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MICROBIOME IN PANCREATIC CANCER – WHAT IS NEW IN 2024?

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Abstract – Despite great efforts to improve understanding and treatment of pancreatic cancer, mainly pancreatic ductal adenocarcinoma (PDAC), the outcome of this malignant disease today is still very poor. With the introduction of modern sequencing methods and improvements in computational possibilities, microbiome research is an improving and promising field with a link to many possible diseases. In this article, the results of how the microbiome and PDAC influence each other are reviewed, and future directions are discussed.

Keywords: Pancreatic cancer, Pancreatic ductal adenocarcinoma, PDAC, Microbiome.

INTRODUCTION

The role of the microbiome is a field of growing interest with evidence of a link to many conditions in health and disease. As a secretory organ with a direct connection to the gut, the interplay between the microbiome, mainly of the gut, and the pancreas is bidirectional and impacts each other. To demonstrate how the pancreas and the gut microbiome are functionally interwoven, which can be described as the pancreas-microbiome-axis, the excretion of antimicrobial peptides by the exocrine pancreas modulating bacterial composition in the gut and vice versa the activation of secretion of these peptides in the pancreas by microbial products of the gut microbiome is a good example. Ahuja et al[1] showed that Orai1-depleted mice, which lack a calcium channel in pancreatic acini that is responsible for the secretion of antimicrobial peptides like cathleicidin related antimicrobial peptide (CRAMP), suffer from intestinal bacterial overgrowth eventually causing death. Conversely, CRAMP secretion is influenced by microbial fermentation and its production of short-chain fatty acids, of which Butyrate acts on G protein-coupled receptors GPR43 and GPR4[2]. These receptors are expressed by ß-cells of pancreatic islets to induct CRAMP production[3]. The role of the microbiome in pancreatic cancer, of which pancreatic ductal adenocarcinoma is the subtype in 90% of cases, has been extensively researched in recent years as a new possible mechanism in understanding oncogenesis, progression, chemotherapy response and prognosis of this malignant disease which is characterized by low survival. The interplay of the microbiome, not only of the gut but also intratumoral and oral microbiomes, with pancreatic ductal adenocarcinoma was further researched in the past year and the results are summarized in this article.

METHODS

Articles listed on PubMed published between March 2023 and March 2024 with the search terms “microbiome” AND “pancreatic ductal adenocarcinoma” were identified.

RESULTS –WHAT IS NEW IN 2023?

Microbiome in Early Detection and as a Diagnostic Tool

Being characterized by a late onset of symptoms in a state of advanced local and systemic infiltration of tumor cells, early detection methods of pancreatic ductal adenocarcinoma (PDAC), in a state where surgical resection can offer a curative therapy, are urgently needed or can support other diagnostic tools like imaging, laboratory testing, biopsy, and histopathology. By using mendelian randomization, Zhong et al[4] looked for associations between blood metabolites, gut microbiome and risk for PDAC. They found that an increase of *Flavonifractor sp90199495*, a flavonoid degrading bacterium, is present in PDAC patients microbiome. Furthermore, the authors found an inverse association of this species with a metabolite called X-21849. Flavonoids are polyphenolic secondary metabolites and have shown health-promoting effects, with tumor growth inhibition and apoptosis promotion among these effects. Whether this metabolite has a direct impact on cancerogenesis in PDAC remains to be seen and could be an interesting approach for further research. Another study by Zhang et al[5] used Multi-Omics Co-training Graph Convolutional Networks (MOGCN), which integrates multiple omics data and graph convolutional networks (GCN), like microbiome data and exposome data to adjust microbiome data to lifestyle factors for the diagnosis of pancreatic cancer and validated the results on a German cohort. The exposome data included 23 known risk factor variables and 125 bacterial species. The integration of exposome and microbiome data aimed to predict PDAC. This process resulted in the identification of 3 exposome data and 42 bacterial species as the most significant entities. It was shown that among the significantly important species, 61.9% belonged to *Firmicutes phylum*, demonstrating a higher abundance of *Firmicutes phylum* in general and specifically increased species being *Fusobacterium hwasookii nucleatum*, *Alloscardovia omnicolens*, *Veillonella spp*. (*Veillonella atypica* and *Veillonella parvula*) and decreased species of *Clostridiales*, *Bacteroides coprocola*, *Faecalibacterium prausnitzii*, and *Bifidobacterium bifidum* in PDAC patients' microbiomes. Altogether, the importance of Firmicutes phylum to diagnostically distinguish between PDAC and controls was shown. Furthermore, future microbiome studies should interpret microbiome data along with exposome data to specify results and minimize confounding factors, as done in the aforementioned project.

Microbiome in Development and Prognosis of PDAC

In recent years, the impact of bacterial metabolites, both locally within the gut and systemically upon absorption, has been demonstrated. Schwarcz et al[6] uncovered that Lithocholic acid (LCA), which was first thought to be carcinogenic, possesses anti-tumor effects in PDAC. Using a Capan-2 cell culture model, a cell line to study PDAC biology, LCA exposure in serum-like concentrations inhibited proliferation on tumor cells exclusively. Furthermore, epithelial-mesenchymal transition (EMT), a process that allows tumor cells to dissociate and form metastasis, was significantly decreased in cells treated with LCA in comparison to dimethyl sulfoxide (DMSO). This effect of LCA in preventing EMT could be linked to the induction of oxidative stress in PDAC cells and was attenuated when adding the antioxidants thiol reductant glutathione and pegylated catalase, which mimics the higher abundance of antioxidants in PDAC. This is linked to a poorer outcome due to the cytostatic effect of oxidative stress. To test which receptors mediate the different effects, receptor antagonists to block downstream activation pathways and siRNA silencing were used, unveiling that a decrease of cell invasion is mediated by LCA acting on constitutive androstane receptor (CAR), farnesoid X receptor (FXR) and vitamin D receptors (VDR) suppressing mesenchymal marker ß-catenin and Snail expression and increasing epithelial marker expression of Zonula occludens-1 protein (ZO1). Finally, the impact of LCA in different chemotherapy regimens, Gemcitabine, 5-fluoruracil, paclitaxel, oxaliplatin and irinotecan, was investigated and it was shown[7] that LCA has neither a positive nor negative effect here. The study demonstrated interesting results of how a microbial metabolite might impact PDAC progression and provides an interesting approach for further studies, given the translational limits of cell culture models to human models.

As pathogen microbes mediate an immune response, commensal non-pathogenic bacteria are in a constant immune crosstalk with the host in the gut, also exerting distant effects. Chandra et al[7] demonstrated that enteric Interleukin 17 receptor A (IL-17RA) deficiency leads to gut dysbiosis and induces PDAC growth by increasing Th17 development, which leads to a higher production of IL-17, affecting Dual oxidase 2 (DUOX2) signaling in PDAC cells. This finding goes along with observations of delayed tumor initiation and growth with microbial ablation and improved survival of PDAC patients undergoing antibiotic treatment. By using IL17RA deficient mice, a significantly higher tumor growth after implantation of PDAC cells was seen, which was linked to a microbially driven IL-17 elevation affecting tumor growth-promoting genes.

Microbiome in PDAC Therapy

Part of the poor outcome of PDAC is its low response to chemotherapy in less than 50% of patients undergoing treatment[8,9]. Since diet was shown to influence response rates, Tintelnot et al[10] used shotgun metagenomic sequencing and metabolomic screening and found that indole-3-acetic acid (3-IAA), a tryptophan metabolite of gut bacterial origin, was elevated in therapy-responsive patients. 3-IAA producers found in this study[10] are *Bacteroides fragilis* and *Bacteroides thetaiotaomicron* in 22 PDAC patients. It was shown that 3-IAA and FIRINOX, a chemotherapy drug combination, cause oxidative stress in cancer cells by generating high levels of reactive oxygen species (ROS), with 3-IAA being a substrate for ROS-producing myeloperoxidase (MPO), which leads to reduced proliferation of PDAC cells. Moreover, dietary supplementation and fecal microbiome transfer were shown to mimic this effect in mice and could thereby be a beneficial, cheap and easy way to improve chemotherapy[10]. A review article by Mendes and Vale[11] summarizes what is known about microbiome-acquired gemcitabine resistance in PDAC. With gemcitabine being a common treatment for many, PDAC patients, a reduction in response, due to the development of PDAC cancer cell resistance may limit therapeutic success. The microbiome can affect efficacy due to the ability of certain bacteria from the class of *Gammaproteobacteria* to convert the active form of gemcitabine to its inactive form (2',2'-difluorodeoxyuridine). Following this rationale, an improvement in the efficacy of antibiotic treatment along with gemcitabine chemotherapy in PDAC patients is reported in two different studies and cohorts[12,13]. It was found that antibiotic treatment, along with gemcitabine, leads to an improvement in overall survival and cancer-specific survival. Takaori et al[14] did not check the impact of the microbiome on chemotherapy, but the effect of chemotherapy on the gut microbiome itself with 16S ribosomal RNA sequencing in patients undergoing neoadjuvant chemotherapy before and after treatment. The cohort included 20 patients, 18 of which received gemcitabine and the remaining two a combinational therapy. No differences were found in alpha and beta diversity before or after treatment. However, it was found that a decrease in *Bifidobacterium* is associated with a lower response to chemotherapy. With many side effects immanent in chemotherapy, Takaori et al[14] demonstrated a relative stability of microbiome composition.

Intratumoral Microbiome

Whether an intratumoral microbiome exists is highly debatable due to the low biomass environment in PDAC and the likelihood of microbial detection because of contamination from the surroundings. Decontamination is an important step in researching intratumoral microbiome, and many studies have been questioned because of incorrect decontamination. The infiltration of microbes to the pancreas in a disease state might be facilitated by a loss of integrity and barrier functions, which could lead to an intratumoral microbiome in pancreatic cancer. However, Pushalkar et al[15] and Aykut et al[16] demonstrated that bacteria and fungi rather pass the major duodenal papilla in a retrograde manner. Several groups did research on this matter in the past year. Abe et al[17] applied qPCR and *in-situ* hybridization of 16S rRNA in 162 surgically resected PDAC to look for bacterial colonization of pancreatic cancer tissue and then further specified with 16S sequencing after DNA extraction of PDAC tissue. It was found that 32% of tumors harbor bacteria, and when compared to clinical outcomes,

a link between shorter survival and the presence of anaerobic bacteria is found, which could be explained by a decrease in immune cell infiltration in the cancer tissue. Khan et al[18] also did a metagenomic analysis on PDAC tissues and adjacent normal tissue and found that in tumor tissues, there was a higher abundance of P*roteobacteria* and *Actinomycetota*. Programmed death-ligand 1 (PD-L1) plays a key role in the ability of cancer cells to evade the immune system and an overexpression of this protein facilitates immune evasion of tumors. Looking at the correlation of PD-L1 protein expression and bacterial abundance, a positive correlation with *Streptomyces*, *Cutibacterium* and *Delftia* was found. As is often the case in microbiome research, a potential causal relationship remains unclear; however, these findings may aid in the diagnosis and assessment of PDAC, providing a foundation for future studies. Li et al[19] demonstrated spatial heterogeneity of microbes in tumors. Microbial presence wa detected in stromal areas rather than in cancer cell areas, and it consisted mostly of gram-negative bacteria. How these bacteria impact immune infiltration in pancreatic cancer was further evaluated, and an effect on CD8+ T-cells was found. In areas with high levels of Lipopolysaccharide (LPS), a marker for gram-negative bacteria, CD8+ T-cells were more abundant. This could be explained by the observation of prior studies where bacteria can be found intracellular in cancer cells but also in immune cells and might be actively transported to cancer localizations by immune cells[20-22]. In summary, it was found in the present study that a higher diversity of an intratumoral pancreatic microbiome is linked to an increase in long-term survival due to a better anti-tumor immune response mediated by tumor-residing bacteria in a mouse model. Li et al[19] furthermore demonstrates the impact of bacteria on the tumor microenvironment and, thereby, might offer a therapeutic approach. As demonstrated in recent years, not only the gut microbiome has an impact on pancreatic health and disease, but also a link of oral bacteria, especially in PDAC, was found[23,24]. Whether potential lifestyle risk factors like smoking and diet might be the actual cause of PDAC progression and also cause changes in the oral microbiome composition remains to be discovered. McKinley et al[24] compared salivary and tumor microbiomes and found a high abundance of *Veillonella* and *Streptococcus* in PDAC tissue and *Veillonella atypica* in salivary and PDAC tissues. Genetic similarity between oral-derived and pancreas-derived *V. atypical* makes a translocation from the oral cavity to the pancreas probable and could explain enrichment in PDAC. Considering the high amount of lactate produced by PDAC cells, a beneficial environment for *Veillonella*, a lactate fermenter, is given in PDAC. By producing LPS, colonization with *Veillonella* might increase inflammation and tumor progression. This interesting finding unravels a potential mechanism in the tumor progression of PDAC and underlines poor oral health and smoking, which goes along with higher *Veillonella* abundance in smokers[25], as a risk factor for PDAC and other diseases distant from the oral cavity.

CONCLUSIONS

Microbiome research has the potential to aid in the detection, diagnosis, and therapy of pancreatic ductal adenocarcinoma. This potential has been built on the foundation of great research efforts in recent years. With consistent findings of species linked to PDAC, like *Veillonella spp*. in two publications reviewed in this article[5,24], some contradictory data still remains to be clarified, and conservatism in the field of microbiome research should not be forgotten, considering that correlation *vs.* causation in this area can be misleading. As aforementioned, the existence and role of an intratumoral microbiome remain debatable, and future research should be aware of thorough decontamination or find new ways to show reliable results. Integrating lifestyle factors could be a good approach for future studies to minimize confounders and better explain observed effects and phenotypes.

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Conflict of Interest

The authors declare no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

Ethics Approval and Informed Consent

Not applicable due to the type of study.

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Data Availability

All literature sources used for the review are listed accordingly as references.

Authors' Contributions

EH and SS drafted and wrote the article, CAH and CS revised it critically for important intellectual content and have given final approval of the version.

AI Disclosure

No AI assistance was used in the present review article.

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