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Emerging role of bacteria in oral carcinogenesis: a review with special reference to perio-pathogenic bacteria

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Oral cancer, primarily oral squamous cell carcinoma (OSCC), continues to be a major global health problem with high incidence and low survival rates. While the major risk factors for this malignancy, mostly lifestyle related, have been identified, around 15% of oral cancer cases remain unexplained. In light of evidence implicating bacteria in the aetiology of some cancer types, several epidemiological studies have been conducted in the last decade, employing methodologies ranging from traditional culture techniques to 16S rRNA metagenomics, to assess the possible role of bacteria in OSCC. While these studies have demonstrated differences in microbial composition between cancerous and healthy tissues, they have failed to agree on specific bacteria or patterns of oral microbial dysbiosis to implicate in OSCC. On the contrary, some oral taxa, particularly Porphyromonas gingivalis and Fusobacterium nucleatum, show strong oral carcinogenic potential in vitro and in animal studies. Bacteria are thought to contribute to oral carcinogenesis via inhibition of apoptosis, activation of cell proliferation, promotion of cellular invasion, induction of chronic inflammation, and production of carcinogens. This narrative review provides a critical analysis of and an update on the association between bacteria and oral carcinogenesis and the possible mechanisms underlying it.

Keywords: bacteria; carcinoma; dysbiosis; inflammation; microbiome; mouth; squamous cell

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Oral cancer encompasses malignant neoplasms, predominantly oral squamous cell carcinoma (OSCC), evolving from the lining mucosae of the lips and the mouth (oral cavity) including the anterior two-thirds of the tongue as defined by the International Classification of Disease (1). Along with oropharyngeal cancer, it constitutes an important global public health problem, ranking the eighth most common cancer in the world. In 2012, an estimated 300,400 new cases of and 145,400 deaths from oral cancer were reported (2). Two-thirds of all cases are recorded in developing countries; the Indian subcontinent alone accounts for one-third of the global burden (2). Oral cancer has a predilection for males and socially disadvantaged population groups in developing countries as well as minority ethnic groups in developed countries (1, 2). Although the oral cavity is accessible to direct examination, oral cancer is usually detected at late stages (3). Despite advances in surgery, radiation and chemotherapy, the 5-year survival rates remain below 50%, one of the lowest for major cancers (4, 5). Furthermore, patients who survive a first cancer of the oral cavity are at very high risk of developing a recurrent or second primary oral cancer (4, 6).

Risk factors for oral cancer

Oral cancer is of multifactorial origin; several risk factors act individually or in combinations in the pathogenesis of the disease, and the mix varies from one population to another. While major risk factors for OSCC are extensive sun exposure, tobacco use, and alcohol consumption (3), other factors such as use of betel quid, betel quid with areca nut, and areca nut have been reported as significant risk factors in many studies (6-8). Studies have consistently shown that patients with OSCC have higher plaque index, gingival index, and bleeding index compared to healthy controls (9-11). The presence of a high concentration of bacteria, which are associated with OSCC, in biofilms on the tongue has also been documented (9, 12). Additionally, accumulating evidence implicates a dysbiotic oral microbiome in the development and progression of OSCC (13).

1/ Both authors contributed equally to the manuscript.
another. The major risk factors of OSCC, the most extensively studied, include smoking of cigarettes, cigars, beedies and pipes, smokeless tobacco usage, betel quid (with or without tobacco) chewing, other forms of areca nut usage, heavy alcohol consumption and human papillomavirus (HPV) infection (7–10). Tobacco smoking and alcohol consumption alone account for a population attributable risk of 74% (11). Viral infections particularly explain the elevated incidence of cancer among the young population (12, 13) and non-smoking females (14). Genetic susceptibility of the individual arising from polymorphisms in carcinogen metabolising enzymes and DNA repair mechanisms (15–17), often in a background of dietary micronutrient deficiencies, is another factor associated with oral carcinogenesis (13). Lesser known risk factors include exposure to excessive solar radiation/UV light, sulphur dioxide, pesticides, mists from strong inorganic acids and burning of fossil fuels (18, 19). Periodontitis has also been shown in several studies to be associated with increased risk of OSCC (20), with a possible underlying mechanism being long-term chronic inflammation (21).

Can bacteria be a risk factor of oral cancer?
Approximately 15% of oral cancers cannot be explained by the aforementioned major risk factors (22), which has evoked the need to explore for other potential risk factors. In the last two decades, significant evidence has emerged implicating bacteria in the aetiology of some cancer types such as Helicobacter pylori in gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphomas (23), Chlamydia trachomatis in cervical cancer (24), Salmonella typhi in gallbladder cancer (25) and both Bacteroides fragilis and Fusobacterium nucleatum in colon cancer (26, 27). This has recently inspired and triggered a considerable amount of research into the possible role of bacteria in oral oncogenesis, which has indeed provided some evidence to support such a role. Relevant work has been already reviewed in a number of publications (28–30). However, the overwhelmingly growing literature keeps calling for new, updated reviews.

Therefore, this narrative review aims to dissect existing literature on the association between bacteria and oral cancer and to provide an in-depth update about the underlying mechanisms, with particular focus on the work done on Porphyromonas gingivalis and F. nucleatum.

The healthy oral microbiome
Bacteria living within the body of an average healthy adult human outnumber the human cells by at least 10-folds (31, 32). Furthermore, the collective genome of these bacteria (metagenome) surpasses the human genome by orders of magnitude (> 150-folds in terms of gene content) (33, 34). Therefore, humans can be thought of as ‘Super-organisms whose metabolism represents an amalgamation of microbial and human attributes’ (35). The ‘human microbiome’ is a term used to define all microorganisms in the human body and their collective genomes (36). Microbial communities found in the different body habitats (e.g. upper respiratory tract, mouth, skin, gut and urogenital tract) contribute to the overall microbiome.

The oral cavity has several distinct niches that provide unique conditions and nutrients for populating microbes, predominately bacteria (37). Each niche (e.g. hard palate, soft palate, lateral and dorsal surfaces of the tongue, and tooth surfaces above and below the gingival margin) displays site specificity and distinct bacterial profile (38). Once established, the oral microbial communities maintain a stable composition ‘microbial homeostasis’ and exhibit commensal and mutualistic relation with the host (39). The host provides its microbial communities with an environment where they can flourish; in return, microbes protect the host as they colonise specific surfaces and prevent adherence and/or hinder growth of pathogenic bacteria (40).

The human mouth harbours one of the most diverse microbiota; at least 687 species/phylotypes have been identified, for each of which a reference 16S rRNA sequence is available in the Human Oral Microbiome Database (HOMD 14.5; www.homd.org) (41). These species belong to 185 genera and 12 phyla, namely Firmicutes, Fusobacteria, Proteobacteria, Actinobacteria, Bacteroidetes, Chlamydiae, Chloroflexi, Spirochaetes, SR1, Synergistetes, Saccharibacteria (TM7) and Gracilibacteria (GN02). Thirty-two percent of these species remain uncultivated and 14% have not been named.

Prior to 2000, the composition of the oral microbiome had been assessed via culture methods or close-ended molecular techniques, mainly checkerboard DNA–DNA hybridisation and polymerase chain reaction (PCR). After 2000, clonal analysis of 16S rRNA gene with Sanger sequencing was employed to identify uncultivable species in oral samples and assess diversity of the oral microbiota (42). Recently, the advent of high throughput, next-generation sequencing (NGS) has enabled analysis of the microbial communities at unprecedented depth and breadth, providing unmatched opportunity for profiling the oral microbiome in health and identifying microbial shifts associated with disease (43).

Oral microbiome dysbiosis and disease
Under certain circumstances, the homeostatic state of oral bacterial flora can be lost, resulting in an ‘ecological shift’ or ‘dysbiosis’ characterised by increased abundance of pathogenic bacteria and expression of virulence properties (39, 40). Subsequently, these pathogens become capable of causing diseases within and beyond the oral cavity, reverting the oral microbiota relationship with the host from mutualistic to parasitic (44). A consortium of microbes rather than one species is usually involved in
causing disease (45). Classic examples of oral microbial dysbiosis include dental caries and periodontal infections. In caries, the ecological shift favours growth of acidogenic and aciduric species, namely mutans streptococci, lactobacilli and Bifidobacteria (46). In periodontal diseases, proteolytic bacteria that challenge the host inflammatory response are in play (47). The leading bacteria at periodontal destruction sites include members of the so-called red complex, namely P. gingivalis, Tannerella forsythia and Treponema denticola (48), as well as a number of new taxa such as oral Synergistetes and Saccharibacteria (TM7) (49). Currently, there is increasing interest in the possible role of microbial dysbiosis in cancers.

**Oral microbial dysbiosis in oral cancer**

The relationship between bacterial profiles and OSCC has been thoroughly studied (Table 1). In the first association study, Nagy et al. (50) performed culture-based analysis of surface swabs and found the levels of Porphyromonas spp., Fusobacterium spp. and other bacterial species to be significantly higher on OSCC tissue compared with adjacent healthy mucosa. In a subsequent study using immunohistochemistry for detection of P. gingivalis, Katz et al. (51) found that sections of gingival squamous cell carcinoma displayed higher staining intensity than those of healthy gingival tissue samples, indicating a higher colonisation of cancerous tissues by this bacterium. Interestingly and consistent with the above findings, both P. gingivalis and F. nucleatum have been linked recently to pancreatic and colorectal cancers (CRC), respectively (26, 52–54).

**Streptococcus anginosus** has also been implicated in OSCC. In one study, *S. anginosus* was detected by PCR in all head and neck squamous cell carcinoma, including OSCC, samples studied (55). Consistently, Sasaki et al. (56) detected *S. anginosus* in 19/42 (45%) OSCC samples, but in none of the leukoplakia, lymphoma or rhabdomyosarcoma samples tested. However, Morita et al. (57) identified *S. anginosus* in only 5/38 (13%) of their OSCC samples. In addition, more recent studies have identified *S. anginosus* in non-tumorous oral tissue at equal and even higher frequency compared with tissue from the tumours, suggesting that *S. anginosus* is a normal coloniser of the oral mucosa (58, 59).

Mager et al. (60) studied the differences in salivary counts of 40 common oral bacteria between 45 OSCC cases and 229 cancer-free controls using checkerboard DNA–DNA hybridisation. They found Capnocytophaga gingivalis, Prevotella melaninogenica and *S. mitis* to be significantly elevated in the saliva of cases. When used as diagnostic markers, these three bacterial species were found to differentiate between cases and controls with 80% sensitivity and 83% specificity. However, these results have not been replicated in any subsequent study.

In 2006, Hooper et al. (61), using culture methods, isolated 80 bacterial species from within the tissue of 20 OSCC biopsies. Further analysis of 10 specimens using 16S rRNA Sanger sequencing identified an additional 28 bacterial species (59). These studies provided some evidence for tumour specificity of bacteria, as some species were exclusively found in either the cancerous or non-cancerous tissues. However, the majority of bacteria identified in the tumours were saccharolytic and aciduric (Table 1), suggesting a selection process by the tumour microenvironment rather than a potential association with carcinogenesis. In 2012, Pushalkar et al. (58), also using 16S rRNA Sanger sequencing, detected 80 bacterial species in 10 specimens of OSCC, 35 species of which reported for the first time in OSCC, thus expanding the bacterial diversity within OSCC tissues to 140 species. In this study, however, a totally different panel of species was found to be associated with the tumours (Table 1).

A caveat of conventional culture methods and Sanger sequencing is low analysis depth, that is, the limited number of strains/clones that can be affordably analysed, which hampers reproducible detection of possibly relevant species, particularly those with relatively low abundance. This limitation has been surpassed by the advent of NGS, which allows the study of microbial communities at unprecedented depth and breadth (43). To date, three studies have employed NGS to characterise the oral microbiome associated OSCC. In one study, Pushalkar et al. (62) identified in salivary samples from three OSCC cases and two healthy controls 860 operational taxonomic units (OTUs) matching known species; 244 and 398 of these were exclusively present in the OSCC and control samples, respectively. However, the comparison between the two groups was limited to the genus level; the genera *Streptococcus, Rothia, Gemella, Peptostreptococcus, Porphyromonas, Micromonas and Lactobacillus* were found to be more abundant in the OSCC cases, whereas *Prevotella, Netisseria, Leptotrichia, Capnocytophaga, Actinobacillus* and *Oribacterium* were higher in the controls. In a larger-scale study, Schmidt et al. (63) analysed swabs of lesion surface and contra-lateral normal mucosa from 18 OSCC patients, eight pre-cancer cases and nine healthy controls. In contrast to the study by Pushalkar et al., the abundance of genera *Streptococcus* and *Rothia* was significantly lower in the tumour samples compared with contra-lateral normal as well as to the pre-cancer samples; instead, the tumours were associated with significantly higher proportions of the genus *Fusobacterium*. In addition, the phylum Bacteroidetes was notably more abundant in both cancerous and normal tissues of OSCC patients compared with pre-cancer and healthy control subjects, suggesting that higher colonisation with this phylum may be associated with increased risk of OSCC, and thus serve as a biomarker.
Table 1. Summary of epidemiological studies that assessed the association between bacteria and oral cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Technology used</th>
<th>Case sample</th>
<th>Control sample</th>
<th>Taxa associated with oral cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nagy et al., 1998 (50)</td>
<td>21</td>
<td>Cultivation; biochemical identification</td>
<td>Tumour surface swabs</td>
<td>Contagious mucosa surface swabs</td>
<td>Fusobacterium, Porphyromonas, Actinomyces, Propionibacterium spp. and Candida albicans</td>
</tr>
<tr>
<td>Katz et al., 2011 (51)</td>
<td>15</td>
<td>Immunohistochemical staining</td>
<td>FFPE gingival carcinoma tissue</td>
<td>FFPE normal tissue</td>
<td>Porphyromonas gingivalis</td>
</tr>
<tr>
<td>Tateda et al., 2000 (55)</td>
<td>270</td>
<td>Cultivation, PCR and Southern-blot</td>
<td>Tumour tissue, gingival smears &amp; oropharyngeal swabs</td>
<td>None</td>
<td>Streptococcus anginosus</td>
</tr>
<tr>
<td>Sasaki et al., 2005 (56)</td>
<td>49</td>
<td>PCR</td>
<td>Fresh tumour tissue, dental plaque &amp; saliva</td>
<td>Fresh tissue, leukoplakia, lymphoma &amp; rhabdomyosarcoma</td>
<td>S. anginosus</td>
</tr>
<tr>
<td>Morita et al., 2003 (57)</td>
<td>63</td>
<td>Real-time PCR</td>
<td>Fresh tumour tissue</td>
<td>Fresh non-cancerous tissue</td>
<td>Association with S. anginosus ruled out</td>
</tr>
<tr>
<td>Mager et al., 2005 (60)</td>
<td>274</td>
<td>Checkerboard DNA–DNA hybridisation</td>
<td>Unstimulated saliva</td>
<td>Unstimulated saliva</td>
<td>Capnocytophaga gingivalis, Prevotella melaninogenica and S. mitis</td>
</tr>
<tr>
<td>Hooper et al., 2006 (61)</td>
<td>51</td>
<td>Cultivation; 16S rRNA gene sequencing</td>
<td>Fresh tumour tissue</td>
<td>Fresh contagious tissue</td>
<td>Micrococcus luteus, P. melaninogenica, Exiguobacterium oxidotolerans, Fusobacterium naviforme, Staphylococcus aureus and Veillonella parvula</td>
</tr>
<tr>
<td>Hooper et al., 2007 (59)</td>
<td>20</td>
<td>16S rRNA metagenomics (Sanger sequencing)</td>
<td>Fresh tumour tissue</td>
<td>Fresh contagious tissue</td>
<td>Ralstonia insidiosa, Fusobacterium naviforme, Peptostreptococcus micros, Clavibacter michiganensis subsp. tesselarius, Capnocytophaga sp. oral strain S3 and Prevotella sp. oral clone BED73</td>
</tr>
<tr>
<td>Pushalkar et al., 2012 (58)</td>
<td>20</td>
<td>16S rRNA metagenomics (Sanger sequencing) &amp; DGGE</td>
<td>Fresh tumour tissue</td>
<td>Fresh contagious tissue</td>
<td>Parvimonas sp. oral taxon 110, Eubacterium infirmum, Eubacterium brachy, Gemella haemolysans, Gemella morbillorum, Gemella sanguinis, Johnsonella ignava, Peptostreptococcus stomatis, S. gordonii, S. parasanguinis I and S. salivarius</td>
</tr>
<tr>
<td>Pushalkar et al., 2011 (62)</td>
<td>05</td>
<td>16S rRNA metagenomics (NGS; Roche’s 454) &amp; DGGE</td>
<td>Stimulated saliva</td>
<td>Stimulated saliva</td>
<td>Genera Streptococcus, Rothia, Gemella, Peptostreptococcus, Lactobacillus, Micromonas and Porphyromonas</td>
</tr>
<tr>
<td>Schmidt et al., 2014 (63)</td>
<td>94</td>
<td>16S rRNA metagenomics (NGS; Illumina)</td>
<td>Tumour surface swabs</td>
<td>Surface swabs: contra-lateral normal; healthy and pre-cancer subjects</td>
<td>Genus Fusobacterium and phylum Bacteroidetes (Streptococcus and Rothia showed inverse association)</td>
</tr>
<tr>
<td>Al-Hebshi et al., 2015 (64)</td>
<td>03</td>
<td>16S rRNA metagenomics (NGS; Roche’s 454)</td>
<td>Fresh tumour tissue</td>
<td>None</td>
<td>Bacteroides fragilis</td>
</tr>
</tbody>
</table>

PCR: polymerase chain reaction; FFPE: formalin-fixed, paraffin-embedded; DGGE: denaturing gradient gel electrophoresis; NGS: next-generation sequencing.
A limitation to the studies by Pushalkar et al. (62) and Schmidt et al. (63), as the case with most oral microbiome studies employing NGS, was the low taxonomic resolution, that is, failure to reliably classify NGS sequences beyond the genus level, which hinders accurate assessment of the possible association between bacteria and oral cancer. This limitation been overcome in a third study by Al-hebshi et al. (64) utilising a novel bioinformatic algorithm that exploits well-curated databases of 16S rRNA reference sequences, including the HOMD, for the classification of individual NGS reads to the species level. By applying the algorithm to three samples of OSCC DNA, the study revealed the presence of 228 bacterial species, of which 35 species were present in all samples. Two of the samples contained \textit{B. fragilis}, a bacterium seldom detected in the oral cavity. Interestingly, six proteins from this species had been identified in saliva of OSCC in an earlier study (65). Furthermore, \textit{B. fragilis} has been recently implicated in colon cancer (27). Together, these findings are suggestive of a role of \textit{B. fragilis} in oral carcinogenesis, a possibility worth further investigation.

It is evident, therefore, that there is currently no consensus among studies on which bacterial species are linked to oral cancer. In addition, due to the cross-sectional nature of these studies, it is not possible to tell whether any microbial dysbiosis identified is involved in the aetiology of oral cancer or just a consequence of it. Nevertheless, and despite the lack of strong epidemiological evidence to support a role for \textit{P. gingivalis} and \textit{F. nucleatum} in oral cancer, the carcinogenic properties of these two species, particularly \textit{P. gingivalis}, have been extensively demonstrated in vitro and in experimental animals as detailed in the section below.

Possible mechanisms by which oral bacteria contribute to oral carcinogenesis

Oral bacteria possibly contribute to oral carcinogenesis via a number of mechanisms including inhibition of apoptosis, activation of cell proliferation, promotion of cellular invasion, induction of chronic inflammation and production of carcinogens (Fig. 1).

Inhibition of apoptosis

\textit{P. gingivalis} represses chemically induced apoptosis in primary cultures of gingival epithelial cells (GECs) (66), which seems to be mediated by various mechanisms. \textit{P. gingivalis} stimulates JAK1/STAT3 and PI3K/Akt signalling, which controls intrinsic mitochondrial apoptosis pathways (67, 68). At the mitochondrial membrane, the activity of proapoptotic BAD (BCL-2-associated death promoter) is suppressed resulting in elevated BCL2 (antiapoptotic): BAX (proapoptotic) ratio, which in turn deceases the release of the apoptosis effector cytochrome c (69). Downstream, activity of both caspase-9 and the executioner caspase-3 is blocked (68, 69). In a different mechanism, \textit{P. gingivalis} has been shown to upregulate microRNA-203 in GECs, which through downregulation

\textbf{Fig. 1.} The possible mechanisms by which oral bacteria contribute to oral carcinogenesis. ROS, reactive oxygen species; RNI, reactive nitrogen intermediates, MMPs, matrix metalloproteinases; PAR, protease-associated receptor; EMT, epithelial to mesenchymal transition.

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of SOCS3 (suppressor of cytokine signalling 3) increases the activity of STAT3 (signal transducer and activator of transcription 3) and, in turn, inhibits apoptosis (70). Furthermore, P. gingivalis secretes a nucleoside diphosphate kinase (NDK), which prevents ATP-dependent apoptosis mediated through purinergic receptor P2X7 on GECs (71); NDK might also interfere with an anticancer immune response mediated by ATP activation of P2X7 receptors on dendritic cells (29, 72). In 2015, Binder Gallimidi et al. (73) reported that chronic coinfection with P. gingivalis and F. nucleatum promotes progression of chemically induced oral cancer in a murine model via activation of the IL6/STAT3 axis.

Activation of cell proliferation
In addition to its anti-apoptotic properties, P. gingivalis accelerates progression of GECs through the S and G2 phases of the cell cycle via upregulation of cyclins (A, D and E), activation (phosphorylation) of cyclin-dependent kinases (CDKs) and diminishing the level of p53 tumour suppressor (74, 75). These effects are dependent on the possession of fimbriae (FimA adhesin). Since bacterial lipopolysaccharide (LPS) has been reported to dysregulate p53, a similar role by P. gingivalis LPS is possible (29). P. gingivalis may also contribute to a proliferative phenotype in GECs through activation of β-catenin via a gingipain-dependent proteolytic process (76).

F. nucleatum also promotes cell proliferation. In human epithelial cells, infection by F. nucleatum results in upregulation of 12 kinases, the majority of which are involved in cell proliferation and cell survival signalling as well as DNA repair (77). A strong relationship between F. nucleatum and CRC is particularly evident. In vitro, fusobacterial adhesion FadA has been found to bind to E-cadherin on CRC cells and in turn activate the β-catenin signalling pathway (78). Downstream, this results in elevated transcriptional activity of oncogenes and pro-inflammatory cytokines, and subsequently enhanced CRC cell proliferation. Consistently, expression levels of the FadA gene in colon tissue from patients with CRC have been shown to be >10-fold higher than those in normal individuals (78).

Promoting cellular migration and invasion
Both P. gingivalis and F. nucleatum promote cellular invasion in OSCC. Using an OSCC cell line, P. gingivalis infection was demonstrated to upregulate expression of pro-matrix metalloproteinase-9 (pro-MMP-9) by the activation of the ERK1/2-ETS1, p38/HSP27 and PAR/NF-κB pathways; gingipains then cleave the proenzyme into active MMP-9, enhancing cellular invasion (79, 80). Repeated exposure to P. gingivalis can also increase invasiveness of OSCC cells by triggering epithelial to mesenchymal transition (EMT), acquisition of stemness and enhanced production of MMP-1 and MMP-10 (81). In a similar fashion, the infection of human epithelial cells by F. nucleatum increases the production of MMP-13 (collagenase 3) through the activation of mitogen-activated protein kinase p38 and promotes cellular migration, possibly via stimulation of Etk/BMX, S6 kinase p70 and RhoA kinase (77).

Induction of inflammation
Chronic inflammation, triggered by infections or environmental exposures, plays a pivotal role in all stages of carcinogenesis including induction, progression, invasion and metastasis (82). Reactive oxygen species (ROS), reactive nitrogen intermediates (RNI) and cytokines produced by inflammatory cells are believed to contribute to initiation of cancer by inducing mutations, genomic instability and epigenetic alterations. Inflammatory cytokines then activate key transcription factors such as STAT3 and NF-κB within the premalignant cells; this in turn supports pro-malignant processes including proliferation, angiogenesis, and invasion and metastasis, and most importantly, results in a sustained tumour-promoting inflammation within the tumour microenvironment (82, 83).

Chronic inflammation has, therefore, been anticipated as one of the potential pathways by which bacteria contribute to oral carcinogenesis (30). Indeed, this provides a plausible explanation for the strong association between periodontitis and higher risk of OSCC (20). A pro-inflammatoryatory potential is documented for some oral bacterial species. F. nucleatum has been found to be associated with high cytokine levels in CRC and to create an inflammatory microenvironment supportive of tumour progression (84, 85). In GECs as well as OSCC cell lines, P. gingivalis upregulates B7-H1 and B7-DC receptors, both of which are known to contribute to chronic inflammation (72). Increased production of IL-1, IL-6, IL-8 and TNF-α in response to P. gingivalis infection has been documented in engineered human oral mucosa (86).

Similar pro-inflammatory properties have been reported for other oral bacteria including Eikenella corrodens, S. anginosus and S. mitis (87, 88).

Production of carcinogens
Ethanol itself is not a carcinogen but its metabolites acetaldehyde, hydroxyl ethyl radicals and hydroxyl radicals are carcinogenic (89, 90). The International Agency for Research on Cancer classified acetaldehyde associated with alcohol consumption as a Group 1 carcinogen in humans, with the capability to cause sister chromatid exchanges, point mutations, DNA adducts and hyperproliferation of epithelium (8, 91). It is well established that certain bacteria and Candida spp. in the oral cavity possess the enzyme alcohol dehydrogenase (ADH), which catalyses the production of mutagenic amounts of acetaldehyde under aerobic or microaerophilic conditions (30, 92–94). Examples of such oral bacteria include
S. salivarius, S. intermedius, S. mitis (95) and non-pathogenic Neisseria spp. (96).

**Conclusion and future directions**

There is an increased interest in the potential role of bacteria in oral cancer as evident from the increasing number of publications addressing the topic. However, published studies disagree on which specific bacteria or patterns of oral microbial dysbiosis to associate with oral cancer. This is probably due to the significant methodological variation between studies with respect to sampling (e.g. deep tissue biopsy vs. surface swab), selection of control tissues and microbial profiling technology. 16S rRNA metagenomics with NGS has great potential in exploring the oral microbiome shifts associated with oral cancer, but currently lacks adequate standardisation, particularly in terms of DNA extraction, selection of primers and hypervariable region for amplification, and bioinformatics. In addition, 16S rRNA microbial profiling does not inform about microbial gene expression and microbial function. Therefore, future work should, in addition to methodological standardisation, embody full metagenomics or metatranscriptomics analysis into case-control studies to fully explain the role of the oral microbiome in oral cancer and generate information of translational value, for example, identify diagnostic and prognostic markers.

The oral carcinogenic properties of P. gingivalis and F. nucleatum are well documented in vitro as well as in experimental animals; many of the mechanisms underlying these have been elucidated. It has been suggested, therefore, that the detection of P. gingivalis and F. nucleatum in oral potentially malignant conditions could be used as a marker of risk of malignant transformation. Virulence factors such as FimA of P. gingivalis and FadA of F. nucleatum may also serve as novel targets for therapeutic intervention of oral cancer. However, it is important to note that a strong epidemiological evidence for the association of P. gingivalis and F. nucleatum with oral cancer is still lacking. Large-scale, well-designed longitudinal studies that involve follow-up of microbiota associated with precancerous lesions while adequately control for the confounding effects of established risk factors are required to demonstrate if these two or other species contribute or even have an exclusive role in oral carcinogenesis in a subset of patients. Such an approach will also help resolve the legitimate hen and egg question: which species, if any, is involved in carcinogenesis (driver) and which is simply an adapter to the cancer tissue (passenger)?

There are a number of well-established risk factors for oral cancers and a presumed multifactorial aetiology. The balance of risk factors varies from individual to individual. In the age of personalised medicine, it is important to isolate causes and the molecular damage caused, in order to plan personalised therapy. Further work on the oral bacteriome associated with oral cancers has an exciting future and can generate hypotheses for exploring mechanisms in cell and animal models.

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