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Review of Experimental Data *in vivo* and *in vitro* Supporting Carcinogenesis of Oral Squamous Cell Carcinoma by Periodontal Disease and Possible Molecular Mechanisms

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Abstract

Objective: to describe different *in vivo* and *in vitro* experimental models and summarize evidence that proposes molecular mechanisms or demonstrate a cause and effect correlation between periodontal disease (PD) and oral squamous cell carcinoma development. **Material and Methods:** a Literature search of various electronic databases (Medline, SCOPUS, Web of Science) using appropriate keywords (e.g., periodontal disease, periodontitis, squamous cell carcinoma, oral cancer, transformation, carcinogenesis, mouth neoplasms, and carcinoma) was performed. Then, a comprehensive literature review of the current understanding of this link was elaborated. **Results:** the experimental data suggest that PD induces initiation, promotion, and oral squamous cell carcinoma progression. Periodontal disease models here revised demonstrate a direct relation to carcinogenesis and propose several possible molecular mechanisms of action, such as via activation of Toll-like receptors (TLRs), activation of transcription 3 activator (STAT3) and Notch1 pathways and increased interleukin-6 (IL-6), TNF-α, TGF-β1, cyclin D1, metalloproteinase matrix-9 (MMP-9) and heparinase production as well as regulating the host immune response and induce epithelial-mesenchymal transition (EMT). **Conclusion:** the use of *in vivo* and *in vitro* studies is very significant for understanding the casual association of these biological processes. The current findings highlight the importance of further studies in this area to understand the cause and effect relationship of these pathologies and the development of new strategies for the prevention and treatment of this type of cancer.

Keywords: Mouth neoplasms; Periodontal disease; Molecular mechanism; *P. gingivalis;* Inflammation

Introduction

According to the International Agency for Research on Cancer, the global impact of cancer has more than doubled in the last 30 years.[1] With the number of cancer cases increasing, expanding knowledge about neoplasms is of worldwide importance. Oral squamous cell carcinoma (OSCC) is the sixth most common cancer in the world,[2] with 345,864 new cases and 177,384 deaths occurring in 2018 alone. The disease is more common in males above 60 years.[3] The survival rate for OSCC is around 5 years and is considered the lowest among malignant neoplasms. The prognosis of the disease has not altered in the last 30 years, thus justifying the search for new substances with antineoplastic effects that overcome the side effects and the high costs of the current treatments.[4, 5]

Tobacco, excessive alcohol consumption, and HPV infection are some of the main risk factors associated with oral cancer development.[6] However, several epidemiological studies define a correlation between oral cancer and periodontal disease (PD), which is a chronic inflammatory disease.[7, 8] Such studies have correlated the presence of visible plaque, level of gingival bleeding, tooth loss, prosthetic use, tooth mobility, furcation involvement and lost marginal bone to the emergence of OSCC.[9, 10]

Recently, several reviews analyzing the clinical correlation of PD with the appearance of OSCC have been made [11, 12], and can be a valuable source of information on the topic. The present work aims to establish a cause and effect relationship and determine the molecular and cellular mechanisms related to PD and periodontopathogens in the process of tumorigenesis of oral cancer. Therefore, we focus on reviewing and analyzing experimental works, *in vitro* and *in vivo,* related to the influence of PD on the incidence and evolution of oral cancer, thus establishing a possible cause and effect relationship between these two pathologies, in addition to making a comprehensive study for both clinical and experimental biology professionals.

Material and Methods

First, a short descriptive revision of the leading experimental models *in vitro* and *in vivo* for periodontal disease and oral cancer was performed to introduce both clinicians and experimental biologists to the subject. After, a literature search was performed independently by 2 reviewers (RCDA and GFA). A search was performed on the following databases: Medline, SCOPUS and Web of Science. Studies published in the fifteen years before 31 November 2019, with full text available, were considered for

inclusion. For MEDLINE/ PubMed, the following keywords as MESH terms or free words were used: (periodontal disease OR periodontitis OR periodontal infection OR periodontopathogens) AND (squamous cell carcinoma OR oral cancer OR transformation OR carcinogenesis OR mouth neoplasms OR carcinoma). Additionally, included publications were screened from citations of selected papers, recovered from the excluded group, or resulted from manual searches of the most relevant periodontics and cancer journals. Paper selection and data extraction were performed independently by the aforementioned researchers.

Inclusion criteria were: 1) studies involving mouse models of PD or oral cancer; 2) studies involving cell culture *in vitro* experimentation; 3) a combination of both.

Exclusion criteria were: 1) epidemiological studies; 2) clinical studies; 3) articles that did not meet the inclusion criteria.

For further evaluation, we selected research articles describing: 1) implications of periodontal-related disease and periodontopathogens in cancer development, 2) the role of PD in cancer progression associated with migration and invasion and immune evasion and 3) the molecular and cellular mechanisms induced by PD that corroborate with carcinogenesis. Studies in languages other than English and article abstracts were excluded. Then, evidence was presented in the form of a narrative review.

Results and Discussion

Experimental Models in vivo and in vitro of Periodontal Disease and Oral Cancer: Methods to Study a Causal Effect

Periodontal disease refers to inflammation of the gingival tissues associated with loss of root cement, alveolar bone, and periodontal ligament. PD is described as a process originating from the imbalance between mechanisms of bacterial aggression and defense of the host resulting in the collapse of the supporting tissues around the tooth. PD is usually associated with a chronic infection of certain periodontopathogens such as *Treponema denticola, Tannerella forsythensis,* and *Porphyromonas gingivalis*. Other microorganisms that may be involved include *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, *Campylobacter rectus and Fusobacterium nucleatum*.[13]

Against different aggressions, the immune system mounts an inflammatory defensive response. It is long known that chronic inflammation is a risk factor for developing human cancer since such inflammation generates wear on cells in the affected tissue accompanied by proliferation, which can happen for decades.[14] For discussion of factors of a possible causal link between PD and cancer, it is necessary a brief description of experimental animals and cell models of PD and oral cancer. Such studies made it possible to analyze the existence of a possible causal relationship between both diseases.

Animal Models of PD

Currently, several animal models mimic the development of PD in humans. These models allow the study of both the evolution and possible treatments for periodontal disease and diseases associated with it. Among these models, the main ones used are described below.

Experimental Models of Ligature-Induced PD: Periodontal disease induced by ligation in rats was described in 1966 [15] and is currently one of the most used models in periodontal research. In this model, a cotton, silk or nylon thread is placed in the cervical area of the maxillary or mandibular molars of rats to induce bacterial plaque accumulation.[16] The bacterial plaque adhered to the wire facilitates the invasion of connective tissue. It leads to changes in periodontal tissue, as in human PD, such as rupture and apical migration of the junctional epithelium, influx of inflammatory cells and loss of periodontal ligament fibers and bone destruction.[17] As in humans PD, alveolar bone loss in ligature models is dependent on the presence of bacteria. In order to provide a faster and more evident induction of periodontal disease, there is the option of placing bandages soaked in the culture of periodontopathogenic bacteria.[18, 19] The relevance of the ligature model in rats has been questioned with the justification that the mechanical injury of the segment could aggravate periodontal destruction. In addition, bone loss caused by physiological bone remodeling in rats and mice has been considered as another disadvantage of experimentally ligation-induced PD.[20, 21] Ligature models trigger acute disease since bone destruction is rapid and severe and usually shows insertion loss above 50%, in a period between one to two weeks.[22] After this period, a decrease in the intensity of the destruction is expected to become a chronic reaction.

Experimental models of oral gavage-induced PD of periodontopathogenic bacteria: The introduction of human bacterial strains by oral gavage and the subsequent impact on the periodontium have been studied in several rodent models.[17] An experimental model of PD was developed with the introduction of *P. gingivalis* strains in rats to induce alveolar bone loss.[23, 24] Subsequently, this model was replicated using other pathogens, including *Aggregatibacter actinomycetemcomitans* [25], *Tannerella forsythia,* and *Porphyromonas gulae* [26]. Bone loss usually takes more than four weeks and is measured histologically by morphometric analysis or by computed tomography.[25, 27] The use of oral gavage models to study a wide variety of hypotheses related to the pathogenesis of PD was evaluated by varying the role of the host's response to the virulence characteristics of suspected periodontopathogens, in addition to the connection of these factors with systemic parameters.[28]

Animal Models of Oral Cancer

Several experimental models of oral cancer induction are described in the literature using chemical induction (carcinogens), such as 9,10-dimethyl-1,2- benzanthracene (DMBA) or nitroquinoline oxide (4NQO), cell transplantation of cystic adenoid carcinoma in immunosuppressed mice and the transplantation of squamous cell carcinoma cell lines.

Models of bone metastases in the maxillofacial region are performed through the intracardiac injection of human breast cancer cells.[29, 30]

In an attempt to induce oral carcinogenesis in animals, a variety of chemical carcinogens was used; however, many failed, as the incidence in the formation of malignant neoplasia (penetrance) was very low. Among them, DMBA, which, despite being still used, is becoming obsolete due to the low efficiency of the method.[31] Currently, 4NQO, a water-soluble synthetic carcinogen, is the best carcinogen available for induction of oral tumorigenesis in rodents.[32] 4NQO induces the formation of sequential stages of carcinogenesis (initiation, promotion and progression) and the histological and molecular changes induced by this compound are similar to those that occur in humans. It is believed that the carcinogenic effects of 4NQO are due to the enzymatic reduction of its nitro group present in one of its aromatic rings, followed by the formation of an intermediate compound known as 4-hydroxyaminoquinolone-N-oxide (4HAQO), which is a metabolite cancer involved in the formation of DNA errors.[32]

Cell Lines of Oral Cancer

Cell culture is characterized by allowing the maintenance of live cells (*in vitro*) in a laboratory independent of the organism that originated them. Cell culture is an extension of the tissue culture technique, a generic term used to include *in vitro* cultures of organs, tissues and cells. Cell lines established from neoplastic and non-neoplastic cells are used extensively in preclinical studies to develop new diagnostic approaches and studies on the biology of diseases, such as cancer. Several human OSCC strains have been used in the literature to study this pathology, such as SCC4, SCC9 , SCC15, SCC25 , CAL27, BICR 10, BICR 16, BICR 3, BICR 31, BICR 56, BICR 78, BICR 82, DOK, H103, H157 , H314, H357, H376, H400, H413, PE / CA-PJ15, PE / CA-PJ34, PE / CA-PJ41, PE / CA-PJ49, among others. Bearing in mind that the genetic alterations present in malignant cell lines are similar to the alterations existing in the original tumors, the use of transformed cells finds wide application in the development of cancer treatments. Development of new diagnostic approaches and studies on the biology of diseases such as cancer.

Therefore, biological models that better represent the organism *in vivo*, both in physiological and pathological processes, will allow researchers more conclusive results and safer therapies.

Experimental Findings that Support the Cause and Effect Relationship of PD and its Periodontopathogens with Oral Cancer Carcinogenesis

The last decades have contributed a lot to the understanding of cancer biology and the mechanisms involved in tumor initiation, development, and progression. In the various stages that constitute the process of carcinogenesis, each acquired genetic modification gives tumor cells a type of advantage, thus constituting the characteristics of cancer. These capacities acquired by the tumor cells during the development process favor their maintenance. Among them, we can highlight the process of tumor initiation and progression, the evasion of the immune system, activation of invasion mechanisms and metastases.[33]

Chronic infections are increasingly recognized as an important epidemiological / environmental factor in the development of oral cancer. However, there is still lack of experimental evidence to prove the cause and effect relationship between these two pathologies for oral cancer. Below described are all information found on PD and periodontopathogens and their influence on oral carcinogenesis. All major biological findings, molecular pathways, and models found are summarized in Table 1.

Carcinogenesis and PD: Initiation and Promotion

The carcinogenesis can be divided into three different stages: initiation, promotion and progression.[33] Tumor initiation is the process that involves the exposure of normal cells to chemical, physical or biological agents that cause irreversible damage to genes, leading to activation of oncogenes and/or inactivation of tumor suppressor genes. As examples of such agents are radiation in the form of ultraviolet rays from sunlight and electromagnetic radiation (x-rays, gamma rays) and particulate (alpha, beta, proton, and neutron) or viruses such as papillomavirus (HPV), Epstein- Barr virus (EBV), hepatitis B virus (HBV).[34] The human papillomavirus, the hepatitis B and C virus, and the *Helicobacter pylori* bacteria are implicated in the pathogenesis of cervical, liver and stomach cancer, respectively.[35] This stage has a low frequency and depends directly on the dose and time of carcinogen agent exposure. The cells can remain stationary in the "initiated" state for varying times if they are not stimulated to divide in the process called promotion. At this stage, initiated cells can remain dormant for weeks, months or years, or they can grow autonomously and clonally.[10, 36] The initiated cell is not a neoplastic cell but has taken its first step towards this state that can be achieved only after successive genotypic and phenotypic changes.[10] However, initiated cells respond in an increased manner to some stimuli, such as growth factors. The promotion process comprises the clonal expansion of the initiated cells until they form visible tumors, usually benign lesions or foci of pre-neoplastic cells. Promotion can occur as a result of exogenous exposure to different agents. Tumor promoters are characterized by their ability to reduce the latency period in the formation of a tumor after exposing the tissue to an initiator or by increasing the number of tumors formed in that tissue, as an example the proinflammatory cytokine TNF-α induction which has a close correlation with tumor promotion.[37] Results provide significant evidence that TNF-α is the first player (major cytokine) in tumor promotion with other cytokines such as IL-1 and IL-6 as contributors or retro [38] and the route of signaling via NF-κB transcription factor

activation the main pro-inflammatory pathway involved in human carcinogenesis [37, 38]. Thus, tumor promoters are not able, by themselves, to produce mutations in DNA, that is, they are not carcinogenic. They can induce tumor formation when combined with an initiator. Progression is the step where the tumor cells gets increasingly aggressive characteristics (increased proliferation, invasion and tumor metastasis), because of the huge amount of change experienced by cells at this stage. The genomic instability experienced by the cell provides a favorable environment for genetic exchange, causing the amplification of some genes and the altered expression of others, which may result in a higher growth rate and the acquisition of properties such as local invasion and metastatic dissemination. Not all cells exposed to promoters participate in the promotion stage, only cells stimulated to divide (initiated), undifferentiated and that have survived apoptosis, can contribute to the instability between cell growth and death and lead to the appearance of a malignant neoplasm. In this stage, as the acquisition of multiple genetic alterations occurs, there is the triggering of tumor proliferation, uncontrolled, and irreversible multiplication of the altered cells.[11]

Experimental data suggest that the process of PD may directly contribute to tumor initiation, as chemical induction of PD in animal models (*in vivo*) was capable of inducing the spontaneous generation of OSCC.[39] The drug antagonist of calcium channels (mibefradil dihydrochloride), known to induce excessive gum growth *in vivo,* associated with chronic intake of a diet rich in fiber was able to induce PD characteristics leading to the significant and spontaneous generation of OSCC in mice. Simultaneously, this drug was not directly carcinogenic in genotoxicity assays indicating that a direct mutational effect of the new compound in the development of OSCC was highly unlikely.[39] Excessive gingival growth induced by the drug increased the opportunity for trapping and penetration of foreign bodies (for example, food particles and hair), because the swollen gums were more susceptible to mechanical injury. Based on the data, gingival inflammation, which occurs constantly and repeatedly in mice, likely contributed to the induction and exacerbation of periodontal lesions, which corroborated with tumor development. These results indicate that the formation of tumors is attributable to severe periodontal disease favored by diet and excessive growth/gingival inflammation (Table 1).

Table 1. Summary of the main transformation phenotypes and suggested mechanisms of induction divided by periodontal disease and oral cancer model.

Model	Phenotype	Mechanism	References
In vitro*:** **Tumor cells infected with *P. gingivalis* and *F. nucleatum	• Increased proliferation • Increased migratory capacity of cells for invasion and migration • Increased angiogenesis • Degradation and remodeling of the extracellular matrix • EMT (epithelial-mesenchymal transition) characteristics	• Overexpression of cyclin D1 • Production of matrix metalloproteinase-9 (MMP-9) • Increased heparinase • Production of transforming growth factor beta 1 (TGF-β1) and tumor necrosis factor alpha (TNF-α) •Reduced expression of E-caderin and increased vimentin	40, 63
In vitro*:** **Tumor and Normal Human Epithelial cells infected with *P. gingivalis	• Evasion of the immune system	• Overexpression of B7-H1 e B7-DC	66
In vitro*:** **Normal Human Epithelial cells infected with *P. gingivalis	•Transf ormed morphology • Increased proliferation • Increased migration • EMT (epithelial-mesenchymal transition) induction	• Up or down regulation of NNMT, FLI1, GAS6, lncRNA CCAT1, PDCD1LG2, CD274, IL6, STAT1, LYN, BDNF, C3, CD274, DCD1LG2 e CXCL10 genes • Activation of glycogen synthase kinase-3 beta (p-GSK3β)	46, 52
***In vivo*:** **Periodontitis Induction by Calcium Channel Antagonist**	• Increased cell proliferation and inflammation of epithelial cells • Spontaneous OSCC occurrence	•None proposed	39
In vivo*:** **Periodontitis: Gavage of *P. gingivalis* and *F. nucleatum **Tumor: 4NQO orally**	• Increased number of tumors and OSCC • Increased invasion and tumor Score • Altered lipid metabolism	• Increased IL-6 • Overexpression of cyclin D2 • Activation of STAT3 pathway	39, 40, 59
In vivo*:** **Periodontitis: Gavage of *P. gingivalis **Tumor: Tumor xenografts**	• Increased tumor growth	• Increased IL-6	41
In vivo*:** **Xenograft of tumor cells infected with *P. gingivalis	• Larger tumors • Increased number of Metastases • Resistance to Taxol Treatment	• Notch1 activation	59

Corroborating the initiator and promoter role of PD in OSCC carcinogenesis, [40] demonstrated *in vivo* the association of PD induction of OSCC in synergy with carcinogens induced tumors. PD and chronic infection induced by gavage of *P. gingivalis* and *F. nucleatum*, together with oral administration of the carcinogen 4NQO, promoted the malignant transformation of oral cells (OSCC) and increased expression of interleukin-6 (IL-6) and the activity of transcription activator 3 (STAT3). Corroborating result observed in another model in which serum level of IL-6 was significantly increased in mice treated with *P. gingivalis*.[41] Interleukin-6 is a potent inflammatory cytokine, with redundant and pleiotropic activity that mediates a series of physiological functions, including lymphocyte differentiation, cell proliferation and survival [42, 43] and its continuous and unregulated synthesis is related to several diseases, including cancer [44]. The role of chronic PD in chemoresistance has also been traced to IL6 as the most important factor, where the serum level of only IL-6 was significantly impacted by the administration of *P. gingivalis* and after treatment with anti-inflammatory ibuprofen that reduced the levels of IL-6 and reverted resistance to paclitaxel, a drug used to treat cancer.[41] The serum levels of antibodies against periodontopathogens and the levels of IL-6 in patients with OSCC were compared with healthy controls. Serum IgG against *P. gingivalis* and IL-6 levels were significantly higher in a group of 62 patients with OSCC that in 46 patients healthy controls.[45] These data suggest a correlation in humans between IL-6 in the inflammatory process and the OSCC development. Cytokines such as IL-6 promote tumor initiation, elevating reactive intracellular oxygen species (ROS) and reactive nitrogen intermediates (RNI), in addition to causing epigenetic changes in several tumor related genes. In addition, cytokines as IL-6 facilitate tumor progression by activating transcription factors related to tumorigenesis as STAT3.[46] STAT3 regulates a diversity of cellular processes, as it promotes the transcription of genes related to cell survival and growth, angiogenesis, as well as pro- and anti- apoptotic genes.[47, 48] It was demonstrated that activation of STAT3 by IL-6 drives the growth and invasiveness of cells malignant agreeing to reports demonstrating that the signal of this signaling pathway is pro-tumorigenic.[49] In addition, it has been shown, *in vitro,* that infection by *P. gingivalis* and *F. nucleatum* stimulates tumorigenesis by direct interaction with oral epithelial cells through the activation of Toll-like receptors (TLRs), causing the production of IL-6. Furthermore, these oral pathogens stimulate proliferation of OSCC and expression of key molecules in tumorigeneses, such as cyclin D1, matrix metalloproteinase-9 (MMP-9) and heparinase.[40] Cyclin D1 acts on the cell cycle accelerating G1 phase entrance leading to proliferation and tumor growth.[50] These periodontopathogens were also capable of inducing in cancerous tongue epithelium-derived cells, SCC-25, an increase in tumor progression characterized by increased proliferation and invasiveness of adjacent tissues.

Chronic infection in the oral cavity can contribute to the initiation process of tumorigenesis by several mechanisms including aberrant activation of immune cells, induction of DNA damage by reactive oxygen species (ROS) and increased levels of derived bioactive immunocytes which also facilitate tumor progression. The above data shows that PD induction and periodontopathogens can directly stimulate cancer cells or adjacent skin, resulting in the initiation and promotion of OSCC suggesting a relationship of cause and effect. Thus, during the evolutionary history of microbial/ mucosa / immunity- interaction in the pathogenesis of OSCC, this data adds a new level of complexity, highlighting the role of a direct interaction between pathogens and epithelial cells.

Demonstrating the initiator and promoter potential of PD and its periodontopathogens, *in vitro* tests suggests that this stimulus is sufficient to promote the transformed phenotype of normal cells. Using human oral non-tumorigenic immortalized keratinocytes shown that persistent exposure to the *P. gingivalis* bacteria-induced cell morphological changes of transformation, increased proliferation capacity, induce changes in microenvironment to an inflammatory phenotype, and promoted migration and invasion.[40, 51, 52] It has been suggested that tumor-related genes like NNMT, FLI1, GAS6, lncRNA CCAT1, PDCD1LG2 and CD274 are key regulators in transformation under prolonged exposure to *P. gingivalis*. The positive regulation of CCAT1 (cancer-associated transcript-1) was identified for the first time related to oral epithelial cells transformation. CCAT1 is a recently discovered lncRNA that is upregulated in various types of cancer and is associated with the migration and proliferation of tumor cells.[53-56]

Thus, chronic infection with this bacterium can be considered a potential risk factor for oral cancer. Another upregulated gene that was confirmed in the mRNA levels was the one encoding the Nicotinamide N-methyltransferase (NNMT). NNMT is an enzyme closely related to biotransformation and is highly expressed in several malignant lesions.[57] Infection by *P. gingivalis* in oral cancer cells induces a more aggressive transformation, and the increased expression of NNMT was associated with the development of OSCC. Another study identified IL-6, STAT1, LYN, BDNF, C3, CD274, PDCD1LG2, and CXCL10 as important candidates associated with OSCC and illustrates the role of promotion by *P. gingivalis* infection in the initiation and progression process of OSCC.[46]

All these data support the close relationship between infection with periodontopathogens and oral cancer development and are reinforced by the identification of biomarkers of bacteria associated with OSCC evolution in patients. The analysis of oral microbiota of 51 healthy and 197

OSCC patients at different tumor stages was investigated using 16S rRNA V3V4 amplicon sequencing. The oral microbiota communities of patients showed significantly greater complexity than those of healthy controls. *Fusobacterium periodonticum, Parvimonas micra, Streptococcus constellatus, Haemophilus influenza* and *Filifactora locis*, that have already been correlated with the development of OSCC, have progressively increased in abundance from stage 1 to stage 4 of this cancer.[58] Among several oral pathogens, chronic infection by *P. gingivalis* was one of the most cited in several studies and presented results suggestive of oral cancer causality. In a mouse model of OSCC induced by 4NQO when associated with chronic infection with *P. gingivalis* demonstrated a greater proportion of mice with tumors on the tongue with a significant increase in lesion size and invasion compared to the 4NQO treatment alone. In addition, 80% of the mice in the 4NQO + *P. gingivalis* group had a higher risk of oral carcinogenesis, compared with 50% in the 4NQO group. These results indicated that *P. gingivalis* promoted oral cancer induced by the 4NQO.[59]

Together, these data suggest that these bacteria, that are the major responsible for the destruction of periodontal tissues, act in the process of carcinogenesis of OSCC.

Tumor progression and PD: invasion and metastasis

Cancers can spread throughout the body through two mechanisms: invasion and metastasis. Invasion refers to direct cell migration and penetration into neighboring tissues. And in addition to locally spread from its point of origin, the cancer cells can penetrate blood and lymphatic vessels and be transported to distant places, where they start new cancerous growth *foci* in a process called metastasis.[33]

We showed above recent studies that have positively correlated PD with the increased risk and severity OSCC. Interestingly, the presence of *P. gingivalis* increases tumorigenic properties and, therefore, has been proposed as a potential etiological agent for OSCC. One possibility is that the initial molecular changes induced by *P. gingivalis* promotes transformation by epithelial-mesenchymal transition (EMT) once infected cells display decreased numbers of desmosomes and weakened cell junction which is a precondition for migration and invasion.[60] Furthermore, *P. gingivalis* infection significantly increased the expression of an important regulator of EMT, heparanase, and of different matrix metalloproteinases as MMP-9. MMP-9 belongs to a family of matrix metalloproteinases (MMPs) capable of cleaving many proteins of the extracellular matrix (ECM) regulating it´s remodeling increasing migratory ability of cell, invasion, metastasis, and angiogenesis.[61] Heparanase which is an endoglycosidase that cleaves heparan sulfate (HS) participates in the degradation and remodeling of ECM as well. It is highly expressed in human tumors and when artificially overexpressed in tumor cells lead to an invasive phenotype in experimental animals. Heparanese also releases angiogenic factors from the ECM and thus induces angiogenesis leading to increase in tumor vascularization and the low postoperative survival of cancer patients.[62] Furthermore, analysis of E-cadherin adhesion protein showed a significant decrease in its expression and a loss of membrane location of β-catenin and an increase in vimentin expression. This set of data suggests that infection by *P. gingivalis, in vitro,* can induce EMT as well as promote increased cell migration. The same effects of increased migration and invasion were reproduced in H400 strain of OSCC infected with *P. gingivalis* and *F. nucleatum* where a significant upregulation in transcription of different mesenchymal markers (vimentin) and negative regulation of epithelial markers (E- cadherin). All cytokines examined, such as transforming growth factor beta 1 (TGF-ß1) and tumor necrosis factor alpha (TNF-α), were significantly increased by periodontopathogens, suggesting that both were involved in the induction of EMT.[63] Thus, these periodontopathogens caused changes OSCC cells at the molecular, structural and behavioral levels in conformation with EMT.

Data *in vitro* and *in vivo* corroborate that infection by *P. gingivalis* promotes distant metastasis of oral cancer and affects tumor growth.[59] To investigate whether chronic PD could affect the growth of oral cancer *in vivo*, OSCC cells (human OSCC cell line, OSC-20) infected or not with *P. gingivalis* were inoculated in mice to form xenograft tumors. It was observed that tumors induced by uninfected cells were significantly larger than those induced by *P. gingivalis* infected cells. However, metastatic lesions were detected in greater quantities and in larger areas in mice that received infected cells. The size of metastatic *foci* was variable, ranging from microscopic lesions to large nodules that replaced large portions of the lung lobes. Repetitive exposure of OSC-20 cells to *P. gingivalis* for 5 weeks resulted in metastatic *foci* in the lung tissues of all six tested mice. In contrast, only one mouse injected with uninfected OSC-20 cells developed macronodules in the lungs.

Oral Cancer Immune Evasion Induced by PD

Periodontal disease, characterized by chronic inflammation, is a significant risk factor for oral carcinogenesis. *P. gingivalis* is proposed as a fundamental pathogen in chronic PD, causing a discordant immune response.[64] The bacterium regulates specific receptors on OSCC cells and keratinocytes, induces EMT of normal oral epithelial cells and activates MMP -9 and IL - 6 in carcinoma cell cultures.[40, 52, 60, 63]

The immune system plays a major role in the surveillance against tumors. To avoid attack from the immune system, tumor cells develop different strategies to escape immune surveillance. Evidence of immune surveillance comes from

both animal models and clinical observations.[65] PD may play a major role in immune evasion by oral tumor cells. The involvement of the immune response on OSCC tumor progression was assessed by analyzing B7-H1 and B7-DC receptors, which play important roles in the immune response, being co-signaling molecules that mediate the regulation of T cell activation and tolerance and are capable of negatively regulate the functions and survival of activated T cells.[66] High expression of B7-H1 in host cells can contribute to chronic inflammatory disorders and represents a possible mechanism of immune evasion that frequently precede the development of human cancers. *P. gingivalis* infection of human OSCC cell lines (SCC-25) induced the expression of B7-H1 and B7-DC, which would justify a greater evasion of the immune system in oral cancer, demonstrating and justifying, thus, one more causal correlation between PD and cancer and a possibly evasion of the immune.

Conclusions

PD is associated with oral cancer. The data presented suggest a probable casual effect of periodontal disease development with the incidence and progression of oral cancer and possible molecular mechanisms. The use of experimental animals and *in vitro* cell culture studies are very useful to understand the association of these biological processes.

The diversity of models found in the literature often prevents a direct comparison between the results and conclusions. This review comparatively evaluates different models of PD and cancer induction and shows a major role of the interaction between these diseases in development and progression and cellular/molecular characteristics of cancer. It also suggests that the inflammatory condition associated with periodontal diseases has a possible important role in the process of carcinogenesis, regardless of other associated risk factors. Although additional studies are needed to unravel completely the complex intricate network of molecular and cellular events underlying the action of PD in tumorigenesis of oral cancer, the current findings highlight the importance of further studies in this area to deepen the understanding of the correlation between PD and cancer, but also for the design of new prevention / treatment strategies for oral cavity OSCC in the scenario of chronic oral infection.

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