Oral microbiota and oral cancer: Review

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Abstract

In this review, we draw attention and discuss the risk factors and causes of the development of oral squamous cell carcinoma (OSCC) focusing on oral microbiota. Recently, a breakthrough in the study of cancer has been the discovery of the relationship between the presence of certain types of bacteria and the development of cancer in the human body. Studies have shown that, *Porphyromonas gingivalis* (*P. gingivalis*) bacteria that is responsible for the destructive processes in the oral cavity, could play an important role in the development of OSCC. In our continuing search for bacteria that causes oral squamous cell carcinoma, we came across the *Pseudomona aeruginosa*, which due to its metabolite properties, may play important role in carcinogenesis of oral cancer. One possible mechanism is the ability of *Pseudomonas* to synthesize nitric oxide (NO) that modulates different cancer-related appearances such as apoptosis, cell cycle, angiogenesis, invasion, and metastasis. We think that *P. aeruginosa* increases the concentration of NO by converting salivary nitrite to nitric oxide, and this is how it contributes to NO-related carcinogenesis. Early diagnosis and treatment of periodontitis are very important not only for patients' oral health, but also for the prevention of OSCC development. Screening test for OSCC based on determination of salivary NO levels could be appealing and may prove to be useful assay for diagnosis and early detection of disease progression in oral cancer.

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Introduction

Head and neck cancers originating from the nasal cavity and paranasal sinuses, oral cavity, pharynx, including the nasopharynx, oropharynx, larynx and salivary glands, are the sixth most common malignant tumors in the world.[1,2] Most common oral cancers emerge from the oral cavity and it incorporates lip, tongue, gums, oral mucosa, floor of the mouth, hard palate, maxilla, mandible and the pharyngeal cancers. The latter includes all malignant tumors arising in the nasopharynx, oropharynx, or hypopharynx. These cancers are most commonly squamous cell carcinomas.[3] A web-based search for all types of articles published was initiated using MEDLINE/PubMed (since 1992 to 2020), with the key words such as '*oral squamous cell carcinoma (OSCC)*', '*oral microbiota*,' '*bacteria and cancer*' and '*bacterial toxins*', '*nitric oxide (NO)*', '*nitric oxide and cancer*', '*nitric oxide synthase*,' '*Porphyromonas gingivalis*' and '*Pseudomonas aeruginosa*'.

Terms microbiome and microbiota have subtle differences. In this article the term microbiota is used to refer to specific microorganisms that are found within a specific environment.

Study analysis has shown that, 657,000 new cases of cancers of the oral cavity and pharynx appear each year, with more than 330,000 deaths annually.[4] American cancer society has shared an estimated data of expected new cancer cases of the oral cavity and pharynx in the United States for the year 2020. In total, there will be approximately 53,260 cancers of oral cavity and pharynx diagnosed, which is the equivalent of approximately 148 new cases each day.[5]

The primary risk factors for the development of OSCC are the consumption of tobacco, heavy drinking of alcoholic beverages, poor oral hygiene and inappropriate dietary habits. However, approximately 15-20% of all oral cancer cases occur in patients that are non-smokers and non-alcohol drinkers.[6,7] Some of the other important risk factors include chronic inflammatory processes in the oral cavity, various viruses and bacteria, trauma to the mucosa with prostheses or while brushing teeth, and chewing tobacco. In addition, infections with high-risk human papillomavirus (HPV) genotypes and bacteria have also recently been implicated in the etiopathogenesis of OSCC.

Since the carcinogenic potential of tobacco, alcohol, and viral agents has been reviewed extensively elsewhere, we will not describe it in a great detail here. We only note that, throughout the world, there are more than 1.3 billion smokers.[8] The World Health Organization has estimated that annually, tobacco causes about 6.4 million deaths and does economic damage of thousands of billions of dollars in the world.[9] The strong connection between smoking and the development of oral cancer has been firmly established.[10,11] A cigarette is usually made with a lot of ingredients, which contain more than 60 toxic chemicals such as cancer-promoting substances and carcinogens[12] that can invade the various systems of the body.[13] The most important carcinogens in tobacco

are the certain flavor additives that are added by some tobacco companies to make their products more attractive. Aromatic hydrocarbon benz-pyrene and the tobacco-specificnitrosamines (TSNs), specifically 4-(nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-nitrosonornicotine (NNN) are the most common additives. The studies in animals have demonstrated that NNK and NNN in the tobacco products, that covalently bind with deoxyribonucleic acid (DNA) of keratinocyte stem cells forming DNA adducts that are responsible for critical mutations involved in the DNA replication, cause tumors of the oral cavity . The metabolism of these carcinogens involves oxygenation with P450 enzymes in cytochromes and conjugation by glutathione-S-transferase (GST).[14] It is suspected that genetic polymorphisms in the genes coding for these enzymes play a key role in genetic predisposition to the tobacco-induced cancers of the head and neck.[15] At the same time, it has been reported that in the patients with head and neck cancer, the ability to repair carcinogen-induced DNA damage is reduced.[16]

Alcohol

Beverages containing alcohol have been considered to be carcinogenic to humans specifically causing the tumors of the oral cavity; however, alcohol by itself has not been proved to be carcinogenic in animal studies. Alcohol consumption has been linked with the tobacco in increasing the risk of developing oral cancer. There are very few studies that have been able to analyze the patients who consume alcohol but are nonsmokers, and who smoke but are nondrinkers.[17] A study has demonstrated that alcohol can be an independent risk factor for oral leukoplakia.[18] However, in a similar study which evaluated the oral epithelial dysplasia occurrence in alcohol consumers who were nonsmokers, has shown that the role of alcohol in the development of oral epithelial dysplasia was crucial only in the cases when considered in conjunction with tobacco.[19]

Considering the abovementioned, the role of alcohol as an independent factor for the development of oral tumors is still unclear, although, epidemiological evidence establishes a synergistic role played by alcohol with tobacco. It is known that alcohol increases the permeability of oral mucosa, altering the morphology that is characterized by epithelial atrophy, which subsequently can lead to easier penetration of carcinogens into the oral mucosa].[19]

There are substances which have been seen in alcohol beverages such as N-nitroso compounds, inorganic arsenic, mycotoxins, urethane, and others that have been believed to be carcinogenic to humans. The major metabolite of alcohol is acetaldehyde, the transformation of which is mainly carried out by the alcohol dehydrogenase (ADH) enzyme. Afterwards, acetaldehyde is oxidized to acetate by means of aldehyde dehydrogenase (ALDH). Acetaldehyde causes DNA damage in cultured mammalian cells, and then interferes with the DNA synthesis and repair. Also, it induces sister chromatid exchanges and specific gene mutations,[20] inhibits the enzyme 6-methylguanitransferase that is responsible for repairing injuries caused by alkylating agents. Additionally, acetaldehyde initiates or promotes tumor formation, following which it leads to the increase in acetaldehyde accumulation in the body either due to increase in its production or due to decrease in its elimination, which subsequently is considered to be maleficent. Alternately, the reduction in ALDH enzyme can also lead to the accumulation of acetaldehyde. Genetic polymorphisms have been observed in these two enzymes (ADH and ALDH), which have been related to the increased risk of alcohol-related cancers.[21]

There are reports that alcohol drinking habits are associated with changes in the oral microbial community. The authors note that, alcohol intake may impact the oral microbiota in several ways: by direct cytotoxic effects on bacteria,[22] by disturbing saliva-bacterium interactions,[23,24] and by providing ethanol as a substrate for bacterial metabolism.[25] Additionally, the connection between excessive drinking and poor oral health was observed in a population study.[26]

The authors tested the relationship of level and types of alcoholic beverages with the oral microbiome in 1044 individuals from two large US national cohorts.[27] It was found that oral microbiome was characterized by bacterial 16S rRNA gene sequencing. The authors also argue that alcohol consumption is related to overall oral microbiome community composition and to the abundance of specific oral taxa. Heavy drinking may influence bacterial composition, including potential depletion of beneficial commensal bacteria and increased colonization of potentially pathogenic bacteria. It is clear that, such changes potentially contribute to alcohol-related diseases, including periodontal disease, head and neck cancer, and digestive tract cancers. We agree with the majority of authors' statements that improved understanding of the causes and health impacts of oral dysbiosis can lead to microbiome-targeted approaches for disease prevention.

The relationship between oral microbiota and tumor development

The results of the studies that are based on genome sequencing have begun to shed light on the presence of microbial patterns that are site-specific, which might be considered as normal oral microbiota.[28] However, dysbiosis, which is a term for the microbial imbalance, has been broadly studied both in animal and human models. It is characterized by the general loss of microbial diversity, loss of beneficial microbes and the expansion of pathogenic microbes.[29-31] According to some authors, dysbiosis can eventually lead to the development of cancer.[32] The three most common archetypes proposed to describe the pathogenetic processes involving microbiota in the development of cancer are: trigger of chronic inflammation and immune responses that can promote carcinogenesis and tumor growth, alteration of metabolic activity which leads to the increased production of toxic metabolites, and virus latency abrogation that lead to malignancies.[33]

The number of studies demonstrating that the oral microbiota plays an important role in the development of oral diseases is vastly increasing.[34-36] Furthermore, it has been suggested that poor oral hygiene and periodontal diseases play a role in the development of oral cancer,[28] and recently, studies also indicated that oral microbiota may be involved in the development of distant organ tumors as well.[37]

Although different pathogenic mechanisms have been proposed, the reverse causality cannot be excluded and the direct evidence is still not available. Specifically, as it was stated earlier, oral microbiota could be involved in cancer development, but specific tissue tropism for microbial translocation and molecular mechanisms of microbiota-driven carcinogenesis at distal sites are yet to be proven.[38]

It should still be underlined that the evidences which are linking oral microbiota with different tumors are promising. Furtherly, meta-transcriptomic and metabolomic analyses are making a significant contribution to the understanding of the relationship between oral microbiota metabolism and human health.[28]

If the direct connection between the oral dysbiosis and risk of tumor development is demonstrated, it could lead to new preven-

tive strategies. Furthermore, the link between oral microbiota and cancer could contribute to the search for new therapeutic anti-cancer strategies, such as the use of pre- and pro-biotics.[39]

Viruses and oral cancer

Approximately 20% of all malignant neoplasms of human are associated with viral or bacterial infections.[40] At the beginning of the 20th century, Peyton Rous established that viruses can cause cancer. He demonstrated that chicken sarcomas could be transmitted by a virus which we call *rous sarcoma virus*.[41] Today, however, it is recognized that Epstein-Barr virus (hepatitis B virus, hepatitis C virus, human papilloma virus, human T-cell lymphotropic virus and Kaposi's associated sarcoma virus contribute to 10-15% of the cancers worldwide.[42] The HPV is well known as an oncogenic agent in the pathogenesis of cervical cancer.[43] In recent years, the role of viruses in the development of oral cancer has been supported by many studies.[44-47] Considering the association with the OSCC and oral premalignant lesions, certain types of HPV (types 16, 18, 31, 33, 35, and 39) are known to have high oncogenic potential.[48,49]A large number of studies describe how HPVs induce and maintain the malignant phenotype. The major evidence supporting the role of HPV in the development of cancer is that their genes and gene products are capable of disturbing the cell cycle process. HPV encodes two major oncoproteins, specifically E6 and E7 proteins. The E6 and E7 proteins have mechanisms for down regulating expression of histocompatibility genes and having effects both on innate and adaptive immunity. The increased evidence of HPV-related oral cavity cancer in man is demonstrated in many studies.

At the same time, it has been demonstrated that the HPV was present in normal oral mucosa, which in turn suggests that the role of HPV in oral carcinogenesis might be speculative.[50] There are reports of a study of sixty histopathologically confirmed OSCC samples, tested by polymerase chain reaction for HPV. Studies have shown that none of the samples were HPV positive.[51] Other authors used ultra-high-throughput sequencing of the cancer transcriptome to assess whether papilloma virus transcripts are present in cutaneous SCCs. These data demonstrated that papillomavirus mRNA expression is not a factor in the maintenance of cutaneous SCCs.[52]

Bacteria and oral cancer

More than 700 kinds of bacteria exist in the normal oral cavity, among which red complex (a group of bacteria: *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*) is the most important for periodontal disease.[53]

These three bacteria are Gram-negative anaerobic bacteria and usually located in the periodontal pockets. They can express virulence factors to interfere in the defense system, and invade and destroy periodontal tissue and host immune function.[54]

Our particular interest was aroused by the authors' work concerning comparative assessment of the microbiota compositions between tumor sites and opposite normal tissues in buccal mucosal of 50 patients with OSCC using the 16S rDNA sequencing.[19] It is noted that, richness and diversity of bacteria were significantly higher in tumor sites than in the control tissues. Cancer tissues were enriched in six families (*Prevotellaceae, Fusobacteriaceae, Flavobacteriaceae, Lachnospiraceae, Peptostreptococcaceae,* and *Campylobacteraceae*) and 13 genera, including *Fusobacterium, Alloprevotella* and *Porphyromonas*. At the species level, the abundances of *Fusobacterium nucleatum, Prevotella intermedia, Aggregatibacter segnis, Capnocytophaga leadbetteri, Peptostreptococcus stomatis*, and another five species were significantly increased, suggesting a potential association between these bacteria and OSCC. Here, the authors report that genes involved in bacterial chemotaxis, flagellar assembly and lipopolysaccharide (LPS) biosynthesis which are associated with various pathological processes, were significantly increased in the OSCC group. Overall, oral bacterial profiles showed significant difference between cancer sites and normal tissue of OSCC patients, which might be considered diagnostic markers and treatment targets. In recent years, there have been reports that, that *P. gingivalis* were found in the brain of patients suffering from Alzheimer's disease, Parkinson's disease.[55-57]Additionally, they were found in joints affected by arthritis,[58,59] in myocardium during the myocardial infarction,[60,61] atherosclerotic plaques[62,63] and others.

There is a suggestion that, if *P. gingivalis* gains access to peripheral blood, then it can colonize any part of the body or organ and cause pathological processes in it. There is an assumption that, after entering the brain, *P. gingivalis* may spread slowly over many years from neuron to neuron along anatomically connected pathways,[64] and can contribute to intracerebral inflammation, and compromise vascular and microvascular integrity.[65] Some authors suggest that infectious factors such as viruses or bacteria can lead to cytokine dysregulation and brain cell injury.[66] The DNA of *P. gingivalis* have been detected in the synovial fluid of patients with rheumatoid arthritis.[67,68] *P. gingivalis* were also found in patients with endocarditis, and in heart specimens of cardiovascular patients.[69]

The authors'(20) bibliographic research was carried out selecting articles published until 2020, on PubMed, Web of Science, and Scopus databases, with the following keywords: *P. gingivalis*, oral cancer, oral squamous cell carcinoma, and periodontal pathogen. Seventeen articles, 14 *in vitro* and three in animal models were selected. Models mimicking OSCC were OSCC pre-established cell lines (11 studies), OSCC/healthy human biopsies (three studies), and animals with OSCC (three studies). *P. gingivalis* strains used to cause infection in those studies were ATCC 33277, 381, and W83. According to authors, *P. gingivalis* could play an important role in OSCC development and could be involved in three different stages: epithelial-mesenchymal transition of malignant cells, neoplastic proliferation, and tumor invasion.[70]

A breakthrough in the study of cancer in recent years has been the discovery of the relationship between the presence of certain types of bacteria and the development of cancer in the human body. The major evidence supporting the role of bacteria in the development of cancer is Helicobacter pylori, which is closely related to the development of gastric cancer, gastric mucosa-associated lymphoid tissue lymphoma, and other cancers.

It has been reported that, Fusobacteria living in the oral cavity can trigger the development of colon cancer. Fusobacteria do not settle on healthy tissues, but on colorectal tumors. After settling, they begin to actively multiply, following which the disease continues to progress.[71]

It is known that, *Streptococcus gordonii*, *Streptococcus sanguinis* and *Streptococcus oligofermentans* cause infective endocarditis[72] and *Streptococcus mutans* is involved in the development of caries.[73] It is suspected that *Streptococcus gallolyticus* causes colorectal cancer.[74,75] Interestingly, *Streptococcus mitis* were suggested as diagnostic marker which were able to predict 80% of oral cancer cases.[76]

Recently, there has been an increased interest in the possible role of oral microbiota in the development of OSCC. Numerous

bacterial species and their metabolic by-products may induce permanent genetic alterations in epithelial cells, which can lead to development of oral carcinogenesis.

In gingival carcinoma tissues *P. gingivalis* was reported to be more than 33% higher than that in normal gingival.[77] Interestingly, in malignant tissues, *P. gingivalis* gathered around cell nuclei with obvious heterogeneity. However, it was undefined whether *P. gingivalis* indeed played a stimulating role in the early stages of OSCC or only invaded into the transformed malignant cells.[78] In addition, in gingival epithelial cells, infection by *P. gingivalis* in the early stage can regulate the production of reactive oxygen species the key factors inducing DNA damage and genomic instability within an inflammatory microenvironment.[79,80]

In our continuing search for the oral bacteria causing OSCC, we came across the very interesting bacteria from our point of view *Pseudomonas aeruginosa* which presented a common encapsulated, Gram-negative, rod-shaped bacterium.

We hypothesized that, due to its metabolite properties, *P. aeruginosa* may play important role in carcinogenesis of OSCC. One possible mechanism is *Pseudomonas* ability to synthesize NO.

In recent years, a great interest related to NO induced carcinogenesis has appeared. NO is a known bio product in almost all types of organisms, ranging from bacteria to plants, fungi, and animal cells.[81] From the initiation to the progression of carcinogenesis, NO seems to play a role throughout various stages. It has been suggested that NO modulates different cancer-related appearances such as apoptosis, cell cycle, angiogenesis, invasion, and metastasis. The expression of NOs has been observed in various human cancers.[82] It is known, that the release of NO can play an important role in angiogenesis.[82-84] Understanding the role of nitric oxide in the tumor biology could help reduce the controversy and confusion that is observed in various publications.

Salivary NO concentrations are increased in patients with OSCC.[85] It was found that the increased salivary nitric oxide in pre-cancer patients is associated with the progression of diseases.[86] In the oral cavity of a human, the nitrate that is secreted as a salivary component is reduced by certain bacteria to nitrite and NO.[87]

There are reports that many bacterial species have nitrate and nitrite reductases, but some have been especially implicated in the reduction of nitrate to nitrite including *Streptococcus salivarius, S. mitis, S. bovis, Veionella* spp., *Staphyloccocus aureus* and *S. epidermidis*, *Nocordia* spp., *Corynebacterium* sp..[88-90]

The authors report that, many of these bacteria have additional reductases that enable denitrification of nitrate all the way to nitrogen gas (N2) *via* formation of NO and N2O.[91] Denitrification process has been found in dental plaques and, as suggested by the authors may relate to the pathophysiology of periodontitis and caries.[92]

The *P. aeruginosa* deserves our attention since *P. aeruginosa* can catalyze the reduction of nitrite to NO by cd1 nitrite reductase.[93] We think that *P. aeruginosa* increases the concentration of NO by converting salivary nitrite to NO, and this is how it contributes to NO-related carcinogenesis.

Conclusions

Despite the steady, annual increase in the number of patients with OSCC, it has not received adequate attention neither from the medical nor from dental professionals. The visual examination of the oral cavity is the most common method used to detect squamous cell carcinoma. However, the problem of early diagnosis of squamous cell carcinoma is that the doctors of rather narrow specialties, for example, dentists, focus their attention on more common diseases of the oral cavity such as caries and periodontal infections. The lack of sufficient oncological alertness among these specialists, as well as the poor awareness of the population itself, often leads to the development of advanced stages of the disease. Moreover, small lesions are often asymptomatic or may present with vague symptoms.

Screening test for oral squamous cell carcinoma based on determination of salivary nitric oxide levels could be appealing and may prove to be useful assay for diagnosis and early detection of disease progression in oral cancer.

References

1. Mascolo M, Siano M, Ilardi G, et al. Epigenetic disregulation in oral cancer. Int J Mol Sci 2012;93:2331-53.
2. Shah JP, Gil Z. Current concepts in management of oral cancer - Surgery. Oral Oncol 2009;45:394-401.
3. Chi AC, Day TA, Neville BW. Oral cavity and oropharyngeal squamous cell carcinoma-an update. CA Cancer J Clin 2015; 65:401-21.
4. Montero PH, Patel SG. Cancer of the Oral Cavity. Surg Oncol Clin North Am 2015;24:491-508.
5. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin 2020;70:7-30.
6. Cao SM, Simons MJ, Qian CN. The prevalence and prevention of nasopharyngeal carcinoma in China. Chin J Cancer 2011;30:114-9.
7. Vargas-Ferreira F, Nedel F, Etges A, et al. Etiologic factors associated with oral squamous cell carcinoma in non-smokers and non-alcoholic drinkers: A brief approach. Braz Dent J 2012;23:586-90.
8. Walt G. WHO's World Health Report 2003. BMJ 2004;324:6.
9. Samim D, Méan M, Clair C, Marques-Vidal P. A 10-year observational study on the trends and determinants of smoking status. PLoS One 2018;13.
10. Gupta P, Murti P, Bhonsle R, et al. Effect of cessation of tobacco use on the incidence of oral mucosal lesions in a 10–yr follow□up study of 12 212 users. Oral Dis 1995;1:54-8.
11. Llewelyn J, Mitchell R. Smoking, alochol and oral cancer in South East Scotland: A 10-year experience. Br J Oral Maxillofac Surg 1994;32:146-52.
12. Ezzati M, Lopez AD. Estimates of global mortality attributable to smoking in 2000. Lancet 2003;362:847-52.
13. Proctor RN. The global smoking epidemic: a history and status report. Clin Lung Cancer 2004;5:371-6.
14. Warnakulasuriya KAAS, Johnson NW, Linklater KM, Bell J. Cancer of mouth, pharynx and nasopharynx in Asian and Chinese immigrants resident in Thames regions. Oral Oncol 1999;35:471-5.
15. Scully C, Field JK, Tanzawa H. Genetic aberrations in oral or head and neck squamous cell carcinoma (SCCHN): 1. Carcinogen metabolism, DNA repair and cell cycle control. Oral Oncol 2000;36:256-63.
16. Warnakulasuriya KAAS, Ralhan R. Clinical, pathological, cellular and molecular lesions caused by oral smokeless tobacco - A review. J Oral Pathol Med 2007;36:63-77.
17. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, World Health Organization, International Agency for Research on Cancer. Betel-quid and areca-nut chewing and some areca-nut derived nitrosamines. IARC Monogr Eval Carcinog Risks Hum 2004;85:1-334.
18. Jafarey NA, Mahmood Z, Zaidi SH. Habits and dietary pattern of cases of carcinoma of the oral cavity and oropharynx. J Pak

Med Assoc 1977;27:340-3.
19. IARC monographs on the evaluation of carcinogenic risks to humans. IARC Monogr Eval Carcinog Risks to Humans 2010;93:9-38. Available from: http://monographs.iarc.fr/
20. Evstifeeva TV, Zaridze DG. Nass use, cigarette smoking, alcohol consumption and risk of oral and oesophageal precancer. Eur J Cancer Part B Oral Oncol 1992;28:29-35.
21. Murti PR, Bhonsle RB, Pindborg JJ, et al. Malignant transformation rate in oral submucous fibrosis over a 17□year period. Community Dent Oral Epidemiol 1985;13:340-1.
22. Ingram LO. Ethanol tolerance in bacteria. Crit Rev Biotechnol 1989;9:305-19.
23. Enberg N, Alho H, Loimaranta V, Lenander-Lumikari M. Saliva flow rate, amylase activity, and protein and electrolyte concentrations in saliva after acute alcohol consumption. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001;92:292-8.
24. Scannapieco FA. Saliva-bacterium interactions in oral microbial ecology. Crit Rev Oral Biol Med 1994;5:203-48.
25. Homann N, Tillonen J, Meurman JH, et al. Increased salivary acetaldehyde levels in heavy drinkers and smokers: A microbiological approach to oral cavity cancer. Carcinogenesis 2000;21:663-8.
26. Jansson L. Association between alcohol consumption and dental health. J Clin Periodontol 2008;35:379-84.
27. Fan X, Peters BA, Jacobs EJ, et al. Drinking alcohol is associated with variation in the human oral microbiome in a large study of American adults. Microbiome 2018;6:59.
28. García-Castillo V, Sanhueza E, McNerney E, et al. Microbiota dysbiosis: a new piece in the understanding of the carcinogenesis puzzle. J Med Microbiol 2016;65:1347-62.
29. Mascitti M, Togni L, Troiano G, et al. Beyond head and neck cancer: The relationship between oral microbiota and tumour development in distant organs. Front Cell Infect Microbiol 2019:9.
30. Petersen C, Round JL. Defining dysbiosis and its influence on host immunity and disease. Cell Microbiol 2014;16:1024-33.
31. Meurman JH, Uittamo J. Oral micro-organisms in the etiology of cancer. Acta Odontol Scand 2008;66:321-6.
32. Schwabe RF, Jobin C. The microbiome and cancer. Nature Rev Cancer 2013;13:800-12.
33. Plottel CS, Blaser MJ. Microbiome and malignancy. Cell Host Microbe 2011;10:324-35.
34. Zhang L, Liu Y, Zheng HJ, Zhang CP. The Oral Microbiota May Have Influence on Oral Cancer. Front Cell Infect Microbiol 2020;9.
35. Santarelli A, Mascitti M, Rubini C, et al. Active inflammatory biomarkers in oral lichen planus. Int J Immunopathol Pharmacol 2015;28:562-8.
36. Zhang Y, Wang X, Li H, et al. Human oral microbiota and its modulation for oral health. Biomed Pharmacother 2018;99:883-93.
37. Klimesova K, Jiraskova Zakostelska Z, Tlaskalova-Hogenova H. Oral Bacterial and Fungal Microbiome Impacts Colorectal Carcinogenesis. Front Microbiol 2018;9:774.
38. Chen J, Domingue JC, Sears CL. Microbiota dysbiosis in select human cancers: Evidence of association and causality. Semin Immunol 2017;32:25-34.
39. Rosier BT, Marsh PD, Mira A. Resilience of the Oral Microbiota in Health: Mechanisms That Prevent Dysbiosis. J Dent Res 2018;97:371-80.
40. Howley PM. Gordon Wilson Lecture: Infectious Disease Causes of Cancer: Opportunities for Prevention and Treatment. Trans Am Clin Climatol Assoc 2015;126:117-32.
41. Meissner HC. From Peyton Rous to the HPV Vaccine: A Journey of Discovery and Progress. Pediatrics 2019;144.
42. Martin D, Gutkind JS. Human tumor-associated viruses and new insights into the molecular mechanisms of cancer. Oncogene 2008;S31-42.
43. Findik S, Findik S, Abuoğlu S, et al. Human papillomavirus (HPV) subtypes and their relationships with cervical smear results in cervical cancer screening: a community-based study from the central Anatolia region of Turkey [Internet]. Int J Clin Exp Pathol 2019;12.
44. Rooper LM, Windon MJ, Hernandez T, et al. HPV-positive Squamous Cell Carcinoma of the Larynx, Oral Cavity, and Hypopharynx. Am J Surg Pathol 2020;1.
45. Yang L-Q, Xiao X, Li C-X, et al. Human papillomavirus genotypes and p16 expression in oral leukoplakia and squamous cell carcinoma. Int J Clin Exp Pathol 2019;12:1022-8.
46. Shillitoe EJ. The Microbiome of Oral Cancer. Crit Rev Oncog 2018;23:153-60.
47. Gholizadeh P, Eslami H, Yousefi M, et al. Role of oral microbiome on oral cancers, a review. Biomed Pharmacother 2016;84:552-8.
48. Chang F, Syrjänen S, Kellokoski J, Syrjänen K. Human papillomavirus (HPV) infections and their associations with oral disease. J Oral Pathol Med 1991;20:305-17.
49. Chen S-F, Yu F-S, Chang Y-C, et al. Role of human papillomavirus infection in carcinogenesis of oral squamous cell carcinoma with evidences of prognostic association. J Oral Pathol Med 2012;41:9-15.
50. Sankaranarayanan R, Mathew B, Varghese C, et al. Chemoprevention of oral leukoplakia with vitamin A and beta carotene: An assessment. Eur J Cancer Part B Oral Oncol 1997;33:231-6.
51. Rajesh D, Azeem Mohiyuddin SM, Moideen Kutty AV, Balakrishna S. Prevalence of human papillomavirus in oral squamous cell carcinoma: A rural teaching hospital-based cross-sectional study. Indian J Cancer 2017;54:498-501.
52. Arron ST, Ruby JG, Dybbro E, et al. Transcriptome sequencing demonstrates that human papillomavirus is not active in cutaneous squamous cell carcinoma. J Invest Dermatol 2011;131:1745-53.
53. Suzuki N, Yoneda M, Hirofuji T. Mixed red-complex bacterial infection in periodontitis [Internet]. Int J Dent 2013; 2013:587279.
54. Bodet C, Chandad F, Grenier D. Potentiel pathogénique de Porphyromonas gingivalis, Treponema denticola et Tannerella forsythia, le complexe bactérien rouge associé à la parodontite. Pathol Biol 2007;55:154-62.
55. Carter CJ, France J, Crean SJ, Singhrao SK. The Porphyromonas gingivalis/Host Interactome shows enrichment in GWASdb genes related to Alzheimer's disease, diabetes and cardiovascular diseases. Front Aging Neurosci 2017;9(DEC).
56. Hayashi K, Hasegawa Y, Takemoto Y, et al. Continuous intracerebroventricular injection of Porphyromonas gingivalis lipopolysaccharide induces systemic organ dysfunction in a mouse model of Alzheimer's disease. Exp Gerontol 2019;120:1-5.
57. Dominy SS, Lynch C, Ermini F, et al. Porphyromonas gingivalis in Alzheimer's disease brains: Evidence for disease causation and treatment with small-molecule inhibitors. Sci Adv 2019;5.
58. Adams B, Nunes JM, Page MJ, et al. Parkinson's disease: A systemic inflammatory disease accompanied by bacterial inflammagens. Front Aging Neurosci 2019;10.

59. Bennett JP, Keeney PM, Brohawn DG. RNA Sequencing Reveals Small and Variable Contributions of Infectious Agents to Transcriptomes of Postmortem Nervous Tissues From Amyotrophic Lateral Sclerosis, Alzheimer's Disease and Parkinson's Disease Subjects, and Increased Expression of Genes From D. Front Neurosci 2019;13:235.
60. Akamatsu Y, Yamamoto T, Yamamoto K, et al. Porphyromonas gingivalis induces myocarditis and/or myocardial infarction in mice and IL-17A is involved in pathogenesis of these diseases. Arch Oral Biol 2011;56:1290-8.
61. Ashigaki N, Suzuki J-I, Ogawa M, et al. Periodontal bacteria aggravate experimental autoimmune myocarditis in mice. Am J Physiol Heart Circ Physiol 2013;304:H740-8.
62. Atarbashi-Moghadam F, Havaei SR, Havaei SA, et al. Periopathogens in atherosclerotic plaques of patients with both cardiovascular disease and chronic Periodontitis. ARYA Atheroscler 2018;14:53-7.
63. Kramer CD, Simas AM, He X, et al. Distinct roles for dietary lipids and Porphyromonas gingivalis infection on atherosclerosis progression and the gut microbiota. Anaerobe 2017;45:19-30.
64. Li L, Michel R, Cohen J, et al. Intracellular survival and vascular cell-to-cell transmission of Porphyromonas gingivalis. BMC Microbiol 2008;8:26.
65. Harding A, Robinson S, Crean S, Singhrao SK. Can Better Management of Periodontal Disease Delay the Onset and Progression of Alzheimer's Disease? J Alzheimer's Dis 2017;58:337-48.
66. Sochocka M, Zwolińska K, Leszek J. The Infectious Etiology of Alzheimer's Disease. Curr Neuropharmacol 2017;15.
67. Kempsell KE, Cox CJ, Hurle M, et al. Reverse transcriptase-PCR analysis of bacterial rRNA for detection and characterization of bacterial species in arthritis synovial tissue. Infect Immun 2000;68:6012-26.
68. Moen K, Brun JG, Valen M, et al. Synovial inflammation in active rheumatoid arthritis and psoriatic arthritis facilitates trapping of a variety of oral bacterial DNAs. Clin Exp Rheumatol 2006;24:656-63.
69. Nakano K, Inaba H, Nomura R, et al. Distribution of Porphyromonas gingivalis fimA genotypes in cardiovascular specimens from Japanese patients. Oral Microbiol Immunol 2008;23:170-2.
70. Lafuente Ibáñez de Mendoza I, Maritxalar Mendia X, García de la Fuente AM, et al. Role of *Porphyromonas gingivalis* in oral squamous cell carcinoma development: A systematic review. J Periodontal Res 2020;55:13-22.
71. Sun C-H, Li B-B, Wang B, et al. The role of Fusobacterium nucleatum in colorectal cancer: from carcinogenesis to clinical management. Chronic Dis Transl Med 2019;5:178-87.
72. Ercibengoa M, Goenaga MA, Ardanuy C, et al. Epidemiological and clinical characteristics of Streptococcus tigurinus endocarditis. BMC Infect Dis 2019;19.
73. Esberg A, Sheng N, Mårell L, et al. Streptococcus Mutans Adhesin Biotypes that Match and Predict Individual Caries Development. EBioMedicine 2017;24:205-15.
74. McAuliffe A, Bhat V, Cox J. Streptococcus gallolyticus group bacteremia and colonic adenocarcinoma. J Am Osteopath Assoc 2019;119:65.
75. Butt J, Blot WJ, Teras LR, et al. Antibody responses to Streptococcus Gallolyticus subspecies Gallolyticus proteins in a large prospective colorectal cancer cohort consortium. Cancer Epidemiol Biomarkers Prev 2018;27:1186-94.
76. Mager DL, Haffajee AD, Delvin PM, et al. The salivary microbiota as a diagnostic indicator of oral cancer: A descriptive, non-randomized study of cancer-free and oral squamous cell carcinoma subjects. J Transl Med 2005;3.
77. Nagy KN, Sonkodi I, Szöke I, et al. The microflora associated with human oral carcinomas. Oral Oncol 1998;34:304-8.
78. Geng F, Liu J, Guo Y, et al. Persistent exposure to Porphyromonas gingivalis promotes proliferative and invasion capabilities, and tumorigenic properties of human immortalized oral epithelial cells. Front Cell Infect Microbiol 2017;7(FEB).
79. Choi CH, Spooner R, Deguzman J, et al. Porphyromonas gingivalis-nucleoside-diphosphate-kinase inhibits ATP-induced reactive-oxygen-species via P2X7 receptor/NADPH-oxidase signalling and contributes to persistence. Cell Microbiol 2013;15:961-76.
80. Grivennikov SI, Greten FR, Karin M. Immunity, Inflammation, and Cancer. Cell 2010;140:883-99.
81. Culotta E, Koshland DEJ. NO news is good news [see comments]. Science 1992;258:1862-5.
82. Korde Choudhari S, Chaudhary M, Bagde S, Gadbail AR, Joshi V. Nitric oxide and cancer: A review. World J Surg Oncol 2013;11.
83. Brennan PA, Umar T, Wilson AW, Mellor TK. Expression of type 2 nitric oxide synthase and vascular endothelial growth factor in oral dysplasia. J Oral Maxillofac Surg 2002;60:1455-60.
84. Shang ZJ, Li JR, Li ZB. Effects of exogenous nitric oxide on oral squamous cell carcinoma: An in vitro study. J Oral Maxillofac Surg 2002;60:905-10.
85. Bahar G, Feinmesser R, Shpitzer T, et al. Salivary analysis in oral cancer patients: DNA and protein oxidation, reactive nitrogen species, and antioxidant profile. Cancer 2007;109:54-9.
86. Mishra A, Mishra A. Estimation of Salivary Nitric Oxide Level in Various Stages of Precancerous Lesion. Int J Oral Care Res 2018;6:56-9.
87. Deng D-J. progress of gastric cancer etiology: N-nitrosamides 1999s. World J Gastroenterol 2000;6:613-8.
88. Li H, Duncan C, Townend J, et al. Nitrate-reducing bacteria on rat tongues. Appl Environ Microbiol 1997;63:924-30.
89. Palmerini CA, Palombari R, Perito S, Arienti G. NO synthesis in human saliva. Free Radical Res 2003;37:29-31.
90. Hezel MP, Weitzberg E. The oral microbiome and nitric oxide homoeostasis. Oral Dis 2015;21:7-16.
91. Zetterquist W, Pedroletti C, Lundberg JON, Alving K. Salivary contribution to exhaled nitric oxide. Eur Respir J 1999;13:327-33.
92. Schreiber F, Stief P, Gieseke A, et al. Denitrification in human dental plaque. BMC Biol 2010;22:8.
93. Silvestrini MC, Falcinelli S, Ciabatti I, et al. Pseudomonas aeruginosa nitrite reductase (or cytochrome oxidase): An overview. Biochimie 1994;76:641-54.