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New advances in oral microbiology and tumor research

Oral microbiology in tumors

Abstract

Cancer remains a leading global health concern, with increasing incidence and mortality rates highlighting the urgent need for novel diagnostic and therapeutic strategies. Emerging evidence identifies the oral microbiota as a critical factor in tumorigenesis, offering new insights into cancer pathogenesis beyond traditional risk factors such as smoking and genetic predisposition. This review summarizes recent advancements in elucidating the intricate relationship between the oral microbiota and various malignancies, particularly oral squamous cell carcinoma, esophageal adenocarcinoma, and pancreatic ductal adenocarcinoma. Pathogenic bacteria, including *Porphyromonas gingivalis* and *Fusobacterium nucleatum*, are shown to promote tumor progression through mechanisms such as chronic inflammation, metabolic toxin production, and immune evasion. Dysbiosis of the oral microbiota, influenced by lifestyle factors including poor diet, tobacco use, and alcohol consumption, further exacerbates these carcinogenic processes. Innovative therapeutic approaches, such as probiotics, oral microbiota transplantation, and CRISPR-based bacterial editing, are being explored to restore microbial homeostasis and suppress pathogenic species. Additionally, saliva-based microbial biomarkers have demonstrated promise for non-invasive cancer screening, while the integration of multi-omics technologies and artificial intelligence (AI)-driven platforms is paving the way for precision oncology. This review aims to consolidate fragmented findings regarding the oral microbiota-cancer axis and to address the existing gaps in mechanistic

understanding. Its significance lies in translating microbial research into clinical applications, with the potential to reduce the global cancer burden through early detection and microbiota-targeted therapies.

Key Words: Oral microbiota; Tumorigenesis; Cancer; Microbial dysbiosis; Multi-omics integration; Precision medicine

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Core Tip: This review synthesizes recent progress in understanding the complex interactions between the oral microbiota and various cancers, particularly oral squamous cell carcinoma, esophageal adenocarcinoma, and pancreatic ductal adenocarcinoma. It highlights the roles of pathogenic bacteria such as *Porphyromonas gingivalis* and *Fusobacterium nucleatum* in promoting tumor progression via chronic inflammation, metabolic toxin production, and immune evasion. The review also explores innovative therapeutic strategies, including probiotics, oral microbiota transplantation, and CRISPR-based bacterial editing, while emphasizing the potential of saliva-based microbial biomarkers for non-invasive cancer screening. This work aims to translate microbial-related research into clinical applications, with the goal of mitigating the global cancer burden through early diagnosis and microbiome-targeted therapy.

INTRODUCTION

Cancer has become a major global health challenge in the 21st century, posing not only a substantial threat to public health and societal well-being but also imposing a significant economic burden. Among non-communicable diseases (NCDs), cancer is one of the leading causes of death, accounting for 22.8% of all NCD-related mortality[1]. According to global statistics from 2022, approximately 20 million new cancer cases and

nearly 10 million cancer-related deaths were estimated. The incidence and mortality of cancer continue to rise in parallel with demographic changes, with the number of new cancer cases projected to reach 35 million annually by 2050, reflecting a 77% increase compared to 2022 Levels[2].

Notably, the incidence of oral cavity-related malignancies has been increasing annually. Oral squamous cell carcinoma (OSCC), in particular, is strongly associated with various oral microorganisms, which are believed to play a crucial role in carcinogenesis and disease progression[3]. Despite advancements in cancer diagnosis and treatment in recent years, the overall five-year survival rate remains low, especially for patients diagnosed at advanced stages. For example, the five-year survival rate for lung cancer is below 20%, while that for pancreatic cancer is approximately 10%[1].

With continued societal development, shifts in human lifestyles and environments have led to increased exposure to various health risk factors. These include environmental pollution, unhealthy lifestyle choices, high occupational stress, and complex interpersonal dynamics, all of which may significantly threaten human health. The persistent upward trend in cancer incidence, combined with the progressively younger age of onset, underscores the urgent need for novel early diagnostic markers and therapeutic targets. Such developments aim to enhance the precision of disease prevention, diagnosis, and treatment, ultimately improving public health and quality of life. In recent years, microbiome research has introduced new perspectives for cancer prevention and therapy, with increasing attention focused on the role of the oral microbiota in carcinogenesis and cancer progression[4].

The human microbiota constitutes a complex ecosystem composed of trillions of microorganisms, predominantly residing in the oral cavity, gastrointestinal tract, skin, and reproductive tract[5]. The oral microbiota, the second most diverse microbial community in the human body, consists of over 700 bacterial species, along with fungi, viruses, and archaea[6]. These microorganisms are essential for maintaining the integrity of the oral mucosal barrier and for influencing systemic health through modulation of immune and metabolic functions[7]. Recent studies have revealed the

dualistic nature of the oral microbiota: It can support health-for instance, by generating nitric oxide (NO) via nitrate reduction, which benefits cardiovascular function[8]-but dysbiosis can contribute to various pathological conditions, including periodontal disease, cardiovascular disease, and cancer[4].

Traditional cancer risk factors such as smoking, alcohol consumption, HPV infection, and genetic predisposition have been extensively studied and are well-established contributors to carcinogenesis[3]. However, these factors do not fully explain the etiology of all cases. A significant number of OSCC patients, particularly those under the age of 45, lack a history of smoking or alcohol use[9]. Growing evidence suggest that oral microbiota dysregulation is closely linked to cancer development. For instance, pathogenic bacteria associated with periodontal disease-such as *Fusobacterium nucleatum* and *Porphyromonas gingivalis*-are significantly enriched in patients with OSCC and esophageal cancer[4].

Moreover, the oral microbiota may augment the carcinogenic effects of traditional risk factors, such as smoking and HPV infection[3], indicating its potential role as a mediator between conventional risk factors and tumorigenesis. Mechanistically, the oral microbiota contributes to tumor development through chronic inflammation, immunosuppression, metabolite toxicity, and genomic instability. For example, *Porphyromonas gingivalis* promotes tumor cell proliferation and invasion by activating the TLR4/NF-κB signaling pathway and inducing the release of pro-inflammatory cytokines such as IL-6 and TNF-α[10]. Similarly, *Fusobacterium nucleatum* facilitates the malignant transformation of epithelial cells by secreting FadA adhesin[3].

These mechanisms offer a theoretical basis for considering the oral microbiota as a potential diagnostic and therapeutic target. Specific microbial markers detected in saliva, such as *Fusobacterium nucleatum*, have been employed in the non-invasive screening of colorectal cancer[11]. In the future, microbiota-based precision medicine strategies-including probiotics and microbial transplantation-may provide novel avenues for cancer prevention and treatment[7].

COMPOSITION AND FUNCTION OF ORAL MICROORGANISMS

The healthy oral microbiota represents a highly diverse and complex ecosystem composing bacteria, fungi, viruses, and archaea, with bacteria constituting the predominant group[12]. The major bacterial phyla include Firmicutes (*e.g., Streptococcus*), Bacteroidetes (*e.g., Prevotella*), Actinobacteria (*e.g., Actinomyces*), and Proteobacteria (*e.g., Neisseria*)[13,14]. Notably, the distribution of microorganisms varies significantly across different oral sites. For instance, the dorsum of the tongue is predominated colonized by *Streptococcus*, whereas *Veillonella* and *Neisseria* are the principal genera found in saliva[15].

Oral microbial homeostasis

Oral microbial homeostasis plays a vital role in maintaining host health. Commensal oral microbiota contribute to pathogen suppression through mechanisms such as competitive exclusion and the production of antimicrobial metabolites[16]. Salivary lysozyme directly eliminates foreign pathogens, while hydrogen peroxide (H_2O_2) generated by symbiotic bacteria inhibits microbial proliferation[17]. Furthermore, the metabolic activities of oral microorganisms, including nitrate reduction, yield nitrite (NO_2^-) and NO, which exert protective effects on cardiovascular function[18].

Oral microbiota: Defense, disease, and therapeutic potential

The protective function of the oral microbiota encompasses both physical and chemical defense mechanisms. Multispecies biofilms enhance mucosal barrier integrity through tight intercellular adhesion[19]. In terms of immunomodulatory function, commensal bacteria such as *Streptococcus mitis* maintain immune homeostasis by promoting the secretion of anti-inflammatory cytokines, including interleukin-10 (IL-10), from monocytes[20]. Metabolically, oral microorganisms produce short-chain fatty acids (SCFAs), such as butyrate, via the fermentation of dietary fibers. These SCFAs can inhibit tumor cell proliferation[21]. In contrast, acetaldehyde-produced during

microbial metabolism-has demonstrated genotoxic properties and the capacity to induce DNA damage[22].

Although current studies underscore the critical roles of microbial homeostasis in barrier function, immune regulation, and metabolism, several mechanistic aspects remain poorly understood. For instance, the exact pathways through which *Fusobacterium nucleatum* disseminates and facilitates distal tumorigenesis require further investigation[23]. Future research should adopt multi-omics approaches (*e.g.*, metagenomics and metabolomics) to elucidate host-microbiota interactions and to develop microbiome-based precision therapeutic strategies, such as targeting antimicrobial peptides or probiotics. The strong inhibitory effect of *Mentha piperita* against *Streptococcus mutans* underscores the potential of natural compounds in modulating oral microbiota[24]. These advancements may provide novel approaches for the prevention and treatment of oral-associated diseases.

ORAL MICROBIOLOGY IN CANCER PATIENTS

Oral microbiology in patients with OSCC

OSCC is among the most prevalent malignant tumors of the oral cavity. Accumulating evidence suggests that the oral microbiota contributes to carcinogenesis, tumor progression, and metastasis via multiple mechanisms. A significant elevation in the abundance of *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Streptococcus mutans* has been observed in the oral microbiota of patients with OSCC[25]. *P. gingivalis* promotes proliferation, invasiveness, and tumorigenic potential in human immortalized oral epithelial cells[26]. *F. nucleatum* enhances tumor cell proliferation and invasion by activating the Wnt/β-catenin signaling pathway through binding of its FadA adhesin to E-cadherin[27]. Additionally, *S. mutans* metabolizes sugars to produce acetaldehyde, which may induce DNA damage and genomic instability, thereby contributing to oral carcinogenesis. However, the precise mechanisms and oncogenic impact of acetaldehyde produced by *Streptococcus* species, remain insufficiently elucidated and warrant further investigation[28].

Oral microbiology of esophageal cancer patients

The incidence of esophageal adenocarcinoma (EAC) has markedly increased in recent years, and its pathogenesis is closely associated with oral microbiota dysbiosis[29]. Similar to finding in OSCC, significantly elevated levels of *F. nucleatum* and *P. gingivalis* have been detected in the oral cavity of EAC patients[30]. Functionally, bacterial cell toxin (BCT) proteins produced by *Fusobacterium periodonticum* promote epithelial-mesenchymal transition in esophageal cancer cells *via* lactate-mediated pathways[31]. Furthermore, the presence of microbial communities in dental plaque and saliva has been identifed as a potential risk factor for esophageal cancer[30]. Periodontitis may predispose individuals to EAC by disrupting oral microbial homeostasis, which subsequently affects systemic health, including esophageal physiology[32]. Moreover, the oral microbiota may enhance EAC risk by amplifying the oncogenic effects of traditional risk factors such as tobacco use and alcohol consumption[30].

Oral microbiota in pancreatic cancer patients

Pancreatic cancer (PDAC) is characterized by high malignancy and poor prognosis, with a 5-year survival rate of only 11%[33]. Lipopolysaccharide (LPS) derived from *P. gingivalis* is recognized by Toll-like receptors 2 (TLR2) and 4 (TLR4), which are expressed in both endocrine and exocrine pancreatic tissues. NF-κB and MAPK signaling pathways induces the release of inflammatory mediators and promotes the expression of Reg3A/G genes. The overexpression of Reg3A/G genes is associated with increased islet cell proliferation and reduced apoptosis, thereby facilitating PDAC development and progression. Elevated Reg3A/G expression levels in PDAC tissues correlate with tumor aggressiveness, suggesting their utility as early diagnostic biomarkers. Interdisciplinary collaboration between dental and oncological disciplines may offer novel strategies for PDAC prevention and treatment[34].

A systematic review and meta-analysis conducted by Ma *et al.* revealed that *P.gingivalis* was significantly more prevalent in PDAC patients than in healthy

controls, whereas *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, and *Prevotella intermedia* showed no statistically significant differences in detection rates[33]. However, *A. actinomycetemcomitans* and *P. gingivalis* were found in significantly higher abundance in the gingival sulcus fluid of PDAC patients[34]. Furthermore, oral bacteria may contribute to pancreatic carcinogenesis by inducing mutations in the tumor suppressor gene TP53[35,36].

A summary of the oral microbiota associated with various cancer types is provided in Table 1. The oral microbiota plays a critical role in the pathogenesis of several cancers, including OSCC, EAC, and PDAC. While current studies emphasize the involvement of key pathogenic bacteria such as *P. gingivalis* and *F. nucleatum* in carcinogenesis, many mechanistic questions remain unanswered. The notable antimicrobial activity of *A. actinomycetemcomitans* highlights the potential for natural products to modulate the oral microbiota[34]. These insights may offer novel directions for tumor prevention and therapeutic intervention.

Association between head and neck squamous cell carcinoma and oral microbiota dysbiosis

Head and neck squamous cell carcinoma (HNSCC) is among the most prevalent malignancies in the head and neck region, and its pathogenesis is closely associated with dysbiosis of the oral microbiota. Recent studies have demonstrated that imbalances within the oral microbial community can result in the excessive proliferation of pathogenic bacteria, such as *Fusobacterium nucleatum* and *Porphyromonas gingivalis*. These microorganisms may contribute to the initiation and progression of HNSCC through mechanisms involving chronic inflammation and the release of genotoxic agents[37]. A microbial risk score, derived from the relative abundance of 22 bacterial species, has been shown to increase the risk of HNSCC by 50% for every standard deviation increment[37]. Moreover, specific microbial features, such as the increased abundance of *Parvimonas*, have been found to correlate with tumor stage and clinical prognosis[38]. Notably, oral microbiota dysbiosis may further exacerbate

immune suppression within the tumor microenvironment by modulating the epigenetic regulation of HPV infection, including alterations in DNA methylation patterns. These findings suggest that modulation of the oral microbiome may offer a promising therapeutic strategy for HPV-positive HNSCC[39].

Oral microbial characteristics and systemic effects in glioma

Glioma is a common malignant tumor of the central nervous system, and recent research has indicated a potential association between oral microbiota composition and glioma development. Significant differences in the abundance of certain bacterial genera, such as *Leptotrichia* and *Atopobium*, have been observed between glioma patients and healthy controls, with these variations appearing to exhibit gender specificity. This phenomenon may be attributed to hormonal influences on the salivary microbiota composition[40]. Animal studies have also revealed pronounced dysbiosis in the gut microbiota of glioma-bearing mice compared to healthy counterparts, particularly in terms of altered β-diversity. Similar physiological patterns have been identified in human glioma patients[41], suggesting a possible link between glioma and microbioal dysregulation, thereby offering critical insights into the systemic microbial mechanisms implicated in gliomagenesis.

Gastric cancer and oral microbiota dysbiosis

Gastric cancer remains a leading cause of cancer-related mortality worldwide and is strongly associated with *Helicobacter pylori* infection. Increasing evidence indicates that the oral microbiota also plays a significant role in gastric carcinogenesis[42]. Oral microorganisms may colonize the gastric mucosa *via* mechanisms such as gastroesophageal reflux, thereby altering the gastric microbiome and contrbuting to tumor progression[43]. Dysbiosis of the oral microbiota can influence the tumor microenvironment in the stomach by altering microbial community structure, modulating metabolic pathways, inducing chronic inflammation, and affecting immune

responses. Collectively, these factors may elevate the risk of gastric cancer and facilitate its progression[42].

Oral-vaginal microbiome synergy in cervical cancer

Cervical cancer (CC) is one of the most common malignancies in women, and its pathogenesis has been increasingly linked to dysbiosis of both the oral and vaginal microbiota. A study involving 82 women reported significantly elevated levels of *Fusobacterium* and *Veillonella* in the oral cavities of CC patients, with these increases positively correlated with serum C-reactive protein (CRP) levels, suggesting a systemic inflammatory link between microbial dysregulation and cancer progression. Additionally, amino acid metabolism by the oral microbiota was significantly enhanced in CC patients, potentially contributing to genomic instability through the toxic effects of metabolic byproducts. Notably, a synergistic pattern between oral and vaginal microbiota characterized by *Lactobacillus* depletion and anaerobic bacterial enrichment has emerged as a promising biomarker for non-invasive cancer screening. However, the precise mechanisms underlying microbial translocation between oral and vaginal sites remain to be elucidated[44].

POTENTIAL MECHANISM OF ACTIONS

Chronic inflammation drives carcinogenesis

Chronic inflammation represents a fundamental mechanism through which the oral microbiota contributes to carcinogenesis. Oral pathogens initiate persistent inflammatory responses by activating host immune signaling pathways, thereby disrupting microenvironmental homeostasis and promoting malignant transformation[45].

Activation of TLR/NF-κB signaling pathway: Lipopolysaccharides produced by *Porphyromonas gingivalis* activate the NF-κB signaling pathway via binding to Toll-like receptors TLR2 and TLR4, leading to the excessive release of pro-inflammatory cytokines such as IL-6, TNF-α, and IL-1β[34]. These cytokines stimulate epithelial cell

proliferation and inhibit apoptosis, thus creating a microenvironment conducive to tumorigenesis[46]. Likewise, *Fusobacterium nucleatum* interacts with host E-cadherin through FadA adhesin, thereby activating the Wnt/β-catenin signaling pathway and inducing IL-8 secretion, which further amplifies the inflammatory response[27].

Tumor microenvironment remodeling: Microbe-driven infiltration of inflammatory cells such as M2 macrophages and myeloid-derived suppressor cells into the tumor microenvironment results in the secretion of immunosuppressive cytokines, including TGF-β and IL-10, which inhibit anti-tumor immune responses. A study on oral squamous cell carcinoma demonstrated that *Fusobacterium nucleatum* significantly enhances tumor progression through CXCL2-mediated interactions between tumor cells and macrophages[47].

Metabolic toxicants

Oral microorganisms produce various carcinogenic metabolites that can directly or indirectly damage DNA and promote malignant cellular transformation[48].

Acetaldehyde: *Streptococcus mutans* and certain lactic acid bacteria convert ethanol to acetaldehyde *via* alcohol dehydrogenase (ADH). Acetaldehyde is a highly potent genotoxin that induces DNA adduct formation and chromosomal breaks. Clinical evidence indicates a significant elevation in salivary acetaldehyde concentrations among chronic alcohol consumers, which is positively correlated with an increased risk of OSCC in a dose-dependent manner[49].

Genotoxins: Certain oral bacteria, such as *Escherichia coli*, contain polyketide synthase (pks) gene clusters encoding the genotoxin colibactin, which induces DNA double-strand breaks and genomic instability[50]. Additionally, other genotoxins have been implicated in microbe-cancer associations. For example, *Helicobacter pylori* produces cytotoxicity-associated gene A (CagA) and vacuolating cytotoxin A (VacA), both of which impair host DNA repair mechanisms and promote genomic instability[51].

Short-chain fatty acids and PCWBR2 protein: SCFAs produced by *Peptostreptococcus anaerobius* have been shown to modulate immune responses. Furthermore, the PCWBR2 protein expressed on the surface of *P. anaerobius* interacts with integrin α2/β1 on colorectal cancer cells, activating the PI3K/AKT/FAK signaling pathway. This cascade promotes tumor cell proliferation and facilitates colorectal cancer development[52].

Immune escape

The oral microbiota constitutes a highly complex and diverse community that plays a pivotal role in the initiation and progression of various cancers. In breast cancer, *Fusobacterium nucleatum* has been demonstrated to promote immune evasion by upregulating PD-L1 expression in tumor cells *via* the NF-κB signaling pathway, thereby enabling these cells to evade $CD8^+$ T cell-mediated cytotoxicity[53]. Similarly, *Porphyromonas gingivalis* exhibits potent immune-evasive properties. This bacterium activates PARP9 through the TLR2 signaling pathway, inducing the production of type I interferon. Consequently, the host's antibacterial immune response is suppressed, allowing the pathogen to persist within the host[54]. Moreover, *P. gingivalis* induces the secretion of thrombospondin-1 (TSP-1) through a co-signaling mechanism involving CD47 and TLR2, which inhibits neutrophil bactericidal activity, thereby enabling the bacterium to survive in inflammatory environments and contributing to microbial dysbiosis[55]. Simultaneously, the oral microbiota maintains a dynamic, symbiotic relationship with the oral microenvironment, collectively supporting the delicate balance of oral microecology. The oral microbiota facilitates tumor immune escape by functions across multiple dimensions[56]. In terms of immune modulation, *Fusobacterium nucleatum* within polymorphonuclear neutrophils (PMNs) reduces intracellular reactive oxygen species (ROS) through Fn1792, a lysozyme inhibitor, allowing the bacterium to survive within phagocytes. Fn1792 also induces the expression of CX3CR1, thereby activating the NF-κB/STAT3 signaling pathway and inhibiting phagocyte apoptosis, which further supports the intracellular survival of *F. nucleatum*. These $CX3CR1^+$ phagocytes also upregulate PD-L1 and enhance immune

evasion. The $CX3CR1^{+}PD\text{-}L1^{+}$ phagocytes, including macrophages and monocytes, migrate into the bloodstream, transferring intracellular *F. nucleatum* to tumor cells. These tumor cells subsequently express Fn-Dps, a virulence factor of *F. nucleatum*, and PD-L1. Within the tumor microenvironment, tumor cells secrete chemokines such as CXCL2/8 and CCL5, respectively, recruiting PMNs and macrophages. Fn-Dps promotes PD-L1 expression in tumor cells, suppressing T cell activity and enhancing immunosuppression. The combination of Fn-induced immune evasion and PD-L1 upregulation facilitates tumor metastasis. Figure 1 illustrates the central role of *F. nucleatum* in modulating the tumor microenvironment and promoting cancer progression[56].

At the level of immune function suppression, *Porphyromonas gingivalis* secretes specific gingival proteases that degrade complement components C3 and C5, which are crucial for chemitaxis and immune activation. This degradation weakens immune-mediated pathogen clearance, favoring the formation of a tumor-supportive microenvironment[57]. More critically, *F. nucleatum* binds to inhibitory receptors on immune cells, impairing their antigen-presenting capacity and anti-tumor responses. Tumors may exploit the presence of *F. nucleatum* to enhance their immune escape and establish a permissive microenvironment for growth and survival[58]. Collectively, these findings reveal the molecular basis for the synergistic promotion of tumor immune escape by oral microorganisms via a tripartite mechanism: immune cell regulation, immune function inhibition, and suppression of antigen presentation.

Tumor cell dissemination

Oral microorganisms contribute not only to immune evasion but also play a crucial role in tumor invasion and metastasis. *F. nucleatum* has been shown to enhance proliferation and migration across various tumor cell types. In colorectal cancer, it promotes tumor progression by upregulating PD-L1 through IFIT1 protein modification mediated by m6A methylation, thus enhancing immune evasion and promoting tumor proliferation and metastasis[59]. Similarly, *Porphyromonas gingivalis* infection in esophageal cancer

cells leads to Fas protein degradation through YTHDF2-mediated mechanisms, inhibiting T cell cytotoxicity and accelerating tumor progression[60]. Furthermore, oral microbes may disseminate to distant organs *via* the bloodstream or lymphatic system, where they can induce vascular inflammation and lipid deposition. These effects not only exacerbate atherosclerosis but may also contribute to the establishment of tumor-permissive microenvironments[61].

Epigenetic regulation driven by oral microbiota

Epigenetic regulation constitutes another critical mechanism by which oral microbiota influence tumor progression. Infection with *F. nucleatum* has been shown to induce extensive transcriptomic and epigenetic alterations in host cells, including the downregulation of genes associated with histone modification and the activation of enhancer elements. These changes collectively promote the expression of inflammation-related genes such as TNF and CXCL8[62]. Additionally, oral microbiota-particularly *Porphyromonas gingivalis*-modulate host gene expression through epigenetic regulation of the PADI4 gene and peptidyl-arginine deiminase (PPAD)-mediated protein citrullination. In rheumatoid arthritis, environmental factors such as smoking and microbial infection have been implicated in DNA methylation and tumor gene silencing, thereby facilitating chronic inflammation and tumorigenesis[63]. These epigenetic modifications not only affect immune responses but may also promote systemic cancer progression by stabilizing cancer stem cell phenotypes or activating oncogenic signaling pathways.

FACTORS INFLUENCING THE ORAL FLORA AND REGULATORY STRATEGIES

Influence of oral hygiene and lifestyle habits on oral microbiota

Oral hygiene and lifestyle habits—including diet, smoking, and alcohol consumption—significantly influence the compositionfunction of the oral microbiota, thereby affecting the risk of tumorigenesis[64].

Diet: Diets high in sugar promote the proliferation of *Streptococcus mutans*, which metabolizes sugars into acid and acetaldehyde. Acetaldehyde is highly genotoxic and induces DNA damage and mutations. Conversely, a diet rich in dietary fiber can suppress the retention, proliferation, and migration of pathogenesis microbiota by enhancing intestinal peristalsis and increasing bowel movement frequency, thus maintaining a relatively healthy physiological state[65].

Smoking: Smoking significantly alters both the composition and diversity of the oral microbiota. Smokers exhibit a marked increase in certain bacterial populations and a reduction in others, likely due to alterations in the oral environment such as oxygen levels, pH, and nutrient availability. For instance, *Porphyromonas gingivalis* is significantly more abundant in the saliva of smokers (P = 0.035), and its presence correlates with the severity of alveolar bone loss. Although the increase in *Fusobacterium nucleatum* abundance is not statistically significant, its elevated levels in smokers may still reflect the impact of tobacco use on the oral microbiota[66].

Nicotine, the primary active compound in tobacco, exhibits immunosuppressive properties and promotes chronic inflammation, thereby fostering an environment conducive to the colonization and proliferation of pathogenic bacteria. This microbial imbalance contributes to periodontal disease and other oral health disorders. Nicotine also impairs neutrophil phagocytosis and reduces antioxidant levels in saliva, further compromising host defense mechanisms against pathogenic invasion[67,68].

Alcoholism: Chronic alcoholism significantly alters the oral microenvironment by affecting pH levels, moisture content, and nutrient availability, thereby disrupting the balance of the oral microbiota. These alterations compromise the survival and colonization of beneficial oral flora. Chronic alcohol abuse leads to a reduction in the abundance of lactic acid bacteria, diminishing their capacity to competitively inhibit pathogenic microorganisms. This microbial imbalance exacerbates oral health conditions and heightens the risk of oral diseases[69]. Additionally, alcohol is metabolized into acetaldehyde by oral bacteria such as *Streptococcus mutans*, which

induces direct DNA damages and activates oncogenes[49]. Therefore, restricting alcohol intake is essential for preserving oral health.

Intervention of antibiotics and probiotics on oral microbiota

Antibiotics and probiotics function as double-edged swords in modulating the oral microbiota, necessitating careful evaluation of their therapeutic benefits and associated risks[70].

Adverse effects of antibiotics: Broad-spectrum antibiotics (*e.g., amoxicillin*) not only target pathogenic bacteria but also disrupt commensal microbiota (*e.g., Streptococcus* species), resulting in dysbiosis[71]. Prolonged antibiotic administration may facilitate the colonization of drug-resistant strains, such as *methicillin-resistant Staphylococcus aureus*, further compromising oral health and microbial equilibrium[72].

Protective effects of probiotics: Lactobacillus species are key constituents of the oral microbiota and are widely recognized as beneficial probiotics. These organism ferment carbohydrates produce lactic acid, thereby reducing the pH of the oral environment and inhibiting the growth of pathogenic species. For example, *Lactobacillus reuteri* suppresses the proliferation of harmful bacteria such as *Fusobacterium nucleatum* by secreting bacteriocins, including reuterin, and enhances mucosal barrier function[73]. Additionally, *Lactobacillus* species play a pivotal role in maintaining the ecological balance of the oral microbiota, thereby supporting oral health[69].

Emerging therapies

Emerging therapeutic strategies offer promising avenues for cancer prevention and treatment through precise modulation of the oral microbiota[74].

Oral microbiome transplantation: Oral microbiome transplantation (OMT) is a novel therapeutic approach aimed at restoring microbial diversity and suppressing the colonization of pathogenic bacteria by introducing oral microbiota from healthy donors into the patient's oral cavity. OMT has demonstrated promising potential in treating oral diseases and improving oral health outcomes[75]. Although preclinical studies

have shown the efficacy of OMT in treating halitosis[76], its clinical application in oncological settings remains limited and warrants further investigation. Additional research is necessary to elucidate the underlying mechanisms of OMT, establish evidence-based protocols, and comprehensively assess its safety profile. While no OMT-related adverse events have been reported in clinical trials to date, potential risks—including opportunistic infections and microbiome dysbiosis—must be rigorously evaluated, particularly in the context of insufficient pathogen screening. Furthermore, regulatory and ethical guidelines concerning donor eligibility and long-term monitoring of recipients require clear definition to ensure both safety and feasibility. Addressing these challenges will necessitate multidisciplinary collaboration to close current knowledge gaps and develop clinical guidelines.

Targeted regulation of dominant bacterial species: The use of CRISPR-Cas9 technology to genetically modify commensal bacteria, such as *Streptococcus salivarius*, has been shown to enhance the secretion of antimicrobial peptides, thereby inhibiting the progression of precancerous lesions[77]. In addition, phage therapy offers a targeted approach to eliminate pathogenic bacteria (*e.g., Porphyromonas gingivalis*) while preserving beneficial species, thus supporting oral microbial homeostasis. Owing to its specificity and minimal disruption of the native microbiota, phage therapy has garnered considerable interest. Nonetheless, several critical challenges must be addressed before it can be widely adopted in clinical practice. These include the development of robust phage screening and characterization techniques, strategies to counteract bacterial resistance, and the establishment of comprehensive regulatory frameworks to facilitate the approval and commercialization of phage-based therapies[78].

Microbial markers and vaccine development: Specific microbial markers, such as DNA fragments from *Fusobacterium nucleatum* found in saliva, have been proposed as non-invasive biomarkers for early colorectal cancer screening, with a reported area under the curve (AUC) of 0.85[79]. Furthermore, a vaccine targeting the FadA adhesin

of *F. nucleatum* has demonstrated a 60% reduction in tumor volume in animal models, indicating its potential as an effective therapeutic strategy[69].

Synergistic carcinogenic effects of comorbidities and gut microbiota dysbiosis

Recent evidence suggests that oral microbiota can migrate to and colonize the gastrointestinal tract, integrating into the gut microbiome and contributing to systemic diseases[80]. Opportunistic pathogens from the oral cavity may reach the intestines *via* swallowing or breaches in periodontal tissue, where they disrupt the gut epithelial barrier, induce chronic inflammation, and contribute to disease pathogenesis. For instance, Niu *et al.* (2024) demonstrated in an animal model that oral-derived Porphyromonas gingivalis actively colonizes the gut, exacerbates insulin resistance, and induces dysbiosis by suppressing the aryl hydrocarbon receptor (AhR) signaling pathway involved in tryptophan metabolism, thereby promoting obesity-related tumorigenesis[80]. Similarly, Knop-Chodyła *et al.* (2024) reported that the colonization of oral microbes in the gastrointestinal tract can enhance the production of proinflammatory cytokines (*e.g.*, IL-6, TNF-α) and carcinogenic metabolites (*e.g.*, acetaldehyde), which impair T cell function, cause DNA damage, and promote the malignant transformation of gastrointestinal epithelial cells[81].

Mechanisms underlying the synergistic tumor-promoting effects of comorbidities and microbial dysbiosis

Metabolic disorders, such as diabetes and obesity, are closely linked to dysbiosis of both oral and gut microbiota, which together accelerate tumorigenesis through multiple mechanisms. Noureldein *et al.* (2022) reported a significant reduction in butyrate-producing bacteria in the gut microbiota of diabetic mice, which was associated with elevated levels of IL-1β and NADPH oxidase 4 (NOX4), contributing to colorectal cancer progression[82]. Similarly, Soeda *et al.* (2023) demonstrated that mice with diabetes and non-alcoholic steatohepatitis (NASH) exhibited impaired intestinal insulin signaling and microbiota imbalance. The microbial profile of these mice resembled that

of human diabetic patients and was associated with an elevated risk of hepatocellular carcinoma[83]. Additionally, Biragyn and Ferrucci proposed that age-related microbial dysbiosis, characterized by a reduction in short-chain fatty acids, may promote "inflammaging"—a state of chronic low-grade inflammation that impairs immune surveillance and facilitates the accumulation of mutated cells[84]. Collectively, these findings suggest the existence of a vicious cycle wherein comorbidities exacerbate microbial dysbiosis, which in turn promotes tumorigenesis through chronic inflammation, immune suppression, and the production of carcinogenic metabolites.

Regulatory strategies

With the advancement of systems biology, research on oral microbe—tumor interactions has transitioned from single-target interventions toward comprehensive precision regulatory systems[85]. Molecular network models derived from multi-omics technologies have elucidated critical pathways, such as the TLR4/NF-κB signaling cascade, through which *Porphyromonas gingivalis* promotes tumor cell proliferation and invasion[10]. More importantly, these models provide multidimensional biomarkers with potential for clinical translation.

In the context of precision medicine, multi-omics data are transforming the design of clinical trial. The integration of gene-centered and genome-wide metagenomic approaches has deepened our understanding of microbial diversity and functionality[86]. Such insights enable personalized interventions based on individual physiological and microbial profiles, which is particularly essential given the substantial inter-individual variability in microbiota composition. Consequently, the development of tailored probiotic or antibiotic regimens is critical to achieving optimal clinical outcomes[70].

Looking forward, artificial intelligence (AI)-driven platforms for multi-omics integrationare anticipated to expedite the clinical application of these findings[87]. For instance, tools such as Vaxign-ML and DeepVacPred have demonstrated significant utility in predicting vaccine candidates by integrating genomic, proteomic, and

metabolomic data. These platforms employ machine learning algorithms to identify antigenic epitopes and prioritize potential vaccine targets, thereby streamlining the vaccine development process[88].

In the near future, the implementation of a closed-loop system that combines AI-powered computational predictions with experimental validation in human subjects may offer novel avenues for the development of vaccines targetingpathogenic oral microorganisms. For example, the TGSD algorithm has been effectively utilized to identify key proteins from multi-omics datasets, underscoring the importance of integrating diverse data types to refine predictive accuracy[89]. This approach not only accelerates the discovery of therapeutic targets but also improves the precision of vaccine design.

By integrating these technological advancements, AI-driven platforms can substantially enhance both the efficiency and accuracy of vaccine development, particularly for oral microbial pathogens. The integration of computational modeling with experimental validation represents a promising advancement toward more effective and individualized medical interventions.

CONCLUSION

PROBLEMS AND SOLUTIONS

Epidemiological insights and oral microbiota's role in tumor development

The oral microbiota plays a crucial role in the pathogenesis of various cancers, including oral squamous cell carcinoma, esophageal cancer, and pancreatic cancer. Epidemiological studies havedemonstrated a significant correlation between oral microbial dysbiosis—particularly the overrepresentation of *Porphyromonas gingivalis* and *Fusobacterium nucleatum*—and an increased risk of tumor development. For instance, individuals with periodontal disease have been reported to exhibit a 43% higher risk of esophageal cancer compared to healthy controls. Nonetheless, further studies are required to more precisely quantify this risk and elucidate the underlying

mechanisms[90]. Additionally, elevated levels of *Aggregatibacter actinomycetemcomitans* have been observed in the gingival sulcus fluid of patients with pancreatic cancer[32].

Oral microorganisms facilitate tumorigenesis through several mechanisms, including the induction of pro-inflammatory mediators, direct or indirect production of cytotoxic substances, and suppression of immune cell functions. These findings contribute to a deeper understanding of the complex interactions among oral microbes, the host immune system, and tumor development. However, additional research is necessary to clarify causality and identify the molecular pathways involved.

Experimental validation

Although numerous studies have identified associations between oral microbiota and tumorigenesis, the causal relationship remains insufficiently understood. It is yet to be determined whether microbial alterations initiate tumorigenesis or if changes in the tumor microenvironment drive microbial dysregulation. Experimental studies are thus essential to elucidate these mechanisms.

Cohort study design: A bidirectional cohort study should be established, involving the longitudinal collection of oral microbiota samples. Participants would undergo regular physical examinations, imaging assessments, and other diagnostic procedures to monitor tumor development. For those who develop tumors, detailed information on tumor type, stage, location, and treatment outcomes should be recorded. By analyzing temporal changes in oral microbiota and correlating them with tumor progression, researchers can investigate whether microbial dysbiosis precedes or results from tumorigenesis. Statistical analyses, including survival and time-series models, should be applied to determine causality.

Animal model experiments: To investigate the role of the oral microbiota in tumorigenesis, animal models may be employed to simulate the effects of specific pathogenic bacteria or the absence of beneficial commensals. Tumor development can be monitored over time by colonizing animals with pathogenic bacteria or by administering antibiotics to deplete commensal microorganisms. Subsequently, the size,

quantity, and growth rate of tumors can be systematically measured. Oral samples should be collected at multiple time points to assess the composition and relative abundance of the oral microbiota. Analytical techniques such as 16S rRNA gene sequencing, metagenomics, and quantitative PCR may be utilized to evaluate microbial diversity and compositional shifts. These experiments serve to elucidate the influence of particular pathogens or the absence of beneficial microorganisms on tumor initiation and progression.

Interventional studies: A randomized controlled trial (RCT) represents another approach for evaluating the impact of oral microbiota modulation on cancer development. In an RCT, participants are randomly allocated to either an experimental group or a control group. The experimental group receives OMT, whereas the control group may receive a placebo or standard medical care. The efficacy of the intervention can be assessed through clinical examinations, imaging modalities such as magnetic resonance imaging (MRI) or computed tomography (CT), and, when necessary, pathological analysis of biopsy specimens. Key indicators of therapeutic success include the prevention or retardation of tumor progression, thereby providing insight into whether oral microbiota transplantation may influence the trajectory of cancer development.

Integrating multi-omics data to promote precision medicine

The increasing recognition of the oral microbiota's involvement in cancer development and progression offers a valuable opportunity to advance diagnostic and therapeutic strategies. The integration of multi-omics technologies—including metagenomics, metabolomics, and single-cell sequencing—can substantially contribute to the evolution of precision medicine[12]. These technologies provide comprehensive insights into the complex interplay between the oral microbiota and host physiology, particularly regarding how microbial alterations influence tumorigenesis. By elucidating the molecular mechanisms underlying the association between the oral microbiota and

cancer, future research may facilitate the development of more targeted and personalized diagnostic and therapeutic interventions.

PROSPECTS FOR ORAL MICROBIAL APPLICATIONS

Development of microbial markers

Microbial signatures present in saliva and gingival sulcus fluid have demonstrated potential as non-invasive biomarkers for the early detection and monitoring of tumor development. These oral fluids are emerging as valuable diagnostic media, offering the advantages of easy accessibility and the feasibility of frequent sampling. However, the precise roles of these fluids in various disease contexts remain incompletely understood, necessitating extensive clinical investigation. Continued research is essential to validate the clinical applicability of oral fluid biomarkers across different malignancies and to facilitate their integration into routine diagnostic practice[91].

Precision intervention strategies

The application of oral microbiota in precision medicine, particularly in antibody-based therapies, represents a promising direction for the development of innovative treatment modalities. One notable approach involves the genetic modification of probiotics to synthesize specific antibodies, which are subsequently encapsulated within nanoparticles for targeted delivery. This method effectively addresses challenges associated with oral drug administration, thereby enhancing the therapeutic precision and efficacy of such interventions. These therapies hold significant potential not only for the treatment of cancer but also for combating infectious and other systemic diseases, offering a more efficient and patient-centered alternative to conventional modalities. Future investigations should prioritize the optimization of antibody-producing probiotics, the resolution of safety and regulatory issues, and the implementation of clinical trials to evaluate their therapeutic utility[92].

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