Review Article

Impact of Oral Microbiome on Head and Neck Cancer Development: A Critical Review.

ABSTRACT

A significant contributor to cancer-related morbidity and mortality worldwide is head and neck cancer (HNC). Depending on the affected site, its etiology may include risk factors like geographic differences, genetic predisposition, gender, age, type of diet, smoking and/or alcohol consumption habits, and sexual preferences. Dysbiosis, or disruption of the oral microbiota balance, is another proposed causative factor for HNC. The oral microbiota is home to over 1,000 microbial species, including pathogenic and commensal strains. Disruption of the healthy balance of the oral cavity microbiota triggers the carcinogenic mechanisms by inducing chronic inflammation in the affected site, which acts as a pro-carcinogenic factor by inducing an immune-suppressed microenvironment that induces cell proliferation and inhibits apoptosis. Recent studies have also found that members of the human microbiome are strongly linked to a wide range of cancer types, with well-established associations between the oral microbiota and chronic inflammation and cell proliferation. Aside from chronic inflammation, the bacterial genome, toxins, and metabolites have been proposed as additional factors that induce or facilitate carcinogenesis and its progression in the head and neck region. However, the precise mechanisms remain largely unknown. However, a greater understanding of the underlying mechanisms may lead to the development of novel preventive or customized treatments that can be used in routine clinical practice. The link between oral dysbiosis and the development of HNC is a relatively new and well-received topic in the oncological and dental communities. Therefore, this review intends to provide a comprehensive summary of the currently available information on the potential

mechanistic relationships between changes in the oral microbiota and the development of HNC, which is still in the research phase.

Keywords: Oral microbiota, dysbiosis, carcinogenesis, head and neck cancer

1. INTRODUCTION

Approximately 30 trillion bacteria reside within each human body (1). The oral cavity has been home to more than 700 different bacterial species (sp), and cutting-edge sequencing technologies are enabling us to uncover even more. Different from other body parts, the structural anatomy of the oral cavity has some distinctive features. Hard tissues (teeth and jaws), exocrine gland tissues (major and minor salivary glands), and highly specialized mucosal surfaces (tongue, buccal mucosa, gingiva, and palate) all contribute to the composition of the oral cavity's microbiota.

A healthy homeostasis between the host and microbiota is important for normal human body function (3). The phrases "microbiota" and "microbiome" are frequently used interchangeably; however, they are not exact synonyms. The microbiota is a diverse group of microorganisms that dwell in a specific habitat; hence, the "human microbiota" encompasses all bacteria, viruses, fungi, and other single-celled organisms that exist in the human body. These organisms can be found in any organ or tissue throughout the human body, including the skin, nasal cavity, oral cavity, gut, urogenital system, lungs, and others. The phrase “microbiome” refers to the collective genomes of microorganisms in a specific environment, which includes all of their genetic resources: RNA and DNA (4). The microbiota interacts with many bodily processes. It can, for instance, regulate immune responses, alter food intake, influence appetite, take part in vitamin biosynthesis, shield the hosts from external pathogens, and produce some antimicrobial substances. (5). Dysbiosis, or a disruption in the balance of microbiota members, can result in serious repercussions such as recurring and persistent

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infections, loss of certain functions like vitamin synthesis, and even the onset and progression of carcinogenic processes.

Cancer poses a homeostatic challenge to the host system, and both its onset and progression are influenced by numerous factors including chemicals, radiation, and physical agents (6). The role of microbes in the carcinogenesis process was long underappreciated before Helicobacter pylori (H. Pylori) was identified as a causative agent of gastric cancer in the early 1990s. Infectious agents have finally been acknowledged as notable causes of gastric cancer thanks to this paradigm shift (7). Although the role of microbes and the microbiota in carcinogenesis was not fully understood during this time, later studies estimated the global cancer risk from microbial infections to be 16.1%, emphasizing the importance of the microbiota even more (8). Microbes and cancer have a complicated relationship; a host's microbiota may affect a person's susceptibility to developing cancer more or less, or not at all. The most recent research indicates that while a small number of microbes have an antitumor effect, a large group of microorganisms associated with tumors are involved in tumorigenesis (9–11).

The oral cavity is one of the most prominent microbiological reservoirs in the human body, housing a significant number of microorganisms such as bacteria, fungi, viruses, and bacteriophages (12). Recently, some well-researched periodontal organisms have come to the forefront of our understanding of the relationship between oral microbiota and cancer (13). The relationship between the oral microbiota and carcinogenesis has illuminated ongoing research in this area, supporting the idea that the oral microbiota may significantly contribute to tumorigenesis through various mechanisms and that a thorough understanding of these mechanisms may aid in the prevention of tumor progression and/or anti-cancer treatments.

There is a great deal of interest in identifying specific microbes and microbiotas that play causal roles in cancer, figuring out how these interactions with environmental factors and

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the host microbiota contribute to carcinogenesis and using this information to diagnose and treat cancer. The research on the connection between particular bacteria and head and neck cancers (HNCs) is still in its infancy. Hence, the main goal of this review is to provide an extensive summary of the available research findings in this area, with a particular emphasis on distinct bacteria and related cancers in this region.

2. COMPONENTS OF ORAL MICROBIOTA

The human oral microbiota is made up of about 700 sp of microorganisms that reside in our mouths, making it one of the body's most complex microbial communities (14). A species-rich heterogeneous ecological system is formed in the oral cavity by small microbial habitats like the tongue, soft and hard palates, buccal mucosa, and teeth (15). Bacteria, fungi, and viruses are among the microorganisms that live in the oral cavity, with bacteria being the most common (16). Firmicutes, Bacillus, Proteobacteria, and Actinomycetes are the most prevalent of these bacteria (17), and unlike the gut microbiota, these bacterial sp do not show significant variations over time in the absence of triggering factors. Although environmental and dietary factors influence the composition of the gut microbiota (18), they have only a minor impact on the bacterial types of the oral cavity.

Nearly 85 fungi sp are found in the oral microbiota of healthy people from various geographic regions of the world. The most significant of these fungi, candida (19), exhibits neutral behavior when the oral microbiota is in good health. On the other hand, when the oral microbiota's delicate balance is disrupted, candida scrutinizes for opportunities to attack oral tissues. Candida forms a biofilm with streptococci that functions as a pathogen (20). Another component of the oral microbiota is viruses, primarily phages (21). The type of phages in the mouth is constant at almost all stages of life (22). Other viruses may also manifest in the mouth in certain disease states; the most prevalent ones are the mumps virus (23) and HIV (24).

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The majority of the oral microbiota's constituents are oral bacteria. Streptococcus mutans, Porphyromonas gingivalis (P. gingivalis), Staphylococcus, and Lactobacillus are the most prevalent oral bacteria (25). Streptococcus mutans is the main constituent of the oral microbiota and one of the most common components of dental plaque (26). P. gingivalis is a gram-negative, anaerobic, non-glycolytic periodontal pathogenic bacterium. P. gingivalis has the potential to result in tooth gum recession if left untreated.

Lactobacillus is a bacterium capable of fermenting sugars to produce lactic acid, which is a type of microorganism that lives in the body and helps to promote host health (27).

Adjustments in microbial biomass in the oral cavity are influenced by the interaction of different microbial sp in the biofilm. Oral biofilm maturation is caused by interactions between early colonizing microorganisms, and the mature biofilm is established later by these colonizers through a variety of mechanisms such as coagulation, metabolic exchange, small molecule-mediated communication, and genetic material exchange. In a healthy oral cavity, the numerous bacteria are not evenly distributed over all surfaces and instead multiply in various ecological niches according to their metabolism (28). The ecology of these habitats is influenced by multiple oral anatomical sites, resulting in significant microbial environmental differences. The most prevalent sp of buccal epithelium include Streptococcus, Gemella, Eubacterium, Selenomonas, Veillonella, Actinomyces, Atopobium, Rothia, Neisseria, Eikenella, Campylobacter, Porphyromonas, Prevotella, Granulicatella, Capnocytophaga, Fusobacterium, Leptotrichia, and Streptococcus mitis. Another resident of the healthy buccal mucosa known as Granulicatella adiacens, which is frequently regarded as an opportunistic pathogen, is typically found in bacteremia/septicemia samples taken from patients with infective endocarditis or atheroma (29). Streptococcus mitis (S. mitis), Streptococcus australis, Streptococcus parasanguinis, Streptococcus salivarius, Streptococcus sp. clone FP015, Streptococcus sp. clone FN051, Granulicatella adiacens, and Veillonella species plural

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(spp). were found on the tongue dorsum between the keratinized filiform papillae. The anatomical distinctions between the lateral margin and dorsum of the tongue have an impact on the bacterial profiles. The lateral margin of the tongue has a smooth non-keratinized surface, and the predominant bacteria are S. mitis, Streptococcus mitis bv. 2, Streptococcus sp. clone DP009, Streptococcus australis, Granulicatella adiacens, Gemella haemolysans, and Veillonella spp (30,31). Streptococcus salivarius, Rothiamucila ginosa (Stomatococcus mucilaginosus), and an unidentified, cultivable sp of Eubacterium (strain FTB41) are the tongue sp most associated with health (32). Streptococcus mitis, Streptococcus mitis biovar 2, Streptococcus sp. clone FN051, Streptococcus infantis, Granulicatella elegans, G. hemolysans, and Neisseria subflava are the main bacterial sp on the hard palate. Streptococcus mitis constitutes the most prevalent strain in almost all healthy sites and subjects. S. mitis and Streptococcus oralis, on the other hand, have been linked to bacterial endocarditis in patients with prosthetic valves (33,34). Aside from the typical bacteria composition of a healthy oral cavity, some bacteria sp associated with periodontal diseases, such as P. gingivalis, Tannerella forsythia, and Treponema denticola, also exist (35).

According to the data, there are some common bacteria as well as many site-specific bacteria that live in the entire or sub-regions of the oral cavity. Although the majority of them are regular residents with no negative impact on oral cavity health, it appears that the balance between the distribution and quantity of these bacteria is critical for the health of the oral cavity and, in some cases, the entire human body. Any imbalance between these bacteria, known as dysbiosis, can cause mild to severe health problems, including HNCs.

3. ORAL MICROBIOTA AND CARCINOGENESIS

The oral microbiota influences a variety of human body functions: it modulates immune responses, alters food intake, affects appetite, participates in vitamin biosynthesis, protects against exogenous pathogens, and produces some types of microbial substances (5). These

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effects are thought to be beneficial, and life without bacteria would be different, if not impossible (3). Diseases may occur if microbiota homeostasis is disrupted and some pathogenic bacteria become more prevalent. The term dysbiosis is used to describe abnormalities that arise in a normally balanced system. However, both internal and external stimuli cause the microbiota to change continuously. As a result, it is challenging to research and comprehend the intricate nature of microbiota-host interactions and the inciting factors of disease in the human body (2).

The role of microbes among the causes of cancer, the most troubling disease affecting nearly every part of the body, was overlooked for a long time (7) before the publication of the groundbreaking studies in the early 1990s that identified Helicobacter pylori (H. pylori) as the catalyst of gastric cancer (36). Later studies highlighted the importance of the microbiota by estimating that microbial infections were responsible for 16.1% of cancer cases worldwide (8).

Marshall and Warren's study provided the first proof that bacteria directly contribute to the development of cancer (37). Contrary to the prevailing belief at the time, their findings indicated that H. pylori was playing a critical role in the development of gastric cancer. Then it has been amply demonstrated by numerous researchers around the world that H. pylori is the root cause of more than 90% of duodenal ulcers and up to 80% of gastric ulcers. As a result, the World Health Organization has classified it as a class I carcinogen due to its capacity to promote gastric cancer following chronic infection (38-40).

The microbiota not only performs crucial local tasks like establishing mucosal immunity and enriching barriers, but it also has systemic effects like regulating immunity, inflammation, and metabolism. Inflammation, immunity, and epithelial and stromal cell homeostasis are all influenced by the microbiota composition and abundance at epithelial barrier surfaces (41). Several factors, including infections, trauma, dietary considerations, and

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germline mutations, can harm the mucosal barriers within the body. The majority of the time, barrier injuries and tissue homeostasis are quickly and correctly fixed. Persistent barrier damage and failure to restore homeostasis are caused by weakened host defenses or harmed microbiome composition. In these circumstances, the microbiota can facilitate cancer development by altering the immune system's performance, host metabolism, and tumor cell proliferation and death (42). In a brief, oral microbiota contributes to cancer primarily by the following mechanisms: (1) inducing mutagenesis, oncogene activation, and angiogenesis; (2) inducing chronic inflammation; (3) promoting cell proliferation and inhibiting cellular apoptosis; and (4) producing carcinogens (43).

According to the literature, chronic inflammation is a risk factor for about 25% of human cancers, indicating that it is the seventh hallmark and one of the main characteristics of cancer (44). This evidence suggests a logical possibility: that the oral microbiota influences the initiation and progression of cancer by causing chronic local and systemic inflammation. Several processes, such as cell proliferation, angiogenesis, mutagenesis, and oncogene activation, can be either caused by or aided by chronic inflammatory mediators by altering the normal homeostasis of cells and tissues (44). Fusobacterium, Porphyromonas, and Prevotella sp are a few anaerobic oral bacteria that can cause chronic inflammation and are linked to periodontal diseases. Bacterial cells can interact with cells from different tissues and release inflammatory mediators that cause widespread inflammatory reactions. Interleukins (IL-1, IL-6, IL-17, and IL-23), tumor necrosis factor (TNF), and proteinases that degrade extracellular matrix (MMP-8, -9, and 13) are just a few examples of proinflammatory mediators that are affected by periodontal bacteria (45,46). Numerous cancers are caused by an increase in the production of these inflammatory proteins, and their elevated expression levels (particularly IL-6 and MMP-9) are associated with an aggressive tumor phenotype and a worse prognosis (47-49).

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The upregulation of cytokines and other inflammatory factors causes changes in a variety of molecular metabolic pathways, including those responsible for modulating cellular metabolism and proliferation. For instance, changes in the oral microbiota caused by periodontal disease result in a significant increase in the expression of the protein RAGE, which causes carcinogenesis (50). Furthermore, gram-negative bacteria produce a proinflammatory lipopolysaccharide endotoxin (LPS) that can stimulate the production of IL1-β, IL-6, and TNF-α by binding to leukocyte TLR receptors (51,52). These inflammatory cytokines, in particular, cause the overexpression of other proinflammatory proteins that stimulate the release of phospholipase A2, prostaglandins (PGs), and acute phase proteins (53,54). Other studies have shown that IL-6 overexpression promotes tumor spread by upregulating matrix metalloproteinases, adhesion molecules, and endothelial leukocyte adhesion molecules (55,56). All of these findings suggest that interleukins, particularly IL-6, are linked to neoplastic transformation (57). Aside from interleukins, altered TNF-α levels caused by changes in the Wnt and NF-κB signaling pathways have been linked to tumor development (58,59). Although NF-B acts as an immune stimulator factor against neoplastic cells, its protein expression is increased in several cancers and acts as an oncogene (60,61).

Another way the oral microbiota contributes to carcinogenesis is through the production of carcinogens. Numerous bacteria-produced substances are suspected of being carcinogenic. Bacterial metabolism generates sulfur compounds, acids, and free radicals, particularly reactive nitrogen and oxygen sp, which can damage DNA in a way that promotes the development of tumors. Additionally, some bacteria have an alcoholic metabolism that results in the production of acetaldehyde, which aids in the development of cancer (52). Reactive oxygen and reactive nitrogen sp are known to accumulate as a result of altered nicotinamide adenine dinucleotide phosphate oxidase and nitric oxide synthase activities, which stimulate cancer development and chronic inflammation (62,63). Bacterial metabolism

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may directly or indirectly play a significant role in these pro-carcinogenic processes. While performing these functions, some oral peroxygenase bacteria, such as Bifidobacterium adolescentis, Lactobacillus acidophilus, Lactobacillus. fermentum, Lactobacillus jensenii, and Leptodiaptomus minutus, may also produce hydrogen peroxide (H_2O_2) (52,64).

These dangerous substances have the potential to damage oncogenes or onco-suppressor genes' DNA (65,66). Bacteria, including sp of the genus Bacteroides and the class Firmicutes, can ferment extra protein from the host into sulfides and nitrosamines. These harmful substances are capable of causing DNA damage in oncogenes or onco-suppressor genes (65,66). Furthermore, lactic, acetic, butyric, isobutyric, isovaleric, and isocaproic acids produced by oral microorganisms like Bifidobacterium, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Peptostreptococcus stomatis, and Streptococcus cause the environment's pH to decrease (67-69). These acids help to create an ideal microenvironment in the tissue, which promotes cancer cell proliferation and metastasis (70,71). Other research has emphasized the significance of superoxide dismutase activity and expression by analyzing microbiomes found in cancer samples and normal mucosa from oral cancer patients (72). Superoxide dismutases are a group of enzymes that catalyze the dismutation of superoxide radicals (O_2^-) to molecular oxygen (O_2) and H2O2, providing cellular defense against reactive oxygen sp. Hence, superoxide dismutase activity is critical for preventing the harmful effects of oxygen. It has been demonstrated that the presence of Fe2+ in tumor samples reacts with H_2O_2, resulting in the production of deleterious reactive sp that promote neoplastic transformation by inducing DNA mutations affecting key genes involved in cell cycle regulation (73).

The breakdown of the epithelial barrier brought on by an unbalanced oral microbiota, specifically by proinflammatory conditions sustained by microbial changes, is also a notable factor in the development of cancer and its progression (74). The epithelial barrier can

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become dysfunctional as a result of alterations in the microbial composition and mucus production, which can also modify the microenvironment (75). One of the main causes of infections and other microbial diseases, such as tumors, is the resulting imbalance between the epithelia and the microbiota (76,77).

Some of the oral microbiota components may induce carcinogenesis by stimulating cell growth and inhibiting cellular death. Recent research has shown that bacteria can influence apoptosis, as seen in oral squamous cell cancers (78). P. gingivalis is mostly known as an oral pathogen capable of suppressing apoptosis of the oral cell epithelium, albeit it may cause apoptosis via arginine or lysine gingipains (79). Some bacteria may trigger immune cell apoptosis in various ways to protect cancer cells from immune attack, which is a vital process in the carcinogenesis cascade and its progression (80). For instance, P. gingivalis infection improves the survival and proliferation of gingival epithelial cells in the mouth by boosting phosphatidylinositol 3-kinase /Akt signaling and modifying Blc-2 family proteins, so indirectly suppressing intrinsic apoptosis (81,82). P. gingivalis, however, may also activate Jak1/Akt/Stat3 signaling, which would improve tumor cell survival. This bacterium has the ability to release the NDK enzyme, which cleaves ATP and inhibits the activation of the proapoptotic P2X7 receptor, regulating ATP/P2X7 signaling. Fusobacterium nucleatum (F. nucleatum) modulates a myriad of anti-apoptotic pathways. Bacteria stimulate NF-kB signaling as a result of TLR activation. When F. nucleatum stimulates p38, MMP-9 and MMP-13 are secreted, which promotes cancer cell invasion and metastasis (83). Another oral cavity bacterium, Lactobacillus plantarum, has been shown to induce apoptosis in oral cancer cells KB by upregulating PTEN and downregulating MAPK signaling pathways, which may play a crucial role in preventing the development of oral cavity cancers (84).

It has been demonstrated that certain oral bacteria have numerous mechanisms for fostering cell cycle progression and proliferation (80). For instance, oral bacteria like P.

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gingivalis were proven to have secondary impacts on proliferation rates by modifying the expression levels of α-defensin genes linked to oncogenesis and tumor cell proliferation in an in vitro study by Hoppe et al. (85). By promoting infection, oral bacteria such as P. gingivalis activate certain genes involved in the downstream signaling cascade of proinflammatory active transcription, as well as processes connected to cyclins (86, 87). Bacteria that cause cell proliferation either induce normal oral epithelial cell proliferation by provoking kinases such as Chk2, aurora A, CK1 delta, and proinflammatory factors, or increase IL-6 production by activating STAT3, which then prompts important effectors such as cyclinD1, allowing cancer cells to grow (88, 89).

Finally, another role of oral microbiota in carcinogenesis is angiogenesis promotion. Cancer cells can proliferate and invade quickly, necessitating large amounts of nutrition and oxygen. In other words, with the exception of dormancy, cancer cells are always hyper-metabolic. Vascular endothelial growth factor (VEGF) is a crucial angiogenesis mediator that promotes the creation of abnormal blood vessels around cancer cells in order to supply them with sufficient nutrients and oxygen (90). VEGF has also been linked to the differentiation and prognosis of oral cavity cancers (50). Mirkeshavarz et al. (91), for example, demonstrated that IL-6 increases VEGF production in oral squamous cell cancers in experimental research. Furthermore, P. gingivalis and F. nucleatum have been shown to promote IL-6 and IL-8 in the development of oral squamous cell cancers, activating the JAK/STAT signaling pathway implicated in angiogenesis, resulting in VEGF production and neoangiogenesis (91, 92). As a result, while the precise mechanism for cancer-induced angiogenesis is likely to be more complex, there is likely to be a strong association between the bacterial components of oral microbiota and neoangiogenesis, which is guided to some extent by the bacterial tissue invasion-related local inflammatory response and its systemic effects (Figure 1).

4. ORAL BACTERIA and HEAD & NECK CANCER

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The majority of tumors in the head and neck region are head and neck squamous cell carcinomas (HNSCCs), which frequently develop from the mucosal endothelium of the oral cavity, pharynx, and larynx (93,94). As research on the oral microbiota and its connection to HNSCCs has grown, so has the understanding of the role played by bacteria in the susceptibility and development of this type of cancer (95).

Microbial colonies in the oral cavity can grow anywhere on hard and soft tissues such as the tongue, buccal mucosa, tonsils, and palate. Because these surfaces provide different growth conditions, biofilms can differ significantly in composition in a site-specific manner (96). There is, however, a strict balance between the components of the oral cavity microbiota, which is requisite for the host's health. Any disruption in this balance, or dysbiosis, can result in a variety of disease conditions in the oral cavity and throughout the body, including the HNCSS. In dysbiosis, there is a compositional shift in favor of pro-inflammatory commensals and a decrease in beneficial microorganisms, which can result in chronic and exacerbated inflammatory conditions like periodontitis (97). Although some potentially pre-malignant and malignant oral diseases have been linked to dysbiosis in studies (1, 98), information on the relationship between oral microbiota and cancer is not always reliable. Moreover, it has been demonstrated that some oral microbiota components can mediate antitumor effects by inactivating carcinogens, which confuses our ability to ascribe and comprehend precise mechanisms (1,75). For example, some bacterial outer membrane vesicles have immunomodulatory properties and have thus been proposed as novel cancer therapeutics (99,100).

Chronic periodontitis, gingivitis, and HNSCC carcinogenesis and progression have all been linked to the altered oral bacterial microbiome and associated chronic inflammation (101-103). Recent research has reported alterations in the microbiome of HNSCC patients with an apparent loss of microbial diversity (104-107), as well as changes in the relative

abundance of some oral bacteria such as Fusobacteria and Streptococci. What's more, chronic infections and inflammation are increasingly being recognized as factors in carcinogenic feedback loops involving the resident microbiota in HNSCC patients who do not have traditional risk factors such as alcohol and tobacco use. Such evidence suggests that F. nucleatum may act as a key driver in premalignant and/or early lesions of HNSCC, which initiates the carcinogenesis process and is later replaced by opportunistic bacterial passengers, resulting in a lower abundance in later stages of the disease (108,109). This hypothesis is related to F. nucleatum's known pro-carcinogenic properties associated with bacterial invasion and inflammation (e.g., flagellation and bacterial chemotaxis) (110,111), metabolic pathways (e.g., homolactic fermentation) (112), production of DNA-damaging compounds (113), and promotion of cell proliferation (e.g., E-cadherin/-catenin signaling via its FadAadhesin) (114). Such proof establishes a solid basis for the supposition that F. nucleatum accumulation during the early stages of tumorigenesis is an independent risk factor in patients who do not have major risk factors for HNSCC.

Metsäniitty et al. conducted a systematic review of 34 studies involving 2294 HNSCC patients and 4432 healthy participants (115). The authors found that patients with HNSCC had higher concentrations of bacteria from the genera Fusobacterium, Peptostreptococcus, Alloprevotella, Capnocytophaga, and Prevotella. Aside from that, it was discovered that HNSCC patients had higher concentrations of Prevotella melaninogenica, F. nucleatum, and Prevotella intermedia. Certain bacterial genera, on the other hand, such as Streptococcus, Haemophilus, Rothia, and Veillonella, decreased. Nonetheless, in patients with HNSCC, either Veillonella dispar, Aggregatibacter segnis, or Streptococcus pneumoniae were elevated or decreased, indicating that the results were not always consistent (115). Hayes et al. examined mouthwash samples from 129 HNSCC patients, including pharyngeal, laryngeal, and oral cavity cancers. Higher levels of the genera Corynebacterium, Kingella, Neisseria,

Abiotrophia, Capnocytophaga, and the sp Kingella denitrificans and Streptococcus sanguinis, as well as the sp Kingella denitrificans and Streptococcus sanguinis, were linked to a lower risk of laryngeal cancer (96). In contrast, Debelius et al. investigated the relationship between nasopharyngeal cancer (NPC) and the oral microbiota in 499 patients using 16S rRNA sequencing and discovered that a pair of Granulicatellaa diacensamplicon sequence variants was strongly associated with NPC status (116). Similarly, a decrease in Lactobacillus sp levels may cause changes in the microbial ecosystem of patients with oral tongue cancer, which may alter the conditions in favor of microbial dysbiosis such as pH and micronutrients (117).In another study, Kakabedze et al. discovered a significant increase in the abundance of F. nucleatum, Prevotella intermedia, Aggregatibacter segnis, Capnocytophaga leadbetteri, Peptostreptococcus stomatis, and five other sp, implying a possible link between these bacteria and oral squamous cell carcinoma (OSCC) (118).

Investigations have also been made into the various oral microbiota members' diagnostic potential. Mager et al. suggested Streptococcus mitis as a diagnostic marker that could reliably predict 80% of cases of oral cancer (119). The abundance of Firmicutes and Actinobacteria genera was significantly decreased in cancer samples, according to the results of a larger study by Schmidt et al. that examined swabs of lesion surfaces and contralateral normal mucosa from 18 patients with OSCC (120). Moreover, pyrosequencing was used to elucidate the bacterial communities in the saliva of six OSCC patients, and it was suggested that paired taxa within the family Enterobacteriaceae together with the genus Oribacterium could be used to distinguish OSCC specimens from oropharyngeal squamous cell carcinoma (OPSCC) and normal specimens (121).

Zu and colleagues examined the salivary microbiomes of 70 OSCC patients to investigate the tumor-promoting properties of Capnocytophaga gingivalis (C. gingivalis) (122). According to the study's researchers, Streptococcus, Capnocytophaga, and

Peptostreptococcus were found in higher concentrations in OSCC tissue sections than in the nearby non-tumorous tissues. Additionally, Capnocytophaga's fluorescence signal was also much stronger than that of Peptostreptococcus and Streptococcus. It was demonstrated that C. gingivalis additionally facilitated OSCC invasion and migration, which would then suggest the emergence of a more aggressive tumor phenotype in the presence of C. gingivalis infection P. gingivalis is an invasive opportunistic pathogen that belongs to the "red complex" category of oral infections and can be found in advanced periodontitis (123,124). Pro-matrix metalloproteinase 9 (proMMP-9) and proMMP-8 are expressed and activated more frequently when P. gingivalis activates the host response (125–127). On the other hand, gingipains convert proMMP-9 and proMMP-8 to active forms, which hasten extracellular matrix disintegration and cell penetration. In order to ascertain the role played by P. gingivalis in the pathogenesis of OSCC, Kylmä et al. examined the prevalence of R gingipain, a well-known virulence factor of the oral pathogen Pg, as well as the prevalence of MMP-8 and 9 expression in 202 unselected consecutive patients with OPSCC (128). The authors postulated two mechanisms for R gingipain's involvement in the development of OPSCC: 1) activation of MMP-9 and 2) induction of inflammatory cell responses and inhibition of tumor growth.

However, despite the fact that specific sp or even strains are frequently linked to the disease, the aforementioned studies have been unable to provide sp-level bacterial composition, leaving the results murky due to the sparse sample sizes. Nonetheless, Al-hebshi et al. profiled bacterial communities at the sp level in OSCC tissue samples from Yemeni patients, providing the first epidemiological evidence for F. nucleatum and Pseudomonas aeruginosa associations with OSCC (129) (Figure 2).

Given the accessible evidence, it is reasonable to assume that various oral microbiota members play critical roles in HNSCC genesis and progression, either alone or in collaboration with others. It is, however, prudent to design future studies to identify the

specific bacteria, bacterial load, genomic properties, related tissue alterations, metabolites, or other bacterial catalysts that initiate malignant transformation and facilitate its progression to more advanced cancer stages resistant to known therapeutic interventions in the oral cavity.

5. POTENTIAL MECHANISM OF HEAD AND NECK CARCINOGENESIS INDUCED BY BACTERIA

A bacterial imbalance in the affected tissue may lead to chronic infections and exacerbated inflammation. In such conditions, instead of responding to the bacteria, the immune system of the host tissue does so in response to the tissue damage brought on by the infection (130). During the carcinogenesis process, the microbes and/or microbial metabolites' invasion into the tumor microenvironment promotes neoplasia progression by activating tumor-potentiating immune cell responses (131). Numerous bacteria have been demonstrated to produce toxins that disrupt the cell cycle and alter cell growth in theory or cause long-lasting infections (132,133). Through the activation of the cyclin D1 and mitogen-activated kinase (MAPK) pathways, chronic infections promote cell proliferation and DNA replication. They also increase the frequency of cell transformation and the rate of tumor development through an increase in genetic mutations (134,135). Multiple infections cause the pathogen to accumulate inside the cell, suppressing apoptosis primarily by altering the expression of Bcl2 family proteins or deactivating the retinoblastoma protein pRb (136,137). Despite efforts by the host immune system to kill the infected cells through apoptosis, this tactic creates a niche in which the intracellular pathogen can survive. In this way, the partially transformed cells can advance to a higher level of transformation and eventually become tumorigenic (138), avoiding the self-destructive process.

Numerous pathogenic microorganisms that have intracellular access and result in chronic infection alter host cell signaling pathways, thereby assisting pathogen survival (139). To either promote or prevent tumor growth, these signaling factors must be regulated

appropriately. Some infections may mimic the variable gross effects seen in tumorigenesis, and the precancerous lesion formed may regress with antibiotic treatment and bacterial elimination (138). However, if the bacteria-induced carcinogenesis process is not interrupted promptly and effectively, its initiation and progression may become unavoidable. Another possible mechanism is the bacteria's secretion or metabolism of potentially carcinogenic substances. This is significant in the oral cavity, where pre-existing local microflora may promote tumorigenesis by converting ethanol to its carcinogenic derivative, acetaldehyde, in amounts sufficient to cause DNA damage, mutagenesis, and secondary epithelial hyperproliferation (140,141). Increased microbial acetaldehyde production in heavy drinkers and smokers provides additional evidence for the link between bacterial metabolism and the commencement of the carcinogenesis process (142).

Nitrosation may also play a role in microbial carcinogenesis, in which microbial cells catalyze the formation of N-nitroso compounds from nitrite and amines, amides, or other nitrosatable compounds. Many bacterial sp, most notably Escherichia coli, contain strains that can catalyze nitrosation. Other sources of nitrosating organisms include yeasts and fungi. In addition to foods and drinks like cured meat, bacon, fish, and beer, nitrosamines have also been discovered in cosmetics, medications, and the materials used to build new cars' front passenger compartments. They are also common environmental contaminants. Consuming smokeless or burned tobacco also exposes people to high non-occupational levels of nitrosamines (143, 144), and the International Agency for Research on Cancer has classified two specific N-nitroso compounds, NDMA and NDEA, as "probably carcinogenic to humans" (145). Nitrosamines have been linked to a considerable increase in the incidence of esophageal cancer by generating DNA strand breaks (146). No matter the precise cancer-causing mechanisms at play, certain nitro-compounds seem to be relevant candidates for the growth of carcinoma in a variety of sites, including the esophagus and oral cavity (147,148).

Recently, it was reported that the DNA sequence of Streptococcus anginosus (S. anginosus) was found in DNA samples collected from esophageal cancer. Infection with S. anginosus may contribute to the development of HNC since smoking and alcoholism are risk factors for both esophageal cancer and these cancers (149). This carcinogenesis-triggering effect may be related to the incorporation of the exogenous S. anginosus DNA into the host genome. While the others act as inflammatory agents, spread infections, and trigger carcinogenesis in the host genome, they result in a hit-and-run injury (149).

Furthermore, the outcomes of a pilot investigation by Anand et al. raise the possibility that H. pylori and an elevated risk of oral cancer are related (150). High salivary counts of C. gingivalis, Prevotella melaninogenica, and Streptococcus mitis may serve as diagnostic markers for OSCC, according to a different study by Mager et al (119).A review by Chocolatewala et al. reviewed the studies that revealed diversity in isolated bacterial taxa between oral cancer tissue samples and control samples and discovered that Exiguobacterium oxidotolerans, Prevotella melaninogenica, Staphylococcus aureus, and Veillonella parvula were specific to cancerous tissue (138).It's also possible for some bacterial sp to exhibit cancer-causing behaviors that are site-specific. S. anginosus, for instance, has no bearing on oral cancers despite being frequently associated with esophageal and pharyngeal cancers. Analogously, substantial salivary specificity for C. gingivalis, Prevotella melaninogenica, and Streptococcus mitis has been observed in oral cancer patients, making these sp robust salivary markers for early detection of oral cancers, potentially improving the patient's prognosis significantly if appropriately intervened (151).

Bacteria may also employ additional techniques to promote the growth of cancer in different human tissues, including the oral cavity. In a laboratory experiment, Wen et al. investigated whether P. gingivalis invades the oral precancerous and cancerous lesions of a well-established 4-nitroquinoline-1 oxide (4NQO)-induced carcinogenesis mouse model (13).

P. gingivalis was revealed to be an intracellular colonizing bacterium sp that invades, multiplies, and survives in human primary gingival epithelial cells. According to the authors, this bacterium may start and hasten the tumorigenesis process by invading and colonizing oral tissues in mice. The idea that P. gingivalis influences intratumoral immune cells and causes carcinogenesis was also put forth, supporting the idea that bacteria and tumor cells can interact and enable immune escape. In the same study, they additionally discovered that P. gingivalis invades oral precancerous lesions and draws in myeloid-derived suppressor cells by producing chemokines like CCL2 and CXCL2 and cytokines like IL-6 and IL-8 (131). Additional in vitro research has shed light on the role that P. gingivalis plays in accelerating the development of oral cancer, including activating cell proliferation, inhibiting apoptosis, and increasing cellular invasion (152).

The available research results offered vital support for the theories regarding the possible mechanisms used by the bacteria to cause cancer at different HNC sites. It is clear from the research findings that bacteria may employ similar or unique mechanisms to promote the development of the carcinogenic process and its progression. However, the precise mechanisms and initiating factors have yet to be determined in suitably conducted future studies focusing on these particular issues, which may offer essential knowledge on the prevention, early diagnosis, improvement of individualized patient management strategies, and prognosis forecasting of HNC.

6. FUTURE PERSPECTIVES

Numerous studies have been undertaken on the oral microbiome and its connection to head and neck cancers to better understand the function of bacteria in the pathogenesis and vulnerability of cancer. The majority of research was able to discover distinctive bacterial profiles in cancer patients. However, these studies were typically restricted to oral cavity

tumors, had small sample sizes, and only a limited number of bacterial sp were examined in most of them.

In the past, the influence of bacterial sp or oral microbiota on specific malignancies received less attention than the influence of viruses. Epstein-Barr virus (EBV) and human papillomavirus (HPV) are the viruses that are most frequently studied in relation to head and neck cancers. EBV-infected individuals are more likely to develop certain types of fast-growing lymphomas and HNCs, such as Burkitt lymphoma and NPC. Furthermore, some incidences of stomach cancer and Hodgkin's lymphoma may be linked (154 Although EBV genomes are only discovered in NPCs, the relevance of HPV and EBV viruses in the development of laryngeal cancer has been proven (153, 154). It has been demonstrated that the presence of viruses in some cancers has a favorable impact on the prognosis of the diseases. An investigation into the presence of HPV and its subtypes in 41 patients with NPC, for instance, revealed that these patients had a better prognosis and experienced fewer recurrences (155). An intriguing subgroup of etiologically diverse HNCs with distinct epidemiological, clinical, and molecular characteristics from non-HPV-associated cancers is the HPV-associated OPSCC (156). OPSCC is a separate tumor type that is more common in middle-aged white males who are nonsmokers or former/light smokers, as opposed to older men with considerable smoking and alcohol history who have HPV-unrelated OPSCC. Additionally, OPSCC exhibits a distinct pattern of distant metastases, has a better prognosis, and has fewer subsequent primary cancers (156). As a result, research findings indicate that viruses contribute to the etiology of the illness and may be a significant factor in determining local spread, distant metastasis, and disease prognosis. Such findings not only established some viruses as causative and prognostic factors in some HNCs but also resulted in paradigm shifts in their staging methodologies as well as the treatment options and intensities in an individualized manner.

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In contrast to viruses, the data regarding the relationship between the various bacterial sp and head and neck carcinogenesis has not yet been fully elucidated, even though some authors have hypothesized that these bacteria play a significant role in the initiation and progression of oral cancers (65). Although bacterial infections are probably one of the factors contributing to head and neck carcinogenesis (43), it is not clear whether these infections are the underlying cause of the cancers themselves or merely their companions due to the diminished immunity. To determine the precise connection between the carcinogenesis process, its progression, and the detected bacterial sp, it is necessary to conduct genome analyses and lengthy follow-up studies with a large number of healthy individuals and counterparts with cancer. There are still many unanswered questions. For example, is there a bacterial sp that is unique to site-specific carcinogenesis? It is crucial to provide an answer to this question because different bacterial sp predominate in distinct regions of the oral cavity, each of which contains a large number of bacterial members. Another question is 'how much bacterial load is required to induce cancer and facilitate its progression?' To the best of our knowledge, this matter has never been investigated, despite the fact that bacterial burden may have a considerable impact on cancer initiation and progression. Aside from their possible diagnostic utility, it is questionable whether any specific bacterial sp, their quantities, or metabolites play any prognostic or predictive functions that could guide tailored therapies in such individuals. Potential collaborations or counter-actions between different bacteria kinds in cancer initiation or disease progression must also be determined in order to adopt preventive or treatment measures on time. In response to these and other concerns, innovative and ample clinical studies should be conceived to dispel any remaining questions about cancer prevention, early detection, tailored treatment, and prognosis prediction.

7. CONCLUSION

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Several bacterial genera and sp of the oral microbiota appear to play critical roles in HNC patients in terms of cancer initiation, progression, metastasis, recurrence, and/or patient survival. But, despite the recent emergence of encouraging research results, it is still unclear precisely which bacterial combinations, genera, or sp matter most for particular head and neck subtypes. Therefore, it is imperative to encourage and subsidize additional research in this emerging field, which may lead to the development of reliable and advantageous diagnostic and prognostic targets and customized therapeutic approaches for patients with HNC.

Conflicts of Interest

We have no personal or financial conflict of interest and have not entered into any agreement that could interfere with our access to the available literature, or upon our ability to analyze the data independently, to prepare manuscripts, and to publish them.

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FIGURE LEGENDS

Figure 1. The role of oral microbiota on the development of head and neck cancer

Figure 2. Bacterial species that cause various head and neck cancers

UNDER PEER REVIEW

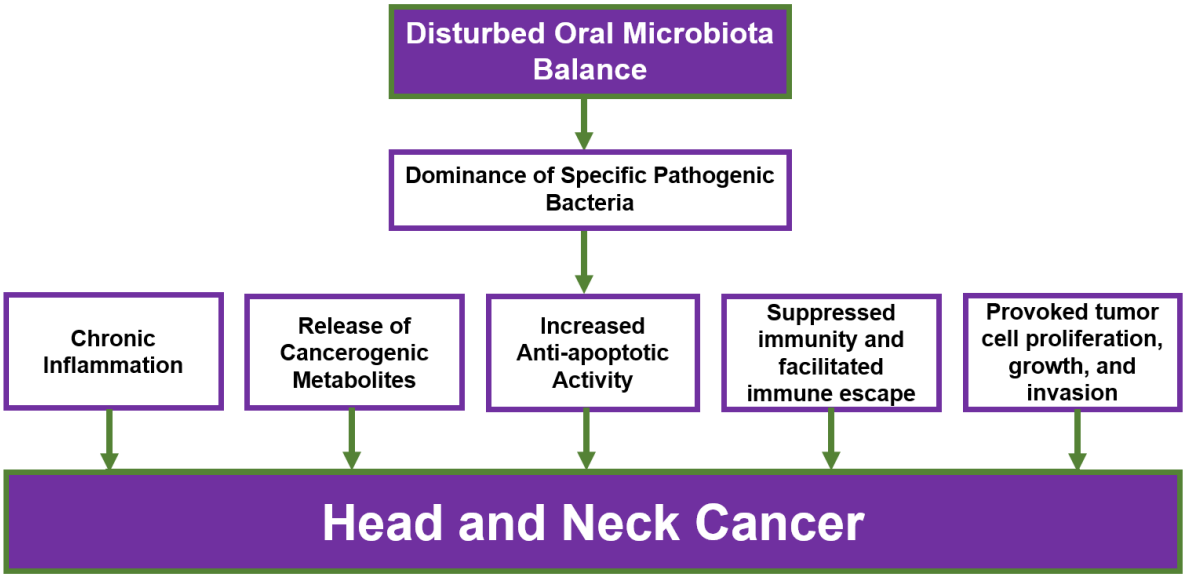
Disturbed Oral Microbiota Balance
Dominance of Specific Pathogenic Bacteria
Chronic Inflammation
Release of Cancerogenic Metabolites
Increased Anti-apoptotic Activity
Suppressed immunity and facilitated immune escape
Provoked tumor cell proliferation, growth, and invasion
Head and Neck Cancer

Figure 1.

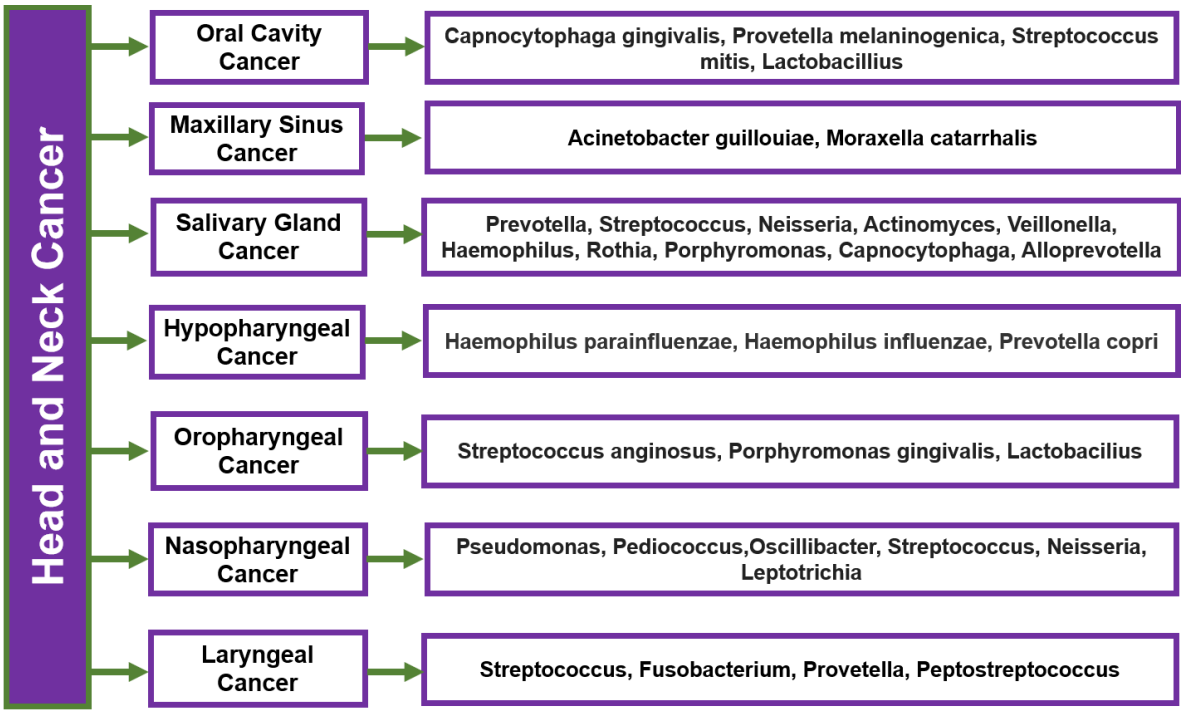

Figure 2.

UNDER PEER