The Open Dentistry Journal

Content list available at: <https://opendentistryjournal.com>

REVIEW ARTICLE

The Role of Periodontopathogens and Oral Microbiome in the Progression of Oral Cancer. A Review

Julián F. Beltrán¹, SM Viafara-García^{2,3,4}, Alberto P. Labrador^{5,*} and Johan Basterrechea⁶

¹Department of Odontopediatric, School of Dentistry, UNICOC University Colegio Odontológico, Bogotá, Colombia

²Unit of Basic Oral Investigation, Faculty of Dentistry (UIBO), Universidad El Bosque, Bogotá, Colombia

³Cellular and Molecular Immunology Group, Faculty of Dentistry (INMUBO), Universidad El Bosque, Bogotá, Colombia

⁴Centro de Investigación e Innovación Biomédica (CIIB), Universidad de los Andes, Santiago, Chile

⁵Department of Oral Surgery and oral Pathology, Centro de Odontología Integral (COI) Acarigua-Portuguesa, Venezuela

⁶Department of Oral and Maxillofacial Surgery, Borden Dental Clinic, Chia, Colombia

Abstract:

Chronic periodontal disease and oral bacteria dysbiosis can lead to the accumulation of genetic mutations that eventually stimulate Oral Squamous Cell Cancer (OSCC). The annual incidence of OSCC is increasing significantly, and almost half of the cases are diagnosed in an advanced stage. Worldwide there are more than 380,000 new cases diagnosed every year, and a topic of extensive research in the last few years is the alteration of oral bacteria, their compositional changes and microbiome. This review aims to establish the relationship between bacterial dysbiosis and OSCC. Several bacteria implicated in periodontal disease, including *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia*, and some *Streptococcus* species, promote angiogenesis, cell proliferation, and alteration in the host defense process; these same bacteria have been present in different stages of OSCC. Our review showed that genes involved in bacterial chemotaxis, the lipopolysaccharide (LPS) of the cell wall membrane of gram negatives bacteria, were significantly increased in patients with OSCC. Additionally, some bacterial diversity, particularly with *Firmicutes*, and *Actinobacteria* species, has been identified in pre-cancerous stage samples. This review suggests the importance of an early diagnosis and more comprehensive periodontal therapy for patients by the dental care professional.

Keywords: Oral bacteria, Oral cancer, Chronic periodontitis, Oral microbiome, Oral dysbiosis, Anaerobic bacteria.

Article History

Received: November 29, 2020

Revised: May 27, 2021

Accepted: June 10, 2021

1. INTRODUCTION

Oral bacteria dysbiosis is known to act as a precursor for diseases such as periodontitis [1, 2]. This pathologic process, which involves chronic inflammatory responses that increase the possibility for tooth loss, can also affect systemic health because its accumulative effect increases the patients' risk for neoplastic processes [3 - 5]. Head and neck cancer is the sixth most common human cancer [6], representing 3% of all types of cancer. Lesions of the oral cavity represent 48% of head and neck cases, with 90% of oral cavity lesions being squamous cell carcinoma (OSCC), which is one of the most common causes of death among all oral cancers [7]. Prognosis is always dependent on age, lymph node involvement, tumor size and location [7]. The development of OSCC is statistically linked to several classical risk factors, including tobacco, alcohol abuse, Human Papillomavirus (HPV), poor hygiene and predi-

sposing conditions, which induce pro-oncogenic and epigenetic alterations [8 - 10]. However, other factors, including oral inflammatory diseases, infections, and bacterial dysbiosis, are recognized as risk factors for cancer development [11, 12].

Evidence has demonstrated that a combination of risk factors such as smoking, alcohol abused, and poor oral hygiene increases the risk of oral cancer by inducing chronic inflammation and infection; these risk factor modify the local microbiota and host interactions, altering homeostasis of the oral environment [11, 13 - 15]. On the other hand, the development of OSCC in subjects who had never used tobacco or alcohol suggests that chronic periodontitis might be an independent risk factors for cancer development [16]. The causal relationship between specific oral bacterial infections and the development of OSCC, has been supported by the presence of some oral periodontopathogens such as *Porphyromonas gingivalis* (*P. gingivalis*), or *Fusobacterium nucleatum* (*F. nucleatum*); these periodontopathogens are known to stimulate different cytokines which facilitate cell proliferation, mutagenesis, apoptosis inhibition, oncogene activation, and angiogenesis [15, 17 - 20].

* Address correspondence to this author at the Centro de Odontología Integral (COI) Acarigua-Portuguesa, Venezuela, Av 5 de Diciembre C.C Metropolitano local 6 zip code 3301, Acarigua-Portuguesa, Venezuela;
E-mail: ajpl26@yahoo.com

The most abundant microbiota is found in the gut, followed by the oral cavity; Oral Cancer (OC) has been associated with oral and gut microbiota dysbiosis [11, 12]. This complex interaction also lays the foundation of the crosstalk between oral microbiota and gut microbiota [21, 22]. Oral bacteria can be transported into the gut and change the gut microbiota affecting the immune defense system [23 - 25], especially in patients diagnosed with chronic periodontitis, comorbidities, systemic diseases and immunocompromised individuals [3, 26, 27]. Therefore, oral microbiota is emerging as a complex factor influencing oral microenvironments, oral diseases and OSCC, but also affecting the gut microbiota in both direct and indirect ways [11, 21]. The most representative bacteria associated with OSCC are *F. nucleatum*, *P. gingivalis*, and *Prevotella intermedia* [17, 28 - 31]. Furthermore, other bacterial species, such as *Actinomyces*, *Clostridium*, *Enterobacteriaceae*, *Fusobacterium*, *Haemophilus*, *Porphyromonas*, *Prevotella*, *Streptococcus spp.* and *Veillonella* have been associated with pre-cancerous lesions and OSCC [31, 32]. However, the cellular and molecular events that these bacteria promote in the host are not totally understood.

To understand the influence in the oral carcinogenesis process associated with oral dysbiosis, a narrative review was performed to summarize the microbiological, cellular, molecular and clinical aspects from *in-vitro* and clinical studies, thus providing an updated and structured overview of the oral microbiota and oral cancer relationship.

2. THE CLINICAL ASSOCIATION BETWEEN PERIODONTAL DISEASES AND ORAL CANCER

OSCC constitutes almost 90% of all oral cancers and is the leading cause of death among all oral cancers [33]. OSCC originates from the oral epithelial mucosa with more than 380,000 cases reported annually worldwide and more than 175,000 recorded deaths worldwide in 2018 [34]. More than 50% of the cases are diagnosed in advanced stages [35]. It occurs more frequently in men, with a higher incidence in South Asia, Western and Eastern Europe [36]. In Colombia, more than 140 to 160 new cases of OSCC occur annually, with 50% occurring in the tongue, and it is threefold more frequent in men than in women [37].

Chronic Periodontal Disease (CPD) is highly prevalent in adults, and its severity increases with age. The relationship between CPD and oral cancer has been examined for some years. Numerous case-controlled studies have addressed the role of CPD in head and neck cancer, and several cohort studies have examined its association with other types of cancers [38]. PubMed, Embase, Science direct, and Scopus searches were performed using keywords such as “oral bacteria” OR “oral cancer” AND “dysbiosis” OR “chronic periodontal disease” AND “oral microbiome”. This review included studies only published in English.

To date, six meta-analyses or reviews have been published about the associations between periodontal disease and/or tooth loss, and the risk of head and neck cancers [38 - 44] (no

randomized controlled trials exist, with relevant exposure categories and outcomes). In fact, given the importance of smoking as a potential promoter for OSCC, only papers with estimated adjustment for smoking were considered [38].

Only one cohort study reports smoking-adjusted associations for periodontal disease and head and neck cancer, which showed low risk, and was not statistically significant (HR = 1.15, 95% CI: 0.73, 1.81) [1]. In the same cohort, after the analyses were limited to non-smokers, a strong association was observed for periodontitis, oropharyngeal and esophageal cancers combined (HR = 2.25, 95% CI: 1.30, 3.90) [44]. Regarding the number of teeth, many studies have been conducted to evaluate general oral health concerning to head, neck and esophageal cancers. However, most of these reports are case-controlled studies with several limitations, including large clinical heterogeneity and incompatible measures of periodontal disease; this has led to the impossibility of conducting accurate and feasible meta-analysis studies. Therefore, cohort studies of periodontitis using standardization of measurements are required to overcome statistical heterogeneity, publication bias and not unified adjusted factors. Although recent studies with improved measurements of periodontitis support strong positive associations for periodontitis and oral cancer risk [45], well-designed cohort studies are still needed to determine a more accurate relationship between these entities [38, 39].

2.1. Oral and Subgingival Dysbiosis in Periodontitis and Cancer

The onset of oral diseases as CPD involves significant changes in the composition of subgingival bacterial communities. Chronic inflammation and the anaerobic nature of the periodontal pocket results in gradual progression to subgingival dysbiosis, which is significantly promoted by keystone pathogens as *P. gingivalis*. In a murine study, it has been established that *P. gingivalis*-induced periodontitis requires the presence of the commensal microbiota because *P. gingivalis* alone is unable to cause periodontitis despite colonizing this host [40 - 46]. In addition, *P. gingivalis* is also influenced by the inflammatory environment orchestrated by pathobiont, such as *F. nucleatum*, *Prevotella spp.*, and even commensal bacteria such as *Streptococcus gordonii*, *Firmicutes* and *Actinobacteria* [47 - 49].

Significant shifts in the composition of the oral microbiome have been observed in oral cancers [50 - 57]. Several oral commensal bacteria, and keystone pathogens are correlated with OSCC and have been isolated from tumoral microenvironments (Table 1) [17, 18, 56, 58]. In this regard, three key microorganisms have been identified as being involved in subgingival dysbiosis, *F. nucleatum*, *P. gingivalis*, and *Prevotella intermedia* which are also considered the most representative types of bacteria in OSCC [17, 28 - 31, 59 - 63], (Fig. 1); An interesting feature is that *P. gingivalis* appears to occur in the early stage of OSCC [53], and *F. nucleatum* has also been identified as a key player in non-oral cancers (gastric, digestive, colorectal and pancreatic cancer) [64 - 67].

Table 1. The composition of the oral microbiome in oral cancers.

Cancer (Subjects)	Bacteria Detected	Method	Site Isolation	Ref	Country, Year
OSCC (46)	<i>Streptococcus anginosus</i>	PCR	Intra tumoral & Dental Plaque	[65]	2005
OSCC (45)	<i>Capnocytophaga gingivalis</i> , <i>Prevotella melaninogenica</i> <i>Streptococcus mitis</i> ,	DNA-DNA hybridization.	Saliva	[30]	2005
OSCC (125)	<i>Bacillus</i> , <i>Enterococcus</i> , <i>Parvimonas</i> , <i>Peptostreptococcus</i> ,	16S rRNA sequencing	Saliva	[66]	Taiwan, 2017
OSCC (10)	<i>Streptococcus sp.</i> , <i>Peptostreptococcus</i> , <i>Gemella spp.</i> , <i>Johnsonella ignava</i>	16S rRNA sequencing	Intra tumoral	[67]	USA 2012
OSCC(5)	<i>Streptococcus</i> and <i>Rothia</i>	16S rRNA sequencing	Intra tumoral	[54]	USA 2014
OSCC (10)	<i>P. gingivalis</i>	immunohistochemical staining	Intra tumoral	[29]	USA 2011
OSCC (30)	<i>Streptococcus spp</i>	Bacterial culture	Lymph nodes	[60]	Japan 1999
OSCC (6)	<i>P. gingivalis</i> and <i>F. nucleatum</i>	16S rRNA sequencing	Intra tumoral and subgingival plaque	[2]	Germany 2019
OSCC (60)	<i>T. denticula</i>	Immunohistochemical staining	Intratumoral	[61]	Finland 2018
OSCC (197)	<i>Fusobacterium periodonticum</i> , <i>Parvimonas micra</i> , <i>Streptococcus constellatus</i> , <i>Haemophilus influenza</i> , and <i>Filifactor</i>	16S rRNA sequencing	Saliva	[53]	Taiwan 2018
OSCC (40)	<i>Fusobacterium</i> , <i>Dialister</i> , <i>Peptostreptococcus</i> , <i>Filifactor</i> , <i>Peptococcus</i> , <i>Catonella</i> and <i>Parvimonas</i>	16S rRNA sequencing	Intra tumoral	[49]	China 2017
OSCC (7852)	<i>P. gingivalis</i>	Serum IgG antibody	Blood	[62]	USA 2012
OSCC (21)	<i>Veillonella</i> , <i>Fusobacterium</i> , <i>Prevotella</i> , <i>Porphyromonas</i> , <i>Actinomyces</i> and <i>Clostridium</i> (anaerobes), and <i>Haemophilus</i> , <i>Enterobacteriaceae</i> and <i>Streptococcus spp.</i>	Bacterial culture	Intra tumoral	[31]	Hungary 1998

Composition of oral microbiome obtained from samples of saliva, blood, tumor, and subgingival plaque in patients with

OSCC. We highlight *P.* and *F. nucleatum* as the main ones (Fig. 1).

Fig (1). Composition of oral microbiome obtained from samples of saliva, blood, tumor and subgingival plaque in patients with OSCC.

3. ORAL MICROBIOME AND ITS RELATIONSHIP WITH ORAL CANCER STAGES

In terms of the composition of the oral microbiota, *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, *Fusobacteria*, and *Actinobacteria* were the five dominant phylum in the oral cavity. Six families (*Prevotellaceae*, *Fusobacteriaceae*, *Flavobacteriaceae*, *Lachnospiraceae*, *Peptostreptococcaceae*, and *Campylobacteraceae*) and 13 genera, including *Fusobacterium*, *Alloprevotella*, and *Porphyromonas*, were increased in OSCC. Species like *F. nucleatum*, *P. intermedia*, *Aggregatibacter segnis*, *Peptostreptococcus stomatis*, and *Catonella morbi*, showed a significant increase in oral cancer lesions [49 - 52]. Interestingly, *Streptococcus*, along with *Actinobacteria* (early oral colonizers), decrease the proinflammatory response orchestrated by *F. nucleatum* in the epithelial cells. Moreover, low levels of *Firmicutes* and *Actinobacteria* also promote a pro cancer environment [59]. Noteworthy, *F. nucleatum* increases substantially in OSCC cases [50, 68].

Different bacteria have been identified at different oral cancer stages. The clinical cancer stages of the 7th AJCC [69], and the oral microbiome present at each stage are depicted in Fig. (2). Yang C, et al. 2018, analyzed the microbiota in 248 samples from oral rinses that were collected within 1-3 weeks after the disease diagnosis in 51 healthy individuals and 197 OSCC patients at different stages, 53 using 16S rRNA V3V4 amplicon sequencing [53]. *Fusobacteria* increased significantly with the progression of oral cancer in comparison to healthy

controls (2.98%) compared to OSCC stage 1 (4.35% $p = 0.0015$), stages 2 and 3 (6.24%, $p < 0.0001$), and compared to stage 4 (7.92% $p < 0.0001$), *Fusobacteria* was the most abundant in stage 4 [53]. As oral cancer progresses, *Bacteroidetes* and *Actinobacteria* decrease from stage 1 to stage 4 [67]. The main bacteria in OSCC stage 4 were: *Streptococcus* (28.21%), *Veillonella* (11.01%), *Neisseria* (9.65%), *Haemophilus* (9.37%), and *Rothia* (4.42%). The genera that increased the most was (*Fusobacterium*) ($p < 0.0001$), and the genera that decreased were: *Streptococcus*, *Haemophilus*, *Porphyromonas*, and *Actinomyces* ($p < 0.0001$) [53]. *Firmicutes* were the most dominant phylum in the oral rinse samples; being found in 58.40% of healthy patients, 59.65% in OSCC stage 1 patients, 59.76% in stage 2 and 3 patients, and 58.43% in OSCC stage 4.53 (Fig. 2).

4. THE ROLE OF THE ORAL MICROBIOME ON CARCINOGENESIS ASSOCIATED WITH ALCOHOL OR TOBACCO

Tobacco and alcohol are the main etiological factors in the development of OSCC [70 - 72]. Tobacco contains several carcinogenic molecules, especially polycyclic hydrocarbon, nitrosamines such as N'-nitrosornicotine (NNN) and nitrosamine 4- (methyl nitrosamine) -1 (-3-pyridyl) -1-butanone (NNK) [73]. The aforementioned molecules are known to be involved in DNA damage, interfering with replication, transcription and DNA repair. The nitrosamines induce chronic inflammatory processes due to the impairment of the defense mechanisms of the host and its microbiome [73 - 76].

Presence of bacteria in healthy controls and at different stages in oral cancer

Fig. (2). Oral bacteria stages in oral cancer progression.

Tobacco also promotes alterations in the oral microbiota, causing a decrease in bacteria such as *Granulicatella*, *Staphylococcus*, *Veillonella*, *Capnocytophaga* and an even greater decrease in *Peptostreptococcus* [77 - 80]; furthermore, species like *Neisseria* are not only inhibited by tobacco but also by alcohol consumption [78].

Tobacco use has been associated with the alteration of the surfaces of oral epithelial cells, allowing the growth of certain pathogenic bacteria [81, 82]. Showing significant increases of *Prevotella parvimonas*, *Fusobacterium*, *Campylobacter*, *Bacteroides*, *Dialister*, *Treponema spp* and *Porphyromonas*, the oral microbiota have been identified with tobacco use [79, 80, 83]. Tobacco consumption affects bacterial growth in several ways, altering the cytotoxic effects on some bacteria [84], impairing saliva-bacterial interaction [85], and producing ethanol as a substrate for bacterial metabolism [86].

Acetaldehyde, the first metabolite of ethanol, is derived from alcohol consumption; its presence is associated with cellular damage, affecting the DNA repair process and has the potential to produce mutations in the DNA. Thus, acetaldehyde is considered a mutagenic and carcinogenic element. It has been associated with liver and upper aerodigestive tract cancer [85, 87].

In contrast alcohol dehydrogenase 2 (ALDH2) is an enzyme that decontaminates acetaldehyde products, but 2(ALDH2) polymorphisms may modify the association between alcohol use and pathogenic periodontal bacteria, as indicated by a higher percentage of pathogenic periodontal bacteria in saliva [88]. Some bacteria, such as *Neisseria*, *Streptococcus* and *Actinomyces*, reportedly have the ability to produce acetaldehyde [87, 89], although this is in contrast with a study by Thomas and Yokohama, which reported a low capacity for acetaldehyde production from these bacteria [78, 90]. Other bacterial species such as *S. gordonii*, *S. mitis*, *S. oralis*, *S. salivarius*, *S. sanguinis*, *R. mucilaginosa*, *P. histicola*, *S. salivarius* and *R. mucilaginosa*, also have the capacity to produce acetaldehyde [89, 91, 92]. It has also been demonstrated that the alteration of the oral microbiome with alcohol, along with poor hygiene, enrich bacterial species such as *Aggregatibacter*, *Actinomyces*, *Kingella*, *Leptotrichia*, *Cardiobacterium*, *Bacteroidales* and *Prevotella*; while at the same time decreasing bacterial families such as *Lactobacillales*, *Bacteroidetes*, *Firmicutes* and *Peptostreptococcaceae* [92].

4.1. Molecular Mechanisms Implicated in the Pathogenesis of Oral Cancer

OSCC is a multiple-step molecular processes that develops from an individual genetic predisposition and/or exposure to external carcinogens. Some bacteria appear to be more prone to produce such changes. TP53 is a tumor suppressor gene that prevents carcinogenesis by activating G1 cell cycle arrest and promoting a p53 product [93], which induces apoptosis. However, 40–70% of oral cancers have mutations in the TP53 gene, with more than 90% of these mutations between exons 5 and 8 of TP [53, 93, 94]. These mutations can be separated into 2 categories: 1) disruptive, promoting an alteration in the DNA binding domains or damaging the p53 gene, with a subsequent

failure of apoptosis and cell cycle arrest [95], and 2) non-disruptive, which only partially affects p53 function [96], producing deficient DNA repair, facilitating genomic instability, cellular proliferation, invasion, migration and dysregulating metabolism, ultimately leading to therapeutic resistance of cancer cells [97]. Another process is the loss of chromosomal region 9p21, which occurs in 70-80% of dysplastic lesions of the oral mucosa, being an early event in oral cancer [98]. The p16 tumor suppressor gene binds to the cyclin-dependent kinases CDK4 and CDK6, inhibiting cellular proliferation and avoiding synthesis phase entry in the cell cycle [99]. Expression of p16 is lost in 83% of oral cancers and 60% of premalignant lesions, heralding a poor prognosis [99]. On the other hand, Retinoblastoma 1 (RB1) is a tumor suppressor gene belonging to the RB family, which regulates the cell cycle at the G1-S checkpoint [100]. This gene is located on the long arm of the chromosome [16], an area with almost 17% of loss of heterozygosity in oral cancer [100, 101]. When hypophosphorylated it inhibits E2F activation, whereas its hyperphosphorylation by cyclin-dependent kinase complexes leads to pRb inactivation, releasing E2F activating target genes (c-MYC, n-MYC, CDC-2, p21WAF-1, cyclin A, c-MYB, and EGFR) involved in DNA synthesis, cell cycle regulation and cell growth [102]. This process can be altered by HPV E7 oncogenes, dysregulating E2F1 and promoting inhibition of apoptosis, and the p53 mediated pathway, facilitating cancer cell progression [103]. Epidermal growth factor receptor (EGFR/ErbB1/HER1) is a proto-oncogene that belongs to the tyrosine kinase receptor family [104]. Somatic mutations in EGFR are reported in over 90% of HNSCC and are linked with the inhibition of apoptosis in tumor cells, increased cell growth, angiogenesis and metastasis with poor prognosis [105]. Almost 3% of oral cancers have mutations in the tyrosine kinase domain of EGFR [106]. The Bcl-2 family comprises at least 15 proteins with either anti-apoptotic (Bcl-2, Bcl-XL) or pro-apoptotic effects (Bax, Bak). Alterations in the ratio of pro-apoptotic to antiapoptotic proteins promote tumor progression and can lead to oncologic therapy resistance [107]. Rat sarcoma virus (RAS) is a protooncogene, that is frequently mutated in oral cancer and the mutations occur mostly in H-Ras [108], being more common in smokers, even in some ethnic particular groups [109]. PTEN/mTOR/AKT/PI3K pathway appears to be a repeatedly dysregulated pathway in oral cancer, inducing some signaling pathways that suppress autophagy, thus reducing cell death [110]. These disorganized events result in activation of several intracellular messenger signaling systems, with an increased production of growth factors, along with evading inhibitory signals, leading to autonomous growth of tumor cells.

5. CELLULAR AND MOLECULAR PRINCIPLES OF MICROBIAL CARCINOGENESIS FOR *PORPHYROMONAS GINGIVALIS*

In primary cultures, *P. gingivalis* inhibited apoptosis by up-regulating the anti-apoptotic molecule Bcl-2 and down-regulating the pro-apoptotic Bax protein [111], thus promoting survival of primary gingival epithelial cells through activation of the pPI3K/Akt pathway [112], also improved the levels of PI3K and phosphoinositide-dependent protein-serine kinase 1

(PKD1) activated by Akt, promoting cell proliferation [113]. Furthermore, *P. gingivalis* aids gingival epithelial cells (GEP) progression through the G1 phase with less control, and more speed by down-regulating kinases such as Chk2, CK1delta, and CK1epsilon [114]. The suppression of the proapoptotic activity of Bax (Bcl-2-associated death promoter), results in blockage of the apoptosis effector cytochrome C [115], leading downstream inhibition of caspase-9 and the executioner caspase-3 [115, 116]. Hoppe *et al.* found an increase in tumor cell proliferation with *P. gingivalis* dysregulates α -defensins [117]. *P. gingivalis* dysregulates some genes that intervene at the proinflammatory pathway of NF- κ B with members of the MAPK family (like MAPK14 (p38), MAPK8 (JNK1), and NFKB1 (p50), which could promote cancer progression [118]. *P. gingivalis* activates a nucleoside diphosphate kinase (NDK), inhibiting apoptosis through purinergic receptor P2X7 in GEP [119]; NDK might also interfere with an anticancer immune response mediated by ATP activation of P2X7 receptors on dendritic cells [18, 120]. In this regard, the regulation of the cell cycle by miR-203 may affect the pathological expression in some carcinomas [121]. In addition, co-infection with *P. gingivalis* and *F. nucleatum* stimulates progression of chemically induced oral cancer in a murine model, via activation of the IL6/STAT3 axis; the suppression of mitochondrial-dependent apoptosis may be an important stratagem for the survival of *P. gingivalis* in periodontal tissues and a key feature of its pathogenicity [122].

6. CELLULAR AND MOLECULAR PRINCIPLES OF MICROBIAL CARCINOGENESIS FOR FUSOBACTERIUM NUCLEATUM

F. nucleatum LPS contain heptose, and 2-keto-3-deoxyoctonate which may inhibit the intrinsic apoptotic pathway of oral epithelial cells [20]. Moreover, *F. nucleatum* enhance a pro-inflammatory response NF- κ B gene activation and mitogen-activated protein kinase (p38). Accordingly, it drives the production of inflammatory cytokines, such as IL-1 α , IL-1 β , IL-6, IL-8, and MMPs, specially MMP13 (collagenase 3) [121 - 124]. *In vitro* studies have shown that *F. nucleatum* triggers Toll-Like Receptor (TLR) activation, stimulating IL-6 production, and activating STAT3, which induced cancer cells to grow by the cyclin D1 effector [123 - 125]. Interestingly, IL-6 was reported to induce vascular endothelial growth factor (VEGF) production in OSCC [126]. Thus, both *P. gingivalis* and *F. nucleatum* promote resistance to apoptosis by the activation of TLR2, which was seen more frequently in OSCC tissues than in normal tissues [125, 126]. *F. nucleatum* promotes cellular migration through stimulation of Etk/BMX, S6 kinase p70, and RhoA kinase [121, 124]. On the other hand, *F. nucleatum* promoted E-cadherin-expressing colorectal cancer cell (CRC) proliferation by modulating the E-cadherin/ β -catenin signaling pathway or by activating TLR4 signaling to NF- κ B and upregulating the expression of microRNA-21 [127 - 129]. *F. nucleatum* induces apoptosis in lymphocytes, an ability mediated by heat-labile outer membrane protein(s), thus allowing the microorganisms to evade the immune system [130] (Fig. 3).

Fig. (3). Molecular process in oral bacteria promoting the evolution of cancer.

CONCLUSION

Chronic periodontal disease facilitates bacteria to alter the microenvironment in OSCC. The resulting inflammatory process modifies the immune response, inhibits cell apoptosis, stimulates angiogenesis, increases the IL-6 or TNF- α effects, and alters the cell cycle function. It has been found that bacteria such as *P. gingivalis*, or *F. nucleatum* differ quantitatively in each cancer stage, which could open an opportunity to consider these bacteria as possible diagnostic or prognostic markers in the management of OSCC. Moreover, oral native bacteria, such as *Streptococcus* and *Neisseria* could produce acetaldehyde affecting epithelial cell phenotype and its protective functions. This review also suggests the importance of raising awareness of prompt periodontal therapy and maintaining good oral hygiene by the dental care professional as a strategy for decreasing cancer risk. In summary, oral dysbiotic communities could promote oral cancer progression. However, cohort studies are still required to overcome statistical heterogeneity, bias, and confusion factors.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

We want to thank Dr. Octavio Gonzalez from Kentucky University for his support and motivation with this research. And to Dr. Harvey Kessler (Retired) from Texas A&M School of Dentistry for his academic collaboration and loyal friendship.

REFERENCES

- Michaud DS, Liu Y, Meyer M, Giovannucci E, Josphura K. Periodontal disease, tooth loss and cancer risk in a prospective study of male health professionals. *Lancet Oncol* 2008; 9(6): 550. [http://dx.doi.org/10.1016/S1470-2045(08)70106-2] [PMID: 18462995]
- Chang C, Geng F, Shi X, *et al.* The prevalence rate of periodontal pathogens and its association with oral squamous cell carcinoma. *Appl Microbiol Biotechnol* 2019; 103(3): 1393-404. [http://dx.doi.org/10.1007/s00253-018-9475-6] [PMID: 30470868]
- Williams HK. Molecular pathogenesis of oral squamous carcinoma. *Mol Pathol* 2000; 53(4): 165-72. [http://dx.doi.org/10.1136/mp.53.4.165] [PMID: 11040937]
- Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *CA Cancer J Clin* 2009; 59(4): 225-49. [http://dx.doi.org/10.3322/caac.20006] [PMID: 19474385]
- Hunter KD, Parkinson EK, Harrison PR. Profiling early head and neck cancer. *Nat Rev Cancer* 2005; 5(2): 127-35. [http://dx.doi.org/10.1038/nrc1549] [PMID: 15685196]
- Ogden GR. Alcohol and oral cancer. *Alcohol* 2005; 35(3): 169-73. [http://dx.doi.org/10.1016/j.alcohol.2005.04.002] [PMID: 16054978]
- Warnakulasuriya S, Sutherland G, Scully C. Tobacco, oral cancer, and treatment of dependence. *Oral Oncol* 2005; 41(3): 244-60. [http://dx.doi.org/10.1016/j.oraloncology.2004.08.010] [PMID: 15743687]
- de Pablo P, Chapple IL, Buckley CD, Dietrich T. Periodontitis in systemic rheumatic diseases. *Nat Rev Rheumatol* 2009; 5(4): 218-24. [http://dx.doi.org/10.1038/nrrheum.2009.28] [PMID: 19337286]
- Benjamin RM. Oral health: the silent epidemic. *Public Health Rep* 2010; 125(2): 158-9. [http://dx.doi.org/10.1177/003335491012500202] [PMID: 20297740]
- Bhatt AP, Redinbo MR, Bultman SJ. The role of the microbiome in cancer development and therapy. *CA Cancer J Clin* 2017; 67(4): 326-44. [http://dx.doi.org/10.3322/caac.21398] [PMID: 28481406]
- La Rosa GRM, Gattuso G, Pedullà E, Rapisarda E, Nicolosi D, Salmeri M. Association of oral dysbiosis with oral cancer development. *Oncol Lett* 2020; 19(4): 3045-58. [http://dx.doi.org/10.3892/ol.2020.11441] [PMID: 32211076]
- Vivarelli S, Salemi R, Candido S, *et al.* Gut microbiota and cancer: From pathogenesis to therapy. *Cancers (Basel)* 2019; 11(1): 38. [http://dx.doi.org/10.3390/cancers11010038] [PMID: 30609850]
- Marshall JR, Graham S, Haughey BP, *et al.* Smoking, alcohol, dentition and diet in the epidemiology of oral cancer. *Eur J Cancer B Oral Oncol* 1992; 28B(1): 9-15. [http://dx.doi.org/10.1016/0964-1955(92)90005-L] [PMID: 1422474]
- Graham S, Dayal H, Rohrer T, *et al.* Dentition, diet, tobacco, and alcohol in the epidemiology of oral cancer. *J Natl Cancer Inst* 1977; 59(6): 1611-8. [http://dx.doi.org/10.1093/jnci/59.6.1611] [PMID: 926184]
- Lamont RJ, Koo H, Hajishengallis G. The oral microbiota: dynamic communities and host interactions. *Nat Rev Microbiol* 2018; 16(12): 745-59. [http://dx.doi.org/10.1038/s41579-018-0089-x] [PMID: 30301974]
- Tezal M, Sullivan MA, Hyland A, *et al.* Chronic periodontitis and the incidence of head and neck squamous cell carcinoma. *Cancer Epidemiol Biomarkers Prev* 2009; 18(9): 2406-12. [http://dx.doi.org/10.1158/1055-9965.EPI-09-0334] [PMID: 19745222]
- Atanasova KR, Yilmaz O. Looking in the Porphyromonas gingivalis cabinet of curiosities: The microbium, the host and cancer association. *Mol Oral Microbiol* 2014; 29(2): 55-66. [http://dx.doi.org/10.1111/omi.12047] [PMID: 24506890]
- Whitmore SE, Lamont RJ. Oral bacteria and cancer. *PLoS Pathog* 2014; 10(3): e1003933. [http://dx.doi.org/10.1371/journal.ppat.1003933] [PMID: 24676390]
- Vesty A, Gear K, Biswas K, Radcliff FJ, Taylor MW, Douglas RG. Microbial and inflammatory-based salivary biomarkers of head and neck squamous cell carcinoma. *Clin Exp Dent Res* 2018; 4(6): 255-62. [http://dx.doi.org/10.1002/cre2.139] [PMID: 30603107]
- Chattopadhyay I, Verma M, Panda M. Role of oral microbiome signatures in diagnosis and prognosis of oral cancer. *Technol Cancer Res Treat* 2019; 181533033819867354. [http://dx.doi.org/10.1177/1533033819867354] [PMID: 31370775]
- Hajishengallis G. Periodontitis: From microbial immune subversion to systemic inflammation. *Nat Rev Immunol* 2015; 15(1): 30-44. [http://dx.doi.org/10.1038/nri3785] [PMID: 25534621]
- Olsen I, Yamazaki K. Can oral bacteria affect the microbiome of the gut? *J Oral Microbiol* 2019; 11(1): 1586422. [http://dx.doi.org/10.1080/20002297.2019.1586422] [PMID: 30911359]
- Olsen I. From the acta prize lecture 2014: The periodontal-systemic connection seen from a microbiological standpoint: Summary of the acta odontologica scandinavia prize lecture 2014 presented at the meeting of the IADR/pan european region in dubrovnik, september 10-13. 2014. *Acta Odontol Scand* 2015; 73(8): 563-8. [http://dx.doi.org/10.3109/00016357.2015.1007480] [PMID: 25891035]
- Seedorf H, Griffin NW, Ridaura VK, *et al.* Bacteria from diverse habitats colonize and compete in the mouse gut. *Cell* 2014; 159(2): 253-66. [http://dx.doi.org/10.1016/j.cell.2014.09.008] [PMID: 25284151]
- Segata N, Haake SK, Mannon P, *et al.* Composition of the adult digestive tract bacterial microbiome based on seven mouth surfaces, tonsils, throat and stool samples. *Genome Biol* 2012; 13(6): R42. [http://dx.doi.org/10.1186/gb-2012-13-6-r42] [PMID: 22698087]
- von Troil-Lindén B, Torkko H, Alaluusua S, Jousimies-Somer H, Asikainen S. Salivary levels of suspected periodontal pathogens in relation to periodontal status and treatment. *J Dent Res* 1995; 74(11): 1789-95. [http://dx.doi.org/10.1177/00220345950740111201] [PMID: 8530742]
- Saygun I, Nizam N, Keskiner I, *et al.* Salivary infectious agents and periodontal disease status. *J Periodontal Res* 2011; 46(2): 235-9.

- [<http://dx.doi.org/10.1111/j.1600-0765.2010.01335.x>] [PMID: 21261620]
- [28] Yilmaz Ö. The chronicles of *Porphyromonas gingivalis*: The microbiom, the human oral epithelium and their interplay. *Microbiology* 2008; 154(Pt 10): 2897-903. [<http://dx.doi.org/10.1099/mic.0.2008/021220-0>] [PMID: 18832296]
- [29] Katz J, Onate MD, Pauley KM, Bhattacharyya I, Cha S. Presence of *Porphyromonas gingivalis* in gingival squamous cell carcinoma. *Int J Oral Sci* 2011; 3(4): 209-15. [<http://dx.doi.org/10.4248/IJOS11075>] [PMID: 22010579]
- [30] Mager DL, Haffajee AD, Devlin PM, Norris CM, Posner MR, Goodson JM. 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(1): 27. [<http://dx.doi.org/10.1186/1479-5876-3-27>] [PMID: 15987522]
- [31] Nagy KN, Sonkodi I, Szöke I, Nagy E, Newman HN. The microflora associated with human oral carcinomas. *Oral Oncol* 1998; 34(4): 304-8. [[http://dx.doi.org/10.1016/S1368-8375\(98\)80012-2](http://dx.doi.org/10.1016/S1368-8375(98)80012-2)] [PMID: 9813727]
- [32] Hu X, Zhang Q, Hua H, Chen F. Changes in the salivary microbiota of oral leukoplakia and oral cancer. *Oral Oncol* 2016; 56: e6-8. [<http://dx.doi.org/10.1016/j.oraloncology.2016.03.007>] [PMID: 27026576]
- [33] Gupta B, Johnson NW, Kumar N. Global epidemiology of head and neck cancers: A continuing challenge. *Oncology* 2016; 91(1): 13-23. [<http://dx.doi.org/10.1159/000446117>] [PMID: 27245686]
- [34] Ferlay J, Colombet M, Soerjomataram I, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *Int J Cancer* 2019; 144(8): 1941-53. [<http://dx.doi.org/10.1002/ijc.31937>] [PMID: 30350310]
- [35] Vasicek SM, Pondorfer P, Holzmeister C, et al. Head and neck cancer in Styria: An epidemiologic and clinical audit. *Wien Klin Wochenschr* 2020; 132(15-16): 444-51. [<http://dx.doi.org/10.1016/j.canep.2016.03.012>] [PMID: 31940091]
- [36] Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. *Oral Oncol* 2009; 45(4-5): 309-16. [<http://dx.doi.org/10.1016/j.oraloncology.2008.06.002>] [PMID: 18804401]
- [37] Perdomo S, Martin Roa G, Brennan P, Forman D, Sierra MS. Head and neck cancer burden and preventive measures in Central and South America. *Cancer Epidemiol* 2016; 44(Suppl. 1): S43-52. [<http://dx.doi.org/10.1016/j.epiro.2016.03.012>] [PMID: 27678322]
- [38] Michaud DS, Fu Z, Shi J, Chung M. Periodontal disease, tooth loss, and cancer risk. *Epidemiol Rev* 2017; 39(1): 49-58. [<http://dx.doi.org/10.1093/epirev/mxx006>] [PMID: 28449041]
- [39] Wang RS, Hu XY, Gu WJ, Hu Z, Wei B. Tooth loss and risk of head and neck cancer: a meta-analysis. *PLoS One* 2013; 8(8): e71122. [<http://dx.doi.org/10.1371/journal.pone.0071122>] [PMID: 23990929]
- [40] Zeng XT, Luo W, Huang W, Wang Q, Guo Y, Leng WD. Tooth loss and head and neck cancer: a meta-analysis of observational studies. *PLoS One* 2013; 8(11): e79074. [<http://dx.doi.org/10.1371/journal.pone.0079074>] [PMID: 24260154]
- [41] Zeng XT, Deng AP, Li C, Xia LY, Niu YM, Leng WD. Periodontal disease and risk of head and neck cancer: A meta-analysis of observational studies. *PLoS One* 2013; 8(10): e79017. [<http://dx.doi.org/10.1371/journal.pone.0079017>] [PMID: 24194957]
- [42] Yao QW, Zhou DS, Peng HJ, Ji P, Liu DS. Association of periodontal disease with oral cancer: A meta-analysis. *Tumour Biol* 2014; 35(7): 7073-7. [<http://dx.doi.org/10.1007/s13277-014-1951-8>] [PMID: 24756759]
- [43] Javed F, Warnakulasuriya S. Is there a relationship between periodontal disease and oral cancer? A systematic review of currently available evidence. *Crit Rev Oncog* 2016; 97: 197-205. [<http://dx.doi.org/10.1016/j.critrevonc.2015.08.018>] [PMID: 26343577]
- [44] Michaud DS, Kelsey KT, Papathanasiou E, Genco CA, Giovannucci E. Periodontal disease and risk of all cancers among male never smokers: an updated analysis of the Health Professionals Follow-up Study. *Ann Oncol* 2016; 27(5): 941-7. [<http://dx.doi.org/10.1093/annonc/mdw028>] [PMID: 26811350]
- [45] Moergel M, Kämmerer P, Kasaj A, et al. Chronic periodontitis and its possible association with oral squamous cell carcinoma - a retrospective case control study. *Head Face Med* 2013; 9(1): 39. [<http://dx.doi.org/10.1186/1746-160X-9-39>] [PMID: 24321243]
- [46] Hajishengallis G, Liang S, Payne MA, et al. Low-abundance biofilm species orchestrates inflammatory periodontal disease through the commensal microbiota and complement. *Cell Host Microbe* 2011; 10(5): 497-506. [<http://dx.doi.org/10.1016/j.chom.2011.10.006>] [PMID: 22036469]
- [47] Hajishengallis G, Lamont RJ. Beyond the red complex and into more complexity: the polymicrobial synergy and dysbiosis (PSD) model of periodontal disease etiology. *Mol Oral Microbiol* 2012; 27(6): 409-19. [<http://dx.doi.org/10.1111/j.2041-1014.2012.00663.x>] [PMID: 23134607]
- [48] Polak D, Wilensky A, Shapira L, et al. Mouse model of experimental periodontitis induced by *Porphyromonas gingivalis*/Fusobacterium nucleatum infection: bone loss and host response. *J Clin Periodontol* 2009; 36(5): 406-10. [<http://dx.doi.org/10.1111/j.1600-051X.2009.01393.x>] [PMID: 19419440]
- [49] Zhao H, Chu M, Huang Z, et al. Variations in oral microbiota associated with oral cancer. *Sci Rep* 2017; 7(1): 11773. [<http://dx.doi.org/10.1038/s41598-017-11779-9>] [PMID: 28924229]
- [50] Al-Hebshi NN, Nasher AT, Maryoud MY, et al. Inflammatory bacteriome featuring *Fusobacterium nucleatum* and *Pseudomonas aeruginosa* identified in association with oral squamous cell carcinoma. *Sci Rep* 2017; 7(1): 1834. [<http://dx.doi.org/10.1038/s41598-017-02079-3>] [PMID: 28500338]
- [51] Guerrero-Preston R, Godoy-Vitorino F, Jedlicka A, et al. 16S rRNA amplicon sequencing identifies microbiota associated with oral cancer, human papilloma virus infection and surgical treatment. *Oncotarget* 2016; 7(32): 51320-34. [<http://dx.doi.org/10.18632/oncotarget.9710>] [PMID: 27259999]
- [52] Zhang L, Liu Y, Zheng HJ, Zhang CP. The oral microbiota may have influence on oral cancer. *Front Cell Infect Microbiol* 2020; 9: 476. [<http://dx.doi.org/10.3389/fcimb.2019.00476>] [PMID: 32010645]
- [53] Yang CY, Yeh YM, Yu HY, et al. Oral microbiota community dynamics associated with oral squamous cell carcinoma staging. *Front Microbiol* 2018; 9: 862. [<http://dx.doi.org/10.3389/fmicb.2018.00862>] [PMID: 29774014]
- [54] Schmidt BL, Kuczynski J, Bhattacharya A, et al. Changes in abundance of oral microbiota associated with oral cancer. *PLoS One* 2014; 9(6): e98741. [<http://dx.doi.org/10.1371/journal.pone.0098741>] [PMID: 24887397]
- [55] Li Y, He J, He Z, et al. Phylogenetic and functional gene structure shifts of the oral microbiomes in periodontitis patients. *ISME J* 2014; 8(9): 1879-91. [<http://dx.doi.org/10.1038/ismej.2014.28>] [PMID: 24671083]
- [56] Sahingur SE, Yeudall WA. Chemokine function in periodontal disease and oral cavity cancer. *Front Immunol* 2015; 6: 214. [<http://dx.doi.org/10.3389/fimmu.2015.00214>] [PMID: 25999952]
- [57] Cugini C, Klepac-Ceraj V, Rackaityte E, Riggs JE, Davey ME. *Porphyromonas gingivalis*: keeping the pathos out of the biont. *J Oral Microbiol* 2013; 5(1): 19804. [<http://dx.doi.org/10.3402/jom.v5i0.19804>] [PMID: 23565326]
- [58] Han YW, Wang X. Mobile microbiome: Oral bacteria in extra-oral infections and inflammation. *J Dent Res* 2013; 92(6): 485-91. [<http://dx.doi.org/10.1177/0022034513487559>] [PMID: 23625375]
- [59] Takahashi N. Oral microbiome metabolism: From "who are they?" to "what are they doing?". *J Dent Res* 2015; 94(12): 1628-37. [<http://dx.doi.org/10.1177/0022034515606045>] [PMID: 26377570]
- [60] Sakamoto H, Naito H, Ohta Y, et al. Isolation of bacteria from cervical lymph nodes in patients with oral cancer. *Arch Oral Biol* 1999; 44(10): 789-93. [[http://dx.doi.org/10.1016/S0003-9969\(99\)00079-5](http://dx.doi.org/10.1016/S0003-9969(99)00079-5)] [PMID: 10530911]
- [61] Listyarifah D, Nieminen MT, Mäkinen LK, et al. *Treponema denticola* chymotrypsin-like proteinase is present in early-stage mobile tongue squamous cell carcinoma and related to the clinicopathological features. *J Oral Pathol Med* 2018; 47(8): 764-72. [<http://dx.doi.org/10.1111/jop.12729>] [PMID: 29747237]
- [62] Ahn J, Segers S, Hayes RB. Periodontal disease, *Porphyromonas gingivalis* serum antibody levels and orodigestive cancer mortality. *Carcinogenesis* 2012; 33(5): 1055-8. [<http://dx.doi.org/10.1093/carcin/bgs112>] [PMID: 22367402]
- [63] Yang SF, Huang HD, Fan WL, et al. Compositional and functional variations of oral microbiota associated with the mutational changes in oral cancer. *Oral Oncol* 2018; 77: 1-8. [<http://dx.doi.org/10.1016/j.oraloncology.2017.12.005>] [PMID: 29362114]
- [64] Karpinski TM. Role of oral microbiota in cancer development. *Microorganisms* 2019; 7(1): 20. [<http://dx.doi.org/10.3390/microorganisms7010020>] [PMID: 30642137]

- [65] Sasaki M, Yamaura C, Ohara-Nemoto Y, *et al.* Streptococcus anginosus infection in oral cancer and its infection route. *Oral Dis* 2005; 11(3): 151-6.
[http://dx.doi.org/10.1111/j.1601-0825.2005.01051.x] [PMID: 15888105]
- [66] Lee WH, Chen HM, Yang SF, *et al.* Bacterial alterations in salivary microbiota and their association in oral cancer. *Sci Rep* 2017; 7(1): 16540.
[http://dx.doi.org/10.1038/s41598-017-16418-x] [PMID: 29184122]
- [67] Pushalkar S, Ji X, Li Y, *et al.* Comparison of oral microbiota in tumor and non-tumor tissues of patients with oral squamous cell carcinoma. *BMC Microbiol* 2012; 12(1): 144.
[http://dx.doi.org/10.1186/1471-2180-12-144] [PMID: 22817758]
- [68] Bronzato JD, Bomfim RA, Edwards DH, Crouch D, Hector MP, Gomes BPFA. Detection of Fusobacterium in oral and head and neck cancer samples: A systematic review and meta-analysis. *Arch Oral Biol* 2020; 112104669
[http://dx.doi.org/10.1016/j.archoralbio.2020.104669] [PMID: 32028171]
- [69] Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann Surg Oncol* 2010; 17(6): 1471-4.
[http://dx.doi.org/10.1245/s10434-010-0985-4] [PMID: 20180029]
- [70] Montero PH. MD* and snehal G. Patel, MD* CANCER OF THE ORAL CAVITY* Head and Neck Surgery Service, Department of Surgery, Memorial Sloan-Kettering Cancer Center). *Surg Oncol Clin N Am* 2015; 24(3): 491-508.
[http://dx.doi.org/10.1016/j.soc.2015.03.006] [PMID: 25979396]
- [71] Stornetta A, Guidolin V, Balbo S. Alcohol-derived acetaldehyde exposure in the oral cavity. *Cancers (Basel)* 2018; 10(1): 20.
[http://dx.doi.org/10.3390/cancers10010020] [PMID: 29342885]
- [72] Bagnardi V, Blangiardo M, La Vecchia C, Corrao G. Alcohol consumption and the risk of cancer: A meta-analysis. *Alcohol Res Health* 2001; 25(4): 263-70.
[PMID: 11910703]
- [73] Sharma AK, DeBusk WT, Stepanov I, Gomez A, Khariwala SS. Oral microbiome profiling in smokers with and without head and neck cancer reveals variations between health and disease. *Cancer Prev Res (Phila)* 2020; 13(5): 463-74.
[http://dx.doi.org/10.1158/1940-6207.CAPR-19-0459] [PMID: 32071121]
- [74] Bartsch H, Nair J. Accumulation of lipid peroxidation-derived DNA lesions: potential lead markers for chemoprevention of inflammation-driven malignancies. *Mutat Res* 2005; 591(1-2): 34-44.
[http://dx.doi.org/10.1016/j.mrfmmm.2005.04.013] [PMID: 16099477]
- [75] Nair U, Bartsch H, Nair J. Lipid peroxidation-induced DNA damage in cancer-prone inflammatory diseases: A review of published adduct types and levels in humans. *Free Radic Biol Med* 2007; 43(8): 1109-20.
[http://dx.doi.org/10.1016/j.freeradbiomed.2007.07.012] [PMID: 17854706]
- [76] Johannsen A, Susin C, Gustafsson A. Smoking and inflammation: Evidence for a synergistic role in chronic disease. *Periodontol* 2000 2014; 64(1): 111-26.
[http://dx.doi.org/10.1111/j.1600-0757.2012.00456.x] [PMID: 24320959]
- [77] Ertel A, Eng R, Smith SM. The differential effect of cigarette smoke on the growth of bacteria found in humans. *Chest* 1991; 100(3): 628-30.
[http://dx.doi.org/10.1378/chest.100.3.628] [PMID: 1889244]
- [78] Thomas AM, Gleber-Netto FO, Fernandes GR, *et al.* Alcohol and tobacco consumption affects bacterial richness in oral cavity mucosa biofilms. *BMC Microbiol* 2014; 14(1): 250.
[http://dx.doi.org/10.1186/s12866-014-0250-2] [PMID: 25278091]
- [79] Charlson ES, Chen J, Custers-Allen R, *et al.* Disordered microbial communities in the upper respiratory tract of cigarette smokers. *PLoS One* 2010; 5(12): e15216
[http://dx.doi.org/10.1371/journal.pone.0015216] [PMID: 21188149]
- [80] Shchipkova AY, Nagaraja HN, Kumar PS. Subgingival microbial profiles of smokers with periodontitis. *J Dent Res* 2010; 89(11): 1247-53.
[http://dx.doi.org/10.1177/0022034510377203] [PMID: 20739702]
- [81] Brook I, Gober AE. Recovery of potential pathogens in the nasopharynx of healthy and otitis media-prone children and their smoking and nonsmoking parents. *Ann Otol Rhinol Laryngol* 2008; 117(10): 727-30.
[http://dx.doi.org/10.1177/000348940811701003] [PMID: 18998498]
- [82] El Ahmer OR, Essery SD, Saadi AT, *et al.* The effect of cigarette smoke on adherence of respiratory pathogens to buccal epithelial cells. *FEMS Immunol Med Microbiol* 1999; 23(1): 27-36.
[http://dx.doi.org/10.1111/j.1574-695X.1999.tb01713.x] [PMID: 10030544]
- [83] Morris A, Beck JM, Schloss PD, *et al.* Comparison of the respiratory microbiome in healthy nonsmokers and smokers. *Am J Respir Crit Care Med* 2013; 187(10): 1067-75.
[http://dx.doi.org/10.1164/rccm.201210-1913OC] [PMID: 23491408]
- [84] Ingram LO. Ethanol tolerance in bacteria. *Crit Rev Biotechnol* 1990; 9(4): 305-19.
[http://dx.doi.org/10.3109/07388558909036741] [PMID: 2178781]
- [85] Scannapieco FA. Saliva-bacterium interactions in oral microbial ecology. *Crit Rev Oral Biol Med* 1994; 5(3-4): 203-48.
[http://dx.doi.org/10.1177/10454411940050030201] [PMID: 7703323]
- [86] 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(4): 663-8.
[http://dx.doi.org/10.1093/carcin/21.4.663] [PMID: 10753201]
- [87] Moritani K, Takeshita T, Shibata Y, Ninomiya T, Kiyohara Y, Yamashita Y. Acetaldehyde production by major oral microbes. *Oral Dis* 2015; 21(6): 748-54.
[http://dx.doi.org/10.1111/odi.12341] [PMID: 25809116]
- [88] Hsiao JR, Chang CC, Lee WT, *et al.* The interplay between oral microbiome, lifestyle factors and genetic polymorphisms in the risk of oral squamous cell carcinoma. *Carcinogenesis* 2018; 39(6): 778-87.
[http://dx.doi.org/10.1093/carcin/bgy053] [PMID: 29668903]
- [89] Muto M, Hitomi Y, Ohtsu A, *et al.* Acetaldehyde production by non-pathogenic Neisseria in human oral microflora: Implications for carcinogenesis in upper aerodigestive tract. *Int J Cancer* 2000; 88(3): 342-50.
[http://dx.doi.org/10.1002/1097-0215(20001101)88:3<342::AID-IJC4>3.0.CO;2-I] [PMID: 11054661]
- [90] Yokoyama S, Takeuchi K, Shibata Y, *et al.* Characterization of oral microbiota and acetaldehyde production. *J Oral Microbiol* 2018; 10(1): 1492316
[http://dx.doi.org/10.1080/20002297.2018.1492316] [PMID: 30013723]
- [91] Pavlova SI, Jin L, Gasparovich SR, Tao L. Multiple alcohol dehydrogenases but no functional acetaldehyde dehydrogenase causing excessive acetaldehyde production from ethanol by oral streptococci. *Microbiology* 2013; 159(Pt 7): 1437-46.
[http://dx.doi.org/10.1099/mic.0.066258-0] [PMID: 23637459]
- [92] 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(1): 59.
[http://dx.doi.org/10.1186/s40168-018-0448-x] [PMID: 29685174]
- [93] Hientz K, Mohr A, Bhakta-Guha D, Efferth T. The role of p53 in cancer drug resistance and targeted chemotherapy. *Oncotarget* 2017; 8(5): 8921-46.
[http://dx.doi.org/10.18632/oncotarget.13475] [PMID: 27888811]
- [94] Rogha M, Berjis N, Lajevardi SM, Alamdaran M, Hashemi SM. Identification of R249 mutation in P53 gene in tumoral tissue of tongue cancer. *Int J Prev Med* 2019; 10: 129.
[http://dx.doi.org/10.4103/ijpvm.IJPVM_50_17] [PMID: 31516670]
- [95] Poeta ML, Manola J, Goldwasser MA, *et al.* TP53 mutations and survival in squamous-cell carcinoma of the head and neck. *N Engl J Med* 2007; 357(25): 2552-61.
[http://dx.doi.org/10.1056/NEJMoa073770] [PMID: 18094376]
- [96] Sandulache VC, Michikawa C, Kataria P, *et al.* High-risk TP53 mutations are associated with extranodal extension in oral cavity squamous cell carcinoma. *Clin Cancer Res* 2018; 24(7): 1727-33.
[http://dx.doi.org/10.1158/1078-0432.CCR-17-0721] [PMID: 29330202]
- [97] Braakhuis BJ, Rietbergen MM, Buijze M, *et al.* TP53 mutation and human papilloma virus status of oral squamous cell carcinomas in young adult patients. *Oral Dis* 2014; 20(6): 602-8.
[http://dx.doi.org/10.1111/odi.12178] [PMID: 24112103]
- [98] Mao L, Lee JS, Fan YH, *et al.* Frequent microsatellite alterations at chromosomes 9p21 and 3p14 in oral premalignant lesions and their value in cancer risk assessment. *Nat Med* 1996; 2(6): 682-5.
[http://dx.doi.org/10.1038/nm0696-682] [PMID: 8640560]
- [99] Bova RJ, Quinn DI, Nankervis JS, *et al.* Cyclin D1 and p16INK4A expression predict reduced survival in carcinoma of the anterior tongue. *Clin Cancer Res* 1999; 5(10): 2810-9.
[PMID: 10537346]
- [100] Goodrich DW. The retinoblastoma tumor-suppressor gene, the

- exception that proves the rule. *Oncogene* 2006; 25(38): 5233-43. [http://dx.doi.org/10.1038/sj.onc.1209616] [PMID: 16936742]
- [101] Kim TM, Benedict WF, Xu HJ, *et al.* Loss of heterozygosity on chromosome 13 is common only in the biologically more aggressive subtypes of ovarian epithelial tumors and is associated with normal retinoblastoma gene expression. *Cancer Res* 1994; 54(3): 605-9. [PMID: 8306317]
- [102] Malumbres M, Barbacid M. To cycle or not to cycle: A critical decision in cancer. *Nat Rev Cancer* 2001; 1(3): 222-31. [http://dx.doi.org/10.1038/35106065] [PMID: 11902577]
- [103] Pützer BM. E2F1 death pathways as targets for cancer therapy. *J Cell Mol Med* 2007; 11(2): 239-51. [http://dx.doi.org/10.1111/j.1582-4934.2007.00030.x] [PMID: 17488475]
- [104] Costa V, Kowalski LP, Coutinho-Camillo CM, *et al.* EGFR amplification and expression in oral squamous cell carcinoma in young adults. *Int J Oral Maxillofac Implants* 2018; 47(7): 817-23. [http://dx.doi.org/10.1016/j.ijom.2018.01.002] [PMID: 29395668]
- [105] Cassell A, Grandis JR. Investigational EGFR-targeted therapy in head and neck squamous cell carcinoma. *Expert Opin Investig Drugs* 2010; 19(6): 709-22. [http://dx.doi.org/10.1517/13543781003769844] [PMID: 20415598]
- [106] Perisanidis C. Prevalence of EGFR tyrosine kinase domain mutations in head and neck squamous cell carcinoma: Cohort study and systematic review. *In Vivo* 2017; 31(1): 23-34. [http://dx.doi.org/10.21873/invivo.11020] [PMID: 28064216]
- [107] Wilson GD, Saunders MI, Dische S, Richman PI, Daley FM, Bentzen SM. bcl-2 expression in head and neck cancer: an enigmatic prognostic marker. *Int J Radiat Oncol Biol Phys* 2001; 49(2): 435-41. [http://dx.doi.org/10.1016/S0360-3016(00)01498-X] [PMID: 11173138]
- [108] Akiyama H, Nakashiro K, Tokuzen N, Tanaka H, Hino S, *et al.* Abstract 1137: Mutation and function of PIK3CA and HRAS in human oral squamous cell carcinoma cells. *Cancer Res* 2016; 76: 1137.
- [109] Krishna A, Singh S, Singh V, Kumar V, Singh US, Sankhwar SN. Does Harvey-Ras gene expression lead to oral squamous cell carcinoma? A clinicopathological aspect. *J Oral Maxillofac Pathol* 2018; 22(1): 65-72. [PMID: 29731559]
- [110] Harsha C, Banik K, Ang HL, *et al.* Targeting AKT/mTOR in oral cancer: Mechanisms and advances in clinical trials. *Int J Mol Sci* 2020; 21(9): 3285. [http://dx.doi.org/10.3390/ijms21093285] [PMID: 32384682]
- [111] Nakhjiri SF, Park Y, Yilmaz O, *et al.* Inhibition of epithelial cell apoptosis by *Porphyromonas gingivalis*. *FEMS Microbiol Lett* 2001; 200(2): 145-9. [http://dx.doi.org/10.1111/j.1574-6968.2001.tb10706.x] [PMID: 11425466]
- [112] Yilmaz O, Jungas T, Verbeke P, Ojcius DM. Activation of the phosphatidylinositol 3-kinase/Akt pathway contributes to survival of primary epithelial cells infected with the periodontal pathogen *Porphyromonas gingivalis*. *Infect Immun* 2004; 72(7): 3743-51. [http://dx.doi.org/10.1128/IAI.72.7.3743-3751.2004] [PMID: 15213114]
- [113] García Z, Kumar A, Marqués M, Cortés I, Carrera AC. Phosphoinositide 3-kinase controls early and late events in mammalian cell division. *EMBO J* 2006; 25(4): 655-61. [http://dx.doi.org/10.1038/sj.emboj.7600967] [PMID: 16437156]
- [114] Vousden KH. Outcomes of p53 activation-spoilt for choice. *J Cell Sci* 2006; 119(Pt 24): 5015-20. [http://dx.doi.org/10.1242/jcs.03293] [PMID: 17158908]
- [115] Yao á, Jermanus C, Barbetta B, *et al.* *Porphyromonas gingivalis* infection sequesters pro-apoptotic bad through akt in primary gingival epithelial cells. *Molecular Oral Microbiology* 2010; 25(2): 89-101.
- [116] Mao S, Park Y, Hasegawa Y, *et al.* Intrinsic apoptotic pathways of gingival epithelial cells modulated by *porphyromonas gingivalis*. *Cell Microbiol* 2007; 9(8): 1997-2007. [http://dx.doi.org/10.1111/j.1462-5822.2007.00931.x] [PMID: 17419719]
- [117] Hoppe T, Kraus D, Novak N, *et al.* Oral pathogens change proliferation properties of oral tumor cells by affecting gene expression of human defensins. *Tumour Biol* 2016; 37(10): 13789-98. [http://dx.doi.org/10.1007/s13277-016-5281-x] [PMID: 27481514]
- [118] Groeger S, Jarzina F, Domann E, Meyle J. *Porphyromonas gingivalis* activates NFkB and MAPK pathways in human oral epithelial cells. *BMC Immunol* 2017; 18(1): 1. [http://dx.doi.org/10.1186/s12865-016-0185-5] [PMID: 28056810]
- [119] Yilmaz O, Yao L, Maeda K, *et al.* ATP scavenging by the intracellular pathogen *Porphyromonas gingivalis* inhibits P2X7-mediated host-cell apoptosis. *Cell Microbiol* 2008; 10(4): 863-75. [http://dx.doi.org/10.1111/j.1462-5822.2007.01089.x] [PMID: 18005240]
- [120] Groeger S, Domann E, Gonzales JR, Chakraborty T, Meyle J. B7-H1 and B7-DC receptors of oral squamous carcinoma cells are upregulated by *Porphyromonas gingivalis*. *Immunobiology* 2011; 216(12): 1302-10. [http://dx.doi.org/10.1016/j.imbio.2011.05.005] [PMID: 21723642]
- [121] Moffatt CE, Lamont RJ. *Porphyromonas gingivalis* induction of microRNA-203 expression controls suppressor of cytokine signaling 3 in gingival epithelial cells. *Infect Immun* 2011; 79(7): 2632-7. [http://dx.doi.org/10.1128/IAI.00082-11] [PMID: 21536793]
- [122] Binder Gallimidi A, Fischman S, Revach B, *et al.* Periodontal pathogens *Porphyromonas gingivalis* and *Fusobacterium nucleatum* promote tumor progression in an oral-specific chemical carcinogenesis model. *Oncotarget* 2015; 6(26): 22613-23. [http://dx.doi.org/10.18632/oncotarget.4209] [PMID: 26158901]
- [123] Gholizadeh P, Eslami H, Kafil HS. Carcinogenesis mechanisms of *fusobacterium nucleatum*. *Biomed Pharmacother* 2017; 89: 918-25. [http://dx.doi.org/10.1016/j.biopha.2017.02.102] [PMID: 28292019]
- [124] Perera M, Al-Hebshi NN, Speicher DJ, Perera I, Johnson NW. Emerging role of bacteria in oral carcinogenesis: A review with special reference to perio-pathogenic bacteria. *J Oral Microbiol* 2016; 8(1): 32762. [http://dx.doi.org/10.3402/jom.v8.32762] [PMID: 27677454]
- [125] Mirkeshavarz M, Ganjibakhsh M, Aminishakib P, *et al.* Interleukin-6 secreted by oral cancer associated fibroblast accelerated VEGF expression in tumor and stroma cells. *Cell Mol Biol* 2017; 63(10): 131-6. [http://dx.doi.org/10.14715/cmb/2017.63.10.21] [PMID: 29096753]
- [126] Ikehata N, Takanashi M, Satomi T, *et al.* Toll-like receptor 2 activation implicated in oral squamous cell carcinoma development. *Biochem Biophys Res Commun* 2018; 495(3): 2227-34. [http://dx.doi.org/10.1016/j.bbrc.2017.12.098] [PMID: 29269299]
- [127] Eisele NA, Ruby T, Jacobson A, *et al.* *Salmonella* require the fatty acid regulator PPAR δ for the establishment of a metabolic environment essential for long-term persistence. *Cell Host Microbe* 2013; 14(2): 171-82. [http://dx.doi.org/10.1016/j.chom.2013.07.010] [PMID: 23954156]
- [128] Kang W, Jia Z, Tang D, *et al.* *Fusobacterium nucleatum* facilitates apoptosis, ROS generation, and inflammatory cytokine production by activating AKT/MAPK and NF- κ B signaling pathways in human gingival fibroblasts. *Oxidative medicine and cellular longevity* 2019; 2019
- [129] Yang Y, Weng W, Peng J, *et al.* *Fusobacterium nucleatum* increases proliferation of colorectal cancer cells and tumor development in mice by activating toll-like receptor 4 signaling to nuclear factor- κ B, and up-regulating expression of microRNA-21. *Gastroenterology* 2017; 152(4): 851-66.: e24.
- [130] Jewett A, Hume WR, Le H, *et al.* Induction of apoptotic cell death in peripheral blood mononuclear and polymorphonuclear cells by an oral bacterium, *Fusobacterium nucleatum*. *Infect Immun* 2000; 68(4): 1893-8. [http://dx.doi.org/10.1128/IAI.68.4.1893-1898.2000] [PMID: 10722579]