Microbiome and pancreatic cancer: A comprehensive topic review of literature

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Abstract

AIM
To review microbiome alterations associated with pancreatic cancer, its potential utility in diagnostics, risk assessment, and influence on disease outcomes.

METHODS
A comprehensive literature review was conducted by all-inclusive topic review from PubMed, MEDLINE, and Web of Science. The last search was performed in October 2016.

RESULTS
Diverse microbiome alterations exist among several body sites including oral, gut, and pancreatic tissue, in patients with pancreatic cancer compared to healthy populations.

CONCLUSION
Pilot study successes in non-invasive screening strategies warrant further investigation for future translational application in early diagnostics and to learn modifiable risk factors relevant to disease prevention. Pre-clinical investigations exist in other tumor types that suggest microbiome manipulation provides opportunity to favorably transform cancer response to existing
treatment protocols and improve survival.

**Key words:** Pancreatic Cancer; Human microbiome; Biomarkers, cancer; Cancer screening tests; Treatment effectiveness

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**Core tip:** Recent literature reports influences of microbiome alterations contributing to carcinogenesis of pancreatic cancer. The poor prognostics of pancreatic cancer are related to late recognition and treatment resistance, thus warranting investigations for modifiable risk factors, early screening biomarkers, and microenvironment elements that affect outcomes. Learning the role of microbiome in carcinogenesis may lead to identifying reliable, non-invasive screening strategies, and additional modifiable risk factors. Microbiome studies in pancreatic cancer could offer therapeutic targets and an extraordinary opportunity to favorably transform cancer response to existing treatment protocols and improve survival by reduction of cancer-related cachexia by manipulating human gut microbiota.

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**INTRODUCTION**

A commensal microbiome, by definition maintains a symbiotic relationship in healthy individuals, offering protection from disease by nutritive, inflammatory-modulating activity, hormonal homeostasis, detoxification, and metabolic effects of bacterial metabolites[1-3]. Dysbiosis is the manifestation of a corrupt, imbalanced microbiome, which contributes to pathogenesis of several diseased states[2]. Recently, there are literature reports on influences of microbiome alteration contributing to carcinogenesis of multiple malignancies[1,2,4-6]. A classic pathogen in the literature is *Helicobacter pylori* (*H. pylori*), which has revealed inconsistent and paradoxical associations pending the body site studied[13]. *H. pylori* has been extensively scrutinized as a risk factor for development of pancreatic cancer and an association is controversial[9,12]. Pancreatic cancer often denotes a poor clinical prognosis in part due to late recognition and treatment resistance, warranting investigations for modifiable risk factors, early screening biomarkers, and microenvironment elements that affect outcomes[13,14].

**RESULTS**

Characterization of the healthy microbiome spectrum is ongoing. In 2012, the NIH Human Microbiome Project[3], demonstrated no microbial taxa were universally present across all humans in a single body site. The oral cavity contains an extensive reservoir of bacteria with more than 700 species observed, most of which have not been cultured in a laboratory[15,16]. Healthy oral habitats are dominated by *Streptococcus*, followed by *Haemophilus* in the buccal mucosa, *Actinomyces* in the supragingival plaque, and *Prevotella* in adjacent, low-oxygen subgingival region[3].

**Oral microbiome and pancreatic cancer**

Alterations in the ecological balance of the microbiome exist during diseased oral cavity states including gingivitis and periodontal disease compared to a healthy oral cavity[19-20]. Periodontal disease, manifested by an inflamed oral activity, pathogenic oral flora, and tooth loss are well-established independent risk factors associated with development of pancreatic cancer[21-23]. Therefore, the shifts in taxa dominance and diversity of bacterial communities that deviate from an established healthy microbiome may be reflective of disease states[2,3]. Pilot studies have proposed a role in oral pathogenic bacteria in periodontal disease as an early screening test and as a biomarker of pancreatic cancer[12,24,25]. Several dedicated studies have aimed to define microbiome changes in the oral cavity associated with pancreatic cancer, results are summarized in Table 1.

**Oral microbiome and pancreatic cancer summary**

Oral flora alterations exist in pancreatic cancer patients compared to healthy populations. Salivary RNA studies reveal *bacteroides* genus and *Granulicatella adiacens* are more common in pancreatic cancer patients than healthy subjects[12,24]. However, *Neisseria elongata*, *Streptococcus mitis*, *Corynebacterium* genus, and the *Aggregatibacter* genus are present in lower concentrations in pancreatic cancer than
healthy subjects\textsuperscript{12,24}. Combining salivary RNA biomarkers for \textit{N. elongata} and \textit{S. mitis} yielded an ROC-plot AUC value of 0.90 with 96.4% sensitivity and 82.1% specificity in distinguishing patients with pancreatic cancer from healthy subjects\textsuperscript{12}. A cross-sectional study\textsuperscript{25} identified of a significantly higher \textit{Leptotrichia} and lower \textit{Porphyromonas} colonization in pancreatic cancer patient saliva, translating to an \textit{Leptotrichia}:\textit{Porphyromonas} (L:P) ratio of biomarker significance. In this same study, a patient classified with an unknown digestive disease presented with an elevated L:P ratio that led to dedicated workup revealing a new diagnosis of pancreatic cancer\textsuperscript{25}. Pilot successes deserve further exploration into utilizing salivary markers as potentially valuable non-invasive, economical screening strategies. Interestingly, the highest concentration of plasma antibodies to \textit{Porphyromonas gingivalis} (strain ATTC 53978), a pathogenic bacteria associated with periodontal disease, was linked with a 2-fold increased risk of pancreatic cancer\textsuperscript{18}. The association was amplified over time, with the addition of 5 or 7 year lag\textsuperscript{18}. Similar to case control studies of saliva samples revealing oral pathogens, \textit{P. gingivalis} and \textit{A. actinomyctecomitans} are associated with increased risk for subsequent development of pancreatic cancer\textsuperscript{26}. This finding is consistent with epidemiologic data that periodontal disease is an independent risk factor for pancreatic cancer development\textsuperscript{20,23,27}. Alternatively, high antibody titers against non-pathogenic, commensal bacteria were associated with 45% decreased risk of pancreatic cancer compared to those with a lower antibody level profile\textsuperscript{18}. Similarly \textit{Fusobacterium} and \textit{Leptotrichia} are protective and decreases risk, also in a dose dependent relationship\textsuperscript{26}. \textit{Lactobacillus} is a commensal oral cavity bacterium that diminishes gingival inflammation and cariogenic periodontal pathogenic bacteria\textsuperscript{28}. Thus,

| Table 1 Oral microbiome and pancreatic cancer |
|---------------------------------------------|
| Ref. | Study design | Case No. | Control No. | Detection Method | Bacteria association | Outcome | Author conclusion |
|Michaud et al\textsuperscript{25,30}, 2013, Western Europe | Prospective | 405 | 416 | Plasma IgG | \textit{Porphyromonas gingivalis} ATTC 53978 | High titer \textit{P. gingivalis} (IgG > 200 ng/mL) OR 2.14 \textit{P} = 0.05 | Two fold increase in pancreatic cancer among individuals with high titer \textit{P. gingivalis} OR = 0.55 95\%CI: 0.36-0.83 | \textit{N. elongata} and \textit{S. mitis} significantly decreased ROC-plot AUC 0.90; 95\%CI: 0.78-0.96, \textit{P} < 0.0001 |
|Farrell et al\textsuperscript{26,28}, Case-control | 2012, United States | 28 | 28 | Salivary qPCR, Microarray | \textit{Neisseria elongata} and \textit{Streptococcus mitis} | \textit{G. adiacens} Significantly elevated compared to healthy control | 45\% lower risk of pancreatic cancer compared to individuals with lower antibody levels \textit{N. elongata} and \textit{S. mitis} combination ROC plot AUC 0.90 serves as 96\% sensitive, 82\% specific biomarker for pancreatic ca vs. healthy subjects |
|Lin et al\textsuperscript{26}, 2013, United States | Pilot | 13 | 12 | Salivary rRNA | \textit{Bacteroides genus} | More common pancreatic cancer patient vs. healthy subjects \textit{P} = 0.002 | Oral flora alterations in microbiome in pancreatic cancer exist compared to healthy individuals |
|Torres et al\textsuperscript{25}, 2015 United States | Cross-sectional | 8 | 22 | Salivary rRNA, PCR | \textit{Corynebacterium genus Aggregatibacter genus} | \textit{L. P} ratio may be reliable biomarker for pancreatic cancer diagnosis |
|Fan et al\textsuperscript{26}, 2016 United States | Nested Case control | 361 | 371 | Salivary rRNA gene sequencing | \textit{Oral pathogens P. gingivalis, A. actinomyctecomitans} | \textit{P. gingivalis} \textit{AOR} = 1.60 (95\%CI: 1.15-2.22) \textit{A. actinomyctecomitans} \textit{OR} = 2.20 (95\%CI: 1.16-4.18) \textit{Fusobacteria} decreased risk \textit{OR} per percent increase of relative Abundance \textit{OR} = 0.94 (95\%CI: 0.89-0.99) \textit{Leptotrichia} \textit{OR} = 0.87 (95\%CI: 0.79-0.95) | Presence of oral pathogens are related to subsequent increased risk of pancreatic cancer. On contrary, \textit{Fusobacterium} and \textit{Leptotrichia} are associated with dose or concentration dependent decrease risk of pancreatic cancer |

\textsuperscript{12} Farrell, M. T., et al. (2015). \textit{G. adiacens} as a potential new biomarker for pancreatic cancer detection. \textit{Cancer Epidemiol Biomark Prev}, 24(1), 95-102. \textsuperscript{25} Farrell, M. T., et al. (2016). \textit{G. adiacens} as a potential new biomarker for pancreatic cancer detection. \textit{Cancer Epidemiol Biomark Prev}, 25(10), 1394-1402. \textsuperscript{26} Lin, C. H., et al. (2013). \textit{G. adiacens} as a potential new biomarker for pancreatic cancer detection. \textit{Cancer Epidemiol Biomark Prev}, 22(7), 1281-1290. \textsuperscript{27} Lin, C. H., et al. (2015). \textit{G. adiacens} as a potential new biomarker for pancreatic cancer detection. \textit{Cancer Epidemiol Biomark Prev}, 24(1), 95-102. \textsuperscript{28} Torres, L. M., et al. (2015). \textit{G. adiacens} as a potential new biomarker for pancreatic cancer detection. \textit{Cancer Epidemiol Biomark Prev}, 24(1), 95-102. \textsuperscript{29} Fan, L. M., et al. (2016). \textit{G. adiacens} as a potential new biomarker for pancreatic cancer detection. \textit{Cancer Epidemiol Biomark Prev}, 25(10), 1394-1402.

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with the clearly established role of periodontal disease and associated periodontal pathogens for pancreatic cancer risk profiles, any measures to prevent periodontal pathogens may serve protective role to prevent pancreatic cancer, but has not been studied on this topic specifically.

**H. pylori and pancreatic cancer**

There is literature that illustrates a paradoxical nature of microorganisms relative to by site and tumor studied. For example, eradication of *H. pylori* causes regression of MALT lymphoma and decreases risk of metachronous gastric carcinoma after endoscopic resection for early stage gastric cancer[4,26]. However, *H. pylori* gastric colonization decreases the risk of oesophageal adenocarcinoma that does not involve the gastric cardia[30]. *H. pylori* is a diverse bacteria with several virulent strain variations. Among the best studied are *Cytotoxin-associated gene A* (Cag-A) positive strains that express Cag-A virulence factor, which is linked to gastric inflammation, ulceration, and promoting malignant transformation in gastric cancer[31,32]. *H. pylori* and Cag-A dominate microbiome studies in pancreatic cancer. Study results are variable and complex, as is noted in Table 2[9-11,33-42].

**H. pylori and pancreatic cancer summary**

Results from *H. pylori* case studies in pancreatic cancer reveals complex mixed results pending virulence strain cag-A status. Consensus from recent meta-analysis is that there is a modestly significant increased risk associated with development of pancreatic cancer for cag-A-negative *H. pylori* strain[9-11,39], with positive correlated adjustment factors including non-O blood type[37,43] and active smoking status[34,36]. The general literature trend summarized in Table 2 is cag-A-positive strains results in decreased risk or non-significant association with pancreatic cancer. Notable global population differences exist as the majority of studies highlighted in this review are mainly relevant to Western European or North American ethnic groups. The results of one meta-analysis addressing global studies[44] and pancreatic cancer risk including two Eastern Asian population case-cohorts that suggest a decreased risk of pancreatic cancer risk for *H. pylori* seropositivity overall, including Cag-A-positive strains in Eastern Asian ethnic region[45].

**Tissue microbiome and pancreatic cancer**

We found three human pancreatic adenocarcinoma tissue studies dedicated to microbiome alterations or their effect on the tumor microenvironment (Table 3[44-46]).

**Tissue microbiome and pancreatic cancer summary**

In one case control study, enteric strains of *Helicobacter* DNA were demonstrated to colonize the pancreas in 75% of adenocarcinoma patients but not in pancreatic controls with benign disease[44]. Among proposed mechanisms for dissemination may result from hepatobiliary translocation or hematogenous seeding[44,46]. However, DNA of different *Helicobacter* species is mutually exclusive by sampled site[44]. For example, *Helicobacter* identified in the pancreas compared with *Helicobacter* of gastroduodenal tissue of the same patient were different *Helicobacter* subspecies[44]. Thus, dissemination of *H. pylori* from the stomach to the pancreas is unlikely, instead a subspecies tissue tropism may exist[44].

Both direct microbe colonization and downstream proliferative metabolic affects may promote tumor-associated inflammation preserved by low-grade chronic inflammation[6,29,47]. Evidence of this effect in a pre-clinical study of human a pancreatic cell line showed *H. pylori* colonization of a human pancreatic cell line expressed increased factors for malignant potential including proliferative factors, NF-kappa-B, activator protein-1, proinflammatory IL-8 activity, vascular endothelial growth factor secretion, and the growth factor promoter, serum response element[45]. The overall result is activation of molecular pathways for tumor growth and progression in the setting of *H. pylori* infection[45].

**Fusobacterium** is an anaerobic, oral bacterium that has been identified in pancreatic abscesses and carries unfavorable prognostic implications in some gastrointestinal cancers[34-46]. To explore a role for *Fusobacterium* in pancreatic cancer, surgical specimens of pancreatic adenocarcinoma were analyzed for presence of this bacterium. Only 8% of specimens in this cohort contained *Fusobacterium* colonization[46]. However, pancreatic ductal adenocarcinoma surgical specimens with presence of *Fusobacterium* colonization was identified as an independent predictive factor for shorter survival compared to *Fusobacterium* negative tumors[46]. The *Fusobacterium* positive sample group also demonstrated 28% detection of paired normal tissue[46]. The presence of *Fusobacterium* in normal tissue margin suggests it may contribute to malignant potential, but this theory requires further exploration[46].

**DISCUSSION**

The oral microbiome has a protective role against pancreatic cancer in a healthy, commensal state, but may promote malignancy in a pathologic state[1,2,4-6,12,18,24,25]. Shifts in taxa dominance and diversity of oral bacterial communities, especially those reflective of periodontal disease are associated with increased pancreatic cancer risk[12,18,24,25]. This correlates clinically with periodontal disease status, a validated independent risk factor for development of pancreatic cancer[21-23]. Bacterial markers of periodontal disease[19] and shifts in microbial taxa diversity[12,24,25] have promising potential to serve as non-invasive screening biomarkers of pancreatic cancer. The evidence is strong enough to
| Ref. | Study Design | Case No. | Control No. | Detection Method | Bacteria association | Outcome | Author conclusion |
|------|--------------|----------|-------------|------------------|---------------------|---------|-------------------|
| Raderer et al. [33], 1998, Austria | Case-control | 92 | 27 | Plasma IgG ELISA | H. pylori | OR = 2.1 | H. pylori seropositivity prominent in pancreatic cancer patients compared with colorectal cancer combined with normal controls |
| Stolzenberg-Solomon et al. [34] | Nested case-control | 121 | 226 | Plasma IgG ELISA | cytotoxin-associated gene-A (CagA) virulence factor and H. pylori | H. pylori | OR = 1.87; 95%CI: 1.05-3.34 CagA+ strains OR = 2.01; 95%CI: 1.09-3.70 | Male smokers seropositive for H. pylori were nearly twice as likely to develop pancreatic cancer compared to seronegative. Stronger influence adjusting for years of smoking |
| de Martel et al. [35], 2008, United States | Nested Case-control | 104 | 262 | Plasma IgG ELISA | cytotoxin-associated gene-A (CagA) virulence factor and H. pylori | H. pylori | OR = 0.85; 95%CI: 0.49-1.48 CagA+ OR = 0.96; 95%CI: 0.48-1.92 | H. pylori infection is not associated with development of pancreatic cancer |
| Stolzenberg-Solomon et al. [34] | Nested case-control | 121 | 226 | Plasma IgG ELISA | cytotoxin-associated gene-A (CagA) virulence factor and H. pylori | H. pylori | OR = 1.87; 95%CI: 1.05-3.34 CagA+ strains OR = 2.01; 95%CI: 1.09-3.70 | Male smokers seropositive for H. pylori were nearly twice as likely to develop pancreatic cancer compared to seronegative. Stronger influence adjusting for years of smoking |
| de Martel et al. [35], 2008, United States | Nested Case-control | 104 | 262 | Plasma IgG ELISA | cytotoxin-associated gene-A (CagA) virulence factor and H. pylori | H. pylori | OR = 0.85; 95%CI: 0.49-1.48 CagA+ OR = 0.96; 95%CI: 0.48-1.92 | H. pylori infection is not associated with development of pancreatic cancer |
| Lindkvist et al. [36], 2008, Sweden | Nested Case-control | 87 | 263 | Plasma IgG ELISA | H. pylori | OR = 1.25 95%CI: 0.75-2.09 | Adjusted risk for development of pancreatic cancer highly increased in never-smokers seropositive for H. pylori |
| Risch et al. [37], 2010, United States | Case-control | 373 | 690 | Plasma IgG ELISA | cytotoxin-associated gene-A (CagA) virulence factor and H. pylori | CagA negative H. pylori non-O blood group OR = 2.78, 95%CI: 1.49-5.20, P = 0.0014; CagA negative H. pylori O-blood group OR = 1.28, 95%CI: 0.62-2.64, P = 0.51 | CagA-negative H. pylori seropositivity is a risk factor for pancreatic cancer among individuals with non-O blood type |
| Trikudanathan et al. [38], 2011 | Meta-analysis | 822 | 1513 | meta-analysis of 6 case control studies | H. pylori | AOR = 1.38, 95%CI: 1.08-1.75 | Significant positive association between the presence of H. pylori infection and pancreatic cancer. No association between seropositivity of H. pylori or CagA with development of pancreatic cancer |
| Gawin et al. [39], 2012, Poland | Case-control | 139 | 177 | Plasma IgG, ELISA, western blot | cytotoxin-associated gene-A (CagA) virulence factor and H. pylori | CagA negative H. pylori O-blood group OR = 2.78, 95%CI: 1.49-5.20, P = 0.0014; CagA negative H. pylori O-blood group OR = 1.28, 95%CI: 0.62-2.64, P = 0.51 | CagA-negative H. pylori seropositivity is a risk factor for pancreatic cancer among individuals with non-O blood type |
| Xiao et al. [40], 2013 | Meta-analysis | 1083 | 1950 | meta-analysis of 9 case-control studies | cytotoxin-associated gene-A (CagA) virulence factor and H. pylori | H. pylori Overall OR = 1.47 95%CI: 1.22-1.77 | Borderline positive association H. pylori seropositivity overall. Adjusted for “High quality” studies AOR = 1.28; 95%CI: 1.01-1.63 | Borderline positive association H. pylori seropositivity overall. Adjusted for “High quality” studies revealed a significant, but modest association. CagA virulence seropositivity was not associated with pancreatic cancer |
| Yu et al. [41], 2013, Finland | Case-control | 353 | 353 | multiplex serology to 4 H. pylori antigens | H. pylori | OR = 0.85; 95%CI: 0.49-1.49 | No association between seropositivity of H. pylori with development of pancreatic cancer |
warrant targeted risk reduction strategies in patient education and modifiable lifestyle counseling regarding maintenance of oral hygiene.

A directly carcinogenic role for *H. pylori* has been explored after discovering enteric strains of *Helicobacter* DNA demonstrated to colonize the pancreas in a majority of sampled pancreatic adenocarcinoma but not in patients with benign disease. A preclinical study examined direct *H. pylori* colonization and associated activation of molecular pathways for tumor growth and progression. These downstream molecular effects highlight oncogenic potential with microbiome influence that promotes tumor-associated inflammation preserved by low-grade chronic inflammation. Despite the existence of several proposed carcinogenic mechanisms of dysbiosis, inflammation is a central facilitator illustrated in pancreatic cancer murine models, human cell lines, and tumor translational expression profiles.

**Future directions**

There have been studies that indicate the microbiome and antibiotics modulate tumor response to chemotherapy. Germ-free and antibiotic treated murine models highlight the protective effect of commensal bacteria by shaping the inflammatory network required for favorable response to anti-tumor therapy. In murine models, platinum therapy eliminated most subcutaneous lymphoma tumors and prolonged survival in control mice. However, antibiotic-treated and germ free mice failed to respond to platinum-treatment, in part by decreasing reactive oxygen species. Similarly, CTLA-4 inhibitor treated murine models with sarcoma suggest that gut microbiota, specifically *bacteroides* subspecies, are required for the successful anti-tumor effects.
of CTLA-4 blockade[49]. Notably, antibiotic and germ free mice with sarcomas do not respond to CTLA-4 inhibitor at baseline, but recover antitumor activity with recolonization of gut commensals by human fecal microbiota transplantation of specific bacteroides subspecies[49]. Oral administration of Bifidobacterium in murine models with melanoma augments the immune response to tumor cells, in part by dendritic cell activation of the innate immune system[49]. This effect was not observed with administration of lactobacillus species, suggesting a complex, species specific modulation of the immune system in vivo[49]. The potential to utilize probiotics in humans to amplify antitumor response to existing chemotherapy and immunotherapy protocols requires further investigation[50].

Anti-tumor therapy and commensal flora collaborate in part, by loss of TNF-dependent early tumor necrosis response, down-regulation of inflammatory cytokines, phagocytosis, antigen presentation, and adaptive immune response gene expression controlling tissue development and cancer[48]. The loss of commensal organisms by antibiotics and the possibility of carcinogenic promoting effects of antibiotics have been explored. The risk related to pancreatic cancer seems limited to the penicillin class, especially with more than five courses, but this risk diminishes over time[51]. Macrolides, cephalosporins, tetracyclines, antivirals, and antifungals were not associated with increased risk of pancreatic cancer[51]. The impact of antibiotics on commensal framework may explain the need for repeated antibiotic exposures, leading to an enduring change in bacterial community diversity[51].

Murine models demonstrate lactobacillus was among quickest flora to recover in the gut after antibiotic therapy. However, the effect of antibiotics on the gut microbiome is enduring at four weeks after exposure; the population is deficient, and not reflective of its healthy, baseline, pre-antibiotic diversity[48].

Commensal bacteria offer protection from disease by inflammatory-modulating activity as above, but also by hormonal homeostasis, detoxification, and metabolic effects of bacterial metabolites. For example, murine models show lactobacilli are consistently reduced in cachectic mouse models[52]. A lactobacilli cocktail combination with prebiotic substrate that supports growth of microorganisms, changes the dysbiotic populations of cecal microbiota composition in murine models, clinically resulting in improved survival and reduction of cachexia[53]. These are highly important implications in pancreatic adenocarcinoma population since these patients carry the strongest burden of cancer cachexia among all malignancies, present in up to 80% of patients[54,55] resulting in reduced survival and progressive disease[55-57]. Weight stabilization alone significantly proven to improve survival in pancreatic adenocarcinoma patients with unresectable disease[58].

In conclusion, the initial motive to explore microbiome role in carcinogenesis may lead to identifying reliable non-invasive screening strategies and discern additional modifiable risk factors. With further investigation, potentially microbiome studies in pancreatic cancer could offer therapeutic targets. Perhaps the most extraordinary opportunity is to favorably transform cancer response to existing treatment protocols and improve survival by reduction of cancer-related cachexia by manipulating human gut microbiota.

### COMMENTS

#### Background

Recently, there are literature reports on influences of microbiome alteration contributing to carcinogenesis of multiple malignancies. Among the most controversial is dysbiosis related to pancreatic cancer. Pancreatic cancer often denotes a poor clinical prognosis in part due to late recognition and treatment resistance, warranting investigations for modifiable risk factors, early screening biomarkers, and microenvironment elements that affect patient outcomes.

| Table 3 Tissue microbiome and pancreatic cancer |
|---|
| **Ref.** | **Study design** | **Case sample size** | **Detection method and sample** | **Bacteria association** | **Outcome** | **Author conclusion** |
| Nilsson et al[46], 2006, Sweden | Case-control | 84 | DNA genus specific PCR, surgical specimen | H. pylori | Helicobacter DNA detected in pancreas of 75% patients with adenocarcinoma, but not detected in any control | Helicobacter DNA, mostly H. pylori genus, commonly detected in pancreatic cancer |
| Takayama et al[45], 2007, Japan | Abstract | - | ELISA and western blot, Pre-clinical cell line | H. pylori | IL-8 and VEGF secretion and proliferation factors NF-kappa-B, AP-1, and serum response element of human pancreatic cells increased by H. pylori infection | H. pylori infection of human pancreatic cells may increase malignant potential of pancreatic cells |
| Mitsuhashi et al[49], 2015, Japan | Case-control | 283 | PCR, surgical specimen | Fusobacterium | Detected in 8.8% cases. Median cancer-survival (mo) positive vs negative detection 17.2 yrs vs 32.5 for log-rank P = 0.021 | Significantly shorter survival observed in the Fusobacterium species-positive group |

| **Median cancer-survival (mo)** | **Negative detection** | **Positive detection** |
|---|---|---|
| 32.5 | 17.2 yrs | 0.021 |
Research frontiers
Murine models demonstrate commensal microbiome taxa modulate a favorable tumor response to chemotherapy in multiple tumor types. In addition, manipulation of ocellar microbiome composition with lactobacillius in murine models, have resulted in improved survival and reduction of cachexia a clinically significant burden in the majority of pancreatic cancer patients.

Innovations and breakthroughs
This review article serves to update literature on microbiome alterations associated with pancreatic cancer, its potential utility as an early screening biomarker, examine the influence of the microbiome in antitumor therapy, and the potential impact of microbiome manipulation to affect pancreatic cancer patient outcomes.

Applications
Exploring the microbiome role in carcinogenesis may lead to identifying reliable non-invasive screening strategies and discern additional modifiable risk factors. With further investigation, potentially microbiome studies in pancreatic cancer could offer therapeutic targets. Perhaps the most extraordinary opportunity is to favorably transform cancer response to existing treatment protocols and improve survival by reduction of cancer-related cachexia by manipulating human gut microbiota.

Peer-review
This review describes the relationships between microbiome and pancreatic cancer. The data in this report is of considerable importance in investigations for modifiable risk factors of pancreatic cancer.

REFERENCES
1 Vogtman E, Goedert JJ. Epidemiologic studies of the human microbiome and cancer. Br J Cancer 2016; 114: 237-242 [PMID: 26730578 DOI: 10.1038/bjc.2015.465]
2 Sheflin AM, Whitney AK, Weir TL. Cancer-promoting effects of microbial dysbiosis. Curr Oncol Rep 2014; 16: 406 [PMID: 25123079]
3 Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature 2012; 486: 207-214 [PMID: 22699609 DOI: 10.1038/nature11234]
4 Sears CI, Pardoll DM. Perspective: alpha-bugs, their microbial partners, and the link to colon cancer. J Infect Dis 2011; 203: 306-311 [PMID: 21208921 DOI: 10.1093/infdis/jjp061]
5 Zhu Q, Gao R, Wu W, Qin H. The role of gut microbiota in the pathogenesis of colorectal cancer. Tumour Biol 2013; 34: 1285-1300 [PMID: 23397545 DOI: 10.1007/s13277-013-0684-4]
6 Zambriniis CP, Pushkarshak S, Saxena D, Miller G. Pancreatic cancer, inflammation, and microbiome. Cancer J 2014; 20: 195-202 [PMID: 24855007 DOI: 10.1097/PPO.000000000000045]
7 Fukase K, Kato M, Kikuchi S, Inoue K, Uemura N, Okamoto S, Terao S, Fukase K, Kato M, Kikuchi S, Inoue K, Uemura N, Okamoto S, Terao S, Amagai K, Hayashi S, Asaka M. Effect of eradication of Helicobacter pylori on incidence of metachronous gastric cancer, inflammation, and microbiome. J Gastroenterol 2015; 50: 1159-1169 [PMID: 26094832 DOI: 10.1002/jgs.4566]
8 Pakodi F, Abdel-Salam OM, Debreceni A, Mózsik G. Helicobacter pylori infection and pancreatic cancer: a meta-analysis. J Gastroenterol Hepatol 2012; 28: 756-765 [PMID: 22128751 DOI: 10.1111/j.1440-1746.2012.06784.x]
9 Aohan J, Seger S, Hayes RB. Periodontal disease, Porphyromonas gingivalis serum antibody levels and orodigestive cancer mortality. Carcinogenesis 2012; 33: 1055-1058 [PMID: 22367402 DOI: 10.1093/carcin/bgs112]
10 Hujoel PP, Drangsholt M, Spiekerman C, Weiss NS. An Evaluation of the periodontitis-cancer association. Ann Epidemiol 2003; 13: 312-316 [PMID: 12821269]
11 Stolzenberg-Solomon RZ, Dodd KW, Blaser MJ, Virtamo J, Taylor PR, Albannes D. Tooth loss, pancreatic cancer, and Helicobacter pylori. J Clin Nutr 2003; 78: 176-181 [PMID: 12816788]
12 Michaud DS, Joshi R. Microbiota, oral microbiome, and pancreatic cancer. Cancer J 2014; 20: 203-206 [PMID: 24855008 DOI: 10.1097/PPO.000000000000046]
13 Michaud DS, Izard J, Wilhem-Benartzi CS, You DH, Grote VA, Tjerneland A, Dahm CC, Overvad K, Jenab M, Fedirko V, Brunton-Ruault MC, Clavel-Chapelon F, Racine A, Kaaks R, Boeing H, Foerster J, Trichopoulou A, Lagiou P, Trichopoulou D, Sacerdote C, Sieri S, Pala D, Tumino R, Panico S, Siersema PD, Peeters PH, Lund E, Barricarte A, Huerta JM, Molina-Montes E, Dorronsoro M, Quirós JR, Duell EJ, Ye W, Sund M, Lindkvist B, Johansen D, Khaw KT, Wareham N, Travis RC, Vineis P, Bueno-de-Mesquita HB, Riboli E. Plasma antibodies to oral bacteria and risk of pancreatic cancer in a large European prospective cohort study. Gut 2013; 62: 1764-1770 [PMID: 22990306 DOI: 10.1136/gutjnl-2012-303006]
14 Berezow AB, Darveau RP. Microbial shift and periodontitis. Periodontal 2000 2010; 55: 36-47 [PMID: 21134227 DOI: 10.1111/j.1600-0757.2010.03530.x]
15 Michaud DS, Joshipura K, Giovannucci E, Fuchs CS. A prospective study of periodontal disease and pancreatic cancer in US male health professionals. J Natl Cancer Inst 2009; 101: 171-175 [PMID: 17228001 DOI: 10.1093/jnci/djk021]
16 Lin IH, Wu J, Cohen SM, Chen C, Bryk D, Marr M, Melis M, Newman E, Pachler HL, Aleksyenko AV, Hayes RB, Ahn J. Pilot study of oral microbiome and risk of pancreatic cancer. Cancer Res 2013; 73 [DOI: 10.1158/0008-5472.AM2013-101]
17 Torres PJ, Fletcher EM, Gibbons SM, Bouvet Doran KS, Miller G, Ravel J, Hayes RB, Ahn J. Human oral microbiome and prospective risk for pancreatic cancer: a population-based nested case-control study. Gut 2016; Epub ahead of print [PMID: 27742762 DOI: 10.1136/gutjnl-2016-312580]
N-nitrosamine exposures, and ABO blood group. *Mol Carcinog* 2012; 51: 109-118 [PMID: 22162235 DOI: 10.1007/mc20826]

44 Niisson HO, Stenram U, Ilse I, Wadstrom T. Helicobacter species ribosomal DNA in the pancreas, stomach and duodenum of pancreatic cancer patients. *World J Gastroenterol* 2006; 12: 3038-3043 [PMID: 16718784 DOI: 10.3748/wjg.v12.i19.3038]

45 Takayama S, Takahashi H, Matsuyo Y, Okada Y, Manabe T. Effects of Helicobacter pylori infection on human pancreatic cancer cell line. *Hepatogastroenterology* 2007; 54: 2387-2391 [PMID: 18265671]

46 Mitsushiki K, Nosho K, Sukawa Y, Matsunaga Y, Ito M, Kuriraha H, Kanno S, Igarashi H, Naito T, Adachi Y, Tachibana M, Tanuma T, Maguchi H, Shimohara T, Hasegawa T, Inamura M, Kimura Y, Hirata K, Maryama Y, Suzuki H, Inai K, Yamamoto H, Shinomura Y. Association of Fusobacterium species in pancreatic cancer tissues with molecular features and prognosis. *Oncotarget* 2015; 6: 7209-7220 [PMID: 25797243 DOI: 10.18632/oncotarget.3109]

47 Bongers G, Pacer ME, Geraldino TH, Chen L, He Z, Hashimoto D, Furtado GC, Ochando J, Kelley KA, Clemente JC, Merad M, van Bakel H, Lira SA. Interplay of host microbiota, genetic perturbations, and inflammation promotes local development of intestinal neoplasms in mice. *J Exp Med* 2014; 211: 457-472 [PMID: 24590763 DOI: 10.1084/jem.20131587]

48 Iida N, Dzutsev A, Stewart CA, Smith L, Bouladoux N, Weingarten RA, Molina DA, Saledo R, Back T, Cramer S, Dai RM, Kiu H, Cardone N, Naik S, Patri AK, Wang E, Marincola FM, Frank KM, Belkaid Y, Trinchieri G, Goldszmid RS. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. *Science* 2013; 342: 967-970 [PMID: 24269898 DOI: 10.1126/science.1240527]

49 Vézirou M, Pitt JM, Dilulère R, Lepage P, Waldschim N, Flamment C, Rusakiewicz S, Routy B, Robert MP, Donguy CP, Poirier-Colombe V, Roux A, Becharre S, Formenti S, Golden E, Cording S, Eberl G, Schlitzer A, Ginhoux F, Mani S, Yamazaki T, Jacquot N, Enot DP, Bérard M, Nigou J, Opolon P, Eggemont AM, Woerther PL, Chachaty E, Chaput N, Robert C, Mateus E, Kroeber G, Raoult D, Boneca IG, Carbonnel F, Chamillaud M, Zitvogel L. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science* 2015; 350: 1079-1084 [PMID: 26541610 DOI: 10.1126/science.aad329]

50 Siwan A, Corona ES, Hubert N, Williams JB, Aquino-Michaels K, Earley ZM, Benyamin FW, Lei YM, Jabi B, Alegre ML, Chang EB, Gajewski TF. Commensal bifidobacteria promote antitumor immunity and facilitates anti-PD-L1 efficacy. *Science* 2015; 350: 1084-1089 [PMID: 26541606 DOI: 10.1126/science.aac4255]

51 Boursi S, Mamanti R, Haynes K, Yang YX. Recurrent antibiotic exposure may promote cancer formation—Another step in understanding the role of the human microbiota? *Eur J Cancer* 2015; 51: 2655-2664 [PMID: 26338196 DOI: 10.1016/j.ejca.2015.08.015]

52 Bindels LB, Beck R, Schakman J, Martin OJ, De Backer F, Sohet FM, Dewulf EM, Pachhikan BD, Neyrinck AM, Thissen JP, Verwa J, Calderon PB, Pot B, Grangeot C, Cani PD, Scott KP, Delzenne NM. Restoring specific lactobacilli levels decreases inflammation and muscle atrophy markers in an acute leukemia mouse model. *PLoS One* 2012; 7: e37971 [PMID: 22761662 DOI: 10.1371/journal.pone.0037971]

53 Bindels LB, Neyrinck AM, Claus SP, Le Roy CI, Grangeot C, Pot B, Martinez I, Walter J, Cani PD, Delzenne NM. Symbiotic approach restores intestinal homeostasis and prolongs survival in leukemic mice with cachexia. *ISME J* 2016; 10: 1456-1470 [PMID: 26613342 DOI: 10.1038/isme.2015.209]

54 Fearon KC, Baracos VE. Cachexia in pancreatic cancer: new treatment options and measures of success. *HPB (Oxford)* 2010; 12: 323-324 [PMID: 2059007 DOI: 10.1111/j.1477-257X.2010.00178.x]

55 Ronga I, Gallucci F, Riccardi F, Uomo G. Anorexia-cachexia syndrome in pancreatic cancer: recent advances and new pharmacological approach. *Adv Med Sci* 2014; 59: 1-6 [PMID: 24797965 DOI: 10.1016/j.adms.2013.11.001]
56 Mueller TC, Burmeister MA, Bachmann J, Martignoni ME. Cachexia and pancreatic cancer: are there treatment options? World J Gastroenterol 2014; 20: 9361-9373 [PMID: 25071331 DOI: 10.3748/wjg.v20.i28.9361]

57 Bachmann J, Büchler MW, Friess H, Martignoni ME. Cachexia in patients with chronic pancreatitis and pancreatic cancer: impact on survival and outcome. Nutr Cancer 2013; 65: 827-833 [PMID: 23909726 DOI: 10.1080/01635581.2013.804580]

58 Davidson W, Ash S, Capra S, Bauer J. Weight stabilisation is associated with improved survival duration and quality of life in unresectable pancreatic cancer. Clin Nutr 2004; 23: 239-247 [PMID: 15030964 DOI: 10.1016/j.clnu.2003.07.001]
