid in mitigating the substantial variability observed when considering the composition of bacteria across different cohorts. Overall, in this review, we have highlighted and summarized the microbial-related functions reported thus far in PDAC (Figure 1) and discussed how these may pave the way for the development of novel diagnostic therapeutic options aiming at improving PDAC prognosis.

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