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Several epidemiological investigations have found associations between poor oral health and different types of cancer, including colorectal, lung, pancreatic, and oral malignancies. The oral health parameters underlying these relationships include deficient oral hygiene, gingival bleeding, and bone and tooth loss. These parameters are related to periodontal diseases, which are directly and indirectly mediated by oral bacteria. Given the increased accessibility of microbial sequencing platforms, many recent studies have investigated the link between the oral microbiome and these cancers. Overall, it seems that oral dysbiotic states can contribute to tumorigenesis in the oral cavity as well as in distant body sites. Further, it appears that certain oral bacterial species can contribute to carcinogenesis, in particular, Fusobacterium nucleatum and Porphyromonas gingivalis, based on results from epidemiological as well as mechanistic studies. Yet, the strength of the findings from these investigations is hampered by the heterogeneity of the methods used to measure oral diseases, the treatment of confounding factors, the study design, the platforms employed for microbial analysis, and types of samples analyzed. Despite these limitations, there is an overall indication that the presence of oral dysbiosis that leads to oral diseases may directly and/or indirectly contribute to carcinogenesis. Proper methodological standardized approaches should be implemented in future epidemiological studies as well as in the mechanistic investigations carried out to explore these results. © International & American Associations for Dental Research 2020

Keywords

cancers, DNA sequencing, host microbial interactions, microbiota, oral cancer

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Association or Causation? Exploring the Oral Microbiome and Cancer Links

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Abstract

Several epidemiological investigations have found associations between poor oral health and different types of cancer, including colorectal, lung, pancreatic, and oral malignancies. The oral health parameters underlying these relationships include deficient oral hygiene, gingival bleeding, and bone and tooth loss. These parameters are related to periodontal diseases, which are directly and indirectly mediated by oral bacteria. Given the increased accessibility of microbial sequencing platforms, many recent studies have investigated the link between the oral microbiome and these cancers. Overall, it seems that oral dysbiotic states can contribute to tumorigenesis in the oral cavity as well as in distant body sites. Further, it appears that certain oral bacterial species can contribute to carcinogenesis, in particular, *Fusobacterium nucleatum* and *Porphyromonas gingivalis*, based on results from epidemiological as well as mechanistic studies. Yet, the strength of the findings from these investigations is hampered by the heterogeneity of the methods used to measure oral diseases, the treatment of confounding factors, the study design, the platforms employed for microbial analysis, and types of samples analyzed. Despite these limitations, there is an overall indication that the presence of oral dysbiosis that leads to oral diseases may directly and/or indirectly contribute to carcinogenesis. Proper methodological standardized approaches should be implemented in future epidemiological studies as well as in the mechanistic investigations carried out to explore these results.

Keywords: oral cancer, DNA sequencing, microbiota, host microbial interactions, cancers

Introduction

According to the National Cancer Institute, 1,735,350 new cancer cases will be diagnosed in 2020 in the United States, with breast, lung, colorectal, and pancreatic cancer being the most common malignancies. While early diagnosis, better prevention, and treatment strategies are needed, they are hampered by gaps in knowledge regarding the molecular mechanisms of carcinogenesis.

Epidemiological studies suggest a link between poor oral health (deficient oral hygiene, gingival bleeding, periodontitis, bone, tooth loss) and cancers (Hujoel et al. 2003), and the overall risk of developing cancer is reduced by dental visits and periodontal treatment (Hwang et al. 2014). It is plausible that oral bacteria accumulation and/or bacteria-induced chronic inflammation can foster microbial translocation to other body sites and that periodontal treatment and dental visits control their potential carcinogenic effects.

The exploration of the potential procarcinogenic role of the oral microbiome is an important follow-up to such epidemiological studies. In the present article, we aim at reviewing the current evidence for the carcinogenic role of oral bacteria, with emphasis on how oral bacteria can contribute to different types of cancer, particularly in the oral cavity.

The Oral Microbiome and Nonoral Cancers: Current Evidence of Associations and Proposed Carcinogenic Mechanisms

The association between poor oral health and cancer is supported by scientific reports using germ-free, antibiotic-treated, and genetically engineered mice with disrupted recognition of microorganism-associated molecular patterns (MAMPs). The

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carcinogenic role of bacteria is associated with specific species as well as dysbiotic communities (Schwabe and Jobin 2013). The microbiome can contribute to carcinogenesis by inducing DNA damage, epigenetic modifications of genes involved in phagocytosis, immune response and chromatin organization, cellular proliferation, and increased DNA mutation rates (Allen and Sears 2019). Disruption of signaling pathways, increased local inflammation, and epithelial barrier function impairment can also be microbiome induced (Schwabe and Jobin 2013; Pang et al. 2018). Bacterial toxins, such as *Aggregatibacter actinomycetemcomitans*–secreted cytolethal distending toxin (CDT), exert direct DNA damage and genomic instability, can cause host response imbalance affecting antigen-presenting cells, and inhibit lymphocyte proliferation (Oscarsson et al. 2019).

Bacterial metabolites such as reactive oxygen species (ROS) sulfides, nitrosamines, butyrate, and acetaldehydes (ACHs) have been linked to carcinogenesis. These oncometabolites can lead to inflammation and tumor formation via DNA alkylation, mutations, damage, and impaired repair. ROS and hydrogen sulfide produced by *Porphyromonas* and *Fusobacterium*, respectively, are associated with colorectal neoplasia. *Firmicutes* and *Bacteroides* sp. ferment excessive protein of the host into sulfides and nitrosamines, which may induce DNA alkylation and host cells mutations. Glycosulfatase in *Bacteroides* can catalyze sulfomucins to release sulfides, contributing to mucins degradation and carcinogenesis. *Streptococcus*- and *Neisseria*-derived acetaldehyde (ACH) can lead to DNA damage. Hydrogen peroxide can diffuse into epithelial cells and form hydroxyl radicals, leading to DNA double-strand break, base modification, and DNA-protein crosslinking by iron-catalyzed reactions (Pang et al. 2018). Butyrate can affect DNA methylation and the expression of demethylation pathways genes (Allen and Sears 2019).

Another potential microbiome procarcinogenic mechanism stems from Toll-like receptor (TLR)–mediated microbial pattern recognition. TLR is a key piece in the microbiome/immunity interplay and a powerful proinflammatory stimulus through nuclear factor (NF)–κB activation. TLR4, a lipopolysaccharide (LPS) ligand, promotes carcinogenesis in the colon, liver, pancreas, and skin. It promotes epithelial carcinogenesis through epithelial cells, stromal fibroblasts, and bone marrow–derived cells. Interestingly, mice expressing constitutively activated epithelial-derived TLR4 have increased tumor load, while TLR4-deficient mice show reduced tumor development (Schwabe and Jobin 2013).

Most host-microbial interactions linked to cancer development occur at epithelial barriers. When intact, they can detect and eliminate invading bacteria and may have additional protective features, such as a mucous layer. Anatomic disruption, microbial composition, and mucus production changes can lead to epithelial barrier dysfunction and microenvironment disruption, granting pathogens and commensals access through the epithelium. Thus, barrier failure resulting from genetic defects, infection, or inflammation can increase procarcinogenic host-microbial interactions. In fact, the absence of mucins can increase the proliferation, increase the migration, and decrease the apoptosis of intestinal epithelial cells, and

mucin 2 knockout mice spontaneously develop colorectal cancer (Schwabe and Jobin 2013; Pang et al. 2018).

The basis of the relationship between the oral microbiome and carcinogenesis is that periodontal diseases and poor oral hygiene facilitate bacterial translocation to other body sites. It is in line with the tenet of microbiome-driven carcinogenesis: dysbiosis, chronic inflammation, and epithelial barrier breach, events that may synergize toward a cumulative carcinogenic milieu. In this review, we will focus on the evidence for oral microbiome-driven carcinogenesis (summarized in Table 1 and Fig. 1) and its mechanisms in colorectal, lung, pancreatic, and oral cancer (summarized in Table 2 and Fig. 2), as they have been the subject of the majority of the publications.

Colorectal Carcinoma

Colorectal Carcinoma (CRC) is the second leading cause of cancer death in the United States, while over 1 million new cases are diagnosed each year worldwide. *Fusobacterium nucleatum*, a common oral bacterium, has been increasingly associated with CRC. Higher *F. nucleatum* levels are present in CRC samples compared with normal mucosa and associated with lymph node metastasis, high-grade dysplasia (Flanagan et al. 2014), and shorter survival (Kunzmann et al. 2019). Conversely, *Lachnospiraceae*, which is negatively correlated with the "Western diet," is protective in CRC development by resisting the colonization of *F. nucleatum* (Flemer et al. 2018). Curiously, oral samples from CRC patients did not reveal significant associations between this species and the malignancy (Kato et al. 2016). It is plausible that cellular changes specific to colorectal tissue favor *F. nucleatum* colonization. For instance, a tumor-enriched bacterial signature is associated with overexpression of cathepsin Z, a tumor-associated protease and interleukin 8, an inflammatory cytokine, and a mediator of innate immunity, all of which might also be cofactors in the process of oncogenesis (Warren et al. 2013). Although less common, other oral taxa have been associated with CRC, including *Parvimonas micra* and *Peptostreptococcus stomatis* as well as *Treponema denticola* and *Prevotella intermedia*.

Mechanistic evidence for a role for *F. nucleatum* in CRC tumorigenesis includes immune modulation (expansion of myeloid-derived immune cells, which inhibit T-cell proliferation and induce T-cell apoptosis), epigenetic modification (present in tumors that exhibit CpG island methylator phenotype, microsatellite instability, methylation positivity, and high mutation burden, all molecular hallmarks of CRC), virulence factors (such as *Fusobacterium* adhesin A [FadA] and the outer membrane protein Fap2), microRNAs (such as miR-21), and bacterial metabolism (Abed et al. 2016). Natural killer (NK) cell killing of various tumors is inhibited in the presence of *F. nucleatum* via the T-cell immunoglobulin and ITIM domain (TIGIT), an inhibitory receptor present on all human NK cells and on various T cells. Fap2-TIGIT interaction leads to tumor immune evasion through inhibition of NK cell cytotoxicity and T-cell activities (Gur et al. 2015).

Furthermore, Gal-GalNAc, a host polysaccharide that is recognized by the fusobacterial lectin Fap2, is overexpressed

Table 1. Association Studies of Oral Microbiome and Cancers.

| Author	Year	Cancer Type	Study Design	Sample Type	Number of Samples	Microbial Assessment Platform	Major Findings
Warren et al.	2013	CRC	Cross sectional	CRC and matched normal control tissues	Tumor/matched normal samples (n=65)	RNA-seq, anerobic culture	A polymicrobial signature of Gram-negative anaerobic bacteria is associated with colorectal carcinoma tissue.
Kato et al.	2016	CRC	Case control	Oral rinse DNA	68 CRCs, 122 controls	16S rRNA gene sequencing	No association was found between *Fusobacterium* abundance or presence and colorectal cancer. CRC was associated with increased presence of genus *Lactobacillus* and increased relative abundance of *Rothia*.
Flemer et al.	2018	CRC	Cross sectional	Oral swabs, colonic mucosae tissue, and stool	CRC (99 subjects), colorectal polyps (32), or controls (103)	16S rRNA gene sequencing	Combining the data from fecal microbiota and oral swab microbiota can distinguish individuals with CRC or polyps (with a sensitivity of 76% [CRC]/88% [polyps]) from controls.
Michaud et al.	2013	PC	Prospective cohort	Prediagnosis blood	405 PCs, 416 matched controls	Immunoblotting	High levels of antibodies to *Porphyromonas gingivalis* were associated with 2 times increased risk for pancreatic cancer. However, increased antibodies to some commensal organisms might reduce risk of pancreatic cancer.
Mitsuhashi et al.	2015	PC	Cross-sectional	Formalin- fixed, paraffin-embedded (FFPE) specimens	283 pancreatic ductal adenocarcinomas, 25 paired specimens of normal tissues	Genomic DNA and qPCR	*Fusobacterium* species were detected in pancreatic cancer tissue. Tumor *Fusobacterium* species status is independently associated with a worse prognosis of pancreatic cancer. *Fusobacterium* species may be a prognostic biomarker of pancreatic cancer.
Fan et al.	2017	PC	Nested case control	Prediagnostic oral wash	361 adenocarcinomas + 371 matched controls from 2 prospective cohort studies	16S rRNA gene sequencing	Carriage of *P. gingivalis* and *Aggregatibacter actinomycetemcomitans* was associated with higher risk of pancreatic cancer.
Gaiser et al.	2019	PC	Cross-sectional	Paired cyst fluid and plasma	Patients with suspected pancreatic cystic neoplasm (n=105)	qPCR 16S rRNA gene sequencing	Enrichment of oral bacteria taxa in cystic precursors of pancreatic cancer with high-grade dysplasia. Pancreatic invasive endoscopic procedures could be a potential iatrogenic route of bacterial translocation.
Apostolou et al.	2011	LC	Cross-sectional	Tissue	32 LC patients	RT-PCR	A diversity of pathogens could be identified in surgically extracted tissue samples of patients with lung cancer, with mycoplasma strains being present in all samples.
Yan et al.	2015	LC	Cross-sectional	Saliva	20 LC patients (10 SCC and 10 adenocarcinoma) and control subjects (n=10)	16S rDNA sequencing, qPCR	Salivary *Capnocytophaga, Selenomonas, Veillonella*, and *Neisseria* were found to be significantly altered in patients with SCC and AC when compared to that in control subjects.
Yang et al.	2018	LC	Cross-sectional	Saliva	75 nonsmoking female LC patients and 172 matched healthy individuals	16S rRNA sequencing	There was significantly lower microbial diversity and richness in lung cancer patients when compared to the control group. The composition of the microbiota in lung cancer patients also differed from that of the control group. Functional analysis from inferred metagenomes indicated that oral microbiome in nonsmoking female lung cancer patients was related to cancer pathways, p53 signaling pathway, apoptosis, and tuberculosis.
Bebek et al.	2012	HNSCC	Cross-sectional	Tissue	Matched tumor and adjacent normal tissue specimens in 42 individuals with HNSCC	16S rRNA sequencing	MDR1 promoter methylation associates with specific microbiomic profiles in tumor over normal mucosa. MDR1 methylation correlates with regional nodal metastases in the context of 2 specific bacterial subpopulations, *Enterobacteriaceae* and *Tenericutes*.
Guerrero-Preston et al.	2016	HNSCC	Longitudinal cohort	Tumor and saliva rinses	19 HNSCC patients 25 normal controls	16S rRNA sequencing	Relative salivary abundance of members of the genera *Streptococcus, Dialister*, and *Veillonella* could significantly discriminate HNSCC from control samples. Longitudinal analyses of salivary samples taken before and after surgery revealed a reduction in the alpha diversity measure after surgery, together with an increase of this measure in patients who recurred.

Table 1. (continued)

Author	Year	Cancer Type	Study Design	Sample Type	Number of Samples	Microbial Assessment Platform	Major Findings
Al-Hebshi et al.	2017	HNSCC	Cross-sectional	Tissue and swabs	20 fresh OSCC and 20 deep-epithelium swabs matched control subjects	16S rRNA sequencing	*Fusobacterium nucleatum* subsp. *polymorphum* was the most significantly overrepresented species in the tumors followed by *P. aeruginosa* and *Campylobacter* sp. oral taxon 44, while *Streptococcus mitis, Rothia mucilaginosa,* and *Haemophilus parainfluenzae* were the most significantly abundant in the controls. Functional prediction showed that genes involved in bacterial mobility, flagellar assembly, bacterial chemotaxis, and LPS synthesis were enriched in the tumors while those responsible for DNA repair and combination, purine metabolism, phenylalanine, tyrosine and tryptophan biosynthesis, ribosome biogenesis, and glycolysis/gluconeogenesis were significantly associated with the controls.
Banerjee et al.	2017	HNSCC	Cross-sectional	FFPE tumor tissues	100 FFPE oral cavity and oropharyngeal SCC	Pan-pathogen array technology (PathoChip) coupled with 16S rRNA sequencing	A distinct OCSCC microbiome signature consisting primarily of HPV16 viral signatures; bacterial signatures of *Escherichia, Brevundimonas, Comamonas, Alcaligenes, Caulobacter, Cardiobacterium, Plesiomonas, Serratia, Edwardsiella, Haemophilus,* and *Frateuria* along with *Rothia* and *Peptoniphilus;* fungal signatures of *Rhodotorula, Geotrichum,* and *Pneumocystis;* and parasitic signatures of *Hymenolepis, Centrocestus,* and *Trichinella* to be associated only with OCSCC and not the controls.
Mukerjee et al.	2017	HNSCC	Cross-sectional	Tissue	Matched tumor and adjacent normal tissue specimens in 39 individuals with SCC	16S rRNA sequencing	Abundance of 22 bacterial and 7 fungal genera was significantly different between the TT and NTT, including *Streptococcus*, which was the most abundant and significantly increased in the tumor group, as well as *Rothia* and *Actinomyces.*
Wolf et al.	2017	HNSCC	Cross-sectional	Saliva	11 HNSCC patients, 11 normal controls	16S rRNA sequencing	*Actinomyces, Schwartzia, Treponema,* and *Selenomonas* were higher abundant in SSC patients. HPV+ patients demonstrated microbiome composition that resembled healthy controls.
Bornigen et al.	2017	HNSCC	Cross-sectional	Oral rinse	121 oral cancer patients to 242 age- and gender- matched controls	16S rRNA sequencing	Significant shifts in composition and function of the oral microbiome were observed with poor oral hygiene, tobacco smoking, and oral cancer.
Shin et al.	2017	HNSCC	Cross-sectional	Tissue	Healthy normal and tumorous (primary and metastatic) human tissues from the oral cavity, larynx-pharynx, and lymph nodes from 34 HNSCC patients	16S rRNA sequencing	Relative abundance of *Fusobacterium* and *Treponema* increased, whereas *Streptococcus* and *Actinomyces* decreased in both primary and metastatic samples. *Streptococcus, Actinomyces,* and *Fusobacterium* genera constituted a significant portion of the differential OTUs. All of the *Streptococcus* and *Actinomyces* OTUs were differentially abundant in the same direction: all were more abundant in the metastatic tissue samples.
Amer et al.	2017	Oral leukoplakia	Cross-sectional	Tissue	36 patients compared to healthy mucosal tissue from the same patients and healthy control subjects	16S rRNA sequencing	Bacterial colonization patterns on oral leukoplakia were highly variable, and 5 distinct bacterial clusters were discerned. These clusters exhibited co-occurrence of *Fusobacterium, Leptotrichia,* and *Campylobacter* species. Increased abundance of the acetaldehydogenic microorganism *Rothia mucilaginosa* was also apparent on oral leukoplakias from lingual sites. Severe dysplasia was associated with elevated levels of *Leptotrichia* spp. and *Campylobacter concisus.* Oral leukoplakia exhibits an altered microbiota that has similarities to the microbiome of colorectal cancer.

(continued)

Table I. (continued)

Author	Year	Cancer Type	Study Design	Sample Type	Number of Samples	Microbial Assessment Platform	Major Findings
Lee et al.	2017	HNSCC	Cross-sectional	Saliva	127 normal, 124 epithelial precursor lesions, 125 OSCC	16S rRNA sequencing	*Bacillus, Enterococcus, Parvimonas, Peptostreptococcus,* and *Slackia* revealed significant differences between epithelial precursor lesion and cancer patients and correlated with their classification into 2 clusters, showing potential to represent markers for prediction of OSCC.
Wang et al.	2017	HNSCC	Cross-sectional	Tissue	Paired normal and tumor resection specimens (*n* = 121 patients)	16S rRNA sequencing	Depletion of *Actinomyces* and enrichment of *Parvimonas* species were observed in tumors. Low-stage patients had increased percentage of *Actinomyces* and low *Parvimonas*.
Zhao et al.	2017	HNSCC	Cross-sectional	Oral swab	OSCC lesion and anatomically matched normal sites (*n* = 40)	16S rRNA sequencing	Periodontitis-related taxa significantly enriched in OSCC samples, including *Treponema, Campylobacter, Eikenella, Alloprevotella, Fusobacterium, Selenomonas, Dialister, Peptostreptococcus, Filifactor, Peptococcus, Catonella, Parvimonas, Capnocytophaga,* and *Peptostreptococcaceae*.
Desci et al.	2019	Oral potentially malignant disorders	Cross-sectional	Tissue	7 individuals with OPMD: samples from lesions and paired adjacent normal tissue sample	Ion torrent metagenomic sequencing	Relative abundance of *S. mitis* decreased in the OPMD lesions compared to the uninvolved tissue. The relative abundance of *F. nucleatum,* implicated in carcinogenesis, was elevated in OPMD. Bacterial diversity in the OPMD compared to the healthy oral mucosa markedly increased. The ratio of *S. mitis* and *F. nucleatum* is altered in the OPMD lesions compared to the healthy mucosa.
Lim et al.	2018	HNSCC	Cross-sectional	Oral rinse	Healthy controls (*n* = 10, 20–30 y old; *n* = 10, >50 y old), high-risk individuals (*n* =, >50 y old with bad oral hygiene and/or oral diseases) and OCC and OPC patients (*n* = 31, HPV+; *n* = 21, HPV−).	16S rRNA sequencing	Oral microbiome predicted the presence of OCC and OPC with sensitivity and specificity of 100% and 90%, respectively. *Actinomyces, Parvimonas, Selenomonas,* and *Prevotella* have higher abundance in OCC compared with OPC; HPV has a positive correlation with *Haemophilus* abundance. *Actinomyces, Actinobacillus, Lautropia, Fusobacterium,* and *Aggregatibacter* are significantly more abundant in high-risk individuals . Oral microbiome panel of *Rothia, Haemophilus, Corynebacterium, Paludibacter, Porphyromonas, Oribacterium,* and *Capnocytophaga* discriminated age-matched normal healthy controls from OCC and OPC patients with high accuracy.
Hayes et al.	2018	HNSCC	Nested case control	Oral wash	129 HNSCC patients, 254 matched controls	16S rRNA sequencing	Overall microbiome composition was not associated with risk of HNSCC. This study demonstrates that greater oral abundance of commensal *Corynebacterium* and *Kingella* is associated with decreased risk of HNSCC, with potential implications for cancer prevention.
Hsiao et al.	2018	HNSCC	Case control	Saliva	138 OSCC cases, 151 controls	16S rRNA sequencing	Three species of periodontopathogenic bacteria, *Prevotella tannerae, F. nucleatum,* and *Prevotella intermedia,* were associated with an increased OSCC risk. This association was modified by the genetic polymorphisms of TLR2 and TLR4.
Perera et al.	2018	HNSCC	Case control	Tissue	25 OSCC cases and 27 FEP	16S rRNA sequencing	*Capnocytophaga, Pseudomonas,* and *Atopobium* were overrepresented in OSCC, while *Lautropia, Staphylococcus,* and *Propionibacterium* were the most abundant in FEP. *Campylobacter concisus, Prevotella salivae, Prevotella loeschii,* and *Fusobacterium* oral taxon 204 were enriched in OSCC, while *S. mitis, Streptococcus* oral taxon 070, *Lautropia mirabilis,* and *Rothia dentocariosa* were more abundant in FEP. Functionally, proinflammatory bacterial attributes, including lipopolysaccharide biosynthesis and peptidases, were enriched in the OSCC tissues.
Yang, Huang, et al.	2018	HNSCC	Cross-sectional	Saliva	39 male OSCC patients	16S rRNA sequencing	Oral microbiota is compositionally and functionally associated with the mutational changes in oral cancer.
Yang, Yeh, et al.	2018	HNSCC	Cross-sectional	Oral rinse	197 OSCC patients, 51 healthy controls	16S rRNA sequencing	Oral microbiota communities dynamically changed with the cancer's progression from stage 1 to stage 4.

AC, adenocarcinoma; CRC, colorectal carcinoma; FEP, fibroepithelial polyp; FFPE, formalin fixed, paraffin embedded; HNSCC, head and neck squamous cell carcinomas; HPV, human papillomavirus; LC, lung cancer; LPC, lipopolysaccharide; OPC, oropharyngeal cancer; OCSCC, oral cavity squamous cell carcinoma; OSCC, oral squamous cell carcinoma; OTU, operational taxonomic unit; PC, pancreatic cancer; qPCR, quantitative polymerase chain reaction; rRNA, ribosomal RNA; RT-PCR, reverse transcription polymerase chain reaction; SCC, squamous cell carcinoma; TT, tumor tissue.
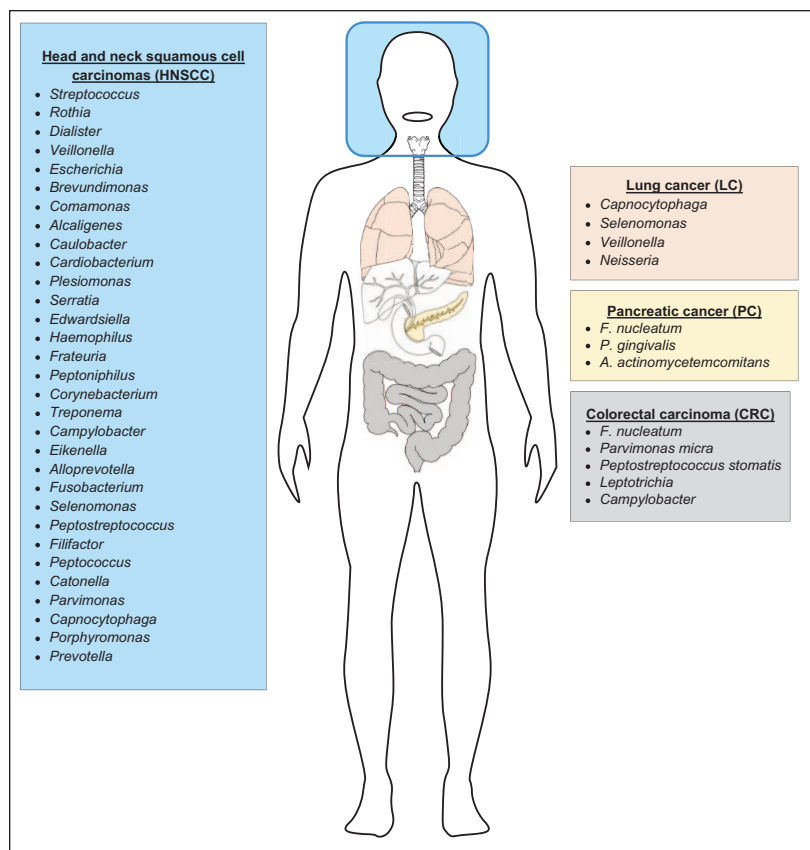
Figure 1. Oral microbiome signature and cancers. The potential polymicrobial signature of the oral microbiome in significantly related cancers, including HNSCC, LC, PC, and CRC. CRC, colorectal carcinoma; HNSCC, head and neck squamous cell carcinomas; LC, lung cancer; PC, pancreatic cancer.

in CRC (Abed et al. 2016). *F. nucleatum* binding to clinical adenocarcinomas correlates with Gal-GalNAc expression and is reduced upon O-glycanase treatment. *Fusobacterium* strains naturally lacking Fap2 or inactivated Fap2 mutants show reduced binding to Gal-GalNAc-expressing CRC cells and established CRCs in mice. In addition, intravenously injected *F. nucleatum* localizes to mouse tumor tissues in a Fap2-dependent manner, suggesting that *Fusobacteria* use a hematogenous route to reach colon adenocarcinomas. *F. nucleatum* may also increase the expression of inflammatory mediators through miRNA-mediated activation of TLR2/TLR4 and increase the frequency of KRAS (Kirsten rat sarcoma viral oncogene homolog) mutations (Proenca et al. 2018).

F. nucleatum's causal role in CRC may also occur via modulation of cadherin/β-catenin signaling. *F. nucleatum* adheres to, invades, and induces oncogenic and inflammatory responses to stimulate the growth of CRC cells through its unique FadA adhesin. FadA binds to E-cadherin on the surface of epithelial cells, activates β-catenin signaling, and differentially regulates the inflammatory and oncogenic responses. The increased FadA expression in CRC correlates with increased expression of oncogenic and inflammatory genes. Furthermore, *F. nucleatum* stimulates the growth of CRC cells. Annexin A1, a

modulator of Wnt/β-catenin signaling, is a key component through which *F. nucleatum* exerts its stimulatory effect. Annexin A1 is expressed in proliferating colorectal cancer cells and activates cyclin D1. Its expression level in colon cancer is an independent predictor of poor prognosis. The FadA adhesin from *F. nucleatum* upregulates annexin A1 expression through E-cadherin. A positive feedback loop between FadA and annexin A1 is identified in the cancerous cells and is absent in the noncancerous cells (Gur et al. 2015; Abed et al. 2016; Rubinstein et al. 2019).

F. nucleatum promotes an LPS-mediated proinflammatory microenvironment via upregulation of NF-κB-driven inflammatory genes and cytokine and chemokine secretion. It stimulates cell proliferation and migration, as well as activates P38, which leads to the secretion of matrix metalloproteinase (MMP)–9 and MMP-13, favoring cell invasion and metastasis. Resulting necrotic and apoptotic cells interact with HMGB1 (high mobility group box 1) protein to generate a proinflammatory milieu that fosters tumor progression. Hence, a "2-hit" model in colorectal carcinogenesis has been proposed, with somatic mutation(s) serving as the first hit and *F. nucleatum* as the second hit, exacerbating cancer progression (Rubinstein et al. 2019). Finally, using the ApcMin/+ mouse model of intestinal tumorigenesis, *F. nucleatum* increases tumor multiplicity and selectively recruits tumor-infiltrating myeloid cells, which can promote tumor progression. Tumors from *F. nucleatum*–exposed ApcMin/+ mice exhibit a proinflammatory expression signature that is shared with human fusobacteria-positive CRC (Kostic et al. 2013), via a TLR4/p-PAK1/ p-β-catenin S675 cascade (Wu, Wu, et al. 2018). These data suggest that, through recruitment of tumor-infiltrating immune cells, *Fusobacteria* generate a proinflammatory microenvironment that is conducive for colorectal neoplasia progression.

Pancreatic Cancer

Pancreatic cancer (PC) is highly lethal, as 93% of patients die within 5 y of diagnosis, and little is known about its prevention. Recent studies indicate that periodontitis and poor oral hygiene may increase PC risk (Hujoel et al. 2003). *Porphyromonas gingivalis* and *A. actinomycetemcomitans* oral carriage, decreased relative abundance of *Leptotrichia*, and high levels of antibodies against *P. gingivalis* are related to increased PC risk (Michaud et al. 2013; Fan et al. 2017). Pancreatic cysts, which are early precursors of invasive PC, are enriched for oral microbiome members. High-grade dysplasia cysts have higher bacterial load and *F. nucleatum* and *Granulicatella adiacens*

Table 2. Studies Investigating Mechanisms of Oral Microbiome-Induced Carcinogenesis.

Author	Year	Cancer Type	Study Design	Sample Type	Number of Samples	Major Findings
Kostic et al.	2013	CRC	In vitro, animal model	In vitro cancer progression model	Samples from adenoma (n = 32) and carcinoma (n = 27) cases	In ApcMin/+ mouse model, F. nucleatum increases tumor multiplicity and selectively recruits tumor-infiltrating myeloid cells, which can promote tumor progression. Tumors from ApcMin/+ mice exposed to *Fusobacterium nucleatum* exhibit a proinflammatory expression signature that is shared with human fusobacteria-positive CRC.
Gur et al.	2015	CRC	In vitro	Cell lines	Cells	*F. nucleatum* specifically targets the inhibitory receptor TIGIT, via its Fap2 protein, to inhibit immune cell activities. NK cell killing of tumors is inhibited by hemagglutinating *F. nucleatum* strains.
Abed et al.	2016	CRC	In vitro, animal model	Human CRC samples, orthotopic mouse CRC model	CRC metastases from 5 frozen and 7 formalin-fixed, paraffin-embedded blocks, 7 tumor-free samples	Fusobacterial Fap2 and host Gal-GalNAc are involved in fusobacterial CRC localization and enrichment.
Proenca et al.	2018	CRC	In vitro	Clinical specimens	Diseased and adjacent normal tissues of 27 CRA and 43 CRC patients.	mRNA expression of IL-1B, IL-6, IL-8, and miR-22 was positively correlated with *F. nucleatum* quantification in CRC tumors. mRNA expression of miR-135b and TNF was inversely correlated. The miRNA/mRNA interaction network suggested that the upregulation of miR-34a in CRC proceeds via aTLR2/TLR4-dependent response to *F. nucleatum*. KRAS mutations were more frequently observed in CRC samples infected with *F. nucleatum* and were associated with greater expression of miR-21 in CRA, while IL-8 was upregulated in MSI-high CRC.
Wu, Wu, et al.	2018	CRC	In vitro, animal model	Animal specimens	C57BL/6-ApcMin/+	*F. nucleatum* and antibiotics treatment altered gut microbial structures in mice. *F. nucleatum* invaded intestinal mucosa in large amounts but were less abundant in the feces of *F. nucleatum*–fed mice. The average number and size of intestinal tumors in *F. nucleatum* groups were increased compared to control groups in ApcMin/+ mice. The expression of TLR4, PAK1, p-PAK1, p-β-catenin S675, and cyclin D1 was increased in *F. nucleatum* groups compared to the controls. TAK-242 decreased number and size of tumors compared to *F. nucleatum* groups. p-PAK1, p-β-catenin S675, and cyclin D1 expression was decreased in the TAK-242-treated group compared to *F. nucleatum* groups.
Rubinstein et al.	2019	CRC	In vitro, animal model	Cancer progression model	18 CRC cases, ApcMin/+ mouse model	Annexin A1 is specifically expressed in proliferating colorectal cancer cells and involved in activation of cyclin D1. Its expression level in colon cancer is a predictor of poor prognosis independent of cancer stage, grade, age, and sex. The FadA adhesin from *F. nucleatum* upregulates annexin A1 expression through E-cadherin. A positive feedback loop between FadA and annexin A1 is identified in the cancerous cells, absent in the noncancerous cells.
Martilla et al.	2013	HNSCC	Clinical	Microbial samples taken from the mucosa using filter paper	30 OSCC; 30 oral lichenoid disease; 30 controls	The majority (68%) of cultures produced carcinogenic levels of acetaldehyde (>100 mM) when incubated with ethanol (22 mM). The mean acetaldehyde production by microbes cultured from smoker samples was significantly higher (213 mM) than from nonsmoker samples (141 mM).
Moritani et al.	2015	HNSCC	Clinical	Saliva, bacterial strains	28 species; 166 orally healthy subjects	All *Neisseria* species tested produced conspicuous amounts of ACH from ethanol, and *Rothia mucilaginosa, Streptococcus mitis*, and *Prevotella histicola* exhibited the ability to produce ACH. In addition, xylitol and sorbitol inhibited ACH production by *Neisseria mucosa* by more than 90%.
Gallimidi et al.	2015	HNSCC	In vitro, animal model	Mouse tongue	n = 14 mice	*Porphyromonas gingivalis* and *F. nucleatum* stimulate tumorigenesis via direct interaction with oral epithelial cells through Toll-like receptors.
Sztutskowa et al.	2016	HNSCC	In vitro	Human TIGKs	Cells infected with *P. gingivalis, Streptococcus gordonii*, and *F. nucleatum*	*P. gingivalis* induced expression and nuclear localization of the ZEB1 transcription factor, which controls epithelial-mesenchymal transition. *P. gingivalis* also caused an increase in ZEB1 expression as a dual-species community with *F. nucleatum* or *S. gordonii*. Increased ZEB1 expression was associated with elevated ZEB1 promoter activity. *P. gingivalis* strains lacking the FimA fimbrial protein were attenuated in their ability to induce ZEB1 expression. ZEB1 levels correlated with an increase in expression of mesenchymal markers, including vimentin and MMP-9, and with enhanced migration of epithelial cells. Knockdown of ZEB1 with siRNA prevented the *P. gingivalis*–induced increase in mesenchymal markers and epithelial cell migration. Oral infection of mice by *P. gingivalis* increased ZEB1 levels in gingival tissues, and intracellular *P. gingivalis* was detected by antibody staining in biopsy samples from OSCC.

Table 2. (continued)

Author	Year	Cancer Type	Study Design	Sample Type	Number of Samples	Major Findings
Groenger et al.	2017	HNSCC	In vitro	Cell lines	PHGK and SCC-25 cells infected with *P. gingivalis*	After infection with *P. gingivalis* membranes, the RNA of 16 to 33 of 84 key genes involved in the antibacterial immune response was upregulated; among them were IKBKB (NF-κB signaling pathway), IRF5 (TLR signaling), and JUN, MAP2K4, MAPK14, and MAPK8 (MAPK pathway) in SCC-25 cells and IKBKB, IRF5, JUN, MAP2K4, MAPK14, and MAPK8 in PHGK. Significant upregulation of IKBKB, MAP2K4, MAPK14, and IRF5 was demonstrated in SCC-25 cells and IKBKB, MAP2K4, MAPK 14, IRF5, and JUN in PHGK. *P. gingivalis* membrane upregulated the expression of genes involved in downstream TLR, NF-κB, and MAPK signaling pathways involved in the proinflammatory immune response in primary and malignant oral epithelial cells.
Geng et al.	2017	HNSCC	In vitro	HIOECs	*P. gingivalis*–infected HIOECs	Persistent exposure to *P. gingivalis* caused cell morphological changes, increased proliferation ability with higher S phase fraction in the cell cycle, and promoted cell migratory and invasive properties. Tumor-related genes such as NNMT, FLI1, GAS6, lncRNACCAT1, PDCD1LG2, and CD274 may be considered the key regulators in tumor-like transformation in response to long-time exposure of *P. gingivalis*.
Woo et al.	2017	HNSCC	In vitro	OSCC cell line (OSC-20)	*P. gingivalis*–infected OSC-20	Sustained infection with *P. gingivalis* could modify the response of OSCC cells to chemotherapeutic agents and their metastatic capability in vivo. Tumor xenografts composed of *P. gingivalis*–infected OSCC cells demonstrated a higher resistance to Taxol through Notch1 activation, as compared with uninfected cells. *P. gingivalis*–infected OSCC cells formed more metastatic foci in the lung than uninfected cells.
Wu, Zheng, et al.	2018	HNSCC	In vitro, animal model	Wild-type C57BL/6 mice	4NQO-induced oral carcinoma and *P. gingivalis*–induced chronic periodontitis model	*P. gingivalis* infection increased the tongue lesion size and multiplicity of each mouse and promoted oral cancer development. *P. gingivalis* treatment significantly increased the level of free fatty acids and altered the fatty acid profile in tongue tissues and the serum of mice. *P. gingivalis* induced the formation of fatty liver of the mice. Expression of fatty acid synthase and acetyl-CoA carboxylase 1 were increased in the tongue and liver tissues of 4NQO-treated mice infected with *P. gingivalis*.
Yost et al.	2018	HNSCC	In vitro	community-wide metatranscriptome	Oral swab samples from OSCC tumor ($n=4$), a healthy adjacent site ($n=4$), matching sites ($n=4$), and buccal mucosa ($n=3$) from healthy subjects ($n=4$)	Fusobacteria showed a higher number of transcripts at tumor sites and tumor-adjacent sites of cancer patients compared to the healthy controls. Specific metabolic signatures were consistently found in disease. Activities such as iron ion transport, tryptophanase activity, peptidase activities, and superoxide dismutase were overrepresented in tumor and tumor-adjacent samples when compared to the healthy controls. OSCC-oral communities showed that activities related to capsule biosynthesis, flagellum synthesis and assembly, chemotaxis, iron transport, hemolysins, and adhesins were upregulated at tumor sites. Activities associated with protection against reactive nitrogen intermediates, chemotaxis, and flagellar and capsule biosynthesis were also upregulated in nontumor sites of cancer patients.
Abdulkareem et al.	2018	HNSCC	In vitro	OSCC cell line (H400)	Cells were treated separately with heat-killed periodontal pathogens *F. nucleatum, P. gingivalis,* or *Escherichia coli* LPS	Upregulation after 1, 5, and 8 d in transcription of mesenchymal markers and downregulation of epithelial ones compared with unstimulated controls. Periodontal pathogens caused increase in level of all cytokines investigated, which could be involved in EMT induction and Snail activation. Exposure of cells to the bacteria increased migration and the rate of wound closure. Downregulation of epithelial markers also resulted in decrease in impedance resistance of cell monolayers to passage of electrical current.
Song et al.	2019	HNSCC	In vitro, animal model	OSC-20 cell line, BALB/c mice	BALB/c mice grafted with OSC-20 and treated with *P. gingivalis*	Compared with uninfected mice, the mice that were chronically administered *P. gingivalis* showed increased resistance to paclitaxel and a decreased tumor growth rate. *P. gingivalis*–treated mice exhibited higher serum IL-6 than uninfected mice. The sensitivity of tumor xenografts to paclitaxel in mice administered *P. gingivalis* was increased when the mice were administered ibuprofen.
Ohshima et al.	2019	HNSCC	In vitro	Human TIGKs, OKF6/TERT2 keratinocytes, SCC9 and HeLa cells	Cells infected with *P. gingivalis, S. gordonii, Streptococcus sanguinis, Streptococcus cristatus,* and *F. nucleatum*	*P. gingivalis* can upregulate expression of ZEB2, a transcription factor that controls epithelial-mesenchymal transition and inflammatory responses. ZEB2 regulation by *P. gingivalis* was mediated through pathways involving β-catenin and FOXO1. *S. gordonii* was capable of antagonizing ZEB2 expression. *S. gordonii* suppressed FOXO1 by activating the TAK1-NLK negative regulatory pathway, even in the presence of *P. gingivalis*.

Table 2. (continued)

Author	Year	Cancer Type	Study Design	Sample Type	Number of Samples	Major Findings
Stashenko et al.	2019	HNSCC	In vitro, animal model	4NQO-induced model of OSCC in gnotobiotic mice	Microbiome inocula from healthy mice and mice with 4NQO-induced tumor, 16S rRNA gene sequencing, and metatranscriptomic	Mice colonized with different oral microbiomes and exposed to 4NQO had increased tumor numbers and sizes compared to controls exposed to 4NQO but lacking a microbiome. In the 2 groups that were inoculated with OSCC-associated microbiome, opposite profiles of abundance in *Parabacteroides* and *Corynebacterium* were observed. While the percentage of *Parabacteroides* bacteria decreased in the control group, it increased in the OSCC group, and the opposite was observed for *Corynebacterium*. The metatranscriptomic analysis revealed overexpression of the same metabolic signatures associated with OSCC regardless of the community profile. These included nitrogen transport, response to stress, interspecies interactions, Wnt pathway modulation, and amino acid and lipid biosynthesis.

ACH, acetaldehyde; CRA, colorectal adenoma; CRC, colorectal carcinoma; EMT, epithelial-mesenchymal transition; HIOEC, human immortalized oral epithelial cell; HNSCC, head and neck squamous cell carcinomas; IL, interleukin; miRNA, microRNA; mRNA, messenger RNA; MSI, microsatellite instability; NK, natural killer; OSCC, oral squamous cell carcinoma; rRNA, ribosomal RNA; siRNA, small interfering RNA; TIGK, telomerase immortalized gingival keratinocyte; TNF, tumor necrosis factor; 4NQO, 4-nitroquinoline-1-oxide.

levels (Gaiser et al. 2019). *F. nucleatum*–positive pancreatic ductal adenocarcinomas are associated with higher cancer-specific mortality rates (Mitsuhashi et al. 2015).

F. nucleatum, P. gingivalis, and *A. actinomycetemcomitans* contributions to PC development remain unknown. Mechanistically, their LPS may accelerate carcinogenesis via TLR4 binding (which is upregulated within the tumor microenvironment) and NF-κB signaling. Also, *F. nucleatum* and *P. gingivalis* are broadly immune disruptive and evasive, as well as strongly antiapoptotic. *P. gingivalis* accelerates progression through the S-phase of the cell cycle and reduces p53 tumor suppressor levels (Wright et al. 2014). *A. actinomycetemcomitans* Y4 can induce genomic instability, a critical step in cancer development, through the introduction of DNA double-strand breaks through CDT activity (Teshima et al. 2018). Collectively, the microbial differences between PC as well as early PC precursor cases and healthy controls, along with immune modulation and genomic damage, suggest a mechanistic role for oral microbiome members on PC development.

Lung Cancer

Lung cancer (LC) is the most common cause of cancer-related deaths in North America and worldwide. Periodontal disease patients are at increased LC risk (Hujoel et al. 2003). Oral inflammatory changes may modify the respiratory epithelium and promote carcinogenesis (Zeng et al. 2016), and the microbiome of LC tissues indicates an etiologic role for chronic infection (Apostolou et al. 2011). Oral bacteria may colonize the lung via the pharynx and may contribute to chronic obstructive pulmonary disease (COPD), also linked to lung cancer. *Capnocytophaga* and *Veillonella* levels are significantly higher in LC saliva than in control samples (Yan et al. 2015). Nonsmoking female LC patients exhibit salivary dysbiosis and lower microbial diversity and richness when compared to controls. Functional analysis of their oral microbiome points to apoptosis, p53 signaling, and cancer pathway gene enrichment (Yang, Mu, et al. 2018). While those studies overall support a role for oral species in lung cancer, specific epidemiological and mechanistic studies are needed.

The Oral Microbiome and Oral Cancers: Current Evidence of Associations and Proposed Carcinogenic Mechanisms

Oral squamous cell carcinomas (OSCCs) are a major cause of cancer morbidity and mortality. Most cases arise in the oral cavity and oropharynx, and while tobacco usage, alcohol consumption, and human papillomavirus (HPV) infection are established risk factors, only a subset of exposed individuals develops OSCC. Thus, other factors might be involved in oral carcinogenesis, including the oral microbiome.

Next-generation sequencing (NGS) platforms increased the depth and breadth of oral microbiome analyses in OSCC. While CRC studies point to the *F. nucleatum* as a major player, in OSCC, the results are heterogeneous and conflicting. Certain surveys found higher levels of *Rothia* and *Actinomyces* in cases (Banerjee et al. 2017; Mukherjee et al. 2017; Wolf et al. 2017), while others reported opposite results (Shin et al. 2017; Wang et al. 2017). These differences are likely due to the variety of methods employed for analysis and sampling (Table 1).

OSCC "signatures" revealed diverse bacterial combinations: *Streptococcus, Dialister*, and *Veillonella* (Guerrero-Preston et al. 2016); *Cardiobacterium, Serratia, Haemophilus, Rothia*, and *Peptoniphilus* (Banerjee et al. 2017); and *Rothia, Haemophilus, Corynebacterium, Paludibacter, Porphyromonas, Oribacterium*, and *Capnocytophaga* (Lim et al. 2018) (Fig. 1). Periodontally pathogenic genera, including *Parvimonas, Treponema, Campylobacter, Filifactor, Prevotella*, and *Fusobacterium* (Lee et al. 2017; Shin et al. 2017; Wang et al. 2017; Wolf et al. 2017; Zhao et al. 2017; Hsiao et al. 2018), are more abundant in OSCC than in controls, with *Porphyromonas endodontalis, Filifactor alocis*, and *Dialister pneumosintes*
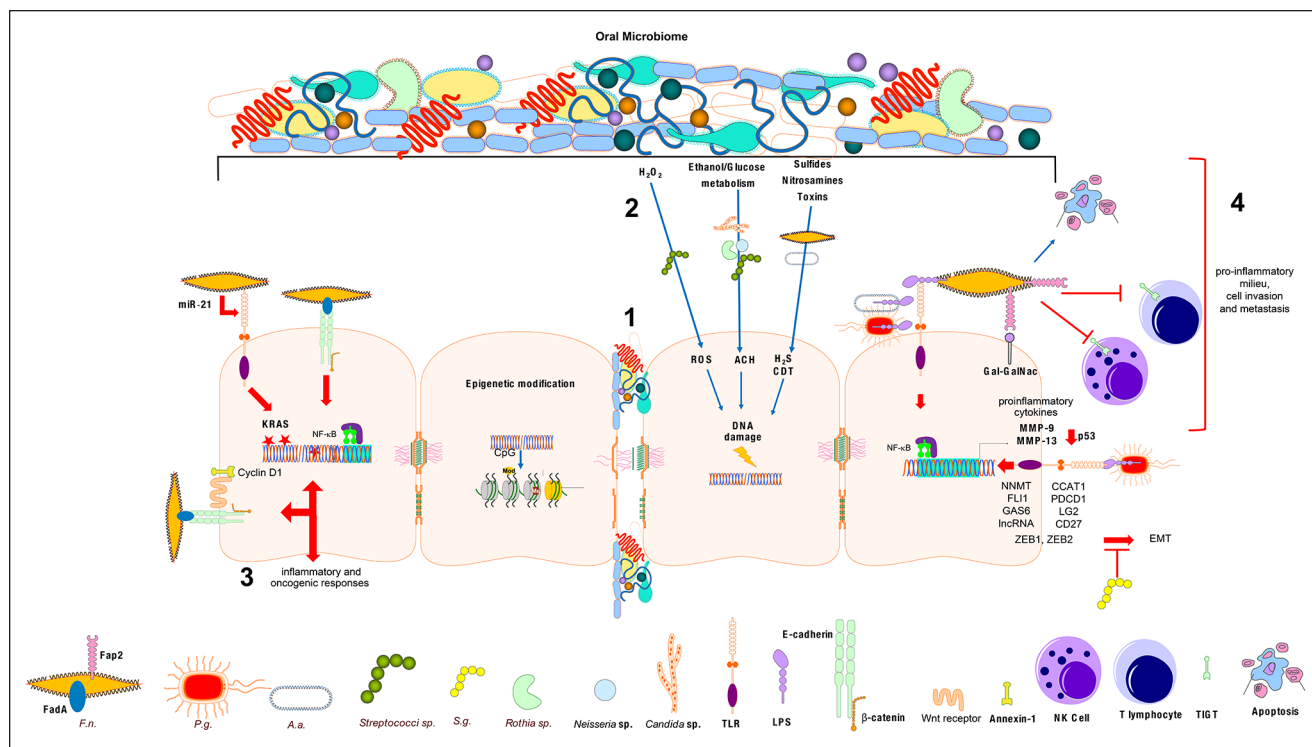
Figure 2. Mechanisms linked between oral microbiome and carcinogenesis. 1. Impairment of barrier function. Bacteria induced impairment of epithelial barrier function, which increases the exposure of immune cells to bacterial endotoxins and antigens and future leads to cancer progression. 2. Synthesis of carcinogenic metabolites and bacterial toxins. Microbial metabolites, including sulfides, nitrosamines, hydroxyl radical, ACH and DCA, and bacterial toxins, such as CDT and colibactin, can further induce DNA damage and trigger oncogenesis. 3. Induction of inflammation. ROS and cytokines produced by inflammatory cells are believed to contribute to initiation of cancer by inducing mutations, genomic instability, and epigenetic alterations. 4. Activation of cell proliferation. FadA of *F.n.* binds to E-cadherin on colorectal carcinoma (CRC) cells and activates β-catenin signaling and subsequently enhanced CRC cell proliferation. *A.a., Aggregatibacter actinomycetemcomitans*; ACH, acetaldehyde; DCA, deoxycholic acid; CDT, cytolethal distending toxin; *F.n., Fusobacterium nucleatum; P.g., Porphyromonas gingivalis*; ROS, reactive oxygen species; *S.g., Streptococcus gordonii.*

abundant in cancer lesions. *Prevotella tannerae, F. nucleatum*, and *P. intermedia* are associated with a 2.3 times increase in OSCC risk, which is modified by the genetic polymorphisms of TLR2 and TLR4. OSCC risk factors, such as alcohol consumption and cigarette use, along with poor oral hygiene, were associated with a higher percentage of periodontopathogenic bacteria (Hsiao et al. 2018). Although several reports have shown the correlation between specific microbiome communities with tumors, other researchers failed in identifying such correlation (Hayes et al. 2018). The conflicting result may be due to having a single timepoint oral sample collection for this prospective study before the occurrence of OSCC and that microbial dysbiosis that precedes OSCC might not coincide with the time of collection.

Function prediction revealed that tumor samples have increased abundance of porphyrin (cofactor and vitamin production), pentose phosphate, pentose and glucuronate interconversion (carbohydrate metabolism), sporulation (microbial endurance and dormancy), and ascorbate and aldarate (sugar metabolism) metabolism genes (Wolf et al. 2017). Carbohydrate- and energy-metabolism-related parameters, such as oxidative phosphorylation, are enriched in late-stage OSCC. Conversely, amino acid metabolism genes, such as folate biosynthesis and

valine, leucine, and isoleucine biosynthesis, were associated with healthy controls (Yang, Huang, et al. 2018). Thus, the tumor microbiome emphasizes sugar metabolism (including biofilm matrix formation) and the stress response (spore formation). Furthermore, significant oral microbiome composition and function shifts occur upon poor oral hygiene and smoking, both risk factors for OSCC (Bornigen et al. 2017).

OSCC samples are enriched for periodontitis-associated taxa, including *Fusobacterium, Dialister, Peptostreptococcus, Filifactor, Peptococcus, Catonella*, and *Parvimonas* (Zhao et al. 2017). Proinflammatory attributes (LPS biosynthesis and peptidases) were enriched in OSCC, along with high *Capnocytophaga, Pseudomonas*, and *Atopobium* levels, while *Lautropia, Staphylococcus*, and *Propionibacterium* were abundant in controls. *Campylobacter concisus, Prevotella salivae, Prevotella loeschii*, and *Fusobacterium* sp. human oral taxon (HOT) 204 were enriched in OSCC, while *Streptococcus mitis, Streptococcus* sp. HOT 070, *Lautropia mirabilis*, and *Rothia dentocariosa* were more abundant in controls (Perera et al. 2018). Functionally, an "inflammatory bacteriome" colonizes OSSC (Al-Hebshi et al. 2017), with mobility, chemotaxis, flagellar assembly, and LPS synthesis enriched in tumor samples. Conversely, DNA repair and combination, purine metabolism,

phenylalanine, tyrosine and tryptophan biosynthesis, ribosome biogenesis, and glycolysis/gluconeogenesis genes were abundant in controls.

NGS studies also considered additional contributors to carcinogenesis, such as HPV status (Guerrero-Preston et al. 2016; Wolf et al. 2017), precancer, disease staging (Yang, Yeh, et al. 2018), and epigenetic profiles (Bebek et al. 2012). HPV+ patients demonstrated a "normal" microbiome, in line with the more favorable prognosis of HPV+ OSCC (Wolf et al. 2017). Oral leukoplakia (OL) harbors increased abundance of *F. nucleatum* and *Rothia mucilaginosa* and reduced levels of *Firmicutes* (Amer et al. 2017; Decsi et al. 2019). Severe dysplasia has elevated levels of *Leptotrichia* spp. and *Campylobacter concisus* (Amer et al. 2017), while *Bacillus, Enterococcus, Parvimonas, Peptostreptococcus*, and *Slackia* have revealed significant differences between epithelial precursor lesion and OSCC (Lee et al. 2017). Regarding disease stage, *F. nucleatum, Fusobacterium periodonticum, P. micra*, and *F. alocis* progressively increased in abundance from stage 1 to stage 4, with *Acinetobacter* and *Fusobacterium* related to late-stage tumors. The opposite was observed for *Actinomyces* sp., *S. mitis, H. parainfluenzae*, and *Porphyromonas pasteri* (Guerrero-Preston et al. 2016; Wang et al. 2017; Yang, Huang, et al. 2018). Specific microbial signatures have been associated with epigenetic modifications in inflammatory- and OSCC-associated genes (Bebek et al. 2012). Multidrug resistance 1 (MDR1) promoter methylation associated with *Enterobacteriaceae*- and *Tenericutes*-enriched microbial signatures correlated with regional nodal metastases. Further, the correlation between different microbiomes with the accumulation of tumor mutations also suggests the potential role of a specific microbiome as a pro-oncogenic factor during cancer development (Yang, Huang, et al. 2018).

Evidence for Specific Mechanisms of Microbiome-Induced Oral Carcinogenesis

Acetaldehyde

ACH is a known carcinogen that can derive from alcohol degradation and microbial metabolism. Oral microbial ACH production and the correlation between microbial communities and OSCC staging are strong indications of the microbiome's role in tumor progression. OSCC samples harbor higher levels of bacteria than potentially malignant oral lichenoid disease (OLD). OSCC and OLD samples contain greater numbers of bacterial species than their control sites as well as healthy individuals. Yet, microbial cultures from all clinical groups can synthesize carcinogenic levels of ACH, with higher levels observed in samples from smokers (Marttila et al. 2015). Several oral taxa, including *Neisseria, Streptococcus*, and *Rothia* species, can produce ACH from ethanol, and many species can generate ACH from glucose (Moritani et al. 2015). Further ACH metabolism in the mouth is limited, leading to a 10 to 100 times higher concentration than in the blood. Interestingly, germ-free rats have lower concentrations of ACH, supporting the role of the oral microbiome in carcinogenesis through ACH.

Periodontal Bacteria-Mediated Cellular Changes: Cell Lines Studies

P. gingivalis membrane upregulates the expression of genes involved in downstream TLR, NF-κB, and mitogen-activated protein kinase (MAPK) signaling pathways involved in the proinflammatory immune response in primary and malignant oral epithelial cells (Groeger et al. 2017). Persistent *P. gingivalis* exposure promotes tumorigenic properties of human immortalized oral epithelial cells (HIOECs), causing morphological changes; increases proliferation ability with higher S phase fraction in the cell cycle; and promotes cell migratory and invasive properties. Tumor-related genes such as NNMT (nicotinamide N-methyltransferase), FLI1 (friend leukemia integration 1), GAS6 (growth arrest–specific 6), lncRNA (long noncoding RNA), CCAT1 (colon cancer–associated transcript 1), PDCD1LG2 (programmed cell death 1 ligand 2), and CD274 (cluster of differentiation 274) may be considered the key regulators in tumor-like transformation in response to long-time exposure of *P. gingivalis* (Geng et al. 2017).

Epithelial-mesenchymal transition (EMT) is potentially involved in increasing metastasis OSCC. Heat-killed *F. nucleatum* and *P. gingivalis* cause significant upregulation of transcription of mesenchymal markers and downregulation of epithelial ones. This downregulation also results in a decrease in impedance resistance. These taxa also increase levels of several cytokines, which could be involved in EMT induction and Snail activation and the rate of migration and wound closure (Abdulkareem et al. 2018). *P. gingivalis* also induces expression and nuclear localization of the ZEB1 (zinc finger E-box-binding homeobox 1) transcription factor, which controls EMT. ZEB1 levels correlate with an increase in expression of mesenchymal markers, including vimentin and MMP-9 and with enhanced migration of epithelial cells, both of which are prevented with small interfering RNA (siRNA) knockdown of ZEB1. *P. gingivalis* strains lacking the FimA fimbrial protein have had less ability to induce ZEB1 expression. Interestingly, oral infection of mice by *P. gingivalis* increases ZEB1 levels in gingival tissues, and intracellular *P. gingivalis* has been detected by antibody staining in biopsy samples from OSCC, indicating that FimA-driven ZEB1 expression can provide a mechanistic basis for a *P. gingivalis* contribution to OSCC (Sztukowska et al. 2016). *P. gingivalis* can also upregulate expression of ZEB2, a transcription factor that controls EMT and inflammatory responses, via pathways involving β-catenin and FOXO1 (forkhead box O1) (Ohshima et al. 2019). Interestingly, *Streptococcus gordonii* antagonizes ZEB1 and ZEB2 expression. Mechanistically, *S. gordonii* suppresses FOXO1 by activating the TAK1 (transforming growth factor β–activated kinase 1)–NLK (nemo-like kinase negative regulatory pathway). Thus, EMT may be induced in response to periodontal pathogen stimulation.
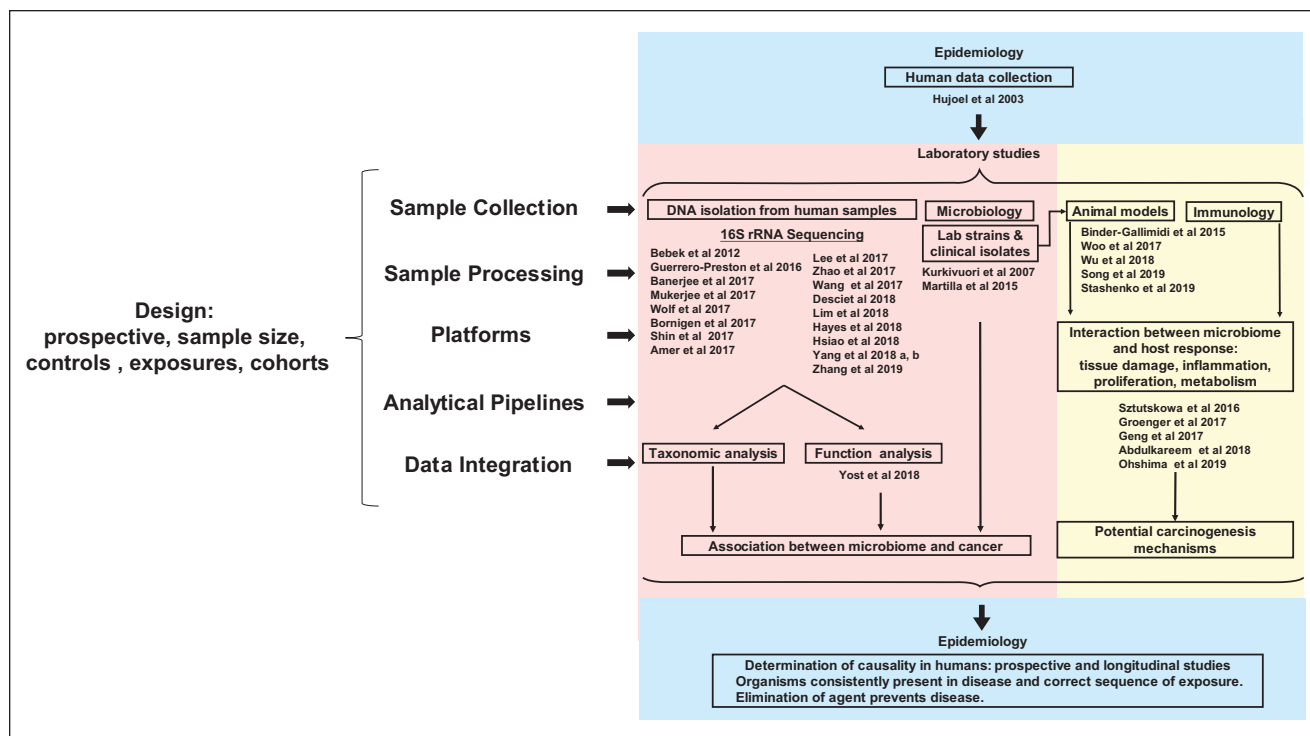
Figure 3. Framework for studying etiology of microbiome-driven carcinogenesis. To better understand the impact of the microbiome on carcinogenesis in humans, assembly of adequately sized human populations for study is needed. DNA from the human tissue or other samples can then be extracted and sequenced for taxonomic and functional analyses of the microbiome. In parallel, culturing of isolated microbes, studies in animal models, and profiling of the immune responses to potential carcinogenic microbes will allow for the discovery of microbiome-associated mechanisms driving carcinogenesis. From what is discovered in the laboratory, prospective and longitudinal human studies will be required to confirm the causal effects of the microbiome on human cancers. Critical study approaches include, for example, studies to confirm that the person is exposed to the microbe of interest prior to disease onset and approaches to prevent disease such as through vaccination against the implicated microbe. Current evidences have been added into this framework (adapted from Chen et al. 2017).

Periodontal Bacteria-Mediated Changes: Animal Models

P. gingivalis and *F. nucleatum* increased tumor severity in mice under a chemical carcinogen protocol (4NQO). Tumor invasion properties, tumor area, and cyclin D1–positive cells were enhanced in mice receiving 4NQO along with the inoculation of *P. gingivalis* and *F. nucleatum* (Binder Gallimidi et al. 2015; Wu, Zheng, et al. 2018). Tumor xenografts composed of *P. gingivalis*–infected OSCC cells demonstrated higher resistance to Taxol through Notch1 activation than uninfected cells. *P. gingivalis*–infected OSCC cells formed more metastatic foci in the lung than uninfected cells (Woo et al. 2017). Similarly, mice chronically administered *P. gingivalis* showed higher serum levels of IL-6 than uninfected mice and increased resistance to paclitaxel, which was increased when the mice were administered ibuprofen, supporting the modulatory effect of periodontal pathogen–induced inflammation in chemoresistance (Song et al. 2019).

The inoculation of gnotobiotic mice with microbiome inocula from healthy mice and from mice bearing a 4NQO-induced tumor shows that mice colonized with different oral microbiomes and exposed to 4NQO had increased tumor numbers and sizes compared to those lacking a microbiome. Specific

microbial patterns emerged during OSCC progression. While the percentage of *Parabacteroides* bacteria decreased in the control group, it increased in the OSCC group, and the opposite was observed for *Corynebacterium*. Metatranscriptomic analysis revealed overexpression of the same metabolic signatures associated with OSCC regardless of the community profile, including nitrogen transport, response to stress, interspecies interactions, Wnt pathway modulation, and amino acid and lipid biosynthesis. Thus, certain collective physiological activities are critical for microbiome-mediated OSCC progression (Stashenko et al. 2019). By comparison, in humans, *Fusobacteria* showed a significantly higher number of transcripts at tumor sites and tumor-adjacent sites of cancer patients compared to the healthy controls analyzed. Regardless of the community composition, specific metabolic signatures were consistently found in disease. Activities such as iron ion transport, tryptophanase activity, peptidase activities, and superoxide dismutase were overrepresented in tumor and tumor-adjacent samples when compared to the healthy controls. The expression of putative virulence factors in the oral communities associated with OSCC showed that activities related to capsule biosynthesis, flagellum synthesis and assembly, chemotaxis, iron transport, hemolysins, and adhesins were upregulated at tumor sites. Moreover, activities associated with

protection against reactive nitrogen intermediates, chemotaxis, and flagellar and capsule biosynthesis were also upregulated in nontumor sites of cancer patients. *Fusobacteria* may be the leading phylogenetic group responsible for the increase in expression of virulence actors in the oral microbiome of OSCC patients (Yost et al. 2018).

Concluding Remarks and Future Directions

Associations between the oral microbiome and colorectal, lung, pancreatic, and head and neck cancer are frequent but of modest magnitude. The literature on mechanistic contributions of the oral microbiome to carcinogenesis is rather provocative but inconclusive. Thus, there is a need to definitively determine the role of bacteria as promoters or passengers in the tumorigenesis process. Published studies have limitations, including relying on self-reported history of periodontal diseases or their surrogate measures (gingival bleeding, tooth mobility, tooth loss). They are often case control or retrospective, which cannot establish temporality, a crucial criterion for determining causality. A framework for the study of the oral microbiome carcinogenic potential is presented in Figure 3 (Chen et al. 2017). Large prospective cohort studies, including collection of clinical data and blood, tissue, and microbial samples longitudinally, are needed. Sample collection, processing, and analytical platforms should be standardized and properly selected. Assessment of the host genetics, inflammatory response, their pan-microbiome (viruses, phages, eukaryotes), exposures (HPV, diet), and their interactions must be considered. Then, findings from human studies should be explored in murine models of cancer development. Only once those steps are taken into consideration will it be possible to realize the potential of the oral microbiome to contribute to cancer development and to be used as a biomarker for progression, recurrence, and as a therapeutic target.

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