The Microbiome in Pancreatic Ductal Adenocarcinoma

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Pancreatic cancer is a highly lethal cancer and less than 10% of patients survive the 5-year mark. The molecular and biological underpinnings leading to this dismal prognosis are well-described, however, translation of these findings with subsequent improvement of the poor prognosis has been slow. The complex and dynamic accumulation of microbes, also called the microbiome, has attracted scientific interest in the pathogenesis of several diseases including pancreatic cancer. Since then, a limited number of significant findings were published pointing towards an important role of the microbiome in cancer, in particular pancreatic cancer.

Keywords: microbiome ; pancreatic ductal adenocarcinoma ; biomarker

1. The Role of the Microbiome in Pancreatic Carcinogenesis

In 2012, the International Agency for Research on Cancer (IARC) Working Group on the Evaluation of Carcinogenic Risks to Humans reported that about 13% of all global cancer cases are caused by so-called “oncomicrobes”. Eleven distinctly defined microbes evidently induce cancer, and there is experimental evidence for even more [1]. Contrary to these well-defined oncomicrobes in certain tumor entities, there is emerging evidence that the tumoral microbiome contributes to carcinogenesis in different ways. **Figure 1** illustrates the established and putative associations between the microbiota and oncogenesis.

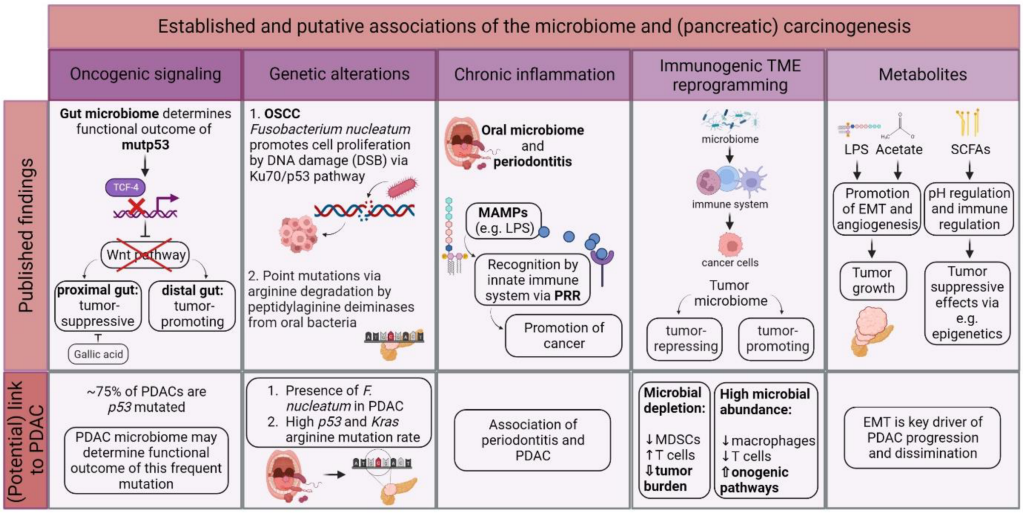

Figure 1. Potential involvement of the microbiome in (pancreatic) oncogenesis. There is growing evidence on how different microbiomes contribute to carcinogenesis, e.g., via promoting oncogenic signaling, direct and indirect genetic alterations, chronic inflammation, and interaction with the immune system and secretion of microbe-derived metabolites. However, most of these theories have yet to be validated in PDAC patients. Tumor microenvironment (TME); mutant p53 (mutp53); pancreatic ductal adenocarcinoma (PDAC); oral squamous cell carcinoma (OSCC); desoxyribonucleic acid (DNA); double-strand break (DSB); microbe-associated molecular pattern (MAMP); lipopolysaccharide (LPS); pattern recognition receptor (PRR); myeloid-derived suppressor cell (MDSC); short-chain fatty acid (SCFA); epithelial-to-mesenchymal transition (EMT); and pondus hydrogenii (pH).

2. Diagnostic Aspects of the Microbiome in PDAC

2.1. Difficulties in Establishing Screening Tools for PDAC

Considering the available descriptive and preliminary mechanistic findings on the PDAC tumor microbiome, the question of its potential diagnostic value and possible implication as a biomarker may arise. One of the main problems with PDAC

is most often the late-stage diagnosis as the tumor is often locally advanced or metastasized. This is mostly due to a lack of early-stage symptoms. To date, a reliable screening method for pancreatic cancer is not available in the clinical routine [2]. Studies investigating different site-specific microbiomes, such as the oral and fecal microbiome, point towards a possible application of the microbiome as a diagnostic biomarker in PDAC [3][4].

2.2. The Orointestinal Microbiome as PDAC Biomarker

Indeed, there are numerous publications addressing the microbiome in the oral cavity and its diagnostic potential for PDAC, of which the latest are summarized in **Table 1**. One of the largest studies was published by Fan et al., which was a population-based nested case-control study on the predictive power of the oral microbiome to assess the risk for pancreatic cancer [5]. Over 730 oral wash samples from two prospective cohort studies were evaluated. The authors found oral pathogens such as *Porphyromonas gingivalis* to be associated with an increased pancreatic cancer risk. The pitfall of the microbial patterns of the oral cavity, however, is their rather pronounced heterogeneity and low specificity, as they may also be present in other cancer entities [6]. Microbiome studies present contradictory results concerning the microbial composition and differential abundances of these microbes (**Table 1**). This can be mainly ascribed to the different kinds of sampling methods, e.g., sputum, dorsal tongue, buccal, or gingival swabs. Furthermore, due to different sequencing approaches, i.e., depending on the selected variable (V) region of the 16S rRNA gene, the results significantly vary [7].

Table 1. Summary of studies regarding the oral, intestinal, and fecal microbiome of patients as a non-invasive biomarker for pancreatic cancer.

Year	Authors	Study Design; Country of Conduction	Sample Type	Detection Method	Number of Patients	Change in Bacterial Composition	Ref.
2012	Farrell et al.	Prospective study; USA	Saliva	Microarray, qRT-PCR	38 PC 27 CP 38 HC	***Neisseria elongata, Streptococcus mitis*** increased in PC cases	[3]
2013	Lin et al.	Cross-sectional study; USA	Oral wash samples	16S rRNA amplicon sequencing (NGS)	13 PC 3 CP 12 HC	***Bacteroides*** increased in PC cases as compared with HC; ***Corynebacterium, Aggregatibacter*** decreased in PC cases as compared with HC	[8]
2013	Michaud et al.	Prospective study; European countries	Blood	Immunoblot array	405 PC 416 HC	Plasma IgG against ***Porphyromonas gingivalis*** ATCC 53978 increased in PC cases	[9]
2015	Torres et al.	Cross-sectional study; USA	Saliva	16S rRNA amplicon sequencing (V4 region) (NGS); qRT-PCR	8 PC 78 other diseases (including pancreatic disease, non-pancreatic digestive disease/cancer, and non-digestive disease/cancer) 22 HC	***Leptotrichia:Porphyromonas*** ratio increased in PC cases; ***Neisseria, Aggregatibacter*** decreased in PC cases	[10]
2016	Fan et al.	Case-control study; USA	Oral wash samples	16S rRNA amplicon sequencing (V3–V4 region) (NGS)	361 PDAC 371 HC	***Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans*** increased in PDAC cases; ***Leptotrichia*** decreased in PDAC cases	[5]

Year	Authors	Study Design; Country of Conduction	Sample Type	Detection Method	Number of Patients	Change in Bacterial Composition	Ref.
2017	Ren et al.	Prospective study; China	Feces	16S rRNA amplicon sequencing (V3–V5 region) (NGS)	85 PC 57 HC	*Veillonella, Klebsiella, Selenomonas*, LPS-producing bacteria (*Prevotella, Hallella, Enterobacter, Cronobacter*) increased in PC cases; *Bifidobacterium*, butyrate-producing bacteria (*Coprococcus, Clostridium IV, Blautia, Flavonifractor, Anaerostipes bifidum, Butyricicoccus, Dorea, Gemmiger*) decreased in PC cases	[11]
2017	Olson et al.	Cross-sectional study; USA	Saliva	16S rRNA amplicon sequencing (V4–V5 region) (NGS)	40 PDAC 39 IPMN 58 HC	*Firmicutes* (e.g., *Streptococcus*) increased in PDAC cases; *Proteobacteria* (e.g., *Haemophilus, Neisseria*) decreased in PDAC cases as compared with HC	[12]
2018	Pushalkar et al.	Case-control study; USA	Rectal swabs	16S rRNA amplicon sequencing (V3–V4 region) (NGS)	32 PDAC 31 HC	*Proteobacteria, Actinobacteria, Fusobacteria, Verrucomicrobia, Synergistetes, Euryarchaeota* increased in PDAC cases	[13]
2018	Mei et al.	Case-control study; China	Duodenal mucosa	16S rRNA amplicon sequencing (V3–V4 region) (NGS)	14 PC (pancreatic head cancer) 14 HC	*Acinetobacter, Aquabacterium, Oceanobacillus, Rahnella, Massilia, Delftia, Deinococcus, Sphingobium* increased in PC cases; *Porphyromonas, Paenibacillus, Enhydrobacter, Escherichia, Shigella, Pseudomonas* decreased in PC cases	[14]
2019	Lu et al.	Case-control study; China	Tongue coat samples	16S rRNA amplicon sequencing (V3–V4 region) (NGS)	30 PC (pancreatic head cancer) 25 HC	*Leptotrichia, Fusobacterium, Rothia, Actinomyces, Corynebacterium, Atopobium, Peptostreptococcus, Catonella, Oribacterium, Filifactor, Campylobacter, Moraxella, Tannerella* increased in PC cases; *Haemophilus, Porphyromonas, Paraprevotella* decreased in PC cases	[15]
2019	del Castillo et al.	Cross-sectional study; USA	Tissue samples (pancreatic duct, duodenum, pancreas); swabs (bile duct, jejunum, stomach); feces	16S rRNA amplicon sequencing (V3–V4 region) (NGS)	39 PC 12 periampullary cancer 18 non-cancer pancreatic conditions 8 non-cancer gastrointestinal conditions 34 HC	*Porphyromonas, Prevotella, Selenomonas, Gemella, Fusobacterium spp.* increased in cancer cases as compared with non-cancer cases; *Lactobacillus* decreased in cancer cases as compared with non-cancer cases	[16]

Year	Authors	Study Design; Country of Conduction	Sample Type	Detection Method	Number of Patients	Change in Bacterial Composition	Ref.
2019	Half et al.	Case-control study; Israel	Feces	16S rRNA amplicon sequencing (V3–V4 region) (NGS)	30 PDAC 6 pre-cancerous lesions 16 NAFLD 13 HC	***Veillonellaceae, Akkermansia, Odoribacter*** increased in PDAC cases as compared with HC; ***Clostridiacea, Erysipelotrichaeceae, Ruminococcaceae, Lachnospiraceae, Anaerostipes*** decreased in PDAC cases as compared with HC	[17]
2020	Vogtmann et al.	Case-control study; Iran	Saliva	16S rRNA amplicon sequencing (V4 region) (NGS)	273 PDAC 285 HC	***Enterobacteriaceae, Lachnospiraceae*** G7, ***Bacteroidaceae, Staphylococcaceae*** increased in PDAC cases; ***Haemophilus*** decreased in PDAC cases	[18]
2020	Sun et al.	Case-control study; China	Saliva	16S rRNA amplicon sequencing (V3–V4 region) (NGS)	10 PC 17 BPD 10 HC	***Fusobacteria*** (e.g., ***Fusobacterium periodonticum***), ***Bacteroidetes, Firmicutes*** increased in PC cases; ***Proteobacteria*** (e.g., ***Neisseria mucosa***) decreased in PC cases	[19]
2020	Kohi et al.	Case-control study; USA	Duodenal fluid	16S and 18S rRNA amplicon sequencing (16S V3–V4 rRNA region, 18S ITS1 rRNA region) (NGS)	74 PDAC 98 pancreatic cysts 134 HC	***Fusobacterium, Bifidobacterium*** genera, ***Enterococcus*** increased in PDAC cases as compared with HC; ***Escherichia/Shigella, Enterococcus, Clostridium sensu stricto 1, Bifidobacterium*** increased in PDAC cases as compared with pancreatic cysts; ***Fusobacterium, Rothia, Neisseria*** increased in PDAC cases with short-term survival	[20]
2020	Wei et al.	Case-control study; China	Saliva	16S rRNA amplicon sequencing (V3–V4 region) (NGS)	41 PDAC 69 HC	***Leptotrichia, Actinomyces, Lachnospiraceae, Micrococcaceae, Solobacterium, Coriobacteriaceae, Moraxellaceae, Streptococcus, Rothia, Peptostreptococcus, Oribacterium*** increased in PDAC cases; ***Porphyromonas gingivalis, Fusobacteriaceae, Campylobacter, Spirochaetaceae, Veillonella, Neisseria, Selenomona, Tannerella forsythia, Prevotella intermedia*** decreased in PDAC cases	[21]

Year	Authors	Study Design; Country of Conduction	Sample Type	Detection Method	Number of Patients	Change in Bacterial Composition	Ref.
2021	Zhou et al.	Case-control study; China	Feces	Metagenomic shotgun sequencing (NGS)	32 PDAC 32 AIP 32 HC	*Gammaproteobacteria* (e.g., *Escherichia coli*), *Veillonella* (*V. atypica, V. parvula, V. dispar*), *Clostridium* (e.g., *Clostridium bolteae, Clostridium symbiosum*), *Fusobacterium nucleatum, Streptococcus parasanguinis, Prevotella stercorea* increased in PDAC cases as compared with HC; Butyrate-producing bacteria (*Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Coprococcus*), *Ruminococcus, Dialister succinatiphilus* decreased in PDAC cases as compared with HC	[22]
2021	Matsukawa et al.	Case-control study; Japan	Feces	Whole-genome sequencing (including PCR) (NGS)	24 PC (thereof 22 PDAC) 18 HC	*Klebsiella pneumoniae, Clostridium bolteae, Clostridium symbiosum, Streptococcus mutans, Alistipes shahii, Bacteroides, Parabacteroides, Lactobacillus* increased in PC cases	[23]
2021	Sugimoto et al.	Case-control study; Japan	Duodenal fluid	16S rRNA terminal restriction fragment length polymorphism method (5' FAM-labeled 516F and 1510R primers)	22 benign pancreaticobiliary diseases (thereof 16 BPD) 12 pancreaticobiliary cancer (thereof 9 PC)	*Bifidobacterium, Clostridium* cluster XVIII increased in PC cases as compared with BPD	[24]
2022	Petrick et al.	Prospective study; USA	Oral wash samples	Metagenomic shotgun sequencing (NGS)	148 PDAC (thereof 122 of African Americans, 26 of Caucasians) 441 HC (thereof 354 of African Americans, 87 of Caucasians)	No significant changes in PDAC cases among African Americans; *Porphyromonas gingivalis* increased in PDAC cases among Caucasians; *Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia* increased in PDAC cases among never-smokers	[25]

Year	Authors	Study Design; Country of Conduction	Sample Type	Detection Method	Number of Patients	Change in Bacterial Composition	Ref.
2022	Kartal et al.	Case-control study; Spain, Germany	Saliva	Metagenomic shotgun sequencing (NGS) (43 PDAC, 12 CP, 45 HC) 16S rRNA amplicon sequencing (V4 region) (NGS) (59 PDAC, 28 CP, 55 HC)	59 PDAC 28 CP 55 HC (Spanish cohort only)	No significant changes in PDAC cases	[4]
			Feces	Metagenomic shotgun sequencing (NGS) (101 PDAC, 29 CP, 82 HC) 16S rRNA amplicon sequencing (V4 region) (NGS) (51 PDAC, 23 CP, 46 HC)	101 PDAC 29 CP 82 HC (thereof 57 PDAC, 29 CP and 50 HC from Spanish cohort; 44 PDAC and 32 HC from German cohort)	*Streptococcus, Akkermansia, Veillonella atypica, Fusobacterium nucleatum/hwasookii, Alloscardovia omnicolens* increased in PDAC cases as compared with HC; *Romboutsia timonensis, Faecalibacterium prausnitzii, Bacteroides coprocola, Bifidobacterium bifidum* decreased in PDAC cases as compared with HC	
2022	Guo et al.	Case-control study; China	Feces	16S rRNA amplicon sequencing (27F, 1492R primer) (NGS)	36 resectable PDAC 36 unresectable PDAC	*Pseudonocardia, Cloacibacterium, Mucispirillum, Anaerotruncus* increased in unresectable PDAC cases; *Alistipes, Anaerostipes, Faecalibacterium, Parvimonas* decreased in unresectable PDAC cases	[26]

Year	Authors	Study Design; Country of Conduction	Sample Type	Detection Method	Number of Patients	Change in Bacterial Composition	Ref.
2022	Nagata et al.	Case-control study; Japan, Spain, Germany	Saliva	Metagenomic shotgun sequencing (NGS)	90 PDAC 280 HC (thereof 47 PDAC and 235 HC from Japanese cohort; others from Kartal et al., 2022)	*Firmicutes* (unknown *Firmicutes, Dialister* and *Solobacterium spp.*), *Prevotella spp.* (*Prevotella pallens, Prevotella sp.* C561) increased in PDAC cases among Japanese cohort; *Streptococcus* spp. (e.g., *Streptococcus salivarius, Streptococcus thermophilus, Streptococcus australis*) decreased in PDAC cases among Japanese cohort; No significant changes in PDAC cases among Spanish cohort; No correlation for oral species between the Japanese and Spanish datasets	[27]
			Feces	Metagenomic shotgun sequencing (NGS)	144 PDAC 65 CP 150 IPMN 317 HC (thereof 43 PDAC, 65 CP, 150 IPMN and 235 HC from Japanese cohort; others from Kartal et al., 2022)	*Streptococcus oralis, Streptococcus vestibularis, Streptococcus anginosus, Veillonella atypica, Veillonella parvula, Actinomyces spp., Clostridium symbiosum,* unknown *Mogibacterium, Clostridium clostridioforme* increased in PDAC cases as compared with HC among Japanese cohort; Unknown *Lachnospiraceae, Eubacterium ventriosum,* unknown *Butyricicoccus, Faecalibacterium prausnitzii* decreased in PDAC cases as compared with HC among Japanese cohort; *Clostridium symbiosum, Streptococcus oralis*, unknown *Mogibacterium* increased in PDAC cases as compared with IPMN and CP among Japanese cohort; Significant correlation for gut species between the Japanese and Spanish datasets and between the Japanese and German datasets; *Streptococcus* spp. (*S. anginosus* and *S. oralis*), *Veillonella spp.* (*V. parvula* and *V. atypica*) increased in PDAC cases among all three cohorts; *Faecalibacterium prausnitzii* decreased in PDAC cases among all three cohorts	

United States of America (USA), quantitative real-time polymerase chain reaction (qRT-PCR), pancreatic cancer (PC), chronic pancreatitis (CP), healthy control (HC), Svedberg unit (S), ribosomal ribonucleic acid (rRNA), next-generation sequencing (NGS), immunoglobulin G (IgG), American Type Culture Collection (ATCC), variable (V), pancreatic ductal adenocarcinoma (PDAC), lipopolysaccharide (LPS), intraductal papillary mucinous neoplasm (IPMN), non-alcoholic fatty liver disease (NAFLD), benign pancreatic disease (BPD), internal transcribed spacer between 18S and 5.8S rRNA (ITS1), autoimmune pancreatitis (AIP), polymerase chain reaction (PCR), fluorescein amidite (FAM), forward (F), reverse (R).

Many studies have demonstrated a positive correlation between the pancreas and gut microbiome. For example, Ren et al. reported that the gut microbiome analyzed via stool samples was unique in PDAC and may serve as a non-invasive

biomarker for the diagnosis of this disease [11]. Recently, Kartal et al. explored the fecal and salivary microbiota in PDAC patient samples from a Spanish and German case-control study as potential biomarkers; they found 27 fecal species that could be employed to identify PDAC throughout early and late stages with high accuracy. Thus, the authors suggested the fecal microbiome as a feasible early-stage PDAC biomarker, particularly in combination with carbohydrate antigen 19-9 [4]. However, these findings require validation in larger patient cohorts. Only a few months later, Nagata et al. reused the data from Kartal et al. and added their Japanese cohort dataset, which also included oral and gut bacteriophages [27]. Their aim was to further identity oral and gut metagenomic microbial signatures to predict PDAC. The authors found 30 gut and 18 oral species to be significantly associated with PDAC in their newly introduced Japanese cohort, and their metagenomic classifiers were also able to predict PDAC accurately. Consistently with Kartal et al., Nagata et al. found the gut microbiomes of European and Asian patients to present a globally robust and powerful biomarker for identifying PDAC.

Taken together, the orointestinal microbiome might be used as a non-invasive screening tool. However, the translational implication to the clinical setting remains unclear at present. Given the high cost of sequencing, a multiplex PCR or microarray for those identified bacteria might be more feasible. Furthermore, it must be discussed who will be screened, whether it be only high-risk patients or a broader screening population. Further studies with high sample numbers, such as one already completed in the U.S. (NCT03302637), will hopefully provide answers to these questions.

2.3. Blood-Derived Microbial Signatures as PDAC Biomarker

One of the most common sampling techniques in the clinical routine is blood drawing. Bacterial extracellular vesicles (bEV) are nano-sized, lipid membrane-delimited particles that contain different molecules, such as DNA, metabolites, proteins, and lipids. Recently, there is growing evidence that bEVs play an important role in bacteria–bacteria and bacteria–host communication [28][29]. These bEVs can be detected in the host's blood, urine, bile, and stool. The exploitation of these vesicles for therapeutic and diagnostic purposes is still in its infancy [30]. One recent study revealed the diagnostic value of bEVs for differentiating between benign and malignant tumors [31]. Another Korean study performed a retrospective propensity score matching analysis showing a distinguishable composition of bEVs in blood by 16S rRNA sequencing [32]. Here again, environmental bacteria were detected in peripheral blood, emphasizing the need for thorough decontamination protocols for blood samples as well, in cases where bacterial DNA is found in very low concentrations [28]. Poore et al. demonstrated that microbial plasma profiles in over 10,000 patients, which were different from their respective healthy tissue signature, can predict different cancer types [33]. The authors used whole-genome and whole-transcriptome sequencing studies from TCGA. Moreover, pre-diagnosis blood samples from PDAC patients were subject to oral microbiota antibody measurements in a study by Michaud et al. Indeed, high levels of antibodies against *Porphyromonas gingivalis*, the pathogen responsible for periodontitis, was correlated with a two-fold increased PDAC risk [9].

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