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Research Article

Porphyromonas Gingivalis and *Fusobacterium nucleatum* in oral Oncogenesis and Tumour

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Abstract

Oncological pathology of the oral cavity is a subject of study due to its significant impact on the quality of life of patients, caregivers and on public health. According to the National Institute of Statistics, oncological disease is the second leading cause of death in Portugal, with oral and pharyngeal cancer having an incidence rate of 16.7% and a 50% mortality rate. Periodontal disease is one of the most prevalent infectious conditions in the world, affecting 25-40% of the adult population. This pathology is often associated with the presence of *Porphyromonas gingivalis* and *Fusobacterium nucleatum*, being a consequence of the complex interactions between these microorganisms and their products, triggering a host inflammatory response that leads to tissue destruction, increasingly associated with oral oncogenesis and tumor progression.

This work goal is to study the involvement of *Porphyromonas gingivalis* and *Fusobacterium nucleatum* in oral oncogenesis and tumor progression to understand how these bacteria influence the pathophysiology of oral cavity cancer.

The conducted systematic review used Cochrane guidelines through the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) diagram and based on the Population, Intervention, Comparison, Outcome (PICO) criteria. Literature search covered four databases – B On, Science Direct, PubMed, Cochrane Library - and grey literature (master's theses, conference proceedings, world organizations). Publications between 2013 and 2023 were selected for full reading.

Several mechanisms explain the association between *Porphyromonas gingivalis* and *Fusobacterium nucleatum* and oral oncogenesis and tumor progression. These bacteria can promote cell proliferation by modulating various signaling pathways, and facilitate cellular invasion, allowing the dissemination of cancer cells. Another important mechanism is the induction of chronic inflammation. Prolonged inflammatory response creates a favorable environment for tumor progression. Pro-inflammatory cytokines (interleukin-6 and interleukin-8) play key roles in the inflammatory process, promoting cell proliferation, invasion, and metastasis. Immune evasion is the mechanism by which cancer cells avoid detection and destruction by the immune system. These bacterial strains modulate host immune response, favoring survival and dissemination of cancer cells. This systematic review highlights the complex interaction between chronic inflammation induced by periodontal bacteria and oral oncogenesis. The results underline the need for future investigations to deepen the molecular mechanisms involved and to develop effective therapeutic approaches. Early identification and targeted treatment of chronic inflammation, as well as modulation of the oral microbiome, may be promising strategies to improve clinical outcomes for oral cancer patients, highlighting the importance of preventive measures in oral oncology.

Keywords: "*Porphyromonas Gingivalis*"; "*Fusobacterium Nucleatum*"; "Periodontal Disease"; "Oral Microbiome"; "Oral Oncogenesis"; "Risk Factors"

Abbreviation

ATP: Adenosine Triphosphate; DAMP: Damage-Associated Molecular Patterns; EMT: Epithelial-Mesenchymal Transition; FN: *Fusobacterium Nucleatum;* HSP27: Heat-Shock Protein 27; IL-1β: Interleukin 1β; IL-6: Interleukin 6; IL-8: Interleukin 8; JAK1: Janus Kinase 1; MMP: Metaloproteinase; NDK: Nucleoside Diphosphate Kinase; NF-κB: Nuclear Factor-κB; OC: Oral Cancer; OSCC: Oral Squamous Carcinoma Cells; P2X7: Purinergic Receptor; PG: *Porphyromonas Gingivalis;* PI3K: Phosphoinositide Kinase; RNI: Reactive Nitrogen Intermediates; ROS: Reactive Oxygen Species; TGF-β1: Transforming Growth Factor β1; TNF-α: Tumour Necrosis Factor α; VEGF: Vascular Endothelial Growth Factor

Introduction

The cancer of the oral cavity or oral cancer (OC) is one of the most frequent types of cancer in the world, with 377,000 new cases diagnosed in 2020 [1,2]. Almost 90% of all oral cancers are squamous cell carcinomas [3].

According to the definitions of the International Statistical Classification of Diseases and Related Health Problems, OC comprises malignant tumors of the lip, oral mucosa, alveolar ridge and gingiva, tongue, floor of the mouth and/or unspecified parts of the mouth, tonsils, retromolar trigone, oropharynx, hard and soft palate [4].

In recent decades, the incidence of lip and oral cavity cancer has increased considerably worldwide in both sexes in young age groups, particularly in females [5]. Portugal is no exception to this trend, as data from the Global Cancer Observatory on mortality and incidence in 2020 recorded 1103 cases and 382 deaths [6].

Overall survival rates of oral cancer at 5 years are low (around 40%), however, if diagnosed in the early stages (stages I and II), patients can have a survival rate of over 80%, while if diagnosed at an advanced stage (stages III and IV) the survival rate for OC is less than 50%. This is due to the fact that most patients don't show symptoms at an early stage and don't look for medical help, seeking it only when signs and symptoms such as pain, bleeding or a mass in the mouth or neck appear, and regional metastasis is already present [4,7].

It is a complex disease, multifactorial in origin, with various etiological factors that have different origins: chemical such as tobacco, physical such as radiation and biological such as viruses, bacteria, hormones, chronic inflammation and oxidative stress [8,9].

Currently, the triad - oral microbiome, periodontal disease and oral oncogenesis - has been investigated. The oral microbiome refers to the complex community of microorganisms that colonize the oral cavity, playing a fundamental role in oral health, and maintaining a delicate balance with the human host. It is known that imbalances in the oral microbiome, associated with various periodontal risk factors, can lead to the development of periodontal disease. These inflammatory conditions result from the host's response to pathogenic microorganisms that accumulate mainly in dental plaque. In addition to the direct impact on oral health, with deterioration of the soft and hard structures of the oral cavity, emerging studies also suggest a possible link between periodontal disease and oral oncogenesis. The chronic inflammation associated with periodontal disease can create a favourable microenvironment for the growth of cancer cells and promote the progression of pre-neoplastic lesions to OC. Understanding these interactions between the oral microbiome, periodontal disease and oncogenesis is crucial for developing more effective preventive and therapeutic strategies to protect oral health and reduce the risk of OC [10,11].

Several bacteria in the oral cavity have been identified as potential bacterial aetiological agents in oral oncogenesis. The oral microbiota is made up of a diverse and constantly changing set of bacteria that reside within the oral cavity and develop interactions with the host and the surrounding environment. The oral microbiota influences the health/disease binomial of the oral cavity, being interconnected with systemic disorders and involved in carcinogenesis [12].

The notion that bacteria may be involved in the development of benign and malignant oral tumours is not new. There are several broadly defined pathways by which bacteria can contribute to tumour growth and development. These include modulating the balance of proliferation and death of host cells, disrupting immune surveillance, promoting an inflammatory microenvironment and altering the metabolism of compounds produced by the host [13].

The transformation of the normal oral mucosa into a malignant neoplasm involves several stages, with more than 80% of cases developing from potentially malignant oral lesions, the most common of which are at high risk of transformation: oral leukoplakia, proliferative verrucous leukoplakia, erythroplakia, oral lichen planus and oral submucosal fibrosis [14].

Porphyromonas gingivalis (PG)

The genus Porphyromonas is made up of Gram-negative, asaccharolytic, obligate anaerobic, non-spore-forming, non-motile and pleomorphic bacilli. One species of this genus is PG, which belongs to the Porphyromonadaceae family. This bacterium is normally found in deep periodontal pockets [12].

PG is a well-known periodontal pathogen, associated with the beginning and progression of periodontitis. Some studies have demonstrated that this bacterial strain can colonize malignant tissues in the oral cavity [15-17].

PG capacity of survival and causing disease is strongly dependent on a wide range of virulent factors. These factors include both structural components inherent to the bacterium itself, such as lipopolysaccharides, fímbrias and heat-shock proteins, as well as secretory components such as gingipains and outer membrane vesicles. The presence of these virulent factors allows PG to effectively escape the host's immune system and create a persistent infection, resulting in the development of periodontal disease [18].

This bacterium uses several strategies to compromise the integrity of tissues, impairing the host's immune response, including inhibition of cell apoptosis, stimulation of cell proliferation, initiation of chronic inflammation and generation of oncometabolites. PG, through its virulent factors, induces cytokines and chemokines, such as interleukin 6 (IL-6), interleukin 8 (IL-8), tumour necrosis factor α (TNF-α) e interleukin 1β (IL-1β) [18,19]. These inflammatory mediators can induce or facilitate cell proliferation, mutagenesis, oncogenic activation and angiogenesis [20,21].

PG can promote carcinogenesis by interfering with several signaling pathways

- After host infection by PG, the B7-H1 receptor can be activated, facilitating apoptosis of activated T cells. The increase in the expression of these receptors can lead to the persistence of inflammatory diseases [11].
- Nucleoside diphosphate kinase (NDK), effector protein produced by PG, can block the signaling pathway of adenosine triphosphate (ATP)/ purinergic receptor (P2X7) in macrophages, avoiding the secretion of IL-1β and helping the process of carcinogenesis. The enzyme NDK also phosphorylates the heat-shock protein 27 (HSP27) and can trigger apoptotic processes after the phosphorylation [11].
- PG activates apoptotic pathways such as Janus kinase 1 (JAK1)/ signal transducer and transcription activator 3 and phosphoinositide kinase-3 (PI3K) in oral epithelial cells, promoting, in this way, OC. Besides inhibiting apoptosis of invaded cells, it can also increase the progression of the S phase of cell cycle by inhibiting the gene coding for the tumour suppressor p53 through adesine fimA. Due to the induction of T regulatory cells by the B7-H1 receptor, the immune system is partially inactivated [22].
- The activation of the pathways ETS1, p38/HSP27 and PAR2/ NF-κB of protein kinase 1/2 regulated by extracellular signal (ERK1/2), was observed in response to PG infection, leading to the induction of the expression of matrix metaloproteinase-9 (MMP-9) and increased cellular invasion [23,24].

PG causes persistent intracellular infections. Due to the production of inflammatory molecules such as IL-6, that cause hypomethylation of DNA and hypermethylation of the promotor region, chronic inflammation has been associated to cancer. IL-8 can regulate zinc-dependent MMPs that degrade extracellular matrix to induce metastisation of malignant cells. Through the activation of epidermic growth factor, IL-8 can also promote cell growth [13].

Transforming growth factor β1 (TGF-β1) regulates various cellular functions. Some studies link TGF-β1 to epithelial-mesenchymal transition, tumour angiogenesis and metastasis. Like IL-8, TGF-β1 can increase cell invasiveness by activating MMPs. The cytokine TNF-α affects carcinogenesis in several stages. Reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI) of that protein cause genome instability and cancer causing mutations. Other pathways of TNF-α include the induction of epithelial-mesenchymal transition and the production of vascular endothelial growth factor (VEGF), that promotes tumour angiogenesis [20].

PG infection promotes the expression of B7-H1 receptor, that regulates immune responses mediated by cells, meaning that PG can direct malignant cells to distant places. However, co-stimulatory signals mediated by the B7-H1 receptor can induce anergy and apoptosis in activated T cells, allowing malignant cells to avoid the immune response [12].

PG metabolites, such as oxygen radicals, butyrate and acetaldehyde, can also cause cancer. Butyrate is a strong bacterial carcinogen that can trigger apoptosis of T and B lymphocytes and decrease their function. Mutations and genomic instability, induced by oxygen radicals, can result in DNA damage such as double strand breaks or nucleotide changes. Moreover, as a dangerous by-product of ethanol metabolism, acetaldehyde can damage DNA and promote aberrant epithelial cell growth. Consequently, through the production of these toxic products, PG can cause OC [16].

Fusobacterium nucleatum (FN)

The genus Fusobacterium comprises several species of obligate anaerobic, non-spore-forming, mobile or non-mobile Gram-negative rods, which are considered late colonising bacteria of the healthy oral cavity. The appearance of OC has been associated with the FN strain, which is commensal to the human oral cavity [12].

FN is another Gram-negative anaerobic bacterium frequently found in the oral microbiome and in periodontal disease sites. It is known for its adhesive properties, which facilitate attachment to other bacterial species and host cells [25].

Like PG, high levels of FN are associated with the onset and progression of OC. Through a variety of processes, including the production of toxins, enzymes and signalling molecules, this bacterium can attach itself to the oral epithelium, cause inflammation, alter the immune system and trigger carcinogenesis [26].

The possible mechanisms by which FN may contribute to the development of cancer include the following pathways

- Proliferation of oral epithelial cells is induced by FN FadA [27,28]. The FadA factor attaches to E-cadherin, that activates β-catenin. Translocation of β-catenin to the nucleus leads to the overexpression of oncogenes, such as Myc and cyclin D, inducing cell proliferation [29].
- FN infection can lead to the increase in the synthesis of MMP-13, also known as collagenase 3, facilitating the movement of cells by activation of Etk/BMX and kinase RhoA. It is also capable of activating protein kinase p38 that will trigger the activation of HSP-27, leading to the production of MMP-9 and MMP-13 [30,31], that favour tumour invasion and metastatisation.

FN strongly induces collagenase 3, a MMP that degrades extracellular matrix and helps tissue degradation and cell migration. Cells infected by FN, produce collagenase 3 through p38, a signalling system that controls proliferation, survival and cellular inflammation. By increasing the synthesis of collagenase 3, FN can cause periodontal disturbances and other infections [12].

FN infection causes translocation of nuclear factor-κB (NF-κB) to the nucleus, resulting in the induction of cytokine coding genes. FN infection also activates the NLRP3 inflammasome, which causes activation of caspase-1 and production of mature IL-1β. Unlike other pathogens, FN may be able to activate the inflammasome in gingival epithelial cells without ATP. At the same time as caspase-1 is activated, FN infections release other DAMPs that cause inflammation, such as high mobility group protein 1 and apoptosis-associated speck-like protein. FN is known to produce pathogen-associated molecular patterns that activate NF-κB and act as an endogenous DAMP to activate the inflammasome, causing further inflammation through secondary DAMPs [12,29,32].

FN infection also decreases the expression of the cyclin-dependent kinase inhibitor p27, DNA repair protein Ku70 and p53, which suggests that FN infection promotes OC, affecting DNA repair via the Ku70/p53 pathway and creating genomic instability [33].

Although several studies have revealed the coexistence of PG and FN in cancer patients, the interaction between these two strains has not been well studied so far [11].

PG and FN are two bacteria associated with periodontal disease, that often act in association and have been implicated in oral oncogenesis. These microorganisms can generate a favorable microenvironment for the development of OC through multiple mechanisms. PG produces enzymes and toxins that promote chronic

inflammation and tissue damage in the oral cavity, facilitating the progression of pre-neoplastic lesions. On the other hand, FN has the ability to adhere to epithelial cells and infiltrate tissues, promoting the formation of bacterial biofilms and contributing to the local inflammatory response.

Objective

Given the relevance of this topic, this systematic review aims to discuss and analyse the impact of biological risk factors on oral oncogenesis. More specifically, the aim is to study the involvement of the bacteria Porphyromonas gingivalis and Fusobacterium nucleatum in oral oncogenesis and tumour progression, when present in cell cultures of human tissues, in order to better understand how they influence the pathophysiology of OC.

Understanding the joint role of PG and FN in oral oncogenesis is crucial for developing targeted therapeutic strategies aimed at interrupting these processes and reducing the risk of OC associated with periodontal disease.

Materials and Methods

Study design/protocol

This systematic review was prepared using the Cochrane guidelines and recommendations for systematic reviews (Version 6.4, 2023), following the Population, Intervention, Comparison, Outcome (PICO) search strategy [34] and systematised using the Preferred Reporting Items for Systematic Reviews in Meta-analysis (PRISMA) analysis diagram [35].

Investigation question and inclusion and exclusion criteria

To start the investigation process, the following question was proposed:

"What is the relationship between exposure of human tissue cell cultures to the bacteria Porphyromonas gingivalis and Fusobacterium nucleatum and oncogenesis and tumour progression in the oral cavity?"

Table 1 shows the PICO criteria (Patient/Population, Intervention, Comparison and Outcome) used to formulate the research questions for the systematic review [36].

The PICO criteria are defined in the following way

- P (Population/Patient): Characteristics of participants included in the study.
- I (Intervention): Intervention or investigated treatment.
- C (Comparison): Absence of treatment or other standard treatment.
- (Results/Outcome): Main evaluated results.

The use of these criteria aims to ensure the clarity and specificity of the research questions, facilitating the search for the available evidence.

Criteria	
Population	Cellular cultures exposed to PG and/or FN.
Intervention/ exposition	Infection of human cells with PG and FN and its relationship with oral oncogenesis and tumour progression.
Comparison	Compare tumour development and progression of cells exposed to PG and/or FN, with cells not exposed to these bacteria.
Results (Outcomes)	In cells exposed to PG and/or FN oncogenesis and tumour progression in oral cavity are exacerbated by the infection.

Table 1: Formulation of the research question according with PICO criteria.

Inclusion criteria

- Studies performed in the last 10 years (between 2013 and 2023)
- Studies that address OC
- *In vitro* studies
- Human cells
- Full text available
- Presence of PG and/or FN in the paper
- Presence of oncogenic markers

Exclusion criteria

- Studies addressing other types of cancer besides OC
- Human studies
- *In vivo* studies
- Animal studies

Keywords

- *Porphyromonas gingivalis*
- *Fusobacterium nucleatum*
- Periodontal disease
- Oral cancer
- Oral microbiome
- Oral oncogenesis
- Epidemiology
- Risk factors
- Tumoral markers

Research strategy and papers selection

The bibliographic search for this systematic literature review was carried out in 4 electronic databases: b-On, Science Direct, PubMed, Cochrane Library, and also includes consultation of grey literature (master's theses, minutes of scientific events, world organisations) between June 2023 and March 2024, using the keywords "*Porphyromonas gingivalis*", "*Fusobacterium nucleatum*", "Periodontal disease", "Oral cancer", "Oral microbiome", "Oral oncogenesis", "Risk factors", "Tumour markers" and the combination of these keywords.

The following combination was used

(*Porphyromonas gingivalis* OR *Fusobacterium nucleatum*) AND (oral cancer or mouth cancer or oropharyngeal cancer or oral squamous cell carcinoma or tongue cancer) AND (periodontal disease or periodontitis or chronic periodontitis or oral microbioma) AND (oncological markers or risk factors)

The Mendeley application was used to organize and group the articles according to the search criteria and to exclude repeated articles.

Articles made available for reading in the aforementioned databases between 2013 and 2023 were selected. Additional searches and full reading were carried out after searching for articles that were in the bibliographical references of others that had already been read (manual search). Figure 1 shows the selection process, carried out by 4 researchers.

Evaluation of the methodology quality of the studies

The quality of the studies used for this review was analysed taking into account the parameters of the QUIN Tool's risk of bias table, which consists of 12 points, with scoring and classification options, allowing the quality of in vitro studies to be assessed.

The twelve essential criteria for evaluating the methodology of a QUIN Tool study [37] are

- **Clarity of objectives:** The study must have clear and specific objectives that must be followed during the study.
- **Detailed explanation of the calculation of the sample size:** Details about how the sample size was calculated, including used software, formula and parameters should be presented.
- **Detailed explanation of sampling technique:** The method of sample selection, including the pre-defined population, sampling technique and inclusion and exclusion criteria, must be completely presented.
- **Details of the control group:** The control group, being a positive, a negative or a standard control, must be specified.
- **Detailed explanation of the methodology:** The procedure used, the method of standardisation and details of universal standards (if applicable) must be clear.
- **Operator details:** The number of operators and details of their training and calibration must be specified.
- **Randomization:** Details on sequence generation and allocation concealment must be provided.
- **Method of measuring the result:** The procedure and reason for choosing the measurement method should be clear. If applicable, the method of standardisation and details of universal standards should be specified.
- **Details of the results assessor:** The number of assessors and details of their testing and calibration should be specified.
- **Blinding:** It must be specified whether there was blinding of the operator, results assessor and statistician.
- **Statistical analysis:** The used software programme and details of the statistical analysis must be specified.
- **Presentation of results:** The results should be based on the pre-defined objectives and all data should be properly tabulated, including baseline data, if applicable.

To each of the mentioned points is given a score ranging from 0 (zero), 1 (one) and 2 (two) "Adequately specified (score = 2)": Indicates that the criterion in question has been clearly defined and explained in the study, resulting in a score of 2.

"Inadequately specified (score = 1)": Indicates that the criterion was mentioned in the study, but insufficiently or unclearly, resulting in a score of 1.

"Non specified (score = 0)": Indicates that the criterion was not addressed in the study, which corresponds to a score of 0.

"Not applicable": Indicates that the criterion does not apply to the study in question [37].

Results

All the recommendations of the PRISMA flow chart were followed when selecting the articles, respecting the exclusion criteria.

From a total of 126 potentially relevant articles, 109 remained after removing duplicates. A subsequent screening excluded 98 articles after careful evaluation. This led to the selection of 11 articles that make up the results of this systematic review (Figure 1).

Table 2 shows the assessment of the risk of bias of the studies selected for this systematic review, according to the QUIN Tool criteria.

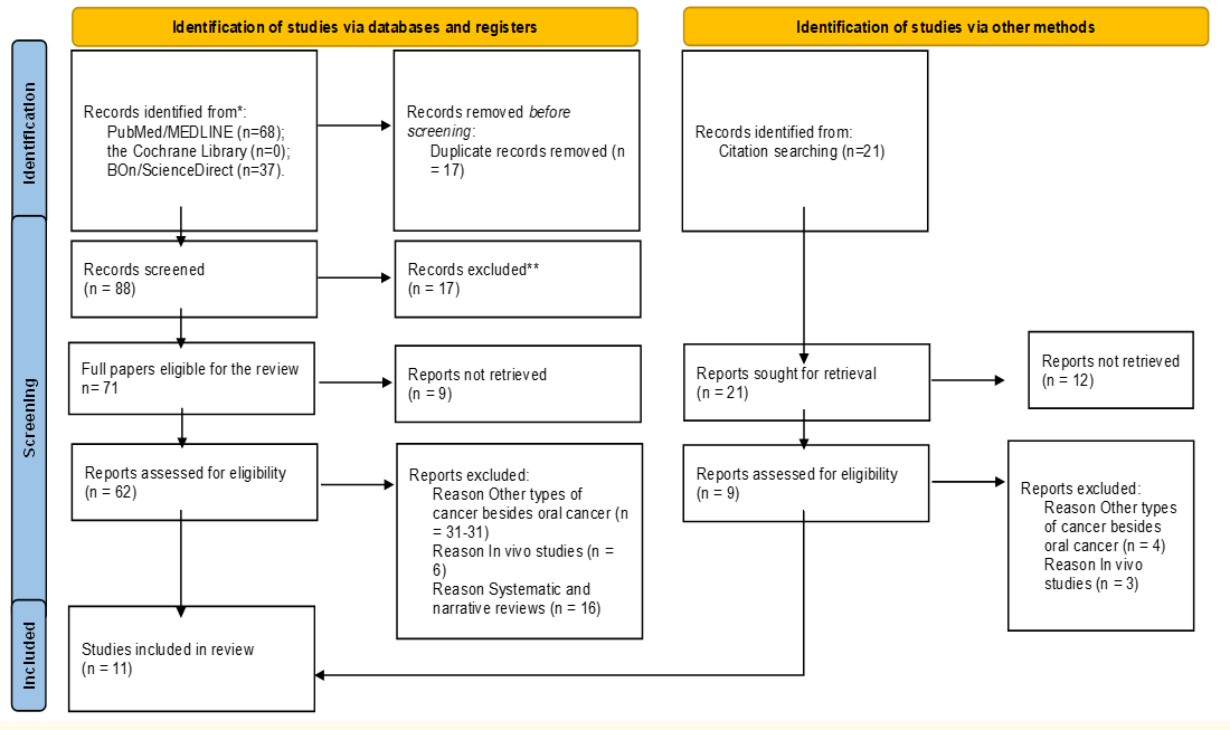

Figure 1: The flowchart of the article's selection process (adapted from Page., et al. [35]).

*Consider, if feasible to do so, reporting the number of records identified from each database or register searched (rather than the total number across all databases/registers).

**If automation tools were used, indicate how many records were excluded by a human and how many were excluded by automation tools.

Criterium Study	Inaba., et al. [23]	Cho., et al. [38]	Inaba., et al. [39]	Ha., et al. [40]	Inaba., et al. [41]	Ha., et al. [24]	Cho., et al. [42]	Geng., et al. [43]	Groeger., et al. [44]	Zhang., et al. [19]	Shao., et al. [45]	Average
Clearly defined Goals/ objectives	2	2	0	1	1	2	2	2	2	1	1	1.46
Detailed explanation of the calculation of sample size	0	0	0	0	0	0	2	2	1	1	2	0.77
Detailed explanation of the sampling technique	1	1	1	1	0	2	2	2	2	2	2	1.46
Details of the comparison group	1	1	1	1	1	2	2	2	2	2	2	1.55
Detailed explanation of methodology	1	1	2	2	1	2	2	2	2	2	2	1.73
Operator details	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Randomization	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Method of measurement of the result	1	1	2	2	2	2	2	2	2	2	2	1.82
Details of the results evaluator	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Blinding	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Statistical analysis	0	0	1	2	0	1	2	2	1	0	1	0.91
Presentation of results	1	1	2	2	1	2	1	2	2	1	2	1.55
Average	0.88	0.88	1.13	1.38	0.75	1.63	1.88	2	1.75	1.38	1.75	

Table 2: Risk of bias.

Most of the studies' goals/objectives were clearly defined, and the average for this parameter is close to 1.46 when judged between 0 and 2. However, looking at the results of the explanation of the sample size calculation, it can be seen that it has lower values, with an average of around 0.73. The average of 1.46 for the sampling technique reflects that this parameter is generally of good quality. The same can be seen for the details of the comparison group, which were adequate, with an average of 1.55.

As for the methodology used, it was explained in detail in most of the studies, with an overall average of 1.73, indicating clarity and objectivity. The method used to measure results was also of high quality in most of the studies, with an average of 1.82. On the other hand, the statistical analysis showed a significant variation between the 11 studies analysed, with an average of 0.91, indicating the need for improvement in many studies. The quality of the presentation of the results in most of the studies is notable, with an average of 1.55.

To make the selected studies easier to understand, the objectives of each one are detailed in Table 3, as well as the used materials and methods, the main results and the conclusions, which will then be analysed and discussed. This table brings together and summarises the 11 in vitro studies analysed in this systematic review, the main aim of which is to investigate the influence and impact of periodontal bacteria, PG and FN, on the cells of the oral cavity, paying particular attention to aspects of oncogenesis, proliferation, invasion and the expression of OC-related genes.

The studies vary in terms of cell samples, and this diversity allows for a comprehensive perception of the impact of PG and FN bacteria on different tissues.

Author/ Year	Objective	Sample	Presence of bacteria		Results
			PG	FN	
Inaba., *et al*. [23]	Examine the effects of PG in the expression and maturation of MMP2 and MMP9 by oral squamous carcinoma cells (OSCC).	Cells "human tongue squamous cell carcinoma" (SAS) highly invasive incubated with PG and FN	X	X	PG activated the pathways ERK1/2-Ets1, p38/HSP27 and (PAR2) /NF-κB to induce the production of proMMP9.
Cho., *et al*. [38]	Analyses the effect of PG in OC cells.	Cells infected with PG.	X		Infected cells showed reduced proliferative rate and an arrest in G1 when compared with non-infected cells. PG triggered the production of Reactive Oxygen Species (ROS) that induced cells autophagia.
Inaba., *et al*. [39]	Evaluate the pathways that mediate proMMP9 induced by PG.	SAS cells infected by PG with low multiplicity of infection during 24h.	X		Ribonucleic acid (RNA) of the gene PAR4 cancelled both the expression of proMMP9 and the invasion. Phosphorylation of p38 and ERK1/2 was reduced while nuclear translocation of NF-κB was not inhibited.
Ha., *et al*. [40]	Investigate the possible connection between periodontitis and OC through the creation of an experimental model that mimics the clinical course of PG in oral cancer cells.	CEO cells were infected with PG 2x/week for 5 weeks.	X		The repetitive infection by PG resulted in morphological changes of cancer cells, promoted migratory and invasive properties. It also induced a rise in the level of expression of CD44 and CD133. The increase in invasiveness was associated with the increased production of MMP-1 and MMP-10 stimulated by the release of IL-8.
Inaba., *et al*. [41]	Evaluate the effect of polyphenols as inhibitors of cellular invasion caused by PG in SAS cells.	SAS cells were infected with PG including gingipain mutants to evaluate the inhibitors: apple polyphenols, hop bract polyphenols (HBP), PAM-PAE, BPM-PAE, EGCg, KYT-1 and KYT-36.	X		KYT-1/KYT-36, AP, HBP and HMW-HBP significantly inhibited the expression of mRNA of PAR2 and PAR4, the activation of proMMP-9 and cell invasion. Apple polyphenols, HBP and HMW-HBP reduced the activation of HS27 and Ets1 and the nuclear translocation of NF-κB.
Ha., *et al*. [24]	Examine the effect of PG in CEO cells invasiveness including SCC-25, OSC-20 and SAS.	Cells SCC-25, OSC-20 and SAS infected with PG.	X		Exposure to PG promoted the invasive capacity of OSC-20 and SAS cells. Secretion of IL-8 was increased in these cells. Negative regulation negativa de IL-8 atenuou o potencial invasivo das células e os níveis de MMP.
Geng., *et al*. [43]	Explore the inner effect of chronic infection by PG in CEO and identify relevant markers.	Human immortalised oral epithelial cells (HIOECs) exposed to PG with low multiplicity of infection for 5-23 weeks.	X		Persistent exposure to PG caused cell morphological changes, increased proliferation capacity and promoted cell migratory and invasive properties.

Groeger., *et al*. [44]	Investigating the activation of signalling cascades in primary epithelial cells and CO cell lines.	Cells infected with whole bacterial cells and also with bacterial fractions.	X		In SAS cells genes IKBKB (signalling pathway NF-κB), IRF5 (pathway TLR) and JUN, MAP2K4, MAPK14 and MAPK8 (via MAPK) were positively regulated. In primary cells genes IRF5, JUN, MAP2K4, MAPK14 and MAPK8 were positively regulated.
Cho., *et al*. [42]	To investigate the anti-invasive effect of acetylshiconine on PG-infected cancer cells and the mechanisms involved.	Cells were co-cultivated with low multiplicity PG.	X		The cancer cells became more aggressive when infected with PG. Acetylshiconine significantly inhibited cell invasion by suppressing IL-8 release and IL-8-dependent MMP release.
Zhang., *et al*. [19]	To analyse the transcriptome profile of HIOECs exposed to FN infection.	RNA from HIOECsm infected with FN and not infected.		X	3307 mRNAs were identified as differentially expressed, including FYN, RAF1, ATM, FOS, CREB, NCOA3, VEGFA, JAK2, CREM and ATF3.
Shao., *et al*. [45]	To examine the regulation of p-EMT by FN in CEO cells.	Cells with epithelial phenotype, p-EMT or EMT cultured with live or heat inactivated FN.		X	Both live and inactivated FN positively regulated the expression of p-EMT-related genes in cells with an epithelial phenotype, but not with a p-EMT or EMT phenotype. FN promoted the invasion of SAS cells with an epithelial phenotype.

Table 3: Compilation of the results of the studies analysed.

(X: Presence of Bacteria; *Porphyromonas Gingivalis* (PG); *Fusobacterium Nucleatum* (FN).

Analysing the results (Table 3) shows the variation in the protocols used, and it can be seen that in 7 studies there was cell exposure only to PG, in 2 studies there was cell exposure only to FN and only 1 study shows cell exposure to both bacteria. The complexity of the interactions between periodontal bacteria and oral cells is well known, and according to studies, these interactions are influenced by factors such as bacterial virulence and the host's immune response.

Discussion

During the course of this systematic review, several pieces of scientific evidence were found that prove the relationship between chronic inflammation and OC. More specifically, we report on the influence of PG and FN bacteria (present in higher concentrations in the oral microbiome when periodontal disease is diagnosed) and their association with oral oncogenesis and tumour progression.

Although one of the used exclusion criteria was human studies, it is pertinent to address in this section the relevance of a study found in which the author demonstrates the usefulness of serum antibody levels against periodontal microorganisms as biomarkers for OC. In addition, there is a notable increase in IL-6, which is a representative inflammatory cytokine, in the serum of patients with OSCC compared to normal controls. It further demonstrates that inflammation is implicated in the pathogenesis of OSCC. A higher level of serum IL-6 in OSCC patients was also highly correlated with a worse prognosis, indicating the possibility of using the serum IL-6 level as a prognostic factor. The strong association between infection and the inflammatory component, which are closely related, is noteworthy [46].

Supporting this interconnection, IL-8 is a pro-inflammatory cytokine that plays a crucial role in the immune response, acting as a chemotactic to attract neutrophils to sites of inflammation and infection. On the other hand, IL-6 is a multifunctional cytokine that regulates a variety of biological processes, including inflammation, immune response and metabolic regulation. Both interleukins play important roles in modulating the body's inflammatory response to various stimuli. These cytokines have been widely studied due to their importance in the pathogenesis of various conditions, including inflammatory diseases and cancer [47].

Studies have shown that high levels of IL-6 are associated with the progression of OC, contributing to cell proliferation, invasion and metastasis. In addition, IL-8 has been implicated in promoting angiogenesis and recruiting inflammatory cells to the tumour microenvironment, thus facilitating the progression of OC [48].

In the results of the studies used for this review, there is an impact of microbial infection and inflammation on cancer cells, specifically on the oncogenesis and progression of OC.

According to the study by Cho., *et al*. [38], by triggering infection and inflammation in the tumour microenvironment, microorganisms manipulate host cells to promote their own survival and persistence. As a result, after microbial invasion, host cells often face 2 mechanisms: apoptosis or cell proliferation. Some researchers have reported that PG inhibits cell proliferation by inducing apoptosis. Others have observed an increase in cell proliferation due to the acceleration of the cell cycle or the activation of pro-survival signals by modulating apoptosis. This study shows that active PG can modulate the behaviour of malignant neoplastic cells, inhibiting cell cycle progression and subsequent cell proliferation, without interfering with apoptosis. Therefore, different factors will contribute to different outcomes, such as: tissue specificity, the state of the cells, the presence of inflammation or other associated risk factors. Analysing these variables as a whole, makes possible to understand their interconnectedness more consistently.

Inaba., *et al*. [23], identified a new molecular mechanism by which a microorganism responsible for periodontal disease can simultaneously be important for the invasion and metastatisation of malignant neoplastic cells, thus establishing a concrete link between periodontitis and OSCC. PG induces the production of proMMP-9 through multiple signalling cascades, including the ERK1/2-Ets1, p38/HSP27 and PAR2/NFκB pathways. Subsequently, the proenzyme is activated by bacterial gingipains, resulting in invasion of SAS cells. These results suggest the possible involvement of PG in various systemic diseases and provide a basis for understanding its role in the progression and metastasisation of OSCC.

Ha., *et al.* [40] concluded that prolonged PG infection of oral cells present in malignant neoplasms induces changes similar to epithelial-mesenchymal transition (EMT) and the emergence of OSCC properties. The results of the study suggest that PG is one of the most significant risk modifiers that can transform OC cells into more aggressive populations and emphasise the clinical importance of controlling periodontitis in the progression and prognosis of OC at a molecular level.

According to the conclusions of the study by Inaba., *et al*. [39], also analysed in this review, infection of human squamous cell carcinoma cells of the tongue by PG leads to an increase in cell invasion, due to the high expression of PAR2 and PAR4. This study suggests that the activation of these receptors by PG infection in OSCC, together with the subsequent production of proMMP-9, may be a key mechanism contributing to OSCC invasion in response to this periodontal microorganism.

The following year, the same researchers, Inaba., *et al*. [41] carried out another study in which it was found that carcinomas that develop in the superficial epithelium of the oral cavity, known as squamous cell carcinomas, are predominantly of the OSCC type. These tumours have the ability to spread to the lymph nodes. Metastatisation, especially initial invasion, plays a crucial role in the unfavourable prognosis associated with various forms of solid neoplasms, including OSCC, significantly affecting patient morbidity and mortality. This metastatisation process can be divided into five distinct stages, with invasion standing out as the first critical step, which in turn has three associated causes: alterations in cell-extracellular matrix interactions, cell disconnection and degradation of the extracellular matrix. This degradation is mediated by the secretion of proteolytic enzymes by the cancerous tissue, promoting the destruction of the basement membrane and, consequently, stimulating malignancy and metastatic spread. Therefore, effective control of cell invasion has the potential to provide antimetastatic effects and improvements in patient survival, thus standing out as a highly relevant therapeutic target. The findings of this study

also indicate that PG infection is associated with increased OSCC invasion, while gingipain-activated MMP-9, as well as apple- and hop-derived polyphenols, may be considered potential cytostatic agents to prevent this invasion.

Ha et al. [24] in their study show that PG exacerbates the invasion capacity of OSCC through IL-8-dependent up-regulation of MMPs. These scientific findings promote a better understanding of the correlation between periodontitis and oral carcinoma, presenting clinical and significant relevance for future OSCC prevention and treatment strategies.

Geng et al. [43], through their study, confirmed the active role of PG in tumour transformation under conditions of prolonged infection. Bioinformatic analyses have shown a new perspective on some biomarkers with potential application in the early detection of OSCC. Based on the current model, PG emerges as a motivating agent in tumour transformation under a specific inflammatory microenvironment. For future research, the use of animal models is essential to explore the role of chronic PG infection in the development of OC, while possible molecular regulators need further validation. PG can be detected not only in periodontal tissues, but also in other regions of the human body, such as respiratory, vascular and oesophageal tissues, and it is therefore extremely important to focus attention on the potential effect of the presence of PG as a biomarker in various types of tumours.

According to Groeger et al. [44] the main cause of periodontitis is the oral microbial biofilm, however, the progression of the disease is regulated by the immune-inflammatory reaction and the destruction of the supporting tissues of the teeth. PG plays a crucial role in the pathogenesis and progression of periodontitis. Among various mechanisms, PG has been shown to differentially activate the NF-κB pathway. The data from this study suggest a possible relationship between PG infection and oral squamous cell carcinomas, given that periodontal disease has been associated with the risk of oral tumours. Huynh et al. [49] reported that, in human oral epithelial cells, the expression of interleukin 6 regulatory factor was significantly increased after challenge with PG.

Cho et al. [42] observed that PG infection led to morphological changes in YD10B oral carcinogenic cells, as well as EMT characteristics indicative of greater cell aggressiveness. In addition, PG infection resulted in the production of cytokines and chemokines, namely IL-6 and IL-8, which are associated with tumour invasion and metastasis. Particularly, IL-8 plays a crucial role in cell invasion and the activation of MMPs, which are crucial in both periodontal tissue destruction and tumour invasion. Acetylshikonin, a flavonoid with anti-inflammatory potential, was shown to be effective in suppressing IL-8 induction and MMP release, as well as inhibiting the invasion of PG-infected YD10B oral carcinogenic cells, without direct toxic effects. These findings suggest that acetylshikonin may be a promising option as a therapeutic adjuvant in the treatment of OC associated with chronic inflammation.

Zhang et al. [19] recognised FN as a commensal bacterium, frequently found in the human oral cavity. This study found that the amount of invasive FN gradually increased in pre-malignant adenomatous lesions to carcinomas in the course of colorectal carcinogenesis, suggesting that FN could be understood as a new risk factor for disease progression from adenoma to cancer. According to these authors, a recent meta-analysis showed that FN was more abundant in head and neck oncological pathology. Compared to non-tumour lesions, the prevalence of FN in tumour lesions increased by 6%, and the possibility of FN being present in tumour lesions was 2.93 times higher, suggesting that FN infection may contribute to head and neck cancer pathology. Although FN has been detected in OSCC, the clinical characteristics associated with the high amount of FN are not yet conclusive.

Shao et al. [45], concluded that it is widely recognised that the ability of cancer cells to undergo p-EMT, as opposed to full EMT, is associated with an increased risk of metastatisation. Recently, an analysis of the transcriptome of single cells in OSCC identified several genes involved in the p-EMT program. Among these p-EMT-related genes, we identified some associated with prognosis, such as SERPINE1, TGFBI, ITGA5, CDH13, P4HA2 and LAMC2. Thus, the p-EMT program is correlated with an unfavourable prognosis for OSCC. It is important to note that FN enrichment is frequently observed in OSCC tissues compared to healthy oral mucosal tissues. The transformation of the epithelial phenotype to p-EMT, due to altered gene expression by FN infection, may increase the metastatic capacity of OSCC. Given that FN is an oral commensal bacterium known to be involved in periodontal diseases, oral hygiene measures aimed at reducing the amount of FN may contribute to reducing the risk of increasing the metastatic capacity of OSCC.

The systematic review carried out provided a comprehensive, confirmed and up-to-date overview of the relationship between chronic inflammation and OC, with a critical focus on the influence of PG and FN bacteria that cause periodontal disease and their association with oral oncogenesis and tumour progression.

When analysing the studies included in the review, it is clear that there is a convergence of results highlighting the crucial role of inflammation and the oral microbiome in the pathogenesis and development of OC, especially in OSCC.

The studies covered in this review provide consistent evidence that chronic inflammation, induced by the presence of PG and FN, is closely linked to the progression of OC. These pathogenic bacteria not only exacerbate local inflammation, but also promote changes in the tumour microenvironment, which favours the survival, proliferation and invasion of cancer cells. The complex interaction between oral bacteria, the host's inflammatory response and the molecular pathways involved in oral oncogenesis represents a promising field of research to better understand the mechanisms underlying the development of OC.

One of the most intriguing aspects highlighted by the included studies is the role of pro-inflammatory cytokines, such as IL-6 and IL-8, in modulating the inflammatory response and the progression of OC. IL-6 has been associated with cell proliferation, invasion and metastasis of OSCC, while IL-8 plays a crucial role in promoting angiogenesis and recruiting inflammatory cells to the tumour microenvironment. These cytokines have emerged as potential prognostic biomarkers and therapeutic targets in the treatment of OC, highlighting the importance of understanding the molecular mechanisms underlying chronic inflammation in oral oncogenesis.

The studies suggest a relationship between the presence of periodontal bacteria and tumour progression, which highlights the importance of the oral microbiome in oncogenesis and indicates that PG and FN periodontal bacteria may play a significant role in oncogenesis and tumour progression.

In addition, the studies analysed provided important information on the molecular mechanisms by which periodontal bacteria, especially PG, promote OSCC invasion and metastasis. The activation of specific signalling pathways and the differential regulation of genes associated with tumour progression highlight the multifaceted role of oral bacteria in modulating the tumour microenvironment and promoting malignancy. These findings not only expand knowledge about the interaction between the oral microbiome and OC, but also offer potential therapeutic targets for the treatment of OC.

Conclusion

Throughout this systematic review, scientific evidence were found that confirms the relationship between infection, chronic inflammation and OC. Of particular relevance is the influence of PG and FN bacteria present in the oral microbiome in cases of periodontal disease, and their contribution to oncogenesis and the progression of OC.

Studies in cell cultures with human tissue show that exposure to PG and FN is associated with the expression of markers of oncogenesis and progression of OC, especially oral squamous cell carcinoma. As well as intensifying local inflammation, these bacteria also alter the tumour microenvironment, promoting the survival, proliferation and invasion of cancer cells.

The roles of pro-inflammatory cytokines such as IL-6 and IL-8 stand out. IL-6 has been associated with cell proliferation, invasion and metastasis, while IL-8 promotes the formation of new blood vessels and the recruitment of inflammatory cells to the tumour microenvironment, making them potential biomarkers and therapeutic targets in the treatment of OC.

The molecular mechanisms by which PG promotes invasion and metastasis have been explored, revealing the activation of specific signalling pathways and the regulation of genes related to tumour progression. These findings increase the understanding of the interaction between the oral microbiome and OC, offering new therapeutic targets.

However, it is important to recognise the limitations of the studies included in this review, such as the lack of standardisation in the analysis methods and the heterogeneity of the populations studied. In addition, most of the studies were carried out in cellular and animal models, which highlights the need for well-controlled clinical studies to validate these findings in relevant clinical contexts.

The complexity of the interaction between the oral microbiome, inflammation and OC requires interdisciplinary and collaborative approaches to fully elucidate the underlying mechanisms and develop effective OC prevention and treatment strategies.

The path looks promising and opens the door to research studies that are absolutely crucial to changing the epidemiology of OC around the world.

Conflict of Interest

The authors declare no financial interest or conflicts of interest.

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