THE TRANSLATIONAL AND THERAPEUTIC POTENTIAL OF THE TUMOR MICROENVIRONMENT IN ORAL CANCER,
2nd Edition

EDITED BY: Keith David Hunter, Daniel Lambert and Ricardo D. Coletta
PUBLISHED IN: Frontiers in Oral Health

frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-83250-723-0
DOI 10.3389/978-2-83250-723-0

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

THE TRANSLATIONAL AND THERAPEUTIC POTENTIAL OF THE TUMOR MICROENVIRONMENT IN ORAL CANCER, 2nd Edition

Topic Editors:
Keith David Hunter, The University of Sheffield, United Kingdom
Daniel Lambert, The University of Sheffield, United Kingdom
Ricardo D. Coletta, Campinas State University, Brazil

Publisher's note: This is a 2nd edition due to an article retraction.

Citation: Hunter, K. D., Lambert, D., Coletta, R. D., eds. (2022). The Translational and Therapeutic Potential of the Tumor Microenvironment in Oral Cancer, 2nd Edition. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-83250-723-0

Table of Contents

frontiers in Oral Health

EDITORIAL
published: 27 September 2021
doi: 10.3389/froh.2021.763731

Editorial: The Translational and Therapeutic Potential of the Tumor Microenvironment in Oral Cancer

Keith D. Hunter[1], *Daniel W. Lambert*[1] *and Ricardo D. Coletta*[2,3]*

[1] The School of Clinical Dentistry, The University of Sheffield, Sheffield, United Kingdom, [2] Department of Oral Diagnosis, School of Dentistry, University of Campinas, São Paulo, Brazil, [3] Graduate Program in Oral Biology, School of Dentistry, University of Campinas, São Paulo, Brazil

Keywords: tumor microenvironment, oral cancer, inflammation, fibroblasts, therapeutic potential

Editorial on the Research Topic

The Translational and Therapeutic Potential of the Tumor Microenvironment in Oral Cancer

It is now recognized that the tumor microenvironment (TME) can influence all the essential hallmarks of cancer, contributing to growth, progression and treatment response of tumors. The TME is composed of many cellular components including a vast repertoire of immune/inflammatory cells, blood and lymphatic vessels, peripheral nervous structures, fibroblasts and an array of non-cellular components such as extracellular matrix proteins, cytokines and growth factors. As the TME is a crucial part of the tumor, its components have become one of the key targets for tumor treatment. Although many strategies to target the TME have been developed, the results so far are disappointing, with limited impact observed on tumor burden or overall survival. This special collection encompassed 7 reviews and 2 original articles dedicated to the translational and therapeutic potential of the TME in oral cancer, highlighting opportunities and the need to concentrate efforts to overcome the barriers to develop new and efficient TME-targeted therapeutic strategies.

Several of the studies discuss emerging findings related to the tumor-infiltrating immune/inflammatory cells. It is becoming clear that a cause-effect relationship exists between inflammation and cancer, and Elebyary et al. performed an extensive review in the putative connection between oral carcinogenesis and the chronic inflammation associated with periodontitis. The authors provided evidence that the inflammatory milieu in periodontitis is ideal for cancer cell seeding, migration, proliferation and immune escape. Moreover, the authors showed evidence that dental biofilm (bacterial)-derived substances may contribute to the induction of permanent genomic alterations. Niklander extensively reviewed the literature to characterize the major TME-associated inflammatory cells and mediators that may contribute to proliferation and spread of oral cancer cells. This study also highlighted the rationale for detection of salivary inflammatory factors as biomarkers for the diagnosis of oral squamous cell carcinoma (OSCC).

Evasion of immune surveillance and induction of an immunosuppressive TME are common features of the head and neck squamous cell carcinomas (HNSCC), and one of the main immune escape mechanisms includes the overexpression of the programmed death ligand-1 (PD-L1) in the surface of the tumor cells. The article by Wondergem et al. reviewed the currently approved immune checkpoint inhibitors (drugs that block immune checkpoint proteins such as PD-L1) in HNSCC, including nivolumab and pembrolizumab (anti-PD-1 antibodies), and highlighted the potential benefits of modulating STAT3 and in PI3K/AKT/mTOR and Wnt pathways to boost the response to these inhibitors and prevent drug resistance. In this context, Dobriyan et al. reported the differences in the immune/inflammatory cells

OPEN ACCESS

Edited and reviewed by:
Marco Magalhaes,
University of Toronto, Canada

***Correspondence:**
Ricardo D. Coletta
coletta@unicamp.br

Specialty section:
This article was submitted to Oral Cancers, a section of the journal Frontiers in Oral Health

Received: *24 August 2021*
Accepted: *30 August 2021*
Published: *27 September 2021*

Citation:
Hunter KD, Lambert DW and Coletta RD (2021) Editorial: The Translational and Therapeutic Potential of the Tumor Microenvironment in Oral Cancer. Front. Oral. Health 2:763731. doi: 10.3389/froh.2021.763731

and cancer-associated fibroblasts (CAFs) between patients with OSCC treated with neoadjuvant pembrolizumab that displayed complete or incomplete remission. In the patients with complete remission, the tumor was replaced by a granulomatous type of inflammation, enriched with T lymphocytes, with approximately equal amounts of $CD4^+$ and $CD8^+$ cells, numerous $CD68^+$ and $CD163^+$ macrophages and absence of CAFs. The tumors with incomplete remission showed a moderate inflammatory response, with a variable $CD4^+/CD8^+$ ratio, and the presence of CAFs. These encouraging results warrant further investigation in large cohorts.

Hypoxia is an important feature of the TME, and the review by Chaudhary et al. explored its effects on the crosstalk between OSCC cells and immune cells. The authors reported that in the hypoxic microenvironment, OSCC cells and other TME components secrete immunosuppressive onco-metabolites that regulate immune escape, via disturbing redox balance, mitochondrial function and ATP production through aerobic glycolysis.

The connection between inflammation and oral carcinogenesis was also reviewed in Niklander et al. by exploring the roles of interleukin 1 (IL-1) signaling, which has been shown to be activated in several types of tumors, in HNSCC. After reviewing important roles of the main IL-1 family members in the growth, differentiation and aging of normal oral keratinocytes, the authors showed that the dysregulated expression of IL-1 and IL-1R are associated with the acquisition of malignant phenotypes and reduced survival in HNSCC. The authors also provided evidence of the potential of IL-1 family members as diagnostic biomarkers and as attractive therapeutic targets for HNSCC. The participation of the IL-1/IL-1R axis in oropharyngeal squamous cell carcinomas (OPSCC) was investigated in Al-Sahaf et al.. Initially the authors demonstrated that HPV-negative OPSCC contained significantly more neutrophils than HPV-positive tumors. Applying a 3D cell culture model with HPV-negative or HPV-positive OPSCC cells in the presence of anakinra, an IL-1R inhibitor, the authors showed that both chemokine secretion and neutrophil recruitment are dependent on IL-1β/IL-1R paracrine signaling. These features make IL-1/IL-1R axis promising biomarkers and therapeutic targets for HNSCC.

The role of CAFs in OSCC is extensively reviewed in Bienkowska et al.. Besides summarizing our current understanding of CAF subtypes and function in controlling tumor proliferation, invasion, metabolic switch, angiogenesis, immune surveillance and therapy resistance, which all contribute to poor patient survival, the article discusses CAF-targeting therapies. The potential strategies discussed in the article include inhibiting CAF activation or function (e.g., inhibiting TGF-β or CXCL12/CXCR4 signaling pathways), "normalizing" CAF (e.g., NOX4 inhibitors) or killing CAF (e.g., FAP-based depletion). Although these strategies are promising, caution is required because those molecules are widely expressed in different conditions and tissues and their targeting may result in many side effects.

The participation of the sympathetic nervous system in the development and progression of HNSCC was explored in Vincent-Chong and Seshadri, by investigating the neurovascular interactions mediated via the adrenergic signaling. This study also brought a basis to support the therapeutic potential for HNSCC of directly targeting adrenergic signaling in tumor cells or indirectly targeting neurovascular interactions.

This Research Topic is a useful reference of the current understanding about the vital role of TME in oral cancer development and progression, highlighting its potential to development of new strategies for oral cancer treatment.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Oral Health

MINI REVIEW
published: 16 December 2020
doi: 10.3389/froh.2020.585710

Hypoxic Transformation of Immune Cell Metabolism Within the Microenvironment of Oral Cancers

Amrita Chaudhary[1], Swarnendu Bag[1], Neeraj Arora[2], Vivek S. Radhakrishnan[3], Deepak Mishra[2] and Geetashree Mukherjee[1]**

[1] Department of Histopathology, Tata Medical Center, Kolkata, India, [2] Department of Laboratory Hematology and Molecular Genetics, Tata Medical Center, Kolkata, India, [3] Department of Clinical Hematology, Tata Medical Centre, Kolkata, India

Oral squamous cell carcinoma (OSCC) includes tumors of the lips, tongue, gingivobuccal complex, and floor of the mouth. Prognosis for OSCC is highly heterogeneous, with overall 5-year survival of ∼50%, but median survival of just 8–10 months for patients with locoregional recurrence or metastatic disease. A key feature of OSCC is microenvironmental oxygen depletion due to rapid growth of constituent tumor cells, which triggers hypoxia-associated signaling events and metabolic adaptations that influence subsequent tumor progression. Better understanding of leukocyte responses to tissue hypoxia and onco-metabolite expression under low-oxygen conditions will therefore be essential to develop more effective methods of diagnosing and treating patients with OSCC. This review assesses recent literature on metabolic reprogramming, redox homeostasis, and associated signaling pathways that mediate crosstalk of OSCC with immune cells in the hypoxic tumor microenvironment. The likely functional consequences of this metabolic interface between oxygen-starved OSCC and infiltrating leukocytes are also discussed. The hypoxic microenvironment of OSCC modifies redox signaling and alters the metabolic profile of tumor-infiltrating immune cells. Improved understanding of heterotypic interactions between host leukocytes, tumor cells, and hypoxia-induced onco-metabolites will inform the development of novel theranostic strategies for OSCC.

Keywords: hypoxia, immune cells, metabolism, microenvironment, oral cancer

OPEN ACCESS

Edited by:
Daniel Lambert,
The University of Sheffield,
United Kingdom

Reviewed by:
Samapika Routray,
All India Institute of Medical Sciences Bhubaneswar, India
Craig Murdoch,
The University of Sheffield,
United Kingdom

****Correspondence:***
Geetashree Mukherjee
geetashree.mukherjee@tmckolkata.com
Amrita Chaudhary
chaudharybt8@gmail.com

Specialty section:
This article was submitted to Oral Cancers, a section of the journal Frontiers in Oral Health

Received: *21 July 2020*
Accepted: *13 November 2020*
Published: *16 December 2020*

Citation:
Chaudhary A, Bag S, Arora N, Radhakrishnan VS, Mishra D and Mukherjee G (2020) Hypoxic Transformation of Immune Cell Metabolism Within the Microenvironment of Oral Cancers. Front. Oral. Health 1:585710. doi: 10.3389/froh.2020.585710

INTRODUCTION

Squamous cell carcinoma (SCC) accounts for more than 95% of all cancers affecting the head and neck region, with high rates of associated mortality and morbidity that represent a major public health burden worldwide [1, 2]. In oral squamous cell carcinoma (OSCC), the predominant sites involved include the tongue and the gingivobuccal complex, and mortality rates can be substantially reduced by early detection and prevention strategies [3]. However, despite the development of several high-throughput multimodal diagnostic tools, early stage detection is still problematic; hence, 5-year survival rates in recurrent and metastatic disease remain extremely poor [4]. It remains unclear to what extent radiotherapy or chemotherapy exerts stimulatory or suppressive effects on host leukocyte responses [5]. The immune cell composition, function, and metabolic status are strongly influenced by the tumor microenvironment (TME) [6–8]. The complex dynamics of the OSCC microenvironment alter spatiotemporal distribution and effector

functions of infiltrating leukocytes to modify/diminish host defense mechanisms in favor of tumor cell survival [9, 10]. In particular, local oxygen depletion leads to the induction of reactive oxygen species (ROS) that promote cancer cell proliferation and drive autophagic/lysosomal loss of stromal caveolin-1 [an inhibitor of transforming growth factor-β (TGF-β) signaling] in cancer-associated fibroblasts (CAFs), resulting in tumor recurrence and metastasis and affecting patient survival [11–14]. Furthermore, the elevated levels of ROS result in detrimental stabilization of hypoxia-inducible factor (HIF)-1α, which activates pro-angiogenic genes including vascular endothelial growth factor (VEGF) [15–17]. HIF-1α acts as a master regulator of oxygen concentration to stimulate hypoxia-adaptive responses in cells. Immune signaling can be altered through the production of onco-metabolites that may further influence the clinical course of OSCC [18]. Consequently, a better understanding of how hypoxic stress, ROS generation, and onco-metabolites alter immune function in the TME is now a priority issue for the OSCC research community. The present article therefore reviews current knowledge of how redox factors alter leukocyte metabolism to promote the immune suppressive microenvironment of hypoxic OSCC.

HYPOXIA AND REDOX BALANCE IN THE ORAL SQUAMOUS CELL CARCINOMA MICROENVIRONMENT

Hypoxic TME alters local ROS generation and metabolic profile of both constituent tumor cells and infiltrating leukocytes [19–21]. Glycolysis is a metabolic process carried out in cell cytoplasm to generate two ATPs and pyruvates; this pyruvates serves as a fuel for tricarboxylic acid (TCA) cycle and oxidative phosphorylation (OXPHOS) under aerobic conditions [22, 23]. However, under anaerobic conditions, pyruvate is reduced to lactate, and this lactate is secreted into extracellular matrices [24]. The metabolic features of cancer cells are very heterogeneous where OXPHOS and aerobic glycolytic activities are impaired [25].

Intriguingly, cancer cells specifically express pyruvate kinase M (PKM)-2 that oxidizes and generates reduced nicotinamide adenine dinucleotide phosphate (NADPH) to maintain redox buffering; besides, this PKM-2 gene trans-activates HIF-1α target genes, leading to a significant shift in metabolic activity and cancer cell signaling [26, 27]. In solid tumors like OSCC, increased production of ROS, cytokines, and CAFs stimulates the production of pro-angiogenic factors in an attempt to promote neovascularization and enhance survival (**Figure 1**) [28, 29]. In addition, the cancer cells undergo a metabolic shift from OXPHOS to glycolysis, which produces lactate and increases serum levels of lactate dehydrogenase (LDH), which has been linked with poor survival in patients with OSCC [30]. Tumor cells therefore have the capacity to utilize both OXPHOS and aerobic glycolysis for baseline metabolic activity and rapid energy production *via* the lactate pathway (**Figure 1**). In this regard, Otto Warburg proposed that due to mitochondrial defects, the predominant metabolism in cancer cells is aerobic glycolysis rather than OXPHOS. Warburg's historic findings were called Warburg effect [31]. However, the metabolic coupling between OSCC cells and associated stromal cells is mainly determined by growth requirements; these effects are called dual/reverse Warburg effect [32, 33]. A previous NMR-based study suggested that OSCC can contravene the Warburg effect and implicated malonate (a competitive inhibitor of succinate) to induce drastic alterations in the TCA cycle that produce more fatty acid for membrane biogenesis in OSCC [34–36]. Among various glycolytic enzymes, alpha-enolase is crucial to produce phosphoenolpyruvate. As the mortality of OSCC is known to be due to metastasis, enolase in particular seems to play a major role in the malignant transformation of dysplastic epithelium in oral pre-cancer through promoting cell surface receptor enolase [37–40]. In addition to altered glucose metabolism, modified amino acid metabolism also occurs in OSCC. The amplified glutamine catabolism creates glutamine scarcity in hypoxic tumor core and leads to a dramatic histone hypermethylation [41]. In order to better understand the effects of hypoxia on OSCC, we will require new immunological paradigms that consider how dysregulation of crucial metabolic pathways can impact on both tumor growth and host leukocyte responses.

MITOCHONDRIAL HOMEOSTASIS AND IMMUNE DYSFUNCTION IN ORAL SQUAMOUS CELL CARCINOMA

In the hypoxic/acidic TME, reduced OXPHOS and electron transport chain (ETC) activity in local immune cells lead to altered mitochondrial membrane potential and impaired generation of ATP [42, 43]. The immune system not only kills cancerous cells but also modifies the TME in three phases—elimination, equilibrium, and escape [44]. The growing and transformed cells can be eradicated by immune response in the elimination phase; however, immune selection and reorganization create an immune resistant environment, namely, the equilibrium phase [45]. Consequently, immune surveillance escapes to kill tumor cells, and tumor cells grow in an uncontrolled manner [46]. In the last phase of cancer immuno-editing (i.e., "escape" phase), cancer cells produce large amounts of "pro-tolerogenic" kynurenine catalyzed by indoleamine 2,3-dioxygenase (IDO) processing of tryptophan [47]. Tryptophan catabolites have affinity to bind aryl hydrocarbon receptors (AhRs) of mitochondria, which persuade mitochondrial dysfunction in T cells and natural killer (NK) cells [48, 49]. Thus, under conditions of acute tryptophan depletion, central mitochondrial metabolic processes and synthesis of NADPH are disrupted such that infiltrating immune cells will undergo apoptosis rather than eradicating the tumor [50].

HETEROTYPIC IMMUNE MODULATION IN HYPOXIC ORAL SQUAMOUS CELL CARCINOMA

Hypoxic OSCC reprograms cellular metabolism in order to modify the repertoire of infiltrating immune cells toward a

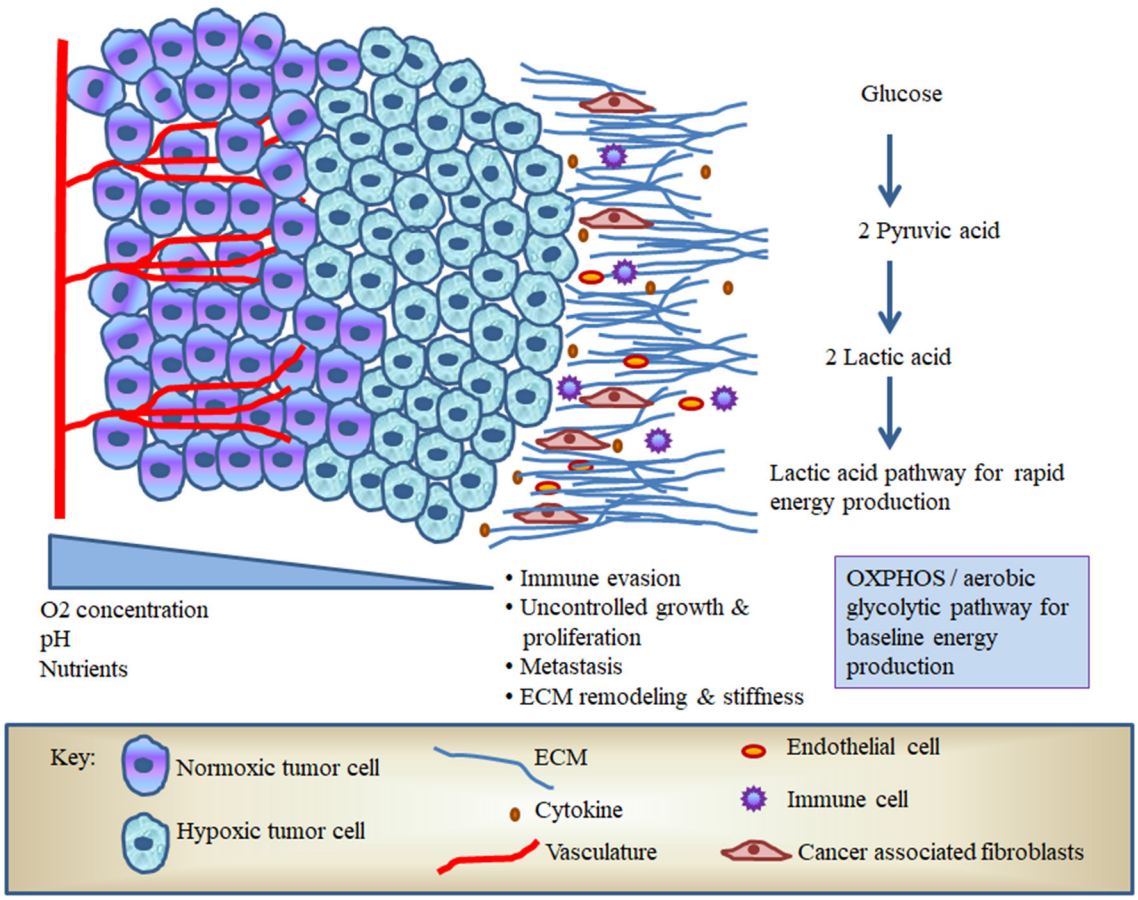

FIGURE 1 | The hypoxic microenvironment of oral squamous cell carcinoma (OSCC).

more tumor-permissive profile [51]. For example, macrophages located within hypoxic tumors tend to polarize toward an "anti-inflammatory" M2 phenotype, whereas cytotoxic T lymphocytes shift from glycolysis to OXPHOS-based metabolism (**Figure 2**) [52, 53]. Since essentially all OSCC tumors are subject to hypoxia upon reaching a certain mass, it is important to study how innate and adaptive immune cells alter their metabolism under these conditions in order to fully understand their influence on disease progression.

Hypoxia "Edits" Immune Signaling

The OSCC microenvironment has two forms of immune responses: innate and adaptive. Innate immune responses are non-specific and instant against pathogens, allergens, and non-self proteins. Phagocytes [myeloid-derived suppressor cells (MDSCs), neutrophils, monocytes, and macrophages] and NK cells are the main cells of innate immunity. Phagocytes engulf the foreign particles and digest through lysosomal enzymes, whereas NK cells kill the foreign bodies using altered major histocompatibility complex I (MHC class I) proteins, perforin, and granzyme-mediated apoptosis [54]. Dendritic cells (DCs) serve as a crucial link between innate and adaptive immune responses under physiological conditions. DCs process antigens and present them to T lymphocytes via MHC class I or II [55]. Adaptive immunity is composed of mainly T and B lymphocytes; B cells are professional antigen-presenting cells (APCs) that can activate T cells in tertiary lymphoid structures, allowing coordination of B and T cell responses in OSCC [56, 57]. In head and neck squamous cell carcinoma (HNSCC), regulatory T cells (T_{reg}) situated in the center of the tumor mass have been reported to be more strongly immunosuppressive than circulating T_{reg} [58]). T_{reg} can impede T effector (T_{eff}) cell function to reduce antitumor activity and contribute to poor prognosis in multiple types of cancer. Activated CD8+ T_{eff} are dominant antitumor cells that secrete granzymes, perforin, and pro-inflammatory cytokines, such as tumor necrosis factor (TNF) and interferon (IFN)-γ, whereas CD4+ T cells can either inhibit or promote tumor cell activity *via* the specific activities listed here [59, 60].

The macrophage subtypes M_1 and M_2 are activated in response to microbial and cancer-derived stimuli, respectively [61]. M_1 polarization of macrophages is induced by T helper type 1 (T_H1) cytokines such as IFN-γ and signaling through signal transducer and activator of transcription 1 (STAT1), whereas M_2 polarization is promoted by T helper type 2 (T_H2) cytokines such as IL-4 and IL-13 that trigger the STAT6 pathway [62]. Functionally, M_1 macrophages produce pro-inflammatory cytokines, ROS and reactive nitrogen species (RNS), while

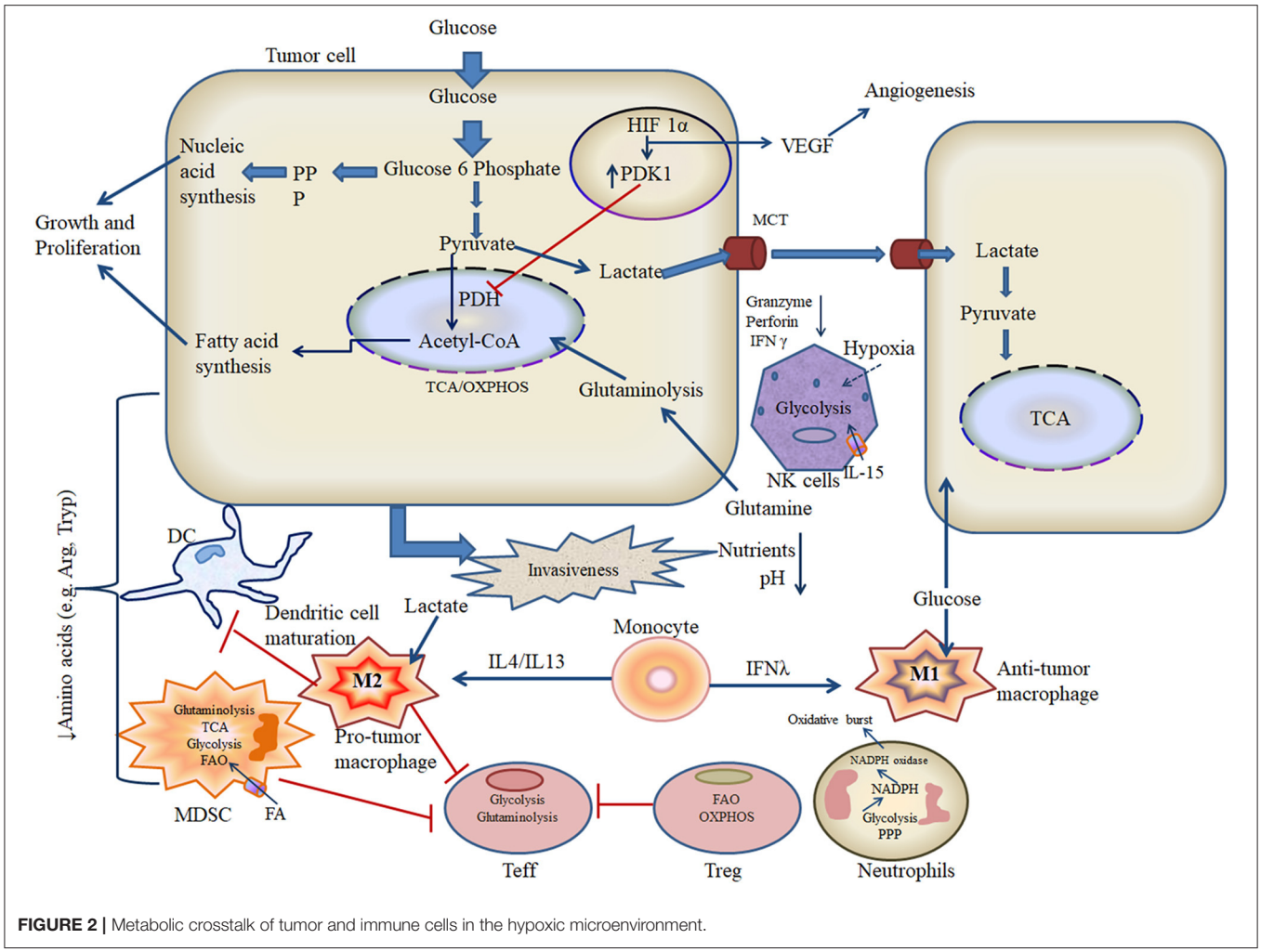

FIGURE 2 | Metabolic crosstalk of tumor and immune cells in the hypoxic microenvironment.

mediating antigen presentation *via* MHC class II molecules. M_1 macrophages also actively phagocytose pathogens and are considered to suppress tumor development [63]. In contrast, M_2 macrophages are activated by cytokines including IL-4/IL-13, IL-10, TGF-β, and glucocorticoids that promote secretion of anti-inflammatory mediators. Further, M2 macrophages inhibit the lytic activity of CD8+ cytotoxic T cells [55]. Despite their opposing roles, both M1 and M2 macrophages can coexist within the same tumor.

Tumor cells can secrete IL-10, colony-stimulating factor (CSF)-1, and various chemokines [C-C motif chemokine ligand (CCL)-2, CCL-18, CCL-17, and C-X-C motif chemokine ligand (CXCL)-4] that appear to favor M_2 polarization [64]. In addition to cytokine expression, hypoxic tumors can further direct macrophage phenotypes and responses via release of exosomes loaded with soluble factors and suppressive micro RNA [65]. In human HNSCC, the acidic TME has been reported to promote HIF-1α activation and tumor-associated macrophage (TAM) expression of M_2-specific markers CSF1R and CD163, as well as driving concomitant production of arginase and VEGF [66, 67]. NK cells have the capacity to kill tumor cells and activate antitumor T cell responses by secreting IFN-λ and cytotoxic molecules such as granzyme and perforin, but these activities can be severely restricted by the concomitant presence of MDSCs [68, 69]. MDSCs are known for their immune suppressive activity for both innate and adaptive immunity. The two subtypes of MDSCs are monocytic (M-MDSCs) and polymorphonuclear (PMN-MDSCs), which have variable capacities to inhibit the function of activated CD8+ T cells [70, 71]. MDSCs are thought to suppress T cell responses by expressing a range of inhibitory factors including arginase, inducible nitric oxide synthase (iNOS), TGF-β, IL-10, cyclooxygenase (COX)-2, and IDO [72]. HIF-1α appears to play a key role in this immunosuppressive process by driving the expression of cytokines that promote MDSC infiltration of the tumor mass [73]. In addition, granulocyte MDSCs (G-MDSCs) are classified as T cell-suppressive neutrophils because of similar morphology and cell surface markers as mature neutrophils [74, 75]. Neutrophils migrate toward and infiltrate tumors under the influence of potent chemokines such as IL-8, after which these cells appear to enhance tumor proliferation and are correlated with poor survival in solid cancers [76, 77]. Several clinical observations indicate that

neutrophil activity is further modulated under the influence of the TME to assist cancer development [78]. However, tumor-associated neutrophils (TANs) may exert a dual role, since these cells appear capable of promoting either CD8+ T cell activity or tumor progression, depending on the prevailing level of TGF-β within the TME [79].

Likewise, with the polarization of TAMs, TANs exhibit two polarization phenotypes, i.e., N1 (antitumor neutrophils) and N2 (pro-tumor neutrophils), where TGF-β signaling plays a vital role [80]. Obstruction of TGF-β signaling or type I IFNs activates N1 phenotype with accretion of TNF-α and type 1 IFN, whereas augmentation in TGF-β signaling leads to N2 phenotype with high levels of neutrophil elastase (NE) and arginase in oral cancers [81]. Immature DCs are activated by pathogen-associated molecular patterns such as toll-like receptor (TLR) ligands, thereafter migrating to lymphoid organs and presenting antigen to T cells in the context of MHC [82]. In this process, phosphoinositide 3-kinase (PI3K)/Akt signaling pathway regulates the metabolic switch through inhibiting AMP-activated protein kinase and promotes glycolysis [83]. While adenosine signaling limits DC activation, ATP detection by P2YR and P2XR promotes DC migration and IL-1 secretion, respectively [84]. Hypoxic TME not only alters the innate immune signaling but also modifies adaptive immune signaling. Likewise, under hypoxic conditions, B cell caspase signaling is activated and kinase complex mammalian target of rapamycin complex 1 (mTORC1) pathway is reduced, leading to cell death via apoptosis [85]. B cells also secrete IL-10 under hypoxic stress; however, in-depth molecular pathway is not well-characterized [86]. The role of HIF-1α transcription factor in tumor-infiltrating T cells remains unclear [87]. However, in cancer cells, HIF-1α interaction with hypoxia response element (HRE) in the programmed cell death ligand 1 (PD-L1) promoter can trigger rapid expression of this immune checkpoint molecule, which is also capable of signaling more effectively in the lactate-rich TME [59, 88]. PD-L1 ligation of programmed cell death protein 1 (PD-1) on T_{eff} can inhibit T cell receptor (TCR) signaling and attenuates the PI3K/Akt and Ras/mitogen activated protein kinase (MEK)/extracellular signal-regulated kinase (ERK) pathways to restrict antitumor responses [89, 90]. Accordingly, antibodies targeting the PD-1: PD-L1 axis and other immune checkpoints have the ability to restore glucose levels in the hypoxic TME and have proven highly effective in the treatment of OSCC [91–93]. In particular, abnormal metabolic processes within cancer cells can generate neo-antigens that are presented by MHC class I molecules on the cell surface of antigen-presenting cells for recognition by CD8+ cytotoxic T cells [94].

Hypoxia Modifies the Resting Metabolic Status of Immune Cells

The resting metabolic status and associated effector functions of local immune cells play vital roles in determining the nature of host antitumor responses. In particular, glucose transport regulates pyruvate flow into the TCA cycle and is essentially "rate-limiting" for host immunity, since leukocytes typically require rapid energy generation in order to achieve full activation. The hypoxic TME is a key driver of M_2 polarization in infiltrating macrophages likely via the expression of specific cytokine signals that activate nuclear factor (NF)-κB, although the underlying mechanism has yet to be precisely defined in OSCC [95, 96]. Like other myeloid lineage cells, macrophage mitochondria can generate both superoxide and NO, which react to form the powerful oxidant peroxynitrite, which is highly toxic to cancer cells [97, 98]. While some investigators have reported that mitochondrial ROS (mROS) stimulate macrophage expression of pro-inflammatory cytokines, other researchers have instead observed the induction of an anti-inflammatory phenotype; hence, further study is required to fully understand these events [99–102]. The glycolytic reprogramming of TAM is regulated by oxygen sensors including prolyl-hydroxylases (PHDs) and is accompanied by proton pumping and acidification of M2 macrophages that subsequently impair antitumor responses [103]. Similarly, while resting NK cells typically utilize OXPHOS, exposure to high doses of tissue damage-associated cytokine IL-15 stimulates conversion to glycolytic activity [104]. For tumor-infiltrating neutrophils, the principal metabolic pathways employed are aerobic glycolysis and the pentose phosphate pathway (PPP), which support chemotaxis and microbicidal activities, respectively (**Figure 2**) [105]. Metabolic shift toward PPP is also required for formation of neutrophil extracellular traps, which envelope and attach to the circulating cancer cells and expedite metastasis to distant sites [106]. Tumor-associated MDSCs predominantly utilize fatty acid (FA)-β oxidation (FAO) and thus display high rates of oxygen consumption (**Figure 2**) [107]. In the hypoxic TME, MDSCs display potent immunosuppressive activity, which depends on the endoplasmic reticulum (ER) stress response transcription factor CCAAT-enhancer-binding protein homologous protein (CHOP) [108]. DCs are the critical components of the immune system against cancer as they have robust antigen-presenting ability to educate T cells [109, 110]. Upon microbial stimulation, DCs typically shift from OXPHOS to glycolysis; however, in TME, DCs promote immune suppression through galectin-1 [111]. In this regard, how metabolic profiles influence DC function and tumor progression *in vivo* is not yet well-defined [56, 112]. Activated B cells can secrete antibodies that can bind and induce tumor cell killing, but these processes can be strongly influenced by mitochondrial generation of ROS and heme synthesis. While the presence of CD20+ B cells within the TME indicates a good prognosis in lung cancer, gastric cancer, and melanoma, the role played by B cells in HNSCC has yet to be fully investigated. Variable hypoxia across the developing OSCC tumor is thought to alter tissue distribution of local B cells, which generate immune complexes and produce cytokines that then modify myeloid cell function to assist tumor progression [113]. B cells utilize glycolytic metabolism during early development in the bone marrow. Later survival, maturation, and functional activity of B cells are instead regulated by HIF-1α and depend on glucose transporters and phosphofructokinase. The oncogenic Myc expression in B cells hindered the oxidation of acetyl-CoA in TCA cycle as it persuades lactate dehydrogenase for conversion

of pyruvate (glycolysis intermediate) to lactate [114]. Naive T cells employ OXPHOS and fatty acid metabolism before shifting glycolysis in order to support T_{eff} functions [112]. Memory T cells also depend on OXPHOS for energy generation in the resting state, whereas T_{reg} favor fatty acid oxidation. It is noteworthy that while glycolytic metabolism predominates among T_{eff} and T_{reg}, both populations have been observed to maintain OXPHOS within the TME (**Figure 2**), which may contribute to detrimental cancer-associated inflammation, further mutation, and eventual metastasis [115, 116]. Metabolic alterations in the T cell pool may also impede the differentiation of T_{eff} while increasing the generation of T_{reg} and "exhausted" populations, thus further supporting cancer evasion of host immunity [50].

Hypoxic Oral Squamous Cell Carcinoma Produces Immunosuppressive Onco-Metabolites

The metabolic products of cancer cells (onco-metabolites) are intimately linked with the control of the immune cell function [117]. For example, the microenvironment of OSCC is characterized by hypoxia, low pH, and elevated lactate levels, which disturb ETC operation and leads to deposition of citrate and succinate [21]. Citrate is converted into acetyl-CoA and utilized in several biosynthetic pathways. Oxidation of succinate produces ROS and promotes HIF-1α activation. Hypoxia-generated lactate also drives macrophage differentiation toward an M2 phenotype [118]. TAMs are unable to utilize extracellular arginine due to rapid enzymatic breakdown by arginase and must instead use extracellular glutamine to produce this "semi-essential" amino acid [119]. TANs also produce high levels of arginase to disrupt TCR signaling. Increased lactate concentration in the hypoxic TME favors decreased NK cell expression of granzyme/perforin and NKp46, leading to reduced anticancer cytolytic activity (**Figure 2**) [120]. The nuclear factor of activated T cells (NFAT) has also been implicated in downregulation of NK cell activity *via* an increase in cancer-associated lactate dehydrogenase expression [121]. Other hypoxia-induced onco-metabolites such as adenosine and lactic acid have previously been reported to impair DC activation (**Figure 2**) [122]. The tumor-associated dendritic cells (TADCs) stimulate arginase, which then depletes arginine in the extracellular matrix (ECM) and arginine scarcity impairs CD8+ T cell responses [123]. Some stable onco-metabolites (like kynurenine and quinolinate) along with specific cytokine milieu promote AhR signaling in non-functional T_{reg}, Foxp3+-induced T_{reg} (iT_{reg}), and T_H17 cells. These signaling pathways further de-differentiate T_{reg}, iT_{reg}, and T_H17 cells into functional iT_{reg} and endorse immune tolerance [124, 125]. IDO contributes to the tolerogenic ability of DCs to inhibit T_{eff} functions and promotes T_{reg} activity [126, 127]. The by-products (highly reactive aldehyde) of anomalous lipid peroxidation (triggered by ROS) create ER stress on TADCs and lower antitumor responses [128]. In addition, acylcarnitine and 2-hydroxyglutarate (2-HG) have been identified as a prominent onco-metabolite in HNSCC [129]. This 2-HG skews T_H17 polarization and alters T_{reg} metabolism by promoting the OXPHOS and destabilizing HIF-1α [130, 131].

IMMUNE-TUMOR METABOLIC SWITCH UNDER HYPOXIA

Tumor hypoxia is characterized by local tissue acidification and nutrient depletion, thereby creating metabolic competition and generating active biomolecules that influence cancer cell interactions with host leukocytes. Competition for key metabolites along with cholesterol esterification, release of adenosine, and expression of prostaglandin E2 inhibits effector T cells [132]. Hypoxic OSCC modifies pro- and antitumoral γδ T cell populations *via* exosomes [11]. OSCC can also express a range of different TLRs, with high levels of TLR-2 and TLR-4 correlating with tumor progression and chemoresistance, respectively. HIF-1 can deregulate *TLR3* and *TLR4* in OSCC cell lines under hypoxia stress, leading to potent effects on tumor cell survival, proliferation, and metastatic potential [133].

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Hypoxia-related metabolic stress inhibits the activity of host immune cells to support oncogenic transformation and inflammation in OSCC. The hypoxia signaling in immune cells not only alters the glycolysis but also modifies other metabolic pathways like amino acid, FAO, PPP, and TCA, resulting in onco-metabolite production. Further, these onco-metabolites disturb the redox balance, mitochondrial function, and ATP production through aerobic glycolysis in OSCC.

In future studies, it will be important to elucidate the correlation between spatial distributions of immune cells in hypoxic/non-hypoxic OSCC. Novel and advanced therapeutic approaches like interfering with HIF-1α signaling in immune cells through antisense or small interfering RNA, modulating the metabolic status of immune cells through gene editing technology [clusters of regularly interspaced short palindromic repeats–caspase 9 (CRISPR-Cas9)], and designing new smart oxygen-sensitive chimeric antigen receptor (CAR) T cell may provide new insight to overcome the challenges associated with hypoxic OSCC in the future.

AUTHOR CONTRIBUTIONS

AC conceptualized, conceived, and wrote the manuscript. SB conceptualized and organized the review. NA guided and revised the manuscript. VR revised and approved the manuscript. DM guided, revised and approved the manuscript. GM guided to write, organized and revised the manuscript. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

AC and SB acknowledged the Department of Biotechnology, Government of India, and New Delhi for providing research fellowship as Scientist C under the project: Systems Medicine Cluster (SyMeC, Project Reference: No./BT/Med-II/NIBMG/SyMeC/2014/Vol II).

REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo MM, et al. Cancer incidence and mortality worldwide, sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer*. (2015) 136:359–86. doi: 10.1002/ijc.29210
2. Gupta B, Johnson NW, Kumar N. Global epidemiology of head and neck cancers: a continuing challenge. Oncology. (2016) 91:13–23. doi: 10.1159/000446117
3. Larsen SR, Johansen J, Sørensen JA, Krogdahl A. The prognostic significance of histological features in oral squamous cell carcinoma. *J Oral Path Med*. (2009) 38:657–62. doi: 10.1111/j.1600-0714.2009.00797.x
4. Rogers SN, Brown JS, Woolgar JA, Lowe D, Magennis P, Shaw RJ, et al. Survival following primary surgery for oral cancer. *Oral Oncol*. (2009) 45:201–11. doi: 10.1016/j.oraloncology.2008.05.008
5. Carvalho HDA, Villar RC. Radiotherapy and immune response, the systemic effects of a local treatment. *Clinics*. (2018) 73:e557s. doi: 10.6061/clinics/2018/e557s
6. Gentles AJ, Newman AM, Liu CL, Bratman SV, Feng W, Kim D, et al. The prognostic landscape of genes and infiltrating immune cells across human cancers. *Nature Med*. (2015) 21:938. doi: 10.1038/nm.3909
7. Lei Y, Xie Y, Tan YS, Prince ME, Moyer JS, Nör J, et al. Telltale tumor infiltrating lymphocytes (TIL) in oral, head and neck cancer. *Oral Oncol*. (2016) 61:159–65. doi: 10.1016/j.oraloncology.2016.08.003
8. Kong H, Chandel NS. Regulation of redox balance in cancer and T cells. *J Biol Chem*. (2018) 293:7499–507. doi: 10.1074/jbc.TM117.000257
9. Chakraborty P, Karmakar T, Arora N, Mukherjee G. Immune and genomic signatures in oral (head and neck) cancer. *Heliyon*. (2018) 4:e00880. doi: 10.1016/j.heliyon.2018.e00880
10. Purohit V, Simeone DM, Lyssiotis CA. Metabolic regulation of redox balance in cancer. *Cancers*. (2019) 11:955. doi: 10.3390/cancers11070955
11. Fu P, Chen F, Pan Q, Zhao X, Zhao C, Cho WCS, et al. The different functions and clinical significances of caveolin-1 in human adenocarcinoma and squamous cell carcinoma. *OncoTargets Ther*. (2017) 10:819. doi: 10.2147/OTT.S123912
12. Bag S, Conjeti S, Das RK, Pal M, Anura A, Paul RR, et al. Computational analysis of p63+ nuclei distribution pattern by graph theoretic approach in an oral pre-cancer (sub-mucous fibrosis). *J Pathol Inform*. (2013) 4:35. doi: 10.4103/2153-3539.124006
13. Chen X, Song M, Zhang B, Zhang Y. Reactive oxygen species regulate T cell immune response in the tumor microenvironment. *Oxid Med Cell Longevity*. (2016) 2016:1580967. doi: 10.1155/2016/1580967
14. Liou GY, Storz P. Reactive oxygen species in cancer. *Free Radic Res*. (2010) 44:479–96. doi: 10.3109/10715761003667554
15. Fribley A, Zeng Q, Wang CY. Proteasome inhibitor PS-341 induces apoptosis through induction of endoplasmic reticulum stress-reactive oxygen species in head and neck squamous cell carcinoma cells. *Mol Cell Biol*. (2004) 24:9695–704. doi: 10.1128/MCB.24.22.9695-9704.2004
16. Perillo B, Di Donato M, Pezone A, Di Zazzo E, Giovannelli P, Galasso G, et al. ROS in cancer therapy, the bright side of the moon. *Exp Mol Med*. (2020) 52:1–12. doi: 10.1038/s12276-020-0384-2
17. Sobhakumari A, Love-Homan L, Fletcher EV, Martin SM, Parsons AD, Spitz K, et al. Susceptibility of human head and neck cancer cells to combined inhibition of glutathione and thioredoxin metabolism. *PLoS ONE*. (2012) 7:e0048175. doi: 10.1371/journal.pone.0048175
18. Li Y, Patel SP, Roszik J, Qin Y. Hypoxia-driven immunosuppressive metabolites in the tumor microenvironment, new approaches for combinational immunotherapy. *Front Immunol*. (2018) 9:1591. doi: 10.3389/fimmu.2018.01591
19. Eckert AW, Wickenhauser C, Salins PC, Kappler M, Bukur J, Seliger B. Clinical relevance of the tumor microenvironment and immune escape of oral squamous cell carcinoma. *J Transl Med*. (2016) 14:85. doi: 10.1186/s12967-016-0828-6
20. Peltanova B, Raudenska M, Masarik M. Effect of tumor microenvironment on pathogenesis of the head and neck squamous cell carcinoma, a systematic review. *Mol Cancer*. (2019) 18:63. doi: 10.1186/s12943-019-0983-5
21. Epstein T, Gatenby RA, Brown JS.The Warburg effect as an adaptation of cancer cells to rapid fluctuations in energy demand. *PLoS ONE*. (2017) 12:e0185085. doi: 10.1371/journal.pone.0185085
22. Zheng JIE. Energy metabolism of cancer: glycolysis versus oxidative phosphorylation. *Oncol Lett*. (2012) 4:1151–7. doi: 10.3892/ol.2012.928
23. Ashton TM, McKenna WG, Kunz-Schughart LA, Higgins GS. Oxidative phosphorylation as an emerging target in cancer therapy. *Clin Cancer Res*. (2018) 24:2482–90. doi: 10.1158/1078-0432.CCR-17-3070
24. Romero-Garcia S, Moreno-Altamirano MMB, Prado-Garcia H, Sánchez-García FJ. Lactate contribution to the tumor microenvironment: mechanisms, effects on immune cells and therapeutic relevance. *Front Immunol*. (2016) 7:52. doi: 10.3389/fimmu.2016.00052
25. Jose C, Bellance N, Rossignol R. Choosing between glycolysis and oxidative phosphorylation: a tumor's dilemma? *Biochim. Biophys. Acta*. (2011) 1807:552–61. doi: 10.1016/j.bbabio.2010.10.012
26. McGarry T, Biniecka M, Veale DJ, Fearon U. Hypoxia, oxidative stress and inflammation. *Free Rad Biol Med*. (2018) 125:15–24. doi: 10.1016/j.freeradbiomed.2018.03.042
27. Hamanaka RB, Chandel NS.Warburg effect and redox balance. *Science*. (2011) 334:1219–20. doi: 10.1126/science.1215637
28. Al Tameemi W, Dale TP, Al-Jumaily RMK, Forsyth NR. Hypoxia-modified cancer cell metabolism. *Front Cell Dev Biol*. (2019) 7:4. doi: 10.3389/fcell.2019.00004
29. Johnson S, De Costa AM, Young M. Effect of the premalignant and tumor microenvironment on immune cell cytokine production in head and neck cancer. *Cancers (Basel)*. (2014) 6:756–70. doi: 10.3390/cancers6020756
30. Wei J, Wu J, Xu W, Nie H, Zhou R, Wang R. Salvanic acid B inhibits glycolysis in oral squamous cell carcinoma via targeting PI3K/AKT/HIF-1α signaling pathway. *Cell Death Dis*. (2018) 9:599. doi: 10.1038/s41419-018-0623-9
31. Ngo H, Tortorella SM, Ververis K, Karagiannis TC. The Warburg effect, molecular aspects and therapeutic possibilities. *Mol Biol Rep*. (2015) 42:825–34. doi: 10.1007/s11033-014-3764-7
32. Fu Y, Liu S, Yin S, Niu W, Xiong W, Tan M, et al. The reverse Warburg effect is likely to be an Achilles' heel of cancer that can be exploited for cancer therapy. *Oncotarget*. (2017) 8:57813. doi: 10.18632/oncotarget.18175
33. Wilde L, Roche M, Domingo-Vidal M, Tanson K, Philp N, Curry J, et al. Metabolic coupling and the Reverse Warburg Effect in cancer, Implications for novel biomarker and anticancer agent development. *Semin Oncol*. (2017) 44:198–203. doi: 10.1053/j.seminoncol.2017.10.004
34. Bag S, Banerjee DR, Basak A, Das AK, Pal M, Banerjee R, et al. NMR (1H and 13C) based signatures of abnormal choline metabolism in oral squamous cell carcinoma with no prominent Warburg effect. *Biochem Biophys Res Commun*. (2015) 459:574–8. doi: 10.1016/j.bbrc.2015.02.149
35. Sant'Anna-Silva ACB, Santos GC, Campos SPC, Oliveira Gomes AM, Pérez-Valencia JA, Rumjanek FD. Metabolic profile of oral squamous carcinoma cell lines relies on a higher demand of lipid metabolism in metastatic cells. *Front Oncol*. (2018) 8:13. doi: 10.3389/fonc.2018.00013
36. Chen X, Yu D. Metabolomics study of oral cancers. *Metabolomics*. (2019) 15:22. doi: 10.1007/s11306-019-1483-8
37. Tsai ST, Chien IH, Shen WH, Kuo YZ, Jin YT, Wong TY. ENO1, a potential prognostic head and neck cancer marker, promotes transformation partly via chemokine CCL20 induction. *Eur J Cancer*. (2010) 46:1712–23. doi: 10.1016/j.ejca.2010.03.018
38. Bag S, Dutta D, Chaudhary A, Sing BC, Pal M, Ray AK, et al. Identification of α-enolase as a prognostic and diagnostic precancer biomarker in oral submucous fibrosis. *J Clin Pathol*. (2018) 71:228–38. doi: 10.1136/jclinpath-2017-204430
39. Bag S, Dutta D, Chaudhary A, Sing BC, Banerjee R, Pal M, et al. NanoLC MALDI MS/MS based quantitative metabolomics reveals the alteration of membrane biogenesis in oral cancer. *RSC Adv*. (2016) 6:62420–33. doi: 10.1039/C6RA07001A
40. Munir R, Lisec J, Swinnen JV, Zaidi N. Lipid metabolism in cancer cells under metabolic stress. *Br J Cancer*. (2019) 120:1090–8. doi: 10.1038/s41416-019-0451-4
41. Pan M, Reid MA, Lowman XH, Kulkarni RP, Tran TQ, Liu X, et al. Regional glutamine deficiency in tumours promotes dedifferentiation

through inhibition of histone demethylation. *Nature Cell Biol*. (2016) 18:1090–101. doi: 10.1038/ncb3410
42. Chattopadhyay E, Roy B. Altered mitochondrial signalling and metabolism in cancer. *Front Oncol*. (2017) 7:43. doi: 10.3389/fonc.2017.00043
43. Chen Y, Zhou Z, Min W. Mitochondria, oxidative stress and innate immunity. *Front Physiol*. (2018) 9:1487. doi: 10.3389/fphys.2018.01487
44. Mittal D, Gubin MM, Schreiber RD, Smyth MJ. New insights into cancer immunoediting and its three component phases—elimination, equilibrium and escape. *Curr Opin Immunol*. (2014) 27:16–25. doi: 10.1016/j.coi.2014.01.004
45. Kim R, Emi M, Tanabe K. Cancer immunoediting from immune surveillance to immune escape. *Immunol*. (2007) 121:1–14. doi: 10.1111/j.1365-2567.2007.02587.x
46. Fridman WH. From cancer immune surveillance to cancer immunoediting: birth of modern immuno-oncology. *J Immunol*. (2018) 201:825–6. doi: 10.4049/jimmunol.1800827
47. Hornyák L, Dobos N, Koncz G, Karányi Z, Páll D, Szabó Z, Halmos G, Székvölgyi L. The role of indoleamine-2,3-dioxygenase in cancer development, diagnostics, and therapy. *Front Immunol*. (2018) 9:151. doi: 10.3389/fimmu.2018.00151
48. Anderson G, Maes M. Interactions of tryptophan and its catabolites with melatonin and the alpha 7 nicotinic receptor in central nervous system and psychiatric disorders: role of the aryl hydrocarbon receptor and direct mitochondria regulation. *Internat J Tryptophan Res*. (2017) 10:1178646917691738. doi: 10.1177/1178646917691738
49. Wang X, Li S, Liu L, Jian Z, Cui T, Yang Y, et al. Role of the aryl hydrocarbon receptor signaling pathway in promoting mitochondrial biogenesis against oxidative damage in human melanocytes. *J Dermatol Sci*. (2019) 96:33–41. doi: 10.1016/j.jdermsci.2019.09.001
50. Angajala A, Lim S, Phillips JB, Kim JH, Yates C, You Z, et al. Diverse roles of mitochondria in immune responses: novel insights into immuno-metabolism. *Front Immunol*. (2018) 9:1605. doi: 10.3389/fimmu.2018.01605
51. Canning M, Guo G, Yu M, Myint C, Groves M, Byrd K, et al. Heterogeneity of the head and neck squamous cell carcinoma immune landscape and its impact on immunotherapy. *Front Cell Dev Biol*. (2019) 7:52. doi: 10.3389/fcell.2019.00052
52. Meng W, Hao Y, He C, Li L, Zhu G. Exosome-orchestrated hypoxic tumor microenvironment. *Mol Cancer*. (2019) 18:57. doi: 10.1186/s12943-019-0982-6
53. Li Y, Zhu B. Metabolism of cancer cells and immune cells in the tumor microenvironment. *Front Immunol*. (2018) 9:3080. doi: 10.3389/fimmu.2018.03080
54. Pandya PH, Murray ME, Pollok KE, Renbarger JL. The immune system in cancer pathogenesis, potential therapeutic approaches. *J Immunol Res*. (2016) 2016:4273943. doi: 10.1155/2016/4273943
55. Veglia F, Gabrilovich DI. Dendritic cells in cancer: the role revisited. *Curr Opin Immunol*. (2017) 45:43–51. doi: 10.1016/j.coi.2017.01.002
56. Cano RLE, Lopera HDE. Introduction to T and B lymphocytes. In: Anaya JM, Shoenfeld Y, Rojas-Villarraga A, Levy RA, Cervera R, editors. *Autoimmunity: From Bench to Bedside [Internet]*. Bogota: El Rosario University Press (2013) 77–95.
57. Lechner A, Schlößer HA, Thelen M, Wennhold K, Rothschild SI, Gilles R, et al. Tumor-associated B cells and humoral immune response in head and neck squamous cell carcinoma. *Oncoimmunol*. (2019) 8:1535293. doi: 10.1080/2162402X.2018.1535293
58. Jie HB, Gildener-Leapman N, Li J, Srivastava RM, Gibson SP, Whiteside TL, et al. Intratumoral regulatory T cells upregulate immunosuppressive molecules in head and neck cancer patients. *Br J Cancer*. (2013) 109:2629–35. doi: 10.1038/bjc.2013.645
59. Cao X, Cai SF, Fehniger TA, Song J, Collins LI, Piwnica-Worms DR, et al. Granzyme B and perforin are important for regulatory T cell-mediated suppression of tumor clearance. *Immunity*. (2007) 27:635–46. doi: 10.1016/j.immuni.2007.08.014
60. Siska PJ, Rathmell JC. T cell metabolic fitness in antitumor immunity. *Trends Immunol*. (2015) 36:257–64. doi: 10.1016/j.it.2015.02.007
61. Henze AT, Mazzone M. The impact of hypoxia on tumor-associated macrophages. *J Clin Inv*. (2016) 126:3672–9. doi: 10.1172/JCI84427
62. Binnemars-Postma K, Bansal R, Storm G, Prakash J. Targeting the Stat6 pathway in tumor-associated macrophages reduces tumor growth and metastatic niche formation in breast cancer. *FASEB J*. (2018) 32:969–78. doi: 10.1096/fj.201700629R
63. Genard G, Lucas S, Michiels C. Reprogramming of tumor-associated macrophages with anticancer therapies, radiotherapy versus chemo-and immunotherapies. *Front Immunol*. (2017) 8:828. doi: 10.3389/fimmu.2017.00828
64. Sica A, Mantovani A. Macrophage plasticity and polarization, *in vivo* veritas. *J Clin Invest*. (2012) 122:787–95. doi: 10.1172/JCI59643
65. Park JE, Dutta B, Tse SW, Gupta N, Tan CF, Low JK. Hypoxia-induced tumor exosomes promote M2-like macrophage polarization of infiltrating myeloid cells and microRNA-mediated metabolic shift. *Oncogene*. (2019) 38:5158–73. doi: 10.1038/s41388-019-0782-x
66. Colegio OR, Chu NQ, Szabo AL, Chu T, Rhebergen AM, Jairam V, et al. Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. *Nature*. (2014) 513:559–63. doi: 10.1038/nature13490
67. Ohashi T, Aoki M, Tomita H, Akazawa T, Sato K, Kuze B, et al. M2-like macrophage polarization in high lactic acid-producing head and neck cancer. *Cancer Sci*. (2017) 108:1128–34. doi: 10.1111/cas.13244
68. Baginska J, Viry E, Paggetti J, Medves S, Berchem G, Moussay E, et al.The critical role of the tumor microenvironment in shaping natural killer cell-mediated anti-tumor immunity. *Front Immunol*. (2013) 4:490. doi: 10.3389/fimmu.2013.00490
69. Krasnova Y, Putz EM, Smyth MJ, Souza-Fonseca-Guimaraes F. Bench to bedside: NK cells and control of metastasis. *Clin Immunol*. (2017) 177:50–9. doi: 10.1016/j.clim.2015.10.001
70. Bronte V, Brandau S, Chen SH, Colombo MP, Frey AB, Greten TF, et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. *Nature Commun*. (2016) 7:12150. doi: 10.1038/ncomms12150
71. Movahedi K, Guilliams M, Van den Bossche J, Van den Bergh R, Gysemans C, Beschin A, et al. Identification of discrete tumor-induced myeloid-derived suppressor cell subpopulations with distinct T cell–suppressive activity. *Blood*. (2008) 111:4233–44. doi: 10.1182/blood-2007-07-099226
72. Veglia F, Perego M, Gabrilovich D. Myeloid-derived suppressor cells coming of age. *Nature Immunol*. (2018) 19:108–19. doi: 10.1038/s41590-017-0022-x
73. Parker KH, Beury DW, Ostrand-Rosenberg S. Myeloid-derived suppressor cells: critical cells driving immune suppression in the tumor microenvironment. *Adv Cancer Res*. (2015) 128:95–139. doi: 10.1016/bs.acr.2015.04.002
74. Aarts CE, Kuijpers TW. Neutrophils as myeloid-derived suppressor cells. *European J Clin Invest*. (2018) 48:e12989. doi: 10.1111/eci.12989
75. Zilio S, Serafini P. Neutrophils and granulocytic MDSC: the janus god of cancer immunotherapy. *Vaccines*. (2016) 4:31. doi: 10.3390/vaccines4030031
76. Egners A, Erdem M, Cramer T.The response of macrophages and neutrophils to hypoxia in the context of cancer and other inflammatory diseases. *Mediat Inflamm*. (2016) 2016:2053646. doi: 10.1155/2016/2053646
77. Manfroi B, Moreaux J, Righini C, Ghiringhelli F, Sturm N, Huard B.Tumor-associated neutrophils correlate with poor prognosis in diffuse large B-cell lymphoma patients. *Blood Cancer J*. (2018) 8:66. doi: 10.1038/s41408-018-0099-y
78. Shen M, Hu P, Donskov F, Wang G, Liu Q, Du J. Tumor-associated neutrophils as a new prognostic factor in cancer, a systematic review and meta-analysis. *PLoS ONE*. (2014) 9:e98259. doi: 10.1371/journal.pone.0098259
79. Grecian R, Whyte MK, Walmsley SR. The role of neutrophils in cancer. *Br Med Bull*. (2018) 128:5–14. doi: 10.1093/bmb/ldy029
80. Dumitru CA, Fechner MK, Hoffmann TK, Lang S, Brandau S. A novel p38-MAPK signaling axis modulates neutrophil biology in head and neck cancer. *J Leukoc Biol*. (2012) 91:591–8. doi: 10.1189/jlb.0411193
81. Jablonska E, Puzewska W, Grabowska Z, Jablonski J, Talarek L. VEGF, IL-18 and NO production by neutrophils and their serum levels in patients with oral cavity cancer. *Cytokine*. (2005) 30:93–9. doi: 10.1016/j.cyto.2004.12.004
82. Metelmann HR, Hyckel P, Podmelle F. Oral cancer treatment and immune targets–A role for dendritic cells? *J CranioMaxill Surg*. (2012) 40:103–4. doi: 10.1016/j.jcms.2011.03.009

83. Krawczyk CM, Holowka T, Sun J, Blagih J, Amiel E, DeBerardinis RJ, et al. Toll-like receptor–induced changes in glycolytic metabolism regulate dendritic cell activation. *Blood*. (2010) 115:4742–9. doi: 10.1182/blood-2009-10-249540
84. Pearce EJ, Everts B. Dendritic cell metabolism. *Nat Rev Immunol*. (2015) 15:18–29. doi: 10.1038/nri3771
85. Gough NR. Hypoxia limits IgG-producing B cells. *Sci Signal*. (2016) 9:ec209. doi: 10.1126/scisignal.aaj1881
86. Meng X, Grötsch B, Luo Y, Knaup KX, Wiesener MS, Chen XX, et al. Hypoxia-inducible factor-1α is a critical transcription factor for IL-10-producing B cells in autoimmune disease. *Nat Commun*. (2018) 9:1–17. doi: 10.1038/s41467-017-02683-x
87. Gerriets VA, Rathmell JC. Metabolic pathways in T cell fate and function. *Trends Immunol*. (2012) 33:168–73. doi: 10.1016/j.it.2012.01.010
88. Wang Y, Wang H, Yao H, Li C, Fang JY, Xu J. Regulation of PD-L1, emerging routes for targeting tumor immune evasion. *Front Pharmacol*. (2018) 9:536. doi: 10.3389/fphar.2018.00536
89. Dan HC, Ebbs A, Pasparakis M, Van Dyke T, Basseres DS, Baldwin AS. Akt-dependent activation of mTORC1 complex involves phosphorylation of mTOR (mammalian target of rapamycin) by IκB kinase α (IKKα). *J Biol Chem*. (2014) 289:25227–40. doi: 10.1074/jbc.M114.554881
90. Kortum RL, Rouquette-Jazdanian AK, Samelson LE. Ras and extracellular signal-regulated kinase signaling in thymocytes and T cells. *Trends Immunol*. (2013) 34:259–68. doi: 10.1016/j.it.2013.02.004
91. Kim J. Regulation of immune cell functions by metabolic reprogramming. *J Immunol Res*. (2018) 2018:8605471. doi: 10.1155/2018/8605471
92. Maruse Y, Kawano S, Jinno T, Matsubara R, Goto Y, Kaneko N, et al. Significant association of increased PD-L1 and PD-1 expression with nodal metastasis and a poor prognosis in oral squamous cell carcinoma. *Int J Oral Max Surg*. (2018) 47:836–45. doi: 10.1016/j.ijom.2018.01.004
93. Mei Z, Huang J, Qiao B, Lam AKY. Immune checkpoint pathways in immunotherapy for head and neck squamous cell carcinoma. *Int J Oral Sci*. (2020) 12:1–9. doi: 10.1038/s41368-020-0084-8
94. Wei X, Chen F, Xin K, Wang Q, Yu L, Liu B, et al. Cancer-testis antigen peptide vaccine for cancer immunotherapy: progress and prospects. *Trans Oncol*. (2019) 12:733–8. doi: 10.1016/j.tranon.2019.02.008
95. Formentini L, Santacatterina F, Núñez de Arenas C, Stamatakis K, López-Martínez D, Logan A, et al. Mitochondrial ROS production protects the intestine from inflammation through functional M2 macrophage polarization. *Cell Rep*. (2017) 19:1202–13. doi: 10.1016/j.celrep.2017.04.036
96. Tripathi C, Tewari BN, Kanchan RK, Baghel KS, Nautiyal N, Shrivastava R, et al. Macrophages are recruited to hypoxic tumor areas and acquire a pro-angiogenic M2-polarized phenotype via hypoxic cancer cell derived cytokines Oncostatin M and Eotaxin. *Oncotarget*. (2014) 5:5350–68. doi: 10.18632/oncotarget.2110
97. Fraszczak J, Trad M, Janikashvili N, Cathelin D, Lakomy D, Granci V, et al. Peroxynitrite-dependent killing of cancer cells and presentation of released tumor antigens by activated dendritic cells. *J Immunol*. (2010) 184:1876–84. doi: 10.4049/jimmunol.0900831
98. Griffiths HR, Gao D, Pararasa C. Redox regulation in metabolic programming and inflammation. *Redox Biol*. (2017) 12:50–7. doi: 10.1016/j.redox.2017.01.023
99. Laskin DL, Sunil VR, Gardner CR, Laskin JD. Macrophages and tissue injury: agents of defense or destruction? *Ann Review Pharmacol Toxicol*. (2011) 51:267–88. doi: 10.1146/annurev.pharmtox.010909.105812
100. Li X, Fang P, Mai J, Choi ET, Wang H, Yang XF. Targeting mitochondrial reactive oxygen species as novel therapy for inflammatory diseases and cancers. *J Hematol Oncol*. (2013) 6:19. doi: 10.1186/1756-8722-6-19
101. Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive oxygen species in inflammation and tissue injury. *Antioxid Redox Sign*. (2014) 20:1126–67. doi: 10.1089/ars.2012.5149
102. Naik E, Dixit VM. Mitochondrial reactive oxygen species drive proinflammatory cytokine production. *J Exp Med*. (2011) 208:417–20. doi: 10.1084/jem.20110367
103. Benmoussa K, Garaude J, Acín-Pérez R. How mitochondrial metabolism contributes to macrophage phenotype and functions. *J Mol Biol*. (2018) 430:3906–21. doi: 10.1016/j.jmb.2018.07.003
104. Yongsheng Li, Wan YY, Zhu B. Immune cell metabolism in tumor microenvironment. *Adv Exp Med Biol*. (2017) 1011:163–96. doi: 10.1007/978-94-024-1170-6_5
105. Azevedo EP, Rochael NC, Guimarães-Costa AB, de Souza-Vieira TS, Ganilho J, Saraiva EM, et al. A metabolic shift toward pentose phosphate pathway is necessary for amyloid fibril-and phorbol 12-myristate 13-acetate-induced neutrophil extracellular trap (NET) formation. *J Biol Chem*. (2015) 290:22174–83. doi: 10.1074/jbc.M115.640094
106. Najmeh S, Cools-Lartigue J, Rayes RF, Gowing S, Vourtzoumis P, Bourdeau F, et al. Neutrophil extracellular traps sequester circulating tumor cells via β1-integrin mediated interactions. *Int J Cancer*. (2017) 140:2321–30. doi: 10.1002/ijc.30635
107. Al-Khami AA, Rodriguez PC, Ochoa AC. Metabolic reprogramming of myeloid-derived suppressor cells (MDSC) in cancer. *Oncoimmunol*. (2016) 5:e1200771. doi: 10.1080/2162402X.2016.1200771
108. Cubillos-Ruiz JR, Bettigole SE, Glimcher LH. Tumorigenic and immunosuppressive effects of endoplasmic reticulum stress in cancer. *Cell*. (2017) 168:692–706. doi: 10.1016/j.cell.2016.12.004
109. Gowda VMA, Smitha T. The dendritic cell tool for oral cancer treatment. *J Oral Maxill Pathol*. (2019) 23:326. doi: 10.4103/jomfp.JOMFP_325_19
110. Ostrand-Rosenberg S, Sinha P, Beury DW, Clements VK. Cross-talk between myeloid-derived suppressor cells (MDSC), macrophages, and dendritic cells enhances tumor-induced immune suppression. *Sem Cancer Biol*. (2012) 22:275–81. doi: 10.1016/j.semcancer.2012.01.011
111. Everts B, Amiel E, Huang SC, Smith AM, Chang CH, Lam WY, et al. TLR-driven early glycolytic reprogramming via the kinases TBK1-IKKε supports the anabolic demands of dendritic cell activation. *Nature Immunol*. (2014) 15:323–32. doi: 10.1038/ni.2833
112. Mu CY, Huang JA, Chen Y, Chen C, Zhang XG. High expression of PD-L1 in lung cancer may contribute to poor prognosis and tumor cells immune escape through suppressing tumor infiltrating dendritic cells maturation. *Nature Rev Immunol*. (2015) 15:18–29. doi: 10.1007/s12032-010-9515-2
113. Krzywinska E, Stockmann C. Hypoxia, metabolism and immune cell function. *Biomed*. (2018) 6:56. doi: 10.3390/biomedicines6020056
114. Osthus RC, Shim H, Kim S, Li Q, Reddy R, Mukherjee M, et al. (2000). Deregulation of glucose transporter 1 and glycolytic gene expression by c-Myc. *J Biol Chem*. 275:21797–800. doi: 10.1074/jbc.C000023200
115. Konjar S, Veldhoen M. Dynamic metabolic state of tissue resident CD8 T cells. *Front Immunol*. (2019) 10:1683. doi: 10.3389/fimmu.2019.01683
116. Jung J, Zeng H, Horng T. Metabolism as a guiding force for immunity. *Nature Cell Biol*. (2019) 21:85–93. doi: 10.1038/s41556-018-0217-x
117. Palazon A, Goldrath AW, Nizet V, Johnson RS. HIF transcription factors, inflammation, and immunity. *Immunity*. (2014) 41:518–28. doi: 10.1016/j.immuni.2014.09.008
118. Saravia J, Raynor JL, Chapman NM, Lim SA, Chi H. Signaling networks in immunometabolism. *Cell Res*. (2020) 30:1–15. doi: 10.1038/s41422-020-0301-1
119. Newsholme P. Why is L-glutamine metabolism important to cells of the immune system in health, postinjury, surgery or infection? *J Nutr*. (2001) 131:2515–22. doi: 10.1093/jn/131.9.2515S
120. Husain Z, Huang Y, Seth P, Sukhatme VP. Tumor-derived lactate modifies antitumor immune response, effect on myeloid-derived suppressor cells and NK cells. *J Immunol*. (2013) 191:1486–95. doi: 10.4049/jimmunol.1202702
121. Chambers AM, Lupo KB, Matosevic S. Tumor microenvironment-induced immunometabolic reprogramming of natural killer cells. *Front Immunol*. (2018) 9:2517. doi: 10.3389/fimmu.2018.02517
122. Wculek SK, Khouili SC, Priego E, Heras-Murillo I, Sancho D. Metabolic control of dendritic cell functions, digesting information. *Front Immunol*. (2019) 10:775. doi: 10.3389/fimmu.2019.00775
123. Tran Janco JM, Lamichhane P, Karyampudi L, Knutson KL. Tumor-infiltrating dendritic cells in cancer pathogenesis. *J Immunol*. (2015) 194:2985–91. doi: 10.4049/jimmunol.1403134
124. Gandhi R, Kumar D, Burns EJ, Nadeau M, Dake B, Laroni A, et al. Activation of the aryl hydrocarbon receptor induces human type 1 regulatory T cell–like and Foxp3+ regulatory T cells. *Nat Immunol*. (2010) 11:846. doi: 10.1038/ni.1915

125. Hill M, Tanguy-Royer S, Royer P, Chauveau C, Asghar K, Tesson L, et al. IDO expands human CD4+ CD25high regulatory T cells by promoting maturation of LPS-treated dendritic cells. *Eur J Immunol*. (2007) 37:3054–62. doi: 10.1002/eji.200636704
126. Gottfried E, Kunz-Schughart LA, Ebner S, Mueller-Klieser W, Hoves S, Andreesen R, et al. Tumor-derived lactic acid modulates dendritic cell activation and antigen expression. *Blood*. (2006) 107:2013–21. doi: 10.1182/blood-2005-05-1795
127. Hou DY, Muller AJ, Sharma MD, DuHadaway J, Banerjee T, Johnson M, et al. Inhibition of indoleamine 2, 3-dioxygenase in dendritic cells by stereoisomers of 1-methyl-tryptophan correlates with antitumor responses. *Cancer Res*. (2007) 67:792–801. doi: 10.1158/0008-5472.CAN-06-2925
128. Cubillos-Ruiz JR, Silberman PC, Rutkowski MR, Chopra S, Perales-Puchalt A, Song M, et al. ER stress sensor XBP1 controls anti-tumor immunity by disrupting dendritic cell homeostasis. *Cell*. (2015) 161:1527–38. doi: 10.1016/j.cell.2015.05.025
129. Mukherjee PK, Funchain P, Retuerto M, Jurevic RJ, Fowler N, Burkey B, et al. Metabolomic analysis identifies differentially produced oral metabolites, including the oncometabolite 2-hydroxyglutarate, in patients with head and neck squamous cell carcinoma. *BBA Clin*. (2017) 7:8–15. doi: 10.1016/j.bbacli.2016.12.001
130. Böttcher M, Renner K, Berger R, Mentz K, Thomas S, Cardenas-Conejo ZE, et al. D-2-hydroxyglutarate interferes with HIF-1α stability skewing T-cell metabolism towards oxidative phosphorylation and impairing Th17 polarization. *Oncoimmunol*. (2018) 7:e1445454. doi: 10.1080/2162402X.2018.1445454
131. Lyssiotis CA, Kimmelman AC. Metabolic interactions in the tumor microenvironment. *Trends Cell Biol*. (2017) 27:863–75. doi: 10.1016/j.tcb.2017.06.003
132. Li L, Cao B, Liang X, Lu S, Luo H, Wang Z, et al. Microenvironmental oxygen pressure orchestrates an anti-and pro-tumoral γδ T cell equilibrium via tumor-derived exosomes. *Oncogene*. (2019) 38:2830–43. doi: 10.1038/s41388-018-0627-z
133. Han S, Xu W, Wang Z, Qi X, Wang Y, Ni Y, et al. Crosstalk between the HIF-1 and Toll-like receptor/nuclear factor-κB pathways in the oral squamous cell carcinoma microenvironment. *Oncotarget*. (2016) 7:37773–89. doi: 10.18632/oncotarget.9329

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oral Health

REVIEW
published: 20 January 2021
doi: 10.3389/froh.2020.584705

The Crossroads of Periodontitis and Oral Squamous Cell Carcinoma: Immune Implications and Tumor Promoting Capacities

Omnia Elebyary[1], *Abdelahhad Barbour*[1], *Noah Fine*[1], *Howard C. Tenenbaum*[1,2] *and Michael Glogauer*[1,2,3]*

[1] Faculty of Dentistry, University of Toronto, Toronto, ON, Canada, [2] Department of Dentistry, Centre for Advanced Dental Research and Care, Mount Sinai Hospital, Toronto, ON, Canada, [3] Department of Dental Oncology, Maxillofacial and Ocular Prosthetics, Princess Margaret Cancer Centre, Toronto, ON, Canada

Periodontitis (PD) is increasingly considered to interact with and promote a number of inflammatory diseases, including cancer. In the case of oral squamous cell carcinoma (OSCC) the local inflammatory response associated with PD is capable of triggering altered cellular events that can promote cancer cell invasion and proliferation of existing primary oral carcinomas as well as supporting the seeding of metastatic tumor cells into the gingival tissue giving rise to secondary tumors. Both the immune and stromal components of the periodontium exhibit phenotypic alterations and functional differences during PD that result in a microenvironment that favors cancer progression. The inflammatory milieu in PD is ideal for cancer cell seeding, migration, proliferation and immune escape. Understanding the interactions governing this attenuated anti-tumor immune response is vital to unveil unexplored preventive or therapeutic possibilities. Here we review the many commonalities between the oral-inflammatory microenvironment in PD and oral-inflammatory responses that are associated with OSCC progression, and how these conditions can act to promote and sustain the hallmarks of cancer.

Keywords: periodontitis, oral pathogens, inflammation, oral squamos cell carcinoma, innate immunity, tumor microenvironment

OPEN ACCESS

Edited by:
Keith David Hunter, The University of Sheffield, United Kingdom

Reviewed by:
Alison Mary Rich, University of Otago, New Zealand
Marilena Vered, Tel Aviv University, Israel

***Correspondence:**
Michael Glogauer
michael.glogauer@utoronto.ca

Specialty section:
This article was submitted to Oral Cancers, a section of the journal Frontiers in Oral Health

Received: *17 July 2020*
Accepted: *16 September 2020*
Published: *20 January 2021*

Citation:
Elebyary O, Barbour A, Fine N, Tenenbaum HC and Glogauer M (2021) The Crossroads of Periodontitis and Oral Squamous Cell Carcinoma: Immune Implications and Tumor Promoting Capacities. Front. Oral. Health 1:584705. doi: 10.3389/froh.2020.584705

PERIODONTAL PATHOGENS AND THE HOST RESPONSES: WAR AND TRUCE

A strong correlative link exists between inflammation and the development and progression of many cancers. Approximately 20% of all human cancers have been related to chronic inflammatory conditions, raising speculation regarding the specific roles of inflammatory processes in driving carcinogenesis [1]. These highly-regulated multicellular responses involve a large network of both immune and stromal cells and result in major alterations in the abundance of reactive oxygen and nitrogen species, prostaglandins, cytokines, and chemokines within the microenvironment encompassing the cellular milieu in various tissues [2]. It can be inferred that initiation of tumourigenesis and tumor progression might be derived by these environmental modifications which can alter the cellular behavior and change the composition of the surrounding extracellular matrix.

In relation to the above, the putative role played by inflammation in tumourigenesis can be observed in the oral cavity [3]. The healthy oral cavity harbors an abundant commensal microbial community with varied microbial floral diversity. It represents one of the most ecologically complex niches within the human body where the oral microbiota–host equilibrium remains balanced in health conditions (truce) and shifts to disease state when immunoresponses are altered in retaliation to dysbiosis (war). Indeed, dysbiosis of the commensal oral microbiota and their subsequent invasion of the tooth supporting structures (e.g., the gingiva, periodontal ligament and bone) leads to the initiation and propagation of an inflammatory condition termed periodontitis or periodontal disease (PD) [4, 5].

PD is considered to be, by far, the most common inflammatory condition affecting the oral cavity and it has been designated as an enabling characteristic of cancer development [6–8]. The presence of PD has been correlated to the presence of several types of malignancies including but not limited to breast, pancreatic and colorectal cancers [9–13] (**Figure 1**). However, whether this is a correlative or a causative association is not clear, and further investigation of the potential role of PD in cancer pathogenesis and potential systemic mechanistic effects is required. One group pointed out that although the link between PD with oral and other cancers has been well-documented in systematic reviews, these are often of limited power, and further research is necessary to fully confirm this association [8].

An association between PD and OSCC, two conditions that co-exist within the oral microenvironment, has been confirmed in many studies, suggesting that the immunologically distinct oral setting in PD could support tumor progression [14, 15]. Based on this observation one might reasonably predict that the most common site of primary squamous cell carcinomas in the oral cavity will be the gingival tissue, from which periodontal disease arises, however this was found not to be the case. Other sites in the oral cavity such as the lateral border of the tongue and the floor of the mouth have been recognized as common sites for OSCC and account for the majority of primary lesions [16]. This does not rule out the involvement of PD in supporting pathogenesis of OSCC at these sites. The specific sites where OSCC is commonly manifested in the mouth are known to have a greater permeability to carcinogenic factors compared to other oral tissues, and therefore are more susceptible to pathologenesis upon exposure to carcinogens [17]. The gingiva is covered by a thick protective layer of keratinized mucosa, which renders it less permeable to external carcinogenic insults, thus making it less affected [18]. Since the lateral border of the tongue and the floor of the mouth are common locations for premalignant oral conditions, the development of malignancies at these same areas may be a reflection of this pattern [19, 20]. Potential mechanisms through which the environmental conditions in the oral cavity associated with the presence of PD could support the progression of these lesions to develop carcinomas are the primary focus of this review.

Periodontal pathogens can contribute to carcinogenesis at locations other than the gingiva especially at the most common site, the lateral border of the tongue. Based on its anatomical location, this area comes in direct contact with the lingual aspect of mandibular molars, sites that have been reported to have the highest index of plaque accumulation and gingivitis [21]. Therefore, it can be inferred that the mucosa of the lateral border of the tongue is constantly exposed to the damaging effects of pathogenic periodontal bacteria and their toxins. Certain bacterial species, such as the putative periodontal pathogen *Porphyromonas. gingivalis* and *Tannerella Forsythia,* where detected in higher amounts in both the subgingival regions and the tongue of periodontally affected patients [22]. This supports the model that PD is a potential contributing factor or an accelerator to the development of primary oral cancer. Further studies are required to determine whether or not periodontitis can directly promote initiation of OSCC.

The gingiva has been identified as the most common site for metastatic carcinomas when compared to all the other soft tissues of the oral cavity [23]. These tumors have been reported as metastatic lesions of other primary cancers from distant sites including lung cancers, renal cell carcinomas, hepatocellular carcinomas and breast cancers [24]. Furthermore, metastatic gingival carcinomas have been strongly linked to the presence of teeth, which highlights the importance of the dentoalveolar structure in this pathogenic process [23]. Thus, the deregulated environment of gingival tissue in PD may form an attractive milieu for the seeding and growth of these metastatic cells.

A recent study suggested that periodontal pathogens can be regarded as a risk factor for PD independent of other well-known risk factors such as smoking, alcohol and human papilloma virus (HPV) infections [25]. Moreover, it has been suggested that periodontal therapy may result in a dramatic decrease in the risk of developing future OSCC [3]. This type of reversibility argues strongly in favor of the presence of a mechanistic link between PD and the development of both primary and secondary OSCCs. Given the high mortality and poor survival rate of OSCC (∼50%), more investigations are being undertaken to determine what could be the underlying mechanisms that link PD with an increased risk of OSCC development [26]. In this review, we will explore the possible role played by the inflammatory environment created by PD in relation to carcinogenesis.

Interaction of Malignant Cells and the Extracellular Matrix: Possible Links to the Development of Cellular Mutagenesis

When epithelial cells accumulate oncogenic mutations, complex interactions of these cells with their surrounding stroma contribute to initiation, progression and metastasis of OSCC [27]. It has been suggested that the deregulated microenvironment that exists in PD could contribute to various steps in carcinogenesis [28]. It has been well-established that chronic inflammatory conditions, including periodontitis, generate a mutagen-enriched environment [29]. Elevated levels of carcinogens such as reactive oxygen species (ROS) and bacterial-derived carcinogenic substances including volatile sulfur compounds, acetaldehyde, lactic acid, acetic acid, butyric acid, and isocaproic acid are associated with PD [30]. These compounds directly predispose the oral environment to promote host-cell DNA damage and the development of cellular

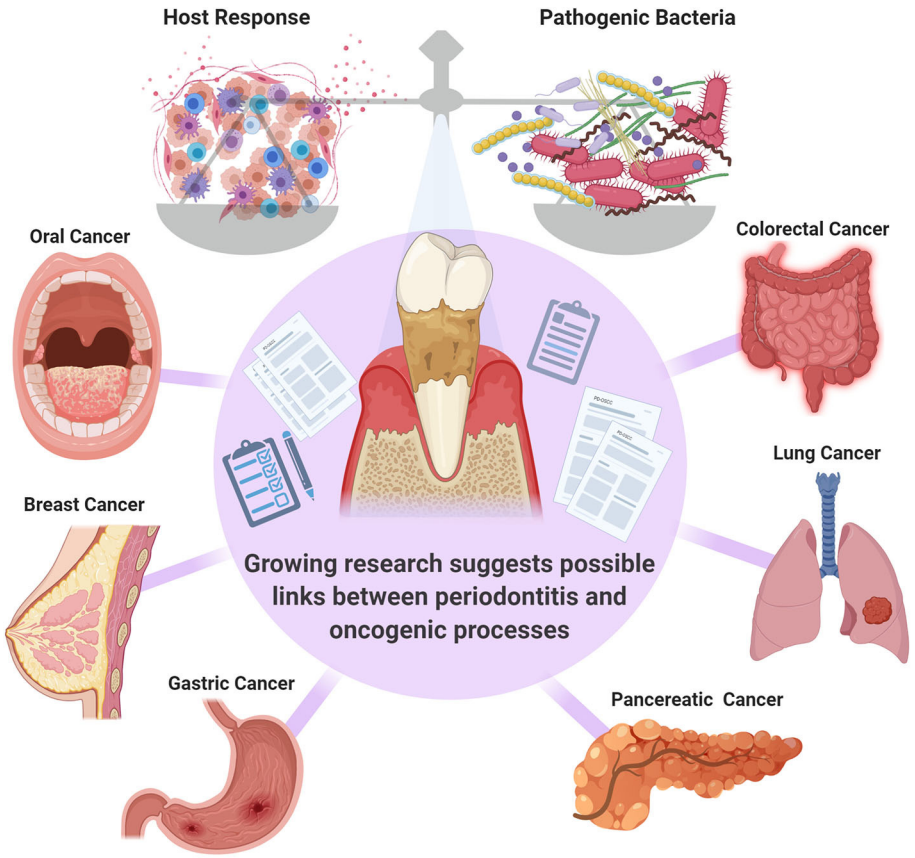

FIGURE 1 | The balance between the pathogenic bacteria and host immune response is critical in governing the transition from health to disease. Systematic reviews suggest a link between periodontal inflammation oral [14], colorectal [11], breast [9], lung [12], gastric [13], and pancreatic [10] cancers. However, further confirmatory studies are required.

abnormalities that may give rise to malignant transformation. The genotoxic effect of PD on buccal mucosa was assessed in an attempt to estimate whether or not PD can be used as a marker for genomic instability in oral tissues [31]. In accordance with the severity of PD, more DNA damage was observed in the form of nuclear bud formation, and chromosomal instability in cells of the buccal mucosa [31]. One of the most recognized genomic alterations in PD is that of the *TP53* gene encoding the p53 tumor suppressor protein, regarded as the *guardian of the genome*. When p53 is upregulated it has destructive effects that compromise periodontal integrity, suggesting its contribution to tumourigenesis [32]. Interestingly, the frequency of *TP53* gene expression in neoplastic conditions is similar to that seen in PD. Overexpression of *TP53* was linked to the pathogenesis of oral malignancies including squamous cell carcinoma and Kaposi's sarcoma, and was suggested to be a poor prognostic marker [33, 34]. Furthermore, the role of PD in cancer initiation has been demonstrated in a murine model where the size and number of OSCC lesions induced by the carcinogen, 4-nitroquinoline-1-oxide (4-NQO), were increased when putative periodontal pathogenic bacteria were co-introduced (orally) as compared to germ-free mice that received the carcinogen only [35].

In terms of the contribution of PD to cancer progression, it has been reported that a key periodontal pathobiont *P. gingivalis* is capable of disrupting immune surveillance by activating STAT3 signaling [36]. This in turn induces the generation of immunosuppressive myeloid-derived dendritic suppressor cells (MDSCs) from monocytes, which help to sustain oncogenic cell-proliferation and sponsor immune escape [36]. Periodontal-associated pathogens can also interfere with the expression of the Notch signaling pathway, the latter playing a major role in the development of chemoresistance in mutated cells [37].

Bidirectional Relationship Between OSCC and PD

Recent findings suggest that the link between PD and OSCC could be reciprocal or bidirectional, with OSCC contributing to the development and/or the exacerbation of PD and *vice versa*. When the carcinogen 4-NQO was administrated to rats in order to induce the development of OSCC, the group of rats that developed OSCCs upon 4-NQO exposure had significant spontaneous alveolar bone resorption compared to both the control group that did not receive 4-NQO and the treated-group that did not develop OSCC. Thus, this enhanced alveolar bone loss could be correlated to the development of squamous

cell carcinoma lesions in the oral cavity [38]. Interestingly, Pushalkar et al. had found significant variations in the microbiota diversity between tumor and non-tumor sites in individuals affected with OSCC [39]. In that study, tumor sites tended to have more distinct pathogenic microbial populations than those found at non-tumor sites in the same subjects. This microbial shift was suggested to induce a chronic inflammatory state at tumor sites, which can support the progression of adjacent tumors. This highlights the interconnectivity of both diseases, and how changes in the oral environment could induce conditions that are permissive to the development of either PD, OSCC or both.

Although the inflammatory oral microenvironment has been recognized to orchestrate a predominantly pro-tumor immune response, there are also paradoxical effects that can be anticarcinogenic one of which is that of the CXCL14 chemokine [40]. CXCL14 is normally expressed in healthy oral tissues yet it is decreased or absent in malignant oral epithelium [41]. CXCL14 was found to completely block OSCC establishment in SCID mouse xenografts [42]. A more recent study, explained that the anti-tumor functions exerted by CXCL14 are carried out by means of supressing PD-L1 expression and NF-*k*B mediated EMT [43]. Periodontal pathogen *P. gingivalis* is able to stimulate the expression of the CXCL14 from oral epithelial cells both directly and by antagonizing epidermal growth factor (EGF)-induced activation of the MEK-ERK1/2 pathway known to repress CXCL14 transcription [44]. This CXCL14 involvement in PD may be considered as one of the methods by which anti-tumor responses are supported. However, further research will be necessary to understand the complex interactions between PD and OSCC.

OSCC IMMUNE PERMISSIVE ENVIRONMENT INDUCED BY PERIODONTAL DISEASE

Intense focal influx of immune cells into sites affected by pathogenic bacteria leads to a cumulative build-up of their numbers within the oral environment [45]. Changes in the behavior of immune cells under the critical influence of pathogenic bacteria, or interactions between these cells and other immune-regulatory networks e.g., cytokines, chemokines, and growth factors can disrupt productive immune surveillance in PD [46]. Any "corruption" of the immune response could hypothetically permit the homing of metastatic tumor cells into these sites or possibly induce the development of primary malignant lesions. Interestingly, in PD many immune cells adopt behaviors or characteristics similar to those observed in immune cells recruited by tumors that favor their progression. Hence, the "re-education" of dysfunctional oral immune cells by means of periodontal therapy could conceivably lead to beneficial results insofar as the development and/or treatment of OSCC are concerned. Herein, we discuss how cells of both the myeloid and lymphoid lineages are affected by PD, and how they contribute to the etiopathology of OSCC (**Figure 2**).

Myeloid Derived Cells

PMNs

Neutrophils, also called polymorphonuclear leukocytes (PMNs), are the most abundant primary innate immune responders in the gingival crevice and periodontal pocket. They play an indispensable role in maintaining periodontal health and their greatly upregulated recruitment is observed in response to oral microbial dysbiosis [47–50]. PMNs have been implicated in prolonging the extent and severity of inflammatory PD leading to substantial damage to bone and soft connective tissues surrounding the teeth [51, 52]. In cancer, different PMN subsets with either anti-tumor or pro-tumor characteristics have been identified. This has led to the emergence of a new classification of PMNs, based on anti-tumor (N1) and pro-tumor (N2) cancer-associated PMNs [53]. In OSCC, it was noted that an increase in the oral PMN infiltrate is correlated with a reduced survival rate and an increased recurrence rate [54]. Furthermore, co-cultures of peripheral blood PMNs and OSCC cells were found to increase the invasiveness of the cancer cells, independently of direct cell contact [55]. Hence, the presence of abundant pro-inflammatory PMNs in PD could contribute to the pathogenesis of oral cancers.

In PD, changes in oral PMN phenotype were observed. This was characterized by PMNs that survived longer in the oral cavity due to upregulation of anti-apoptotic genes of the Bcl-2 family, and downregulation of pro-apoptotic transcripts [56]. Moreover, direct interaction between PMNs and regulatory T-cells (T-regs) that had been stimulated with lipopolysaccharides (LPS) promoted an abnormal production of the immunosuppressive cytokine IL-10 by the PMNs, and these IL-10-producing PMNs were observed in the oral cavity of patients with PD [57]. Interestingly, similar, longer lived, IL-10+ PMN phenotypes were identified in the tumor microenvironment (TME) of OSCC [58, 59]. These PMNs displayed a clear upregulation of the anti-apoptotic genes of both the Bcl-2 and NF-*k*B families upon entering the TME and transitioning into N2 tumor associated neutrophils (TAN).

Another common feature between PMNs associated with both OSCC and PD is the downregulated expression of PMN gelatinase-associated lipocalin (NGAL) [60, 61]. The downregulation of NGAL observed in OSCC has been implicated in improving the survival of cancer cells while upregulating their ability to migrate and invade tissues. This is related to the activation of mTOR signaling and the suppression of autophagy as a result of reduced expression of LC3B [61]. The resemblance between PMN behavior in the microenvironment of both PD and OSCC, suggests that PMNs in PD can augment and potentiate the development of OSCC by exerting the same pro-tumor functions as TANs in the oral microenvironment.

PD deregulates the expression of many PMN-secreted products, which can impair certain PMN functions and provide a favorable environment for cancer cells to thrive. For instance, the defensins, human neutrophil peptides 1, 2 and 3 (HNP1-3), are known to induce cytolytic effects on their target cells, particularly cancer cells. It has been suggested that higher levels of defensins might promote preferential oncolysis and provide a better host response to tumor invasion (i.e., prevention). Suppression

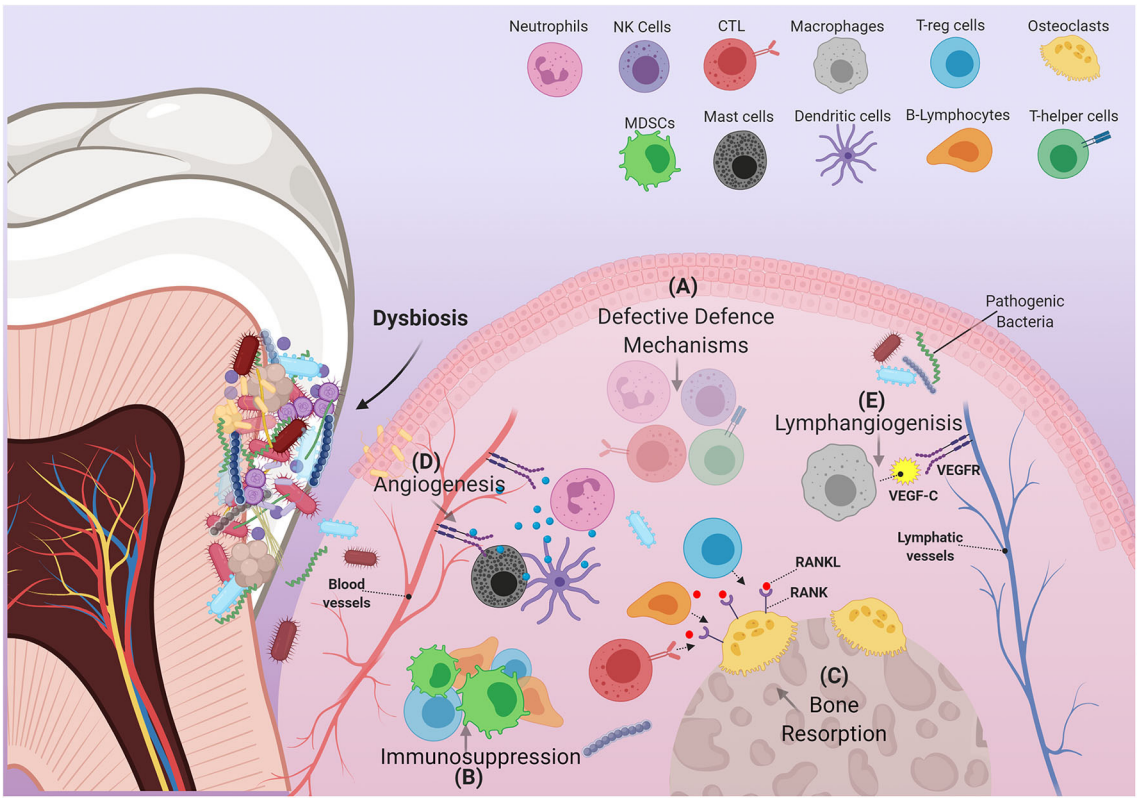

FIGURE 2 | Schematic illustration of immune cells in inflamed periodontium. The figure shows how the immune cell infiltrate in PD can exert several alterations within the gingival tissue environment that are able to support the pathogenicity of OSCC. **(A)** In PD, many immune cells fail to carry out some of their characteristic defensive functions. These cells include, but are not limited to, (1) Neutrophils: decreased defensins and defective NETs formation, (2) cytotoxic T-lymphocytes: decreased IFN-γ production, (3) T-helper cells: suppressed effector Th1 response, and (4) natural killer cells: reduced granzyme and IFN-γ production. **(B)** Immune suppressive cells: MDSCs, T-regs, B-regs (a subtype of B-lymphocytes) are recruited to the site of inflammation, disrupting immune surveillance. **(C)** Increased RANKL expression by several immune components including T-regs, B-lymphocytes and Cytotoxic T-lymphocytes, induces resorption of alveolar bone. **(D)** Expansion of the vascular compartment of the periodontium is mediated by several factors produced by certain immune cells. These include MMP-9, which is mainly produced by neutrophils and dendritic cells, as well as many angiogenic products released by mast cells (VEGF, tryptase, heparin, histamine, IL-8, basic fibroblast growth factor). **(E)** Macrophages contribute to the expansion of lymphatic vessels by producing VEGF-C.

of antimicrobial peptides HNP1-3 was observed in patients with PD [62]. This suppression could therefore be anticipated to cause a loss of PMN mediated oncolysis. Additionally, in PD, PMN-mediated expression of pro-angiogenic matrix metalloproteinase-9 (MMP-9) is accompanied by a substantial decrease in the expression of their inhibitors, tissue inhibitors of matrix metalloproteinases (TIMPs) [63, 64]. In combination, this unopposed upregulated MMP-9 expression can lead to conditions that are permissive for angiogenesis required for tumor growth and intravasation and therefore, progression of tumors such as OSCC.

In chronically inflamed gingival tissue, increased PMN-expression of a proliferation-inducing ligand, known as APRIL, was shown to increase the proliferation, survival, and invasion capacity of tumor cells in oral cancer [65]. An increased rate of tumor growth and aggressiveness was associated with APRIL-rich breast cancers compared to those with reduced expression of APRIL [66]. Other PMN products such as neutrophil elastase (NE), which have the capacity to degrade most extracellular proteins, has been implicated as a biomarker in both PD as well as head and neck squamous cell carcinoma (HNSCC). A direct link between increased levels of NE in PD and its potential role as a co-factor in the initiation and advancement of OSCC has recently been established. A considerable increase in salivary levels of NE in patients with combined PD and OSCC, as compared to those affected by either PD or OSCC, highlights the important role of NE in contribution to oral cancer [67].

One of the strategies that PMNs use to counteract infectious agents is formation of web-like neutrophil extracellular traps (NETs) [68]. These extruded structures, generated by a process referred to as NETosis, are composed of chromatin and are able to trap pathogenic moieties and destroy them by delivering high concentrations of anti-microbial agents such as histones, proteases and PMN granule proteins [69]. Excessive NETosis, evident in exacerbated inflammatory conditions, has been linked to unfavorable consequences such as autoimmune diseases, thrombus formation and cancer metastasis [70]. In HNSCC, it has been proposed that monitoring NET levels can be a

successful approach to predict if patients are at a high risk of developing metastatic tumors [71]. PMN NETs are highly prevalent in PD, and may therefore stimulate the metastatic potential of oral cancer cells [49, 72]. Furthermore, NETs formed under the influence of PD were found to have defective functions. *P. gingivalis*, which can cleave the protease-activated receptor-2 (PAR2) on the surface of PMNs, was found to trigger excessive formation of NETs that lacked their characteristic bactericidal activities [73]. These NETs were unable to restrain and kill *P. gingivalis* and other pathogenic bacterial species, allowing them to thrive. Although it hasn't been extensively explored, it can be suggested that this defective function of NETs can also be beneficial to cancer cells, allowing them to escape PMN-mediated destruction.

Collectively, these findings indicate that PD is associated with functional alterations in the oral PMN population, which suppress PMN immune surveillance by modifying the production of many PMN-secreted products. These changes are not dissimilar to characteristics of the N2 pro-tumor PMN. Accordingly, in the presence of PD, the PMNs that are recruited to the OSCC TME could augment the progression of cancer instead of limiting its progression.

Macrophages

In addition to PMNs, macrophages play a central role in the immune response observed in PD and contribute to both the onset and resolution of inflammation [74]. Functionally, macrophages can be classified into two main phenotypes, M1 and M2, based on the type of activation they acquire upon interacting with the microenvironment at diseased sites. M1, or classically activated macrophages, differentiate in the presence of IFN-γ, LPS, or TNF-α; and are the primary producers of pro-inflammatory cytokines, such as IL-1, IL-12, and TNF-α [75]. Additionally, these cells have potent anti-tumor capabilities and treatments that can induce tumor-associated macrophages to adopt an M1 phenotype hold potential therapeutic promise [76]. M2, or alternatively activated macrophages, are induced in the presence of IL-4, IL-10, or IL-13 [74]. They are involved in immunoregulation, tissue repair, and angiogenesis, and are linked to carcinogenesis and tumor progression [77].

Although PD is more often associated with elevated numbers of M1 anti-tumor macrophages [75, 78], this does not necessarily rule out the presence of M2 pro-tumor macrophages. In a recent study, the effect of periodontal inflammation on macrophages demonstrated the presence of increased numbers of M1 macrophages as well as elevated numbers of M2 macrophages [79]. Furthermore, when screening for changes in cytokine expression in gingival tissue derived from participants with PD, the M1 cytokines, TNF-α and IL-1β, and the M2 cytokine, IL-10, were all significantly increased [79]. These findings have challenged the prevalent concept of macrophages being solely pro-inflammatory within the context of PD, and have proposed the presence of a more heterogeneous macrophage population in PD. Based on this, it cannot be assumed that changes in macrophages in the presence of PD will only favor the pro-tumor response.

Secreted factors produced by macrophages can play a role in the pathogenesis of OSCC. For instance, elevated levels of TGF-β1 in the PD environment can induce vascular endothelial growth factor-C (VEGF-C) production by macrophages, which stimulates the proliferation of new lymphatic vessels through stimulation of VEGF receptor-3 (VEGFR-3) on lymphatic endothelial cells [80]. In the same context, inflammatory macrophages had been recognized previously to be able to transdifferentiate into lymphatic endothelial cells that express endothelial markers such as LYVE-1 and Prox-1, thereby integrating into lymphatic vessels promoting their expansion [81]. In cancer, this can have a negative impact on the overall prognosis for patients with OSCC, as increases in microvascular density might support metastasis of malignant cells to nearby and distant lymph nodes. This was shown when OSCC of the tongue was studied. In this regard, higher levels of VEGF-C were considered to predict poor cancer-specific survival [82].

Macrophage migration inhibitory factor (MIF) has been noted to play a role in both periodontitis and cancer. MIF is a well-known pro-inflammatory effector cytokine expressed by a variety of immune and non-immune cells including macrophages. In inflammation, it promotes the migration and recruitment of leukocytes to the infected sites in response to pathogenic stimuli. Investigations have recognized MIF as a central participant in the cancer-associated immune response, having the capacity to support the development of HNSCC by its pleiotropic roles in mediating hypoxia response, angiogenesis, and epithelial-mesenchymal transition (EMT) [83]. Moreover, gene inactivation of MIF receptor (CD74) or the use of MIF agonist ISO-1 resulted in a significant reduction in the proliferation of gastric cancer cells [84]. Therefore, the higher concentrations of serum and salivary MIF observed in PD might stimulate the proliferation, metastasis and invasion, of oral cancer cells into other tissues [85].

Myeloid-Derived Suppressor Cells

The expansion of immune suppressive cells, including myeloid-derived suppressor cells (MDSCs), and their accumulation at the sites of malignant lesions is one of the main mediators of cancer progression [86]. Not only do these cells contribute significantly to the immune response regulation, but they also provide an environment that is capable of sustaining angiogenesis, stemness, and metastasis [86]. Although these recently discovered cells have not been extensively studied in the context of PD, emerging evidence suggests that they are more involved in PD and in PD induced OSCC than previously suggested. Strains of *P. gingivalis* that express Mfa1-fimbriae were found to mediate immune escape by invading monocytes and inducing their differentiation into myeloid-derived dendritic suppressor cells (MDDSCs), which were characterized by being highly resistant to apoptosis [36]. Additionally, these cells appeared to be compatible with the immune suppressive environment created by the indoleamine 2, 3 dioxygenase (IDO) enzyme found at chronically inflamed sites including PD.

Furthermore, the expansion in the MDSC population has been linked directly to promoting the development of either primary or metastatic cancers in the oral cavity as follows. A

recent study revealed that *P. gingivalis* can induce differentiation of MDSCs from monocytes, and also invade oral pre-malignant lesions thereby inducing a substantial influx of MDSCs [87]. This is achieved by upregulating the expression levels of certain chemokines (CCL2 and CXCL2) and cytokines (IL-6 and IL-8) in dysplastic oral keratinocytes. Accordingly, this "biases" the immune system toward supporting oncogenesis. Hence, this suggests that patients with PD could be more susceptible to developing primary oral cancers from pre-existing oral dysplastic lesions.

It has been suggested that periodontal inflammation can shape the pre-metastatic immune microenvironment in the gingiva. In a very recent study, gingival fibroblasts showed increased production of IL-1β upon sustained exposure to LPS, which resulted in increased expression of CCL5, CXCL12, CCL2, and CXCL5, potent chemotactic factors for MDSCs [88]. This generates a pre-metastatic niche at sites of inflammation, which promotes metastatic events, for example, progression of breast cancer to head and neck tissues [88].

Dendritic Cells

Dendritic cells (DCs) are specialized antigen presenting cells (APCs) that can be activated by different stimuli including bacteria, viruses, and damaged tissues, thereby potentiating their phagocytic activities [89]. These cells have been linked strongly to the induction of T cell responses and therefore it has been suggested that they play a key role in mediating anti-tumor immunity [90]. However, compelling evidence indicates that the function of DC is impaired in cancer. Decreased function of DC is thought to be related to the presence of immunosuppressive conditions that induce a network of factors capable of suppressing these cells [91]. A greater understanding of how DCs respond to PD is necessary in order to determine how this can contribute to tumor immune responses.

Periodontal pathogens can, in many ways, alter the response of DCs to suppress the local immune surveillance mechanism, allowing them to invade periodontal tissues. For example, LPS derived from *P. gingivalis* has been shown to induce alterations in the MMP-9/TIMP-1 ratio in DCs similar to those described previously for PMNs. MMP-9 is expressed in significant amounts in concert with reduced production of TIMP-1 resulting in a higher MMP-9/TIMP-1 ratio that favors local tissue destruction. In laryngeal cancer, higher levels of MMP-9 were correlated with increased counts of tolerogenic DCs [92]. These tolerogenic DCs have altered functionality and produce immune-regulatory molecules, including retinoic acid and TGF-β, so that they appear to induce the development of laryngeal cancer specific T-regs that suppress the production of the cytotoxic T-lymphocyte effector molecule granzyme B [93]. Moreover, it was found that *P. gingivalis* strains having minor fimbria can bind to the DC-SIGN receptor expressed by DCs [94]. This binding is followed by internalization of these bacterial strains which was shown to alter DC function, retarding their maturation and minimizing their production of inflammatory cytokines. This also results in a Th2-biased weak immune response that promotes immune escape [94].

Plasmacytoid dendritic cells (pDCs) are a unique subset of DCs that can detect the nucleic acids of pathogenic microorganisms and respond rapidly by releasing an enormous amount of type 1 interferon (INF) [95]. The negative regulation of these cells by TAM signaling was found to be repressed in PD, which explains their increased influx in the gingiva of patients with PD [96]. Later studies have indicated that the number of tumor-infiltrating pDCs is linked with lymph node metastasis and poorer prognostic outcomes in patients with OSCC as these cells were dysfunctional with relatively low production of IFN-α, IL-6, and TNF-α as compared to circulating pDCs [97]. This functional impairment came as a result of many inhibitory factors present in the TME including VEGF, TGF-β, and IL-10 which inhibit the activation of pDCs and prevent antigen presentation. Therefore, although PD appears to induce higher pDC recruitment in oral cancer, these cells are functionally repressed by the TME, and therefore fail to contribute to anti-tumor immunity. Therefore, current results support the model that PD suppresses anti-tumor functions of DCs, indicating that reduction in periodontal inflammation could restore anti-tumor functions of DCs.

Mast Cells

Mast cells (MCs) are immune cells linked principally to allergic reactions, but they are also functionally associated with many other pathological conditions including cancer and PD [98, 99]. Morphological features include their distinct cytoplasmic granularity related to the presence of unique basophilic granules that are rich in inflammatory mediators including histamine and heparin [100]. These cells reside in all the connective tissue components of the oral cavity, including the periodontal ligament and gingiva, and are distributed mainly along the available vasculature [101]. Their unique location, in close proximity to other immune responders, allows them to rapidly take action whenever they sense changes within their immediate microenvironment. In PD, constant irritation from the plaque biofilm stimulates MCs to proliferate, which denatures the composition of the normal gingival tissue [102]. This increase in number results in differential expression of MC mediators within the oral connective tissues, and the subsequent functional consequences of this have the potential to contribute directly to the pathologic processes of OSCC.

Other than the basophilic granular content of MCs, they have the ability to synthesize and express many mediators that induce several characteristic functions; such as the chymase and tryptase proteolytic enzymes. In PD, the production of MC chymase has the ability to activate many factors including collagenase, MMP-3, and MMP-9 which are known to induce disruption of the basement membrane [102, 103]. As such, the increase in MC chymase levels can reflect one potential means by which MCs promote the progression of oral malignant and premalignant lesions. Although it has not been studied in OSCC, the increase in levels of chymase has been shown to influence prostate cancer progression via downregulation of expression of signals for the androgen receptor expressed in prostate cancer cells thereby increasing the invasive ability of these cells [104]. As for tryptase, the severity of periodontal inflammation was found to be

correlated positively with its expression levels [105]. This elevated expression can impact the role MCs play in cancer as MC-derived tryptase is considered a key fibroblast activator prompting them to adopt a tumor-favoring phenotype [103]. MC-activated cancer-associated fibroblasts (CAF) stimulate the migration, proliferation, and malignant transformation of keratinocytes and have the ability to alter the tumor immune response by their sustained TGF-β production, which regulates the activity of natural killer (NK) cells [106].

Another function of MCs, which might be linked to their role in evolving malignant lesions, is their ability to prompt MC-directed vascular expansion. Given their strategic location in close proximity to the oral vasculature, they are able to drive neovascularization aided by angiogenic factors, including VEGF, or substances that can have angiogenic properties, such as tryptase, IL-8, TNF, basic fibroblast growth factor (bFGF), heparin, and histamine [101]. Angiogenesis facilitates the invasion and metastasis of cancer cells by remodeling the TME, and provides increased oxygen and nutrients for tumor growth. In the context of OSCC, a positive correlation between the MC and vascular densities in OSCC has been recently established, suggesting the ability of these cells to regulate angiogenesis [107]. This may explain the aggressive behavior and metastatic tendencies of these oral tumors. Although the angiogenic effects of MCs are only one of many pathways that induce vascular expansion within the TME, which responds to angiogenic factors from a variety of different sources, it is clear that MCs are effective inducers of this process and their role should be further investigated.

Lymphoid Derived Cells

Regulatory T-Lymphocytes

In PD, when dysbiotic bacteria attack the gingival tissue, an exaggerated host immune response is triggered in an attempt to avoid the dissemination of these micro-organisms, which results in tissue damage [108]. Consequently, Immunosuppressive T-regulatory cells (T-regs) are recruited into these sites to attenuate this aberrant inflammatory response and promote immune homeostasis. The main purpose of these cells is to minimize collateral tissue damage, which in turn promotes the survival of periodontal pathogens [109]. These cells limit the immune response by modulating the activities of other immune cells by a number of mechanisms, including: (1) binding of the T-reg expressed CTLA-4 to CD80/86 on DCs which inhibits co-stimulatory signals; (2) limiting the availability of IL-2 needed for effector T-cell activation; (3) producing immunosuppressive factors (ex. TGF-β, IL-10, IL-35); and (4) IDO-mediated T-cell dysfunction [110]. As one of the central players in PD, the recruitment of these immunosuppressive cells into the oral milieu will impact the development of further diseases including malignancies. Indeed, infiltration of Foxp3+ T-regs into the oral environment was shown to promote malignant transformation of oral premalignant lesions giving rise to OSCC [77].

It has been noted that CCL20, a chemokine linked with recruiting CCR6-expressing cells (including CCR6+ T-regs), is upregulated in both gingival fibroblasts and in periodontal ligament cells during periodontal inflammation [111, 112]. Co-stimulation of gingival fibroblasts by IL-1β and IL-22, present in the inflammatory milieu of PD, enhanced the activation of the C-Jun N-terminal kinase and the NF-κB pathway resulting in the upregulation of CCL20 [111]. In a similar manner, IL-6/sIL-6R stimulation enhanced CCL20 production in IL-1β-stimulated periodontal ligament cells by activating the STAT3 signaling pathway [112]. Although CCL20 has been mainly linked to recruiting Th17 cell, a correlation between the expression of CCL20 and FOXP3 mRNA in OSCC was noted. This led to a further study demonstrating that oral cancers with high CCL20 expression favor the selective recruitment of CCR6+ T-regs [113]. These CCR6+ cells are phenotypically and functionally distinct from CCR6- T-regs and possess enhanced suppressive activity, IL-10 production and FOXP3 expression [113]. Further studies are necessary to determine if the intensified recruitment of CCR6+ T-regs by CCL20/CCR6 signaling in PD can promote the progression OSCC.

In PD, the triad of molecules that regulate bone hemeostasis/pathology, receptor activator of nuclear factor-κB ligand (RANKL), its receptor RANK and its decoy receptor osteoprotegerin (OPG) are imbalanced resulting in bone loss. RANKL, which is upregulated in PD, promotes T-reg induction, while RANKL inhibition interferes with the migration of T-regs, and induces lower expression levels of the T-reg-related cytokines IL-10 and TGF-β [114]. This RANKL-mediated T-reg activation is further sustained in PD by the downregulation of osteoprotegerin that can block RANKL in the saliva and GCF of patients with PD [115, 116]. This RANKL–RANK signaling in OSCC has been shown to stimulate cancer metastasis in concert with the presence of active tumor-infiltrating T-regs [117]. Therefore, the imbalance of the RANKL/RANK/OPG signaling in PD may contribute to developing a T-reg mediated downregulated immune response which allows oral cancer cells to evade immune destruction.

Natural Killer Cells

Natural killer cells (NKs) are innate lymphoid cells that are actively involved in the destruction of foreign, infected and cancerous cells that are deemed to be of danger to the host [118]. Their cytotoxic activity is mediated by trafficking their granular content into the cytosol of their targets resulting in their lysis in a granzyme B and perforin-dependent fashion. Additionally, these cells produce an array of pro-inflammatory cytokines and chemokines (IFN-γ, TNF, IL-6, GM-CSF, and CCL5) that shape both the innate and adaptive immune response [119]. In PD however, the NK population has been linked to unresolved immune responses leading to the progression of PD [120]. This is due to immunosuppressive molecules in the periodontal inflammatory microenvironment that are capable of suppressing these NK cells.

Immune suppressive molecules including (IL-10, TGF-β, and IL-35) were found to be expressed locally as well as systemically under the impact of PD [121, 122]. In OSCC patients, IL-10 and TGF-β have been found to reduce the ability of peripheral NK cells to lyse their targets, and reduced their expression of IFN-γ, their key effector molecule [123]. TGF-β specifically has

been found to severely compromise an important stimulator of the effector function of cytotoxic lymphocytes and NKs. NKG2D, a receptor found on the surface of these cytotoxic cells, upon engaging with their NKG2D ligand expressed on tumor cells a stimulatory signal is transmitted into these cells allowing immunosurveillance of cancer cells [124]. TGF-β was found to interfere with this process by reducing the NKG2DL expression by tumor cells and downregulating NKG2D on NK cells [125]. On the other hand, IL-35 has the ability to reduce granzyme B production in NK cells impeding this cytolytic defense mechanism [126]. Another cytokine readily available in PD, that may contribute to impairing NK function, is IL-17 which plays a role in accelerating the phosphorylation of GSK-3β [127]. This increased GSK-3β phosphorylation was found to be involved in weakening the cytotoxic response of NK cells. These findings highlight several NK suppressive mechanisms adopted in PD that can compromise the effectiveness of the NK mediated anti-tumor immune response.

Pathogenic microorganisms might also contribute to modulating the activities of NKs. This was observed recently when *Fusobacterium nucleatum*, a pathogen strongly associated with PD, was discovered to bind to CEACAM1 (an inhibitory receptor on various immune cell subsets) [128]. The activation of CEACAM1 impairs degranulation of NK cells preventing the release of their lytic content and also diminishes their production of the immune-activating cytokine IFN-γ. Therefore, due to the suppressive influence of cytokines and microorganisms in PD, it is most likely that these cells will not function efficiently to kill tumor cells in OSCC.

Cytotoxic T-Lymphocytes

Cytotoxic T-lymphocytes (CTL) are the key effector arm of the adaptive immune response. They adopt one of three mechanism to mediate the clearance of foreign, infective or malignant cells: (1) releasing cytokines that stimulate killing of their targets, predominantly TNF-α and IFN-γ; (2) binding of their Fas ligand of to the Fas receptor expressed on their targets, triggering apoptosis; and (3) trafficking pre-synthesized destructive granzymes and perforin into the intercellular space of the opposed cells [129]. Increased levels of CTL were observed in PD, yet, these cells were in fact linked to the etiopathogenesis of PD, leading to a more rapid onset of severe tissue destruction [129].

Recent studies postulate that the different cellular and molecular elements found within the inflammatory setting of PD may modify the function of CTLs. For example, the increased frequency of MDSCs, as described earlier in PD, has been confirmed to impede the function of CTLs in OSCC [130]. These MDSCs hamper expression of the CD3-ζ chain on CTLs, modifying the cytoplasmic signaling that leads to their activation [130]. MDSCs have also been implicated in decreasing IFN-γ production from these cytotoxic cells, impairing one of their most prominent destructive mechanisms [130]. Finally, MDSCs suppress the proliferative ability of CTLs, through inhibiting the TCR-driven cycling [130]. Together, this supports the establishment of an inflammatory state that supports the advancement of tumors.

Another mean by which PD can limit functionality of CTLs is through the production of programmed death-ligand 1 (PD-L1), a ligand that signals for T cell apoptosis. The binding of PD-L1, a transmembrane protein belonging to the B7 family, to its receptor (PD-1) is considered an important factor for suppression of the adaptive immune response. It can result in stunting the activities of immune cells leading to their apoptosis. The upregulated expression of both PD-1 and PD-L1 on CTLs, with the concomitant increase of PD-L1 mRNA in saliva of PD patients will, without doubt, affect the immune response in OSCC [131, 132]. The effect of these CD8+PD-1+ cells was observed in OSCC, where they were found to be less responsive than those CD8+ cells lacking PD-1 expression [133]. This is consistent with the observation that higher grades of oral premalignant and malignant lesions were positively linked to higher expression of PD-1/PD-L1 [134].

B-Lymphocytes

B cells are regarded for their role in mediating the humoral part of the adaptive immune response given their chief role in antibody production [135]. They have been subdivided into many different subsets depending on various contacts and cytokine stimuli present within the local issue in which they respond. One of these functional B cell subsets, which is significantly increased in PD is the IL-10 producing regulatory B cells (B-regs) [136]. The conversion of human B cells into B-regs was found to be induced by the immunosuppressive cytokine IL-35, which is, as previously mentioned, upregulated in patients with PD [122, 137]. These recruited cells halt the progressive bone loss associated with PD by modifying the expression of pro-inflammatory cytokines and local proliferation of Th17 cells [136]. The immunosuppressive properties of these cells are likely to impact oral malignancies, for example, in tongue squamous cell carcinoma, the frequency of B-regs within the microenvironment was positively correlated to IL-10 mediated conversion of CD4+T cells to T-regs and thus was an indicator of poor prognosis [138].

One of the most notable features of B cells that were observed in both OSCC and PD was their distribution. In these diseases B cells were mainly arranged on the periphery and peri-lesion areas mostly toward its advancing edge [139, 140]. Furthermore, in PD all B cell subsets had elevated expression of RANKL, promoting osteoclast formation and bone recession [141]. In a parallel phenomenon, B cells promoted invasion and metastasis of oral cancer cells, and increased B cell infiltration in OSCC was significantly correlated with lymph node metastasis [140].

From another perspective, growing evidence supports the involvement of Epstein-Barr virus (EBV) in the pathogenesis of PD. This is linked directly to the function of B cells present in the PD inflammatory microenvironment, as EBV infects B cells promoting their expansion and differentiation before remaining latent in the circulating memory B cells [142]. Through this, PD supports the amplification and viral spreading of EBV in a B cell dependent manner. This may favor the development of OSCC, as the vast majority of OSCC cases are located in B cell rich areas,

with EBV+ tumors having a more rapid onset and more invasive potential [143].

Although the role of B cells in both PD and OSCC has been understudied, the findings summarized here are promising, and suggest an important role of these cells.

T-Helper Cells

T-helper (Th) cells are pivotal moderators of the inflammatory immune response. They assist the other leukocytes in delivering an effective immune response by several means such as: the induction of B-cell maturation; CTL and macrophage activation; and recruitment of PMNs [144]. Several subsets of these CD4+ T-lymphocytes have been characterized including Th1, Th2, Th3, Th17, and T follicular helper (Tfh) cells, with each harboring unique features and functions [145]. Originally, Th1 and Th2 were shown to play a role in the pathogenesis of PD. However, with the emergence of new Th subsets and a deeper understanding of their plasticity under the influence of environmental conditions, this Th1/Th2 model has been altered considerably [146]. Herein, we discuss how the effects of each of the three dominant Th subsets in PD (Th1, Th2, & Th17) might also contribute to OSCC pathogenesis.

Th1 cells functions by enhancing phagocytosis, complement fixation, and opsonization, strengthening the immune response against pathogens. Th1 cells differentiate from naïve T cells under the influence of the inductive signals IL-12 and IFN-α. In PD, an upregulation of GATA-3 signaling was noted [147]. GATA-3 was previously reported to suppress STAT4 signaling and thereby limit the differentiation capacity of Th1 cells and in the meantime enhance that of Th2 cells [148]. Likewise, overexpression of GATA-3 was identified in OSCC samples, consistent with another study reporting that Th1 cytokines (IL2, IFNγ) had lower expression in the sera of OSCC patients [149, 150]. The prior upregulation of GATA-3 in PD patients may augment the GATA-3 suppressive effect on Th1 in OSCC impairing the Th1 mediated immune response.

On the contrary, the Th2 subsets are involved in weaker innate responses and are more prevalent in OSCC facilitating the progression of cancer cells [151]. A very recent study highlighted that, in tongue squamous cell carcinoma, the immune response was biased toward Th2, with their cytokines (IL-4, IL-10) suppressing the cytotoxicity of CTL [152]. The involvement of these Th2 cells has been established in PD; however, in this context it has been suggested that Th2 cells promote a generalized state of immunosuppression through additional means. The elevated levels of the Th2-secreted cytokine, IL-4, within the inflammatory milieu induces CCR4-dependent recruitment of immunosuppressive T-regs into the periodontium [153]. Together, this Th2-mediated suppression of the adaptive immune response in PD is likely to exhibit similar defective mechanisms toward any forthcoming OSCC lesions.

It has only recently been suggested that the Th17 subset might play a role in periodontal diseases. As the principal producers of IL-17, Th17 cells play a central role in recruiting PMNs by upregulating the CXCL8 expression [154]. Th17 cells are now accepted as one of the critical upregulated cellular elements present in periodontally inflamed sites. Indeed, their presence was shown to be directly proportional to probing depths in patients with PD [155], and higher IL-17 levels were detected in the saliva, serum and GCF of diseased patients [155, 156]. Generally, Th17 cells have been linked to unfavorable prognostic outcomes in patients with cancer in the head and neck region [157]. This may come as a result of the tumor promoting activities of IL-17, which regulates the levels of VEGF-A and IL-6 promoting the proliferation of oral cancer cell [157].

CHANGES IN THE PERIODONTAL MICROENVIRONMENT THAT CONTRIBUTE TO THE PATHOGENESIS OF OSCC

The interactions that cancer cells make with their surrounding environment are critical for tumor initiation and progression. Changes in the oral microenvironment in periodontitis can profoundly impact the development of oral cancer. Periodontal inflammation and associated bacterial pathogens cause damage to, and otherwise alter, the specialized tissue that surrounds the teeth (epithelium, connective tissue, vasculature, and bone). These changes can increase the potential for metastatic seeding, survival and growth of cancer cells, explaining why the gingiva, out of all the oral soft tissues, is the most common site for developing metastatic oral carcinomas [23]. Therefore, it is important to discuss some of these PD-induced changes and how they can potentially affect carcinogenesis. Here, we focus on specific changes that may directly influence OSCC [3] (**Figure 3**).

Epithelial Changes

The oral epithelial barrier has a critical function of protecting its underlying structures from pathogenic invaders, exogenous substances and mechanical stresses excreted by masticatory forces [158]. Once pathogenic microorganisms breach this protective barrier, gaining access to the tooth supporting structures, they elicit a chronic, persistent inflammatory state with subsequent connective tissue and bone destruction as the severity of inflammation increases [159]. Therefore, disruption of the epithelial barrier and ingress of pathogenic bacteria can cause further epithelial injury in a feed-forward manner. Downregulated expression of E-cadherin, an adhesion molecule that is fundamental for the cell-cell adhesion in the epithelial monolayer, contributes to the compromised integrity of the epithelium. Reduced E-cadherin expression is partly a consequence of increased macrophage production of TNF-α, a well-known E-cadherin down-regulator [160]. Loss of E-cadherin marks the advancement of premalignant, benign lesions into aggressive, metastatic prone carcinomas and is considered a hallmark of EMT in OSCC [161]. Upon exposure to periodontal pathogens, primary oral keratinocytes show increased nuclear activity of Snail-1 [162], which results in down-regulated E-cadherin, and increased expression of vimentin, another EMT biomarker.

The transcription factor, Twist, also an EMT mediator, increases the motility of epithelial cells allowing them to detach and adopt invasive characteristics of mesenchymal-like cells

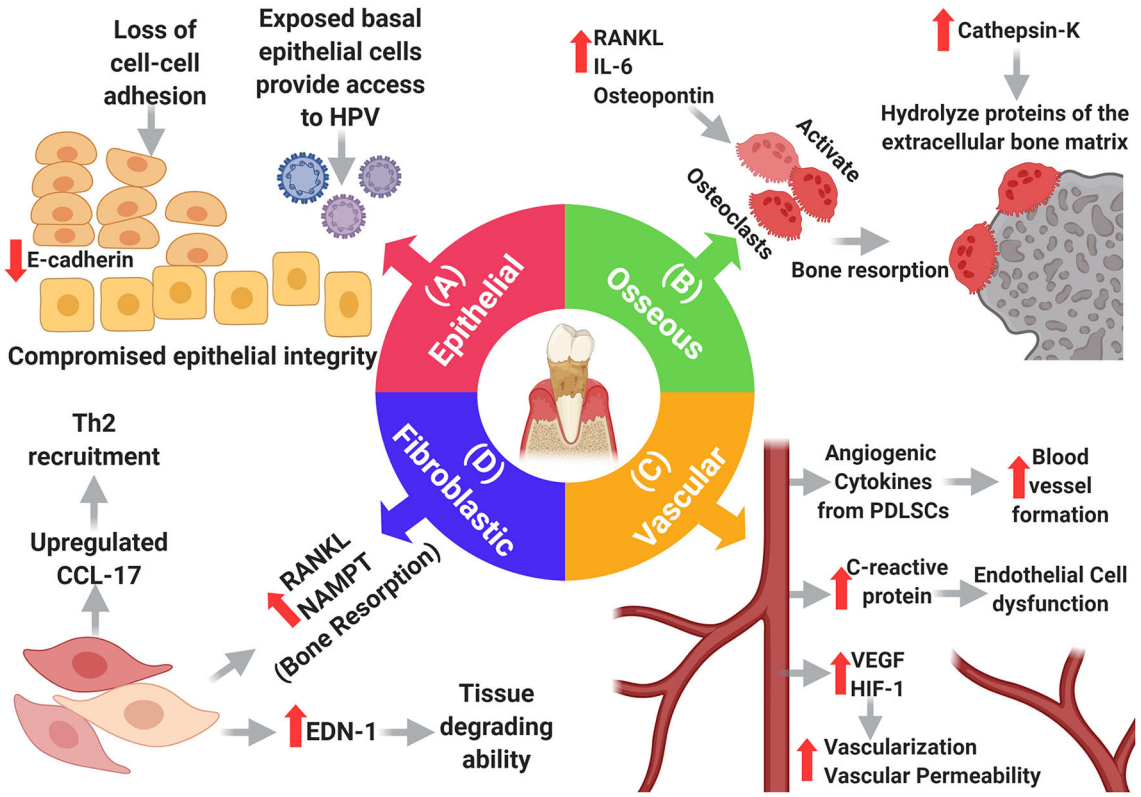

FIGURE 3 | Changes in the main resident cells of the periodontium in periodontitis. **(A)** Epithelial changes: loss of adhesion molecules results in loss of cell-cell adhesion compromising the integrity of the oral epithelium which exposes basal cells providing a portal of entry to HPV. **(B)** Osseous changes: increased RANKL, IL-6, and Osteopontin promote osteoclast-dependent bone resorption, and increased cathepsin-k promotes hydrolysis of extracellular bone proteins. **(D)** Fibroblastic changes: fibroblasts adopt new functions in periodontitis as they upregulate endothelin-1, which in turn increases their tissue degrading ability, moreover, they induce bone resorption by upregulating RANKL and NAMPT. Additionally, fibroblasts upregulate CCL-17, causing expansion of the Th2 cell population, thereby weakening the immune response. **(C)** Vascular changes: many elements within the inflammatory setting induce vascular growth including PDLSCs. VEGF and HIF-1 contribute to angiogenesis, but also, increase the permeability of blood vessels. Increased levels of C-reactive protein cause endothelial cell dysfunction.

[161]. Higher Twist expression is coupled with higher grades of dysplasia in oral premalignant lesions, making it a useful indicator of malignant transformation in oral epithelium [161]. When periodontal pathogens were co-cultured with OSCC cell lines, Twist expression was upregulated in the malignant cells, and these cells had increased migration [163]. This suggests that PD promotes upregulation of Twist expression, and facilitates cell migration and EMT. The inflamed periodontal tissues also show differential expression of several members of the laminin family including (laminin gamma 2 and laminin beta 3) [164, 165]. These glycoproteins contribute to cancer-related cellular processes, and interact with receptors expressed by cancer cells to stimulate production of collagenase IV, which facilitates cancer cell migration [166]. The same laminin members that were upregulated in PD were also found to be upregulated in OSCC [167].

The loss of cellular adhesion, in addition to the continuous deepening of the periodontal pocket, results in successive exposure of basal epithelial cell layers. As basal cells are the exclusive cellular targets for human papillomavirus (HPV) in the oral cavity, these exposed basal cells provide access points to HPV infections [168]. Further proliferation of these cells favors the persistence of the HPV infection by sustaining viral replication, thus making the infected periodontal pockets reservoirs of this virus [168]. As a central risk factor for cancer development, around 70% of oropharyngeal cancers have been linked to HPV infections [169]. Although only a few studies have explored this association, strong evidence supports the involvement PD mediates HPV-induced carcinogenesis in the oral cavity. Additional longitudinal studies to confirm this interaction will help to reinforce the insight that maintaining periodontal health may prevent the deleterious effects of HPV, specifically in relation to cancer.

Osseous Changes

When untreated, the breakdown of the periodontal tissue and the subsequent bone destruction in PD ultimately results in tooth loss. This devastating consequence comes as a result of not only microbial invasion, but most importantly, the elicited host inflammatory response, which is central to this local osteolytic process. Hints for an association between osseous changes that occur in PD and the pathogenesis of OSCC have been suggested.

The bony changes that occur in PD are involved in the underlying molecular and cellular etiopathological mechanisms governing OSCC, and can also be considered significant risk predictors of OSCC. A study reported that there is a 5.2-fold increase in the risk of developing squamous cell carcinoma of the tongue with every millimeter alveolar bone loss observed in patients with PD [170].

Osteoclast regulators, RANKL, its receptor RANK, and decoy receptor OPG are the core elements orchestrating the osteo-immune bone destruction in PD. Elevated RANKL expression by many inflammatory cell types including, but not limited to, B and T lymphocytes and stromal cells, has been shown at sites with destructive periodontal activity [171]. RANKL induces maturation of osteoclasts, which are directly responsible for resorption of the dento-alveolar bone complex. Treating periodontal conditions was found to result in a significant reduction of RANKL levels in the gingival crevicular fluid, confirming the significance of RANKL in the inflammatory periodontal process [172]. In oral cancer, this amplified osteoclastic activity in PD supports the invasion of cancer cells into bone, which promotes their metastatic spread [173].

Many other osteoclastogenic cytokines are predominant in PD, disrupting the balance between bone resorption and bone formation. IL-6, a cytokine that has been identified as being upregulated in both the saliva and serum in PD, was shown to induce the activation of local osteoclasts and as a result potentiate bone resorption in periodontally inflamed sites [174, 175]. As for its role in oral malignancies, cases of OSCC that harbored greater osteoclast-derived IL-6 mRNA expression were associated, to a larger extent, with mandibular invasion, demonstrating its pro-invasive capacity in OSCC [176]. Osteopontin (OPN), a major non-collagenous bone protein, is key for many biological activities and is well-known for its involvement in bone diseases including PD. OPN enhances the proliferative and differentiation abilities of osteoclasts, and also suppresses osteoblasts [177]. Recently, an important role of OPN was revealed when it was found to induce nuclear translocation and phosphorylation NF-κB, which upregulates the transcription of genes that encode the production of bone damaging products in osteoclasts [177]. This OPN rich environment in PD is likely to also boost osteoclasts-mediated bone resorption in the context of OSCC. Furthermore, OPN was shown to have direct effects on cancer cells. When OSCC cells were cultivated in the presence of OPN, they developed greater adhesive, proliferative, and invasive capacities and expressed greater levels of IL-6 and IL-8 [178].

The cysteine protease Cathepsin K, known to hydrolyze proteins of the extracellular bone matrix, was increased in the GCF of PD patients, with its concentration reflecting disease severity [179]. Mice with experimentally induced PD that were treated with odanacatib, a small molecular inhibitor of Cathepsin K, significantly suppressed the activity of osteoclasts, essentially weakening the bone resorptive process [180]. The involvement of Cathepsin K in PD may contribute to more destructive forms of OSCC, since upregulation of Cathepsin K was shown to be linked with lymph node metastasis and unfavorable prognosis in OSCC [181].

Fibroblast Changes

Gingival fibroblasts are the most abundant cellular component of the periodontal connective tissue [182]. They function to shape the structural framework of the tissue by continuously regulating the turnover of the extra cellular matrix. However, they also have functions beyond their structural role, and make important contributions to pathological processes. Fibroblasts are one of the first cells that encounter pathogenic threats, and they are able to produce inflammatory mediators, and induce the expression of RANKL and OPG in PD. Invading periodontal micro-organisms were found to upregulate the expression of RANKL and reduce OPG expression in gingival fibroblasts [183]. Pathogen exposed fibroblast exhibit a similar phenotype to those found in the OSCC TME. Cancer associated fibroblasts (CAF) have also been observed to modulate RANKL expression, contributing to bone invasion by promoting osteoclastogenesis [184].

Several other key GF products have been shown to have altered expression upon encountering pathogenic bacteria, and these changes mirror the CAF phenotype present in the TME of OSCC. *P. gingivalis* prompts the expression of Galectin-1 (Gal-1), a beta-galactoside-binding protein, in gingival fibroblasts [185]. This deregulates the cytokine network in PD, limiting the migration of leukocytes and thereby allowing further colonization by pathogenic bacteria. In OSCC, Gal-1 was found to activate CAF and their expression of alpha-smooth muscle actin (α-SMA) inducing their transdifferentiation into myofibroblasts [186]. This results in upregulated expression of fibroblast monocyte chemotactic protein-1 (MCP-1), which then binds to CCL2 on cancer cells, promoting the metastatic ability of these malignant cells. Knockdown of Gal-1 in CAF results in quiescence of these fibroblasts and reverses their ability to promote migration of cancer cells [186].

Other molecules that govern the expression of different GF products show comparable contributions in both PD and OSCC [187, 188]. For instance, the pleiotropic peptide endothelin-1 (EDN-1) is elevated in both diseases. EDN-1 induces fibroblast tissue degrading capacity and breakdown of the oral stroma, which can promote the invasion by OSCC cells [188]. Nicotinamide phosphoribosyltransferase (NAMPT) regulates GFs in both diseases [189, 190]. In PD, NAMPT is critical for inducing GF expression of bone destructive mediators, including COX2, MMP1, and MMP3, supporting progressive bone loss [189]. In OSCC, upregulation of NAMPT was observed, suggesting it may play a role in oral dysplastic pathologies [190].

Vascular Changes

Aberrant vascularization is one of the most prominent features of inflamed periodontium. Many cells contribute to the vascularization process, which is intensified by the dense inflammatory infiltrate. Periodontal ligament stem cells (PDLSCs) are one of the most prominent cell types involved in this process. In the inflammatory milieu, PDLSCs promote angiogenesis by activating autophagy, which in turn enhanced the production of angiogenic cytokines including basic fibroblast growth factor (bFGF) and angiogenin [191]. Although vascular density was found to be increased in PD, endothelial and microvascular dysfunction has been reported in cases of severe

PD [192]. Moreover, elevated levels of IL-6 and C-reactive protein (CRP) in the peripheral blood of periodontal patients was found to predispose to an increased risk of developing peripheral arterial diseases which are regarded as an independent risk factors for developing cancer [193, 194].

CRP, a plasma protein mainly produced by the liver, is a valuable inflammatory biomarker and has been shown to promote endothelial dysfunction. In PD, levels of this protein were found to be elevated both in the local inflammatory site as well as systemically [193]. It has been recognized that this local increase comes as a result of stimulation of endothelial cells by IL-6 and IL-1β produced by other inflammatory cells. This local increase was augmented by extra-hepatic production of CRP to induce the systemic elevation manifested in PD. High CRP levels are associated with OSCC patients that had metastatic lesions, suggesting that it could be used as a predictor of lymph node metastasis [195]. In addition, it has been suggested that a higher CRP/albumin ratio might be an independent prognostic marker of poor outcomes in patients with OSCC [196]. Since CRP affects the metastatic ability of cancer cells this could represent another potential avenue whereby PD interacts with OSCC.

The expression of hypoxia inducible factor-1 (HIF-1) and vascular endothelial growth factor (VEGF) in periodontal patients was shown to be greater than that of healthy individuals [197]. VEGF, a fundamental growth factor that stimulates the proliferation of endothelial cells and increases vascular permeability, is regulated by HIF-1. The expression of both cytokines was found to correlate with the severity of the periodontal condition [197]. HIF-1α also provokes invasion of OSCC cells, and VEGF-dependent angiogenesis was found to be vital for continuous vascular expansion during the transition from epithelial dysplasia to carcinoma [198, 199].

CONCLUSION

In an era where understanding the putative interconnectivity between cancer and other diseases, particularly inflammatory diseases, unveils new aspects and targets for cancer therapy, it has become clear that PD has the potential to exacerbate all the pathogenic characteristics of OSCC. From the earliest steps of the neoplastic process, PD may, in part, contribute to the induction of permanent genomic alterations, due to the sustained presence of bacterial-derived mutagens. Furthermore, primary and metastatic tumor cells can benefit from the weakened stromal elements due to PD-dependent tissue destruction, and their proliferation in this environment is sustained by its rich growth/survival factors. In addition to potentially mutagenic properties and stromal defects, the oral inflammatory environment in PD is permissive for tumor progression. This is characterized by increased inflammatory infiltrate, which is nevertheless associated with a blunted or suppressed immune response that contributes to the development and progression of OSCCs. PD can therefore aid the development of oral cancers that would otherwise be destroyed in a healthy oral environment by normal immune cell functions. Therefore, monitoring oral health and providing effective treatments for PD might hold promise in normalizing this misdirected immune response, restoring an inflammatory network capable of exerting anti-tumor properties.

AUTHOR CONTRIBUTIONS

OE designed and drafted the manuscript. AB, NF, and HT reviewed and edited the manuscript. MG organized, provided the frame for the manuscript, and critically revised the content. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Singh N, Baby D, Rajguru JP, Patil PB, Thakkannavar SS, Pujari VB. Inflammation and cancer. *Ann Afr Med*. (2019) 18:121–6. doi: 10.4103/aam.aam_56_18
2. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell*. (2010) 140:883–99. doi: 10.1016/j.cell.2010.01.025
3. Moergel M, Kämmerer P, Kasaj A, Armouti E, Alshihri A, Weyer V, et al. Chronic periodontitis and its possible association with oral squamous cell carcinoma - a retrospective case control study. *Head Face Med*. (2013) 9:39. doi: 10.1186/1746-160X-9-39
4. Kinane DF, Stathopoulou PG, Papapanou PN. Periodontal diseases. *Nat Rev Dis Primers*. (2017) 3:17038. doi: 10.1038/nrdp.2017.38
5. Oveisi M, Shifman H, Fine N, Sun C, Glogauer N, Senadheera D, et al. Novel assay to characterize neutrophil responses to oral biofilms. *Infect Immun*. (2019) 87:e00790-18. doi: 10.1128/IAI.00790-18
6. Raitapuro-Murray T, Molleson TI, Hughes FJ. (2014) The prevalence of periodontal disease in a Romano-British population c. 200-400 AD. *Br Dental J*. 217:459–66. doi: 10.1038/sj.bdj.2014.908
7. Yao QW, Zhou DS, Peng HJ, Ji P, Liu DS. Association of periodontal disease with oral cancer: a meta-analysis. *Tumour Biol*. (2014) 35:7073–7. doi: 10.1007/s13277-014-1951-8
8. Corbella S, Veronesi P, Galimberti V, Weinstein R, Del Fabbro M, Francetti L. Is periodontitis a risk indicator for cancer? *a meta-analysis. PLoS ONE*. (2018) 13:e0195683. doi: 10.1371/journal.pone.0195683
9. Sfreddo CS, Maier J, De David SC, Susin C, Moreira C. Periodontitis and breast cancer: a case-control study. *Community Dent Oral Epidemiol*. (2017) 45:545–51. doi: 10.1111/cdoe.12318
10. Chang JS, Tsai CR, Chen LT, Shan YS. Investigating the association between periodontal disease and risk of pancreatic cancer. *Pancreas*. (2016) 45:134–41. doi: 10.1097/MPA.0000000000000419
11. Barton MK. Evidence accumulates indicating periodontal disease as a risk factor for colorectal cancer or lymphoma. *CA Cancer J Clin*. (2017) 67:173–4. doi: 10.3322/caac.21367
12. Zeng XT, Xia LY, Zhang YG, Li S, Leng WD, Kwong JS. Periodontal disease and incident lung cancer risk: a meta-analysis of cohort studies. *J Periodontol*. (2016) 87:1158–64. doi: 10.1902/jop.2016.150597
13. Chou SH, Tung YC, Wu LS, Chang CJ, Kung S, Chu PH. Severity of chronic periodontitis and risk of gastrointestinal cancers: a population-based follow-up study from Taiwan. *Medicine*. (2018) 97:e11386. doi: 10.1097/MD.0000000000011386
14. Karmakar S, Kar A, Thakur S, Rao V. Periodontitis and oral Cancer-a striking link. *Oral Oncol*. (2020) 106:104630. doi: 10.1016/j.oraloncology.2020.104630

15. Ye L, Jiang Y, Liu W, Tao H. Correlation between periodontal disease and oral cancer risk: a meta-analysis. *J Cancer Res Ther*. (2016) 12:C237–40. doi: 10.4103/0973-1482.200746
16. Shulman JD, Gonzales CD. Chapter 3 - epidemiology/biology of oral cancer. *Prevent Clin Oral Health Care*. (2008) 27–43. doi: 10.1016/B978-0-323-03695-5.50007-0
17. Lesch CA, Squier CA, Cruchley A, Williams DM, Speight P. The permeability of human oral mucosa and skin to water. *J Dent Res*. (1989) 68:1345–9. doi: 10.1177/00220345890680091101
18. Squier CA, Kremer MJ. Biology of oral mucosa and esophagus. *JNCI Monographs*. (2001) 29:7–15. doi: 10.1093/oxfordjournals.jncimonographs.a003443
19. Messadi DV. Diagnostic aids for detection of oral precancerous conditions. *Int J Oral Sci*. (2013) 5:59–65. doi: 10.1038/ijos.2013.24
20. Neville BW, Day TA. Oral cancer and precancerous lesions. *CA Cancer J Clin*. (2002) 52:195–215. doi: 10.3322/canjclin.52.4.195
21. Sreenivasan PK, Prasad KVV. Distribution of dental plaque and gingivitis within the dental arches. *J Int Med Res*. (2017) 45:1585–96. doi: 10.1177/0300060517705476
22. Tanner AC, Paster BJ, Lu SC, Kanasi E, Kent R Jr, Van Dyke T, et al. Subgingival and tongue microbiota during early periodontitis. *J Dent Res*. (2006) 85:318–23. doi: 10.1177/154405910608500407
23. Allon I, Pessing A, Kaplan I, Allon DM, Hirshberg A. Metastatic tumors to the gingiva and the presence of teeth as a contributing factor: a literature analysis. *J Periodontol*. (2014) 85:132–9. doi: 10.1902/jop.2013.130118
24. Seoane J, Van der Waal I, Van der Waal RI, Cameselle-Teijeiro J, Antón I, Tardio A, et al. Metastatic tumours to the oral cavity: a survival study with a special focus on gingival metastases. *J Clin Periodontol*. (2009) 36:488–92. doi: 10.1111/j.1600-051X.2009.01407.x
25. Ganly I, Yang L, Giese RA, Hao Y, Nossa CW, Morris L, et al. Periodontal pathogens are a risk factor of oral cavity squamous cell carcinoma, independent of tobacco and alcohol and human papillomavirus. *Int J Cancer*. (2019) 145:775–84. doi: 10.1002/ijc.32152
26. Geum DH, Roh YC, Yoon SY, Kim HG, Lee JH, Song JM, et al. The impact factors on 5-year survival rate in patients operated with oral cancer. *J Korean Assoc Oral Maxillofac Surg*. (2013) 39:207–16. doi: 10.5125/jkaoms.2013.39.5.207
27. Vucicevic Boras V, Fucic A, Virag M, Gabric D, Blivajs I, Tomasovic-Loncaric C, et al. Significance of stroma in biology of oral squamous cell carcinoma. *Tumor*. (2018) 104:9–14. doi: 10.5301/tj.5000673
28. Galvão-Moreira LV, da Cruz MC. Oral microbiome, periodontitis and risk of head and neck cancer. *Oral Oncol*. (2016) 53:17–9. doi: 10.1016/j.oraloncology.2015.11.013
29. Coussens LM, Werb Z. Inflammation and cancer. *Nature*. (2002) 420:860–7. doi: 10.1038/nature01322
30. Karpiński TM. Role of oral microbiota in cancer development. *Microorganisms*. (2019) 7:20. doi: 10.3390/microorganisms7010020
31. Borba TT, Molz P, Schlickmann DS, Santos C, Oliveira CF, Prá D, et al. Periodontitis: genomic instability implications and associated risk factors. *Mutat Res Genetic Toxicol Environ Mutagenesis*. (2019) 840:20–3. doi: 10.1016/j.mrgentox.2019.01.005
32. Memmert S, Gölz L, Pütz P, Jäger A, Deschner J, Appel T, et al. Regulation of p53 under hypoxic and inflammatory conditions in periodontium. *Clin Oral Investig*. (2016) 20:1781–9. doi: 10.1007/s00784-015-1679-x
33. Cutilli T, Leocata P, Dolo V, Altobelli E. p53 as a prognostic marker associated with the risk of mortality for oral squamous cell carcinoma. *Oncol Lett*. (2016) 12:1046–50. doi: 10.3892/ol.2016.4742
34. Alsenani M. *The Requirement of p53* in *Oral MSC, and Differentiation Which Leads to Kaposi's Sarcoma, in Dental*. (Ph. D. Theses). Philadelphia, PA: UPenn (2016).
35. Stashenko P, Yost S, Choi Y, Danciu T, Chen T, Yoganathan S, et al. The oral mouse microbiome promotes tumorigenesis in oral squamous cell carcinoma. *mSystem*. (2019) 4:e00323-19. doi: 10.1128/mSystems.00323-19
36. Arjunan P, Meghil MM, Pi W, Xu J, Lang L, El-Awady A, et al. Oral pathobiont activates anti-apoptotic pathway, promoting both immune suppression and oncogenic cell proliferation. *Sci Rep*. (2018) 8:16607. doi: 10.1038/s41598-018-35126-8
37. Woo BH, Kim DJ, Choi JI, Kim SJ, Park BS, Song JM, et al. Oral cancer cells sustainedly infected with *Porphyromonas gingivalis* exhibit resistance to Taxol and have higher metastatic potential. *Oncotarget*. (2017) 8:46981–92. doi: 10.18632/oncotarget.16550
38. Oballe H, Muniz F, Bueno CC, Klein IP, Carrard VC, Rösing CK, et al. Spontaneous alveolar bone loss after 4NQO exposure in wistar rats. *Arch Oral Biol*. (2018) 89:44–8. doi: 10.1016/j.archoralbio.2018.02.001
39. Pushalkar S, Ji X, Li Y, Estilo C, Yegnanarayana R, Singh B, et al. Comparison of oral microbiota in tumor and non-tumor tissues of patients with oral squamous cell carcinoma. *BMC Microbiol*. (2012) 12:144. doi: 10.1186/1471-2180-12-144
40. Sahingur SE, Yeudall WA. Chemokine function in periodontal disease and oral cavity cancer. *Front Immunol*. (2015) 6:214. doi: 10.3389/fimmu.2015.00214
41. Frederick MJ, Henderson Y, Xu X, Deavers MT, Sahin AA, Wu H, et al. *In vivo* expression of the novel CXC chemokine BRAK in normal and cancerous human tissue. *Am J Pathol*. (2000) 156:1937–50. doi: 10.1016/S0002-94401065067-5
42. Ozawa S, Kato Y, Kubota E, Hata R. BRAK/CXCL14 expression in oral carcinoma cells completely suppresses tumor cell xenografts in SCID mouse. *Biomed Res*. (2009) 30:315–8. doi: 10.2220/biomedres.30.315
43. Liang R, Lie W, Yi S, Hongyan G, Ke L. CXCL14 regulates cell proliferation, invasion, migration and epithelial-mesenchymal transition of oral squamous cell carcinoma. *Biotechnol Biotech Equip*. (2019) 33:1335–42. doi: 10.1080/13102818.2019.1664930
44. Aw J, Scholz GM, Huq NL, Huynh J, O'Brien-Simpson NM, Reynolds EC. Interplay between *Porphyromonas gingivalis* and EGF signalling in the regulation of CXCL14. *Cell Microbiol*. (2018) 20:e12837. doi: 10.1111/cmi.12837
45. Huang N, Gibson FC. *Immuno-pathogenesis of periodontal disease: current and emerging paradigms. Curr Oral Health Rep*. (2014) 1:124–32. doi: 10.1007/s40496-014-0017-8
46. Hajishengallis G, Lambris JD. Complement and dysbiosis in periodontal disease. *Immunobiology*. (2012) 217:1111–6. doi: 10.1016/j.imbio.2012.07.007
47. Sochalska M, Potempa J. Manipulation of neutrophils by porphyromonas gingivalis in the development of periodontitis. *Front Cell Infect Microbiol*. (2017) 7:197. doi: 10.3389/fcimb.2017.00197
48. Uriarte SM, Edmisson JS, Jimenez-Flores E. Human neutrophils and oral microbiota: a constant tug-of-war between a harmonious and a discordant coexistence. *Immunol Rev*. (2016) 273:282–98. doi: 10.1111/imr.12451
49. Fine N, Hassanpour S, Borenstein A, Sima C, Oveisi M, Scholey J, et al. Distinct oral neutrophil subsets define health and periodontal disease states. *J Dent Res*. (2016) 95:931–8. doi: 10.1177/0022034516645564
50. Ley K, Hoffman HM, Kubes P, Cassatella MA, Zychlinsky A, Hedrick CC, et al. Neutrophils: new insights and open questions. *Sci Immunol*. (2018) 3:eaat4579. doi: 10.1126/sciimmunol.aat4579
51. Chakravarti A, Raquil MA, Tessier P, Poubelle PE. Surface RANKL of Toll-like receptor 4-stimulated human neutrophils activates osteoclastic bone resorption. *Blood*. (2009) 114:1633–44. doi: 10.1182/blood-2008-09-178301
52. Ha H, Kwak HB, Lee SW, Jin HM, Kim HM, Kim HH, et al. Reactive oxygen species mediate RANK signaling in osteoclasts. *Exp Cell Res*. (2004) 301:119–27. doi: 10.1016/j.yexcr.2004.07.035
53. Magalhaes MA, Glogauer JE, Glogauer M. Neutrophils and oral squamous cell carcinoma: lessons learned and future directions. *J Leukoc Biol*. (2014) 96:695–702. doi: 10.1189/jlb.4RU0614-294R
54. Shen M, Hu P, Donskov F, Wang G, Liu Q, Du J. Tumor-associated neutrophils as a new prognostic factor in cancer: a systematic review and meta-analysis. *PLoS ONE*. (2014) 9:e98259. doi: 10.1371/journal.pone.0098259
55. Glogauer JE, Sun CX, Bradley G, Magalhaes MA. Neutrophils increase oral squamous cell carcinoma invasion through an invadopodia-dependent pathway. *Cancer Immunol Res*. (2015) 3:1218–26. doi: 10.1158/2326-6066.CIR-15-0017
56. Lakschevitz FS, Aboodi GM, Glogauer M. Oral neutrophil transcriptome changes result in a pro-survival phenotype in periodontal diseases. *PLoS ONE*. (2013) 8:e68983. doi: 10.1371/journal.pone.0068983

57. Lewkowicz N, Mycko MP, Przygodzka P, Cwiklińska H, Cichalewska M, Matysiak M, et al. Induction of human IL-10-producing neutrophils by LPS-stimulated Treg cells and IL-10. *Mucosal Immunol*. (2016) 9:364–78. doi: 10.1038/mi.2015.66
58. Caldeira PC, Vieira É, Sousa AA, Teixeira AL, Aguiar M. Immunophenotype of neutrophils in oral squamous cell carcinoma patients. *J Oral Pathol Med*. (2017) 46:703–9. doi: 10.1111/jop.12575
59. Fridlender ZG, Sun J, Mishalian I, Singhal S, Cheng G, Kapoor V, et al. Transcriptomic analysis comparing tumor-associated neutrophils with granulocytic myeloid-derived suppressor cells and normal neutrophils. *PLoS ONE*. (2012) 7:e31524. doi: 10.1371/journal.pone.0031524
60. Belstrøm D, Eiberg JM, Enevold C, Grande MA, Jensen C, Skov L, et al. Salivary microbiota and inflammation-related proteins in patients with psoriasis. *Oral Dis*. (2020) 26:677–87. doi: 10.1111/odi.13277
61. Monisha J, Roy NK, Padmavathi G, Banik K, Bordoloi D, Khwairakpam AD, et al. NGAL is downregulated in oral squamous cell carcinoma and leads to increased survival, proliferation, migration and chemoresistance. *Cancers*. (2018) 10:228. doi: 10.3390/cancers10070228
62. Türkoglu O, Emingil G, Kütükçüler N, Atilla G. Evaluation of gingival crevicular fluid adrenomedullin and human neutrophil peptide 1-3 levels of patients with different periodontal diseases. *J Periodontol*. (2010) 81:284–91. doi: 10.1902/jop.2009.090517
63. Trellakis S, Bruderek K, Dumitru CA, Gholaman H, Gu X, Bankfalvi A, et al. Polymorphonuclear granulocytes in human head and neck cancer: enhanced inflammatory activity, modulation by cancer cells and expansion in advanced disease. *Int J Cancer*. (2011) 129:2183–93. doi: 10.1002/ijc.25892
64. Hernández M, Martínez B, Tejerina JM, Valenzuela MA, Gamonal J. MMP-13 and TIMP-1 determinations in progressive chronic periodontitis. *J Clin Periodontol*. (2007) 34:729–35. doi: 10.1111/j.1600-051X.2007.01107.x
65. Abe T, AlSarhan M, Benakanakere MR, Maekawa T, Kinane DF, Cancro MP, et al. The B cell-stimulatory cytokines BLyS and APRIL are elevated in human periodontitis and are required for b cell-dependent bone loss in experimental murine periodontitis. *J Immunol*. (2015) 195:1427–35. doi: 10.4049/jimmunol.1500496
66. García-Castro A, Zonca M, Florindo-Pinheiro D, Carvalho-Pinto CE, Cordero A, Gutiérrez del Fernando B, et al. *APRIL promotes breast tumor growth and metastasis and is associated with aggressive basal breast cancer. Carcinogenesis*. (2015) 36:574–84. doi: 10.1093/carcin/bgv020
67. Aral CA, Ölçer SN, Aral K, Kapila Y. Oxidative stress, neutrophil elastase and IGFBP7 levels in patients with oropharyngeal cancer and chronic periodontitis. *Oral Dis*. (2020) 26:1393–401. doi: 10.1111/odi.13370
68. Yuen J, Pluthero FG, Douda DN, Riedl M, Cherry A, Ulanova M, et al. NETosing neutrophils activate complement both on their own NETs and bacteria via alternative and non-alternative pathways. *Front Immunol*. (2016) 7:137. doi: 10.3389/fimmu.2016.00137
69. Petretto A, Bruschi M, Pratesi F, Croia C, Candiano G, Ghiggeri G, et al. Neutrophil extracellular traps NET induced by different stimuli: a comparative proteomic analysis. *PLoS ONE*. (2019) 14:e0218946. doi: 10.1371/journal.pone.0218946
70. Honda M, Kubes P. Neutrophils and neutrophil extracellular traps in the liver and gastrointestinal system. *Nat Rev Gastroenterol Hepatol*. (2018) 15:206–21. doi: 10.1038/nrgastro.2017.183
71. Decker AS, Pylaeva E, Brenzel A, Spyra I, Droege F, Hussain T, et al. Prognostic role of blood NETosis in the progression of head and neck cancer. *Cells*. (2019) 8:946. doi: 10.3390/cells8090946
72. Magán-Fernández A, O'Valle F, Abadía-Molina F, Muñoz R, Puga-Guil P, Mesa F. Characterization and comparison of neutrophil extracellular traps in gingival samples of periodontitis and gingivitis: a pilot study. *J Periodont Res*. (2019) 54:218–24. doi: 10.1111/jre.12621
73. Bryzek D, Ciaston I, Dobosz E, Gasiorek A, Makarska A, Sarna M, et al. Triggering NETosis via protease-activated receptor PAR-2 signaling as a mechanism of hijacking neutrophils function for pathogen benefits. *PLoS Pathog*. (2019) 15:e1007773. doi: 10.1371/journal.ppat.1007773
74. Sima C, Glogauer M. Macrophage subsets and osteoimmunology: tuning of the immunological recognition and effector systems that maintain alveolar bone. *Periodontology*. (2013) 63:80–101. doi: 10.1111/prd.12032
75. Zhou LN, Bi CS, Gao LN, An Y, Chen F, Chen FM. Macrophage polarization in human gingival tissue in response to periodontal disease. *Oral Dis*. (2019) 25:265–73. doi: 10.1111/odi.12983
76. Baer C, Squadrito ML, Laoui D, Thompson D, Hansen SK, Kiialainen A, et al. Suppression of microRNA activity amplifies IFN-γ-induced macrophage activation and promotes anti-tumour immunity. *Nat Cell Biol*. (2016) 18:790–802. doi: 10.1038/ncb3371
77. Kouketsu A, Sato I, Oikawa M, Shimizu Y, Saito H, Tashiro K, et al. Regulatory T cells and M2-polarized tumour-associated macrophages are associated with the oncogenesis and progression of oral squamous cell carcinoma. *Int J Oral Maxillofac Surg*. (2019) 48:1279–88. doi: 10.1016/j.ijom.2019.04.004
78. Almubarak A, Tanagala K, Papapanou PN, Lalla E, Momen-Heravi F. Disruption of monocyte and macrophage homeostasis in periodontitis. *Front Immunol*. (2020) 11:330. doi: 10.3389/fimmu.2020.00330
79. Yu T, Zhao L, Huang X, Ma C, Wang Y, Zhang J, et al. Enhanced activity of the macrophage M1/M2 phenotypes and phenotypic switch to M1 in periodontal infection. *J Periodontol*. (2016) 87:1092–102. doi: 10.1902/jop.2016.160081
80. Mkonyi LE, Bakken V, Søvik JB, Mauland EK, Fristad I, Barczyk MM, et al. Lymphangiogenesis is induced during development of periodontal disease. *J Dent Res*. (2012) 91:71–7. doi: 10.1177/0022034511424747
81. Maruyama K, Ii M, Cursiefen C, Jackson DG, Keino H, Tomita M, et al. Inflammation-induced lymphangiogenesis in the cornea arises from CD11b-positive macrophages. *J Clin Invest*. (2005) 115:2363–72. doi: 10.1172/JCI23874
82. Åström P, Juurikka K, Hadler-Olsen ES, Svineng G, Cervigne NK, Coletta RD, et al. The interplay of matrix metalloproteinase-8, transforming growth factor-β1 and vascular endothelial growth factor-C cooperatively contributes to the aggressiveness of oral tongue squamous cell carcinoma. *Br J Cancer*. (2017) 117:1007–16. doi: 10.1038/bjc.2017.249
83. Wang SS, Cen X, Liang XH, Tang YL. Macrophage migration inhibitory factor: a potential driver and biomarker for head and neck squamous cell carcinoma. *Oncotarget*. (2017) 8:10650–61. doi: 10.18632/oncotarget.12890
84. Zheng YX, Yang M, Rong TT, Yuan XL, Ma YH, Wang ZH, et al. CD74 and macrophage migration inhibitory factor as therapeutic targets in gastric cancer. *World J Gastroenterol*. (2012) 18:2253–61. doi: 10.3748/wjg.v18.i18.2253
85. Ortiz-García YM, García-Iglesias T, Morales-Velazquez G, Lazalde-Ramos BP, Zúñiga-González GM, Ortiz-García RG, et al. Macrophage migration inhibitory factor levels in gingival crevicular fluid, saliva, and serum of chronic periodontitis patients. *BioMed Res Int*. (2019) 2019:7850392. doi: 10.1155/2019/7850392
86. De Sanctis F, Solito S, Ugel S, Molon B, Bronte V, Marigo I. MDSCs in cancer: Conceiving new prognostic and therapeutic targets. *Biochim Biophys Acta*. (2016) 1865:35–48. doi: 10.1016/j.bbcan.2015.08.001
87. Wen L, Mu W, Lu H, Wang X, Fang J, Jia Y, et al. *Porphyromonas gingivalis* promotes oral squamous cell carcinoma progression in an immune microenvironment. *J Dent Res*. (2020) 99:666–75. doi: 10.1177/0022034520909312
88. Cheng R, Billet S, Liu C, Haldar S, Choudhury D, Tripathi M, et al. Periodontal inflammation recruits distant metastatic breast cancer cells by increasing myeloid-derived suppressor cells. *Oncogene*. (2020) 39:1543–56. doi: 10.1038/s41388-019-1084-z
89. Wculek SK, Cueto FJ, Mujal AM, Melero I, Krummel MF, Sancho D. Dendritic cells in cancer immunology and immunotherapy. *Nat Rev Immunol*. (2020) 20:7–24. doi: 10.1038/s41577-019-0210-z
90. Veglia F, Gabrilovich DI. Dendritic cells in cancer: the role revisited. *Curr Opin Immunol*. (2017) 45:43–51. doi: 10.1016/j.coi.2017.01.002
91. Sheng KC, Wright MD, Apostolopoulos V. Inflammatory mediators hold the key to dendritic cell suppression and tumor progression. *Curr Med Chem*. (2011) 18:5507–18. doi: 10.2174/092986711798347207
92. Jotwani R, Eswaran SV, Moonga S, Cutler CW. MMP-9/TIMP-1 imbalance induced in human dendritic cells by *Porphyromonas gingivalis*. *FEMS Immunol Med Microbiol*. (2010) 58:314–21. doi: 10.1111/j.1574-695X.2009.00637.x
93. Wang BQ, Zhang CM, Gao W, Wang XF, Zhang HL, Yang PC. Cancer-derived matrix metalloproteinase-9 contributes to tumor tolerance. *J Cancer Res Clin Oncol*. (2011) 137:1525–33. doi: 10.1007/s00432-011-1010-4

94. Zeituni AE, Jotwani R, Carrion J, Cutler CW. Targeting of DC-SIGN on human dendritic cells by minor fimbriated *Porphyromonas gingivalis* strains elicits a distinct effector T cell response. *J Immunol*. (2009) 183:5694–704. doi: 10.4049/jimmunol.0901030
95. Reizis B. Plasmacytoid dendritic cells: development, regulation, and function. *Immunity*. (2019) 50:37–50. doi: 10.1016/j.immuni.2018.12.027
96. Mizraji G, Nassar M, Segev H, Sharawi H, Eli-Berchoer L, Capucha T, et al. *Porphyromonas gingivalis* promotes unrestrained type I interferon production by dysregulating TAM signaling via MYD88 degradation. *Cell Rep*. (2017) 18:419–31. doi: 10.1016/j.celrep.2016.12.047
97. Han N, Zhang Z, Liu S, Ow A, Ruan M, Yang W, et al. Increased tumor-infiltrating plasmacytoid dendritic cells predicts poor prognosis in oral squamous cell carcinoma. *Arch Oral Biol*. (2017) 78:129–34. doi: 10.1016/j.archoralbio.2017.02.012
98. Khazaie K, Blatner NR, Khan MW, Gounari F, Gounaris E, Dennis K, et al. The significant role of mast cells in cancer. *Cancer Metastasis Rev*. (2011) 30:45–60. doi: 10.1007/s10555-011-9286-z
99. Batista AC, Rodini CO, Lara VS. Quantification of mast cells in different stages of human periodontal disease. *Oral Dis*. (2005) 11:249–54. doi: 10.1111/j.1601-0825.2005.01113.x
100. Krystel-Whittemore M, Dileepan KN, Wood JG. Mast cell: a multi-functional master cell. *Front Immunol*. (2016) 6:620. doi: 10.3389/fimmu.2015.00620
101. Gaje PN, Amalia Ceausu R, Jitariu A, Stratul SI, Rusu LC, Popovici RA, et al. Mast cells: key players in the shadow in oral inflammation and in squamous cell carcinoma of the oral cavity. *BioMed Res Int*. (2016) 2016:9235080. doi: 10.1155/2016/9235080
102. Sheethal HS, Kn H, Smitha T, Chauhan K. Role of mast cells in inflammatory and reactive pathologies of pulp, periapical area and periodontium. *J Oral Maxillofac Pathol*. (2018) 22:92–7. doi: 10.4103/jomfp.JOMFP_278_17
103. Yadav A, Desai RS, Bhuta BA, Singh JS, Mehta R, Nehete AP. Altered immunohistochemical expression of mast cell tryptase and chymase in the pathogenesis of oral submucous fibrosis and malignant transformation of the overlying epithelium. *PLoS ONE*. (2014) 9:e98719. doi: 10.1371/journal.pone.0098719
104. Li L, Dang Q, Xie H, Yang Z, He D, Liang L, et al. Infiltrating mast cells enhance prostate cancer invasion via altering LncRNA-HOTAIR/PRC2-androgen receptor AR-MMP9 signals and increased stem/progenitor cell population. *Oncotarget*. (2015) 6:14179–90. doi: 10.18632/oncotarget.3651
105. Tang YC, Li J, Huang SG. Tryptase and TIM-1 double-positive mast cells in different stages of human chronic periodontitis. *Int J Clin Exp Pathol*. (2018) 11:462–71.
106. Liu T, Han C, Wang S, Fang P, Ma Z, Xu L, et al. Cancer-associated fibroblasts: an emerging target of anti-cancer immunotherapy. *J Hematol Oncol*. (2019) 12:86. doi: 10.1186/s13045-019-0770-1
107. Pereira N, Pinheiro TN. Histomorphometric comparative analysis between oral dysplastic potentially malignant disorders and oral squamous cell carcinoma. *Eur J Dent*. (2019) 13:1–4. doi: 10.1055/s-0039-1688734
108. Lee W, Aitken S, Sodek J, McCulloch CA. Evidence of a direct relationship between neutrophil collagenase activity and periodontal tissue destruction in vivo: role of active enzyme in human periodontitis. *J Periodont Res*. (1995) 30:23–33. doi: 10.1111/j.1600-0765.1995.tb01249.x
109. Alvarez C, Rojas C, Rojas L, Cafferata EA, Monasterio G, Vernal R. Regulatory T lymphocytes in periodontitis: a translational view. *Med Inflamm*. (2018) 2018:7806912. doi: 10.1155/2018/7806912
110. Ohue Y, Nishikawa H. Regulatory T treg cells in cancer: can Treg cells be a new therapeutic target? *Cancer Sci*. (2019) 110:2080–9. doi: 10.1111/cas.14069
111. Hosokawa Y, Hosokawa I, Shindo S, Ozaki K, Matsuo T. IL-22 enhances CCL20 production in IL-1β-stimulated human gingival fibroblasts. *Inflammation*. (2014) 37:2062–6. doi: 10.1007/s10753-014-9939-5
112. Hosokawa Y, Shindo S, Hosokawa I, Ozaki K, Matsuo T. IL-6 trans-signaling enhances CCL20 production from IL-1β-stimulated human periodontal ligament cells. *Inflammation*. (2014) 37:381–6. doi: 10.1007/s10753-013-9750-8
113. Lee JJ, Kao KC, Chiu YL, Jung CJ, Liu CJ, Cheng SJ, et al. Enrichment of human CCR6+ regulatory T cells with superior suppressive activity in oral cancer. *J Immunol*. (2017) 199:467–76. doi: 10.4049/jimmunol.1601815
114. Francisconi CF, Vieira AE, Azevedo M, Tabanez AP, Fonseca AC, Trombone A, et al. RANKL triggers treg-mediated immunoregulation in inflammatory osteolysis. *J Dent Res*. (2018) 97:917–27. doi: 10.1177/0022034518759302
115. Mogi M, Otogoto J, Ota N, Togari A. Differential expression of RANKL and osteoprotegerin in gingival crevicular fluid of patients with periodontitis. *J Dent Res*. (2004) 83:166–9. doi: 10.1177/154405910408300216
116. Teodorescu AC, Martu I, Teslaru S, Kappenberg-Nitescu DC, Goriuc A, Luchian I, et al. Assessment of salivary levels of RANKL and OPG in aggressive versus chronic periodontitis. *J Immunol Res*. (2019) 2019:6195258. doi: 10.1155/2019/6195258
117. Tan W, Zhang W, Strasner A, Grivennikov S, Cheng JQ, Hoffman RM, et al. Tumour-infiltrating regulatory T cells stimulate mammary cancer metastasis through RANKL-RANK signalling. *Nature*. (2011) 470:548–53. doi: 10.1038/nature09707
118. Guillerey C, Huntington ND, Smyth MJ. Targeting natural killer cells in cancer immunotherapy. *Nat Immunol*. (2016) 17:1025–36. doi: 10.1038/ni.3518
119. Chiossone L, Dumas PY, Vienne M, Vivier E. Natural killer cells and other innate lymphoid cells in cancer. *Nat Rev Immunol*. (2018) 18:671–88. doi: 10.1038/s41577-018-0061-z
120. Parisi L, Bassani B, Tremolati M, Gini E, Farronato G, Bruno A. Natural killer cells in the orchestration of chronic inflammatory diseases. *J Immunol Res*. (2017) 2017:4218254. doi: 10.1155/2017/4218254
121. Gao L, Zhao Y, Wang P, Zhang L, Zhang C, Chen Q, et al. Detection of Th17/Treg cells and related factors in gingival tissues and peripheral blood of rats with experimental periodontitis. *Iran J Basic Med Sci*. (2017) 20:294–300. doi: 10.22038/ijbms.2017.8359
122. Mitani A, Niedbala W, Fujimura T, Mogi M, Miyamae S, Higuchi N, et al. Increased expression of interleukin IL-35 and IL-17, but not IL-27, in gingival tissues with chronic periodontitis. *J Periodontol*. (2015) 86:301–9. doi: 10.1902/jop.2014.140293
123. Dutta A, Banerjee A, Saikia N, Phookan J, Baruah MN, Baruah S. Negative regulation of natural killer cell in tumor tissue and peripheral blood of oral squamous cell carcinoma. *Cytokine*. (2015) 76:123–30. doi: 10.1016/j.cyto.2015.09.006
124. Lazarova M, Steinle A. The NKG2D axis: an emerging target in cancer immunotherapy. *Expert Opin Ther Targets*. (2019) 23:281–94. doi: 10.1080/14728222.2019.1580693
125. Lazarova M, Steinle A. Impairment of NKG2D-mediated tumor immunity by TGF-β. *Front. Immunol*. (2019) 10:2689. doi: 10.3389/fimmu.2019.02689
126. Ohshima T, Inoue H, Uchihashi K, Hirano SI, Nishikawa Y Effect of IL¬-12 family cytokines on NK92 cells. *J Osaka Dental Univ*. (2017) 51:115–23. doi: 10.18905/jodu.51.2_115
127. Adachi T, Inoue H, Uchihashi K, Fujimoto T, Nishikawa Y. Inhibitory mechanisms of NK92 cell cytotoxicity by IL-¬17 stimulation. *J Osaka Dental Univ*. (2017) 51:105–13. doi: 10.18905/jodu.51.2_105
128. Gur C, Maalouf N, Shhadeh A, Berhani O, Singer BB, Bachrach G, et al. Fusobacterium nucleatum supresses anti-tumor immunity by activating CEACAM1. *Oncoimmunology*. (2019) 8:e1581531. doi: 10.1080/2162402X.2019.1581531
129. Andersen MH, Schrama D, Thor Straten P, Becker JC. Cytotoxic T cells. *J Invest Dermatol*. (2006) 126:32–41. doi: 10.1038/sj.jid.5700001
130. Dar AA, Patil RS, Pradhan TN, Chaukar DA, D'Cruz AK, Chiplunkar SV. Myeloid-derived suppressor cells impede T cell functionality and promote Th17 differentiation in oral squamous cell carcinoma. *Cancer Immunol Immunother*. (2020) 69:1071–86. doi: 10.1007/s00262-020-02523-w
131. Zhu D, Liu F, Dai F, Luo X, Hong B. Expression of programmed death-1 and programmed death ligand-1 in the peripheral T-lymphocytes from patients with chronic periodontitis. *Chin J Stomatol*. (2014) 49:216–9.
132. Yu J, Lin Y, Xiong X, Li K, Yao Z, Dong H, et al. Detection of exosomal PD-L1 RNA in saliva of patients with periodontitis. *Front Genet*. (2019) 10:202. doi: 10.3389/fgene.2019.00202
133. Malaspina TS, Gasparoto TH, Costa MR, de Melo EF Jr, Ikoma MR, Damante JH, et al. Enhanced programmed death 1 PD-1 and PD-1 ligand PD-L1 expression in patients with actinic cheilitis and oral squamous cell carcinoma. *Cancer Immunol Immunother*. (2011) 60:965–74. doi: 10.1007/s00262-011-1007-5

134. Chen XJ, Tan YQ, Zhang N, He MJ, Zhou G. Expression of programmed cell death-ligand 1 in oral squamous cell carcinoma and oral leukoplakia is associated with disease progress and CD8+ tumor-infiltrating lymphocytes. *Pathol Res Pract*. (2019) 215:152418. doi: 10.1016/j.prp.2019.04.010
135. Figueredo CM, Lira-Junior R, Love RM. T and B cells in periodontal disease: new functions in A complex scenario. *Int J Mol Sci*. (2019) 20:3949. doi: 10.3390/ijms20163949
136. Shi T, Jin Y, Miao Y, Wang Y, Zhou Y, Lin X. IL-10 secreting B cells regulate periodontal immune response during periodontitis. *Odontology*. (2020) 108:350–7. doi: 10.1007/s10266-019-00470-2
137. Wang RX, Yu CR, Dambuza IM, Mahdi RM, Dolinska MB, Sergeev YV, et al. Interleukin-35 induces regulatory B cells that suppress autoimmune disease. *Nat Med*. (2014) 20:633–41. doi: 10.1038/nm.3554
138. Lao XM, Liang YJ, Su YX, Zhang SE, Zhou XI, Liao GQ. Distribution and significance of interstitial fibrosis and stroma-infiltrating B cells in tongue squamous cell carcinoma. *Oncol Lett*. (2016) 11:2027–34. doi: 10.3892/ol.2016.4184
139. Mahanonda R, Champaiboon C, Subbalekha K, Sa-Ard-Iam N, Rattanathammatada W, Thawanaphong S, et al. Human memory B cells in healthy gingiva, gingivitis, and periodontitis. *J Immunol*. (2016) 197:715–25. doi: 10.4049/jimmunol.1600540
140. Taghavi N, Mohsenifar Z, Baghban AA, Arjomandkhah A. CD20+ Tumor infiltrating B lymphocyte in oral squamous cell carcinoma: correlation with clinicopathologic characteristics and heat shock protein 70 expression. *Pathol Res Int*. (2018) 2018:4810751. doi: 10.1155/2018/4810751
141. Demoersman J, Pochard P, Framery C, Simon Q, Boisramé S, Soueidan A, et al. (2018). B cell subset distribution is altered in patients with severe periodontitis. *PLoS ONE*. 13:e0192986. doi: 10.1371/journal.pone.0192986
142. Olivieri CV, Raybaud H, Tonoyan L, Abid S, Marsault R, Chevalier M, et al. Epstein-Barr virus-infected plasma cells in periodontitis lesions. *Microb Pathog*. (2020) 143:104128. doi: 10.1016/j.micpath.2020.104128
143. Guidry JT, Birdwell CE, Scott RS. Epstein-barr virus in the pathogenesis of oral cancers. *Oral Dis*. (2018) 24:497–508. doi: 10.1111/odi.12656
144. Mims CA, Nash A, Stephen J. Chapter 6 - the immune response to infection. In: *Mims' Pathogenesis of Infectious Disease*, *6th Edn*. Boston, MA: Academic Press (2015). p. 119–44.
145. Hirahara K, Nakayama T. CD4+ T-cell subsets in inflammatory diseases: beyond the Th1/Th2 paradigm. *Int Immunol*. (2016) 28:163–71. doi: 10.1093/intimm/dxw006
146. Arun KV, Talwar A, Kumar TS. T-helper cells in the etiopathogenesis of periodontal disease: a mini review. *J Indian Soc Periodontol*. (2011) 15:4–10. doi: 10.4103/0972-124X.82255
147. Qi W, Yang X, Ye N, Li S, Han Q, Huang J, et al. TLR4 gene in the regulation of periodontitis and its molecular mechanism. *Exp Ther Med*. (2019) 18:1961–6. doi: 10.3892/etm.2019.7809
148. Zhou M, Ouyang W. The function role of GATA-3 in Th1 and Th2 differentiation. *Immunol Res*. (2003) 28:25–37. doi: 10.1385/IR:28:1:25
149. Rodrigues MF, de Oliveira Rodini C, de Aquino Xavier FC, Paiva KB, Severino P, Moyses RA, et al. PROX1 gene is differentially expressed in oral cancer and reduces cellular proliferation. *Medicine*. (2014) 93:e192. doi: 10.1097/MD.0000000000000192
150. Gaur P, Singh AK, Shukla NK, Das SN. Inter-relation of Th1, Th2, Th17 and Treg cytokines in oral cancer patients and their clinical significance. *Hum Immunol*. (2014) 75:330–7. doi: 10.1016/j.humimm.2014.01.011
151. Biancardi MR, Fernandes D, Barbeiro OC, Da Silveira HA, Arroyo EA, Bufalino A. Elevated level of Il-2, Il-5 and tnf-α are associated with proliferative verrucous leukoplakia. *Oral Surg Oral Med Oral Pathol Oral Radiol*. (2020) 129:e180. doi: 10.1016/j.oooo.2019.06.765
152. Meehan K, Leslie C, Lucas M, Jacques A, Mirzai B, Lim J, et al. Characterization of the immune profile of oral tongue squamous cell carcinomas with advancing disease. *Cancer Med*. (2020) 9:4791–807. doi: 10.1002/cam4.3106
153. Araujo-Pires AC, Vieira AE, Francisconi CF, Biguetti CC, Glowacki A, Yoshizawa S, et al. IL-4/CCL22/CCR4 axis controls regulatory T-cell migration that suppresses inflammatory bone loss in murine experimental periodontitis. *J Bone Miner Res*. (2015) 30:412–22. doi: 10.1002/jbmr.2376
154. Cheng WC, Hughes FJ, Taams LS. The presence, function and regulation of IL-17 and Th17 cells in periodontitis. *J Clin Periodontol*. (2014) 41:541–9. doi: 10.1111/jcpe.12238
155. Chen XT, Chen LL, Tan JY, Shi DH, Ke T, Lei LH. Th17 and Th1 lymphocytes are correlated with chronic periodontitis. *Immunol Invest*. (2016) 45:243–54. doi: 10.3109/08820139.2016.1138967
156. Awang RA, Lappin DF, MacPherson A, Riggio M, Robertson D, Hodge P, et al. Clinical associations between IL-17 family cytokines and periodontitis and potential differential roles for IL-17A and IL-17E in periodontal immunity. *Inflamm Res*. (2014) 63:1001–12. doi: 10.1007/s00011-014-0776-7
157. Lee MH, Tung-Chieh Chang J, Liao CT, Chen YS, Kuo ML, Shen CR. Interleukin 17 and peripheral IL-17-expressing T cells are negatively correlated with the overall survival of head and neck cancer patients. *Oncotarget*. (2018) 9:9825–37. doi: 10.18632/oncotarget.23934
158. Groeger SE, Meyle J. Epithelial barrier and oral bacterial infection. *Periodontol 2000*. (2015) 69:46–67. doi: 10.1111/prd.12094
159. Wang SS, Tang YL, Pang X, Zheng M, Tang YJ, Liang XH. The maintenance of an oral epithelial barrier. *Life Sci*. (2019) 227:129–36. doi: 10.1016/j.lfs.2019.04.029
160. Abe-Yutori M, Chikazawa T, Shibasaki K, Murakami S. Decreased expression of E-cadherin by *Porphyromonas gingivalis*-lipopolysaccharide attenuates epithelial barrier function. *J Periodont Res*. (2017) 52:42–50. doi: 10.1111/jre.12367
161. de Freitas Silva BS, Yamamoto-Silva FP, Pontes HA, Pinto Júnior D. E-cadherin downregulation and twist overexpression since early stages of oral carcinogenesis. *J Oral Pathol Med*. (2014) 43:125–31. doi: 10.1111/jop.12096
162. Abdulkareem AA, Shelton RM, Landini G, Cooper PR, Milward MR. Potential role of periodontal pathogens in compromising epithelial barrier function by inducing epithelial-mesenchymal transition. *J Periodont Res*. (2018) 53:565–74. doi: 10.1111/jre.12546
163. Abdulkareem AA, Shelton RM, Landini G, Cooper PR, Milward MR. Periodontal pathogens promote epithelial-mesenchymal transition in oral squamous carcinoma cells *in vitro*. *Cell Adh Migr*. (2018) 12:127–37. doi: 10.1080/19336918.2017.1322253
164. Emingil G, Kuula H, Pirilä E, Atilla G, Sorsa T. Gingival crevicular fluid laminin-5 gamma2-chain levels in periodontal disease. *J Clin Periodontol*. (2006) 33:462–8. doi: 10.1111/j.1600-051X.2006.00933.x
165. Gersdorff N, Miró X, Roediger M, Geffers R, Toepfer T, Huels A, et al. *Gene expression analysis of chronically inflamed and healthy human periodontal ligament cells in vivo*. *Dental Res J*. (2009) 5:5–11.
166. Kagesato Y, Mizushima H, Koshikawa N, Kitamura H, Hayashi H, Ogawa N, et al. Sole expression of laminin gamma 2 chain in invading tumor cells and its association with stromal fibrosis in lung adenocarcinomas. *Jpn J Cancer Res*. (2001) 92:184–92. doi: 10.1111/j.1349-7006.2001.tb01081.x
167. Tanis T, Cincin ZB, Gokcen-Rohlig B, Bireller ES, Ulusan M, Tanyel CR, et al. The role of components of the extracellular matrix and inflammation on oral squamous cell carcinoma metastasis. *Arch Oral Biol*. (2014) 59:1155–63. doi: 10.1016/j.archoralbio.2014.07.005
168. Ortiz A, Ramos JM, Garcia S, Andriankaja OM, Pérez CM. Is human papilloma virus infection linked to periodontitis? A narrative review. *Curr Oral Health Rep*. (2019) 6:22–30. doi: 10.1007/s40496-019-0206-6.
169. Bansal A, Singh MP, Rai B. Human papillomavirus-associated cancers: A growing global problem. *Int J Appl Basic Med Res*. (2016) 6:84–9. doi: 10.4103/2229-516X.179027
170. Tezal M, Sullivan MA, Reid ME, Marshall JR, Hyland A, Loree T, et al. Chronic periodontitis and the risk of tongue cancer. *Arch Otolaryngol Head Neck Surg*. (2007) 133:450–4. doi: 10.1001/archotol.133.5.450
171. Chen B, Wu W, Sun W, Zhang Q, Yan F, Xiao Y. RANKL expression in periodontal disease: where does RANKL come from? *BioMed Res Int*. (2014) 2014:731039. doi: 10.1155/2014/731039
172. López Roldán A, García Giménez JL, Alpiste Illueca F. Impact of periodontal treatment on the RANKL/OPG ratio in crevicular fluid. *PLoS ONE*. (2020) 15:e0227757. doi: 10.1371/journal.pone.0227757
173. Sambandam Y, Ethiraj P, Hathaway-Schrader JD, Novince CM, Panneerselvam E, Sundaram K, et al. Autoregulation of RANK ligand in oral squamous cell carcinoma tumor cells. *J Cell Physiol*. (2018) 233:6125–34. doi: 10.1002/jcp.26456

174. Batool H, Nadeem A, Kashif M, Shahzad F, Tahir R, Afzal N. Salivary levels of IL-6 and IL-17 could be an indicator of disease severity in patients with calculus associated chronic periodontitis. *BioMed Res Int*. (2018) 2018:8531961. doi: 10.1155/2018/8531961
175. Dineshkumar T, Ashwini BK, Rameshkumar A, Rajashree P, Ramya R, Rajkumar K. Salivary and serum interleukin-6 levels in oral premalignant disorders and squamous cell carcinoma: diagnostic value and clinicopathologic correlations. *Asian Pac J Cancer Prev/*. (2016) 17:4899–906. doi: 10.22034/APJCP.2016.17.11.4899
176. Matsubara S, Nomura T, Takano N, Shibahara T. Expression of osteoclast-related cytokines in mandibular invasion by gingival squamous cell carcinoma. *Oral Sci Int*. (2013) 10:20–4. 10.1016/S1348-86431200047-X.
177. Dong M, Yu X, Chen W, Guo Z, Sui L, Xu Y, et al. Osteopontin Promotes bone destruction in periapical periodontitis by activating the NF-κB pathway. *Cell Physiol Biochem*. (2018) 49:884–98. doi: 10.1159/000493219
178. Teixeira LN, de Castro Raucci LM, Alonso GC, Coletta RD, Rosa AL, de Oliveira PT. Osteopontin expression in co-cultures of human squamous cell carcinoma-derived cells and osteoblastic cells and its effects on the neoplastic cell phenotype and osteoclastic activation. *Tumour Biol*. (2016) 37:12371–85. doi: 10.1007/s13277-016-5104-0
179. Mogi M, Otogoto J. Expression of cathepsin-K in gingival crevicular fluid of patients with periodontitis. *Arch Oral Biol*. (2007) 52:894–8. doi: 10.1016/j.archoralbio.2007.01.006
180. Hao L, Chen J, Zhu Z, Reddy MS, Mountz JD, Chen W, et al. Odanacatib, A cathepsin K-specific inhibitor, inhibits inflammation and bone loss caused by periodontal diseases. *J Periodontol*. (2015) 86:972–83. doi: 10.1902/jop.2015.140643
181. Leusink FK, Koudounarakis E, Frank MH, Koole R, van Diest PJ, Willems SM. Cathepsin K associates with lymph node metastasis and poor prognosis in oral squamous cell carcinoma. *BMC Cancer*. (2018) 18:385. doi: 10.1186/s12885-018-4315-8
182. Lee IK, Lee MJ, Jang HS. The interrelationship between human gingival fibroblast differentiation and cultivating time. *Tissue Eng Regenerat Med*. (2013) 10:60–4. doi: 10.1007/s13770-013-0371-y
183. Belibasakis GN, Bostanci N, Hashim A, Johansson A, Aduse-Opoku J, Curtis MA, et al. Regulation of RANKL and OPG gene expression in human gingival fibroblasts and periodontal ligament cells by *Porphyromonas gingivalis*: a putative role of the Arg-gingipains. *Microb Pathog*. (2007) 43:46–53. doi: 10.1016/j.micpath.2007.03.001
184. Elmusrati AA, Pilborough AE, Khurram SA, Lambert DW. Cancer-associated fibroblasts promote bone invasion in oral squamous cell carcinoma. *Br J Cancer*. (2017) 117:867–75. doi: 10.1038/bjc.2017.239
185. Herath T, Darveau RP, Seneviratne CJ, Wang CY, Wang Y, Jin L. Heterogeneous *Porphyromonas gingivalis* LPS modulates immuno-inflammatory response, antioxidant defense and cytoskeletal dynamics in human gingival fibroblasts. *Sci Rep*. (2016) 6:29829. doi: 10.1038/srep29829
186. Wu MH, Hong HC, Hong TM, Chiang WF, Jin YT, Chen YL. Targeting galectin-1 in carcinoma-associated fibroblasts inhibits oral squamous cell carcinoma metastasis by downregulating MCP-1/CCL2 expression. *Clin Cancer Res*. (2011) 17:1306–16. doi: 10.1158/1078-0432.CCR-10-1824
187. Ruest LB, Ranjbaran H, Tong EJ, Svoboda KK, Feng JQ. Activation of receptor activator of nuclear factor-κB ligand and matrix metalloproteinase production in periodontal fibroblasts by endothelin signaling. *J Periodontol*. (2016) 87:e1–8. doi: 10.1902/jop.2015.150397
188. Hinsley EE, Kumar S, Hunter KD, Whawell SA, Lambert DW. Endothelin-1 stimulates oral fibroblasts to promote oral cancer invasion. *Life Sci*. (2012) 91:557–61. doi: 10.1016/j.lfs.2012.04.001
189. Park KH, Kim DK, Huh YH, Lee G, Lee SH, Hong Y, et al. NAMPT enzyme activity regulates catabolic gene expression in gingival fibroblasts during periodontitis. *Exp Mol Med*. (2017) 49:e368. doi: 10.1038/emm.2017.116
190. Lin, LW, Lu, YC, Wang CP, Hsu CC, Lu LF, Hsin MC, et al. Visfatin/pre-B cell colony-enhancing factor immunohistochemical overexpression in oral cancers. *J Appl Biomed*. (2014) 12:255–62. doi: 10.1016/j.jab.2014.08.003
191. Wei W, An Y, An Y, Fei D, Wang Q. Activation of autophagy in periodontal ligament mesenchymal stem cells promotes angiogenesis in periodontitis. *J Periodontol*. (2018) 89:718–27. doi: 10.1002/JPER.17-0341
192. Lira-Junior R, Figueredo CM, Bouskela E, Fischer RG. Severe chronic periodontitis is associated with endothelial and microvascular dysfunctions: a pilot study. *J Periodontol*. (2014) 85:1648–57. doi: 10.1902/jop.2014.140189
193. Maekawa T, Tabeta K, Kajita-Okui K, Nakajima T, Yamazaki K. Increased expression of C-reactive protein gene in inflamed gingival tissues could be derived from endothelial cells stimulated with interleukin-6. *Arch Oral Biol*. (2011) 56:1312–8. doi: 10.1016/j.archoralbio.2011.04.010
194. van Kruijsdijk RC, van der Graaf Y, Peeters PH, Visseren FL, Second Manifestations of ARTerial disease (SMART) study group. *Cancer risk in patients with manifest vascular disease: effects of smoking, obesity, and metabolic syndrome. Cancer Epidemiol Biomarkers Prev*. (2013) 22:1267–77. doi: 10.1158/1055-9965.EPI-13-0090
195. Acharya S, Kale J, Hallikeri K, Anehosur V, Arnold D. Clinical significance of preoperative serum C-reactive protein in oral squamous cell carcinoma. *Int J Oral Maxillofac Surg*. (2018) 47:16–23. doi: 10.1016/j.ijom.2017.06.017
196. Wang Q, Song X, Zhao Y, He Q, Shi M, Xu P, et al. Preoperative high c-reactive protein/albumin ratio is a poor prognostic factor of oral squamous cell carcinoma. *Fut Oncol*. (2019) 15:2277–86. doi: 10.2217/fon-2019-0063
197. Ng KT, Li JP, Ng KM, Tipoe GL, Leung WK, Fung ML. Expression of hypoxia-inducible factor-1α in human periodontal tissue. *J Periodontol*. (2011) 82:136–41. doi: 10.1902/jop.2010.100100
198. Indrapriyadharshini K, Sekar B, Saranyan R, Nirmal RM, Murugesan A, Yoithaprabhunath TR. Role of vascular endothelial growth factor VEGF in tumor progression among oral epithelial dysplasia OEDs, verrucous carcinoma VC and oral squamous cell carcinoma OSCC - an immunohistochemical *study*. *Int J Adv Sci Tech*. (2019) 28:720–8.
199. Ryu MH, Park HM, Chung J, Lee CH, Park HR. Hypoxia-inducible factor-1alpha mediates oral squamous cell carcinoma invasion via upregulation of alpha5 integrin and fibronectin. *Biochem Biophys Res Commun*. (2010) 393:11–5. doi: 10.1016/j.bbrc.2010.01.060

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oral Health

ORIGINAL RESEARCH
published: 11 February 2021
doi: 10.3389/froh.2021.604565

Increased Abundance of Tumour-Associated Neutrophils in HPV-Negative Compared to HPV-Positive Oropharyngeal Squamous Cell Carcinoma Is Mediated by IL-1R Signalling

*Sarmad Al-Sahaf[1], Naeima B. Hendawi[1], Bethany Ollington[1], Robert Bolt[1], Penelope D. Ottewell[2], Keith D. Hunter[1] and Craig Murdoch[1]**

[1] School of Clinical Dentistry, University of Sheffield, Sheffield, United Kingdom, [2] Department of Oncology and Metabolism, University of Sheffield, Sheffield, United Kingdom

OPEN ACCESS

Edited by:
Carolina Cavalieri Gomes, Federal University of Minas Gerais, Brazil

Reviewed by:
Tarcilia A. Silva, Federal University of Minas Gerais, Brazil
Steffen Wagner, University of Giessen, Germany
Andre Luiz Sena Guimaraes, Unimontes, Brazil

***Correspondence:**
Craig Murdoch
c.murdoch@sheffield.ac.uk

Specialty section:
This article was submitted to Oral Cancers, a section of the journal Frontiers in Oral Health

Received: 09 September 2020
Accepted: 20 January 2021
Published: 11 February 2021

Citation:
Al-Sahaf S, Hendawi NB, Ollington B, Bolt R, Ottewell PD, Hunter KD and Murdoch C (2021) Increased Abundance of Tumour-Associated Neutrophils in HPV-Negative Compared to HPV-Positive Oropharyngeal Squamous Cell Carcinoma Is Mediated by IL-1R Signalling. Front. Oral. Health 2:604565. doi: 10.3389/froh.2021.604565

The incidence of human papillomavirus (HPV)-associated cancer is increasing and HPV is now implicated in the aetiology of more than 60% of all oropharyngeal squamous cell carcinomas (OPSCC). In OPSCC, innate immune cells such as neutrophils and macrophages generally correlate with poor prognosis, whilst adaptive immune cells, such as lymphocytes, tend to correlate with improved prognosis. This may, in part, be due to differences in the immune response within the tumour microenvironment leading to the recruitment of specific tumour-associated leukocyte sub-populations. In this study, we aimed to examine if differences exist in the levels of infiltrated leukocyte sub-populations, with particular emphasis on tumour-associated neutrophils (TAN), and to determine the mechanism of chemokine-induced leukocyte recruitment in HPV-positive compared to HPV-negative OPSCC. Immunohistochemical analysis showed that HPV-negative OPSCC contained significantly more neutrophils than HPV-positive tumours, whilst levels of CD68+ macrophages and CD3+ lymphocytes were similar. Using a 3D tissue culture model to represent tumour-stromal interactions, we demonstrated that HPV-negative tumour-stromal co-cultures expressed significantly higher levels of CXCL8, leading to increased neutrophil recruitment compared to their HPV-positive counterparts. HPV-negative OPSCC cells have previously been shown to express higher levels of IL-1 than their HPV-positive counterparts, indicating that this cytokine may be responsible for driving increased chemokine production in the HPV-negative 3D model. Inhibition of IL-1R in the tumour-stromal models using the receptor-specific antagonist, anakinra, dramatically reduced chemokine secretion and significantly impaired neutrophil and monocyte recruitment, suggesting that this tumour-stromal response is mediated by the IL-1/IL-1R axis. Here, we identify a mechanism by which HPV-negative OPSCC may recruit more TAN than HPV-positive OPSCC. Since TAN are associated with

poor prognosis in OPSCC, our study identifies potential therapeutic targets aimed at redressing the chemokine imbalance to reduce innate immune cell infiltration with the aim of improving patient outcome.

Keywords: oropharyngeal cancer, human papillomavirus, leukocytes, fibroblasts, chemokine, IL-1, neutrophils

INTRODUCTION

The worldwide increasing incidence of human papillomavirus (HPV)-driven oropharyngeal squamous cell cancer (OPSCC) has raised the profile of these tumours and intensified interest in this area of cancer research [1]. Of particular interest is the finding that HPV-positive OPSCC generally display improved prognosis and response to therapy than their HPV-negative counterparts, whose aetiology is generally linked to DNA damage by classical risk factors such as tobacco use and alcohol consumption rather than viral infection [2]. In addition to aetiology, the tumour microenvironment also has a profound influence on tumour progression. The tumour microenvironment consists of dynamic molecular interactions between cancer cells, fibroblasts, the extracellular matrix and immune cell populations, where pro-tumour factors outweigh those intended to inhibit disease.

In many malignant tumours, a pro-inflammatory tumour microenvironment drives the recruitment of tumour-infiltrating leukocytes that in turn have a major effect on tumour progression [3]. The presence of increased tumour-associated neutrophils (TAN) and tumour-associated macrophages (TAM) have been correlated with poor clinical outcome in many tumours, whilst on many occasions tumour-infiltrating lymphocytes (TIL) have been associated with improved prognosis [4]. There is good evidence to suggest that HPV-positive OPSCC contain abundant CD8+ T-cells that can recognise tumour cell-expressed HPV antigens, enabling activation of adaptive immune responses to eliminate cancer cells and impart improved outcome [5–7]. Although increased levels of TAM and TAN correlate with poor prognosis in oral squamous cell carcinoma (OSCC) [8, 9], the levels of TAM in HPV-positive/negative OPSCC are less well-characterised and to date there are no published studies correlating abundance of TAN in OPSCC with HPV status.

It has been known for many years that the increased gene expression and subsequent secretion of chemokines by cells at infected or inflammatory sites is mediated by pro-inflammatory cytokines, such as IL-1, that are present within the local environment [10]. There are 47 different human chemokines each with specific or overlapping affinities for different leukocyte populations [11]. Leukocytes are recruited to inflammatory sites and tumours from the circulation via chemotactic gradients of chemokines. Although OPSCC cells are known to over-express a number of chemokines, many now believe that the pro-inflammatory paracrine signalling between tumour cells and the surrounding tumour-associated fibroblasts is the driving force behind elevated chemokine expression in the tumour microenvironment [12]. The specific type and proportions of leukocytes recruited to tumours are directly related to the profile of chemokines released. We have recently demonstrated that HPV-negative OPSCC cells stimulate tonsillar fibroblasts to secrete a distinct chemokine-rich profile compared to HPV-positive OPSCC cells. Moreover, this effect is mediated by the actions of tumour cell-secreted IL-1 on the IL-1R expressed by tonsillar fibroblasts [13]. It is therefore plausible that the chemotactic cues driven by the tumour microenvironment in HPV-negative OPSCC is different from those in HPV-positive OPSCC and this may directly account for any differences observed in tumour-associated leukocyte sub-populations. Here we examine the abundance of leukocyte populations in HPV-positive and HPV-negative OPSCC and show for the first time that TAN are preferentially recruited to HPV-negative OPSCC. Moreover, using a 3D culture system consisting of HPV-negative or HPV-positive OPSCC spheroids embedded in a tonsillar fibroblast-populated stromal matrix to mimic the tumour microenvironment, we show that both chemokine secretion and neutrophil recruitment are dependent on IL-1β/IL-1R paracrine signalling. Our data point to possible intervention strategies to inhibit TAN recruitment to tumours that may be beneficial for patient prognosis.

MATERIALS AND METHODS

Human Samples

This study utilised patient samples and associated clinical data within the period of primary diagnosis 2002–2012. Patients were selected using the Chemocare database system (National Health Service, UK) and further cross-referenced against the histopathological database held at Sheffield Teaching Hospitals NHS Trust, UK in order to confirm diagnosis and to identify each tissue biopsy reference number. Patients were included in the study if there was sufficient tissue remaining after clinical histopathological diagnosis for TMA generation and if there was a reasonably complete dataset of associated clinicopathological information. All biopsy material was obtained before commencement of anti-cancer treatment. Paraffin-wax embedded tissue samples were retrieved and collated in a blinded fashion with respect to HPV status. HPV positivity using RNAScope® was determined as described in Hendawi et al. [14], whilst expression of p16 staining was assessed using the H-score system with a 300-point cut-off for positivity [14, 15]. Overall HPV status in the samples was found to be proportionate to the UK prevalence of HPV OPSCC, reflecting that a representative population sample was used in this study. Clinical data regarding tumour recurrence, clinical status and follow-up were retrieved from the patient's medical files at the Sheffield Teaching Hospital NHS Foundation Trust, UK. Overall survival was determined by the difference between the date of treatment and either the date of death due to the tumour or last follow-up. The study was

conducted with National Research Ethical Committee approval (UK 12/LO/2018).

Tissue Microarray Construction, Immunohistochemistry, and Image Analysis

Tissue microarrays (TMA) were constructed by selecting tumour regions displaying more than 70% cellularity with minimal necrosis and marked on haematoxylin and eosin-stained sections. TMA were constructed using a tissue arrayer with 3 × 1.0 mm diameter cores from each tissue block arranged at mapped locations into recipient paraffin blocks. TMA were sectioned (5 μm) by microtome onto Superfrost™ adhesive glass slides (ThermoFisher Scientific) and automated immunohistochemical staining performed for CD3 (T lymphocytes, Agilent, clone F7.2.38), CD68 (macrophages, Agilent, clone KP-1) and myeloperoxidase (MPO, Agilent, code IS511,) by the Histology Department, Sheffield Teaching Hospitals NHS Foundation Trust. Stained TMA sections were imaged using an Aperio ScanScope CS slide scanner (Aperio Technologies, Vista, USA). Digital analysis was performed using QuPath software (https://qupath.github.io) [16]. Further details of the QuPath analytical approach is provided in **Supplementary Figure 1**. Cores with insufficient histology (<80% area) were not included in QuPath analysis. Each OPSCC case was represented by at least two cores in QuPath analysis and the image analysis data was calculated as the mean percent number of positively stained cells as a proportion of the total number of cells in each core. Leukocytes within blood vessels were excluded from the analysis.

Cell Culture

The OPSCC HPV-positive cell line UPCI-SCC90 was provided by Prof. Susanne Gollin, University of Pittsburgh and the HPV-negative cell line FaDu was purchased from the American Type Culture Collection. The authenticity of cell lines was verified by short tandem repeat analysis and their HPV status confirmed by the HPV Cytology Screening Unit using the cobas® qPCR HPV testing kit that detects a 200 bp region within the L1 region of the HPV genome (Sheffield Teaching Hospital NHS Foundation Trust, UK). Normal tonsillar fibroblasts (NTF) were isolated from biopsies obtained from tonsillectomies at the Sheffield Teaching Hospitals NHS Foundation Trust with written, informed consent (ethical approval 09/H1308/66) as described previously [17]. For this study, NTF isolated from one donor was used in all experiments to limit experimental variation. UPCI-SCC90 cells and NTF were cultured in Dulbecco's Modified Eagle's Medium and FaDu in RPMI-1640, both media were supplemented with 10% v/v foetal calf serum (FCS), 2 mM L-glutamine, 100 IU penicillin and 100 μg/ml streptomycin, (all medium and supplements from Sigma-Aldrich) and cells cultured in a humidified incubator with 5% CO_2 at 37°C. All cells were confirmed mycoplasma-free before use in experiments.

Leukocyte Isolation

Human peripheral blood leukocytes were isolated from the venous blood of healthy donors with written, and informed consent (University of Sheffield ethical approval, 012597) as previously described [18]. The donors were two male and one female, none had received the HPV vaccine, none were taking prescription medication or had been ill in the previous 2 weeks, and all were non-smokers. Blood was anti-coagulated using sterile 3.8% sodium citrate (Sigma-Aldrich, W302600) and centrifuged at 400 g for 20 min to separate the plasma from leukocytes. Mononuclear cells were isolated by Ficoll-Paque Plus (GE Healthcare, GE17-1440-02) density-gradient centrifugation, washed twice with Hank's balanced salt solution (HBSS, ThermoFisher Scientific, 14170112) and erythrocytes removed from neutrophils by hypotonic lysis. Total neutrophils and mononuclear cells were re-combined by re-suspension in RPMI-1640 supplemented with 10% v/v FCS and 2 mM L-glutamine then labelled with CellTracker™ Deep Red dye (ThermoFisher Scientific, C34565) according to the manufacturer's instructions. Cell viability was assessed by trypan blue (Sigma-Aldrich, T8154) exclusion and was >95% by light microscopy.

3D Tumour—Stromal *in vitro* Culture Models

Multicellular tumour spheroids (MCTS) were generated as previously described [19]. Briefly, 100 μL of a 1 × 10^5 cells/mL suspension of either FaDu or UPCI-SCC90 cells were added to each well of a 96-well plate previously coated with 100 μL sterile 1.5% w/v type IV agarose (Sigma-Aldrich, 121852) in serum-free medium. Culture plates were incubated for 2 d in a humidified incubator with 5% CO_2 at 37°C to allow MCTS formation. To prepare 3D tumour-stromal *in vitro* models, type 1 rat-tail collagen (produced in-house) was mixed with 10 × RPMI-1640, 10% FCS, 2 mM L-glutamine and reconstitution buffer (2.2% $NaHCO_3$, 4.8% HEPES, 0.25% NaOH in dH_2O, all from Sigma-Aldrich) and pH adjusted to 7.4. One millilitre collagen was added to 2 × 10^5NTF and 60 FaDu or UPCI-SCC90 MCTS, the contents mixed to evenly disperse the NTF/spheroids and the hydrogel allowed to solidify for 1 h at 37°C, 5% CO_2 in a 24-well plate. A ratio of ∼1:3 NTF to tumour cells was used in accordance with our previous 2D work [13]. Once set, 1 mL of serum-containing medium was added and models incubated for a further 24 h. Models were then washed twice with serum-free medium and incubated in the absence or presence of 1 or 10 μg/ml anakinra (Trade name Kineret, Amgen) in 500 μl serum-free medium and further incubated for 24 h. Levels of anakinra were maintained throughout the experiment. Culture medium was then removed and stored at −20°C for further analysis by ELISA.

To generate immune cell-containing 3D tumour-stromal models, 200 μL of CellTracker™-labelled total leukocytes were added to the surface of either FaDu or UPCI-SCC90 3D co-culture models in the absence or presence of 10 μg/ml anakinra, and incubated for 24 h. Models were washed twice with HBSS to remove non-infiltrating leukocytes then total cells dispersed from the models using type I collagenase (2 mg/mL in HBSS, Sigma-Aldrich, SCR103), washed in HBSS, sieved to remove cell aggregates and then fixed with 2% paraformaldehyde. Flow cytometry (FACSCalibur™ with associated CellQuest™

TABLE 1 | Oropharyngeal squamous cell carcinoma patient demographics and clinical characteristics.

Characteristics (%)			HPV-positive (%)	HPV-negative (%)	*P*
Total Subjects	59 (100)		40 (68)	19 (32)	
Sex	Female	14 (24)	10 (25)	4 (21)	0.739
	Male	45 (76)	30 (75)	15 (79)	
Age (year)	Median		57	56	0.651
	Range		29-68	31-70	
Alcohol[a]	Never		3 (7.5)	0 (0)	**0.018**
	Moderate		22 (55)	6 (31.5)	
	Heavy		4 (10)	7 (37)	
	Unknown		11 (27.5)	6 (31.5)	
Smoking[b]	Smoker		13 (32.5)	9 (47)	**0.032**
	Non-smoker		11 (27.5)	0 (0)	
	Ex-smoker		9 (22.5)	7 (37)	
	Unknown		7 (17.5)	3 (16)	
Site	Tonsil		28 (70)	9 (47)	0.205
	Base of tongue		11 (27.5)	7 (37)	
	Post wall of pharynx		0 (0)	1 (5)	
	Soft palate		0 (0)	1 (5)	
	Oropharynx (not otherwise specified)		1 (2.5)	1 (5)	
Disease stage	Stage I		8 (20)	2 (11)	0.271
	Stage II		16 (40)	5 (26)	
	Stage III		5 (12.5)	6 (32)	
	Stage IV		10 (25)	5 (26)	
	Unknown		1 (2.5)	1 (5)	
T-Stage[c]	T1/T2		24 (60)	7 (37)	0.155
	T3/T4		15 (37.5)	11 (58)	
	Unknown		1 (2.5)	1 (5)	
N-stage[c]	N0		3 (7.5)	3 (15.7)	0.654
	N1-N2a		28 (70)	13 (68)	
	N2b-N3		7 (17.5)	3 (15.7)	
	Unknown		2 (5)	0 (0)	
M-Stage[c]			0 (0)	0 (0)	
Grade[c]	Poor		25 (62.5)	9 (48)	0.339
	Moderately		9 (22.5)	8 (42)	
	Well		2 (5)	1 (5)	
	Unknown		4 (10)	1 (5)	
Recurrence	Local		1 (5)	4 (21)	0.495
	Regional		0 (0)	2 (10.5)	
	None		39 (95)	13 (68.5)	

[a] *Moderate alcohol consumption is defined as up to 10–25 units/week, heavy consumption is defined as >25 units/week.*

[b] *Ex-smoker is defined as an individual who has smoked at least 100 cigarettes in their lifetime but who had quit smoking within the last 12 months.*

[c] *Brierly et al. [20].*

Two-sided Chi-squared or Fisher's exact-test was used for comparison of categorical variables and two-tailed Student t-test was used for continuous variables. $P < 0.05$ are given in bold text.

software, BD Biosciences) was used to plot side scatter against fluorescence (650 nm emission) to determine the total number of infiltrating leukocytes into tumour-stromal models. These cells were further gated and side scatter plotted against forward scatter to determine the relative numbers of neutrophils, monocytes and lymphocytes according to their cell size and granularity (see **Figure 5** for gating strategy). Each experiment was performed using the leukocytes isolated from one donor with each test performed in technical triplicates and the entire experiment was repeated three times ($n = 3$) using the blood from a different donor (two male, one female, all non-HPV-vaccinated and non-smokers) on each occasion.

Cell Viability

Cell viability as measured by metabolism of PrestoBlue™ (ThermoFisher Scientific, P50200) was performed according to the manufacturer's instructions. 3D models were incubated with serum-containing medium for 48 h at 37°C, 5% CO_2.

Models were washed with HBSS before addition of fresh medium containing PrestoBlue™ (1:10 v/v) and then incubated for a further 2 h. Medium was removed and the colour change measured by fluorescence excitation at 560 nm and emission at 590 nm.

Chemokine Level Quantification

Conditioned medium collected from 3D models in the absence or presence of anakinra was subjected to ELISA for CXCL8, CCL2 (BD Biosciences 555244 and 559017, respectively) and CCL5 (R&D systems, DRN00B) according to the manufacturer's instructions.

Statistics

All data are expressed as mean ± SD of at least three independent experiments performed in triplicate unless otherwise stated. Statistical analysis was undertaken using GraphPad Prism (v8.4.3, GraphPad Software, San Diego, CA). For demographic and clinical data two-sided Chi-squared or Fisher's exact-test was used for comparison of categorical variables and two-tailed Student *t*-test was used for continuous variables. For TMA data pairwise comparisons were performed using Mann-Whitney *U*-test, and for ELISA data group-wise comparisons were made using one-way ANOVA with Tukey's multiple comparisons test. Flow cytometric data was analysed using one sample *t* and Wilcoxon-test. SPSS (v22, IBM Chicago, IL) was used to test for HPV-status effects in cumulative survival curves according to the Kaplan-Meier method with comparisons between survival curves made using the log rank test. Differences between groups was considered significant when $p < 0.05$.

RESULTS

HPV-Positive Status Is Associated With Improved 5-Year Survival in OPSCC

The patient demographic information is summarised in **Supplementary Table 1**. The median age was 57 years for HPV-positive and 56 years for HPV-negative patients. There was a statistically significant difference in the levels of alcohol ($p = 0.018$) and smoking ($p = 0.032$) between subjects with HPV-positive and HPV-negative OPSCC. However, there was no significant difference between any of the clinical parameters analysed (**Table 1**). OPSCC cases were stratified for HPV-status and overall survival over a 5-year follow-up period examined. Kaplan-Meier survival analysis showed that individuals with HPV-positive OPSCC correlated significantly ($p = 0.04$) with better overall 5-year survival than individuals with HPV-negative OPSCC (**Figure 1**).

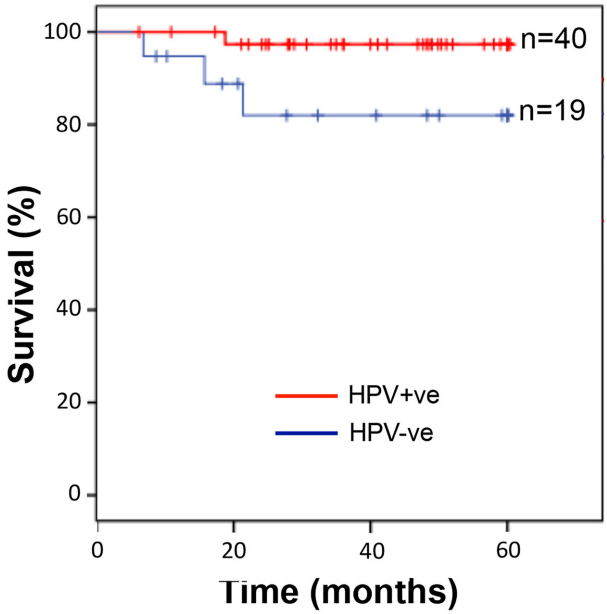

FIGURE 1 | Individuals with HPV-positive OPSCC display significantly better overall 5-year survival than those with HPV-negative OPSCC. Kaplan-Meier analysis followed by log rank-test showed that individuals with HPV-positive OPSCC ($n = 40$) correlated with significantly ($p = 0.04$) improved overall 5-year survival than individuals with HPV-negative OPSCC ($n = 19$).

HPV-Negative OPSCC Contain Elevated Numbers of Tumour-Associated Neutrophils Than HPV-Positive OPSCC but Levels of Other Tumour-Associated Leukocytes Are Similar

The number of tumour-infiltrating leukocytes present in HPV-negative or HPV-positive OPSCC was measured by immunohistochemical staining of TMA for myeloperoxidase (MPO; neutrophils), CD68 (macrophages) or CD3 (pan T cells) followed by QuPath image analysis and the percent number of positively stained cells as a proportion of the total number of cells in each core calculated (**Supplementary Figure 1**). There was a significant ($p = 0.003$) increase in the level of MPO-immunopositive staining in HPV-negative (median 9.04%, $n = 19$) compared with HPV-positive (median 2.98%, $n = 40$) OPSCC, indicating the increased prevalence of neutrophils in HPV-negative compared to HPV-positive OPSCC (**Figures 2A,B**). In contrast, similar levels of macrophages, identified by CD68-positive staining, were observed in both HPV-positive (median 5.49%, $n = 39$) and HPV-negative (median 2.64%; $p = 0.277$) OPSCC (**Figures 2C,D**). Likewise, the levels of T lymphocytes were similar in both HPV-positive (median 4.13%, $n = 38$) and HPV-negative (median 2.14%, $n = 17$; $p = 0.333$) OPSCC (**Figures 2E,F**). The tissue neutrophil to lymphocyte ratio (NLR) for HPV-negative OPSCC was significantly greater (median 2.60) than the ratio for HPV-positive tumours (median 0.773; $p < 0.01$). There were no significant differences observed in the levels of infiltrating leukocyte populations between HPV-positive and HPV-negative OPSCC at disease stage (**Supplementary Figure 2**) or T-stage (**Supplementary Figure 3**) examined. Taken together, these data show that HPV-negative OPSCC contained significantly more TAN compared to HPV-positive OPSCC whereas levels of CD68 and CD3-positive leukocyte populations were similar.

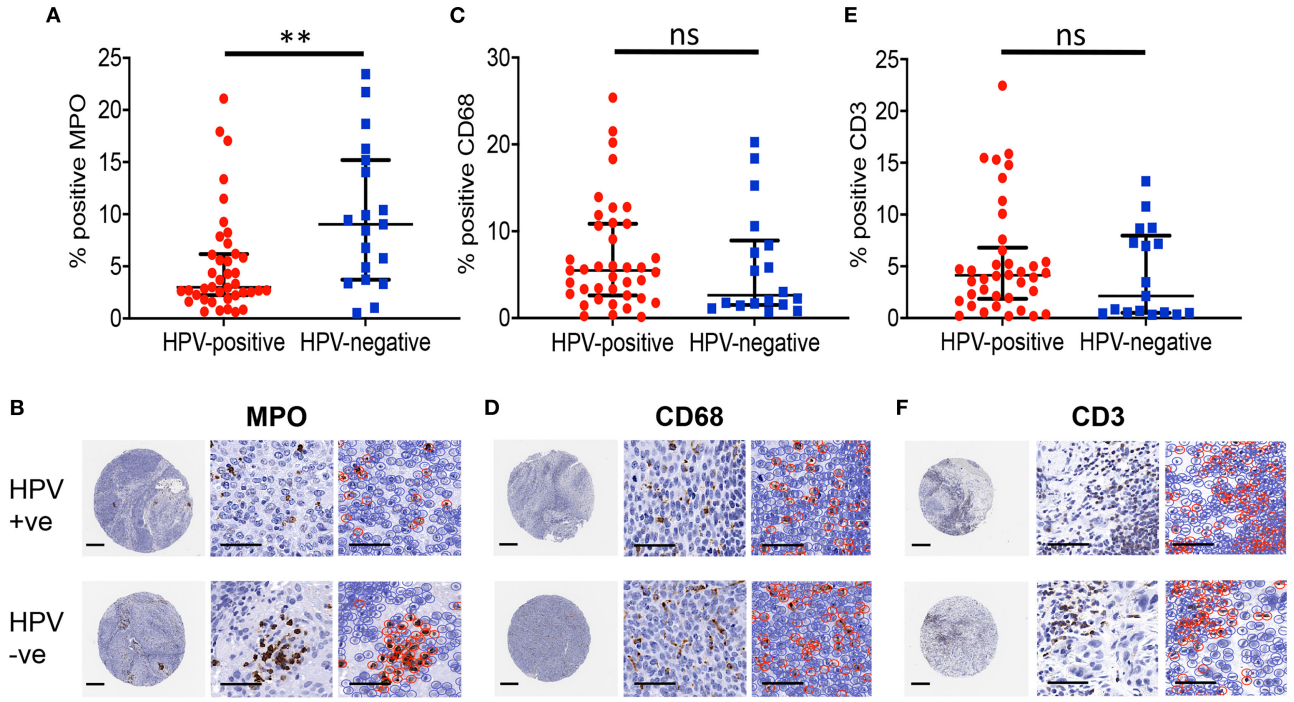

FIGURE 2 | HPV-negative OPSCC contain more neutrophils than HPV-positive OPSCC. Levels of tumour associated-leukocyte populations for HPV-positive and HPV-negative OPSCC tissue sections were analysed by QuPath. **(A,B)** MPO+ neutrophils, **(C,D)** CD68+ macrophages, **(E,F)** CD3+ T lymphocytes. Scale bar = 250 μm for whole cores (left), and 50 μm for magnified core images (centre) and corresponding QuPath image analysis (right). Brown staining shows immuno-positively identified cells within the cores. Data are presented as the median and interquartile range of percent positively stained cells as a proportion of the total number of cells. Differences between groups was determined using a non-parametric Mann-Whitney *U*-test; $^{**}p < 0.01$.

HPV-Negative 3D Tumour-Stromal Culture Models Produce Significantly Higher Levels of Chemokines than their HPV-Positive Counterparts

Our previously published data using a 2D monolayer experimental culture system showed that when cultured with conditioned medium derived from several different HPV-negative OPSCC tumour cells, NTF are stimulated to secrete high levels of chemokines in an IL-1-dependent manner [13]. Here we used a 3D *in vitro* construct containing HPV-negative (FaDu) or HPV-positive (UPCI-SCC90) multi-cellular tumour spheroids (MCTS) (**Supplementary Figures 4A–F**) embedded in a NTF-populated stromal collagen matrix in an attempt to more accurately model the intimate cell-cell OPSCC tumour-stromal cell interactions within a 3D environment, similar to those occurring *in vivo*. Haematoxylin and eosin stained sections of the tumour-stromal 3D model demonstrated an evenly distributed fibroblast-populated matrix containing MCTS that often display a central necrotic core surrounded by several layers of tumour epithelium (**Supplementary Figures 4E,F**). The histological appearance of the tumour-stromal 3D model is comparable to that frequently observed with OPSCC tumours *in vivo* (**Supplementary Figure 4G**) although the density of the fibroblasts within the *in vitro* models appears to be lower than observed *in vivo*.

Cell viability within collagen gels was measured for NTF, FaDu or UPCI-SCC90 spheroids alone or in combination. The mean fluorescence readings for all tests containing cells were increased compared to collagen alone showing that cells remained metabolically active and therefore viable whilst embedded in collagen. When in co-culture with NTF, both UPCI-SCC90 and FaDu displayed an additive and significant ($p < 0.05$) increase in fluorescence compared to when the spheroids or NTF were cultured alone, reflecting the synergistic nature of the co-culture system (**Supplementary Figure 4H**).

We next measured the levels of chemokines released in models containing NTF or MCTS alone or when in combination. We chose to measure CXCL8, CCL2 and CCL5, as these chemokines are well-recognised, potent chemoattractants for neutrophils, monocytes and lymphocyte populations, respectively. When cultured alone, NTF produced low levels for all three of the chemokines tested with 0.12 ± 0.007 ng/ml, 0.20 ± 0.02 ng/ml and 0.06 ± 0.005 ng/ml for CXCL8, CCL2 and CCL5, respectively (**Figures 3A–C**). This was also the case for UPCI-SCC90 MCTS when cultured alone (CXCL8−0.09 ± 0.01 ng/ml, CCL2−0.14 ± 0.02 ng/nl and CCL5−0.13 ± 0.001 ± ng/ml; **Figures 3A–C**). Chemokine secretion was not significantly increased when NTF were co-cultured with UPCI-SCC90 MCTS (CXCL8−3.52 ±

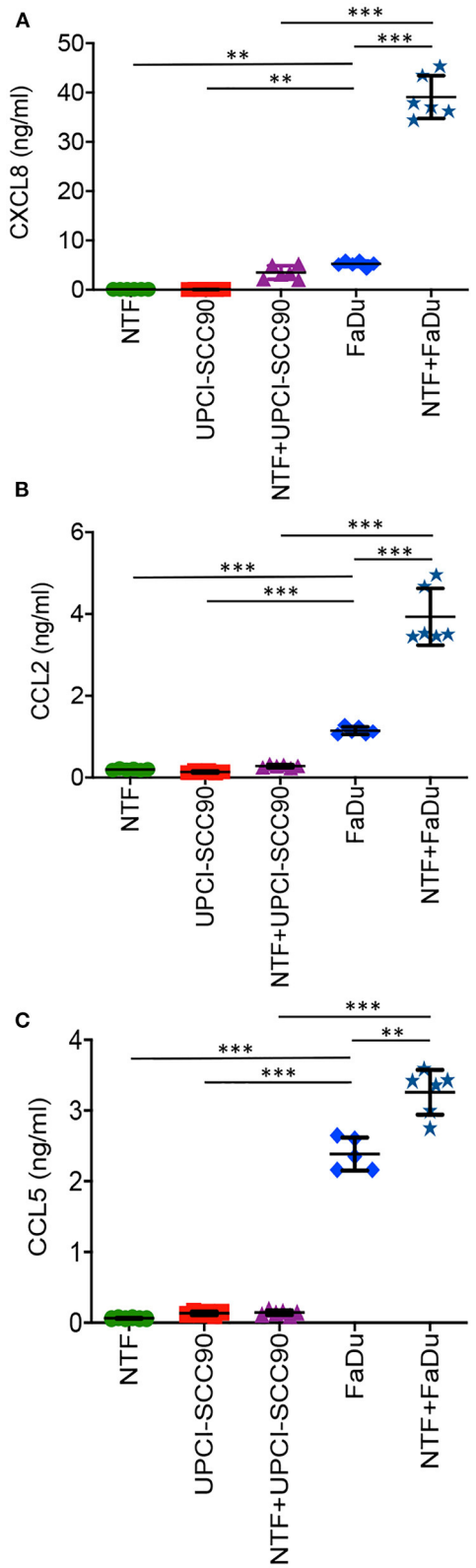

FIGURE 3 | FaDu HPV-negative OPSCC 3D tumour-stromal models display increased chemokine secretion compared to their UPCI-SCC90 HPV-positive counterparts. Chemokine secretion of NTF-populated collagen alone, UPCI-SCC90 and FaDu MCTS alone or NTF/MCTS co-cultures for **(A)** CXCL8, **(B)** CCL2, and **(C)** CCL5. Data are mean ± SD for at least 5 independent experiments. Statistics were performed using One-way ANOVA with Tukey's multiple comparisons test. $^{**}p < 0.01$, $^{***}p < 0.001$.

(Continued)

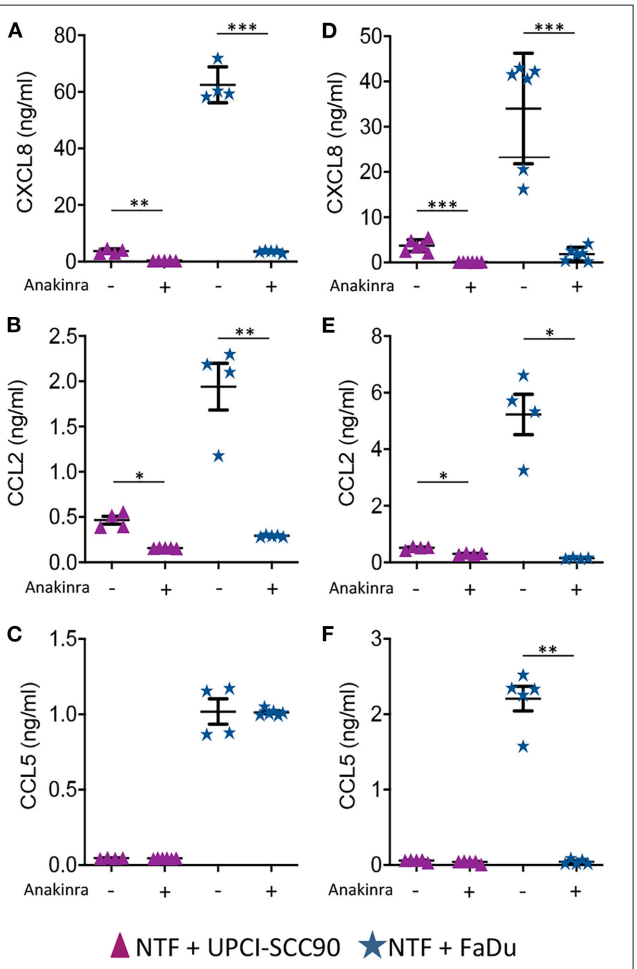

FIGURE 4 | Chemokine secretion by HPV-negative OPSCC 3D tumour-stromal models is reduced by anakinra. **(A)** CXCL8, **(B)** CCL2, and **(C)** CCL5 secretion from HPV-positive UPCI-SCC90/NTF or HPV-negative FaDu/NTF co-cultures in the absence or presence of 1 μg/mL **(A–C)** or 10 μg/mL **(D–F)** anakinra. Data are mean ± SD for at least 4 independent experiments. Statistics were performed using Mann Whitney *U*-test. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

1.38 ng/ml, CCL2−0.28 ± 0.04 ng/nl and CCL5−0.14 ± 0.004 ± ng/ml; **Figures 3A–C**). In contrast, for CXCL8 (5.28 ± 0.6 ng/ml; $p < 0.05$) and CCL2 (1.15 ± 0.09 ng/ml; $p < 0.001$), FaDu MCTS alone secreted significantly more chemokine than NTF or UPCI-SCC90 MCTS alone, and for CCL5 (2.38 ± 0.23 ng/ml; $p < 0.001$) significantly more than NTF/UPCI-SCC90 co-culture models (**Figures 3A–C**). However, when FaDu MCTS were combined with NTF in a 3D tumour-stromal model, levels of CXCL8 increased 7-fold (39.1 ± 4.34 ng/ml; $p < 0.001$) and CCL2 by

3-fold (3.93 ± 0.69 ng/ml; $p < 0.001$, **Figures 3A,B**), showing that NTF and FaDu act synergistically in stimulating production of these chemokines. Levels of CCL5 also increased in the 3D tumour-stromal model compared to MCTS alone, although the difference between the amounts of chemokine released was much less [2.4 ± 0.2 ng/ml compared to 3.3 ± 0.3 ng/ml for FaDu MCTS alone and NTF + FaDu MCTS, respectively ($p < 0.01$); **Figure 3C**]. These data indicate that OPSCC cells within a 3D environment interact with NTF via paracrine signalling.

Synergistic Production of Chemokines by HPV-Negative 3D Tumour-Stromal Culture Models Is Mediated by IL-1/IL-1R

We next tested if the IL-1/IL-1R axis was the main paracrine signalling pathway in mediating elevated chemokine release by inhibiting this pathway using anakinra, a highly specific IL-1R antagonist. Here, 3D models containing either UPCI-SCC90 or FaDu MCTS co-cultured with NTF were pre-incubated with 1 or 10 μg/ml anakinra and levels of CXCL8, CCL2 and CCL5 measured in the conditioned medium after 24 h. Pre-incubation with 1 μg/ml anakinra dramatically reduced the production of CXCL8 and CCL2 but not CCL5 for both UPCI-SCC90 and FaDu 3D tumour-stromal models. Specifically, in FaDu-stromal 3D experimental models, CXCL8 was reduced 18-fold ($p < 0.001$) and CCL2 6-fold ($p < 0.01$) upon treatment with anakinra, whereas CCL5 levels remained the same as untreated models (**Figures 4A–C**). Inhibition of chemokine secretion was even more pronounced when models were pre-incubated with 10 μg/ml anakinra, where levels of CXCL8, CCL2 and CCL5 were almost abolished in anakinra-treated FaDu stromal models ($p < 0.01$; **Figures 4D–F**).

HPV-Positive UPCI-SCC90 Tumour-Stromal Models Recruit Fewer Neutrophils than their HPV-Negative FaDu Counterparts Reproducing What Is Observed *in vivo*

Fluorescently labelled leukocytes isolated from whole blood were added to HPV-positive UPCI-SCC90 or HPV-negative FaDu tumour stromal models and the levels of total infiltrating leukocytes, as well as individual leukocyte subsets, quantified by flow cytometry using a gating strategy to identify neutrophils, monocytes and lymphocytes based on their well-characterised forward and side scatter profiles (**Figures 5A–D**). There were 36.8 ± 14.9% fewer total leukocytes infiltrating into HPV-positive UPCI-SCC90 tumour-stromal models compared to those infiltrating into the HPV-negative FaDu tumour-stromal models ($p = 0.05$; **Figure 5E**, data showing the actual numbers of total leukocytes or leukocyte subsets infiltrating into the tumour-stromal models for each individual experiment is provided in **Supplementary Table 1**). When broken down into leukocyte subsets, the proportion of neutrophils infiltrating into HPV-positive UPCI-SCC90 tumour-stromal models was 52.3 ± 19.9% less ($p = 0.045$) when compared to those infiltrating HPV-negative FaDu tumour-stromal models, whereas, although overall decreased, the proportion of infiltrating monocytes (28.8 ± 27.9%) and lymphocytes (19.8 ± 15.5%) were not statistically different between UPCI-SCC90 and FaDu models (**Figures 5B,C,E**). Although these flow cytometric results have a relatively large standard deviation due to donor-to-donor variability, the data reflect those observed in the immunohistochemical analysis of leukocyte infiltration in the patient OPSCC tumour sections, where greater levels of tumour-associated neutrophils were observed in HPV-negative compared to HPV-positive tumours. Since pre-treatment with anakinra significantly reduced the levels of chemokines (CXCL8 and CCL2) in both FaDu and UPCI-SCC90 tumour-stromal models (**Figure 4**), we reasoned that these anakinra-treated tumour-stromal models would therefore also recruit fewer leukocytes. This was indeed the case with anakinra treatment reducing the numbers of infiltrating leukocytes for both UPCI-SCC90 and more dramatically for FaDu tumour-stromal models (**Supplementary Figure 4**). Here, anakinra-treated FaDu tumour-stromal models recruited 45.2 ± 12.0% ($p = 0.023$) fewer total leukocytes than untreated models (**Figures 5B,D,F**). At the leukocyte subpopulation level, the proportion of neutrophils and monocytes infiltrating FaDu tumour-stromal models was significantly reduced by 45.9 ± 12.3% ($p = 0.023$) and 52.9 ± 16.4% ($p = 0.030$) respectively, upon anakinra treatment, whereas the proportion of lymphocytes infiltrating into anakinra-treated FaDu tumour-stromal models was not significantly different (40.3 ± 17.5%; $p = 0.057$) from untreated models (**Figure 5F**).

DISCUSSION

The tumour microenvironment plays a major role in directing the course of tumour progression, and factors that regulate the immune response and direct the infiltration of tumour-associated leukocytes are key to this process [21]. It is important to understand the overall tumour-associated immune burden and mechanisms of how this is controlled because several lines of evidence show that the abundance of certain leukocyte subsets within tumours correlate with either poor or improved prognosis.

Although our patient cohort was relatively small, HPV status still stratified the patient groups in terms of outcome, where patients with HPV-positive OPSCC displayed significantly improved 5-year survival than those with HPV-negative tumours, as observed in several other studies [2, 6, 22, 23]. The percent 5-year overall survival for HPV-positive patients seen in this study is similar to those observed in previous studies, whereas we observed an increased 5-year overall survival for HPV-negative OPSCC subjects compared to previous studies [2, 6, 22, 23]. This is likely due to the relatively small sample size for the HPV-negative cohort used in this study compared to other larger studies as well as differences in local detection and/or treatment regimens. Upon leukocyte tumour burden assessment, we found TAN to be significantly more abundant in HPV-negative than HPV-positive tumours; the first time that differences in TAN levels in OPSCC has been shown to correlate with HPV status. We used a validated antiserum specifically raised against neutrophil MPO to detect the presence

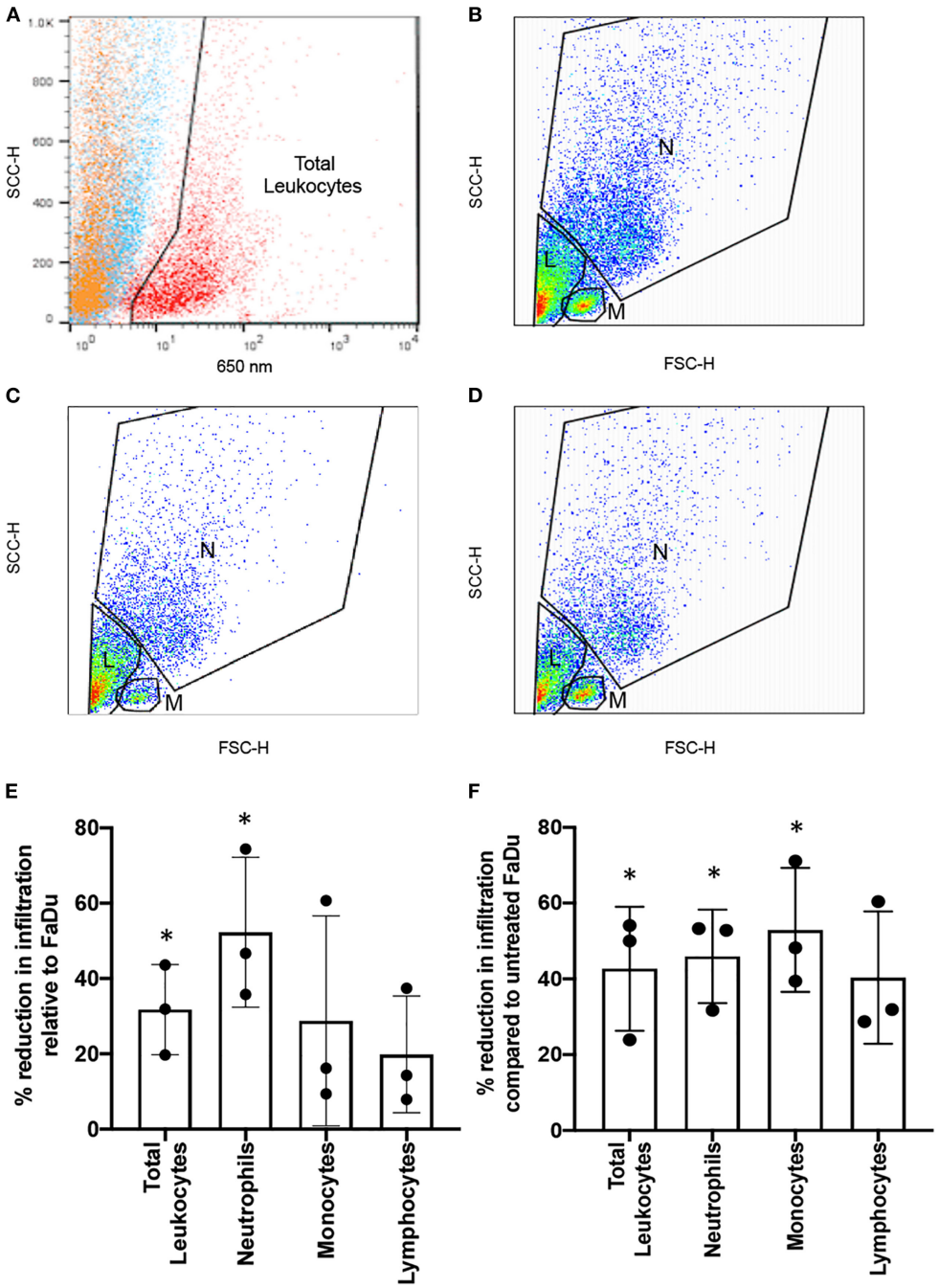

FIGURE 5 | Anakinra reduces leukocyte recruitment in an *in vitro* 3D model of OPSCC. Infiltration of leukocytes into 3D OPSCC tumour-stromal models in the absence or presence of 10 μg/ml anakinra. **(A)** Fluorescence-based gating strategy to identify leukocyte population (red) compared to tumour (orange) or NTF (blue) cell populations. Total leukocytes identified in panel A were re-gated for their well-characterised side and forward scatter properties to determine the number of neutrophils (N), monocytes (M) and lymphocyte (L) populations infiltrating into **(B)** FaDu tumour-stromal models and **(C)** UPCI-SCC90 tumour-stromal models in the absence of anakinra, or **(D)** FaDu tumour-stromal models pre-treated with 10 μg/ml anakinra for 24 h before addition of leukocytes. **(E)** Percent reduction in the number of leukocytes infiltrating HPV-positive UPCI-SCC90 tumour-stromal models relative to those infiltrating HPV-positive FaDu tumour-stromal models. **(F)** Percent reduction in the number of leukocytes infiltrating anakinra treated (10 μg/ml) HPV-negative FaDu tumour-stromal models compared to untreated FaDu tumour-stromal model controls. Data in E and F are mean ± SD for 3 independent experiments with each experiment being performed using the blood from an individual donor. Statistics were performed using one sample *t* and Wilcoxon-test. *$p < 0.05$.

of TAN, a biomarker commonly used by diagnostic pathology laboratories for the detection of neutrophils. MPO may not be as specific as less commonly used markers such as CD15 and CD66b, although these biomarkers too are expressed by other granulocytes. Unfortunately, we were unable to correlate TAN levels with prognosis for HPV status cases due to the sample size. However, similar data have been reported for OSCC [24], although this form of cancer rarely harbours oncogenic HPV [25]. Here, Li et al., observed increased abundance of CD15+ neutrophils in HPV-negative compared to HPV-positive OSCC, as well as finding that high levels of neutrophils correlated with poor 5-year survival, increased lymph node metastasis and were an independent prognostic factor for OSCC [24]. Using The Cancer Genome Atlas (TCGA) data set Chen et al., also found a higher neutrophil genetic imprint for HPV-negative compared to HPV-positive head and neck cancer [26].

In other tumour types, high levels of TAN have been shown to drive tumour progression by producing factors such as reactive oxygen species that initiate further DNA mutations [27], and by secreting potent pro-tumour factors such as vascular endothelial growth factor and matrix metalloproteinases that promote tumour angiogenesis [28, 29], and transforming growth factor beta (TGF-β) that induces myofibroblast formation [30]. Moreover, neutrophils that display an immunosuppressive phenotype upon stimulation by tumour micro-environmental cues [so called polymorphonuclear myeloid-derived suppressor cells (PMN-MDSC)] have been shown to accumulate in both patient biopsies and murine experimental models of head and neck squamous carcinoma (HNSCC), where they inhibit the tumouricidal functions of natural killer cells by secretion of TGF-β, nitric oxide and arginase-1 [31, 32]. Given these findings, it could be speculated that high TAN levels and their associated secreted factors are key in driving tumour immunosuppression, progression, metastasis and therefore poor outcome in HPV-negative OPSCC. Investigation of the TAN phenotypes in HPV-negative and HPV-positive OPSCC in future studies is warranted.

Similar to TAN, increased levels of TAM have been shown to correlate with poor prognosis in several tumours including HPV-negative OSCC [9, 33]. Although we observed high levels of TAM in tumours there was no significant difference in their abundance between HPV-positive and HPV-negative OPSCC. Our data is in line with other studies reporting similarities in overall TAM levels and HPV status in OPSCC and HNSCC [7, 26, 34]. In contrast, Seminerio et al., found increased prevalence of CD68+ macrophages in HPV-positive compared to HPV-negative OPSCC in the intra-epithelial but not stromal tumour component [33]. It is possible that different TAM phenotypes are recruited to OPSCC. Although we did not examine TAM polarisation status, a recent study found a higher M1/M2 TAM ratio in HPV-positive HNSCC that may account for differences [26].

Much attention has been recently paid to TIL that are correlated with improved prognosis in many tumours [35] and are the basis for several current immunotherapy approaches that have had variable success in treating head and neck cancers [36]. Using CD3, a pan T cell marker, we found no difference in the levels of CD3+ lymphocytes in HPV-positive compared to HPV-negative OPSCC in our patient cohort, a similar finding previously observed by others [34, 37]. However, there is now compelling evidence that it is the recruitment of specific lymphocyte subsets that are crucial in driving anti-tumour responses, in particular CD8+ T cells, where the ability to control HPV-positive tumours is likely mediated by CD8+ T cell-specific recognition of HPV-derived antigens [38, 39]. Indeed, numerous reports have shown increased abundance of CD8+ T cells in HPV-positive compared to HPV-negative OPSCC where their presence is correlated with overall improved survival [5–7, 37, 40, 41]. In addition, CD20+ B cells [34, 40, 42] and FoxP3+Treg [5, 34] are also increased in HPV-positive compared to HPV-negative OPSCC, suggesting a complex interplay in adaptive immunity in response to HPV-driven oncogenesis. In light of this substantial evidence we did not pursue further lymphocyte characterisation at the sub-population level.

The ratio of circulating peripheral blood neutrophils in relation to lymphocytes has been suggested as a potential prognostic marker for many cancers including OPSCC [43, 44]. A high circulating neutrophil to lymphocyte ratio (NLR) has been found for both HPV-positive and HPV-negative OPSCC [45]. In HPV-positive OPSCC, a high NLR was associated with higher T classification, lower 5-year overall survival and disease-free survival [46]. Similarly, in a large cohort study, Huang et al. found higher levels of circulating neutrophils in HPV-negative compared to HPV-positive OPSCC, although both correlated with lower overall survival compared to patients with lower numbers of circulating neutrophils [47]. Contrary to this, high circulating lymphocyte levels were associated with improved recurrence-free survival in HPV-positive OSCC. However, following multivariate analysis the investigators found that a high circulating neutrophil count predicted for lower overall and recurrence-free survival for only the HPV-positive cohort [47]. In contrast, Rosculet et al. found that NLR was an indicator for both recurrence-free and overall survival in OPSCC but this association was lost when HPV-status was included in the analysis [48]. In support of this, a high NLR predicted for a worse 5-year overall survival compared to a low NLR in HPV-negative OPSCC [49]. Moreover, Rachidi et al. found that the circulating NLR was significantly lower in HPV-positive OPSCC compared to the HPV-negative counterparts [50].

This study showed that the tissue NLR is significantly greater for HPV-negative than HPV-positive OPSCC, which is supportive of some but not all of the current circulating NLR data. There may be a discourse between the numbers of circulating leukocytes and those recruited to the tumour micro-environment particularly for lymphocytes, as it appears that HPV-positive OPSCC contain greater numbers of specific subsets (CD8+ T cells, CD20-B cells and FoxP3+Treg) than their HPV-negative counterparts that are also associated with overall improved survival in HPV-positive OPSCC [5–7, 37, 40, 41]. Larger scale tissue-based immunohistochemical analysis in concert with measurement of circulating NLR is required to resolve the suitability of this type of analysis for prognostication of OPSCC with HPV-status.

Given the striking increase in TAN numbers in HPV-negative compared to HPV-positive tumours, we decided to examine the mechanisms by which the disparity in TAN abundance may be mediated. Recruitment of leukocytes to tumours is driven by chemokines; with specific chemokines responsible for the recruitment of particular leukocyte subsets and several studies have reported that OPSCC cells express a number of chemokines [13, 41, 51]. We reasoned that HPV-negative tumours would produce more neutrophil-specific chemokines than HPV-positive tumours as a mechanism to recruit increased numbers of TAN. Indeed, using a rudimentary 2D culture medium transfer model we previously showed that NTF were stimulated to secrete elevated levels of several cytokines in response to the culture medium from a number of HPV-negative but not HPV-positive OPSCC cell lines [13, 52]. Moreover, this increase was mediated in an IL-1-dependent manner with IL-1 liberated from HPV-negative OPSCC cell lines acting on the NTF IL-1R to stimulate chemokine production [13]. However, traditional 2D-based monolayer cell culture systems lack the 3D architecture and the spatial complexity that give solid tumours their characteristic features such as gradients for oxygen, pH, nutrition and waste products that give rise to areas of tumour necrosis and hypoxia, and so data generated in 2D often does not reflect what occurs in 3D. Most tumours grow as 3D masses closely surrounded by a fibroblast-populated stroma that communicate with tumour and other cells via paracrine signalling, driving tumour progression. *In vivo* orthotopic tumours cannot be used because the oropharyngeal cavity of rodents is small and any sizable tumour grown at this site would cause rapid asphyxiation and so *in vivo* models of OPSCC are limited to subcutaneously grown tumours that are far removed in terms of tissue structure and microenvironment than the tumours observed in humans. In addition, HPV is not a natural host of rodents and so an inappropriate immune response may ensue giving rise to false-positive data. We therefore endeavoured to recreate the tissue architecture using tumour spheroids [structures that are known to replicate many biophysical features of avascular tumours, [53]], embedded in a NTF-populated collagen matrix to more closely mimic the *in vivo* OPSCC microenvironment.

Using these 3D tumour-stromal models we observed that HPV-negative FaDu tumour/NTF co-cultures produced substantially more chemokines than NTF or FaDu MCTS cultured alone, and importantly, much more than UPCI-SCC90 HPV-positive tumour-stromal models. Furthermore, expression of the neutrophil-specific chemokine CXCL8 was produced at significantly higher levels than CCL2 or CCL5 in HPV-negative tumour-stromal models, suggesting that neutrophil-specific chemokines may out-weigh those for monocytes and lymphocytes, potentially skewing leukocyte subset recruitment in these tumours. We were unable to produce reliable and consistent MCTS from other HPV-positive cells lines and so our 3D analysis is limited to FaDu and UPCI-SCC90 cell lines. However, the chemokine profiles detected using these MCTS directly correlated with the profiles observed for other HPV-positive/negative cell lines in our previous 2D study [13], providing some evidence that these phenomena is not cell line specific but may be a general occurrence for HPV status. In support of this, analysis of the Gene Expression Omnibus (GEO) and TCGA databases showed significantly elevated levels of CXCL8 in HPV-negative compared to HPV-positive tumours [24], consistent with our *in vitro* 3D model data. In addition to CXCL8, increased abundance of other neutrophil-specific chemokines such as CXCL1, CXCL5 and CXCL6 is likely, as evidenced by our previous cytokine array analysis [13], and this may further skew the imbalance in leukocyte recruitment in favour of preferential neutrophil recruitment.

Blocking IL-1R using the highly specific antagonist anakinra almost completely abolished chemokine production, providing further support to the notion that IL-1/IL-1R paracrine signalling between HPV-negative tumour cells and NTF is of paramount importance for chemokine production. In support of this, increased levels of IL-1β have been found in HPV-negative compared to HPV-positive OPSCC patient samples [13, 41].

Tonsillar [54] and oral keratinocytes [55] constitutively express IL-1 at basal levels, but levels significantly increase upon DNA-induced malignant transformation [56, 57] and this appears to be important in tumour progression, as pharmacological inhibition of IL-1β interrupts chemically-induced oral carcinogenesis in rodent models [57]. HPV infection appears to ablate the ability of keratinocytes to express IL-1β; indeed we previously observed low levels of IL-1α and IL-1β in HPV-positive compared to HPV-negative cell lines and tumour biopsies [13]. Moreover, a complete lack of IL-1β gene expression has also been observed in HPV-positive cervical carcinoma cell lines [56]. In HPV infected cervical keratinocytes, Niebler et al., demonstrated that IL-1β is continually degraded in a HPV-16, E6 driven proteasome-dependent process via ubiquitin ligase, leading to complete loss of the cytokine as HPV-induced malignancy develops [56]. It is plausible that such a mechanism also occurs in HPV-positive OPSCC cells. Since IL-1β is known to drive NF-κB-induced chemokine expression in many cell types, it is logical that HPV-negative OPSCC are driven to secrete high levels of chemokines and therefore recruit increased numbers of TAN, whereas chemokines are much less abundant in HPV-positive OPSCC and so TAN recruitment is reduced. Li et al. overexpressed HPV18 E7 in the HPV-negative adenosquamous OSCC cell line Cal27 and showed that this directly reduced CXCL8 gene expression and protein secretion, suggesting that HPV gene products may have a more direct effect on chemokine production [24], although such a mechanism has not been shown for OPSCC cells. HPV infection may act to suppress TAN recruitment to HPV-positive OPSCC, although more research is required to further define this link.

Given the high levels of chemokines produced by the paracrine tumour-stromal interactions in HPV-negative OPSCC models it was no surprise that these co-cultures exhibited substantially more overall leukocyte recruitment than HPV-positive 3D models. However, it is remarkable that we observed significantly more neutrophil infiltration into HPV-negative tumour-stromal models than their HPV-positive counterparts, mirroring that observed in human patient tumour

samples. Treatment with anakinra significantly reduced the recruitment of both neutrophils and monocytes into both HPV-positive and HPV-negative tumour-stromal models, although the treatment was far more pronounced for HPV-negative tumour-stromal models, once again underscoring the importance of IL-1β in immune cell recruitment to tumours. These data are encouraging and validate the usefulness of human 3D *in vitro* models to study complex tumour microenvironment interactions with multiple cell types that may negate some of the problems experienced in the differences between human and murine immune systems encountered during *in vivo* animal experiments and also with 2D culture.

The use of anakinra to inhibit immune cell infiltration is an interesting concept. Indeed, anakinra was found to reduce circulating levels of CXCL8, tumour growth and the number of infiltrating TAM into mice bearing erlotinib-resistant adenosquamous OSCC (Cal27) and laryngeal carcinoma (SQ20B) xenografts grown subcutaneously. Overall survival in these mice was increased further when they were treated with the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, erlotinib, indicating that this adjunct therapy may be effective at overcoming EGFR inhibitor tumour resistance [58]. Anakinra has also been shown to reduce the levels of CXCL8 and number of TAM as well as reducing lymph node metastasis in a murine model of lung cancer [59]. Whereas, in breast cancer blocking IL-1 activity with anakinra or the IL-1β specific antibody, canakinumab, reduced breast cancer metastasis by inhibiting epithelial to mesenchymal transition and preventing metastatic outgrowth of disseminated tumour cells via inhibition of wnt signalling pathways [60–62]. Since blockade of IL-1R by anakinra reduced leukocyte levels in both HPV-positive and HPV-negative OPSCC *in vitro*, it could be argued that this antagonist may be effective for both types of tumour, however, the increased numbers of leukocytes recruited to HPV-negative OPSCC suggests that the treatment would be most effective for this cancer type. Therefore, blockade of the IL-1/IL-1R axis may be a promising adjunct anti-tumour therapy that would particularly affect HPV-negative OPSCC, although there may be inherent side effects in the blocking of this key inflammatory pathway, such as potential changes to inflammatory signalling pathways leading to dysregulated host inflammatory responses to infection or alterations to the activation of important immune cells types such as natural killer and cytotoxic T cells that are crucial in host anti-cancer responses [63, 64].

In summary, our human *in vivo* and 3D tumour-stromal *in vitro* data show, for the first time, that HPV-negative OPSCC contain significantly more TAN than HPV-positive OPSCC. Additionally, the mechanism of neutrophil recruitment appears to be via IL-1-mediated CXCL8 release by NTF. HPV infection appears to prevent IL-1 expression by tumour cells thereby suppressing TAN recruitment. Since TAN have been linked with poor prognosis, their apparent reduced recruitment to HPV-positive tumours may partially explain the improved outcome imparted by HPV-infection in OPSCC. Targeting the IL-1/IL-1R axis may be a viable consideration for the treatment of HPV-negative OPSCC.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by National Research Ethical Committee approval (UK 12/LO/2018). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

CM, KH, and RB conceived and designed the research. SA-S, NH, BO, and CM performed experiments, analysed the data, conducted statistical analysis, and interpreted the results. The manuscript was written and figures prepared by CM, SA-S and BO, and further edited by all the authors. PO contributed essential reagents and expert knowledge. All authors are aware of the content and have read and approved the manuscript for publication.

FUNDING

SA-S was funded by a scholarship from the Iraqi Ministry of Higher Education and Scientific Research and NH was funded by a scholarship from the Libyan Ministry of Higher Education and Scientific Research. RB was supported by a Joint Cancer Research UK and Yorkshire Cancer Research UK Clinical Doctoral Training Fellowship and PO by a Medical Research Council New Investigator Award (MR/P000096/1). BO was supported by a studentship from the Medical Research Council Discovery Medicine North (DiMeN) Doctoral Training Partnership (MR/N013840/1).

ACKNOWLEDGMENTS

The authors would like to thank Dr. Helen Colley, Dr. Vanessa Hearnden, and Dr. Nigel Beasley for supply of tonsil fibroblasts, as well as Dr. Bernie Foran, Dr. Katie Wallace and Dr. Dev Srinivasan (Sheffield Teaching Hospitals NHS Foundation Trust) for clinical data collection.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/froh.2021.604565/full#supplementary-material

REFERENCES

1. Sabatini ME, Chiocca S. Human papillomavirus as a driver of head and neck cancers. *Br J Cancer.* (2020) 122:306–14. doi: 10.1038/s41416-019-0602-7
2. Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tan PF, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med.* (2010) 363:24–35. doi: 10.1056/NEJMoa0912217
3. Wondergem NE, Nauta IH, Muijlwijk T, Leemans CR, van de Ven R. The immune microenvironment in head and neck squamous cell carcinoma: on subsets and subsites. *Curr Oncol Rep.* (2020) 22:81. doi: 10.1007/s11912-020-00938-3
4. Jochems C, Schlom J. Tumor-infiltrating immune cells and prognosis: the potential link between conventional cancer therapy and immunity. *Exp Biol Med (Maywood).* (2011) 236:567–79. doi: 10.1258/ebm.2011.011007
5. Nasman A, Romanitan M, Nordfors C, Grun N, Johansson H, Hammarstedt L, et al. Tumor infiltrating CD8+ and Foxp3+ lymphocytes correlate to clinical outcome and human papillomavirus (HPV) status in tonsillar cancer. *PLoS ONE.* (2012) 7:e38711. doi: 10.1371/journal.pone.0038711
6. Ward MJ, Thirdborough SM, Mellows T, Riley C, Harris S, Suchak K, et al. Tumour-infiltrating lymphocytes predict for outcome in HPV-positive oropharyngeal cancer. *Br J Cancer.* (2014) 110:489–500. doi: 10.1038/bjc.2013.639
7. Oguejiofor K, Galletta-Williams H, Dovedi SJ, Roberts DL, Stern PL, West CM. Distinct patterns of infiltrating CD8+ T cells in HPV+ and CD68 macrophages in HPV- oropharyngeal squamous cell carcinomas are associated with better clinical outcome but PD-L1 expression is not prognostic. *Oncotarget.* (2017) 8:14416–27. doi: 10.18632/oncotarget.14796
8. Trellakis S, Bruderek K, Dumitru CA, Gholaman H, Gu X, Bankfalvi A, et al. Polymorphonuclear granulocytes in human head and neck cancer: enhanced inflammatory activity, modulation by cancer cells and expansion in advanced disease. *Int J Cancer.* (2011) 129:2183–93. doi: 10.1002/ijc.25892
9. Alves AM, Diel LF, Lamers ML. Macrophages and prognosis of oral squamous cell carcinoma: a systematic review. *J Oral Pathol Med.* (2018) 47:460–7. doi: 10.1111/jop.12643
10. Mantovani A, Locati M, Allavena P, Sozzani S. The chemokine superfamily: crosstalk with the IL-1 system. *Immunobiology.* (1996) 195:522–49. doi: 10.1016/S0171-2985(96)80020-9
11. Hughes CE, Nibbs RJB. A guide to chemokines and their receptors. *FEBS J.* (2018) 285:2944–71. doi: 10.1111/febs.14466
12. Raz Y, Erez N. An inflammatory vicious cycle: fibroblasts and immune cell recruitment in cancer. *Exp Cell Res.* (2013) 319:1596–603. doi: 10.1016/j.yexcr.2013.03.022
13. Al-Sahaf S, Hunter KD, Bolt R, Ottewell PD, Murdoch C. The IL-1/IL-1R axis induces greater fibroblast-derived chemokine release in human papillomavirus-negative compared to positive oropharyngeal cancer. *Int J Cancer.* (2019) 144:334–44. doi: 10.1002/ijc.31852
14. Hendawi N, Niklander S, Allsobrook O, Khurram SA, Bolt R, Doorbar J, et al. Human papillomavirus (HPV) can establish productive infection in dysplastic oral mucosa, but HPV status is poorly predicted by histological features and p16 expression. *Histopathology.* (2020) 76:592–602. doi: 10.1111/his.14019
15. Budwit-Novotny DA, McCarty KS, Cox EB, Soper JT, Mutch DG, Creasman WT, et al. Immunohistochemical analyses of estrogen receptor in endometrial adenocarcinoma using a monoclonal antibody. *Cancer Res.* (1986) 46:5419–25.
16. Bankhead P, Loughrey MB, Fernandez JA, Dombrowski Y, McArt DG, Dunne PD, et al. QuPath: open source software for digital pathology image analysis. *Sci Rep.* (2017) 7:16878. doi: 10.1038/s41598-017-17204-5
17. Grayson AK, Hearnden V, Bolt R, Jebreel A, Colley HE, Murdoch C. Use of a Rho kinase inhibitor to increase human tonsil keratinocyte longevity for three-dimensional, tissue engineered tonsil epithelium equivalents. *J Tissue Eng Regen Med.* (2018) 12:e1636–46. doi: 10.1002/term.2590
18. Tazzyman S, Barry ST, Ashton S, Wood P, Blakey D, Lewis CE, et al. Inhibition of neutrophil infiltration into A549 lung tumors *in vitro* and *in vivo* using a CXCR2-specific antagonist is associated with reduced tumor growth. *Int J Cancer.* (2011) 129:847–58. doi: 10.1002/ijc.25987
19. Colley HE, Hearnden V, Jones AV, Weinreb PH, Violette SM, Macneil S, et al. Development of tissue-engineered models of oral dysplasia and early invasive oral squamous cell carcinoma. *Br J Cancer.* (2011) 105:1582–92. doi: 10.1038/bjc.2011.403
20. Brierly JD, Gospodarowicz MK, Wittekind C, editors. *OPSCC were Defined in Accordance with the TNM Classification of Malignant Tumours.* 8th ed. Hoboken NJ: Wiley Blackwell (2016).
21. Barros MR, Jr., de Melo CML, Barros M, de Cassia Pereira de Lima R, de Freitas AC, Venuti A. Activities of stromal and immune cells in HPV-related cancers. *J Exp Clin Cancer Res.* (2018) 37:137. doi: 10.1186/s13046-018-0802-7
22. Fakhry C, Westra WH, Li S, Cmelak A, Ridge JA, Pinto H, et al. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. *J Natl Cancer Inst.* (2008) 100:261–9. doi: 10.1093/jnci/djn011
23. Chakravarthy A, Henderson S, Thirdborough SM, Ottensmeier CH, Su X, Lechner M, et al. Human papillomavirus drives tumor development throughout the head and neck: improved prognosis is associated with an immune response largely restricted to the oropharynx. *J Clin Oncol.* (2016) 34:4132–41. doi: 10.1200/JCO.2016.68.2955
24. Li C, Zhao L, Wang Q, Ma S, Sun J, Ma C, et al. Neutrophils infiltration and its correlation with human papillomavirus status in the oral squamous cell carcinoma. *Cancer Manag Res.* (2019) 11:5171–85. doi: 10.2147/CMAR.S202465
25. Lopes V, Murray P, Williams H, Woodman C, Watkinson J, Robinson M. Squamous cell carcinoma of the oral cavity rarely harbours oncogenic human papillomavirus. *Oral Oncol.* (2011) 47:698–701. doi: 10.1016/j.oraloncology.2011.04.022
26. Chen X, Yan B, Lou H, Shen Z, Tong F, Zhai A, et al. Immunological network analysis in HPV associated head and neck squamous cancer and implications for disease prognosis. *Mol Immunol.* (2018) 96:28–36. doi: 10.1016/j.molimm.2018.02.005
27. Sandhu JK, Privora HF, Wenckebach G, Birnboim HC. Neutrophils, nitric oxide synthase, and mutations in the mutatect murine tumor model. *Am J Pathol.* (2000) 156:509–18. doi: 10.1016/S0002-9440(10)64755-4
28. Jablonska E, Puzewska W, Grabowska Z, Jablonski J, Talarek L. VEGF, IL-18 and NO production by neutrophils and their serum levels in patients with oral cavity cancer. *Cytokine.* (2005) 30:93–9. doi: 10.1016/j.cyto.2004.12.004
29. Bekes EM, Schweighofer B, Kupriyanova TA, Zajac E, Ardi VC, Quigley JP, et al. Tumor-recruited neutrophils and neutrophil TIMP-free MMP-9 regulate coordinately the levels of tumor angiogenesis and efficiency of malignant cell intravasation. *Am J Pathol.* (2011) 179:1455–70. doi: 10.1016/j.ajpath.2011.05.031
30. Dumitru CA, Moses K, Trellakis S, Lang S, Brandau S. Neutrophils and granulocytic myeloid-derived suppressor cells: immunophenotyping, cell biology and clinical relevance in human oncology. *Cancer Immunol Immunother.* (2012) 61:1155–67. doi: 10.1007/s00262-012-1294-5
31. Vasquez-Dunddel D, Pan F, Zeng Q, Gorbounov M, Albesiano E, Fu J, et al. STAT3 regulates arginase-I in myeloid-derived suppressor cells from cancer patients. *J Clin Invest.* (2013) 123:1580–9. doi: 10.1172/JCI60083
32. Greene S, Robbins Y, Mydlarz WK, Huynh AP, Schmitt NC, Friedman J, et al. Inhibition of MDSC Trafficking with SX-682, a CXCR1/2 Inhibitor, Enhances NK-Cell Immunotherapy in Head and Neck Cancer Models. *Clin Cancer Res.* (2020) 26:1420–31. doi: 10.1158/1078-0432.CCR-19-2625
33. Seminerio I, Kindt N, Descamps G, Bellier J, Lechien JR, Mat Q, et al. High infiltration of CD68+ macrophages is associated with poor prognoses of head and neck squamous cell carcinoma patients and is influenced by human papillomavirus. *Oncotarget.* (2018) 9:11046–59. doi: 10.18632/oncotarget.24306
34. Russell S, Angell T, Lechner M, Liebertz D, Correa A, Sinha U, et al. Immune cell infiltration patterns and survival in head and neck squamous cell carcinoma. *Head Neck Oncol.* (2013) 5:24.
35. Gooden MJ, de Bock GH, Leffers N, Daemen T, Nijman HW. The prognostic influence of tumour-infiltrating lymphocytes in cancer: a systematic review with meta-analysis. *Br J Cancer.* (2011) 105:93–103. doi: 10.1038/bjc.2011.189
36. Patel JJ, Levy DA, Nguyen SA, Knochelmann HM, Day TA. Impact of PD-L1 expression and human papillomavirus status in anti-PD1/PDL1 immunotherapy for head and neck squamous cell carcinoma-systematic review and meta-analysis. *Head Neck.* (2020) 42:774–86. doi: 10.1002/hed.26036

37. Wansom D, Light E, Thomas D, Worden F, Prince M, Urba S, et al. Infiltrating lymphocytes and human papillomavirus-16-associated oropharyngeal cancer. *Laryngoscope.* (2012) 122:121–7. doi: 10.1002/lary.22133
38. Albers A, Abe K, Hunt J, Wang J, Lopez-Albaitero A, Schaefer C, et al. Antitumor activity of human papillomavirus type 16 E7-specific T cells against virally infected squamous cell carcinoma of the head and neck. *Cancer Res.* (2005) 65:11146–55. doi: 10.1158/0008-5472.CAN-05-0772
39. Welters MJP, Ma W, Santegoets S, Goedemans R, Ehsan I, Jordanova ES, et al. Intratumoral HPV16-Specific T cells constitute a Type I-oriented tumor microenvironment to improve survival in HPV16-driven oropharyngeal cancer. *Clin Cancer Res.* (2018) 24:634–47. doi: 10.1158/1078-0432.CCR-17-2140
40. Distel LV, Fickenscher R, Dietel K, Hung A, Iro H, Zenk J, et al. Tumour infiltrating lymphocytes in squamous cell carcinoma of the oro- and hypopharynx: prognostic impact may depend on type of treatment and stage of disease. *Oral Oncol.* (2009) 45:e167–174. doi: 10.1016/j.oraloncology.2009.05.640
41. Partlova S, Boucek J, Kloudova K, Lukesova E, Zabrodsky M, Grega M, et al. Distinct patterns of intratumoral immune cell infiltrates in patients with HPV-associated compared to non-virally induced head and neck squamous cell carcinoma. *Oncoimmunology.* (2015) 4:e965570. doi: 10.4161/21624011.2014.965570
42. Wood O, Woo J, Seumois G, Savelyeva N, McCann KJ, Singh D, et al. Gene expression analysis of TIL rich HPV-driven head and neck tumors reveals a distinct B-cell signature when compared to HPV independent tumors. *Oncotarget.* (2016) 7:56781–97. doi: 10.18632/oncotarget.10788
43. Templeton AJ, McNamara MG, Seruga B, Vera-Badillo FE, Aneja P, Ocana A, et al. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. *J Natl Cancer Inst.* (2014) 106:dju124. doi: 10.1093/jnci/dju124
44. Yang L, Huang Y, Zhou L, Dai Y, Hu G. High pretreatment neutrophil-to-lymphocyte ratio as a predictor of poor survival prognosis in head and neck squamous cell carcinoma: Systematic review and meta-analysis. *Head Neck.* (2019) 41:1525–35. doi: 10.1002/hed.25583
45. Fanetti G, Alterio D, Marvaso G, Gandini S, Rojas DP, Gobitti C, et al. Prognostic significance of neutrophil-to-lymphocyte ratio in HPV status era for oropharyngeal cancer. *Oral Dis.* (2020) 26:1384–92. doi: 10.1111/odi.13366
46. So YK, Lee G, Oh D, Byeon S, Park W, Chung MK. Prognostic role of neutrophil-to-lymphocyte ratio in patients with human papillomavirus-positive oropharyngeal cancer. *Otolaryngol Head Neck Surg.* (2018) 159:303–9. doi: 10.1177/0194599818764651
47. Huang SH, Waldron JN, Milosevic M, Shen X, Ringash J, Su J, et al. Prognostic value of pretreatment circulating neutrophils, monocytes, and lymphocytes in oropharyngeal cancer stratified by human papillomavirus status. *Cancer.* (2015) 121:545–55. doi: 10.1002/cncr.29100
48. Rosculet N, Zhou XC, Ha P, Tang M, Levine MA, Neuner G, et al. Neutrophil-to-lymphocyte ratio: Prognostic indicator for head and neck squamous cell carcinoma. *Head Neck.* (2017) 39:662–7. doi: 10.1002/hed.24658
49. De Felice F, Tombolini M, Abate G, Salerno F, Bulzonetti N, Tombolini V, et al. Prognostic significance of the neutrophil/lymphocyte ratio in patients with non-human papilloma virus-related oropharyngeal cancer: a retrospective cohort study. *Oncology.* (2019) 96:8–13. doi: 10.1159/000492389
50. Rachidi S, Wallace K, Wrangle JM, Day TA, Alberg AJ, Li Z. Neutrophil-to-lymphocyte ratio and overall survival in all sites of head and neck squamous cell carcinoma. *Head Neck.* (2016) 38(Suppl. 1):E1068–74. doi: 10.1002/hed.24159
51. St John MA, Li Y, Zhou X, Denny P, Ho CM, Montemagno C, et al. Interleukin 6 and interleukin 8 as potential biomarkers for oral cavity and oropharyngeal squamous cell carcinoma. *Arch Otolaryngol Head Neck Surg.* (2004) 130:929–35. doi: 10.1001/archotol.130.8.929
52. Bolt R, Foran B, Murdoch C, Lambert DW, Thomas S, Hunter KD. HPV-negative, but not HPV-positive, oropharyngeal carcinomas induce fibroblasts to support tumour invasion through micro-environmental release of HGF and IL-6. *Carcinogenesis.* (2018) 39:170–9. doi: 10.1093/carcin/bgx130
53. Hirschhaeuser F, Menne H, Dittfeld C, West J, Mueller-Klieser W, Kunz-Schughart LA. Multicellular tumor spheroids: an underestimated tool is catching up again. *J Biotechnol.* (2010) 148:3–15. doi: 10.1016/j.jbiotec.2010.01.012
54. Schilling V, Negri B, Bujia J, Schulz P, Kastenbauer E. Possible role of interleukin 1 alpha and interleukin 1 beta in the pathogenesis of cholesteatoma of the middle ear. *Am J Otol.* (1992) 13:350–5.
55. Xu Q, Izumi K, Tobita T, Nakanishi Y, Feinberg SE. Constitutive release of cytokines by human oral keratinocytes in an organotypic culture. *J Oral Maxillofac Surg.* (2009) 67:1256–64. doi: 10.1016/j.joms.2009.02.003
56. Niebler M, Qian X, Hofler D, Kogosov V, Kaewprag J, Kaufmann AM, et al. Post-translational control of IL-1beta via the human papillomavirus type 16 E6 oncoprotein: a novel mechanism of innate immune escape mediated by the E3-ubiquitin ligase E6-AP and p53. *PLoS Pathog.* (2013) 9:e1003536. doi: 10.1371/journal.ppat.1003536
57. Wu T, Hong Y, Jia L, Wu J, Xia J, Wang J, et al. Modulation of IL-1beta reprogrammes the tumor microenvironment to interrupt oral carcinogenesis. *Sci Rep.* (2016) 6:20208. doi: 10.1038/srep20208
58. Stanam A, Gibson-Corley KN, Love-Homan L, Ihejirika N, Simons AL. Interleukin-1 blockade overcomes erlotinib resistance in head and neck squamous cell carcinoma. *Oncotarget.* (2016) 7:76087–100. doi: 10.18632/oncotarget.12590
59. Watari K, Shibata T, Kawahara A, Sata K, Nabeshima H, Shinoda A, et al. Tumor-derived interleukin-1 promotes lymphangiogenesis and lymph node metastasis through M2-type macrophages. *PLoS ONE.* (2014) 9:e99568. doi: 10.1371/journal.pone.0099568
60. Holen I, Lefley DV, Francis SE, Rennicks S, Bradbury S, Coleman RE, et al. IL-1 drives breast cancer growth and bone metastasis *in vivo*. *Oncotarget.* (2016) 7:75571–84. doi: 10.18632/oncotarget.12289
61. Eyre R, Alferez DG, Santiago-Gomez A, Spence K, McConnell JC, Hart C, et al. Microenvironmental IL1beta promotes breast cancer metastatic colonisation in the bone via activation of Wnt signalling. *Nat Commun.* (2019) 10:5016. doi: 10.1038/s41467-019-12807-0
62. Tulotta C, Lefley DV, Freeman K, Gregory WM, Hanby AM, Heath PR, et al. Endogenous production of IL1B by breast cancer cells drives metastasis and colonization of the bone microenvironment. *Clin Cancer Res.* (2019) 25:2769–82. doi: 10.1158/1078-0432.CCR-18-2202
63. Dinarello CA. Why not treat human cancer with interleukin-1 blockade? *Cancer Metastasis Rev.* (2010) 29:317–29. doi: 10.1007/s10555-010-9229-0
64. Ridker PM, MacFadyen JG, Thuren T, Everett BM, Libby P, Glynn RJ, et al. Effect of interleukin-1beta inhibition with canakinumab on incident lung cancer in patients with atherosclerosis: exploratory results from a randomised, double-blind, placebo-controlled trial. *Lancet.* (2017) 390:1833–42. doi: 10.1016/S0140-6736(17)32247-X

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oral Health

MINI REVIEW
published: 22 February 2021
doi: 10.3389/froh.2021.642238

Inflammatory Mediators in Oral Cancer: Pathogenic Mechanisms and Diagnostic Potential

*Sven E. Niklander**

Unidad de Patologia y Medicina Oral, Facultad de Odontologia, Universidad Andres Bello, Viña del Mar, Chile

Approximately 15% of cancers are attributable to the inflammatory process, and growing evidence supports an association between oral squamous cell carcinoma (OSCC) and chronic inflammation. Different oral inflammatory conditions, such as oral lichen planus (OLP), submucous fibrosis, and oral discoid lupus, are all predisposing for the development of OSCC. The microenvironment of these conditions contains various transcription factors and inflammatory mediators with the ability to induce proliferation, epithelial-to-mesenchymal transition (EMT), and invasion of genetically predisposed lesions, thereby promoting tumor development. In this review, we will focus on the main inflammatory molecules and transcription factors activated in OSCC, with emphasis on their translational potential.

Keywords: oral cancer, inflammation, oral carcinogenesis, biomarker, OPMD

OPEN ACCESS

Edited by:
Ricardo D. Coletta, Campinas State University, Brazil

Reviewed by:
Rogelio González-González, Juárez University of the State of Durango, Mexico
Mariana Villarroel-Dorrego, Central University of Venezuela, Venezuela

***Correspondence:**
Sven E. Niklander
sven.niklander@unab.cl

Specialty section:
This article was submitted to Oral Cancers, a section of the journal Frontiers in Oral Health

Received: *15 December 2020*
Accepted: *13 January 2021*
Published: *22 February 2021*

Citation:
Niklander SE (2021) Inflammatory Mediators in Oral Cancer: Pathogenic Mechanisms and Diagnostic Potential. Front. Oral. Health 2:642238. doi: 10.3389/froh.2021.642238

INTRODUCTION

The links between cancer and chronic inflammation are well-established. In this process, pro-inflammatory mediators act by stimulating inflammation to either abrogate tumor progression or facilitate tumor growth and metastasis [1, 2]. Approximately 15% of cancers are attributable to the inflammatory process [3]. Such cancers include lung, pancreatic, esophageal, bladder, gastric, cervical, colorectal, and prostate [4]. During the oral malignant transformation process [from non-dysplastic hyperkeratosis, through oral dysplasia to the development of oral squamous cell carcinoma (OSCC)], there is a progressive increase of the inflammatory infiltrate (quality and density) [5], and growing evidence supports an association between OSCC and chronic inflammation [6, 7]. Different oral inflammatory conditions, such as OLP, submucous fibrosis, and oral discoid lupus, are all predisposing for the development of OSCC [8]. The microenvironment of these conditions contains activated cytokines and chemokines, prostaglandins, reactive oxygen species, and various transcription factors. Some of these mediators have the ability to induce proliferation, epithelial-to-mesenchymal transition (EMT), and invasion [9] of genetically predisposed lesions (with mutations in tumor-suppressor genes and/or oncogenes), thereby promoting tumor development. In established OSCC, chronic inflammation is also a common feature, being involved in tumor progression, invasion, and metastasis [5, 6, 10]. Thus, many studies have assessed the utility of different inflammatory molecules as prognostic biomarkers and treatment targets for OSCC.

The aim of this review is to outline the main inflammatory molecules and transcription factors activated in OSCC, with special focus on their translational potential.

INFLAMMATORY MEDIATORS INVOLVED IN ORAL CARCINOGENESIS

There are different inflammatory mediators reported to have a role in the development and progression of OSCC (**Table 1**). In this review, we will focus on the most commonly investigated.

NF-κB

Nuclear factor kappa-beta (NF-κB) is a key inflammatory transcription factor frequently expressed in tumors that regulates the expression of a variety of genes involved in inflammation, proliferation, tumorigenesis, and cell survival [47–49]. NF-κB is canonically activated by tumor necrosis factor alpha (TNF-α), interleukin (IL)-1, and lipopolysaccharide (LPS) [48], and when activated, it enhances the expression of different cytokines, including IL-1, IL-6, and IL-8 [50]. Its aberrant expression is linked to carcinogenesis [51] and EMT induction [52] and is associated with worse survival in solid cancers [53]. In OSCC, NF-κB is constitutively activated and is associated with the upregulation of different inflammatory genes, including *IL-6*, *IL-8*, *CCL5*, and *CXCL10* [6], and is considered a major factor responsible for the inflammatory infiltrate observed in the tumor microenvironment (TME) [54]. NF-κB has an important role in the malignant phenotype of oral cancers, as it participates in the modulation of bone invasion [48]; enhances angiogenesis [55], invasion [40, 56], and metastasis [40, 41]; and induces EMT [52]. The EMT process is a crucial step for the development of OSCC metastasis [57]. It involves the repression of E-cadherin (an important epithelial adhesion molecule) *via* Snail expression, which depends on NF-κB activation *via* AKT [52]. IL-8 and EGF are able to induce EMT *via* NF-κB activation, and IL-8- and EGF-induced EMT can be reversed by blocking AKT or NF-κB [58, 59]. NF-κB importance during OSCC development is well-exemplified by its inactivation, as this inhibits cell survival and growth; expressions of IL-1α, IL-6, and IL-8 [60]; and metastases [41]. Because of this, NF-κB inhibition has been proposed as a possible treatment for head and neck squamous cell carcinoma (HNSCC).

TABLE 1 | Inflammatory pathways and mediators reported to have a role in oral carcinogenesis.

Pro-inflammatory cytokines	References
IL-1	[7, 9, 11, 12]
IL-1R1	[13]
IL-6	[6, 11, 14]
IL-8	[6, 11, 14, 15]
TNF-α	[5, 11, 16]
TGF-β	[17–19]
Immunosuppresive Cytokines	
IL-1RA	[20, 21]
IL-2	[22]
IL-4	[22, 23]
IL-10	[24–26]
IL-12	[27]
IL-13	[24]
IFN-γ	[14, 28]
Chemokines	
CCL	[29]
CXCL1	[30]
CXCL2	[29, 31]
CCL4	[32]
CCL7	[33]
CXCL3	[29]
CXCL10	[34]
CXCL12	[31]
CXCR1	[35, 36]
CXCR2	[36]
Transcription factros	
AP-1	[37–39]
NF-kB	[6, 40–42]
Enzymes	
COX2	[43–45]
Prostanoids	
PGE2	[46]

AP-1 Pathway

Activator protein 1 (AP-1) is a transcription factor complex composed of either homodimers of Jun protein or heterodimers of Jun and Fos proteins [61] that orchestrates the expression of different genes involved in inflammation, embryonic development, lymphoid proliferation, oncogenesis, and apoptosis [62] and is reported to be essential for DNA synthesis [63]. AP-1 activation seems to be of clinical significance in cancer, as high expression levels have been associated with drug resistance [64]. AP-1 is activated during oral keratinocyte carcinogenesis [37], and its expression increases with oral tumor progression [38]. Similar to NF-κB, it can also be activated by IL-1 [65], which induces IL-8 secretion and promotes the cell survival and growth of HNSCC cells [39]. A recent study suggested that AP-1 induces bcl-2 expression (a proto-oncogene related to apoptosis suppression implicated in resistance to chemoradiation therapy in OSCC) in recurrent chemo- and radioresistant oral tumors [38], which suggests that targeting the AP-1 pathway might contribute to overcome resistance to chemoradiation therapy in OSCC. As AP-1 can be activated by IL-1, targeting IL-1 could be beneficial for oral cancer treatment, as this could reduce the activation of NF-κB and AP-1 pathways with subsequent reduction of bcl-2, but this hypothesis needs to be corroborated.

TNF-α

TNF-α is a multifunctional cytokine identified as an important mediator of cancer development [66], as well as induces EMT [67] and enhances tumor angiogenesis [68] and invasion [56]. Therefore, it is considered an important regulator of proliferation, invasion, and metastasis of many cancers [69]. TNF-α is expressed in some oral potentially malignant disorders (OPMDs), such as OLP [70], and is endogenously expressed in oral carcinomas [71]. In OSCC, TNF-α promotes a pro-invasive and pro-inflammatory phenotype in a paracrine manner

[5, 16] by upregulating genes that are associated with neutrophil recruitment, invadopodia, and invasion. The upregulation of these genes was also associated with reductions in both the overall survival and disease-free survival of patients with OSCC [5]. Elevated TNF-α receptor-1 (TNFR-1) signaling has also been associated with metastasis of OSCC [41, 72], attributable to the ability of TNF-α to stimulate the invasion of OSCC cells by enhancing matrix metalloproteinase (MMP)-2 and MMP-9 production [41, 56], which is regulated by the NF-κB, AKT, and PI3K signaling pathways [5, 41, 72]. It has been demonstrated that MMPs play critical roles in OSCC, as elevated expression of MMPs is associated with increased OSCC invasion, metastasis, and poor prognosis [73–75]. MMPs are also reported to have a prominent role in the EMT process [76], as MMP-2, MMP-9, and MMP-7 are able to enhance EMT [77–79]. NF-κB is an important regulator of MMPs [80], and TNF-α can activate NF-κB *via* TNFR1, which results in enhanced MMP secretion [81]. TNF-α is also able to induce EMT *via* p38 MAPK activation [82], and TNF-α-induced EMT has been related to the induction of cancer stem cells (CSCs) [67]. The presence of CSCs in OSCC is of importance as this has been linked to therapy resistance and worst prognosis. In OSCC, the expression of CD44 (a well-known CSC marker) has been associated with increased cell invasion, cell migration, and therapy resistance *via* a mechanism that includes the activation of PI3K/Akt/GSK3β and Raf-MEK-ERK signaling networks [83], and silencing CD47 (a molecule involved in the generation of CSCs in OSCC) has shown to reduce EMT and the presence of CSCs [84]. The activation of the PI3K/Akt/GSK3β signaling pathway has also been associated with increased proliferation, invasion [85], the development of EMT, and distant metastasis [86].

Due to all the beneficial effects of TNF-α during cancer development, targeting TNF-α might be a useful strategy for OSCC treatment. *In vitro* experiments have shown anti-TNF-α therapy to reduce growth and metastasis of OSCC cells [87], but this needs to be investigated further before it can be translated into the clinic.

IL-6 and IL-8

Both IL-6 and IL-8 are considered "oncogenic cytokines," as they are able to cause EMT [88], stimulate angiogenesis and tumor growth [89, 90], disrupt cell–cell communication, impede macrophage function, and promote epithelial and endothelial cell migration and invasion [91]. IL-6 and IL-8 levels are elevated in patients with OPMDs and OSCCs [6, 14, 92, 93], which is likely to be a consequence of aberrant NF-κB activation [94]. IL-6 and IL-8 can be produced by malignant oral keratinocytes themselves, or by other cells of the TME, such as tumor-associated macrophages (TAMs). TAMs are an important source of both of these cytokines, and thus many attempts have been done to target TAMs to restrict their secretion [95]. IL-8 is reported to act as an autocrine growth factor in HNSCC and other cancers [15] and has been proposed as a potential mediator of the development of OSCC. It is constitutively expressed in malignant oral keratinocytes, and its inhibition decreases viability, proliferation [6], and invasion of OSCC cells [16] and enhances the proliferation, angiogenesis, and survival rate of cancer cells [96]. Similarly, IL-6 overexpression in patients with HNSCC is associated with poor prognosis, probably by enabling an immunosuppressive TME by increasing the presence of myeloid-derived suppressor cells and PDL-1 expression, and is considered a significant predictor of treatment outcome [97]. In OSCC, the expressions of IL-6 and IL-8 are associated with a more invasive mode of growth [11].

IL-1 Family Members

IL-1 is the prototype of a pro-inflammatory cytokine and includes IL-1α and IL-1β. IL-1α and IL-1β are both constitutively expressed in OSCC [9, 12, 98, 99], can be found in the saliva of patients with OSCC [100, 101], and have been reported to have important functions in OSCC carcinogenesis and tumor progression [7, 10].

IL-1α expressed by OSCC promotes autocrine activation of NF-κB and AP-1 and upregulates the expression of IL-8 [39] and IL-6 [98]. OSCC cells produce IL-1α [12], which induces the proliferation and cytokine secretion by cancer-associated fibroblasts (CAFs) (CCL7, CXCL1, and IL-8), promoting tumor progression [33]. IL-1α seems to be important for the development of distant metastases, as IL-1α is highly expressed in metastatic HNSCC tumors compared to non-metastatic HNSCC tumors. This is probably achieved by the capability of IL-1α to induce transmigration of tumor cells across the endothelium and to enhance the expression of metastatic genes, such as *MMP*-9, *PGE2*, *VEGF*, and *IL-8* [10]. It has also been reported that IL-1α can act as an oncoprotein by itself, as intranuclear IL-1α has been shown to induce malignant transformation of cells from the bone marrow and perivascular area [102].

IL-1β increases the levels of IL-6 and IL-8 expressed by OD and OSCC cells and promotes the invasiveness of OSCC by inducing EMT [9]. IL-1β has also been identified as a key node gene in the TME of OSCC *in vivo* [7]. The expression of precursor IL-1β mRNA is correlated with the presence of malignant changes (from normal, to mild, through severe dysplasia to OSCC) [9], and elevated IL-1β expression has been related to lymph node metastasis of OSCC [103]. IL-1 produced by HNSCC can also stimulate the production of different cytokines by CAFs and normal fibroblasts, such as CCL-7, CXCL1, IL-8, and CCL-5 [33, 104]. These findings strongly suggest a possible role of IL-1β in the oral carcinogenesis process, which is supported by the fact that IL-1β silencing can reduce tumor size *in vivo* [7].

The IL-1 agonist receptor, IL-1R1, is also overexpressed in OSCCs and, together with IL-1β, was shown to promote cancer growth and metastasis by upregulating CXCR4, which could be reversed by inhibiting IL-1R1 by overexpressing the interleukin 1 receptor antagonist (IL-1RA) [13]. Interestingly, IL-1RA has been reported to be downregulated in OD and in OSCC [20] and is reported to regulate IL-1-induced secretion of IL-6 and IL-8 by inhibiting the p38 MAPK and NF-κB pathways [50, 105].

COX-2

Cyclooxygenase (COX)-2, an inflammation-induced enzyme that converts arachidonic acid into prostaglandins [e.g., prostaglandin E2 (PGE2)], is frequently expressed in many types of cancers. COX-2 is able to induce CSC-like activity and to promote

angiogenesis, proliferation, apoptotic resistance, inflammation, invasion, and metastasis of cancer cells [106]. Importantly, COX-2 inhibition has been shown to reverse cancer progression [107, 108]. COX-2 is induced by a variety of molecules, including IL-1 [109], epithelial growth factor (EGF) [110], transforming growth factor-beta (TGF-β) [111, 112], and TNF-α. COX-2 expression is induced early in the process of oral carcinogenesis [43]. Its level is associated with the degree of dysplasia [44], is overexpressed in OSCC [113, 114], and is correlated with advanced tumor stage, high risk of distant metastasis [115], and worse prognosis in patients with OSCC [116]. In oral cancer, COX-2 is of importance for maintaining a chronic inflammatory state [117], influencing different processes, such as cell migration by upregulating the expression of intercellular adhesion molecule-1 (ICAM-1) *via* PGE2 [46], and lymphoangiogenesis by regulating VEGF production [45, 118, 119]. VEGF is commonly overexpressed in OSCC, and COX-2/VEGF-C co-expression is correlated with lymphoangiogenesis, lymph node metastasis, and TNM stage and is reported as an independent factor for survival [45]. Increased VEGF expression in oral cancer is also a consequence of tumor-associated hypoxia, as VEGF is upregulated in decreasing concentrations of oxygen [120, 121]. In reduced oxygen concentrations, hypoxia-inducible factor-1α (HIF-1α) binds to hypoxia response elements and upregulates *VEGF*, promoting angiogenesis [122]. In OSCC, HIF-1α and HIF-2α correlate positively with clinical–pathological parameters, such as tumor size and micro vessel density, and *in vivo* experiments have shown their knockdown to reduce tumor angiogenesis and tumor growth [123]. In addition, hypoxia will also promote an inflammatory state, as VEGF is able to induce COX-2 expression, which will result in the production of PGE2 and the activation of NF-κB [124].

TGF-β

TGF-β is a multifunctional pro-inflammatory cytokine that can either inhibit or promote tumor formation and progression of many cancers, whether by inducing apoptosis and growth arrest and by inhibiting proliferation or by stimulating angiogenesis, inflammation, EMT, and immune suppression, respectively [125]. If TGF-β acts as a tumor suppressor or tumor promoter depends on the regional and cellular context [126]. In OSCC, TGF-β is reported to promote tumorigenesis [17]. This is supported by the fact that OPMDs [18] and OSCC [19, 127] express higher levels of TGF-β than healthy controls and that high TGF-β levels are associated with disease recurrence and poor prognosis in patients with OSCC [19]. There are different mechanisms by which TGF-β could act as a tumor promoter in oral cancer. TGF-β is probably the most important factor involved in the differentiation of CAFs, and the accumulation of CAFs is reported as an independent prognostic factor in OSCC. CAFs are able to modulate the TME, facilitating cancer progression [128]. An *in vivo* mouse model in which TGF-β1 was transgenically induced revealed that TGF-β1 induced epithelia hyperproliferation, severe inflammation, and angiogenesis at similar levels to those observed in HNSCCs, suggesting that TGF-β1 provides a tumor-promoting microenvironment [17]. In OPMDs, TGF-β promotes a more malignant phenotype by increasing cell motility *via* the protein phosphatase 1 (PP-1) signaling pathway [129]. TGF-β seems to be important for EMT development, as TGF-β induces EMT in endothelial cells, and endothelial cells cultured with TGF-β are able to induce EMT in OSCC cells [130]. Also, TGF-β is able to induce EMT by stimulating the expression of ADAM12 (a desintegrin and metalloprotease associated with cancers) [131].

Immunosuppressive Cytokines

Anti-inflammatory cytokines also have a role in the oral carcinogenesis process. They can act as a double-edged sword, they can counteract the tumorigenic potential of their pro-inflammatory counterpart, and they can act as immunosuppressive molecules by decreasing the anti-tumor immune response [132]. Different immunosuppressive cytokines have been reported to have different roles in OSCC development (**Table 1**), with IL-1RA, IL-4, IL-10, and IL-13 being the most commonly investigated. IL-1RA decreases during the oral carcinogenesis process [20] and *IL1RN* (the gene that codes for IL-1RA) is downregulated in HNSCC [133, 134]. This in theory would allow higher IL-1 activity with the aforementioned effects. Nevertheless, high IL1-RA expression has been reported in advanced and poorly differentiated OSCCs [20, 24], suggesting that IL-1RA expression could increase tumor progression, but this needs further investigation. IL-4, IL-10, and IL-13 have also been reported to increase in OSCC patients compared to healthy controls [24, 25, 135], and high IL-10 expression has been associated with a more aggressive OSCC phenotype [136]. IL-4 induces immune deviation from T_H1 to T_H2 responses, which prevents tumor rejection [137]; IL-10 suppresses the anti-tumor immunity and contributes to tumor immune escape [135] and IL-13 compromises the anti-tumor response by inhibiting IFN-γ secretion and CD8+ T lymphocyte activity [138].

SOURCES OF INFLAMMATION

Within the TME, cancer cells are not the only source of inflammatory molecules. Tumors are complex systems composed not only of neoplastic cells but also of stromal cells, which form the TME. These cells are not innocent bystanders and can interact with tumor cells and modify the extracellular matrix, facilitating and promoting proliferation, invasion, angiogenesis, and metastasis [49, 139]. Single cell profile of 5,578 samples obtained from 18 OSCCs revealed the presence of nine clusters of cells based on known marker genes, such as epithelial cells (malignant and non-malignant, based on CNV and their karyotypes), T cells (four sub-clusters), B/plasma cells, macrophages, dendritic cells, mast cells, endothelial cells, fibroblasts (three sub-clusters), and myocytes [140], which shows how heterogeneous tumors are.

As discussed previously, malignant oral keratinocytes (which are genetically unstable) activate different transcription factors, such as AP-1 and NF-κB, leading to the activation of oncogenes (which induces proliferation by the regulation of apoptosis, angiogenesis, and cell growth), and inflammatory genes [6]. The latter results in the constitutive production of different inflammatory factors, such as IL-1, IL-6, IL-8, TNF-α, and

TGF-β [141], among many others, that can activate the same transcription factors, creating a positive feedback loop.

TAMs, T lymphocytes, neutrophils, and mast cells are also considered important sources of inflammatory mediators [5]. TAMs promote proliferation and invasion in OSCC [142, 143], and their presence correlates with disease progression and is considered an adverse prognostic factor [144]. They act as an important source of cytokines, metalloproteinases, and growth factors [95]. T lymphocytes are considered the most abundant inflammatory cells among the inflammatory infiltrate observed in OSCCs and can be beneficial or detrimental for oral carcinogenesis depending on their secretion profile [145]. They can secrete molecules that favor tumor progression (IL-6, IL-17, IL-23, TNF-α, and TGF-β) or molecules that exhibit an anti-tumor effect (IL-12 and IFN-γ) [49]. Neutrophil infiltration increases OSCC invasion by inducing matrix degradation and invadopodia formation through a paracrine TNF-α-induced mechanism [5, 16]. Mast cells have been associated with angiogenesis and are able to secrete numerous cytokines, chemokines, and angiogenic factors [146, 147], but their role in the development of OSCC is still debatable.

Cancer-associated fibroblasts, the most dominant components of the TME, are recipients of many of these factors (e.g., TGF-β), as well as directly contributing to tumor progression and an inflammatory TME *via* the secretion of VEGF, IL-6, IL-8, PGE2, TGF-β, and activation of NF-κB [148]. The presence and activity of CAFs are associated with poorer prognostic outcomes for OSCC [149] and are involved in bone invasion [150].

Senescent cells (which are more prevalent in older individuals, same as cancer) also contribute to chronic inflammation. When cells senesce, they develop an inflammatory secretory phenotype known as the senescent-associated secretory phenotype (SASP) [151], characterized by the secretion of high levels of "pro-oncogenic cytokines," such as IL-1, IL-6, and IL-8 [152]. In fact, the SASP is currently recognized as a cancer promoter mechanism, as it can induce EMT, invasion, and metastasis [151, 153], and its regulation has been proposed as anti-cancer treatment [154, 155].

DIAGNOSTIC POTENTIAL

Due to the advantages of using saliva samples (non-invasive and cost-efficient) for diagnostic and disease screening purposes, different studies have explored the possibility of using salivary inflammatory factors as biomarkers for the development and progression of OSCC. The most studied inflammatory proteins are TNF-α [5, 14, 42, 141, 156–159], IL-1 [5, 14, 141, 160, 161], IL-6 [5, 14, 34, 42, 92, 141, 158, 159, 162–164], and IL-8 [2, 5, 14, 42, 141, 160, 161, 164] (NF-κB-dependent cytokines), which have been found to be increased in the saliva of patients with different OPMDs (including OLP, oral leukoplakia, verrucous proliferative leucoplakia, and oral submucous fibrosis) and OSCC in comparison to healthy controls. Other cytokines, such as MIP-1β and IFNγ [14], and the anti-inflammatory cytokines IL-10 and IL-13 [24] have also been found to be increased in the saliva of patients with OSCC.

Using an array that analyzed the expression of 50 cytokines present in the saliva of patients with stage I OSCC before and after surgical treatment, Kamatani et al. [101] showed a significant decrease in salivary IL-1β after surgical resection. Similar to this, higher salivary levels of IL-8, IL-6, IL-1β, MIP-1β, and IP-10 before surgical intervention in patients with stage I OSCC were recently reported [165]. These results suggest that salivary inflammatory cytokines might be useful to monitor disease relapse.

A proteomic analysis of 60 saliva samples from healthy individuals and patients with OPMD and OSCC detected 21 proteins differentially expressed in OSCC compared to those in the other groups. Among those, three proteins were selected for further validation using ELISA, which included the interleukin-1 receptor antagonist (IL-1RA). Salivary IL-1RA was significantly decreased in patients with OPMD and control individuals compared to that in patients with OSCC but, alone, was suboptimal for distinguishing patients with OPMDs and healthy controls from patients with OSCC. In combination with other biomarkers (SLC3A2 and S100A2), IL-1RA was able to distinguish patients with OSCC from both healthy individuals and patients with OPMD with a sensitivity and specificity of 83.33/83.33% and of 93.33/70%, respectively [21]. Salivary IL-1β and IL-8 have also been found to discriminate between healthy individuals and patients with OSCC [160, 166] and high sensitivity and specificity values have been reported (86/97% for IL-8 and 83/76% for IL-1β) [166, 167]. Nevertheless, a recent systematic review reported average sensitivity and specificity values of 41/69% for IL-8 and of 26/46% for IL-1β [94], which are suboptimal. Both cytokines performed better when used in combination with other markers [161, 166], so it is likely that their future use will be as part of a panel of salivary biomarkers, rather than as single markers.

IL-6 is also a promising biomarker for the development of OSCC. IL-6 salivary levels are reported to increase with the severity of oral dysplasia [34], to differentiate between OPMD and OSCC (sensitivity and specificity values of 96 and 99%, respectively) [163], and to predict treatment outcomes in patients with OSCC [11, 97]. High salivary expression of TNF-α, IL-1α, and IL-8 has also been associated with a decreased survival rate in tongue squamous cell carcinoma (TSCC) [11].

FUTURE DIRECTION

The understanding of the role and origin of the different inflammatory molecules involved during tumor initiation, promotion, and progression of oral cancers provides an opportunity to target inflammation to improve patient outcomes. This can be done by selectively targeting transcription pathways (e.g., NF-κB), cytokines (e.g., IL-1), or cell types known to contribute to the inflammatory secretome (e.g., CAFs and senescent cells). There are several research lines in this field, but little has been translated into the clinics, especially with regard to OSCC. Although all of the aforementioned reports are pointing in the direction that salivary cytokines could be used for diagnostic

and prognostic aims in patients with oral cancer, more prospective studies are needed before they could be used in clinical settings.

AUTHOR CONTRIBUTIONS

SN performed the review and wrote the manuscript.

REFERENCES

1. Voronov E, Dinarello CA, Apte RN. Interleukin-1alpha as an intracellular alarmin in cancer biology. *Semin Immunol*. (2018) 38:3–14. doi: 10.1016/j.smim.2018.10.006
2. Portier M, Zhang XG, Ursule E, Lees D, Jourdan M, Bataille R, et al. Cytokine gene expression in human multiple myeloma. *Br J Haematol*. (1993) 85:514–20. doi: 10.1111/j.1365-2141.1993.tb03341.x
3. Kuper H, Adami HO, Trichopoulos D. Infections as a major preventable cause of human cancer. *J Intern Med*. (2000) 248:171–83. doi: 10.1046/j.1365-2796.2000.00742.x
4. Apte RN, Dotan S, Elkabets M, White MR, Reich E, Carmi Y, et al. The involvement of IL-1 in tumorigenesis, tumor invasiveness, metastasis, and tumor-host interactions. *Cancer Metastasis Rev*. (2006) 25:387–408. doi: 10.1007/s10555-006-9004-4
5. Goertzen C, Mahdi H, Laliberte C, Meirson T, Eymael D, Gil-Henn H, et al. Oral inflammation promotes oral squamous cell carcinoma invasion. *Oncotarget*. (2018) 9:29047–63. doi: 10.18632/oncotarget.25540
6. Rao SK, Pavicevic Z, Du Z, Kim JG, Fan M, Jiao Y, et al. Pro-inflammatory genes as biomarkers and therapeutic targets in oral squamous cell carcinoma. *J Biol Chem*. (2010) 285:32512–21. doi: 10.1074/jbc.M110.150490
7. Wu T, Hong Y, Jia L, Wu J, Xia J, Wang J, et al. Modulation of IL-1beta reprogrammes the tumor microenvironment to interrupt oral carcinogenesis. *Sci Rep*. (2016) 6:20208. doi: 10.1038/srep20208
8. Speight PM, Khurram SA, Kujan O. Oral potentially malignant disorders: risk of progression to malignancy. *Oral Surg Oral Med Oral Pathol Oral Radiol*. (2018) 125:612–27. doi: 10.1016/j.oooo.2017.12.011
9. Lee CH, Chang JS, Syu SH, Wong TS, Chan JY, Tang YC, et al. IL-1beta promotes malignant transformation and tumor aggressiveness in oral cancer. *J Cell Physiol*. (2015) 230:875–84. doi: 10.1002/jcp.24816
10. Leon X, Bothe C, Garcia J, Parreno M, Alcolea S, Quer M, et al. Expression of IL-1alpha correlates with distant metastasis in patients with head and neck squamous cell carcinoma. *Oncotarget*. (2015) 6:37398–409. doi: 10.18632/oncotarget.6054
11. Korostoff A, Reder L, Masood R, Sinha UK. The role of salivary cytokine biomarkers in tongue cancer invasion and mortality. *Oral Oncol*. (2011) 47:282–7. doi: 10.1016/j.oraloncology.2011.02.006
12. Bates AM, Gomez Hernandez MP, Lanzel EA, Qian F, Brogden KA. Matrix metalloproteinase (MMP) and immunosuppressive biomarker profiles of seven head and neck squamous cell carcinoma (HNSCC) cell lines. *Transl Cancer Res*. (2018) 7:533–42. doi: 10.21037/tcr.2018.05.09
13. Sun Y, Zhu D, Wang G, Wang D, Zhou H, Liu X, et al. Pro-inflammatory cytokine IL-1β up-regulates CXC chemokine receptor 4 *via* notch and ERK signaling pathways in tongue squamous cell carcinoma. *PLoS ONE*. (2015) 10:e0132677. doi: 10.1371/journal.pone.0132677
14. Lee LT, Wong YK, Hsiao HY, Wang YW, Chan MY, Chang KW. Evaluation of saliva and plasma cytokine biomarkers in patients with oral squamous cell carcinoma. *Int J Oral Maxillofac Surg*. (2018) 47:699–707. doi: 10.1016/j.ijom.2017.09.016
15. Cohen RF, Contrino J, Spiro JD, Mann EA, Chen LL, Kreutzer DL. Interleukin-8 expression by head and neck squamous cell carcinoma. *Arch Otolaryngol Head Neck Surg*. (1995) 121:202–9. doi: 10.1001/archotol.1995.01890020064013
16. Glogauer JE, Sun CX, Bradley G, Magalhaes MA. Neutrophils increase oral squamous cell carcinoma invasion through an invadopodia-dependent pathway. *Cancer Immunol Res*. (2015) 3:1218–26. doi: 10.1158/2326-6066.CIR-15-0017
17. Lu SL, Reh D, Li AG, Woods J, Corless CL, Kulesz-Martin M, et al. Overexpression of transforming growth factor beta1 in head and neck epithelia results in inflammation, angiogenesis, and epithelial hyperproliferation. *Cancer Res*. (2004) 64:4405–10. doi: 10.1158/0008-5472.CAN-04-1032
18. Gonçalves AS, Mosconi C, Jaeger F, Wastowski IJ, Aguiar MCF, Silva TA, et al. Overexpression of immunomodulatory mediators in oral precancerous lesions. *Hum Immunol*. (2017) 78:752–7. doi: 10.1016/j.humimm.2017.09.003
19. Lu Z, Ding L, Ding H, Hao F, Pu Y, Wang Y, et al. Tumor cell-derived TGF-β at tumor center independently predicts recurrence and poor survival in oral squamous cell carcinoma. *J Oral Pathol Med*. (2019) 48:696–704. doi: 10.1111/jop.12888
20. Shiiba M, Saito K, Yamagami H, Nakashima D, Higo M, Kasamatsu A, et al. Interleukin-1 receptor antagonist (IL1RN) is associated with suppression of early carcinogenic events in human oral malignancies. *Int J Oncol*. (2015) 46:1978–84. doi: 10.3892/ijo.2015.2917
21. Shan J, Sun Z, Yang J, Xu J, Shi W, Wu Y, et al. Discovery and preclinical validation of proteomic biomarkers in saliva for early detection of oral squamous cell carcinomas. *Oral Dis*. (2019) 25:97–107. doi: 10.1111/odi.12971
22. Manchanda P, Sharma SC, Das SN. Differential regulation of IL-2 and IL-4 in patients with tobacco-related oral squamous cell carcinoma. *Oral Dis*. (2006) 12:455–62. doi: 10.1111/j.1601-0825.2005.01220.x
23. Beppu M, Ikebe T, Shirasuna K. The inhibitory effects of immunosuppressive factors, dexamethasone and interleukin-4, on NF-kappaB-mediated protease production by oral cancer. *Biochim Biophys Acta*. (2002) 1586:11–22. doi: 10.1016/S0925-4439(01)00080-1
24. Aziz S, Ahmed SS, Ali A, Khan FA, Zulfiqar G, Iqbal J, et al. Salivary immunosuppressive cytokines IL-10 and IL-13 are significantly elevated in oral squamous cell carcinoma patients. *Cancer Invest*. (2015) 33:318–28. doi: 10.3109/07357907.2015.1041642
25. Alhamarneh O, Agada F, Madden L, Stafford N, Greenman J. Serum IL10 and circulating CD4(+) CD25(high) regulatory T cell numbers as predictors of clinical outcome and survival in patients with head and neck squamous cell carcinoma. *Head Neck*. (2011) 33:415–23. doi: 10.1002/hed.21464
26. Arantes DA, Costa NL, Mendonça EF, Silva TA, Batista AC. Overexpression of immunosuppressive cytokines is associated with poorer clinical stage of oral squamous cell carcinoma. *Arch Oral Biol*. (2016) 61:28–35. doi: 10.1016/j.archoralbio.2015.10.013
27. Adappa ND, Sung CK, Choi B, Huang TG, Genden EM, Shin EJ. The administration of IL-12/GM-CSF and Ig-4-1BB ligand markedly decreases murine floor of mouth squamous cell cancer. *Otolaryngol Head Neck Surg*. (2008) 139:442–8. doi: 10.1016/j.otohns.2008.05.001
28. Tian S, Jiang C, Liu X, Xu S, Zhang Z, Chen H, et al. Hypermethylation of IFN-γ in oral cancer tissues. *Clin Oral Investig*. (2017) 21:2535–42. doi: 10.1007/s00784-017-2052-z
29. Peng X, Li W, Johnson WD, Torres KE, McCormick DL. Overexpression of lipocalins and pro-inflammatory chemokines and altered methylation of PTGS2 and APC2 in oral squamous cell carcinomas induced in rats by 4-nitroquinoline-1-oxide. *PLoS ONE*. (2015) 10:e0116285. doi: 10.1371/journal.pone.0116285
30. Lee CH, Syu SH, Liu KJ, Chu PY, Yang WC, Lin P, et al. Interleukin-1 beta transactivates epidermal growth factor receptor *via* the CXCL1-CXCR2 axis in oral cancer. *Oncotarget*. (2015) 6:38866–80. doi: 10.18632/oncotarget.5640
31. Prasad G, McCullough M. Chemokines and cytokines as salivary biomarkers for the early diagnosis of oral cancer. *Int J Dent*. (2013) 2013:813756. doi: 10.1155/2013/813756
32. Lien MY, Lin CW, Tsai HC, Chen YT, Tsai MH, Hua CH, et al. Impact of CCL4 gene polymorphisms and environmental factors on oral cancer development and clinical characteristics. *Oncotarget*. (2017) 8:31424–34. doi: 10.18632/oncotarget.15615

33. Bae JY, Kim EK, Yang DH, Zhang X, Park YJ, Lee DY, et al. Reciprocal interaction between carcinoma-associated fibroblasts and squamous carcinoma cells through interleukin-1alpha induces cancer progression. *Neoplasia*. (2014) 16:928–38. doi: 10.1016/j.neo.2014.09.003
34. Sharma M, Bairy I, Pai K, Satyamoorthy K, Prasad S, Berkovitz B, et al. Salivary IL-6 levels in oral leukoplakia with dysplasia and its clinical relevance to tobacco habits and periodontitis. *Clin Oral Investig*. (2011) 15:705–14. doi: 10.1007/s00784-010-0435-5
35. Abdullah Zubir AZ, Whawell SA, Wong TS, Khurram SA. The chemokine lymphotactin and its recombinant variants in oral cancer cell regulation. *Oral Dis*. (2020) 26:1668–76. doi: 10.1111/odi.13500
36. Khurram SA, Bingle L, McCabe BM, Farthing PM, Whawell SA. The chemokine receptors CXCR1 and CXCR2 regulate oral cancer cell behaviour. *J Oral Pathol Med*. (2014) 43:667–74. doi: 10.1111/jop.12191
37. Timmons SR, Nwankwo JO, Domann FE. Acetaldehyde activates Jun/AP-1 expression and DNA binding activity in human oral keratinocytes. *Oral Oncol*. (2002) 38:281–90. doi: 10.1016/S1368-8375(01)00056-2
38. Alam M, Kashyap T, Pramanik KK, Singh AK, Nagini S, Mishra R. The elevated activation of NFκB and AP-1 is correlated with differential regulation of Bcl-2 and associated with oral squamous cell carcinoma progression and resistance. *Clin Oral Investig*. (2017) 21:2721–31. doi: 10.1007/s00784-017-2074-6
39. Wolf JS, Chen Z, Dong G, Sunwoo JB, Bancroft CC, Capo DE, et al. IL (Interleukin)-1 promotes nuclear factor- B and AP-1-induced IL-8 expression, cell survival, and proliferation in head and neck squamous cell carcinomas. *Clin Cancer Res*. (2001) 7:1812–20.
40. Nakayama H, Ikebe T, Beppu M, Shirasuna K. High expression levels of nuclear factor kappaB, IkappaB kinase alpha and Akt kinase in squamous cell carcinoma of the oral cavity. *Cancer*. (2001) 92:3037–44. doi: 10.1002/1097-0142(20011215)92:12<3037::AID-CNCR10171>3.0.CO;2-#
41. Tanaka T, Nakayama H, Yoshitake Y, Irie A, Nagata M, Kawahara K, et al. Selective inhibition of nuclear factor-κB by nuclear factor-κB essential modulator-binding domain peptide suppresses the metastasis of highly metastatic oral squamous cell carcinoma. *Cancer Sci*. (2012) 103:455–63. doi: 10.1111/j.1349-7006.2011.02174.x
42. Zhang Y, Lin M, Zhang S, Wang Z, Jiang L, Shen J, et al. NF-kappaB-dependent cytokines in saliva and serum from patients with oral lichen planus: a study in an ethnic Chinese population. *Cytokine*. (2008) 41:144–9. doi: 10.1016/j.cyto.2007.11.004
43. Liu YC, Ho HC, Lee MR, Lai KC, Yeh CM, Lin YM, et al. Early induction of cytokines/cytokine receptors and Cox2, and activation of NF-κB in 4-nitroquinoline 1-oxide-induced murine oral cancer model. *Toxicol Appl Pharmacol*. (2012) 262:107–16. doi: 10.1016/j.taap.2012.04.023
44. Shibata M, Kodani I, Osaki M, Araki K, Adachi H, Ryoke K, et al. Cyclo-oxygenase-1 and−2 expression in human oral mucosa, dysplasias and squamous cell carcinomas and their pathological significance. *Oral Oncol*. (2005) 41:304–12. doi: 10.1016/j.oraloncology.2004.09.009
45. Morita Y, Morita N, Hata K, Nakanishi M, Kimoto N, Omata T, et al. Cyclooxygenase-2 expression is associated with vascular endothelial growth factor-c and lymph node metastasis in human oral tongue cancer. *Oral Surg Oral Med Oral Pathol Oral Radiol*. (2014) 117:502–10. doi: 10.1016/j.oooo.2013.12.410
46. Yang SF, Chen MK, Hsieh YS, Chung TT, Hsieh YH, Lin CW, et al. Prostaglandin E2/EP1 signaling pathway enhances intercellular adhesion molecule 1 (ICAM-1) expression and cell motility in oral cancer cells. *J Biol Chem*. (2010) 285:29808–16. doi: 10.1074/jbc.M110.108183
47. Postler TS, Ghosh S. Bridging the gap: a regulator of NF-κB linking inflammation and cancer. *J Oral Biosci*. (2015) 57:143–7. doi: 10.1016/j.job.2015.05.001
48. Jimi E, Kokabu S, Matsubara T, Nakatomi C, Matsuo K, Watanabe S. NF-κB acts as a multifunctional modulator in bone invasion by oral squamous cell carcinoma. *Oral Sci Int*. (2016) 13:1–6. doi: 10.1016/S1348-8643(15)00038-5
49. Tampa M, Mitran MI, Mitran CI, Sarbu MI, Matei C, Nicolae I, et al. Mediators of inflammation - a potential source of biomarkers in oral squamous cell carcinoma. *J Immunol Res*. (2018) 2018:1061780. doi: 10.1155/2018/1061780
50. Garat C, Arend WP. Intracellular IL-1Ra type 1 inhibits IL-1-induced IL-6 and IL-8 production in Caco-2 intestinal epithelial cells through inhibition of p38 mitogen-activated protein kinase and NF-kappaB pathways. *Cytokine*. (2003) 23:31–40. doi: 10.1016/S1043-4666(03)00182-0
51. Papa S, Bubici C, Zazzeroni F, Pham CG, Kuntzen C, Knabb JR, et al. The NF-kappaB-mediated control of the JNK cascade in the antagonism of programmed cell death in health and disease. *Cell Death Differ*. (2006) 13:712–29. doi: 10.1038/sj.cdd.4401865
52. Julien S, Puig I, Caretti E, Bonaventure J, Nelles L, van Roy F, et al. Activation of NF-kappaB by Akt upregulates snail expression and induces epithelium mesenchyme transition. *Oncogene*(2007) 26:7445–56. doi: 10.1038/sj.onc.1210546
53. Wu D, Wu P, Zhao L, Huang L, Zhang Z, Zhao S, et al. NF-κB expression and outcomes in solid tumors: a systematic review and meta-analysis. *Medicine*. (2015) 94:e1687. doi: 10.1097/MD.0000000000001687
54. Yang X, Cheng H, Chen J, Wang R, Saleh A, Si H, et al. Head and neck cancers promote an inflammatory transcriptome through coactivation of classic and alternative NF-κB pathways. *Cancer Immunol Res*. (2019) 7:1760–74. doi: 10.1158/2326-6066.CIR-18-0832
55. Zhang J, Peng B. NF-kappaB promotes iNOS and VEGF expression in salivary gland adenoid cystic carcinoma cells and enhances endothelial cell motility *in vitro*. *Cell Prolif*. (2009) 42:150–61. doi: 10.1111/j.1365-2184.2009.00588.x
56. Ikebe T, Takeuchi H, Jimi E, Beppu M, Shinohara M, Shirasuna K. Involvement of proteasomes in migration and matrix metalloproteinase-9 production of oral squamous cell carcinoma. *Int J Cancer*. (1998) 77:578–85. doi: 10.1002/(SICI)1097-0215(19980812)77:4<578::AID-IJC18>3.0.CO;2-2
57. Lin Z, Sun L, Chen W, Liu B, Wang Y, Fan S, et al. miR-639 regulates transforming growth factor beta-induced epithelial-mesenchymal transition in human tongue cancer cells by targeting FOXC1. *Cancer Sci*. (2014) 105:1288–98. doi: 10.1111/cas.12499
58. Wang Y, Lin Z, Sun L, Fan S, Huang Z, Zhang D, et al. Akt/Ezrin Tyr353/NF-κB pathway regulates EGF-induced EMT and metastasis in tongue squamous cell carcinoma. *Br J Cancer*. (2014) 110:695–705. doi: 10.1038/bjc.2013.770
59. Ma T, Zhao Z, Wang Z, Wang C, Zhang L. MiR-940 inhibits migration and invasion of tongue squamous cell carcinoma *via* regulatingCXCR2/NF-κB system-mediated epithelial-mesenchymal transition. *Naunyn Schmiedebergs Arch Pharmacol*. (2019) 392:1359–69. doi: 10.1007/s00210-019-01671-w
60. Duffey DC, Chen Z, Dong G, Ondrey FG, Wolf JS, Brown K, et al. Expression of a dominant-negative mutant inhibitor-kappaBalpha of nuclear factor-kappaB in human head and neck squamous cell carcinoma inhibits survival, proinflammatory cytokine expression, and tumor growth *in vivo*. *Cancer Res*. (1999) 59:3468–74.
61. Shaulian E, Karin M. AP-1 as a regulator of cell life and death. *Nat Cell Biol*. (2002) 4:E131–6. doi: 10.1038/ncb0502-e131
62. Fujioka S, Niu J, Schmidt C, Sclabas GM, Peng B, Uwagawa T, et al. NF-kappaB and AP-1 connection: mechanism of NF-kappaB-dependent regulation of AP-1 activity. *Mol Cell Biol*. (2004) 24:7806–19. doi: 10.1128/MCB.24.17.7806-7819.2004
63. Riabowol K, Schiff J, Gilman MZ. Transcription factor AP-1 activity is required for initiation of DNA synthesis and is lost during cellular aging. *Proc Natl Acad Sci U S A*. (1992) 89:157–61. doi: 10.1073/pnas.89.1.157
64. Bunjobpol W, Dulloo I, Igarashi K, Concin N, Matsuo K, Sabapathy K. Suppression of acetylpolyamine oxidase by selected AP-1 members regulates DNp73 abundance: mechanistic insights for overcoming DNp73-mediated resistance to chemotherapeutic drugs. *Cell Death Differ*. (2014) 21:1240–9. doi: 10.1038/cdd.2014.41
65. Werman A, Werman-Venkert R, White R, Lee JK, Werman B, Krelin Y, et al. The precursor form of IL-1alpha is an intracrine proinflammatory activator of transcription. *Proc Natl Acad Sci USA*. (2004) 101:2434–9. doi: 10.1073/pnas.0308705101
66. Scott KA, Moore RJ, Arnott CH, East N, Thompson RG, Scallon BJ, et al. An anti-tumor necrosis factor-alpha antibody inhibits the development of experimental skin tumors. *Mol Cancer Ther*. (2003) 2:445–51.
67. Zhang L, Jiao M, Wu K, Li L, Zhu G, Wang X, et al. TNF-α induced epithelial mesenchymal transition increases stemness properties in renal cell carcinoma cells. *Int J Clin Exp Med*. (2014) 7:4951–8.
68. Johnston DA, Dong B, Hughes CC. TNF induction of jagged-1 in endothelial cells is NFkappaB-dependent.

Gene. (2009) 435:36–44. doi: 10.1016/j.gene.2009.01.003
69. Balkwill F. Tumour necrosis factor and cancer. *Nat Rev Cancer*. (2009) 9:361–71. doi: 10.1038/nrc2628
70. Mozaffari HR, Ramezani M, Mahmoudiahmadabadi M, Omidpanah N, Sadeghi M. Salivary and serum levels of tumor necrosis factor-alpha in oral lichen planus: a systematic review and meta-analysis study. *Oral Surg Oral Med Oral Pathol Oral Radiol*. (2017) 124:e183–9. doi: 10.1016/j.oooo.2017.06.117
71. Scheff NN, Ye Y, Bhattacharya A, MacRae J, Hickman DN, Sharma AK, et al. Tumor necrosis factor alpha secreted from oral squamous cell carcinoma contributes to cancer pain and associated inflammation. *Pain*. (2017) 158:2396–409. doi: 10.1097/j.pain.0000000000001044
72. Tang D, Tao D, Fang Y, Deng C, Xu Q, Zhou J. TNF-alpha promotes invasion and metastasis *via* NF-Kappa B pathway in oral squamous cell carcinoma. *Med Sci Monit Basic Res*. (2017) 23:141–9. doi: 10.12659/MSMBR.903910
73. Ren ZH, Wu K, Yang R, Liu ZQ, Cao W. Differential expression of matrix metalloproteinases and miRNAs in the metastasis of oral squamous cell carcinoma. *BMC Oral Health*. (2020) 20:24. doi: 10.1186/s12903-020-1013-0
74. de Vicente JC, Fresno MF, Villalain L, Vega JA, Hernández Vallejo G. Expression and clinical significance of matrix metalloproteinase-2 and matrix metalloproteinase-9 in oral squamous cell carcinoma. *Oral Oncol*. (2005) 41:283–93. doi: 10.1016/j.oraloncology.2004.08.013
75. Jimenez L, Jayakar SK, Ow TJ, Segall JE. Mechanisms of invasion in head and neck cancer. *Arch Pathol Lab Med*. (2015) 139:1334–48. doi: 10.5858/arpa.2014-0498-RA
76. Ricci S, Pinto F, Auletta A, Giordano A, Giovane A, Settembre G, et al. The enigmatic role of matrix metalloproteinases in epithelial-to-mesenchymal transition of oral squamous cell carcinoma: implications and nutraceutical aspects. *J Cell Biochem*. (2019) 120:6813–9. doi: 10.1002/jcb.26905
77. Horejs CM. Basement membrane fragments in the context of the epithelial-to-mesenchymal transition. *Eur J Cell Biol*. (2016) 95:427–40. doi: 10.1016/j.ejcb.2016.06.002
78. Chen IC, Chiang WF, Huang HH, Chen PF, Shen YY, Chiang HC. Role of SIRT1 in regulation of epithelial-to-mesenchymal transition in oral squamous cell carcinoma metastasis. *Mol Cancer*. (2014) 13:254. doi: 10.1186/1476-4598-13-254
79. Zhang S, Wang B, Zhou X, Yue K, Wang X. The experimental study of CXC subfamily receptor 4 modulating oral squamous cell carcinoma epithelial-mesenchymal transition to influence lymphatic metastasis *in vitro*. *Zhonghua Kou Qiang Yi Xue Za Zhi*. (2014) 49:171–6.
80. Epanchintsev A, Shyamsunder P, Verma RS, Lyakhovich A. IL-6, IL-8, MMP-2, MMP-9 are overexpressed in Fanconi anemia cells through a NF-κB/TNF-α dependent mechanism. *Mol Carcinog*. (2015) 54:1686–99. doi: 10.1002/mc.22240
81. Tanaka T, Imamura T, Yoneda M, Irie A, Ogi H, Nagata M, et al. Enhancement of active MMP release and invasive activity of lymph node metastatic tongue cancer cells by elevated signaling *via* the TNF-α-TNFR1-NF-κB pathway and a possible involvement of angiopoietin-like 4 in lung metastasis. *Int J Oncol*. (2016) 49:1377–84. doi: 10.3892/ijo.2016.3653
82. Bates RC, Mercurio AM. Tumor necrosis factor-alpha stimulates the epithelial-to-mesenchymal transition of human colonic organoids. *Mol Biol Cell*. (2003) 14:1790–800. doi: 10.1091/mbc.e02-09-0583
83. Kashyap T, Pramanik KK, Nath N, Mishra P, Singh AK, Nagini S, et al. Crosstalk between Raf-MEK-ERK and PI3K-Akt-GSK3β signaling networks promotes chemoresistance, invasion/migration and stemness *via* expression of CD44 variants (v4 and v6) in oral cancer. *Oral Oncol*. (2018) 86:234–43. doi: 10.1016/j.oraloncology.2018.09.028
84. Pai S, Bamodu OA, Lin YK, Lin CS, Chu PY, Chien MH, et al. CD47-SIRPα signaling induces epithelial-mesenchymal transition and cancer stemness and links to a poor prognosis in patients with oral squamous cell carcinoma. *Cells*. (2019) 8:1658. doi: 10.3390/cells8121658
85. Liu M, Gao X, Liu CL. Increased expression of lncRNA FTH1P3 promotes oral squamous cell carcinoma cells migration and invasion by enhancing PI3K/Akt/GSK3b/ Wnt/β-catenin signaling. *Eur Rev Med Pharmacol Sci*. (2018) 22:8306–14. doi: 10.26355/eurrev_201812_16528
86. Wang H, Deng X, Zhang J, Ou Z, Mai J, Ding S, et al. Elevated expression of zinc finger protein 703 promotes cell proliferation and metastasis through PI3K/AKT/GSK-3β signalling in oral squamous cell carcinoma. *Cell Physiol Biochem*. (2017) 44:920–34. doi: 10.1159/000485360
87. Lai KC, Liu CJ, Lin TJ, Mar AC, Wang HH, Chen CW, et al. Blocking TNF-α inhibits angiogenesis and growth of IFIT2-depleted metastatic oral squamous cell carcinoma cells. *Cancer Lett*. (2016) 370:207–15. doi: 10.1016/j.canlet.2015.10.016
88. Coppe JP, Patil CK, Rodier F, Sun Y, Munoz DP, Goldstein J, et al. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biol*. (2008) 6:2853–68. doi: 10.1371/journal.pbio.0060301
89. Sparmann A, Bar-Sagi D. Ras-induced interleukin-8 expression plays a critical role in tumor growth and angiogenesis. *Cancer Cell*. (2004) 6:447–58. doi: 10.1016/j.ccr.2004.09.028
90. Ancrile B, Lim KH, Counter CM. Oncogenic ras-induced secretion of IL6 is required for tumorigenesis. *Genes Dev*. (2007) 21:1714–9. doi: 10.1101/gad.1549407
91. Loaiza N, Demaria M. Cellular senescence and tumor promotion: is aging the key? *Biochim Biophys Acta*. (2016) 1865:155–67. doi: 10.1016/j.bbcan.2016.01.007
92. Panneer Selvam N, Sadaksharam J. Salivary interleukin-6 in the detection of oral cancer and precancer. *Asia Pac J Clin Oncol*. (2015) 11:236–41. doi: 10.1111/ajco.12330
93. Dikova VR, Principe S, Bagan JV. Salivary inflammatory proteins in patients with oral potentially malignant disorders. *J Clin Exp Dent*. (2019) 11:e659–64. doi: 10.4317/jced.55917
94. Gaba FI, Sheth CC, Veses V. Salivary biomarkers and their efficacies as diagnostic tools for oral squamous cell carcinoma: systematic review and meta-analysis. *J Oral Pathol Med*. (2018) 1–9. doi: 10.1111/jop.12791. [Epub ahead of print].
95. Zhou J, Tang Z, Gao S, Li C, Feng Y, Zhou X. Tumor-associated macrophages: recent insights and therapies. *Front Oncol*. (2020) 10:188. doi: 10.3389/fonc.2020.00188
96. Waugh DJ, Wilson C. The interleukin-8 pathway in cancer. *Clin Cancer Res*. (2008) 14:6735–41. doi: 10.1158/1078-0432.CCR-07-4843
97. Tsai MS, Chen WC, Lu CH, Chen MF. The prognosis of head and neck squamous cell carcinoma related to immunosuppressive tumor microenvironment regulated by IL-6 signaling. *Oral Oncol*. (2019) 91:47–55. doi: 10.1016/j.oraloncology.2019.02.027
98. Woods RV, Adler-Storthz K, Dayman GL, Francis GM, Grimm EA. Interleukin-1 regulates interleukin-6 secretion in human oral squamous cell carcinoma *in vitro*: possible influence of p53 but not human papillomavirus E6/E. *Cancer Res*. (1998) 58:3142–9.
99. von Biberstein SE, Spiro JD, Lindquist R, Kreutzer DL. Interleukin-1 receptor antagonist in head and neck squamous cell carcinoma. *Arch Otolaryngol Head Neck Surg*. (1996) 122:751–9. doi: 10.1001/archotol.1996.01890190047012
100. SahebJamee M, Eslami M, AtarbashiMoghadam F, Sarafnejad A. Salivary concentration of TNFα, IL1α, IL6, and IL8 in oral squamous cell carcinoma. *Med Oral Patol Oral Cir Bucal*. (2008) 13:E292–5.
101. Kamatani T, Shiogama S, Yoshihama Y, Kondo S, Shirota T, Shintani S. Interleukin-1 beta in unstimulated whole saliva is a potential biomarker for oral squamous cell carcinoma. *Cytokine*. (2013) 64:497–502. doi: 10.1016/j.cyto.2013.08.011
102. Stevenson FT, Turck J, Locksley RM, Lovett DH. The N-terminal propiece of interleukin 1 is a transforming nuclear oncoprotein. *Cell Biol*. (1997) 94:508–13. doi: 10.1073/pnas.94.2.508
103. Lee CH, Wong TS, Chan JY, Lu SC, Lin P, Cheng AJ, et al. Epigenetic regulation of the X-linked tumour suppressors BEX1 and LDOC1 in oral squamous cell carcinoma. *J Pathol*. (2013) 230:298–309. doi: 10.1002/path.4173
104. Al-Sahaf S, Hunter KD, Bolt R, Ottewell PD, Murdoch C. The IL-1/IL-1R axis induces greater fibroblast-derived chemokine release in human papillomavirus-negative compared to positive oropharyngeal cancer. *Int J Cancer*. (2019) 144:334–44. doi: 10.1002/ijc.31852
105. Banda NK, Guthridge C, Sheppard D, Cairns KS, Muggli M, Bech-Otschir D, et al. Intracellular IL-1 receptor antagonist type 1 inhibits IL-1-induced cytokine production in keratinocytes through binding to the

third component of the COP9 signalosome. *J Immunol*. (2005) 174:3608–16. doi: 10.4049/jimmunol.174.6.3608
106. Hashemi Goradel N, Najafi M, Salehi E, Farhood B, Mortezaee K. Cyclooxygenase-2 in cancer: a review. *J Cell Physiol*. (2019) 234:5683–99. doi: 10.1002/jcp.27411
107. Schellhorn M, Haustein M, Frank M, Linnebacher M, Hinz B. Celecoxib increases lung cancer cell lysis by lymphokine-activated killer cells *via* upregulation of ICAM-1. *Oncotarget*. (2015) 6:39342–56. doi: 10.18632/oncotarget.5745
108. Chu TH, Chan HH, Kuo HM, Liu LF, Hu TH, Sun CK, et al. Celecoxib suppresses hepatoma stemness and progression by up-regulating PTEN. *Oncotarget*. (2014) 5:1475–90. doi: 10.18632/oncotarget.1745
109. Choi YH, Back KO, Kim HJ, Lee SY, Kook KH. Pirfenidone attenuates IL-1β-induced COX-2 and PGE2 production in orbital fibroblasts through suppression of NF-κB activity. *Exp Eye Res*. (2013) 113:1–8. doi: 10.1016/j.exer.2013.05.001
110. Konstantinopoulos PA, Vandoros GP, Karamouzis MV, Gkermpesi M, Sotiropoulou-Bonikou G, Papavassiliou AG. EGF-R is expressed and AP-1 and NF-kappaB are activated in stromal myofibroblasts surrounding colon adenocarcinomas paralleling expression of COX-2 and VEGF. *Cell Oncol*. (2007) 29:477–82. doi: 10.1155/2007/831416
111. Fang L, Chang HM, Cheng JC, Leung PC, Sun YP. TGF-β1 induces COX-2 expression and PGE2 production in human granulosa cells through Smad signaling pathways. *J Clin Endocrinol Metab*. (2014) 99:E1217–26. doi: 10.1210/jc.2013-4100
112. Nakao S, Ogtata Y, Shimizu E, Yamazaki M, Furuyama S, Sugiya H. Tumor necrosis factor alpha (TNF-alpha)-induced prostaglandin E2 release is mediated by the activation of cyclooxygenase-2 (COX-2) transcription *via* NFkappaB in human gingival fibroblasts. *Mol Cell Biochem*. (2002) 238:11–8. doi: 10.1023/A:1019927616000
113. Amirchaghmaghi M, Mohtasham N, Mozaffari PM. Comparison of COX2 expression between oral squamous cell carcinoma, leukoplakia and normal mucosa. *J Contemp Dent Pract*. (2012) 13:205–9. doi: 10.5005/jp-journals-10024-1122
114. Chan G, Boyle JO, Yang EK, Zhang F, Sacks PG, Shah JP, et al. Cyclooxygenase-2 expression is up-regulated in squamous cell carcinoma of the head and neck. *Cancer Res*. (1999) 59:991–4.
115. Yang B, Jia L, Guo Q, Ren H, Hu Y, Xie T. Clinicopathological and prognostic significance of cyclooxygenase-2 expression in head and neck cancer: a meta-analysis. *Oncotarget*. (2016) 7:47265–77. doi: 10.18632/oncotarget.10059
116. Sano Y, Kogashiwa Y, Araki R, Enoki Y, Ikeda T, Yoda T, et al. Correlation of inflammatory markers, survival, and COX2 expression in oral cancer and implications for prognosis. *Otolaryngol Head Neck Surg*. (2018) 158:667–76. doi: 10.1177/0194599817745284
117. Nasry WHS, Rodriguez-Lecompte JC, Martin CK. Role of COX-2/PGE2 mediated inflammation in oral squamous cell carcinoma. *Cancers*. (2018) 10:348. doi: 10.3390/cancers10100348
118. Wang YH, Wu MW, Yang AK, Zhang WD, Sun J, Liu TR, et al. COX-2 gene increases tongue cancer cell proliferation and invasion through VEGF-C pathway. *Med Oncol*. (2011) 28(Suppl. 1):S360–6. doi: 10.1007/s12032-010-9737-3
119. Kono M, Watanabe M, Abukawa H, Hasegawa O, Satomi T, Chikazu D. Cyclo-oxygenase-2 expression is associated with vascular endothelial growth factor C expression and lymph node metastasis in oral squamous cell carcinoma. *J Oral Maxillofac Surg*. (2013) 71:1694–702. doi: 10.1016/j.joms.2013.04.015
120. Shang ZJ, Li ZB, Li JR. VEGF is up-regulated by hypoxic stimulation and related to tumour angiogenesis and severity of disease in oral squamous cell carcinoma: *in vitro* and *in vivo* studies. *Int J Oral Maxillofac Surg*. (2006) 35:533–8. doi: 10.1016/j.ijom.2005.09.006
121. Lee LT, Wong YK, Chan MY, Chang KW, Chen SC, Chang CT, et al. The correlation between HIF-1 alpha and VEGF in oral squamous cell carcinomas: expression patterns and quantitative immunohistochemical analysis. *J Chin Med Assoc*. (2018) 81:370–5. doi: 10.1016/j.jcma.2017.06.025
122. Pouysségur J, Dayan F, Mazure NM. Hypoxia signalling in cancer and approaches to enforce tumour regression. *Nature*. (2006) 441:437–43. doi: 10.1038/nature04871
123. Zhu GQ, Tang YL, Li L, Zheng M, Jiang J, Li XY, et al. Hypoxia inducible factor 1α and hypoxia inducible factor 2α play distinct and functionally overlapping roles in oral squamous cell carcinoma. *Clin Cancer Res*. (2010) 16:4732–41. doi: 10.1158/1078-0432.CCR-10-1408
124. Aguilar-Cazares D, Chavez-Dominguez R, Carlos-Reyes A, Lopez-Camarillo C, Hernadez de la Cruz ON, Lopez-Gonzalez JS. Contribution of angiogenesis to inflammation and cancer. *Front Oncol*. (2019) 9:1399. doi: 10.3389/fonc.2019.01399
125. Wu F, Weigel KJ, Zhou H, Wang XJ. Paradoxical roles of TGF-β signaling in suppressing and promoting squamous cell carcinoma. *Acta Biochim Biophys Sin*. (2018) 50:98–105. doi: 10.1093/abbs/gmx127
126. Massagué J. TGFβ signalling in context. *Nat Rev Mol Cell Biol*. (2012) 13:616–30. doi: 10.1038/nrm3434
127. Taghavi N, Bagheri S, Akbarzadeh A. Prognostic implication of CD57, CD16, and TGF-β expression in oral squamous cell carcinoma. *J Oral Pathol Med*. (2016) 45:58–62. doi: 10.1111/jop.12320
128. Takahashi H, Sakakura K, Kudo T, Toyoda M, Kaira K, Oyama T, et al. Cancer-associated fibroblasts promote an immunosuppressive microenvironment through the induction and accumulation of protumoral macrophages. *Oncotarget*. (2017) 8:8633–47. doi: 10.18632/oncotarget.14374
129. Walsh JE, Young MR. TGF-beta regulation of focal adhesion proteins and motility of premalignant oral lesions *via* protein phosphatase 1. *Anticancer Res*. (2011) 31:3159–64.
130. Yoshimatsu Y, Wakabayashi I, Kimuro S, Takahashi N, Takahashi K, Kobayashi M, et al. TNF-α enhances TGF-β-induced endothelial-to-mesenchymal transition *via* TGF-β signal augmentation. *Cancer Sci*. (2020) 111:2385–99. doi: 10.1111/cas.14455
131. Ruff M, Leyme A, Le Cann F, Bonnier D, Le Seyec J, Chesnel F, et al. The disintegrin and metalloprotease ADAM12 is associated with TGF-β-induced epithelial to mesenchymal transition. *PLoS ONE*. (2015) 10:e0139179. doi: 10.1371/journal.pone.0139179
132. Terabe M, Park JM, Berzofsky JA. Role of IL-13 in regulation of anti-tumor immunity and tumor growth. *Cancer Immunol Immunother*. (2004) 53:79–85. doi: 10.1007/s00262-003-0445-0
133. Lallemant B, Evrard A, Combescure C, Chapuis H, Chambon G, Raynal C, et al. Clinical relevance of nine transcriptional molecular markers for the diagnosis of head and neck squamous cell carcinoma in tissue and saliva rinse. *BMC Cancer*. (2009) 9:370. doi: 10.1186/1471-2407-9-370
134. Koike H, Uzawa K, Nakashima D, Shimada K, Kato Y, Higo M, et al. Identification of differentially expressed proteins in oral squamous cell carcinoma using a global proteomic approach. *Int J Oncol*. (2005) 27:59–67. doi: 10.3892/ijo.27.1.59
135. Sun Y, Liu N, Guan X, Wu H, Sun Z, Zeng H. Immunosuppression Induced by Chronic Inflammation and the Progression to Oral Squamous Cell Carcinoma. *Mediators Inflamm*. (2016) 2016:5715719. doi: 10.1155/2016/5715719
136. Chen CJ, Sung WW, Su TC, Chen MK, Wu PR, Yeh KT, et al. High expression of interleukin 10 might predict poor prognosis in early stage oral squamous cell carcinoma patients. *Clin Chim Acta*. (2013) 415:25–30. doi: 10.1016/j.cca.2012.09.009
137. Fallon PG, Jolin HE, Smith P, Emson CL, Townsend MJ, Fallon R, et al. IL-4 induces characteristic Th2 responses even in the combined absence of IL-5, IL-9, and IL-13. *Immunity*. (2002) 17:7–17. doi: 10.1016/S1074-7613(02)00332-1
138. Terabe M, Matsui S, Noben-Trauth N, Chen H, Watson C, Donaldson DD, et al. NKT cell-mediated repression of tumor immunosurveillance by IL-13 and the IL-4R-STAT6 pathway. *Nat Immunol*. (2000) 1:515–20. doi: 10.1038/82771
139. D'Arcangelo E, Wu NC, Cadavid JL, McGuigan AP. The life cycle of cancer-associated fibroblasts within the tumour stroma and its importance in disease outcome. *Br J Cancer*. (2020) 122:931–42. doi: 10.1038/s41416-019-0705-1
140. Puram SV, Tirosh I, Parikh AS, Patel AP, Yizhak K, Gillespie S, et al. Single-cell transcriptomic analysis of primary and metastatic tumor ecosystems in head and neck cancer. *Cell*. (2017) 171:1611–24.e1624. doi: 10.1016/j.cell.2017.10.044
141. Rhodus NL, Ho V, Miller CS, Myers S, Ondrey F. NF-kappaB dependent cytokine levels in saliva of patients with oral preneoplastic lesions

and oral squamous cell carcinoma. *Cancer Detect Prev*. (2005) 29:42–5. doi: 10.1016/j.cdp.2004.10.003
142. Haque A, Moriyama M, Kubota K, Ishiguro N, Sakamoto M, Chinju A, et al. CD206(+) tumor-associated macrophages promote proliferation and invasion in oral squamous cell carcinoma *via* EGF production. *Sci Rep*. (2019) 9:14611. doi: 10.1038/s41598-019-51149-1
143. Lee CH, Liu SY, Chou KC, Yeh CT, Shiah SG, Huang RY, et al. Tumor-associated macrophages promote oral cancer progression through activation of the Axl signaling pathway. *Ann Surg Oncol*. (2014) 21:1031–7. doi: 10.1245/s10434-013-3400-0
144. Hu Y, He MY, Zhu LF, Yang CC, Zhou ML, Wang Q, et al. Tumor-associated macrophages correlate with the clinicopathological features and poor outcomes *via* inducing epithelial to mesenchymal transition in oral squamous cell carcinoma. *J Exp Clin Cancer Res*. (2016) 35:12. doi: 10.1186/s13046-015-0281-z
145. Greten FR, Grivennikov SI. Inflammation and cancer: triggers, mechanisms, and consequences. *Immunity*. (2019) 51:27–41. doi: 10.1016/j.immuni.2019.06.025
146. Michailidou EZ, Markopoulos AK, Antoniades DZ. VEGF expression from human dysplastic or malignant oral epithelium may be related to mast cell density and the subsequent angiogenetic phenomena. *Int J Oral Maxillofac Surg*. (2012) 41:1467–73. doi: 10.1016/j.ijom.2011.12.038
147. López de Cicco R, Watson JC, Bassi DE, Litwin S, Klein-Szanto AJ. Simultaneous expression of furin and vascular endothelial growth factor in human oral tongue squamous cell carcinoma progression. *Clin Cancer Res*. (2004) 10:4480–8. doi: 10.1158/1078-0432.CCR-03-0670
148. Prime SS, Cirillo N, Hassona Y, Lambert DW, Paterson IC, Mellone M, et al. Fibroblast activation and senescence in oral cancer. *J Oral Pathol Med*. (2017) 46:82–8. doi: 10.1111/jop.12456
149. Dourado MR, Guerra ENS, Salo T, Lambert DW, Coletta RD. Prognostic value of the immunohistochemical detection of cancer-associated fibroblasts in oral cancer: a systematic review and meta-analysis. *J Oral Pathol Med*. (2018) 47:443–53. doi: 10.1111/jop.12623
150. Elmusrati AA, Pilborough AE, Khurram SA, Lambert DW. Cancer-associated fibroblasts promote bone invasion in oral squamous cell carcinoma. *Br J Cancer*. (2017) 117:867–75. doi: 10.1038/bjc.2017.239
151. Davalos AR, Coppe JP, Campisi J, Desprez PY. Senescent cells as a source of inflammatory factors for tumor progression. *Cancer Metastasis Rev*. (2010) 29:273–83. doi: 10.1007/s10555-010-9220-9
152. Niklander S, Bandaru D, Lambert DW, Hunter KD. ROCK inhibition modulates the senescence-associated secretory phenotype (SASP) in oral keratinocytes. *FEBS Open Bio*. (2020) 10:2740–9. doi: 10.1002/2211-5463.13012
153. Faget DV, Ren Q, Stewart SA. Unmasking senescence: context-dependent effects of SASP in cancer. *Nat Rev Cancer*. (2019) 19:439–53. doi: 10.1038/s41568-019-0156-2
154. Lau L, Porciuncula A, Yu A, Iwakura Y, David G. Uncoupling the senescence-associated secretory phenotype from cell cycle exit *via* interleukin-1 inactivation unveils its protumorigenic role. *Mol Cell Biol*. (2019) 39:e00586. doi: 10.1128/MCB.00586-18
155. Velarde MC, Demaria M, Campisi J. Senescent cells and their secretory phenotype as targets for cancer therapy. *Interdiscip Top Gerontol*. (2013) 38:17–27. doi: 10.1159/000343572
156. Pezelj-Ribaric S, Prso IB, Abram M, Glazar I, Brumini G, Simunovic-Soskic M. Salivary levels of tumor necrosis factor-alpha in oral lichen planus. *Mediators Inflamm*. (2004) 13:131–3. doi: 10.1080/09629350410001688530
157. Ghallab NA, el-Wakeel N, Shaker OG. Levels of salivary IFN-gamma, TNF-alfa, and TNF receptor-2 as prognostic markers in (erosive) oral lichen planus. *Mediators Inflamm*. (2010) 2010:847632. doi: 10.1155/2010/847632
158. Kaur J, Jacobs R. Proinflammatory cytokine levels in oral lichen planus, oral leukoplakia, and oral submucous fibrosis. *J Korean Assoc Oral Maxillofac Surg*. (2015) 41:171–5. doi: 10.5125/jkaoms.2015.41.4.171
159. Juretić M, Cerović R, Belušić-Gobić M, Brekalo Pršo I, Kqiku L, Špalj S, et al. Salivary levels of TNF-α and IL-6 in patients with oral premalignant and malignant lesions. *Folia Biol*. (2013) 59:99–102.
160. Singh P, Verma JK, Singh JK. Validation of salivary markers, IL-1β, IL-8 and Lgals3bp for detection of oral squamous cell carcinoma in an Indian population. *Sci Rep*. (2020) 10:7365. doi: 10.1038/s41598-020-64494-3
161. Li Y, St John MA, Zhou X, Kim Y, Sinha U, Jordan RC, et al. Salivary transcriptome diagnostics for oral cancer detection. *Clin Cancer Res*. (2004) 10:8442–50. doi: 10.1158/1078-0432.CCR-04-1167
162. Bagan L, Sáez GT, Tormos MC, Labaig-Rueda C, Murillo-Cortes J, Bagan JV. Salivary and serum interleukin-6 levels in proliferative verrucous leukoplakia. *Clin Oral Investig*. (2016) 20:737–43. doi: 10.1007/s00784-015-1551-z
163. Dineshkumar T, Ashwini BK, Rameshkumar A, Rajashree P, Ramya R, Rajkumar K. Salivary and serum interleukin-6 levels in oral premalignant disorders and squamous cell carcinoma: diagnostic value and clinicopathologic correlations. *Asian Pac J Cancer Prev*. (2016) 17:4899–906. doi: 10.22034/APJCP.2016.17.11.4899
164. Khyani IAM, Qureshi MA, Mirza T, Farooq MU. Detection of interleukins-6 and 8 in saliva as potential biomarkers of oral pre-malignant lesion and oral carcinoma: a breakthrough in salivary diagnostics in Pakistan. *Pak J Pharm Sci*. (2017) 30:817–23.
165. Val M, Sidoti Pinto GA, Manini L, Gandolfo S, Pentenero M. Variations of salivary concentration of cytokines and chemokines in presence of oral squamous cell carcinoma. A case-crossover longitudinal prospective study. *Cytokine*. (2019) 120:62–5. doi: 10.1016/j.cyto.2019.04.009
166. Brinkmann O, Kastratovic DA, Dimitrijevic MV, Konstantinovic VS, Jelovac DB, Antic J, et al. Oral squamous cell carcinoma detection by salivary biomarkers in a Serbian population. *Oral Oncol*. (2011) 47:51–5. doi: 10.1016/j.oraloncology.2010.10.009
167. St John MA, Li Y, Zhou X, Denny P, Ho CM, Montemagno C, et al. Interleukin 6 and interleukin 8 as potential biomarkers for oral cavity and oropharyngeal squamous cell carcinoma. *Arch Otolaryngol Head Neck Surg*. (2004) 130:929–35. doi: 10.1001/archotol.130.8.929

Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oral Health

MINI REVIEW
published: 05 March 2021
doi: 10.3389/froh.2021.647980

At the Crossroads of Molecular Biology and Immunology: Molecular Pathways for Immunological Targeting of Head and Neck Squamous Cell Carcinoma

Niels E. Wondergem[1], *Dennis N. L. M. Nijenhuis*[1], *Jos B. Poell*[1], *C. René Leemans*[1], *Ruud H. Brakenhoff*[1] *and Rieneke van de Ven*[1,2]*

[1] Amsterdam UMC, Vrije Universiteit Amsterdam, Department of Otolaryngology/Head and Neck Surgery, Cancer Center Amsterdam, Amsterdam, Netherlands, [2] Amsterdam Institute for Infection and Immunity, Amsterdam, Netherlands

OPEN ACCESS

Edited by:
Ricardo D. Coletta,
Campinas State University, Brazil

Reviewed by:
Wilfredo Alejandro González-Arriagada,
Universidad de Valparaiso, Chile
Abdelhakim Salem,
University of Helsinki, Finland

***Correspondence:**
Rieneke van de Ven
r.vandeven@amsterdamumc.nl

Specialty section:
This article was submitted to Oral Cancers, a section of the journal Frontiers in Oral Health

Received: 30 December 2020
Accepted: 10 February 2021
Published: 05 March 2021

Citation:
Wondergem NE, Nijenhuis DNLM, Poell JB, Leemans CR, Brakenhoff RH and van de Ven R (2021) At the Crossroads of Molecular Biology and Immunology: Molecular Pathways for Immunological Targeting of Head and Neck Squamous Cell Carcinoma. Front. Oral. Health 2:647980. doi: 10.3389/froh.2021.647980

Background: Recent advances in immunotherapy for head and neck squamous cell carcinoma (HNSCC) have led to implementation of anti-programmed death receptor 1 (PD-1) immunotherapy to standard of care for recurrent/metastatic HNSCC. However, the majority of tumors do not respond to these therapies, indicating that these tumors are not immunogenic or other immunosuppressive mechanisms might be at play.

Aim: Given their role in carcinogenesis as well as in immune modulation, we discuss the relation between the STAT3, PI3K/AKT/mTOR and Wnt signaling pathways to identify potential targets to empower the immune response against HNSCC.

Results: We focused on three pathways. First, STAT3 is often overactivated in HNSCC and induces the secretion of immunosuppressive cytokines, thereby promoting recruitment of immune suppressive regulatory T cells and myeloid-derived suppressor cells to the tumor microenvironment (TME) while hampering the development of dendritic cells. Second, PI3K/AKT/mTOR mutational activation results in increased tumor proliferation but could also be important in HNSCC immune evasion due to the downregulation of components in the antigen-processing machinery. Third, canonical Wnt signaling is overactivated in >20% of HNSCC and could be an interesting pleotropic target since it is related to increased tumor cell proliferation and the development of an immunosuppressive HNSCC TME.

Conclusion: The molecular pathology of HNSCC is complex and heterogeneous, varying between sites and disease etiology (i.e., HPV). The in HNSCC widely affected signaling pathways STAT3, PI3K/AKT/mTOR and Wnt are implicated in some of the very mechanisms underlying immune evasion of HNSCC, thereby representing promising targets to possibly facilitate immunotherapy response.

Keywords: head and neck cancer, molecular targets, immune microenvironment, immunotherapy, signaling pathways

INTRODUCTION

In 2018, 700,000 patients were diagnosed with head and neck squamous cell carcinoma (HNSCC) worldwide, and 350,000 patients died of the disease [1]. HNSCC develops from squamous epithelial cells in the upper-aerodigestive tract, most frequently in the oral cavity, oropharynx, hypopharynx and larynx. The development of HNSCC is caused by alcohol and tobacco use [2]. Besides this, infection with human papillomavirus (HPV) is related to formation of a specific type of HNSCC tumors (HPV^+) which are predominantly localized in the oropharynx and have distinct clinical, molecular and immunological characteristics when compared to HPV-unrelated (HPV^-) tumors [3].

From a molecular point of view HNSCCs are very heterogeneous. Besides mutational changes in oncogenes and most particularly tumor suppressor genes, also epigenetic changes and chromosomal instability add to the overall molecular heterogeneity [3, 4]. This variable genetic background translates into a variety of tumor characteristics, challenges treatment efficacy and demands personalized approaches. Likewise, heterogeneity is observed in the immune composition of the tumor microenvironment (TME) of HNSCC, depending on etiology and/or localization of the tumor as recently reviewed by us and others [5–7]. In general, HPV^+ HNSCC often display a more immune inflamed TME compared to HPV^- HNSCC, which are frequently immunologically cold or immune excluded. In addition, tumors with these separate etiologies seem to use different immune escape mechanisms [6, 7].

Despite advances in treatment, the survival rates for HNSCC have improved very moderately over the past five decades, with the average 5-year overall survival (OS) stabilizing at 40–60% for advanced stage disease [8]. Immunotherapy has been at the forefront of translational cancer research for the last decade and has provided great therapeutic benefits in the treatment of various cancer types [9], and also emerged as novel modality for HNSCC.

The currently available immunotherapies for treatment of HNSCC are nivolumab and pembrolizumab, which are both immune checkpoint inhibitors (ICIs) targeting the programmed death receptor 1 (PD-1). Nivolumab was approved in platinum refractory, recurrent/metastatic (R/M) HNSCC on basis of the results of the Checkmate-141 trial showing superior overall survival (OS) of the nivolumab treated arm when compared to investigator's choice of therapy (7.5 vs. 5.1 months; HR 0.70; 97.73% CI 0.51–0.96; $p = 0.01$) [10]. Pembrolizumab was first approved for platinum refractory R/M HNSCC based on the Keynote-012 trial that showed objective responses in 16% (95% CI 11–22) of patients, of which 5% were complete and 82% were durable ($\geq$6 months) [11, 12]. The Keynote-048 trial has led to the approval of pembrolizumab as monotherapy or in combination with chemotherapy as first-line treatment for programmed death-ligand 1 (PD-L1) positive R/M HNSCC [13].

Many studies are underway investigating the use of these ICIs, and evaluating other immunotherapy regimens [14]. Recently promising results have been reported for pembrolizumab and nivolumab with or without ipilimumab [anti-cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4)] neoadjuvant to surgery with curative intent in oral cavity squamous cell carcinoma [15, 16].

Currently, anti-PD-1 immunotherapy is effective in only a minority of HNSCC patients [17], and evidence accumulates that HNSCC can indeed be highly immune-evasive [18]. One approach is to study expression of immune-related genes in HNSCC that might relate to immune cell infiltration, predict patient outcome and could be applied to guide treatment choice [19]. Another approach to overcome immune-evasion might be to apply targeted therapies directed at molecular pathways known to be affected in HNSCC and that specifically relate to its immunological characteristics. To develop such targeted therapies, it is crucial to understand the specific pathways involved. In this review we relate the molecular landscape of HNSCC to its immunological characteristics, focusing on three of the most frequently affected pathways in HNSCC STAT3, PI3K/AKT/mTOR and Wnt, besides the EGFR pathway as a known target.

IMMUNOLOGICAL IMPLICATIONS OF THE HNSCC MOLECULAR LANDSCAPE

EGFR

One of the major hallmarks of cancer is sustained proliferative signaling [20]. Growth factor signaling is commonly mediated by the family of receptor tyrosine kinase (RTK) cell-surface receptors, of which epidermal growth factor receptor (EGFR) is the most prominent in HNSCC [21]. It signals through the RAS-MAPK-, PI3K/AKT/mTOR-, phospholipase C-gamma-, signal transducers and activators of transcription (STAT)- and Src family kinase pathways [22]. Activation of EGFR promotes cell proliferation, angiogenesis, invasiveness and metastatic potential and has a prominent role in tumor initiation and maintenance. Activation of EGFR is seen in up to 80–90% of HNSCC cases as a result of *EGFR* amplifications or autocrine loops [23]. It should be noted, however, that *EGFR* does not show the typical activating mutations in HNSCC that are found in lung cancer, and whether HNSCC cells are really oncogene addicted remains elusive.

Cetuximab, a monoclonal antibody targeting EGFR, was the first new drug in decades, and the first targeted therapy, to be FDA approved for treatment of HNSCC [24]. Initially, cetuximab seemed quite promising with a favorable toxicity profile compared to chemotherapeutics, but recent phase III trials have demonstrated its inferiority to cisplatin in terms of primary disease control [25]. Cetuximab remains one of the cornerstones in the treatment of patients unfit to receive cisplatin and in the R/M setting [26].

While framed as a targeted drug and not introduced as an immunotherapy agent, cetuximab appeared to have an additional immune-related mode-of-action through the mediation of antibody-dependent cellular cytotoxicity (ADCC) [25]. Cetuximab stimulates the CD16/Fc receptor of natural killer (NK) cells resulting in their activation and the release of granzymes and perforins leading to tumor kill [25]. Increased interferon gamma (IFNγ) production by NK cells can induce PD-L1 expression on tumor cells and immune cells within

the TME, providing a rationale for the possible synergistic potential of combining cetuximab with anti-PD-1 therapy [27–29]. Response to cetuximab treatment in HNSCC was shown to be hampered by treatment-induced recruitment of PD-1$^+$ and TIM-3$^+$ dysfunctional tumor-infiltrating T cells [30]. Also, PD-L1 expression on tumor cells hampered the cytolytic abilities of PD-1$^+$ activated NK cells, reducing cetuximab efficacy in patients [31]. Preliminary data of a phase II trial evaluating the combination of PD-1 and EGFR inhibitory therapy showed promising results in 33 R/M HNSCC patients unfit for or refractory to cisplatin, with an overall response rate of 41% and few adverse events [32]. Results on other trials investigating the potential of this combinational regimen are to be awaited (**Supplementary Table 1**) [33].

STAT3

STAT3 is part of the STAT protein family that regulates the transcription of various proliferative and cytokine-related genes [34]. It is of particular interest in HNSCC because of its near-universal signaling activation, already during early oral carcinogenesis [35], and its ability to be directly activated by EGFR [34]. As STAT3 mutations in HNSCC, leading to gain-of-function, have not been described, activation of STAT3 is presumably the result of enhanced signaling through its positive regulators (cytokines, growth factors and non-receptor TKs), or decreased signaling through its negative regulators (protein tyrosine phosphatase receptors) [36]. There are differences reported between HPV$^+$ and HPV$^-$ HNSCC with regards to STAT3 mutations and activation (**Figure 1**). Gaykalova et al. reported significantly more activated STAT3, as well as activated NF-κB, in HPV$^-$ HNSCC [37].

In addition to supporting tumor cell proliferation, STAT3 activity is related to a variety of immunosuppressive mechanisms and is a key regulator of immune processes (**Figure 2**) [43]. STAT3 inhibition could thus hit two birds with one stone. It is implicated in inhibition of pro-inflammatory mediators production such as IFNγ, antigen presentation, and accumulation and anti-tumor potential of effector T cells [44]. In myeloid-derived suppressor cells (MDSCs), STAT3 activation leads to expression of cyclin D and the S100A9 receptor, resulting in the suppression of cellular maturation and increased MDSC survival, respectively [45]. In the treatment of HNSCC, radiotherapy was shown to cause immune modulation by activating STAT3 in MDSCs [46]. Furthermore, STAT3 has the ability to increase expression of the PD-1/PD-L1/L2 and CTLA-4 checkpoints [44, 47].

Altogether, these data provide a rationale for combining STAT3 inhibitors with ICIs, which has already led to various pre-clinical and clinical studies [36, 44, 48]. AZD9150, a small DNA oligonucleotide which competitively blocks the binding site of STAT3 on its promotor has been tested in a phase I dose escalation study [49]. Results showed stable disease in 44% (n = 11/25) of all patients, and tumor shrinkage in 50% (n = 3/6) of treatment refractory lymphoma patients. In a follow-up study in treatment refractory lymphoma a clinical benefit was observed in 13% of patients [50]. A large study

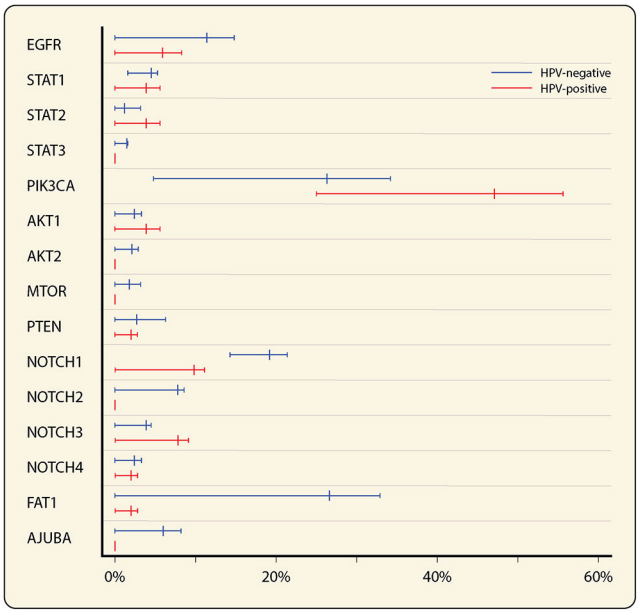

FIGURE 1 | Forest plot of mutation rates of most frequent affected genes in EGFR, PI3K/AKT/mTOR and Wnt-signaling pathways in HNSCC, stratified by HPV status (total n = 385, 334 HPV$^-$ and 51 HPV$^+$). Mean mutation rates were estimated from the mutation rates in three HNSCC datasets [38–40], queried through cBioPortal [41, 42]. Minimum and maximum reported mutation rates are represented by the error bars.

investigating AZD9150 combined with durvalumab anti-PD-L1 immunotherapy in patients with solid tumors including HNSCC is underway [51]. In a recent *in vitro* study in breast cancer sentinel lymph nodes (SLN), van Pul *et al.* showed that STAT3 inhibition in immune cells, combined with immune stimulation through TLR9 using CpG-B, could activate dendritic cell (DC) subsets in SLN cultures and increased tumor-specific T cell responses [52]. Using *in vivo* HNSCC mouse models, Moreira et al. showed that a STAT3-inhibiting oligonucleotide linked to CpG specifically targeted to myeloid cells, increased tumor sensitivity to radiotherapy and increased the anti-tumor immune response, suggesting that this could be a valid pathway to target in HNSCC [53]. In a syngeneic carcinogen-induced immune competent HNSCC mouse model, a small molecule inhibitor HNC0014, targeting cMET/STAT3/CD44 and PD-L1, was shown to reduce tumor growth, pSTAT3 and PD-L1 levels in tumors and increase T cell frequencies in the circulation most efficiently when combined with anti-PD-L1 treatment [48]. Of note, STAT3 inhibition was shown to reduce PD-L1 expression in HNSCC cell lines [47]. Potentially, STAT3 inhibition by itself might already reduce the inhibitory effect of the PD-1/PD-L1 axis, especially when tumor PD-L1 expression is regulated through oncogenic pathway activation often seen in HPV$^-$ HNSCC. Combination therapy with ICIs targeting other immune checkpoints like TIM-3, LAG-3 or TIGIT might prove even more effective [54]. However, little is known at the moment about the effect of STAT3 on the expression of those immune checkpoints.

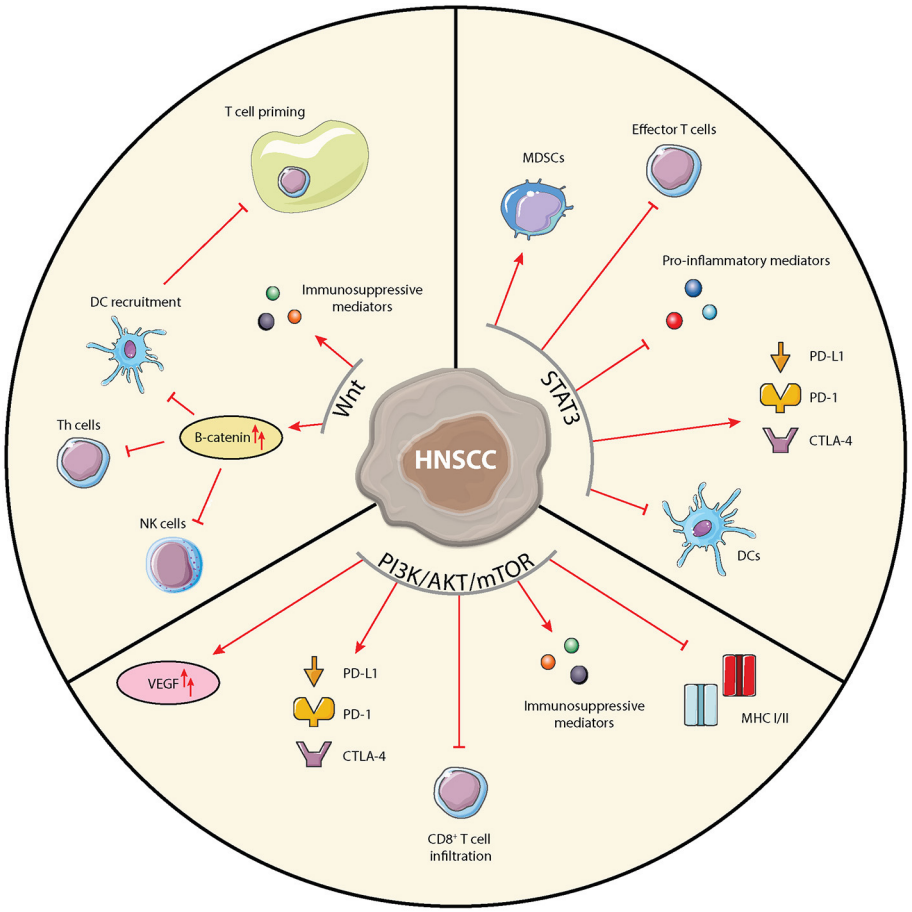

FIGURE 2 | Schematic overview of immunological implications of the STAT3, PI3K/AKT/mTOR, and Wnt-signaling pathways in HNSCC cells. Arrows and bar-headed lines indicate stimulating and inhibiting effects, respectively, where red depicts pro-tumorigenic and green depicts anti-tumorigenic effects.

PI3K/AKT/mTOR Pathway

The PI3K/AKT/mTOR pathway is involved in many cellular processes including cell cycle, survival, proliferation and motility [55]. Phosphatidylinositol 3-kinases (PI3Ks) are heterodimeric kinases formed by a regulatory and catalytic subunit and are activated by RTKs. The p110α catalytic subunit is encoded by a variety of genes of which *PIK3CA* is most important and harbors alterations in 26 and 47% of HPV^- and HPV^+ HNSCC, respectively (**Figure 1**) [38–40]. Upon RTK activation, the regulatory subunit binds the catalytic subunit resulting in lipid phosphorylation and a cascade of events leading to activation of AKT, one of the major effectors of PI3K.

AKT is a serine-threonine kinase comprising three isoforms that are encoded by the *AKT1*, *AKT2,* and *AKT3* genes. Alterations in these genes are uncommon but overexpression of AKT in HNSCC has been reported as the result of a variety of factors such as microenvironmental stimuli, mutations in *PIK3CA* and diminished expression of PTEN [56–59]. PIK3CA and PTEN serve as central regulators of the PI3K/AKT/mTOR pathway and are known as bona fide HNSCC cancer genes.

The serine-threonine kinase mTOR comprises mTORC1 and mTORC2 and is an important downstream effector of AKT. It regulates critical biological functions including growth factor signaling and metabolism [55]. Genetic alterations in *EGFR*, *PIK3CA*, *PTEN*, and *HRAS* are known deregulators of mTOR signaling and are amongst the most frequently affected genes in HNSCC.

Various agents targeting PI3K, AKT, or mTOR have been studied in pre-clinical and clinical studies [60]. Although some studies in HNSCC have shown promising results, no trials have made it to phase III thus far due to unsatisfying efficacy and challenging tolerability. These early clinical trials have, however, provided valuable insights into the various physiological roles of the PI3K/AKT/mTOR pathway. It has become increasingly clear that apart from its functions in cancer cells, the PI3K/AKT/mTOR pathway also regulates many processes within the TME [61]. In various cancer types the PI3K/AKT/mTOR pathway has been implicated in the expression of immunosuppressive chemokines and cytokines [62, 63], expression of the immune suppressive vascular endothelial growth factor (VEGF) [64], reduced tumor $CD8^+$ T cell

infiltration [65], expression of immune checkpoints [66–68], and expression of MHC classes I and II (**Figure 2**) [69, 70]. In HNSCC the association of activated PI3K signaling with suppression of MHC expression was demonstrated using IHC, showing an inverse staining of MHC-I and phospho-AKT [71]. Moore et al. reported that co-inhibition of mTOR and PD-L1 enhanced anti-tumor efficacy in an oral cancer mouse model [72]. Since the PI3K/AKT/mTOR pathway is overactivated in over 90% of HNSCC, through various mechanisms, it is a highly interesting pathway to target in HNSCC, especially combined with ICIs [60].

Wnt-Signaling

The Wnt-signaling cascade is an evolutionary highly conserved cascade important in (embryonic) cell growth, migration and differentiation [73]. About half of breast cancer tumors involve an activated Wnt-signaling pathway and hereditary colon cancer is often induced by a mutation in the adenomatous polyposis coli (*APC*) gene, part of the Wnt-signaling cascade [73]. Also in HNSCC, Wnt-signaling has been recognized as a central player [74].

Canonical Wnt-signaling depends on the APC destruction complex that regulates β-catenin levels, whereas non-canonical Wnt-signaling does not involve the APC complex. Binding of Wnt ligands to the Frizzled receptors leads to activation of the signaling pathway. When the Frizzled receptors are not stimulated by Wnt, cytoplasmic β-catenin levels are regulated by a destruction complex that includes amongst others APC, Axin and glycogen synthase kinase 3β (GSK3β) [73]. β-catenin is phosphorylated by GSK3β and subsequently degraded by the proteasome. Upon Frizzled receptor activation, however, Axin, APC and GSK3β are recruited to the membrane leading to inactivation of the destruction complex. Hence, β-catenin is able to translocate to the nucleus where it drives expression of target genes. These in turn regulate diverse cellular functions including cell proliferation, -survival and -migration [2].

β-catenin levels can be regulated by other processes as well, forming a source of non-canonical pathways. In HNSCC, several genes involved in Wnt-signaling are mutated or inactivated. The Cancer Genome Atlas (TCGA) data showed inactivating *FAT1* mutations in 23% of HNSCCs [38]. FAT1 is a cadherin-related adhesion receptor which can form cellular adhesion structures and therefore plays a role in cell-cell contact. Cadherin receptors can sequester β-catenin to the plasma membrane, thus preventing its translocation to the nucleus [75]. *FAT1* knockdown in glioblastoma cell lines resulted in promotion of cell cycle progression and cellular growth and in a breast cancer xenograft model *FAT1* knockdown resulted in the progression of ductal carcinoma *in situ* to invasive breast cancer [75, 76]. In HNSCC cell lines the effect of *FAT1* knockdown was inconsistent between studies, being associated with both increased [77] and decreased [78] migration and tumorigenesis. In HNSCC patients, *FAT1* mutations and downregulation are independent predictors of shorter disease-free survival [77]. Further studies are required to clarify the role of FAT1 in HNSCC carcinogenesis, including its effect on Wnt signaling.

AJUBA is a scaffold protein which stimulates the phosphorylation and degradation of β-catenin by activating GSK3β. Inactivating *AJUBA* mutations could result in a lower rate of β-catenin phosphorylation which in turn increases β-catenin-mediated gene transcription. Inactivating mutations in *AJUBA* are relatively infrequent in HNSCC with a prevalence of 6% and seem more common in HPV$^-$ disease [38].

Also implicated in the Wnt-signaling pathway is Notch signaling. The Notch family comprises the Notch 1, Notch 2, Notch 3, and Notch 4 receptors, which are cleaved upon binding to their ligand through cell-cell contact, leading to the transfer of their intracellular domain into the nucleus to regulate the expression of target genes. The overall role of Notch signaling in cancer development is still highly debated. Mutational inactivation of Notch has been associated with tumor development, but overexpression has been associated with tumor progression and immune evasion [79]. *Notch 1* mutations are reported in 19% of HNSCC, most common in HPV$^-$ tumors (**Figure 1**), and are correlated to worse outcome [80].

Loganathan et al. performed a CRISPR-Cas study in a HNSCC mouse model, in which they induced mutations in a wide-array of the so-called "long tail" genes [81], genes that are significantly mutated in tumors, but at very low frequency. Strikingly, mutational inactivation of p53 itself did not induce HNSCC, but introduction of mutations in these long tail genes did induce HNSCC tumor growth [81]. When evaluating the specific long tail genes it was found that knockout of *AJUBA* resulted in tumor growth but more importantly, it resulted in a significant decrease in Notch signaling [81]. Induction of Notch expression reversed the increased tumor growth of AJUBA-deficient mice, implicating Notch as the tumor-suppressive factor. Upon investigating the entire long tail gene panel, the authors ultimately concluded that in 67% of HNSCC cases, oncogenic mutations often impact Notch signaling, proposing decreased Notch signaling as a new hallmark of HNSCC [81].

The immunomodulatory role of the Wnt-signaling pathway in cancer has been reviewed extensively [82–85]. In line with its ubiquitous expression and involvement in many diverse cell functions, the Wnt pathway impacts many of the immunological players in the TME (**Figure 2**). Through activation of the Wnt-signaling pathway, DCs are pushed into a regulatory, tumor tolerant state. A recent study by Lopez-Gonzalez *et al.* showed that inhibition of Wnt-signaling through introduction of a constitutively active form of GSK3β restored human DC activation in the presence of immune suppressive cytokines and in an *in vivo* melanoma mouse model resulted in DC recruitment and activation and promoted tumor control [86]. Wnt-pathway activation in tumor cells negatively regulates the production of the chemokine CCL4, inhibiting the recruitment of cross-presenting DCs into the TME [87]. Consequently, infiltration of effector T cells into the TME is impaired, as no nodal T cell priming occurs. In melanoma cells, increased β-catenin expression was shown to even further hamper T cell priming through upregulation of IL-10 [88]. Indeed, analysis of TCGA data indicated that activation of the Wnt-pathway correlated with an immune-excluded TME in 90% of 31 included tumor types [89]. Additionally, upregulated β-catenin expression has been associated with impaired development and function of Th cells, impaired maturation of NK cells, and increased Treg survival

[90]. Colombo et al. reviewed how cancer cells can use Notch signaling to change to cytokine milieu by secreting immune suppressive factors such as TGF-β, IL-10, CXCL12, thereby shaping a pro-tumorigenic microenvironment via cross-talk with stromal cells [91].

Hence, targeting Wnt-signaling seems a promising treatment strategy to augment ICI in HNSCC given its role in carcinogenesis as well as in shaping the TME [92]. Multiple small molecule inhibitors have been developed and some have already entered clinical trials also for HNSCC (**Supplementary Table 1**). LGK974 was shown to effectively inhibit *in vitro* HNSCC tumor growth and metastasis [93] and ICG001 was shown to downregulate HNSCC cancer stem cells and tumor growth *in vitro* [74]. Several other inhibitors of the Wnt-pathway are currently under clinical investigation [94].

Clinical trials targeting the Notch pathway have been performed and are currently ongoing [95]. However, inhibition of Notch is often associated with high toxicity possibly related to the notion that Notch signaling influences a wide array of processes [79, 81]. Clearly these issues need to be addressed, potentially by local rather than systemic inhibition of Notch, before Notch inhibitors could safely make their way to the clinic. To our knowledge, there are currently no targeted therapies available for *FAT1* or *AJUBA* mutated tumors. Obviously the notion that all these genes display inactivating mutations in HNSCC, hampers the exploitation as druggable target, and detailed functional characterization of the signaling pathways is crucial.

CONCLUSION AND FUTURE PERSPECTIVES

We aimed to outline potential targets to aid HNSCC immunotherapy response by linking selected molecular pathways, widely-affected in HNSCC, to their immunological implications. STAT3 and the PI3K/AKT/mTOR- and Wnt-pathways are of particular interest, because they are implicated in some of the very mechanisms believed to be responsible for primary or acquired resistance to ICI therapy. Interfering with the immunomodulatory functions of these pathways could provide a means to boost the response to ICI therapies. Especially for HPV^- HNSCC, which is considered a immunologically "cold" tumor, such strategies may have promise. Cold tumors, characterized by an immune-excluded or -desert phenotype, are known to respond poorly to ICI [96], and could be pushed toward a more inflamed or "hot" phenotype by enabling $CD8^+$ T cell infiltration through inhibition of the PI3K/AKT/mTOR or Wnt-pathways. Inhibiting these pathways would on the one hand inhibit the tumor cells, and on the other hand stimulate the immune cells. Moreover, the milieu of cytokines and other mediators including IFNγ and VEGF seems tailorable through the proposed pathways, as well as the expression of MHCI/II and immune checkpoints like PD-1/L1 and CTLA-4. The TME could additionally be organized into a more pro-inflammatory state through regulating the migration of immunosuppressive players, such as Tregs or MDSCs into the TME as well as directly mitigating their modulatory functions by targeting STAT3 both in the tumor cells and the immune cells. An overview of recent trials investigating agents targeting the discussed pathways is given in **Supplementary Table 1**. Given the difference in prevalence of alterations in particular molecular pathways between HPV^+ and HPV^- HNSCC (**Figure 1**), certain targets might be more relevant for one of the etiologies. Interference with the STAT3 pathway could hold more clinical relevance for HPV^- disease, while in HPV^+ disease the PI3K/AKT/mTOR pathway might be a more suitable target. Future clinical studies should consider building in a comparison between HPV^+ and HPV^- HNSCC when evaluating the efficacy of drugs interfering with these molecular pathways. Moreover, it remains crucial to consider the fact that the functional consequences of targeting the discussed pathways could be unpredictable due to the complex composition of the HNSCC TME [97]. Dissecting the impact of targeted and combination therapies on the various cell populations involved in HNSCC may help explain the success or failure to potentiate an anti-tumor immune response.

AUTHOR CONTRIBUTIONS

NW and DN wrote the manuscript. JP, CL, RB, and RV guided and revised the manuscript. RV conceptualized and guided the project. All authors contributed to the article and approved the submitted version.

FUNDING

NW has recieved funding from the HANARTH Foundation.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/froh.2021.647980/full#supplementary-material

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* (2018) 68:394–424. doi: 10.3322/caac.21492
2. Leemans CR, Snijders PJF, Brakenhoff RH. The molecular landscape of head and neck cancer. *Nat Rev Cancer.* (2018) 18:269–82. doi: 10.1038/nrc.2018.11
3. Leemans CR, Braakhuis BJ, Brakenhoff RH. The molecular biology of head and neck cancer. *Nat Rev Cancer.* (2011) 11:9–22. doi: 10.1038/nrc2982
4. Gaffey MJ, Frierson HF, Mills SE. *Tumors of the Upper Aerodigestive Tract and Ear*. 4th ed. Washington, DC: Armed Forces Institute of Pathology (2000).
5. Bhat AA, Yousuf P, Wani NA, Rizwan A, Chauhan SS, Siddiqi MA, et al. Tumor microenvironment: an evil nexus promoting aggressive head and neck squamous cell carcinoma and avenue for targeted therapy. *Signal Transduct Target Ther.* (2021) 6:12. doi: 10.1038/s41392-020-00419-w

6. Seliger B, Massa C, Yang B, Bethmann D, Kappler M, Eckert AW, et al. Immune escape mechanisms and their clinical relevance in head and neck squamous cell carcinoma. *Int J Mol Sci.* (2020) 21:7032. doi: 10.3390/ijms21197032
7. Wondergem NE, Nauta IH, Muijlwijk T, Leemans CR, van de Ven R. The immune microenvironment in head and neck squamous cell carcinoma: on subsets and subsites. *Curr Oncol Rep.* (2020) 22:81. doi: 10.1007/s11912-020-00938-3
8. Marur S, Forastiere AA. Head and neck squamous cell carcinoma: update on epidemiology, diagnosis, and treatment. *Mayo Clin Proc.* (2016) 91:386–96. doi: 10.1016/j.mayocp.2015.12.017
9. Waldman AD, Fritz JM, Lenardo MJ. A guide to cancer immunotherapy: from T cell basic science to clinical practice. *Nat Rev Immunol.* (2020) 20:651–68. doi: 10.1038/s41577-020-0306-5
10. Ferris RL, Blumenschein G, Jr., Fayette J, Guigay J, Colevas AD, et al. Nivolumab for recurrent squamous-cell carcinoma of the head and neck. *N Engl J Med.* (2016) 375:1856–67. doi: 10.1056/NEJMoa1602252
11. Seiwert TY, Burtness B, Mehra R, Weiss J, Berger R, Eder JP, et al. Safety and clinical activity of pembrolizumab for treatment of recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-012): an open-label, multicentre, phase 1b trial. *Lancet Oncol.* (2016) 17:956–65. doi: 10.1016/S1470-2045(16)30066-3
12. Larkins E, Blumenthal GM, Yuan W, He K, Sridhara R, Subramaniam S, et al. FDA approval summary: pembrolizumab for the treatment of recurrent or metastatic head and neck squamous cell carcinoma with disease progression on or after platinum-containing chemotherapy. *Oncologist.* (2017) 22:873–8. doi: 10.1634/theoncologist.2016-0496
13. Burtness B, Harrington KJ, Greil R, Soulieres D, Tahara M, de Castro G, et al., et al. Pembrolizumab alone or with chemotherapy versus cetuximab with chemotherapy for recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-048): a randomised, open-label, phase 3 study. *Lancet.* (2019) 394:1915–28. doi: 10.1016/S0140-6736(19)32591-7
14. Chow LQM. Head and neck cancer. *N Engl J Med.* (2020) 382:60–72. doi: 10.1056/NEJMra1715715
15. Uppaluri R, Campbell KM, Egloff AM, Zolkind P, Skidmore ZL, Nussenbaum B, et al. Neoadjuvant and adjuvant pembrolizumab in resectable locally advanced, human papillomavirus-unrelated head and neck cancer: a multicenter, phase II trial. *Clin Cancer Res.* (2020) 26:5140–52. doi: 10.1158/1078-0432.CCR-20-1695
16. Schoenfeld JD, Hanna GJ, Jo VY, Rawal B, Chen YH, Catalano PS, et al. Neoadjuvant nivolumab or nivolumab plus ipilimumab in untreated oral cavity squamous cell carcinoma: a phase 2 open-label randomized clinical trial. *JAMA Oncol.* (2020). doi: 10.1001/jamaoncol.2020.2955
17. Cramer JD, Burtness B, Le QT, Ferris RL. The changing therapeutic landscape of head and neck cancer. *Nat Rev Clin Oncol.* (2019) 16:669–83. doi: 10.1038/s41571-019-0227-z
18. Kok VC. Current understanding of the mechanisms underlying immune evasion from PD-1/PD-L1 immune checkpoint blockade in head and neck cancer. *Front Oncol.* (2020) 10:268. doi: 10.3389/fonc.2020.00268
19. Qiu Y, Cui L, Lin Y, Gao B, Li J, Zhao X, et al. Development and validation of a robust immune prognostic signature for head and neck squamous cell carcinoma. *Front Oncol.* (2020) 10:1502. doi: 10.3389/fonc.2020.01502
20. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell.* (2011) 144:646–74. doi: 10.1016/j.cell.2011.02.013
21. Hynes NE, Lane HA. ERBB receptors and cancer: the complexity of targeted inhibitors. *Nat Rev Cancer.* (2005) 5:341–54. doi: 10.1038/nrc1609
22. Yarden Y, Pines G. The ERBB network: at last, cancer therapy meets systems biology. *Nat Rev Cancer.* (2012) 12:553–63. doi: 10.1038/nrc3309
23. Kalyankrishna S, Grandis JR. Epidermal growth factor receptor biology in head and neck cancer. *J Clin Oncol.* (2006) 24:2666–72. doi: 10.1200/JCO.2005.04.8306
24. Bonner JA, Harari PM, Giralt J, Azarnia N, Shin DM, Cohen RB, et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. *N Engl J Med.* (2006) 354:567–78. doi: 10.1056/NEJMoa053422
25. Moy JD, Moskovitz JM, Ferris RL. Biological mechanisms of immune escape and implications for immunotherapy in head and neck squamous cell carcinoma. *Eur J Cancer.* (2017) 76:152–66. doi: 10.1016/j.ejca.2016.12.035
26. Vermorken JB, Mesia R, Rivera F, Remenar E, Kawecki A, Rottey S, et al. Platinum-based chemotherapy plus cetuximab in head and neck cancer. *New Engl J Med.* (2008) 359:1116–27. doi: 10.1056/NEJMoa0802656
27. Chen N, Fang W, Zhan J, Hong S, Tang Y, Kang S, et al. Upregulation of PD-L1 by EGFR activation mediates the immune escape in EGFR-driven NSCLC: implication for optional immune targeted therapy for NSCLC patients with EGFR mutation. *J Thorac Oncol.* (2015) 10:910–23. doi: 10.1097/JTO.0000000000000500
28. Azuma K, Ota K, Kawahara A, Hattori S, Iwama E, Harada T, et al. Association of PD-L1 overexpression with activating EGFR mutations in surgically resected nonsmall-cell lung cancer. *Ann Oncol.* (2014) 25:1935–40. doi: 10.1093/annonc/mdu242
29. Concha-Benavente F, Srivastava RM, Trivedi S, Lei Y, Chandran U, Seethala RR, et al. Identification of the cell-intrinsic and -extrinsic pathways downstream of EGFR and IFNgamma that induce PD-L1 expression in head and neck cancer. *Cancer Res.* (2016) 76:1031–43. doi: 10.1158/0008-5472.CAN-15-2001
30. Jie HB, Srivastava RM, Argiris A, Bauman JE, Kane LP, Ferris RL. Increased PD-1(+) and TIM-3(+) TILs during cetuximab therapy inversely correlate with response in head and neck cancer patients. *Cancer Immunol Res.* (2017) 5:408–16. doi: 10.1158/2326-6066.CIR-16-0333
31. Concha-Benavente F, Kansy B, Moskovitz J, Moy J, Chandran U, Ferris RL. PD-L1 mediates dysfunction in activated PD-1(+) NK cells in head and neck cancer patients. *Cancer Immunol Res.* (2018) 6:1548–60. doi: 10.1158/2326-6066.CIR-18-0062
32. Sacco AG, Chen R, Ghosh D, Wong DJ, Worden FP, Adkins D, et al. An open label, nonrandomized, multi-arm, phase II trial evaluating pembrolizumab combined with cetuximab in patients with recurrent/metastatic (R/M) head and neck squamous cell carcinoma (HNSCC): Results of cohort 1 interim analysis. *Am Soc Clin Oncol.* 37:6033. (2019). doi: 10.1200/JCO.2019.37.15_suppl.6033
33. Saba NF, Chen ZG, Haigentz M, Bossi P, Rinaldo A, Rodrigo JP, et al. Targeting the EGFR and immune pathways in squamous cell carcinoma of the head and neck (SCCHN): forging a new alliance. *Mol Cancer Ther.* (2019) 18:1909–15. doi: 10.1158/1535-7163.MCT-19-0214
34. Lo HW, Hsu SC, Ali-Seyed M, Gunduz M, Xia W, Wei Y, et al. Nuclear interaction of EGFR and STAT3 in the activation of the iNOS/NO pathway. *Cancer Cell.* (2005) 7:575–89. doi: 10.1016/j.ccr.2005.05.007
35. Grandis JR, Drenning SD, Zeng Q, Watkins SC, Melhem MF, Endo S, et al. Constitutive activation of Stat3 signaling abrogates apoptosis in squamous cell carcinogenesis *in vivo*. *Proc Natl Acad Sci USA.* (2000) 97:4227–32. doi: 10.1073/pnas.97.8.4227
36. Geiger JL, Grandis JR, Bauman JE. The STAT3 pathway as a therapeutic target in head and neck cancer: barriers and innovations. *Oral Oncol.* (2016) 56:84–92. doi: 10.1016/j.oraloncology.2015.11.022
37. Gaykalova DA, Manola JB, Ozawa H, Zizkova V, Morton K, Bishop JA, et al. NF-kappaB and stat3 transcription factor signatures differentiate HPV-positive and HPV-negative head and neck squamous cell carcinoma. *Int J Cancer.* (2015) 137:1879–89. doi: 10.1002/ijc.29558
38. Cancer Genome Atlas N. Comprehensive genomic characterization of head and neck squamous cell carcinomas. *Nature.* (2015) 517:576–82. doi: 10.1038/nature14129
39. Agrawal N, Frederick MJ, Pickering CR, Bettegowda C, Chang K, Li RJ, et al. Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. *Science.* (2011) 333:1154–7. doi: 10.1126/science.1206923
40. Stransky N, Egloff AM, Tward AD, Kostic AD, Cibulskis K, Sivachenko A, et al. The mutational landscape of head and neck squamous cell carcinoma. *Science.* (2011) 333:1157–60. doi: 10.1126/science.1208130
41. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov.* (2012) 2:401–4. doi: 10.1158/2159-8290.CD-12-0095
42. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles

using the cBioPortal. *Sci Signal.* (2013) 6:pl1. doi: 10.1126/scisignal.2004088
43. Rebe C, Ghiringhelli F. STAT3, a master regulator of anti-tumor immune response. *Cancers.* (2019) 11:1280. doi: 10.3390/cancers11091280
44. Zou S, Tong Q, Liu B, Huang W, Tian Y, Fu X. Targeting STAT3 in cancer immunotherapy. *Mol Cancer.* (2020) 19:145. doi: 10.1186/s12943-020-01258-7
45. Kumar V, Patel S, Tcyganov E, Gabrilovich DI. The nature of myeloid-derived suppressor cells in the tumor microenvironment. *Trends Immunol.* (2016) 37:208–20. doi: 10.1016/j.it.2016.01.004
46. Sampath S, Won H, Massarelli E, Li M, Frankel P, Vora N, et al. Combined modality radiation therapy promotes tolerogenic myeloid cell populations and STAT3-related gene expression in head and neck cancer patients. *Oncotarget.* (2018) 9:11279–90. doi: 10.18632/oncotarget.24397
47. Bu LL, Yu GT, Wu L, Mao L, Deng WW, Liu JF, et al. STAT3 induces immunosuppression by upregulating PD-1/PD-L1 in HNSCC. *J Dent Res.* (2017) 96:1027–34. doi: 10.1177/0022034517712435
48. Lee JC, Wu ATH, Chen JH, Huang WY, Lawal B, Mokgautsi N, et al. HNC0014, a multi-targeted small-molecule, inhibits head and neck squamous cell carcinoma by suppressing c-Met/STAT3/CD44/PD-L1 oncoimmune signature and eliciting antitumor immune responses. *Cancers.* (2020) 12:3759. doi: 10.3390/cancers12123759
49. Hong D, Kurzrock R, Kim Y, Woessner R, Younes A, Nemunaitis J, et al. AZD9150, a next-generation antisense oligonucleotide inhibitor of STAT3 with early evidence of clinical activity in lymphoma and lung cancer. *Sci Transl Med.* (2015) 7:314ra185. doi: 10.1126/scitranslmed.aac5272
50. Reilley MJ, McCoon P, Cook C, Lyne P, Kurzrock R, Kim Y, et al. STAT3 antisense oligonucleotide AZD9150 in a subset of patients with heavily pretreated lymphoma: results of a phase 1b trial. *J Immunother Cancer.* (2018) 6:119. doi: 10.1186/s40425-018-0436-5
51. Cohen EEW, Harrington KJ, Hong DS, Mesia R, Brana I, Perez Segura P, et al. A phase Ib/II study (SCORES) of durvalumab (D) plus danvatirsen (DAN; AZD9150) or AZD5069 (CX2i) in advanced solid malignancies and recurrent/metastatic head and neck squamous cell carcinoma (RM-HNSCC): updated results. *Ann Oncol.* (2018) 29:viii372. doi: 10.1093/annonc/mdy287
52. van Pul KM, Vuylsteke R, de Beijer MTA, van de Ven R, van den Tol MP, Stockmann H, et al. Breast cancer-induced immune suppression in the sentinel lymph node is effectively countered by CpG-B in conjunction with inhibition of the JAK2/STAT3 pathway. *J Immunother Cancer.* (2020) 8:e000761. doi: 10.1136/jitc-2020-000761
53. Moreira D, Sampath S, Won H, White SV, Su YL, Alcantara M, et al. Myeloid cell-targeted STAT3 inhibition sensitizes head and neck cancers to radiotherapy and T cell-mediated immunity. *J Clin Invest.* (2020) 131:e137001. doi: 10.1172/JCI137001
54. Qin S, Xu L, Yi M, Yu S, Wu K, Luo S. Novel immune checkpoint targets: moving beyond PD-1 and CTLA-4. *Mol Cancer.* (2019) 18:155. doi: 10.1186/s12943-019-1091-2
55. Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. *Nat Rev Cancer.* (2009) 9:550–62. doi: 10.1038/nrc2664
56. Amornphimoltham P, Sriuranpong V, Patel V, Benavides F, Conti CJ, Sauk J, et al. Persistent activation of the Akt pathway in head and neck squamous cell carcinoma: a potential target for UCN-01. *Clin Cancer Res.* (2004) 10(12 Pt 1):4029–37. doi: 10.1158/1078-0432.CCR-03-0249
57. Garcia-Carracedo D, Villaronga MA, Alvarez-Teijeiro S, Hermida-Prado F, Santamaria I, Allonca E, et al. Impact of PI3K/AKT/mTOR pathway activation on the prognosis of patients with head and neck squamous cell carcinomas. *Oncotarget.* (2016) 7:29780–93. doi: 10.18632/oncotarget.8957
58. Bussink J, van der Kogel AJ, Kaanders JH. Activation of the PI3-K/AKT pathway and implications for radioresistance mechanisms in head and neck cancer. *Lancet Oncol.* (2008) 9:288–96. doi: 10.1016/S1470-2045(08)70073-1
59. Stegeman H, Kaanders JH, Wheeler DL, van der Kogel AJ, Verheijen MM, Waaijer SJ, et al. Activation of AKT by hypoxia: a potential target for hypoxic tumors of the head and neck. *BMC Cancer.* (2012) 12:463. doi: 10.1186/1471-2407-12-463
60. Marquard FE, Jucker M. PI3K/AKT/mTOR signaling as a molecular target in head and neck cancer. *Biochem Pharmacol.* (2020) 172:113729. doi: 10.1016/j.bcp.2019.113729
61. O'Donnell JS, Massi D, Teng MWL, Mandala M. PI3K-AKT-mTOR inhibition in cancer immunotherapy, redux. *Semin Cancer Biol.* (2018) 48:91–103. doi: 10.1016/j.semcancer.2017.04.015
62. Ying H, Elpek KG, Vinjamoori A, Zimmerman SM, Chu GC, Yan H, et al. PTEN is a major tumor suppressor in pancreatic ductal adenocarcinoma and regulates an NF-kappaB-cytokine network. *Cancer Discov.* (2011) 1:158–69. doi: 10.1158/2159-8290.CD-11-0031
63. Dong Y, Richards JA, Gupta R, Aung PP, Emley A, Kluger Y, et al. PTEN functions as a melanoma tumor suppressor by promoting host immune response. *Oncogene.* (2014) 33:4632–42. doi: 10.1038/onc.2013.409
64. Peng W, Chen JQ, Liu C, Malu S, Creasy C, Tetzlaff MT, et al. Loss of PTEN promotes resistance to T cell-mediated immunotherapy. *Cancer Discov.* (2016) 6:202–16. doi: 10.1158/2159-8290.CD-15-0283
65. Bucheit AD, Chen G, Siroy A, Tetzlaff M, Broaddus R, Milton D, et al. Complete loss of PTEN protein expression correlates with shorter time to brain metastasis and survival in stage IIIB/C melanoma patients with BRAFV600 mutations. *Clin Cancer Res.* (2014) 20:5527–36. doi: 10.1158/1078-0432.CCR-14-1027
66. Lastwika KJ, Wilson W, 3rd, Li QK, Norris J, Xu H, Ghazarian SR, et al. Control of PD-L1 expression by oncogenic activation of the AKT-mTOR pathway in non-small cell lung cancer. *Cancer Res.* (2016) 76:227–38. doi: 10.1158/0008-5472.CAN-14-3362
67. Zhang C, Duan Y, Xia M, Dong Y, Chen Y, Zheng L, et al. TFEB mediates immune evasion and resistance to mTOR inhibition of renal cell carcinoma via induction of PD-L1. *Clin Cancer Res.* (2019) 25:6827–38. doi: 10.1158/1078-0432.CCR-19-0733
68. Deng L, Qian G, Zhang S, Zheng H, Fan S, Lesinski GB, et al. Inhibition of mTOR complex 1/p70 S6 kinase signaling elevates PD-L1 levels in human cancer cells through enhancing protein stabilization accompanied with enhanced beta-TrCP degradation. *Oncogene.* (2019) 38:6270–82. doi: 10.1038/s41388-019-0877-4
69. Marijt KA, Sluijter M, Blijleven L, Tolmeijer SH, Scheeren FA, van der Burg SH, et al. Metabolic stress in cancer cells induces immune escape through a PI3K-dependent blockade of IFNgamma receptor signaling. *J Immunother Cancer.* (2019) 7:152. doi: 10.1186/s40425-019-0627-8
70. Sivaram N, McLaughlin PA, Han HV, Petrenko O, Jiang YP, Ballou LM, et al. Tumor-intrinsic PIK3CA represses tumor immunogenecity in a model of pancreatic cancer. *J Clin Invest.* (2019) 129:3264–76. doi: 10.1172/JCI123540
71. Chandrasekaran S, Sasaki M, Scharer CD, Kissick HT, Patterson DG, Magliocca KR, et al. Phosphoinositide 3-Kinase signaling can modulate MHC Class I and II expression. *Mol Cancer Res.* (2019) 17:2395–409. doi: 10.1158/1541-7786.MCR-19-0545
72. Moore EC, Cash HA, Caruso AM, Uppaluri R, Hodge JW, Van Waes C, et al. Enhanced tumor control with combination mTOR and PD-L1 inhibition in syngeneic oral cavity cancers. *Cancer Immunol Res.* (2016) 4:611–20. doi: 10.1158/2326-6066.CIR-15-0252
73. Zhan T, Rindtorff N, Boutros M. Wnt signaling in cancer. *Oncogene.* (2017) 36:1461–73. doi: 10.1038/onc.2016.304
74. Alamoud KA, Kukuruzinska MA. Emerging insights into Wnt/beta-catenin signaling in head and neck cancer. *J Dent Res.* (2018) 97:665–73. doi: 10.1177/0022034518771923
75. Morris LG, Kaufman AM, Gong Y, Ramaswami D, Walsh LA, Turcan S, et al. Recurrent somatic mutation of FAT1 in multiple human cancers leads to aberrant Wnt activation. *Nat Genet.* (2013) 45:253–61. doi: 10.1038/ng.2538
76. Lee S, Stewart S, Nagtegaal I, Luo J, Wu Y, Colditz G, et al. Differentially expressed genes regulating the progression of ductal carcinoma in situ to invasive breast cancer. *Cancer Res.* (2012) 72:4574–86. doi: 10.1158/0008-5472.CAN-12-0636
77. Lin SC, Lin LH, Yu SY, Kao SY, Chang KW, Cheng HW, et al. FAT1 somatic mutations in head and neck carcinoma are associated with tumor progression and survival. *Carcinogenesis.* (2018) 39:1320–30. doi: 10.1093/carcin/bgy107
78. Hsu TN, Huang CM, Huang CS, Huang MS, Yeh CT, Chao TY, et al. Targeting FAT1 inhibits carcinogenesis, induces oxidative stress and enhances cisplatin sensitivity through deregulation of LRP5/WNT2/GSS signaling axis in oral squamous cell carcinoma. *Cancers.* (2019) 11:1883. doi: 10.3390/cancers11121883

79. Aster JC, Pear WS, Blacklow SC. The varied roles of notch in cancer. *Annu Rev Pathol.* (2017) 12:245–75. doi: 10.1146/annurev-pathol-052016-100127
80. Zhao YY, Yu GT, Xiao T, Hu J. The notch signaling pathway in head and neck squamous cell carcinoma: a meta-analysis. *Adv Clin Exp Med.* (2017) 26:881–7. doi: 10.17219/acem/64000
81. Loganathan SK, Schleicher K, Malik A, Quevedo R, Langille E, Teng K, et al. Rare driver mutations in head and neck squamous cell carcinomas converge on NOTCH signaling. *Science.* (2020) 367:1264–9. doi: 10.1126/science.aax0902
82. El-Sahli S, Xie Y, Wang L, Liu S. Wnt signaling in cancer metabolism and immunity. *Cancers.* (2019) 11:904. doi: 10.3390/cancers11070904
83. Wang B, Tian T, Kalland KH, Ke X, Qu Y. Targeting Wnt/beta-catenin signaling for cancer immunotherapy. *Trends Pharmacol Sci.* (2018) 39:648–58. doi: 10.1016/j.tips.2018.03.008
84. Goldsberry WN, Londono A, Randall TD, Norian LA, Arend RC. A review of the role of Wnt in cancer immunomodulation. *Cancers.* (2019) 11:771. doi: 10.3390/cancers11060771
85. Martin-Orozco E, Sanchez-Fernandez A, Ortiz-Parra I, Ayala-San Nicolas M. WNT signaling in tumors: the way to evade drugs and immunity. *Front Immunol.* (2019) 10:2854. doi: 10.3389/fimmu.2019.02854
86. Lopez Gonzalez M, Oosterhoff D, Lindenberg JJ, Milenova I, Lougheed SM, Martianez T, et al. Constitutively active GSK3beta as a means to bolster dendritic cell functionality in the face of tumour-mediated immune suppression. *Oncoimmunology.* (2019) 8:e1631119. doi: 10.1080/2162402X.2019.1631119
87. Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity. *Nature.* (2015) 523:231–5. doi: 10.1038/nature14404
88. Yaguchi T, Goto Y, Kido K, Mochimaru H, Sakurai T, Tsukamoto N, et al. Immune suppression and resistance mediated by constitutive activation of Wnt/beta-catenin signaling in human melanoma cells. *J Immunol.* (2012) 189:2110–7. doi: 10.4049/jimmunol.1102282
89. Luke JJ, Bao R, Sweis RF, Spranger S, Gajewski TF. WNT/beta-catenin pathway activation correlates with immune exclusion across human cancers. *Clin Cancer Res.* (2019) 25:3074–83. doi: 10.1158/1078-0432.CCR-18-1942
90. Suryawanshi A, Hussein MS, Prasad PD, Manicassamy S. Wnt signaling cascade in dendritic cells and regulation of anti-tumor immunity. *Front Immunol.* (2020) 11:122. doi: 10.3389/fimmu.2020.00122
91. Colombo M, Mirandola L, Chiriva-Internati M, Basile A, Locati M, Lesma E, et al. Cancer cells exploit notch signaling to redefine a supportive cytokine milieu. *Front Immunol.* (2018) 9:1823. doi: 10.3389/fimmu.2018.01823
92. Qian X, Nie X, Wollenberg B, Sudhoff H, Kaufmann AM, Albers AE. Heterogeneity of head and neck squamous cell carcinoma stem cells. *Adv Exp Med Biol.* 11392019:23–40. doi: 10.1007/978-3-030-14366-4_2
93. Rudy SF, Brenner JC, Harris JL, Liu J, Che J, Scott MV, et al. *In vivo* Wnt pathway inhibition of human squamous cell carcinoma growth and metastasis in the chick chorioallantoic model. *J Otolaryngol Head Neck Surg.* (2016) 45:26. doi: 10.1186/s40463-016-0140-8
94. Jung YS, Park JI. Wnt signaling in cancer: therapeutic targeting of Wnt signaling beyond beta-catenin and the destruction complex. *Exp Mol Med.* (2020) 52:183–91. doi: 10.1038/s12276-020-0380-6
95. Goruganthu MUL, Shanker A, Dikov MM, Carbone DP. Specific targeting of notch ligand-receptor interactions to modulate immune responses: a review of clinical and preclinical findings. *Front Immunol.* (2020) 11:1958. doi: 10.3389/fimmu.2020.01958
96. Wang HC, Chan LP, Cho SF. Targeting the immune microenvironment in the treatment of head and neck squamous cell carcinoma. *Front Oncol.* (2019) 9:1084. doi: 10.3389/fonc.2019.01084
97. Peltanova B, Raudenska M, Masarik M. Effect of tumor microenvironment on pathogenesis of the head and neck squamous cell carcinoma: a systematic review. *Mol Cancer.* (2019) 18:63. doi: 10.1186/s12943-019-0983-5

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oral Health

MINI REVIEW
published: 08 June 2021
doi: 10.3389/froh.2021.689482

Adrenergic-Angiogenic Crosstalk in Head and Neck Cancer: Mechanisms and Therapeutic Implications

Vui King Vincent-Chong[1] *and Mukund Seshadri*[1,2]*

[1] *Center for Oral Oncology, Roswell Park Comprehensive Cancer Center, Buffalo, NY, United States,* [2] *Department of Dentistry and Maxillofacial Prosthetics, Roswell Park Comprehensive Cancer Center, Buffalo, NY, United States*

Head and neck squamous cell carcinomas (HNSCC) are loco-regionally aggressive tumors that often lead to debilitating changes in appearance, speech, swallowing and respiratory function in patients. It is therefore critical to develop novel targeted treatment strategies that can effectively target multiple components within the tumor microenvironment. In this regard, there has been an increased recognition of the role of neural signaling networks as mediators of disease progression in HNSCC. Here, we summarize the current knowledge on the mechanisms of adrenergic signaling in HNSCC specifically focusing on neurovascular crosstalk and the potential of targeting the adrenergic-angiogenic axis through repurposing of FDA-approved drugs against HNSCC.

Keywords: HNSCC, adrenergic signaling, angiogenesis, drug repurposing, propranolol

OPEN ACCESS

Edited by:
Keith David Hunter,
The University of Sheffield,
United Kingdom

Reviewed by:
Rogelio González-González,
Juárez University of the State of Durango, Mexico
Samapika Routray,
All India Institute of Medical Sciences Bhubaneswar, India

***Correspondence:**
Mukund Seshadri
Mukund.Seshadri@roswellpark.org

Specialty section:
This article was submitted to Oral Cancers, a section of the journal Frontiers in Oral Health

Received: *01 April 2021*
Accepted: *14 May 2021*
Published: *08 June 2021*

Citation:
Vincent-Chong VK and Seshadri M (2021) Adrenergic-Angiogenic Crosstalk in Head and Neck Cancer: Mechanisms and Therapeutic Implications. Front. Oral. Health 2:689482. doi: 10.3389/froh.2021.689482

INTRODUCTION

Head and neck squamous cell carcinomas (HNSCC) are loco-regionally aggressive tumors that result in debilitating functional and esthetic sequelae in approximately half a million individuals worldwide [1, 2]. Although chemoradiation and immunotherapy-based approaches have led to improved therapeutic benefit, treatment resistance remains a significant clinical challenge [2]. Additionally, a majority of these patients experience prolonged treatment-induced morbidities including severe xerostomia, dysphagia, loss of dentition, and mandibular osteoradionecrosis [3]. Clearly, there is a need to investigate novel therapies that can exhibit improved therapeutic efficacy against HNSCC with reduced toxicities and treatment-related complications in this patient population.

One strategy to accomplish this goal involves assessing the anticancer activity of existing Food and Drug Administration (FDA) approved drugs used for non-oncologic indications, a concept termed as drug repurposing or repositioning [4, 5]. Given the significant costs associated with drug development, an informed approach focused on identifying and evaluating existing FDA-approved agents that target critical pathways implicated in development, progression or treatment resistance in HNSCC would be beneficial. In this context, there has been an increased recognition of the role of neural signaling networks as mediators of disease progression and therapeutic resistance in several solid tumors including HNSCC [6–8]. In this article, we summarize the current knowledge on the neurovascular talk in HNSCC specifically focusing on the adrenergic signaling within the head and neck tumor microenvironment. The rationale for targeting the adrenergic-angiogenic axis through repurposing of FDA-approved neuroscience drugs against HNSCC is presented.

NEURAL REGULATION OF CANCER

The role of nerves in cancer patients, particularly in the context of cancer pain, has been long recognized [9, 10]. Sympathectomy (localized surgical interruption or removal of nerve fibers/ganglions) has been used to alleviate pain in cancer patients since the 1940s [9]. A study by Batkin et al. in 1970 showed that denervation of sciatic nerves resulted in a reduction in take of neuroblastomas in mice [10]. The infiltration of tumors by growing nerves termed as neoneurogenesis or axonogenesis has been linked to tumor progression [6, 7]. Recent landmark publications in prostate, pancreas and gastric cancers have demonstrated that nerves are not just passive players in carcinogenesis or tumor progression but an integral part of the tumor microenvironment [11–13]. Sympathectomy has been shown to prevent growth and metastasis of transplanted tumors and transgenic models of prostate cancer [11]. Surgical or pharmacologic denervation has been shown to reduce tumor incidence and progression in gastric cancer and enhance chemotherapeutic efficacy [12]. However, the role of nerves in tumor initiation or progression in HNSCC has not been systematically examined until recently.

NEURAL SIGNALING AND ADRENERGIC STRESS IN HNSCC

Head and neck cancers were among the first set of cancers that showed propensity to grow along nerves [14, 15]. Perineural invasion (PNI) is a distinct route of tumor spread that is recognized as a key pathologic feature of many cancers including HNSCC [16]. Over the last decade, experimental studies have implicated the autonomic nervous system, specifically, adrenergic signaling axis in oral cancer progression [17–21]. Chronic stress induced through physical restraint has been shown to promote cancer progression in a mouse model of HNSCC through increased norepinephrine (NE) which upregulates vascular endothelial growth factor (VEGF) and matrix metalloproteinase (MMP2) levels [17, 18]. Adrenergic stimulation has been shown to upregulate interleukin-6 (IL-6) in HNSCC via β-adrenergic receptor (ADRB) activation [19]. Activation of ADRB signaling has also been shown to promote tumor progression and epithelial-to-mesenchymal transition (EMT) in HNSCC [20]. Using the 4NQO carcinogen-induced model of oral squamous cell carcinoma (OSCC), Valente et al. have shown that baseline levels (prior to 4NQO exposure) of NE, cortisone and neurotrophins such as brain-derived neurotrophic factor (BDNF) in normal tongue can be predictive of cancer occurrence in rats exposed to 4NQO [22]. Amit et al. have recently examined the significance and mechanisms involved in neuron reprogramming in head and neck cancer [8]. Using $Krt5^{Cre}Trp53^{flox/flox}$ mice, the authors demonstrated increased nerve density in p53 deficient tumors compared to wild type p53 controls implicating the loss of p53 in epithelial cells with neuritogenesis during oral carcinogenesis. The authors showed that loss of *TP53* leads to phenotypic trans-differentiation of sensory nerves to adrenergic nerves and regulates cancer associated neurons via extracellular vesicle derived signals [8].

In the clinical setting, HNSCC patients have increased circulating levels of NE associated with their bio-behavioral symptoms and anxiety levels [23]. Multiple studies have shown that β-adrenergic receptor-2 (ADRB2) is highly expressed in HNSCC compared to normal epithelium and has been associated with alcohol and tobacco use [24, 25]. The prognostic implications of ADRB2 expression, however, are unclear. Shang and colleagues examined ADRB2 expression in 65 human OSCC specimens and 10 normal oral mucosa samples and observed a higher expression of ADRB2 in OSCC that was positively correlated with tumor size, clinical stage and lymph node metastasis [24]. Similarly, increased TH+ nerve density was associated with lower recurrence-free survival in OSCC [8]. In contrast, strong ADRB2 expression in Brazilian OSCC patients was associated with improved overall survival and cancer-specific survival compared to patients with weak/negative ADRB2 expression [25]. Perhaps due to the global epidemiology of OSCC (higher prevalence in Asia and South East Asia compared to North America or Europe), the prognostic significance of ADRB2 expression in North American or European HNSCC patients has not been reported. However, Amit et al. have reported on the role of adrenergic signaling in 70 head and neck cancer patients treated at MD Anderson Cancer Center (Texas, United States). Although ADRB2 staining was not performed, the authors showed that increased TH+ nerve density was associated with lower overall and recurrence free survival in their patient population [8].

ADRENERGIC-ANGIOGENIC CROSSTALK IN THE TUMOR MICROENVIRONMENT

Angiogenesis is one of the hallmarks of cancer and initiation of the angiogenic switch is recognized as an early and critical event in head and neck cancer [26, 27]. Several studies have shown that overexpression of vascular endothelial growth factor (VEGF) has been associated with poor prognosis in HNSCC [28–31]. Although a considerable body of literature exists on the mechanism(s) of interactions between tumor cells and blood vessels, the literature on reciprocal neurovascular interactions in tumors, especially in HNSCC is limited.

The cross talk between tumor cells, endothelial cells and nerves is mediated by growth factors such as nerve growth factor (NGF) which can regulate VEGF and matrix metalloproteinases (MMPs) within the microenvironment [32–35]. NGF is one of the well-characterized neurotrophins that can serve as autocrine factor to tumor cells [36, 37]. NGF binds to its low affinity receptor, NGFR (p75NTR) or the high affinity receptor, Tropomyosin-related kinase (TrkA) [38, 39]. Work by Ye et al. and Kolokythas et al. has shown that NGF is a critical factor that contributes to oral carcinogenesis [40, 41]. Expression of NGF can induce neovascularization around the nerves in turn promoting tumor growth and proliferation [42]. Conversely, VEGF can induce endothelial cells to secrete collagenase contributing to degradation of the basement membrane, a critical

step in PNI, vascular invasion and metastatic dissemination [16, 42]. Common to these interactions is adrenergic signaling as ADRB2 is expressed both on tumor cells and endothelial cells and activation of adrenergic signaling promotes tumor cell survival, drive angiogenesis through several downstream signaling pathways (**Figure 1A**). ADBR2-mediated signaling (through local release of NE from SNS nerve fibers or from circulation) in tumor cells can upregulate NGF production which in turn can stimulate NGFR/TrkA signaling in an autocrine loop to promote cell survival by preventing apoptosis [13]. Chronic stress-induced release of neurotransmitters can activate ADRB2 and upregulate VEGF levels resulting in enhanced tumor vascularization and aggressive tumor growth [17, 43]. Activation of β-adrenergic signaling through NE has been shown to promote epithelial to mesenchymal transition (EMT) through upregulation of MMP2/9 and VEGF thereby enhancing the invasive and metastatic properties of tumor cells [17, 20]. β-adrenergic signaling is also involved in the regulation of hypoxia [44, 45]. It has been shown that β-adrenergic receptors are fundamental regulators of hypoxia and necessary for hypoxia-inducible factor 1 alpha (HIF-1α) accumulation [44]. In pancreatic cancer cells, binding of NE to ADRB2 has been shown to upregulate HIF-1α expression through Akt and ERK pathways [45]. While the interplay between tumor angiogenesis and hypoxia is well-known, the role of ADBR2 in and regulating tumor hypoxia especially through HIF-1α signaling has not been extensively studied in head and neck cancer and warrants further research. Nevertheless, these observations highlight the cross talk between the vascular and neural components within the HNSCC tumor microenvironment.

TARGETING THE ADRENERGIC-ANGIOGENIC AXIS IN HNSCC

Repurposing Neuroscience Drugs—A Case for Propranolol

Given the role of adrenergic signaling in HNSCC, it would be reasonable to postulate that directly targeting adrenergic signaling in tumor cells or indirectly targeting neuro-vascular interactions (e.g., ADRB2-NGF-VEGF signaling) within the tumor microenvironment could have significant therapeutic benefit in HNSCC. However, safety concerns with anti-NGF antibodies [46] have hampered their clinical use in HNSCC patients. Given the huge cost and time-constraints associated with pharmaceutical development of novel agents, drug repurposing is an attractive approach that can enable successful identification and development of agents that can target the adrenergic-angiogenic axis in head and neck cancer. The availability of pharmacologic and toxicology data of these FDA-approved agents in humans can accelerate clinical evaluation of promising agents [4, 47]. In this regard, the non-selective beta-blocker, propranolol (PRO) is currently being investigated for its therapeutic potential against cancer [48]. PRO is FDA-approved for treating patients with variety of conditions ranging from hypertension and cardiac failure to neurological disorders including anxiety, migraines, tremors and glaucoma [49, 50]. In this section, we summarize the current preclinical and clinical evidence on the effects of PRO on adrenergic signaling and angiogenesis in HNSCC.

PRO has been shown to inhibit NE stimulated migration and invasion of HNSCC *in vitro* [20]. In nasopharyngeal carcinoma (NPC) cell lines, PRO has been shown to inhibit NE-induced MMP-2/9 and VEGF [17]. In Epstein-Barr virus (EBV) associated nasopharyngeal carcinoma, latent membrane protein 1 (LMP1) is the viral oncogene that promotes invasion and metastasis through effects on MMP-2/9 [51, 52]. Using EBV positive (clone 13) and EBV negative (clone 39) of LMP1 expressing HONE-1 cells, Yang et al. showed that NE stimulated the release of VEGF, MMP-2, and MMP-9 in both NPC cells independent of their EBV status. While the direct effects of EBV oncoproteins including LMP1 on adrenergic signaling is unclear, the study showed that both clones of the NPC cell line expressed ADRB2 and were inhibited by PRO through downregulation of MMP-2/9 expression [17]. Similarly, the forkhead box (FOXA) family of transcription factors have been implicated in the biology of NPC [53, 54]. Overexpression of FOXA1 has been shown to suppress proliferation, migration, and invasion of NPC cells in culture [53]. In NPC patients, FOXA1 expression has been correlated with prolonged disease-free survival and overall survival [54]. However, the effects of PRO on FOXA1 signaling has not been previously reported.

Preclinical studies have also examined the interaction between PRO and standard of care chemo- and radiation therapy in HNSCC. Wolter et al. have shown that PRO reduces HNSCC viability, inhibits VEGF production and can enhance the efficacy of cisplatin and radiation against HNSCC cells [55]. Recently, Lucido et al. have shown that PRO exhibits potent antitumor activity against human papillomavirus positive (HPV+) HNSCC that is mediated by a reduction of mitochondrial oxidative phosphorylation [56]. In the study, PRO in combination with chemoradiation resulted in inhibition of primary tumor growth and reduction in metastases.

And finally, studies have also demonstrated the antiangiogenic effects of PRO in experimental tumor models. PRO has been shown to suppress angiogenesis by inhibiting proliferation, migration and differentiation of endothelial cells *in vitro* [57]. Blockade of ADBR2 signaling by PRO inhibits VEGF induced phosphorylation of VEGFR2, extracellular signal-regulated kinase-1/2 (ERK) and pro-MMP2 secretion. PRO exhibits antiangiogenic effects at non-toxic concentrations (<50 μM) and potentiates the antiangiogenic and therapeutic efficacy of chemotherapeutic agents, 5FU and taxol [58]. PRO has been shown to inhibit growth, decrease vessel density and lower VEGF, MMP2/9 levels in neuroblastomas [59] and repress tumor growth in hemangiomas through hypoxia-inducible factor-1 alpha (HIF-1α) and STAT3 signaling [60]. Collectively, these preclinical observations highlight the therapeutic potential of PRO in targeting adrenergic-angiogenic signaling in HNSCC (**Figure 1B**).

Repurposing PRO for use in HNSCC patients presents an attractive strategy considering the cost of the drug and wealth

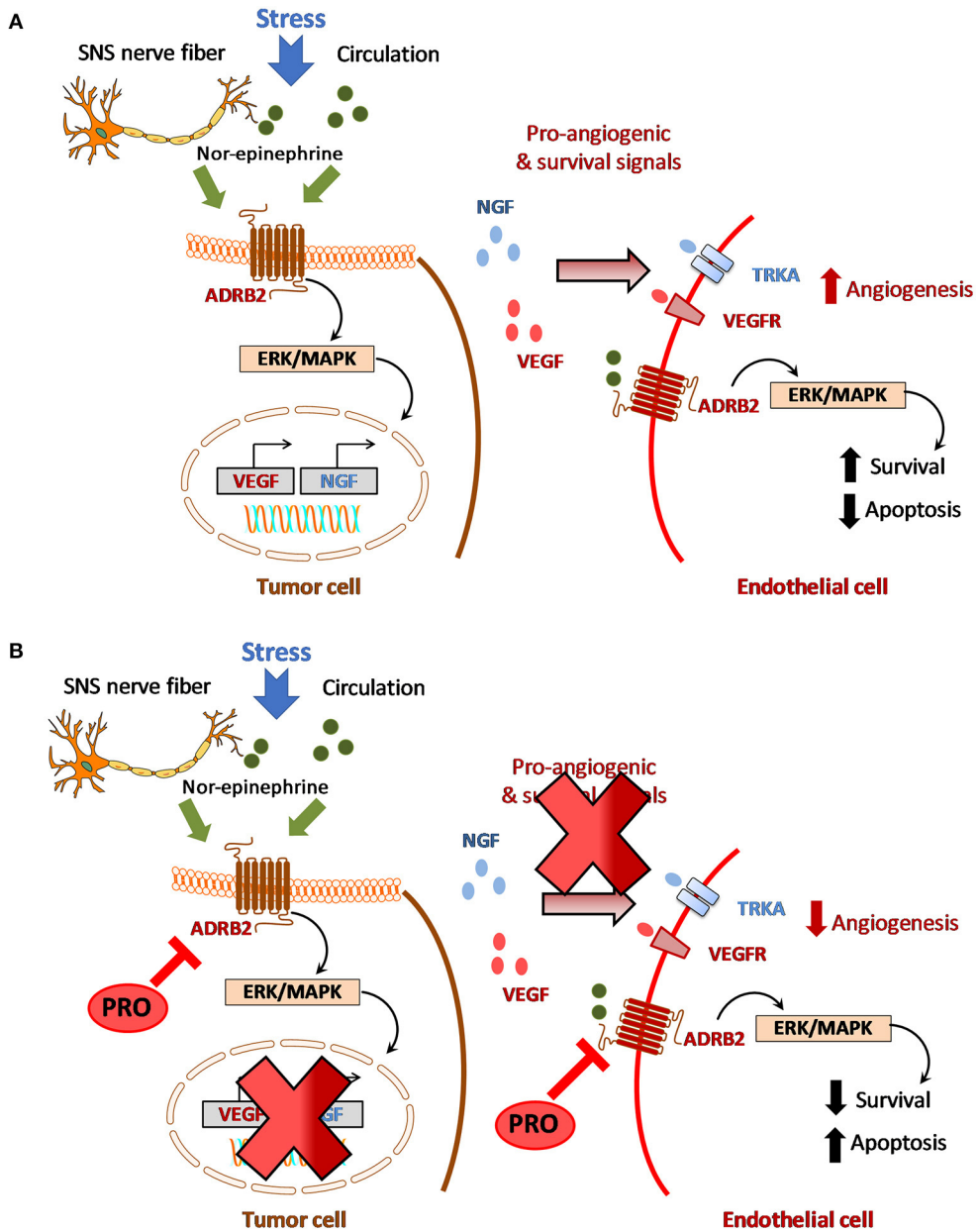

FIGURE 1 | Crosstalk between adrenergic signaling and angiogenesis in HNSCC. **(A)** Stress induced activation of beta-adrenergic signaling within the tumor microenvironment which in turn promotes angiogenesis and disease progression. **(B)** Hypothetical model for targeting adrenergic-angiogenic axis in HNSCC using propranolol.

of pharmacologic and toxicologic data that exists in humans. However, the existing clinical evidence from epidemiologic or retrospective analyses regarding PRO use in HNSCC is conflicting. Chang et al. have shown that long-term PRO use (>1,000 days) was associated with a reduction in risk of HNSCC (HR: 0.58; CI: 0.35–0.95) [61]. In contrast, an observational study in 1,274 patients conduced in South Korea showed that post diagnosis beta-blocker use was associated with decreased survival and increased recurrence in HNSCC patients [62]. A recent meta-analysis of epidemiologic and perioperative studies suggests that benefits associated with beta-blockers are likely to vary across patients with different tumor sites [63].

CONCLUSIONS

In summary, neuronal programming and neurovascular interactions represent relatively understudied mechanisms that contribute to malignant progression in HNSCC. The literature presented in this review highlight the importance and therapeutic potential of targeting adrenergic signaling

pathways within the head and neck tumor microenvironment. However, additional investigation to better understand the role of adrenergic-angiogenic cross talk in head and neck cancer and the potential of targeting this axis using PRO in the current treatment paradigm for HNSCC is warranted. In this regard, 3D organoid models and organoid co-culture systems can serve as a useful platform to dissect the mechanisms of interaction between tumor cells, neurons and endothelial cells and to screen therapeutic agents that can effectively target the adrenergic-angiogenic axis in head and neck cancer. Although limited, the published preclinical evidence on the activity of PRO against HNSCC is encouraging. Studies should therefore investigate the activity of PRO in combination with chemoradiation and immune checkpoint blockade using clinically relevant orthotopic models of HNSCC. Given the known effects of PRO on tumor cells, neural signaling, and blood vessels, such studies could employ clinically relevant imaging methods (e.g., MRI, PET) to assess the metabolic, vascular and hypoxic profiles of tumors. Integration of imaging phenotypes with genomic data and response to PRO would enable identification of patients that could benefit from the addition of PRO to existing standard of care regimens. Investigation into the potential chemopreventive effects of PRO in carcinogen-induced models of HNSCC could also be insightful. Such studies could serve to accelerate the clinical translation of a relatively inexpensive and a readily available drug to treat these esthetically and functionally debilitating cancers.

AUTHOR CONTRIBUTIONS

MS designed and drafted the manuscript. VKV-C and MS edited the manuscript, critically revised the content, and approved the final submitted version of the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

Support from the National Cancer Institute (R01CA243456-01A1; 5P30CA016056) and the Roswell Park Alliance Foundation is acknowledged.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* (2021) 71:209–49. doi: 10.3322/caac.21660
2. Chow LQM. Head and neck cancer. *N Engl J Med.* (2020) 382:60–72. doi: 10.1056/NEJMra1715715
3. Buglione M, Cavagnini R, Di Rosario F, Sottocornola L, Maddalo M, Vassalli L, et al. Oral toxicity management in head and neck cancer patients treated with chemotherapy and radiation: dental pathologies and osteoradionecrosis (Part 1) literature review and consensus statement. *Crit Rev Oncol Hematol.* (2016) 97:131–42. doi: 10.1016/j.critrevonc.2015.08.010
4. Pantziarka P, Bouche G, Meheus L, Sukhatme V, Sukhatme VP, Vikas P. The repurposing drugs in oncology (ReDO) project. *Ecancermedicalscience.* (2014) 8:442. doi: 10.3332/ecancer.2014.485
5. Sleire L, Førde HE, Netland IA, Leiss L, Skeie BS, Enger PØ. Drug repurposing in cancer. *Pharmacol Res.* (2017) 124:74–91. doi: 10.1016/j.phrs.2017.07.013
6. Mancino M, Ametller E, Gascón P, Almendro V. The neuronal influence on tumor progression. *Biochim Biophys Acta.* (2011) 1816:105–18. doi: 10.1016/j.bbcan.2011.04.005
7. Jobling P, Pundavela J, Oliveira SM, Roselli S, Walker MM, Hondermarck H. Nerve-cancer cell cross-talk: a novel promoter of tumor progression. *Cancer Res.* (2015) 75:1777–81. doi: 10.1158/0008-5472.CAN-14-3180
8. Amit M, Takahashi H, Dragomir MP, Lindemann A, Gleber-Netto FO, Pickering CR, et al. Loss of p53 drives neuron reprogramming in head and neck cancer. *Nature.* (2020) 578:449–54. doi: 10.1038/s41586-020-1996-3
9. De Sousa Pereira A. Abdominopelvic sympathectomy for relief of pain of cancer of the cervix. *Arch Surg.* (1946) 52:113–34. doi: 10.1001/archsurg.1946.01230050117001
10. Batkin S, Piette LH, Wildman E. Effect of muscle denervation on growth of transplanted tumor in mice. *Proc Natl Acad Sci USA.* (1970) 67:1521–7. doi: 10.1073/pnas.67.3.1521
11. Magnon C, Hall SJ, Lin J, Xue X, Gerber L, Freedland SJ, et al. Autonomic nerve development contributes to prostate cancer progression. *Science.* (2013) 341:1236361. doi: 10.1126/science.1236361
12. Hayakawa Y, Sakitani K, Konishi M, Asfaha S, Niikura R, Tomita H, et al. Nerve growth factor promotes gastric tumorigenesis through aberrant cholinergic signaling. *Cancer Cell.* (2017) 31:21–34. doi: 10.1016/j.ccell.2016.11.005
13. Renz BW, Takahashi R, Tanaka T, Macchini M, Hayakawa Y, Dantes Z, et al. β2 Adrenergic-neurotrophin feedforward loop promotes pancreatic cancer. *Cancer Cell.* (2018) 33:75–90.e7. doi: 10.1016/j.ccell.2018.10.010
14. Cruveilheir J. *Maladies Des Nerfs Anatomie Pathologique Du Corps Humain*. 2nd ed. Paris, France: JB Bailliere (1835).
15. Neumann E. Secondare cancroid infiltration des nervus mentalis bei einem. *Arch Pathol Anat.* (1862) 24:201. doi: 10.1007/BF01929317
16. Liebig C, Ayala G, Wilks JA, Berger DH, Albo D. Perineural invasion in cancer: a review of the literature. *Cancer.* (2009) 115:3379–91. doi: 10.1002/cncr.24396
17. Yang EV, Sood AK, Chen M, Li Y, Eubank TD, Marsh CB, et al. Norepinephrine up-regulates the expression of vascular endothelial growth factor, matrix metalloproteinase (MMP)-2, and MMP-9 in nasopharyngeal carcinoma tumor cells. *Cancer Res.* (2006) 66:10357–64. doi: 10.1158/0008-5472.CAN-06-2496
18. Xie H, Li C, He Y, Griffin R, Ye Q, Li L. Chronic stress promotes oral cancer growth and angiogenesis with increased circulating catecholamine and glucocorticoid levels in a mouse model. *Oral Oncol.* (2015) 51:991–7. doi: 10.1016/j.oraloncology.2015.08.007
19. Bernabé DG, Tamae AC, Biasoli ÉR, Oliveira SH. Stress hormones increase cell proliferation and regulates interleukin-6 secretion in human oral squamous cell carcinoma cells. *Brain Behav Immun.* (2011) 25:574–83. doi: 10.1016/j.bbi.2010.12.012
20. Liu H, Wang C, Xie N, Zhuang Z, Liu X, Hou J, et al. Activation of adrenergic receptor β2 promotes tumor progression and epithelial mesenchymal transition in tongue squamous cell carcinoma. *Int J Mol Med.* (2018) 41:147–54. doi: 10.3892/ijmm.2017.3248
21. Zhang B, Wu C, Chen W, Qiu L, Li S, Wang T, et al. The stress hormone norepinephrine promotes tumor progression through β2-adrenoreceptors in oral cancer. *Arch Oral Biol.* (2020) 113:104712. doi: 10.1016/j.archoralbio.2020.104712
22. Valente VB, Verza FA, Lopes FYK, Ferreira JZ, Dos Santos PSP, Sundefeld MLMM, et al. Stress hormones concentrations in the normal microenvironment predict risk for chemically induced cancer in rats. *Psychoneuroendocrinology.* (2018) 89:229–38. doi: 10.1016/j.psyneuen.2017.11.001
23. Bastos DB, Sarafim-Silva BAM, Sundefeld MLMM, Ribeiro AA, Brandão JDP, Biasoli ÉR, et al. Circulating catecholamines are associated with biobehavioral factors and anxiety symptoms in head and neck cancer patients. *PLoS ONE.* (2018) 13:e0202515. doi: 10.1371/journal.pone.0202515

24. Shang ZJ, Liu K, Liang DF. Expression of beta2-adrenergic receptor in oral squamous cell carcinoma. *J Oral Pathol Med.* (2009) 38:371–6. doi: 10.1111/j.1600-0714.2008.00691.x
25. Bravo-Calderón DM, Oliveira DT, Marana AN, Nonogaki S, Carvalho AL, Kowalski LP. Prognostic significance of beta-2 adrenergic receptor in oral squamous cell carcinoma. *Cancer Biomark.* (2011–2012) 10:51–9. doi: 10.3233/CBM-2012-0228
26. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell.* (2000) 100:57–70. doi: 10.1016/S0092-8674(00)81683-9
27. Smith BD, Smith GL, Carter D, Sasaki CT, Haffty BG. Prognostic significance of vascular endothelial growth factor protein levels in oral and oropharyngeal squamous cell carcinoma. *J Clin Oncol.* (2000) 18:2046–52. doi: 10.1200/JCO.2000.18.10.2046
28. Lentsch EJ, Goudy S, Sosnowski J, Major S, Bumpous JM. Microvessel density in head and neck squamous cell carcinoma primary tumors and its correlation with clinical staging parameters. *Laryngoscope.* (2006) 116:397–400. doi: 10.1097/01.MLG.0000195286.29613.E1
29. Tse GM, Chan AW, Yu KH, King AD, Wong KT, Chen GG, et al. Strong immunohistochemical expression of vascular endothelial growth factor predicts overall survival in head and neck squamous cell carcinoma. *Ann Surg Oncol.* (2007) 14:3558–65. doi: 10.1245/s10434-007-9632-0
30. Carla C, Daris F, Cecilia B, Francesca B, Francesca C, Paolo F. Angiogenesis in head and neck cancer: a review of the literature. *J Oncol.* (2012) 2012:358472. doi: 10.1155/2012/358472
31. Kyzas PA, Cunha IW, Ioannidis JP. Prognostic significance of vascular endothelial growth factor immunohistochemical expression in head and neck squamous cell carcinoma: a meta-analysis. *Clin Cancer Res.* (2005) 11:1434–40. doi: 10.1158/1078-0432.CCR-04-1870
32. Romon R, Adriaenssens E, Lagadec C, Germain E, Hondermarck H, Le Bourhis X. Nerve growth factor promotes breast cancer angiogenesis by activating multiple pathways. *Mol Cancer.* (2010) 9:157. doi: 10.1186/1476-4598-9-157
33. Magnon C. Role of the autonomic nervous system in tumorigenesis and metastasis. *Mol Cell Oncol.* (2015) 2:e975643. doi: 10.4161/23723556.2014.975643
34. Kuol N, Stojanovska L, Apostolopoulos V, Nurgali K. Role of the nervous system in tumor angiogenesis. *Cancer Microenviron.* (2018) 11:1–11. doi: 10.1007/s12307-018-0207-3
35. Sheng Y, Zhu L. The crosstalk between autonomic nervous system and blood vessels. *Int J Physiol Pathophysiol Pharmacol.* (2018) 10:17–28.
36. Okada Y, Eibl G, Guha S, Duffy JP, Reber HA, Hines OJ. Nerve growth factor stimulates MMP-2 expression and activity and increases invasion by human pancreatic cancer cells. *Clin Exp Metastasis.* (2004) 21:285–92. doi: 10.1023/B:CLIN.0000046131.24625.54
37. Wang L, Sun M, Jiang Y, Yang L, Lei D, Lu C, et al. Nerve growth factor and tyrosine kinase A in human salivary adenoid cystic carcinoma: expression patterns and effects on in vitro invasive behavior. *J Oral Maxillofac Surg.* (2006) 64:636–41. doi: 10.1016/j.joms.2005.12.011
38. Dalal R, Djakiew D. Molecular characterization of neurotrophin expression and the corresponding tropomyosin receptor kinases (trks) in epithelial and stromal cells of the human prostate. *Mol Cell Endocrinol.* (1997) 134:15–22. doi: 10.1016/S0303-7207(97)00165-2
39. Dudás J, Dietl W, Romani A, Reinold S, Glueckert R, Schrott-Fischer A, et al. Nerve growth factor (NGF)-receptor survival axis in head and neck squamous cell carcinoma. *Int J Mol Sci.* (2018) 19:1771. doi: 10.3390/ijms19061771
40. Ye Y, Dang D, Zhang J, Viet CT, Lam DK, Dolan JC, et al. Nerve growth factor links oral cancer progression, pain, and cachexia. *Mol Cancer Ther.* (2011) 10:1667–76. doi: 10.1158/1535-7163.MCT-11-0123
41. Kolokythas A, Cox DP, Dekker N, Schmidt BL. Nerve growth factor and tyrosine kinase A receptor in oral squamous cell carcinoma: is there an association with perineural invasion? *J Oral Maxillofac Surg.* (2010) 68:1290–5. doi: 10.1016/j.joms.2010.01.006
42. Park JA, Choi KS, Kim SY, Kim KW. Coordinated interaction of the vascular and nervous systems: from molecule- to cell-based approaches. *Biochem Biophys Res Commun.* (2003) 311:247–53. doi: 10.1016/j.bbrc.2003.09.129
43. Tilan J, Kitlinska J. Sympathetic neurotransmitters and tumor angiogenesis-link between stress and cancer progression. *J Oncol.* (2010) 2010:539706. doi: 10.1155/2010/539706
44. Cheong HI, Asosingh K, Stephens OR, Queisser KA, Xu W, Willard B, et al. Hypoxia sensing through β-adrenergic receptors. *JCI Insight.* (2016) 1:e90240. doi: 10.1172/jci.insight.90240
45. Hu HT, Ma QY, Zhang D, Shen SG, Han L, Ma YD, et al. HIF-1alpha links beta-adrenoceptor agonists and pancreatic cancer cells under normoxic condition. *Acta Pharmacol Sin.* (2010) 31:102–10. doi: 10.1038/aps.2009.181
46. Chang DS, Hsu E, Hottinger DG, Cohen SP. Anti-nerve growth factor in pain management: current evidence. *J Pain Res.* (2016) 9:373–83. doi: 10.2147/JPR.S89061
47. Huang J, Zhao D, Liu Z, Liu F. Repurposing psychiatric drugs as anti-cancer agents. *Cancer Lett.* (2018) 419:257–65. doi: 10.1016/j.canlet.2018.01.058
48. Pantziarka P, Bouche G, Sukhatme V, Meheus L, Rooman I, Sukhatme VP. Repurposing drugs in oncology (ReDO)-Propranolol as an anti-cancer agent. *Ecancermedicalscience.* (2016) 10:680. doi: 10.3332/ecancer.2016.680
49. DiNicolantonio JJ, Fares H, Niazi AK, Chatterjee S, D'Ascenzo F, Cerrato E, et al. β-Blockers in hypertension, diabetes, heart failure and acute myocardial infarction: a review of the literature. *Open Heart.* (2015) 2:e000230. doi: 10.1136/openhrt-2014-000230
50. Steenen SA, van Wijk AJ, van der Heijden GJ, van Westrhenen R, de Lange J, de Jongh A. Propranolol for the treatment of anxiety disorders: systematic review and meta-analysis. *J Psychopharmacol.* (2016) 30:128–39. doi: 10.1177/0269881115612236
51. Yoshizaki T, Sato H, Furukawa M, Pagano JS. The expression of matrix metalloproteinase 9 is enhanced by Epstein-Barr virus latent membrane protein 1. *Proc Natl Acad Sci USA.* (1998) 95:3621–6. doi: 10.1073/pnas.95.7.3621
52. Wakisaka N, Pagano JS. Epstein-Barr virus induces invasion and metastasis factors. *Anticancer Res.* (2003) 23:2133–8.
53. Wang W, Yi M, Chen S, Li J, Li G, Yang J, et al. Significance of the NOR1-FOXA1/HDAC2-Slug regulatory network in epithelial-mesenchymal transition of tumor cells. *Oncotarget.* (2016) 7:16745–59. doi: 10.18632/oncotarget.7778
54. Ammous-Boukhris N, Ayadi W, Derbel M, Allaya-Jaafar N, Charfi S, Daoud J, et al. FOXA1 expression in nasopharyngeal carcinoma: association with clinicopathological characteristics and EMT markers. *Biomed Res Int.* (2020) 2020:4234632. doi: 10.1155/2020/4234632
55. Wolter NE, Wolter JK, Enepekides DJ, Irwin MS. Propranolol as a novel adjunctive treatment for head and neck squamous cell carcinoma. *J Otolaryngol Head Neck Surg.* (2012) 41:334–44. doi: 10.2310/7070.2012.00047
56. Lucido CT, Callejas-Valera JL, Colbert PL, Vermeer DW, Miskimins WK, Spanos WC, et al. β2-Adrenergic receptor modulates mitochondrial metabolism disease progression in recurrent/metastatic HPV(+) HNSCC. *Oncogenesis.* (2018) 7:81. doi: 10.1038/s41389-018-0090-2
57. Lamy S, Lachambre MP, Lord-Dufour S, Béliveau R. Propranolol suppresses angiogenesis in vitro: inhibition of proliferation, migration, and differentiation of endothelial cells. *Vascul Pharmacol.* (2010) 53:200–8. doi: 10.1016/j.vph.2010.08.002
58. Pasquier E, Ciccolini J, Carre M, Giacometti S, Fanciullino R, Pouchy C, et al. Propranolol potentiates the anti-angiogenic effects and anti-tumor efficacy of chemotherapy agents: implication in breast cancer treatment. *Oncotarget.* (2011) 2:797–809. doi: 10.18632/oncotarget.343
59. Xu T, Xiao X, Zheng S, Zheng J, Zhu H, Ji Y, et al. Antiangiogenic effect of propranolol on the growth of the neuroblastoma xenografts in nude mice. *J Pediatr Surg.* (2013) 48:2460–5. doi: 10.1016/j.jpedsurg.2013.08.022
60. Li P, Guo Z, Gao Y, Pan W. Propranolol represses infantile hemangioma cell growth through the β2-adrenergic receptor in a HIF-1α-dependent manner. *Oncol Rep.* (2015) 33:3099–107. doi: 10.3892/or.2015.3911
61. Chang PY, Huang WY, Lin CL, Huang TC, Wu YY, Chen JH, et al. Propranolol reduces cancer risk: a population-based cohort study. *Medicine.* (2015) 94:e1097. doi: 10.1097/MD.0000000000001097

62. Kim SA, Moon H, Roh JL, Kim SB, Choi SH, Nam SY, et al. Postdiagnostic use of β-blockers and other antihypertensive drugs and the risk of recurrence and mortality in head and neck cancer patients: an observational study of 10,414 person-years of follow-up. *Clin Transl Oncol.* (2017) 19:826–33. doi: 10.1007/s12094-016-1608-8
63. Yap A, Lopez-Olivo MA, Dubowitz J, Pratt G, Hiller J, Gottumukkala V, et al. Effect of beta-blockers on cancer recurrence and survival: a meta-analysis of epidemiological and perioperative studies. *Br J Anaesth.* (2018) 121:45–57. doi: 10.1016/j.bja.2018.03.024

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oral Health

REVIEW
published: 01 July 2021
doi: 10.3389/froh.2021.686337

Cancer-Associated Fibroblasts in Oral Cancer: A Current Perspective on Function and Potential for Therapeutic Targeting

Kamila J. Bienkowska[1], *Christopher J. Hanley*[1] *and Gareth J. Thomas*[1,2]*

[1] School of Cancer Sciences, University of Southampton, Southampton, United Kingdom, [2] Cancer Research UK and National Institute for Health Research (NIHR) Southampton Experimental Cancer Medicine Centre, Southampton, United Kingdom

The role of the tumour microenvironement (TME) in cancer progression and resistance to therapies is now widely recognized. The most prominent non-immune cell type in the microenvironment of oral cancer (OSCC) is cancer-associated fibroblasts (CAF). Although CAF are a poorly characterised and heterogenous cell population, those with an "activated" myofibroblastic phenotype have been shown to support OSCC progression, promoting growth, invasion and numerous other "hallmarks of malignancy." CAF also confer broad resistance to different types of therapy, including chemo/radiotherapy and EGFR inhibitors; consistent with this, CAF-rich OSCC are associated with poor prognosis. In recent years, much CAF research has focused on their immunological role in the tumour microenvironment, showing that CAF shield tumours from immune attack through multiple mechanisms, and particularly on their role in promoting resistance to anti-PD-1/PD-L1 checkpoint inhibitors, an exciting development for the treatment of recurrent/metastatic oral cancer, but which fails in most patients. This review summarises our current understanding of CAF subtypes and function in OSCC and discusses the potential for targeting these cells therapeutically.

Keywords: oral cancer, head & neck squamous cell carcinoma, tumour microenvironment, cancer-associated fibroblasts, myofibroblasts

OPEN ACCESS

Edited by:
Keith David Hunter,
The University of Sheffield,
United Kingdom

Reviewed by:
Sven E. Niklander,
Universidad Andres Bello, Chile
Newell Walter Johnson,
Griffith University, Australia
Jozsef Dudas,
Innsbruck Medical University, Austria

***Correspondence:**
Gareth J. Thomas
g.thomas@soton.ac.uk

Specialty section:
This article was submitted to Oral Cancers, a section of the journal Frontiers in Oral Health

Received: *26 March 2021*
Accepted: *07 June 2021*
Published: *01 July 2021*

Citation:
Bienkowska KJ, Hanley CJ and Thomas GJ (2021) Cancer-Associated Fibroblasts in Oral Cancer: A Current Perspective on Function and Potential for Therapeutic Targeting. Front. Oral. Health 2:686337. doi: 10.3389/froh.2021.686337

INTRODUCTION

Historically, the search for novel therapies has focused on tumour cells, attempting to inhibit oncogenic pathways that drive tumour progression; targeting the EGFR pathway for example. Although such targeted therapies can produce dramatic initial results, acquired resistance, where tumours progress after initial response, seems an almost inevitable consequence of this approach. In recent years, and particularly following the success of anti-PD-1/PD-L1 (programmed cell death protein 1/programmed death ligand 1) checkpoint immunotherapy, there has been a realisation that a tumour is a complex mixture of different cell types that interact to promote tumour progression, and this has generated significant interest in developing therapies that target the tumour microenvironment (TME) [1].

CAF HETEROGENEITY

The most prominent non-immune cells within cancers are cancer-associated fibroblasts (CAF); these can account for up to 80% of tumour mass in late stage head & neck cancers (HNSCC) and are generally assumed to be tumour-promoting [2, 3]. CAF remain a poorly characterised and heterogenous cell population; most studies have focused on myofibroblastic CAF and the terminology within the literature has been confusing with "cancer-associated fibroblast," "myofibroblast" and "peritumoral fibroblast" variably used, and only recently is the term myofibroblastic CAF (myCAF) starting to be used consistently in the literature. These contractile, α-SMA-positive cells are generated principally through TGF-β signalling/mechanotransduction [4], and secrete extracellular matrix (ECM) analogous to myofibroblasts found in healing wounds and fibrotic disorders. In tissue sections these are usually identified using immunochemistry for α-SMA, although this protein is also expressed by pericytes [5] and smooth muscle cells [6]. Indeed, there is no specific single marker that accurately identifies CAF; other markers such as FAP-α (fibroblast activation protein α), FSP1 (fibroblast specific protein 1) and PDGFRB (platelet derived growth factor receptor β) have been used to identify CAF, but also are not CAF specific [7]. Moreover, and despite extensive research, the myCAF cell of origin also remains inconclusive, and, although it is thought that most myCAF originate from local fibroblasts–pericytes, adipocytes, endothelial cells and bone marrow-derived mesenchymal stem cells have all been shown to be potential myCAF progenitors [8, 9]. In recent years, macrophages and cancer stem cells have also been highlighted as potential myCAF precursors [1, 10, 11], and it remains unclear whether cell of origin affects the final myCAF phenotype.

The advent of single cell transcriptomic sequencing (scRNA-seq) is beginning to characterise CAF heterogeneity within HNSCC and other cancers, and it is now accepted that not all CAF subpopulations are characterised by high expression of α-SMA, and conversely, not all α-SMA-positve CAF are myofibroblastic. Elyada et al. [12] and Öhlund et al. [13] analysed pancreatic cancers using scRNA-seq and identified two main CAF populations; myCAF and a subpopulation characterised by expression of inflammatory genes, which were termed iCAF. iCAF subpopulations have subsequently also been identified in breast cancer [14]. Puram et al. [15] used scRNA-seq to characterise HNSCC and described two main fibroblast groups which were termed "myofibroblasts" and "CAF." The latter group could be further divided into two subclusters through differential expression of immediate early response genes, mesenchymal markers, ligands and receptors, and ECM proteins, suggesting further potential subdivisions. It is not yet clear whether iCAF are found in HNSCC as a distinct subpopulation. Patel et al. [16] performed transcriptomic analysis on CAF cultures from oral cancers (OSCC) and also identified two main subgroups: CAF1 (α-SMA^{low}) and CAF2 (α-SMA^{high}). CAF1 was associated with increased proliferation of cancer cells but with suppressed self-renewal growth of oral stem-like cancer cells (oral-SLCCs). Conversely, CAF2 correlated positively with frequency of oral-SLCCs but negatively with tumour cell proliferation. BMP4 (bone morphogenetic protein 4) was differentially expressed between the two CAF populations and was suggested to be at least partially responsible for exerting the suppressive effect on cancer cells' stemness. Costea et al. [17] compared heterogeneity of OSCC CAF with normal fibroblasts, performing transcriptomic analysis on CAF cultured in 2D and 3D-matrices. This study also identified two CAF populations; CAF-N, motile fibroblasts whose transcriptome and secretome were more similar to normal fibroblasts, and CAF-D subpopulation, which had a more divergent expression pattern and secreted high levels of TGF-β1. Both CAF subtypes resulted in higher tumour incidence and deeper invasion in murine models, though intriguingly, CAF-N was best at supporting tumour formation. The two subpopulations were not compared in terms of their α-SMA expression, however 50% of the upregulated genes compared with normal fibroblasts were TGF-β targets, suggesting differentiation towards a myofibroblast-like phenotype. The authors suggest that the two populations may be a spectrum in CAF development, with CAF-N representing an earlier stage of differentiation.

Fibroblast phenotype may also vary according to molecular phenotypes of HNSCC. Hassona et al. [18] compared CAF from genetically stable OSCC (GS-OSCC; maintaining wild-type p53) and genetically unstable OSCC (GU-OSCC) and found that cultured CAF from GU-OSCC were significantly more senescent. They found that malignant keratinocytes from GU-OSCC produce high levels of reactive oxygen species (ROS), associated with increased production of TGFβ1 and TGFβ2, and (myo)fibroblast activation. Consistent with this observation, senescent fibroblasts commonly express α-SMA, are contractile and tumour promoting [19, 20] but differ from myofibroblasts in their ability to deposit ECM [19, 21]. Notably, the generation of intracellular reactive oxygen species plays a major role in CAF (and myofibroblast) differentiation, with the ROS-producing enzyme, NADPH oxidase 4 (NOX4), central to this process [22, 23].

However, despite the progress made in identifying different CAF subtypes in oral cancer (**Table 1**) characterisation of CAF heterogeneity remains incomplete; studies have identified CAF-secreted inflammatory factors as promoting OSCC progression, but it is not yet clear whether iCAF are a distinct subpopulation in HNSCC or whether myCAF also acquire inflammatory properties in certain situations. As novel CAF subgroups are identified, their effect on OSCC progression will be an intriguing area of research, and in order to study function it will be necessary to modify *in vitro* culture conditions to maintain these phenotypes. Standard 2D-tissue culture techniques tend to skew CAF to a myCAF-like phenotype [24], meaning that studies have tended to focus, either deliberately or inadvertently, on myCAF. Future experimental work will require a more detailed characterisation of CAF phenotypes given increasing understanding of CAF heterogeneity and plasticity.

TABLE 1 | Heterogeneity of CAF in oral cancer.

Source	Tumour	CAF subpopulations	Description/Markers
Puram et al. [15]	HNSCC (oral cavity)	Myofibroblasts	ACTA2, MCAM, MYLK, MYL9, IL6, PDGFA
		CAF	FAP, THY1, PDPN, MMP2, MMP11, PDGFRA/L, TGFB3. Could be further divided into CAF1 and CAF2
		Resting fibroblasts	Lack of activation markers
Patel et al. [16]	Gingivobuccal–oral tumour	CAF1 (α-SMA^{low}BMP4^{+ve})	Increased proliferation of cancer cells; supressed self-renewal growth of oral-SLCC
		CAF2 (α-SMA^{high}BMP4^{-ve})	Negative correlation with cancer cells' proliferation; increased frequency of oral-SLCC
Costea et al. [17]	OSCC	CAF-N	Motile; high production of hyaluronan; promoted invasion; secretome similar to the secretome of normal fibroblasts;
		CAF-D	Less motile; high expression of TGF-β1; promoted invasion and EMT; secretome different from the secretome of normal fibroblasts
Hassona et al. [18]	GS-OSCC	Non-senescent CAF	Non-senescent CAF failed to promote keratinocytes' invasion *in vitro*
	GU-OSCC	Senescent CAF	Malignant keratinocytes induced fibroblast activation and senescence through ROS and TGF-β; senescent CAF promoted keratinocytes' invasion *in vitro*

CAF AND OSCC SURVIVAL

Numerous studies have shown that CAF-rich OSCC are associated with significantly shorter patient survival. In the largest study of this type, Marsh et al. [25] performed a retrospective analysis of 282 OSCC patients, analysing a number of tumour cell and stromal cell molecular markers by IHC. They found that the best independent risk factor of early OSCC death was high stromal α-SMA expression, which produced the highest adjusted hazard ratio (HR 3.06, 95% CI 1.65-5.66; $p = 0.002$), and likelihood ratio (3.6; Detection rate:False positive rate) of any feature examined, and was strongly associated with mortality regardless of disease stage. A recent meta-analysis examined correlations between myCAF (assessed by α-SMA immunochemistry) and risk of OSCC death, analysing 1,328 patients from 12 studies [26]. The combined results showed that myCAF predicted poor overall survival (HR 2.16 95% CI 1.60–2.92; $P < 0.00001$) and shortened disease-free survival (HR 3.32 95% CI 2.09–5.26; $P < 0.00001$). The presence of high levels of stromal myCAF was associated with pathological features related to tumour aggressiveness, including depth of invasion, lymphatic invasion and extra-nodal metastatic spread [26].

CAF AND MALIGNANT TRANSFORMATION

Oral potentially malignant disorders (PMDs) include leukoplakia, erythroplakia and oral submucous fibrosis (OSMF) [27]. Approximately 1% of potentially malignant disorders give rise to cancer but the mechanism of the transformation is poorly understood [28]. Changes in stroma, such as myofibroblast activation, are considered as potential promoters of the transformation of pre-malignant lesions. Several studies, using immunohistochemistry staining for α-SMA, have compared the number of myofibroblasts in potentially malignant disorders and OSCC. Generally, studies report a lack of myofibroblasts in leukoplakias and erythroplakias, including a meta-analysis that analysed 19 articles [28, 29]. However, some studies have reported increased myofibroblasts in high-risk epithelial dysplasia [30]. Conversely, studies have consistently reported increased myofibroblasts in OSMF compared to healthy oral mucosa [28, 31–33], with late-stage OSMF reported to contain more myofibroblasts than early-stage OSMF [32]. These findings are perhaps not surprising given that OSMF is a fibrotic disorder, and whether myofibroblasts play an active role in the malignant transformation of this disease remains to be determined.

CAF FUNCTION IN OSCC

Clues as to how CAF function to promote OSCC progression can be found in clinic-pathological correlates. The fact that CAF correlate with many features of tumour aggressiveness, including invasion, metastasis, absence of T-cells and therapy resistance suggest that their role is multifactorial, and this is borne out in numerous functional studies that show that CAF promote many, if not all of the "hallmarks of malignancy" [34]. Understanding these functions will help develop CAF-targeting strategies for OSCC treatment. Tumour promoting functions of CAF in OSCC are summarised in **Figure 1**.

CAF AND TUMOUR CELL INVASION

CAF promote tumour invasion through multiple mechanisms, including deposition and remodelling of ECM, a central myCAF function. Tumour ECM forms a dense meshwork, generally difficult for cells to penetrate. Gaggioli et al. [35] found that CAF create "tracks" in ECM through which tumour cells invade and are led by CAF. Generation of tracks was dependent on a combination of protease- and force-mediated matrix remodelling mechanisms. Expression of integrins α3 and α5, and activity of Rho and ROCK (Rho-associated protein kinase) (drivers of a force-mediated remodelling) in fibroblasts were shown to be crucial for the remodelling process. More recently, Chen et al. [36] demonstrated that HNSCC cell invasion is facilitated by fibroblast-dependent degradation of dense ECM. By varying

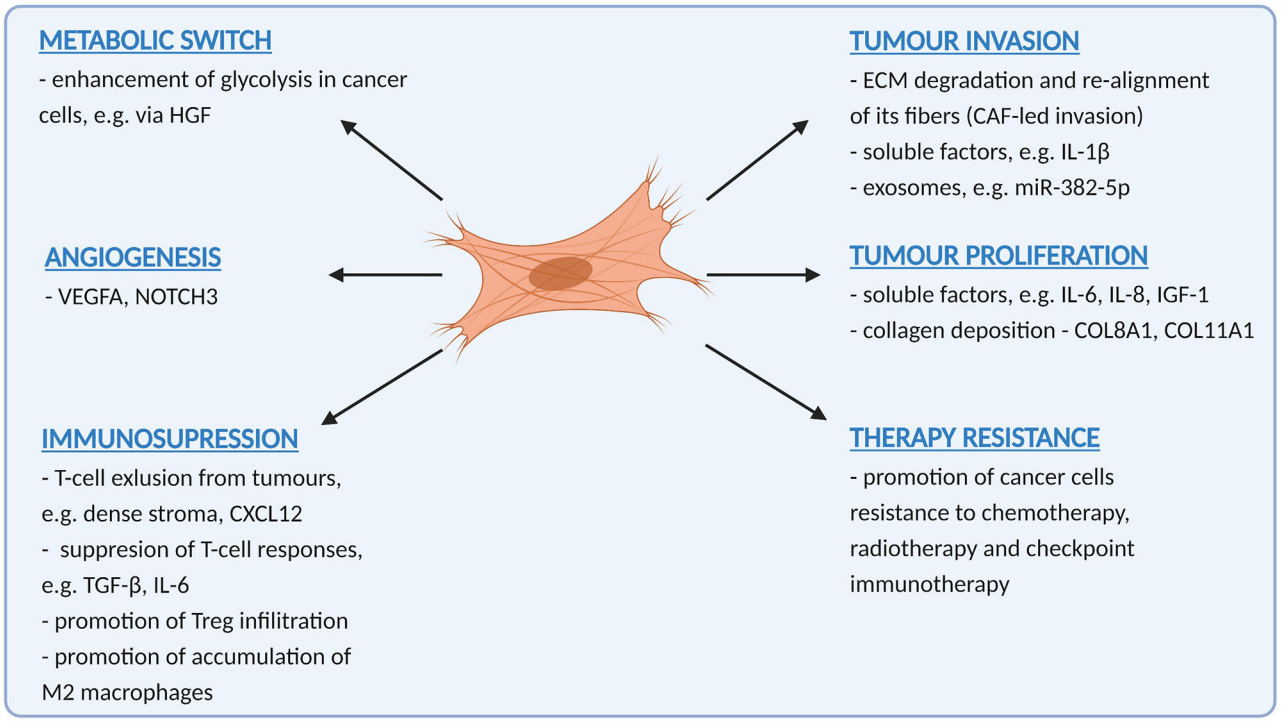

FIGURE 1 | A summary of pro-tumoural functions exerted by cancer-associated fibroblasts in oral cancer (Created with BioRender.com).

collagen concentration in co-culture experiments, they found in lower concentrations of collagen (2 mg/ml), that both tumour cells and fibroblasts remodel the matrix and invade into the surrounding collagen independently. However, tumour cells growing in more concentrated collagen (8 mg/ml) were unable to penetrate the matrix unless fibroblasts were present. Similar to the Gagglioli study, fibroblasts were seen to lead cancer cells through the matrix. Both studies suggest that cancer cells require fibroblasts to remodel dense extracellular matrix in order to invade into surrounding tissues and disseminate.

A similar mechanism has been reported to regulate OSCC invasion into bone [37], where a fibrous CAF stroma characterised by a high expression of α-SMA was observed to sit between tumour cells and bone. Notably, α-SMA-positive CAF were commonly seen infiltrating bone ahead of cancer cells, again suggesting that they are the leading cells in cancer invasion. Two proteins associated with regulation of bone resorption: RANKL (receptor activator of NF κ-B ligand) and OPG (tumour necrosis factor receptor superfamily member 11B) were expressed in OSCC cells and bone-adjacent stroma. Moreover, treatment with conditioned media from both experimentally generated CAF and OSCC-tumour-derived CAF could induce osteoclastogenesis in macrophages – i.e., a transdifferentation of macrophages into bone-resorbing osteoclasts. CAF induced osteoclastogenesis in macrophages to a greater extent than cancer cells, suggesting they may be the main orchestrators of bone invasion in OSCC, modulating macrophages as well as tumour cells in this process.

CAF produce numerous ECM proteins and the composition of the matrix can significantly affect cancer invasion. For example, CAF in OSCC express high levels of hyaluronan synthetase 2 (HAS2), an enzyme producing hyaluronan, which is a major component of ECM. HAS2 promotes invasion of OSCC cells, and its expression correlates with advanced clinical stages and cervical lymph node metastases [38]. The mechanism underlying HAS2-dependent invasion of cancer cells was suggested to depend on regulation of the balance between ECM-degrading matrix metalloproteinases (MMPs) and their inhibitors (tissue inhibitors of metalloproteinases; TIMPs). $HAS2^{+}$ CAF expressed higher levels of MMP1 and lower levels of TIMP1 than normal fibroblasts, and knockdown or inhibition of HAS2 in CAF not only reduced OSCC invasion but also reversed the ratio between MMP1 and TIMP1 expression, reducing expression of MMP1 and upregulating TIMP1. HAS2 has also been reported to play a role in regulating the fibroblast response to TGF-β. Dermal fibroblasts and fibroblast from the oral mucosa respond differently to TGF-β1 treatment [39, 40], which promotes proliferation in dermal fibroblasts but suppresses proliferation in fibroblasts from the oral mucosa. Overexpression of HAS2 in oral fibroblasts resulted in a pro-proliferative response to TGFβ1 stimulation. The presence of hyaluronan in the TME is also known to promote cancer cell and CAF motility [41]. A subtype of hyaluronan-producing CAF could represent a promising drug target.

CAF also modulate tumour cell invasion via secreted soluble factors. Various CAF-secreted factors, have been implicated in

promoting HNSCC invasion and/or proliferation, including IL-1β (interleukin 1β) [42], activin A [43], HGF (hepatocyte growth factor) [44] and EREG (epiregulin) [45], which has additionally been suggested to play an autocrine role in CAF activation [45]. In recent years there has also been significant interest in CAF-tumour cell communication via exosomes. These secreted extracellular vesicles contain proteins, lipids and nucleic acids, such as messenger RNA (mRNA), micro RNA (miRNA), long non-coding RNA (lncRNA) and others [46], and exosomal miR-382-5p and MFAP5 have been shown in separate studies to promote OSCC cell migration and invasion [47, 48]. Intriguingly, Li et al. [49] showed miR-34a-5p could suppress proliferation and metastasis of OSCC cells and that CAF exosomes contained reduced levels of this miRNA, suggesting that the cancer-promoting features of exosomes might not only be due to a transfer of pro-tumoral factors but also due to the lack of suppressive factors.

CAF AND TUMOUR PROLIFERATION

CAF have been shown to promote proliferation of cancer cells in multiple tumour types [50–52]. Co-injection of CAF with HNSCC cells *in vivo* enhances tumour growth [53], and similar observations have been made in 3D co-culture models, where CAF increase proliferation of tumour spheroids [54]. Conditioned medium from CAF promotes HNSCC cell proliferation *in vitro* [53] and consistent with this a number of secreted cytokines and growth factors have been shown to stimulate tumour growth. For example, interleukin-6 (IL-6) has been shown to upregulate expression of osteopontin (SPP1) in HNSCC cells and to increase their proliferation via integrin/NF-κB signalling [55]. Osteopontin may play an important role in bi-directional communication between CAF and HNSCC cells, and in breast cancer models has been shown to induce transformation of mesenchymal stem cells into CAF, in a MZF1(myeloid zinc finger 1)-TGFβ1-dependent manner [56]. Plasma osteopontin has also been suggested as a potential prognostic biomarker in HNSCC patients; negatively correlating with overall and relapse-free survival [57].

Another example of a reciprocal paracrine interaction between tumour cells and CAF was shown by Bae et al. [58], who observed that tumour volume of orthotopic tumours correlated with numbers of CAF co-injected with tumour cells. They found that interleukin-1α (IL-1α) secreted by OSCC cells increased proliferation of CAF and upregulated expression of secretory cytokines, including CCL7 (chemokine ligand 7), CXCL1 (C-X-C motif chemokine 11) and IL-8 (interleukin 8). In turn, these cytokines increased tumour cell proliferation *in vitro*.

Fibroblasts have also shown to be a source of IGF-1 (insulin growth factor 1) in OSCC, which promotes tumour cell proliferation through activation of PI3K-AKT and Hedgehog signalling pathways [59]. The matrix-remodelling capabilities of CAF may also affect tumour proliferation [60]; CAF-secreted collagens, collagen8A1 and collagen11A1 [61], have been shown to modulate tumour cell growth through interaction with DDR1 (Discoidin domain receptor 1), which is overexpressed in HNSCC tissues.

CAF, ANGIOGENESIS, AND THE METABOLIC SWITCH

Rapidly growing tumours create a hypoxic microenvironment and CAF play a major role in neo-angiogenesis, producing angiogenic factors such as VEGFA (vascular endothelial growth factor), and also attracting other cells, such as macrophages, which also contribute to the angiogenic process [62].

In OSCC, CAF are thought to be the main source of interleukin-6, which acts through an autocrine signalling loop to induce the secretion of VEGFA in CAF and also in OSCC cells [63]. Kayamori et al. [64] suggested that Notch signalling in CAF could also promote tumour angiogenesis; around one third of tongue OSCC cases were found to have CAF expressing NOTCH3 (neurogenic locus notch homolog protein 3) which correlated positively with tumour size and was associated with increased micro-vessel density.

CAF can also show metabolic adaptions to meet the energetic demands of tumour cells. In a phenomenon termed the "reverse Warburg effect," CAF and tumour cells are metabolically coupled, whereby CAF metabolism is corrupted to undero aerobic glycolysis, producing metabolites such as lactate and pyruvate that can be used by cancer cells in oxidative phosphorylation. Lactate also has other pro-tumour effects, promoting angiogenesis, metastasis, and generating an immune-suppressive microenvironment [65–68]. Conversely, CAF have also been shown to promote glycolysis in tumour cells. Kumar et al. [3] reported that CAF-secreted HGF induces glycolysis in HNSCC cells and promotes expression of bFGF (basic fibroblast growth factor), which induces oxidative phosphorylation in fibroblasts creating another type of metabolic loop between the cells.

CAF, RADIOTHERAPY, AND CHEMOTHERAPY

Radiotherapy with or without chemotherapy is commonly used in the treatment of OSCC. These treatments can modulate the tumour microenvironment in various ways. Notably, radiotherapy induces a wound healing response, promoting inflammation, CAF modulation/myofibroblast activation and ECM remodelling [69], and while CAF are considered relatively radioresistant [70], the DNA damage induced by radiation can result in a senescent phenotype, which can also be tumour promoting [71]. Kamochi et al. [72] showed that irradiated fibroblasts promote invasive growth of OSCC cells, upregulating expression of TGF-β1, that promote invasion and also potentiate further myofibroblasts differentiation. Indeed, irradiation is a potent activator of latent TGF-β and myofibroblasts which results in the fibrosis which is a well-recognised complication of radiotherapy [73]. CAF may also protect cancer cells from radiation. Huang et al. [74] found that radioresistant nasopharyngeal carcinoma had a higher infiltration of CAF

compared with radiosensitive tissue, and showed that this radioprotective effect was modulated via IL-8/NF-κB signalling. This effect was diminished following treatment with Tranilast – a drug known to inhibit fibrosis and TGF-β signalling.

Chemotherapy, similar to radiotherapy, has been shown to promote the acquisition of CAF phenotype in breast [75], colorectal [76], and head and neck cancer [77]. A number of studies have also reported CAF-mediated chemotherapy resistance in HNSCC. CAF are associated with mediating resistance to cisplatin in head and neck cancer cells, and are generally more intrinsically resistant to this drug. Qin et al. [78] found that CAF transfer an exosomal miR-196a to cancer cells which renders them resistant to cisplatin through a downregulation of CDKN1B and ING5. Resistance to cisplatin has also been shown to be mediated by CAF-secreted collagens [61]; HNSCC cells pre-treated with collagen (or gelatin) prior to cisplatin treatment display significantly reduced cisplatin-induced apoptosis. In the presence of a DDR1 (Discoidin domain receptor 1; collagen receptor expressed by HNSCC cells) inhibitor, this protective effect was abolished.

Yegodayev et al. [79] also demonstrated that resistance to cetuximab is partially mediated by CAF. They found that TGF-β-activated CAF positively correlate with resistance to cetuximab both, *in vitro* and *in vivo*, and inhibiting TGF-β signalling improved cetuximab treatment efficacy. In a separate study, CAF were shown to be non-sensitive to cetuximab, but their conditioned medium protected HNSCC cells from cetuximab in a dose-dependent manner [80]. This CAF-mediated resistance was suggested to be partially driven by an upregulation of metalloproteinases (MMPs) in both, CAF and HNSCC cells, following co-culture. MMP1 was the most upregulated protease, and use of its inhibitor partially restored the sensitivity of the HNSCC to cetuximab. Notably however CAF with silenced MMP-1 could still mediate cetuximab resistance, and the conclusion drawn from the study was that other MMPs (such as MMP-2,−3,−7,−13) were also likely involved in modulating this effect.

CAF AND TUMOUR IMMUNE SUPPRESSION

The microenvironment of HNSCC is immunosuppressive and pro-inflammatory, and is associated with T-cell and NK-cell dysfunction, as well as accumulation of Tregs and pro-tumoral macrophages [81]. In recent years, the role of fibroblasts in promoting this suppressive microenvironment has generated much interest, particularly in the context of resistance to anti-PD-1/PD-L1 checkpoint immunotherapy. CAF contribute to tumour immune evasion through multiple mechanisms, affecting both innate and adaptive immunity [82]. Takahashi et al. [83] found that HNSCC CAF express elevated amounts of PD-L1 (also known as B7H1) and PD-L2 (B7DC); both molecules interact with PD-1 receptor on T-cells to suppress effector functions [84, 85]. CAF also suppress infiltration of CD8 T-cells into tumours; in part this may result from T-cell interactions with the extracellular matrix "barrier" secreted by myCAF [86]. The desmoplastic stroma produced by myCAF is rich in collagen, fibronectin and various proteoglycans (hyaluronan, versican), which have been shown to "trap" T-cells and inhibit T-cell motility [87]. A dense meshwork of collagen fibres has also been shown to limit T-cell penetration into tumours [88] and enhance matrix density; the protease-independent nature of T-cell amoeboid migration leads to contact guidance where T-cells follow a path-of-least-resistance along collagen fibres [89]. CD8 T-cells in myCAF-rich murine and human tumours have been shown to upregulate expression of CTLA-4, which may play a role in modulating T-cell interactions with ECM [23]. In a murine model of pancreatic cancer, CXCL12 (C-X-C motif chemokine ligand 12) produced by CAF has also been show to inhibit T-cell infiltration into tumour islets [90]. CAF-derived TGF-β and IL-6 also have recognised roles in suppressing T-cell responses [91]. Recently, a novel subpopulation of antigen presenting CAF that interact with CD4 T-cells have been identified in pancreatic carcinoma, suggesting even more complex CAF interactions in shaping the adaptive T-cell response [12].

CAF have also been reported to mediate T-cell suppression in OSCC indirectly through attraction and polarisation of macrophages [92]. Clinicopathological analysis has revealed a positive relationship between numbers of CAF and TAMs (tumour-associated macrophages) in OSCC tumour samples, and both cell types correlate with vascular invasion and TNM stage [92]. Several CAF-derived factors have been shown to recruit macrophages into tumours and polarise them towards an M2 tumour promoting phenotype, including CXCL12 and MCP-1 (monocyte chemotactic protein 1) [93, 94], with M2 macrophages having suppressive effect on T-cells mediated by increased expression of arginase I, interleukin-10 and TGF-β [92]. Similarly, CAF-secreted CCL7 that has been shown to increase invasion of OSCC cells is also chemotactic for macrophages [95].

The recruitment of immune cells by CAF is usually described in terms of soluble immunomodulatory factors, such as CXCL10 (C-X-C motif chemokine ligand 10), IL-6, MCP-1 [96], but recently a novel mechanism for recruitment of macrophages was proposed by Pakshir et al. [97], who demonstrated that macrophages can sense ECM deformation resulting from myofibroblast contraction, and migrate towards them. This mechanosensing mechanism could potentially attract macrophages into CAF-rich tumours independent of chemotactic factors.

CAF-TARGETING STRATEGIES

In addition to their tumour-promoting functions, CAF have been shown to confer resistance to different types of cancer therapy, including cetuximab and anti-PD-1/PD-L1 checkpoint immunotherapy, as well as radiotherapy and cisplatin chemotherapy [77–80, 98–101], suggesting that therapeutic CAF targeting could increase response rates for a diverse range of treatments. Potential strategies for CAF-directed therapy include inhibiting CAF activation or function, "normalizing" CAF or killing CAF within the tumour microenvironment

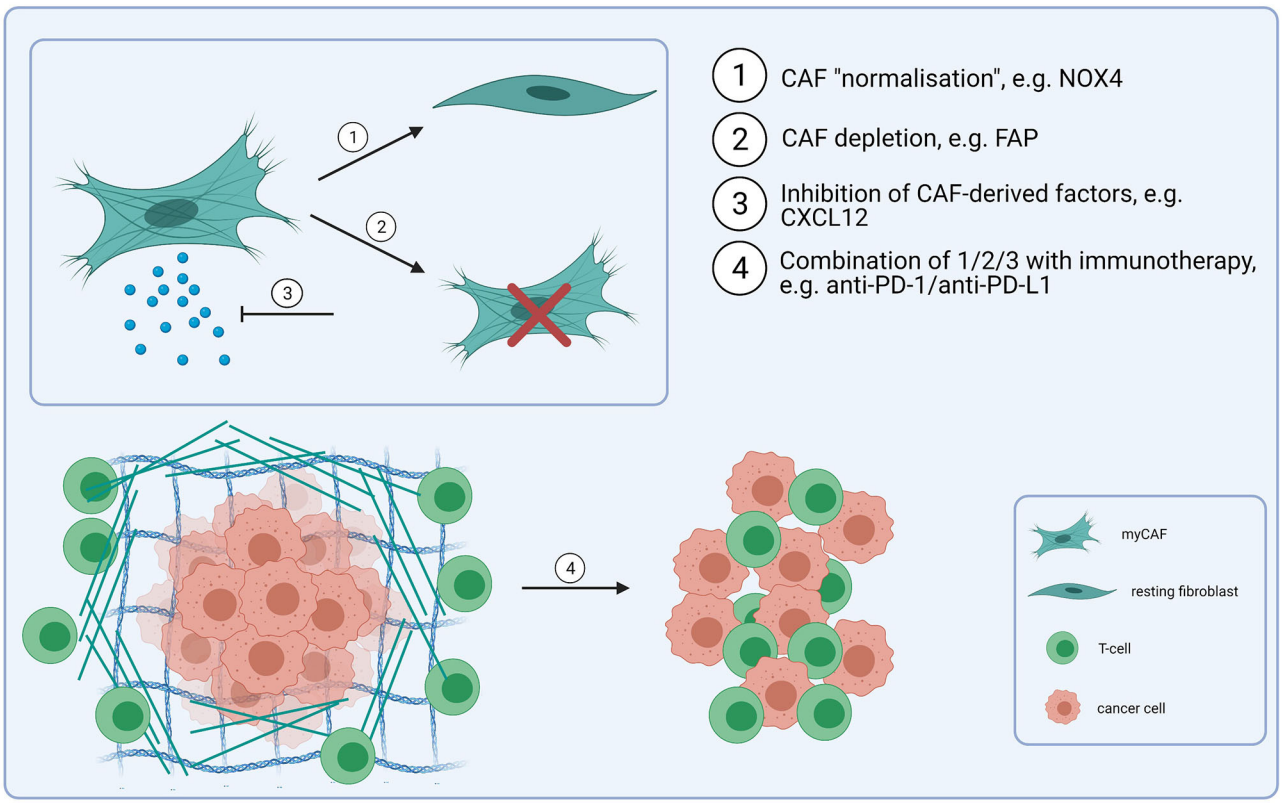

FIGURE 2 | CAF-targeting strategies. Combining immunotherapy-based treatmens (e.g., anti-PD-1/anti-PD-L1) with CAF-targeting, either through CAF "normalisation" (1) (e.g., NOX4 inhibitors), depletion (2) (e.g., FAP-based depletion) or through disrupting a signalling network between CAF and cancer cells/T-cells (e.g., via inhibition of CXCL12) (3). These strategies could improve the response to checkpoint immunotherapy and result in an increased infiltration of T-cells into tumours (4) (Created with BioRender.com).

(**Figure 2**), although clinical testing has generally yielded disappointing results [102, 103]. Given the myriad tumour promoting functions regulated by CAF, the specific context in which a particular pathway is targeted is clearly important. For example, FAP-expressing CAF have been shown to exclude T-cells from tumours through secretion of CXCL12 [90]; targeting the CXCL12/CXCR4 signalling axis using plerixafor (a CXCR4 inhibitor) has been shown to overcome this exclusion effect and promote response to anti-PD-1 [104]. Inhibiting CAF activation has also been investigated as a possible therapy; TGF-β signalling/mechanotransduction pathways are the primary signalling pathway regulating myCAF activation, although other growth factors and signalling molecules, including lysophosphatidic acid, PDGF, FGF, IL-6 and TNF (tumour necrosis factor) have also been implicated in the differentiation process. Targeting TGF-β is potentially problematic for several reasons; in the early stages of tumorigenesis it acts as a tumour suppressor; it also plays an important role in tissue homeostasis, and early testing of small molecule TGF-β1 inhibitors highlighted on-target cardiac toxicities and development of cutaneous carcinomas [105]. However, recognition of its role in promoting resistance to anti-PD-1/PD-L1 checkpoint immunotherapy through both CAF-dependent and independent mechanisms, has led to a resurgence in interest in targeting the pathway [106], including use of neutralising antibodies, receptor inhibitors and ligand traps [107]. With the identification of different fibroblast phenotypes and the recognition of CAF plasticity, "normalizing" CAF is an attractive concept, particularly since certain fibroblast phenotypes may be tumour suppressive [108]. In pancreatic cancer, vitamin D receptor has been shown to act as a master repressor of stellate cell activation, and treatment with calcipotriol (a synthetic derivative of calcitriol) reduces fibrosis and improves response to gemcitabine in tumour-bearing KPC mice [109]. Hanley et al. [22] identified NOX4 as a critical regulator of myCAF activation in multiple tumour types, including HNSCC, and found that inhibiting NOX4 using Setanaxib (GKT137831), a drug developed to treat organ fibrosis, suppressed myCAF activation and also "normalized" established myCAF. More recently, Ford et al. [23] developed myCAF-rich murine tumour models and showed that Setanaxib could be used to effectively overcome myCAF-mediated T-cell exclusion from tumours and potentiate response to anti-tumour vaccination and anti-PD-1 immunotherapies [23]. Grauel et al. [110] also examined the effect of TGF-β inhibition on the CAF phenotype *in vivo*, and found that TGF-β neutralisation reduced myCAF development and promoted an immunomodulatory phenotype characterised by strong response to interferon. CAF depletion could also

be a therapeutic approach. Depletion of fibroblast activation protein (FAP)-positive CAF in murine models has been shown to enhance anti-tumour immunity [111]; Duperret et al. [112] used a DNA vaccine targeting FAP, which induced CD8+ and CD4+ T-cells and synergised with other tumour antigen-specific DNA vaccines to enhance anti-tumour immunity. However, FAP is widely expressed on different cell types [113] and there is still lack of a CAF-specific target that could make this approach effective.

CONCLUSIONS

CAF support HNSCC progression and promote treatment resistance and have emerged as an attractive therapeutic target. Treatments designed to target CAF however, have not been successful clinically, and the identification of specific CAF targets has proven problematic, mostly due to a limited understanding of the molecular and functional phenotypes within a heterogenous CAF population and compounded by murine models that do not accurately recapitulate the stromal micorenvironment of human tumours. However, the advent of new technologies, particularly single-cell RNA-sequencing, is unpicking CAF complexity and there is great optimism in the field that effective CAF-targeted therapies are on the near horizon.

AUTHOR CONTRIBUTIONS

KB, CH, and GT wrote the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was funded through Cancer Research UK (grant nos. A203904, A20256, A27989).

REFERENCES

1. Custódio M, Biddle A, Tavassoli M. Portrait of a CAF: the story of cancer-associated fibroblasts in head and neck cancer. *Oral Oncol.* (2020) 110:104972. doi: 10.1016/j.oraloncology.2020.104972
2. Liu T, Han C, Wang S, Fang P, Ma Z, Xu L, et al. Cancer-associated fibroblasts: an emerging target of anti-cancer immunotherapy. *J Hematol Oncol.* (2019) 12:1–15. doi: 10.1186/s13045-019-0770-1
3. Kumar D, New J, Vishwakarma V, Joshi R, Enders J, Lin F, et al. Cancer-associated fibroblasts drive glycolysis in a targetable signaling loop implicated in head and neck squamous cell carcinoma progression. *Cancer Res.* (2018) 78:3769–82. doi: 10.1158/0008-5472.CAN-17-1076
4. Calvo F, Ege N, Grande-Garcia A, Hooper S, Jenkins RP, Chaudhry SI, et al. Mechanotransduction and YAP-dependent matrix remodelling is required for the generation and maintenance of cancer-associated fibroblasts. *Nat Cell Biol.* (2013) 15:637–46. doi: 10.1038/ncb2756
5. Alarcon-Martinez L, Yilmaz-Ozcan S, Yemisci M, Schallek J, Kiliç K, Can A, et al. Capillary pericytes express α-smooth muscle actin, which requires prevention of filamentous-actin depolymerization for detection. *eLife.* (2018) 7:1–17. doi: 10.7554/eLife.34861
6. Rensen SSM, Doevendans PAFM, Van Eys GJJM. Regulation and characteristics of vascular smooth muscle cell phenotypic diversity. *Netherlands Hear J.* (2007) 15:100–8. doi: 10.1007/BF03085963
7. Nurmik M, Ullmann P, Rodriguez F, Haan S, Letellier E. In search of definitions: cancer-associated fibroblasts and their markers. *Int J Cancer.* (2020) 146:895–905. doi: 10.1002/ijc.32193
8. Ding L, Ren J, Zhang D, Li Y, Huang X, Hu Q, et al. A novel stromal lncRNA signature reprograms fibroblasts to promote the growth of oral squamous cell carcinoma via LncRNA-CAF/interleukin-33. *Carcinogenesis.* (2018) 39:397–406. doi: 10.1093/carcin/bgy006
9. Sahai E, Astsaturov I, Cukierman E, DeNardo DG, Egeblad M, Evans RM, et al. A framework for advancing our understanding of cancer-associated fibroblasts. *Nat Rev Cancer.* (2020) 20:174–86. doi: 10.1038/s41568-019-0238-1
10. Wang S, Meng XM, Ng YY, Ma FY, Zhou S, Zhang Y, et al. TGF-β/Smad3 signalling regulates the transition of bone marrowderived macrophages into myofibroblasts during tissue fibrosis. *Oncotarget.* (2016) 7:8809–22. doi: 10.18632/oncotarget.6604
11. Nair N, Calle AS, Zahra MH, Prieto-Vila M, Oo AKK, Hurley L, et al. A cancer stem cell model as the point of origin of cancer-associated fibroblasts in tumor microenvironment. *Sci Rep.* (2017) 7:1–13. doi: 10.1038/s41598-017-07144-5
12. Elyada E, Bolisetty M, Laise P, Flynn WF, Courtois ET, Burkhart RA, et al. Cross-species single-cell analysis of pancreatic ductal adenocarcinoma reveals antigen-presenting cancer-associated fibroblasts. *Cancer Discov.* (2019) 9:1102–23. doi: 10.1158/2159-8290.CD-19-0094
13. Öhlund D, Handly-Santana A, Biffi G, Elyada E, Almeida AS, Ponz-Sarvise M, et al. Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer. *J Exp Med.* (2017) 214:579–96. doi: 10.1084/jem.20162024
14. Kieffer Y, Hocine HR, Gentric G, Pelon F, Bernard C, Bourachot B, et al. Single-cell analysis reveals fibroblast clusters linked to immunotherapy resistance in cancer. *Cancer Discov.* (2020) 10:1330–51. doi: 10.1158/2159-8290.CD-19-1384
15. Puram S V., Tirosh I, Parikh AS, Patel AP, Yizhak K, Gillespie S, et al. Single-cell transcriptomic analysis of primary and metastatic tumor ecosystems in head and neck cancer. *Cell.* (2017) 171:1611–24.e24. doi: 10.1016/j.cell.2017.10.044
16. Patel AK, Vipparthi K, Thatikonda V, Arun I, Bhattacharjee S, Sharan R, et al. A subtype of cancer-associated fibroblasts with lower expression of alpha-smooth muscle actin suppresses stemness through BMP4 in oral carcinoma. *Oncogenesis.* (2018) 7:78. doi: 10.1038/s41389-018-0087-x
17. Costea DE, Hills A, Osman AH, Thurlow J, Kalna G, Huang X, et al. Identification of two distinct carcinoma-associated fibroblast subtypes with differential tumor-promoting abilities in oral squamous cell carcinoma. *Cancer Res.* (2013) 73:3888–901. doi: 10.1158/0008-5472.CAN-12-4150
18. Hassona Y, Cirillo N, Lim KP, Herman A, Mellone M, Thomas GJ, et al. Progression of genotype-specific oral cancer leads to senescence of cancer-associated fibroblasts and is mediated by oxidative stress and TGF-β. *Carcinogenesis.* (2013) 34:1286–95. doi: 10.1093/carcin/bgt035
19. Mellone M, Hanley CJ, Thirdborough S, Mellows T, Garcia E, Woo J, et al. Induction of fibroblast senescence generates a non-fibrogenic myofibroblast phenotype that differentially impacts on cancer prognosis. *Aging.* (2017) 9:114–32. doi: 10.18632/aging.101127
20. Hassona Y, Cirillo N, Heesom K, Parkinson EK, Prime SS. Senescent cancer-associated fibroblasts secrete active MMP-2 that promotes keratinocyte dis-cohesion and invasion. *Br J Cancer.* (2014) 111:1230–7. doi: 10.1038/bjc.2014.438
21. Hanley CJ, Noble F, Ward M, Bullock M, Drifka C, Mellone M, et al. A subset of myofibroblastic cancer-associated fibroblasts regulate collagen fiber elongation, which is prognostic in multiple cancers. *Oncotarget.* (2016) 7:6159–74. doi: 10.18632/oncotarget.6740
22. Hanley CJ, Mellone M, Ford K, Thirdborough SM, Mellows T, Frampton SJ, et al. Targeting the myofibroblastic cancer-associated fibroblast phenotype through inhibition of NOX4. *J Natl Cancer Inst.* (2018) 110:109–20. doi: 10.1093/jnci/djx121
23. Ford K, Hanley CJ, Mellone M, Szyndralewiez C, Heitz F, Wiesel P, et al. NOX4 inhibition potentiates immunotherapy by overcoming

cancer-associated fibroblast-mediated CD8 T-cell exclusion from tumors. *Cancer Res.* (2020) 80:1846–60. doi: 10.1158/0008-5472.CAN-19-3158
24. Waise S, Parker R, Rose-Zerilli MJJ, Layfield DM, Wood O, West J, et al. An optimised tissue disaggregation and data processing pipeline for characterising fibroblast phenotypes using single-cell RNA sequencing. *Sci Rep.* (2019) 9:9580. doi: 10.1038/s41598-019-45842-4
25. Marsh D, Suchak K, Moutasim KA, Vallath S, Hopper C, Jerjes W, et al. Stromal features are predictive of disease mortality in oral cancer patients. *J Pathol.* (2011) 223:470–81. doi: 10.1002/path.2830
26. Dourado MR, Guerra ENS, Salo T, Lambert DW, Coletta RD. Prognostic value of the immunohistochemical detection of cancer-associated fibroblasts in oral cancer: a systematic review and meta-analysis. *J Oral Pathol Med.* (2018) 47:443–53. doi: 10.1111/jop.12623
27. Parakh MK, Ulaganambi S, Ashifa N, Premkumar R, Jain AL. Oral potentially malignant disorders: clinical diagnosis and current screening aids: a narrative review. *Eur J Cancer Prev.* (2020) 29:65–72. doi: 10.1097/CEJ.0000000000000510
28. Coletta RD, Salo T. Myofibroblasts in oral potentially malignant disorders: is it related to malignant transformation? *Oral Dis.* (2018) 24:84–8. doi: 10.1111/odi.12694
29. de-Assis EM, Pimenta LG, Costa-e-Silva E, Souza PE, Horta MC. Stromal myofibroblasts in oral leukoplakia and oral squamous cell carcinoma. *Med Oral Patol Oral Cir Bucal.* (2012) 17:e733–8. doi: 10.4317/medoral.17834
30. Chaudhary M, Gadbail AR, Vidhale G, Mankar Gadbail MP, Gondivkar SM, Gawande M, et al. Comparison of myofibroblasts expression in oral squamous cell carcinoma, verrucous carcinoma, high risk epithelial dysplasia, low risk epithelial dysplasia and normal oral mucosa. *Head Neck Pathol.* (2012) 6:305–13. doi: 10.1007/s12105-012-0335-x
31. Gandhi P, Prasad UC. Evaluation of myofibroblasts in oral submucous fibrosis and oral squamous cell carcinoma: the pathogenesis and correlation. *Dent Res J.* (2017) 14:314–20. doi: 10.4103/1735-3327.215960
32. Angadi PV, Kale AD, Hallikerimath S. Evaluation of myofibroblasts in oral submucous fibrosis: correlation with disease severity. *J Oral Pathol Med.* (2011) 40:208–13. doi: 10.1111/j.1600-0714.2010.00995.x
33. Bharath Rao K, Malathi N, Narashiman S, Rajan ST. Evaluation of myofibroblasts by expression of alpha smooth muscle actin: a marker in fibrosis, dysplasia and carcinoma. *J Clin Diagnostic Res.* (2014) 8:ZC14–7. doi: 10.7860/JCDR/2014/7820.4231
34. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell.* (2000) 100:57–70. doi: 10.1016/S0092-8674(00)81683-9
35. Gaggioli C, Hooper S, Hidalgo-Carcedo C, Grosse R, Marshall JF, Harrington K, et al. Fibroblast-led collective invasion of carcinoma cells with differing roles for RhoGTPases in leading and following cells. *Nat Cell Biol.* (2007) 9:1392–400. doi: 10.1038/ncb1658
36. Chen YQ, Kuo JC, Wei MT, Wu MC, Yang MH, Chiou A. Fibroblast promotes head and neck squamous cell carcinoma cell invasion through mechanical barriers in 3D collagen microenvironments. *ACS Appl Bio Mater.* (2020) 3:6419–29. doi: 10.1021/acsabm.0c00603
37. Elmusrati AA, Pilborough AE, Khurram SA, Lambert DW. Cancer-associated fibroblasts promote bone invasion in oral squamous cell carcinoma. *Br J Cancer.* (2017) 117:867–75. doi: 10.1038/bjc.2017.239
38. Zhang Z, Tao D, Zhang P, Liu X, Zhang Y, Cheng J, et al. Hyaluronan synthase 2 expressed by cancer-associated fibroblasts promotes oral cancer invasion. *J Exp Clin Cancer Res.* (2016) 35:1–12. doi: 10.1186/s13046-016-0458-0
39. Meran S, Luo DD, Simpson R, Martin J, Wells A, Steadman R, et al. Hyaluronan facilitates transforming growth factor-β1-dependent proliferation via CD44 and epidermal growth factor receptor interaction. *J Biol Chem.* (2011) 286:17618–30. doi: 10.1074/jbc.M111.226563
40. Meran S, Thomas DW, Stephens P, Enoch S, Martin J, Steadman R, et al. Hyaluronan facilitates transforming growth factor-β1- mediated fibroblast proliferation. *J Biol Chem.* (2008) 283:6530–45. doi: 10.1074/jbc.M704819200
41. McCarthy JB, El-Ashry D, Turley EA. Hyaluronan, cancer-associated fibroblasts and the tumor microenvironment in malignant progression. *Front Cell Dev Biol.* (2018) 6:1–13. doi: 10.3389/fcell.2018.00112
42. Zhang X, Hwang YS. Cancer-associated fibroblast stimulates cancer cell invasion in an interleukin-1 receptor (il-1r)-dependent manner. *Oncol Lett.* (2019) 18:4645–50. doi: 10.3892/ol.2019.10784
43. Sobral LM, Bufalino A, Lopes MA, Graner E, Salo T, Coletta RD. Myofibroblasts in the stroma of oral cancer promote tumorigenesis via secretion of activin A. *Oral Oncol.* (2011) 47:840–6. doi: 10.1016/j.oraloncology.2011.06.011
44. Knowles LM, Stabile LP, Egloff AM, Rothstein ME, Thomas SM, Gubish CT, et al. HGF and c-met participate in paracrine tumorigenic pathways in head and neck squamous cell cancer. *Clin Cancer Res.* (2009) 15:3740–50. doi: 10.1158/1078-0432.CCR-08-3252
45. Wang Y, Jing Y, Ding L, Zhang X, Song Y, Chen S, et al. Epiregulin reprograms cancer-associated fibroblasts and facilitates oral squamous cell carcinoma invasion via JAK2-STAT3 pathway. *J Exp Clin Cancer Res.* (2019) 38:1–13. doi: 10.1186/s13046-019-1277-x
46. Dai J, Su Y, Zhong S, Cong L, Liu B, Yang J, et al. Exosomes: key players in cancer and potential therapeutic strategy. *Signal Transduct Target Ther.* (2020) 5:145. doi: 10.1038/s41392-020-00261-0
47. Sun LP, Xu K, Cui J, Yuan DY, Zou B, Li J, et al. Cancer-associated fibroblast-derived exosomal miR-382-5p promotes the migration and invasion of oral squamous cell carcinoma. *Oncol Rep.* (2019) 42:1319–28. doi: 10.3892/or.2019.7255
48. Principe S, Mejia-Guerrero S, Ignatchenko V, Sinha A, Ignatchenko A, Shi W, et al. Proteomic analysis of cancer-associated fibroblasts reveals a paracrine role for MFAP5 in human oral tongue squamous cell carcinoma. *J Proteome Res.* (2018) 17:2045–59. doi: 10.1021/acs.jproteome.7b00925
49. Li Y yin, Tao Y wei, Gao S, Li P, Zheng J mao, Zhang S en, et al. Cancer-associated fibroblasts contribute to oral cancer cells proliferation and metastasis via exosome-mediated paracrine miR-34a-5p. *EBioMed.* (2018) 36:209–20. doi: 10.1016/j.ebiom.2018.09.006
50. Curtis M, Kenny HA, Ashcroft B, Mukherjee A, Johnson A, Zhang Y, et al. Fibroblasts mobilize tumor cell glycogen to promote proliferation and metastasis. *Cell Metab.* (2019) 29:141–55.e9. doi: 10.1016/j.cmet.2018.08.007
51. Sun Y, Fan X, Zhang Q, Shi X, Xu G, Zou C. Cancer-associated fibroblasts secrete FGF-1 to promote ovarian proliferation, migration, and invasion through the activation of FGF-1/FGFR4 signaling. *Tumor Biol.* (2017) 39:1–10. doi: 10.1177/1010428317712592
52. Jahangiri B, Khalaj-kondori M, Asadollahi E, Sadeghizadeh M. Cancer-associated fibroblasts enhance cell proliferation and metastasis of colorectal cancer SW480 cells by provoking long noncoding RNA UCA1. *J Cell Commun Signal.* (2019) 13:53–64. doi: 10.1007/s12079-018-0471-5
53. Wheeler SE, Shi H, Lin F, Dasari S, Bednash J, Thorne S, et al. Enhancement of head and neck squamous cell carcinoma proliferation, invasion, and metastasis by tumor-associated fibroblasts in preclinical models. *Head Neck.* (2014) 36:385–92. doi: 10.1002/hed.23312
54. Magan M, Wiechec E, Roberg K. CAFs affect the proliferation and treatment response of head and neck cancer spheroids during co-culturing in a unique *in vitro* model. *Cancer Cell Int.* (2020) 20:1–11. doi: 10.1186/s12935-020-01718-6
55. Qin X, Yan M, Wang X, Xu Q, Wang X, Zhu X, et al. Cancer-associated fibroblast-derived IL-6 promotes head and neck cancer progression via the osteopontin-NF-kappa B signaling pathway. *Theranostics.* (2018) 8:921–40. doi: 10.7150/thno.22182
56. Weber CE, Kothari AN, Wai PY, Li NY, Driver J, Zapf MAC, et al. Osteopontin mediates an MZF1-TGF-β1-dependent transformation of mesenchymal stem cells into cancer-associated fibroblasts in breast cancer. *Oncogene.* (2015) 34:4821–33. doi: 10.1038/onc.2014.410
57. Petrik D, Lavori PW, Cao H, Zhu Y, Wong P, Christofferson E, et al. Plasma osteopontin is an independent prognostic marker for head and neck cancers. *J Clin Oncol.* (2006) 24:5291–7. doi: 10.1200/JCO.2006.06.8627
58. Bae JY, Kim EK, Yang DH, Zhang X, Park YJ, Lee DY, et al. Reciprocal interaction between carcinoma-associated fibroblasts and squamous carcinoma cells through interleukin-1α induces cancer progression. *Neoplasia.* (2014) 16:928–38. doi: 10.1016/j.neo.2014.09.003
59. Mendes JMF, Valverde L de F, Vidal MTA, Paredes BD, Coelho P, Allahdadi KJ, et al. Effects of igf-1 on proliferation, angiogenesis, tumor stem cell populations and activation of akt and hedgehog pathways in oral squamous cell carcinoma. *Int J Mol Sci.* (2020) 21:1–20. doi: 10.3390/ijms21186487
60. Nissen NI, Karsdal M, Willumsen N. Collagens and cancer associated fibroblasts in the reactive stroma and its relation to cancer biology. *J Exp Clin Cancer Res.* (2019) 38:1–12. doi: 10.1186/s13046-019-1110-6

61. Lai SL, Tan ML, Hollows RJ, Robinson M, Ibrahim M, Margielewska S, et al. Collagen induces a more proliferative, migratory and chemoresistant phenotype in head and neck cancer via DDR1. *Cancers.* (2019) 11:1–16. doi: 10.3390/cancers11111766
62. De Palma M, Biziato D, Petrova T V. Microenvironmental regulation of tumour angiogenesis. *Nat Rev Cancer.* (2017) 17:457–74. doi: 10.1038/nrc.2017.51
63. Mirkeshavarz M, Ganjibakhsh M, Aminishakib P, Farzaneh P, Mahdavi N, Vakhshiteh F, et al. Interleukin-6 secreted by oral cancer- associated fibroblast accelerated VEGF expression in tumor and stroma cells. *Cell Mol Biol.* (2017) 6:131–6. doi: 10.14715/cmb/2017.63.10.21
64. Kayamori K, Katsube KI, Sakamoto K, Ohyama Y, Hirai H, Yukimori A, et al. Notch3 is induced in cancer-associated fibroblasts and promotes angiogenesis in oral squamous cell carcinoma. *PLoS ONE.* (2016) 11:1–19. doi: 10.1371/journal.pone.0154112
65. Warburg O, Wind F, Negelein E. The metabolism of tumors in the body. *J Gen Physiol.* (1927) 8:519–30. doi: 10.1085/jgp.8.6.519
66. de la Cruz-López KG, Castro-Muñoz LJ, Reyes-Hernández DO, García-Carrancá A, Manzo-Merino J. Lactate in the regulation of tumor microenvironment and therapeutic approaches. *Front Oncol.* (2019) 9:1143. doi: 10.3389/fonc.2019.01143
67. Fu Y, Liu S, Yin S, Niu W, Xiong W, Tan M, et al. The reverse Warburg effect is likely to be an Achilles' heel of cancer that can be exploited for cancer therapy. *Oncotarget.* (2017) 8:57813–25. doi: 10.18632/oncotarget.18175
68. Pavlides S, Whitaker-Menezes D, Castello-Cros R, Flomenberg N, Witkiewicz AK, Frank PG, et al. The reverse Warburg effect: aerobic glycolysis in cancer associated fibroblasts and the tumor stroma. *Cell Cycle.* (2009) 8:3984–4001. doi: 10.4161/cc.8.23.10238
69. Barker HE, Paget JTE, Khan AA, Harrington KJ. The tumour microenvironment after radiotherapy: Mechanisms of resistance and recurrence. *Nat Rev Cancer.* (2015) 15:409–25. doi: 10.1038/nrc3958
70. Domogauer JD, de Toledo SM, Howell RW, Azzam EI. Acquired radioresistance in cancer associated fibroblasts is concomitant with enhanced antioxidant potential and DNA repair capacity. *Cell Commun Signal.* (2021) 19:30. doi: 10.1186/s12964-021-00711-4
71. Tsai KKC, Chuang EYY, Little JB, Yuan ZM. Cellular mechanisms for low-dose ionizing radiation-induced perturbation of the breast tissue microenvironment. *Cancer Res.* (2005) 65:6734–44. doi: 10.1158/0008-5472.CAN-05-0703
72. Kamochi N, Nakashima M, Aoki S, Uchihashi K, Sugihara H, Toda S, et al. Irradiated fibroblast-induced bystander effects on invasive growth of squamous cell carcinoma under cancer-stromal cell interaction. *Cancer Sci.* (2008) 99:2417–27. doi: 10.1111/j.1349-7006.2008.00978.x
73. Straub JM, New J, Hamilton CD, Lominska C, Shnayder Y, Thomas SM. Radiation-induced fibrosis: mechanisms and implications for therapy. *J Cancer Res Clin Oncol.* (2015) 141:1985–94. doi: 10.1007/s00432-015-1974-6
74. Huang W, Zhang L, Yang M, Wu X, Wang X, Huang W, et al. Cancer-associated fibroblasts promote the survival of irradiated nasopharyngeal carcinoma cells via the NF-κB pathway. *J Exp Clin Cancer Res.* (2021) 40:87. doi: 10.1186/s13046-021-01878-x
75. Peiris-Pagès M, Sotgia F, Lisanti M. Chemotherapy induces the cancer-associated fibroblast phenotype, activating paracrine Hedgehog-GLI signalling in breast cancer cells. *Oncotarget.* (2015) 6:10728–45. doi: 10.18632/oncotarget.3828
76. Lotti F, Jarrar AM, Pai RK, Hitomi M, Lathia J, Mace A, et al. Chemotherapy activates cancer-associated fibroblasts to maintain colorectal cancer-initiating cells by IL-17A. *J Exp Med.* (2013) 210:2851–72. doi: 10.1084/jem.20131195
77. Schmitz S, Bindea G, Albu RI, Mlecnik B, Machiels JP. Cetuximab promotes epithelial to mesenchymal transition and cancer associated fibroblasts in patients with head and neck cancer. *Oncotarget.* (2015) 6:34288–99. doi: 10.18632/oncotarget.5924
78. Qin X, Guo H, Wang X, Zhu X, Yan M, Wang X, et al. Exosomal miR-196a derived from cancer-associated fibroblasts confers cisplatin resistance in head and neck cancer through targeting CDKN1B and ING5. *Genome Biol.* (2019) 20:1–21. doi: 10.1186/s13059-018-1604-0
79. Yegodayev KM, Novoplansky O, Golden A, Prasad M, Levin L, Jagadeeshan S, et al. TGF-beta-activated cancer-associated fibroblasts limit cetuximab efficacy in preclinical models of head and neck cancer. *Cancers.* (2020) 12:339. doi: 10.3390/cancers12020339
80. Johansson AC, Ansell A, Jerhammar F, Lindh MB, Grénman R, Munck-Wikland E, et al. Cancer-associated fibroblasts induce matrix metalloproteinase-mediated cetuximab resistance in head and neck squamous cell carcinoma cells. *Mol Cancer Res.* (2012) 10:1158–68. doi: 10.1158/1541-7786.MCR-12-0030
81. Canning M, Guo G, Yu M, Myint C, Groves MW, Byrd JK, et al. Heterogeneity of the head and neck squamous cell carcinoma immune landscape and its impact on immunotherapy. *Front Cell Dev Biol.* (2019) 7:52. doi: 10.3389/fcell.2019.00052
82. Monteran L, Erez N. The dark side of fibroblasts: Cancer-associated fibroblasts as mediators of immunosuppression in the tumor microenvironment. *Front Immunol.* (2019) 10:1835. doi: 10.3389/fimmu.2019.01835
83. Takahashi H, Sakakura K, Kawabata-Iwakawa R, Rokudai S, Toyoda M, Nishiyama M, et al. Immunosuppressive activity of cancer-associated fibroblasts in head and neck squamous cell carcinoma. *Cancer Immunol Immunother.* (2015) 64:1407–17. doi: 10.1007/s00262-015-1742-0
84. Xing X, Guo J, Wen X, Ding G, Li B, Dong B, et al. Analysis of PD1, PDL1, PDL2 expression and T cells infiltration in 1014 gastric cancer patients. *Oncoimmunology.* (2018) 7:1–10. doi: 10.1080/2162402X.2017.1356144
85. Kumagai S, Togashi Y, Kamada T, Sugiyama E, Nishinakamura H, Takeuchi Y, et al. The PD-1 expression balance between effector and regulatory T cells predicts the clinical efficacy of PD-1 blockade therapies. *Nat Immunol.* (2020) 21:1346–58. doi: 10.1038/s41590-020-0769-3
86. Wolf K, Müller R, Borgmann S, Bröcker EB, Friedl P. Amoeboid shape change and contact guidance: T-lymphocyte crawling through fibrillar collagen is independent of matrix remodeling by MMPs and other proteases. *Blood.* (2003) 102:3262–9. doi: 10.1182/blood-2002-12-3791
87. Evanko SP, Potter-Perigo S, Bollyky PL, Nepom GT, Wight TN. Hyaluronan and versican in the control of human T-lymphocyte adhesion and migration. *Matrix Biol.* (2012) 31:90–100. doi: 10.1016/j.matbio.2011.10.004
88. Salmon H, Franciszkiewicz K, Damotte D, Dieu-Nosjean MC, Validire P, Trautmann A, et al. Matrix architecture defines the preferential localization and migration of T cells into the stroma of human lung tumors. *J Clin Invest.* (2012) 122:899–910. doi: 10.1172/JCI45817
89. Bougherara H, Mansuet-Lupo A, Alifano M, Ngô C, Damotte D, Le Frère-Belda MA, et al. Real-time imaging of resident T cells in human lung and ovarian carcinomas reveals how different tumor microenvironments control T lymphocyte migration. *Front Immunol.* (2015) 6:500. doi: 10.3389/fimmu.2015.00500
90. Feig C, Jones JO, Kraman M, Wells RJ, Deonarine A, Chan DS, et al. Targeting CXCL12 from FAP-expressing carcinoma-associated fibroblasts synergizes with anti-PD-L1 immunotherapy in pancreatic cancer. *Proc Natl Acad Sci USA.* (2013) 110:20212–7. doi: 10.1073/pnas.1320318110
91. Fearon DT. The carcinoma-associated fibroblast expressing fibroblast activation protein and escape from immune surveillance. *Cancer Immunol Res.* (2014) 2:187–93. doi: 10.1158/2326-6066.CIR-14-0002
92. Takahashi H, Sakakura K, Kudo T, Toyoda M, Kaira K, Oyama T, et al. Cancer-associated fibroblasts promote an immunosuppressive microenvironment through the induction and accumulation of protumoral macrophages. *Oncotarget.* (2017) 8:8633–47. doi: 10.18632/oncotarget.14374
93. Li X, Bu W, Meng L, Liu X, Wang S, Jiang L, et al. CXCL12/CXCR4 pathway orchestrates CSC-like properties by CAF recruited tumor associated macrophage in OSCC. *Exp Cell Res.* (2019) 378:131–8. doi: 10.1016/j.yexcr.2019.03.013
94. Wu MH, Hong HC, Hong TM, Chiang WF, Jin YT, Chen YL. Targeting galectin-1 in carcinoma-associated fibroblasts inhibits oral squamous cell carcinoma metastasis by downregulating MCP-1/CCL2 expression. *Clin Cancer Res.* (2011) 17:1306–16. doi: 10.1158/1078-0432.CCR-10-1824
95. Jung DW, Che ZM, Kim J, Kim K, Kim KY, Williams D, et al. Tumor-stromal crosstalk in invasion of oral squamous cell carcinoma: a pivotal role of CCL7. *Int J Cancer.* (2010) 127:332–44. doi: 10.1002/ijc.25060
96. Ziani L, Chouaib S, Thiery J. Alteration of the antitumor immune response by cancer-associated fibroblasts. *Front Immunol.* (2018) 9:414. doi: 10.3389/fimmu.2018.00414

97. Pakshir P, Alizadehgiashi M, Wong B, Coelho NM, Chen X, Gong Z, et al. Dynamic fibroblast contractions attract remote macrophages in fibrillar collagen matrix. *Nat Commun.* (2019) 10:1–17. doi: 10.1038/s41467-019-10344-4
98. Wang L, Li X, Ren Y, Geng H, Zhang Q, Cao L, et al. Cancer-associated fibroblasts contribute to cisplatin resistance by modulating ANXA3 in lung cancer cells. *Cancer Sci.* (2019) 110:1609–20. doi: 10.1111/cas.13998
99. Long X, Xiong W, Zeng X, Qi L, Cai Y, Mo M, et al. Cancer-associated fibroblasts promote cisplatin resistance in bladder cancer cells by increasing IGF-1/ERβ/Bcl-2 signalling. *Cell Death Dis.* (2019) 10:375. doi: 10.1038/s41419-019-1581-6
100. Zhang H, Yue J, Jiang Z, Zhou R, Xie R, Xu Y, et al. CAF-secreted CXCL1 conferred radioresistance by regulating DNA damage response in a ROS-dependent manner in esophageal squamous cell carcinoma. *Cell Death Dis.* (2017) 8:e2790. doi: 10.1038/cddis.2017.180
101. Wang Y, Gan G, Wang B, Wu J, Cao Y, Zhu D, et al. Cancer-associated fibroblasts promote irradiated cancer cell recovery through autophagy. *EBioMed.* (2017) 17:45–56. doi: 10.1016/j.ebiom.2017.02.019
102. Catenacci DVT, Junttila MR, Karrison T, Bahary N, Horiba MN, Nattam SR, et al. Randomized phase Ib/II study of gemcitabine plus placebo or vismodegib, a hedgehog pathway inhibitor, in patients with metastatic pancreatic cancer. *J Clin Oncol.* (2015) 33:4284–92. doi: 10.1200/JCO.2015.62.8719
103. Narra K, Mullins SR, Lee HO, Strzemkowski-Brun B, Magalong K, Christiansen VJ, et al. Phase II trial of single agent Val-boroPro (Talabostat) inhibiting fibroblast activation protein in patients with metastatic colorectal cancer. *Cancer Biol Ther.* (2007) 6:1691–9. doi: 10.4161/cbt.6.11.4874
104. Chen IX, Chauhan VP, Posada J, Ng MR, Wu MW, Adstamongkonkul P, et al. Blocking CXCR4 alleviates desmoplasia, increases T-lymphocyte infiltration, and improves immunotherapy in metastatic breast cancer. *Proc Natl Acad Sci USA*. (2019) 116:4558–66. doi: 10.1073/pnas.1815515116
105. de Gramont A, Faivre S, Raymond E. Novel TGF-β inhibitors ready for prime time in onco-immunology. *Oncoimmunology*. (2017) 6:e1257453. doi: 10.1080/2162402X.2016.1257453
106. Mariathasan S, Turley SJ, Nickles D, Castiglioni A, Yuen K, Wang Y, et al. TGFβ attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. *Nature.* (2018) 554:544–8. doi: 10.1038/nature25501
107. Huynh LK, Hipolito CJ, Dijke P Ten. A perspective on the development of TGF-β inhibitors for cancer treatment. *Biomolecules.* (2019) 9:743. doi: 10.3390/biom9110743
108. Rhim AD, Oberstein PE, Thomas DH, Mirek ET, Palermo CF, Sastra SA, et al. Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma. *Cancer Cell.* (2014) 25:735–47. doi: 10.1016/j.ccr.2014.04.021
109. Sherman MH, Yu RT, Engle DD, Ding N, Atkins AR, Tiriac H, et al. Vitamin D receptor-mediated stromal reprogramming suppresses pancreatitis and enhances pancreatic cancer therapy. *Cell.* (2014) 159:80–93. doi: 10.1016/j.cell.2014.08.007
110. Grauel AL, Nguyen B, Ruddy D, Laszewski T, Schwartz S, Chang J, et al. TGFβ-blockade uncovers stromal plasticity in tumors by revealing the existence of a subset of interferon-licensed fibroblasts. *Nat Commun.* (2020) 11:6315. doi: 10.1038/s41467-020-19920-5
111. Kraman M, Bambrough PJ, Arnold JN, Roberts EW, Magiera L, Jones JO, et al. Suppression of antitumor immunity by stromal cells expressing fibroblast activation protein-alpha. *Science.* (2010) 330:827–30. doi: 10.1126/science.1195300
112. Duperret EK, Trautz A, Ammons D, Perales-Puchalt A, Wise MC, Yan J, et al. Alteration of the tumor stroma using a consensus DNA vaccine targeting fibroblast activation protein (FAP) synergizes with antitumor vaccine therapy in mice. *Clin Cancer Res.* (2018) 24:1190–201. doi: 10.1158/1078-0432.CCR-17-2033
113. Tran E, Chinnasamy D, Yu Z, Morgan RA, Lee CCR, Restifo NP, et al. Immune targeting of fibroblast activation protein triggers recognition of multipotent bone marrow stromal cells and cachexia. *J Exp Med.* (2013) 210:1065–8. doi: 10.1084/jem.20130110

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oral Health

REVIEW
published: 26 August 2021
doi: 10.3389/froh.2021.722676

IL-1/IL-1R Signaling in Head and Neck Cancer

Sven E. Niklander[1], *Craig Murdoch*[2] *and Keith D. Hunter*[2,3]*

[1] *Unidad de Patología y Medicina Oral, Facultad de Odontologia, Universidad Andres Bello, Viña del Mar, Chile,* [2] *Unit of Oral and Maxillofacial Medicine, Pathology and Surgery, School of Clinical Dentistry, University of Sheffield, Sheffield, United Kingdom,* [3] *Oral Biology and Pathology, University of Pretoria, Pretoria, South Africa*

Decades ago, the study of cancer biology was mainly focused on the tumor itself, paying little attention to the tumor microenvironment (TME). Currently, it is well recognized that the TME plays a vital role in cancer development and progression, with emerging treatment strategies focusing on different components of the TME, including tumoral cells, blood vessels, fibroblasts, senescent cells, inflammatory cells, inflammatory factors, among others. There is a well-accepted relationship between chronic inflammation and cancer development. Interleukin-1 (IL-1), a potent pro-inflammatory cytokine commonly found at tumor sites, is considered one of the most important inflammatory factors in cancer, and has been related with carcinogenesis, tumor growth and metastasis. Increasing evidence has linked development of head and neck squamous cell carcinoma (HNSCC) with chronic inflammation, and particularly, with IL-1 signaling. This review focuses on the most important members of the IL-1 family, with emphasis on how their aberrant expression can promote HNSCC development and metastasis, highlighting possible clinical applications.

Keywords: head and neck cancer, squamous cell carcinoma, oral cancer, tumor microenvironment, IL-1, Anakinra (PubChem CID: 90470007)

OPEN ACCESS

Edited by:
Carolina Cavalieri Gomes, Federal University of Minas Gerais, Brazil

Reviewed by:
Marcelo Lamers, Federal University of Rio Grande do Sul, Brazil
Tarcilia A. Silva, Federal University of Minas Gerais, Brazil
Daniela-Elena Costea, University of Bergen, Norway

***Correspondence:**
Keith D. Hunter
k.hunter@sheffield.ac.uk

Specialty section:
This article was submitted to Oral Cancers, a section of the journal Frontiers in Oral Health

Received: *09 June 2021*
Accepted: *04 August 2021*
Published: *26 August 2021*

Citation:
Niklander SE, Murdoch C and Hunter KD (2021) IL-1/IL-1R Signaling in Head and Neck Cancer. Front. Oral. Health 2:722676. doi: 10.3389/froh.2021.722676

INTRODUCTION

The association between chronic inflammation and cancer has been reported for many years. Fifteen percent of all cancers are attributed to inflammation [1], with a well-recognized association in lung, pancreatic, esophageal, bladder, gastric, cervical, colorectal and prostate cancers [2]. Pro-inflammatory cytokines present in the tumor microenvironment (TME) can have dual effects; they can stimulate inflammation to decrease tumor progression; or they can stimulate inflammation favoring carcinogenesis, tumor growth and metastasis [3]. Cytokines are produced by host cells in response to factors secreted by the tumor cells or by the tumor itself [4, 5]. Interleukin-1 (IL-1) is commonly found at tumor sites and is considered one of the most important cytokines of the TME, where it plays a key role in carcinogenesis and tumor progression [6], and its expression has been associated with poor prognosis in cancer patients [7]. There is a growing association linking head and neck squamous cell carcinoma (HNSCC) with chronic inflammation [8, 9], in which IL-1/IL-1R signaling seems to be a key player [10]. Cumulating evidence suggests that the effects of IL-1 autocrine and paracrine signaling within the TME is central to HNSCC development. This signaling axis not only leads to increased expression of proteases and factors that dramatically alter the extracellular matrix, aiding tumor cell invasion and metastasis [2, 3], but also increases the production of leukocyte chemoattractants [11]. These molecules selectively recruit both innate and adaptive immune cells to the TME that have both anti- and pro-tumorigenic properties.

Innate immune cells, such as macrophages and neutrophils, are recruited in large numbers to the HNSCC TME where they secrete tumor promoting and pro-angiogenic factors that exacerbate inflammation, and increase the supply of nutrients and oxygen that drives tumor progression. It is no surprise that high numbers of macrophages and neutrophils in the TME are associated with poor prognosis in HNSCC patients [12, 13]. In contrast, and particularly for HPV-positive HSNCC, increased numbers of T lymphocytes have been observed and these have been associated with improved prognosis due to direct anti-tumor cell targeting by these cells [14].

Here, we review the most important members of the IL-1 family, with emphasis on how their aberrant expression can promote HNSCC development and metastasis, highlighting possible clinical applications.

IL-1 FAMILY MEMBERS

The IL-1 family consists of several different ligands and receptors (**Table 1**) [16]. The most studied ligands are IL-1α and IL-1β, commonly known collectively as IL-1, and the interleukin-1 receptor antagonist (IL-1RA), which antagonizes the effects of IL-1α and IL-1β [17]. These ligands bind to IL-1 receptor 1 (IL-1R1) and IL-1 receptor 2 (IL-1R2) that are expressed by several cells. IL-1R1 is a biologically active receptor with the ability to bind to either form of IL-1 [18], while IL-1R2 is non-biologically active and acts as a decoy receptor, inhibiting the effects of IL-1 [19, 20].

IL-1α

IL-1α is produced initially as a 31–33 kDa precursor protein (preIL-1α) that is cleaved into its 17 kDa mature C-terminal component (mIL-1 α) and a 16 kDa N-terminal propiece (ppIL-1α) by the calcium-activated cysteine protease, calpain [5, 21–23]. All forms of IL-1α are biologically active [24]. PreIL-1α lacks a leader peptide and therefore cannot be secreted and remains intracellular [17, 25]. Despite not being secreted, preIL-1α can localize on the cell surface of macrophages, endothelial cells, fibroblasts and dendritic cells [26] where is referred to as membrane-bound IL-1α. Here it acts in a juxtracrine manner by activating the IL-1R1 receptor of surrounding cells [17]. Both preIL-1α and mIL-1α are expressed constitutively in epithelial and endothelial cells and are considered to act in an autocrine or paracrine manner [27–29]. IL-1α plays an important role in inflammation acting in a juxtracrine manner [17] and has been related with several other cellular functions, such as onset of senescence [30–32], cell growth, cell differentiation [28, 33], immune response [34] and regulation of gene expression [35–37].

To exert its biological function, mIL-1α binds to IL-1R1 to trigger different cellular functions, but preIL-1α and ppIL-1α can also interact directly with the DNA without binding to IL-1R1 in a variety of cells [28, 38]. This is because ppIL-1α contains a canonical nuclear localization sequence (NLS) that enables it to interact directly within the nucleus in a non-IL-1R1-dependent manner [39, 40]. PreIL-1α, *via* ppIL-1α, interacts with histone acetyltransferases Gcn5, p300, PCAF and with the adaptor component Ada3, inducing protein transcription without activating IL-1R1 [41], and in doing so exerts different intracellular functions, such as NF-κB and AP-1 activation [38], modulation of endothelial proliferation [28], migration [42] and cytokine production [43].

TABLE 1 | IL-1 family of ligands and receptors (adapted from Dinarello, [15]).

IL-1 family member	Receptor	Function
IL-1α	IL-1R1/IL-1R2	PI/AI
IL-1β	IL-1R1/IL-1R2	PI/AI
IL-1RA	IL1-R1	AI
IL-18	IL-1R5	PI
IL-33	IL-1R4	PI
IL-36α, β, γ	IL-1R6	PI
IL-36RA	IL-1R6	AI
IL-37	IL-1R5	AI
IL-38	IL-1R6	AI

IL-1β

IL-1β is the classic inflammatory secreted cytokine produced in response to inflammatory signals and other stimuli and can act in a paracrine or systemic manner [44, 45]. IL-1β is mainly produced by monocytes by intracellular cleavage from its 31 kDa precursor protein (pIL-1β) into a 17.5 kDa mature form (mIL-1β) by caspase-1 or IL-1β converting enzyme (ICE) [5, 46]. The precursor form of IL-1β is considered to be an inactive immature form of the protein [18]. Unlike preIL-1α, pIL-1β is not expressed in health [47]. Many microbial products are able to stimulate IL-1β secretion, and when produced, IL-1β, together with IL-1α, has the ability to upregulate its own gene expression *in vitro* and *in vivo* [48]. IL-1β expression is mainly restricted to inflammatory cells, where it is regulated in response to external stimuli [49]. IL-1β is 25–50-fold more abundant than IL-1α in stimulated human peripheral mononuclear cells [50], and upon activation, around 70% of IL-1β is secreted by these cells after 24 h stimulation [51].

The main functions of IL-1β are to induce upregulation of cytokines, chemokines, adhesion molecules, acute phase proteins and tissue remodeling enzymes [17, 49, 52]; it may also act as an angiogenic factor in tumors [53], inducing the production of vascular endothelial growth factor (VEGF) *via* cyclooxygenase (COX)-2 activation [54]. IL-1β has been associated with the pathobiology of many diseases, such as familial periodic fever syndromes [55], multiple organ failure in sepsis [56], rheumatoid arthritis, type II diabetes [57], chronic obstructive pulmonary disease [58] and growth, vascularization and metastasis of malignant tumors [53].

IL-1 Receptors

IL-1R1

IL-1R1 is the main receptor through which IL-1 exerts its effects and is found on T cells, keratinocytes, fibroblasts, synovial cells, endothelial cells, chondrocytes, and hepatocytes [17, 51]. All active forms of IL-1 (pIL-1α, mIL-1α and mature IL-1β) bind with similar affinity to IL-1R1, triggering biological actions [49, 59]. IL-1R1 is an 80 kDa molecular weight protein and

belongs to the immunoglobulin (Ig) super family. It has a single transmembrane portion with a cytosolic region and an extracellular segment that contains three domains homologous to Igs, with seven N-linked glycosylation sites. To be active, the IL-1R1/IL-1 extracellular complex requires the additional binding of a co-receptor, the IL-1 receptor accessory protein (IL-1RAcP) or IL-1R3, forming a trimeric complex [60, 61]. IL-1RAcP is essential for signal transduction, as murine fibroblasts deficient in IL-1RAcP showed no response after IL-1 stimulation [62]. After IL-1 binds to the extracellular domain, the Toll/interleukin-1 receptor (TIR) domain of the cytoplasmic portion of IL-1R1 triggers a cascade of intracellular signaling events that result in the phosphorylation and degradation of inhibitor nuclear factor κB (IκB) [63]. This releases p50 and p65 NF-κB subunits that upon phosphorylation are transported into the nucleus and bind to specific DNA promotor sequences, initiating gene transcription [49, 64]. Although it is generally accepted that IL-1R1 is localized on the cell membrane, recent reports have shown that the receptor is also present on the nuclear membrane of malignant oral keratinocytes [65, 66], although the relevance of this nuclear localization is unknown.

IL-1R2

IL-1R2 is an inactive IL-1 receptor that acts as a molecular trap, capturing IL-1 on the plasma membrane or within the extracellular space when membrane IL-1R2 is cleaved and present as a soluble receptor, without triggering agonist activity [61, 67]. Together with IL-1RA, these IL-1R2 forms act as IL-1 inhibitors [68]. IL-1R2 is also a member of the Ig super family, consisting of three Ig-like domains in the extracellular portion and a transmembrane segment. It is found on B and T helper 2 cells, neutrophils, monocytes, bone marrow and microglial cells [15]. The main difference between the two IL-1R forms is that, unlike Il-1R1, IL-1R2 has no TIR domain, and is therefore unable to trigger intracellular signaling, rendering it biologically inactive [69].

IL-1RA

IL-1RA blocks the binding of IL-1α and IL-1β to IL-1R1, having no cross-reactivity with IL-1α and IL-1β [70]. When binding to IL-1R1, IL-1RA does not recruit IL-1RAcP. Thus, IL-1RA has no agonist action, acting as a pure antagonist molecule [71]. IL-1RA mainly binds IL-1R1, having little effect on IL-1R2, which is in agreement with their action as IL-1 inhibitor molecules [72]. As IL-1RA competes with IL-1 for the same receptor, it is found in higher concentrations than IL-1. For example, in the skin, IL-1RA expression has been found to be ≈100-fold higher than IL-1α [27]. Similarly, a study with recombinant IL-1RA showed that in order to have 50% inhibition of IL-1-induced actions, IL-1RA had to be present in 5–100-fold excess over both IL-1α and IL-1β [73]. This is because IL-1R1 is very sensitive to small amounts of IL-1. Even 5% IL-1 receptor occupancy is able to trigger a complete biological response [63]. So, for IL-1RA to efficiently block the effects of IL-1, it must be in abundance [15].

Two main forms of IL-1RA are now recognized; a secreted form (sIL-1RA) [74] and an intracellular form (icIL-1RA) [75]. Intracellular IL-1RA has the same amino acid structure as the secreted form, but lacks a leader peptide that prevents its secretion. The intracellular variant is transcribed by alternate splicing of the same gene as sIL-1RA [75]. Three isoforms of the intracellular form have been reported (icIL-1RA1 or transcript variant 3, icIL-1RA2 or transcript variant 2 and icIL-1RA3 or transcript variant 4) [76–78], with icIL-1RA1 being the most studied. Both secreted and intracellular IL-1RA forms are tissue specific. icIL-1RA is constitutively expressed in tissue sites exposed to environmental factors, such as epithelial cells of the skin, oral cavity, vagina, ovaries and upper respiratory tract [65, 75, 79, 80], while sIL-1RA is found in monocytes, neutrophils and other cells [77].

Other IL-1 Family Members

The IL-1 family of proteins comprises of several other members in addition to IL-1 (**Table 1**). IL-18, whose actions are mediated by binding to IL-1R5, is considered an immunomodulatory cytokine important for IFNγ production and is up-regulated by keratinocytes in response to contact sensitizers [81, 82]. IL-33 is a pro-inflammatory cytokine that binds to IL-1R4, but similarly to IL-1α, can also exert its actions directly to the nucleus [83]. IL-36 is an inflammatory protein associated with the development of psoriasis and acts by binding to IL-1R6. Decreased levels of its specific antagonist, IL-36RA, have also been related with the development of pustular psoriasis [84]. Similar to IL-18, IL-37 also binds to and activates IL-1R5. However, unlike IL-18, IL-37 is considered an anti-inflammatory cytokine, where low levels are thought to contribute to disease severity [61]. IL-38 also has predominantly anti-inflammatory actions that are mediated by binding to IL-1R6 [85] where it has been shown to reduce clinical manifestations of systemic lupus erythematosus and arthritis [86].

IL-1 IN KERATINOCYTE BIOLOGY

IL-1α and IL-1RA are constitutively expressed by skin and oral keratinocytes [34, 65, 80, 87]. IL-1β can be found intracellularly [87], but because keratinocytes lack ICE, pIL-1β cannot be cleaved into its 18 kDa mature form and remains inactive and is not reported to be secreted [87, 88]. However, it has been recently reported that normal oral keratinocytes do secrete IL-1β (although in very low levels) that significantly increases with cell aging, although the underlying mechanism is unknown and might be related with the culture conditions (co-culture with irradiated fibroblasts) [65]. To counteract the action of IL-1, keratinocytes mainly express icIL1-RA1 [79, 89], which is localized both in the cytoplasm and inside the nucleus [65], whereas the secreted isoform (sIL-1RA) is either absent or found in very low levels [65, 90]. This makes biological sense as keratinocytes mainly express IL-1α, which is considered an intracellular cytokine, thus an intracellular antagonist is needed to regulate its activity. Although the main actions of icIL1-RA are attributed to its ability to block IL-1R1, icIL1-RA1 is also able to decrease IL-6 and CXCL8 levels by inhibition of the p38 MAPK and NF-κB signaling pathways in an IL-1R1-independent manner [89, 91]. Thus, it is likely that the main icIL-1RA1 functions are related to the regulation of intranuclear

IL-1α. Keratinocytes also express IL-1R2 at higher levels than IL-1R1 (resting and when activated). So, both IL-1R2 and icIL-1RA have synergistic roles in regulating IL-1 action on keratinocytes, protecting these cells from excessive autocrine activation of IL-1α [92].

IL-1α and icIL-1RA may have an important role in keratinocyte growth, differentiation, and aging. An *in vitro* study testing the growth conditions for different epithelial cells found that IL-1α inhibited the proliferation of stratified squamous epithelial cells, whereas IL-1RA enhanced it. Moreover, significant growth promotion in normal epidermal keratinocytes was observed upon addition of exogenous IL-1RA [93]. Changes in the ratio of icIL-1RA:IL-1α might help to control growth and differentiation of human skin, as icIL-1RA accumulates in more differentiated cells and IL-1RA expression in oral keratinocytes is positively correlated with the expression of involucrin (a marker of cell terminal differentiation that is restricted to the granular cell layer) [94], whereas IL-1α is uniformly expressed in all keratinocyte maturation stages [33]. icIL-1RA1 is reported to be an important factor in the regulation of oral keratinocyte senescence and the development of the senescence-associated secretory phenotype (SASP) [65]. Cellular senescence corresponds to a cellular state characterized by permanent cell growth arrest in response to different stressors in order to avoid propagation of genetically damaged cells [95]. When cells senesce, they remain metabolically active a develop a SASP characterized by the presence of multiple pro-inflammatory factors which have been related with the development of age-related disorders, including cancer [96]. Oral keratinocytes lacking icIL-1RA1 have been shown to senesce prematurely when compared with keratinocytes expressing icIL-1RA1, and icIL-RA1 was found to regulate the expression of two important SASP factors, IL-6 and CXCL8 [65], which have been associated with the development of malignancies [96].

IL-1 SIGNALING IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

IL-1α, IL-1β, and IL-1R1 have been reported to be constitutively expressed in HNSCC [66, 97–99] whilst decreased IL-1RA expression has been observed early in the oral carcinogenesis process [65]. As different members of the IL-1 signaling pathway have reported to have important functions in HNSCC carcinogenesis and tumor progression, we will review them separately.

IL-1α

IL-1α expression in HNSCC contributes to cell growth and survival and has been considered by some authors as a prognostic factor. In the study of Leon et al. [100], patients with metastatic HNSCC displayed higher expression of IL-1α than patients without metastases. Constitutive IL-1α over-expression was correlated with that of IL-1 family genes, such as IL-1β and IL-1RA. IL-1α expression also correlated with increased cell transmigration of tumor cells across the endothelium, which was inhibited by addition of IL-1RA. IL-1α expression also correlated with different genes that have been associated with metastasis, particularly MMP-9 (a matrix metalloproteinase associated with EMT), PGE_2 (a product of COX-2 activation associated with metastases of OSCC), VEGF (the most important angiogenic factor in HNSCC) and CXCL8 [100]. The five-year distant metastasis-free survival was 70% for patients with high tumor levels of IL-1α in contrast to 95% for patients with low expression of IL-1α. Patients with increased levels of IL-1α had a 5.3-fold higher risk of developing metastasis and patients with distant metastases had also a significant increase in secreted IL-1α [100].

IL-1α has also been reported to induce the overexpression of IL-6 [97] and CXCL8 in HNSCC cell lines, the latter by inducing NF-κB and AP-1 pathways [36]. This is of importance as IL-6 and CXCL8 are considered important "oncogenic cytokines", as they are able to cause EMT [101], stimulate angiogenesis and tumor growth [102, 103], disrupt cell-cell communication, impede macrophage function and promote epithelial and endothelial cell migration and invasion [104]. NF-κB is considered a key factor in the regulation of the inflammatory infiltrate observed in the TME [105] and has been associated with the acquisition of a malignant phenotype of HNSCC, as is associated with tumor angiogenesis [106], EMT [107], invasion [108, 109] and metastasis [110]. In addition, inactivation of NF-κB in HNSCC suppressed cell survival and expression of IL-1α, IL-6, CXCL8 and GM-CSF in a murine model of head and neck cancer [111] and its aberrant expression is associated with poor prognosis in solid cancers [112]. AP-1 expression increases with HNSCC progression and induces bcl-2 expression that is associated with suppression of apoptosis and resistance to chemoradiation therapy [113] (**Figure 3**).

IL-1β

The mechanism by which IL-1β is constitutively overexpressed in HNSCC is not clear, but a single nucleotide polymorphism of the IL-1β gene could explain this. In fact, IL-1β-511 polymorphism has been reported to be a significant risk factor for the development of OSCC [114]. IL-1β is identified as a key node gene in the tumor microenvironment (TME) of OSCC *in vivo* [115]. Keratinocytes lack ICE and therefore should not be able to produce the mature active form of IL-1β. However, other proteases are able to cleave IL-1β precursor form, suggesting that pIL-1β can be processed after secretion by other proteases that are present in the TME [116]. In agreement with this, IL-1β produced by oral keratinocytes and HNSCC cells is biologically active. Interaction of IL-1β with the TME leads to monocyte recruitment that then differentiate into tumor-associated macrophages (TAMs) whose increased levels in HNSCC are associated with poor prognosis [12, 117]. Also, stimulates the production of numerous cytokines by different cell types, such [116] as cancer-associated fibroblasts (CAFs), normal fibroblast, endothelial cells, neutrophils as well as oral dysplastic and cancer cells, among others [10, 11, 98, 118], through an IL-1-dependent innate immune response.

The IL-1β found in the TME is also produced by other cells in addition to HNSCC cells. A recent report demonstrated that IL-1β produced by CAFs induces CCL22 mRNA overexpression in oral cancer cells. CCL22 is implicated in the recruitment of

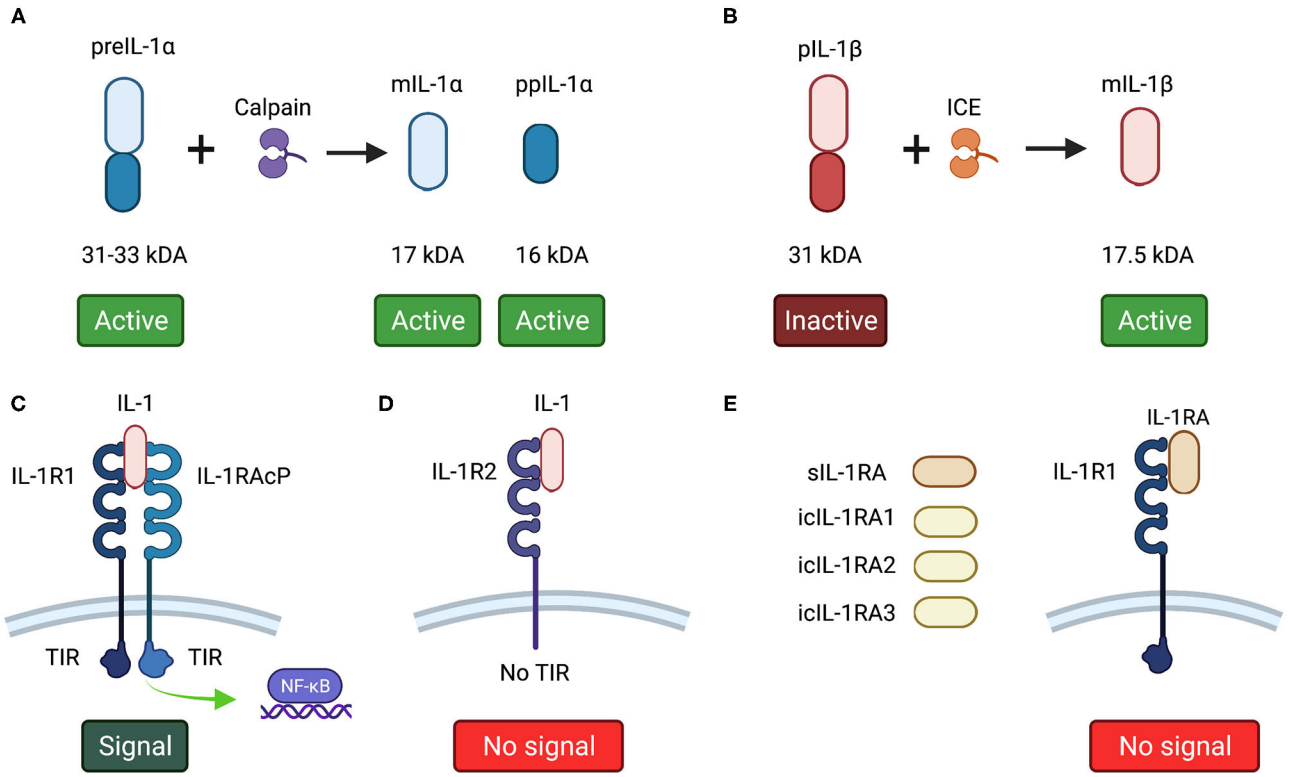

FIGURE 1 | (A) IL-1α and **(B)** IL-1β forms. **(C)** IL-1R1 agonist receptor. To be active, IL-1R1 needs the binding of IL-1RAcP. Both IL-1R1 and IL-1RAcP have a TIR domain, which after a series of phosphorylation following IL-1 binding, activate NF-kB. **(D)** IL-1R2 decoy receptor binds to IL-1 without triggering any agonist action as lacks of a TIR domain. **(E)** IL-1RA variants bind to IL-1R1, blocking the binding of IL-1 without recruiting IL-1RAcP, thus triggers no agonist action. This image was created with Biorender.

T regulatory cells, and its expression in oral cancer patients has been associated with a reduced disease-free survival [119]. Tumor-associated macrophages (TAMs) also secrete IL-1β, which, together with the actions of TNF-α stimulate tumor angiogenesis by inducing the release of VEGF and CXCL8 by HNSCC cells [120]. This creates an inflammatory TME that can predispose to tumor progression [121] (**Figure 1**). For example, in HNSCC IL-1 signaling drives neutrophil and monocyte recruitment [10], and accumulation of these tumor-associated leukocytes has been associated with poor prognosis [13, 122]. The IL-1/IL-1R axis mediates chemokine release from normal tonsillar fibroblasts (NTF) induced by HPV-negative oropharyngeal carcinoma (OPC) cells, which can be reverted with IL-1 inhibition [11]. This is of significance, as for example, CXCL1 and MMP-1 produced by CAFs in response to IL-1β from OSCC cells, increased the invasion and migration capabilities of OSCC cells [123]. Also, IL-1 released from HNSCC cells has been shown to stimulate COX-2 production by CAFs [124] that correlated with lymphangiogenesis [125] and E-cadherin regulation, important factors for epithelial-to-mesenchymal transition (EMT) development [126], and increased risk of distant metastases [127]. In OSCC, IL-1β produced by tumor cells can act in a paracrine manner, inducing the expression of fascine that is associated with ECM degradation and tumor cell invasion [128] (**Figure 2**).

The oncogenic properties of IL-1β have also been demonstrated using *in vivo* models. In a mouse oral cancer model in which carcinogenesis was induced by mimicking tobacco and areca nut carcinogens, an increase in pIL-1β mRNA positively correlated with the presence of malignant change (from normal, to mild, through severe dysplasia to OSCC). In agreement with these findings, OSCC and dysplastic cell lines from smokers and/or betel quid chewers had higher IL-1β levels than controls, with inflammasome components constitutively expressed in OSCC cells that allows the cleavage of pIL-1β into mIL-1β. IL-1β might have an important role in the induction of EMT in OSCC, as in the same study, OSCC cells treated with IL-1β showed upregulation of Snail and Slug (two repressors of E-cadherin expression), increased vimentin expression, and downregulation of E-cadherin. This was also correlated with a change in cell morphology, from a squamous cell-like shape, in cells not exposed to IL-1β, to a spindle-like shape in OSCC cells exposed to IL-1β. The migration capacity of OSCC treated with IL-1β increased significantly after 48 hours, compared to untreated OSCC. These findings strongly suggest a role of IL-1β in EMT in OSCC [98], which has also been reported by

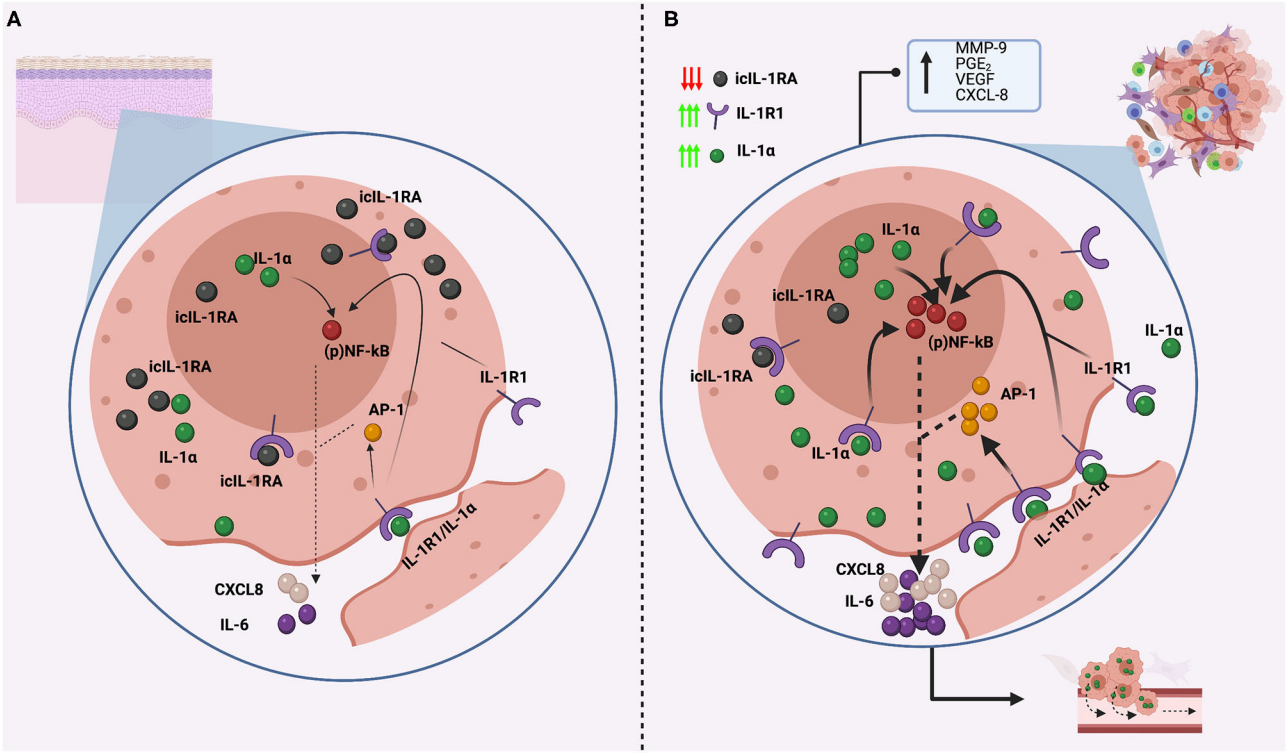

FIGURE 2 | Comparison of IL-1α expression and regulation in normal head and neck keratinocytes **(A)** and in cancerous head and neck keratinocytes **(B)**. **(A)** Normal keratinocytes express low levels of IL-1α, which is not normally secreted, and is regulated by icIL1RA, which is expressed in abundancy to efficiently counteract IL-1α actions. This balance secure low NF-kB activity with low production of other inflammatory molecules, such as IL-6 and CXCL-8. **(B)** In cancerous head and neck keratinocytes, icIL-1RA expression is downregulated, whereas IL-1R1 and IL-1α are constitutively upregulated, generating an imbalance in IL-1 regulation. IL-1α can localize intracellularly and interact directly to the nucleus, can be attached to the cellular membrane (membrane IL-1α) or can be released to the extracellular space. Membrane and secreted IL-1α can both bind to IL-1R1 receptors and activate NF- κB and AP-1 transduction pathways, resulting in the release of IL-6 and CXCL8, which are considered oncogenic cytokines as are associated with tumor growth and metastasis. Also, intranuclear IL-1α can interact directly with the nucleus inducing NF-κB activation in a non-IL-1R1 dependent manner. This also results in the release of IL-6 and CXCL8, which are both overexpressed in HNSCC. This image was created with Biorender.

others [126, 129, 130]. This is also supported by the fact that IL-1β silencing reduces OSCC tumor size *in vivo* [115] and that elevated IL-1β expression has been related with lymph node metastasis of OSCC [131].

IL-1RA

Several gene expression profiles of HNSCC have shown that *IL-1RN* is downregulated in HNSCC when compared to matched normal oral mucosa [132–139]. In addition, *IL-1RN* was reported to be a reliable marker when predicting the presence or absence of HNSCC in tissue samples in a cohort of 46 patients with HNSCC, with sensitivity and specificity of 93.5 and 95.7%, respectively [138]. Despite this, very little is known about the role of IL-1RA in HNSCC.

Von Biberstein et al. [99] reported an imbalance in the IL-1:IL-1RA ratio in HNSCC when compared to healthy patients, that was attributed mainly to an increase in the levels of IL-1α and IL-β, but also to a decrease in the levels of IL-1RA. These authors also reported higher expression of IL-1RA in the more differentiated epithelial cells within the tumors. IL-1RA protein expression decreases progressively during oral carcinogenesis and in HNSCC [65], which is in agreement with a previous report [80] and IL-1R2, the other IL-1 inhibitor, is not able to compensate for IL-1RA lack of expression [65]. Also, IL-1RA levels decrease significantly in immortal normal and dysplastic oral keratinocytes when compared to their mortal counterparts. This suggest that IL-1RA downregulation during the carcinogenic process might be an important step for the acquisition of a malignant phenotype, primarily because the binding of IL-1 to IL-1R1 is not inhibited, allowing dysregulated activation of the IL-1/IL-1R1 axis that can predispose to the carcinogesis process in different ways (**Figures 1**, **2**). Moreover, In oral keratinocytes, icIL-1RA1 regulates IL-6 and CXCL8 secretion, most likely by interfering with NF-κB activation [65]. Both IL-6 and CXCL-8 secretion is canonically regulated by NF-κB, and icIL-1RA1 is able to regulate NF-κB activation wheter by inhibiting IL-1 binding to IL-1R1, or by directly interfering with the NF-kB signaling pathway (see below). Thus, a downregulation of IL-1RA would allow overexpression of these cytokines (**Figure 3**).

There has been much debate about the specific functions of icIL-1RA. icIL-1RA is able to bind to IL-1R1, but as a stricktly intracellular molecule, it is more likely that the

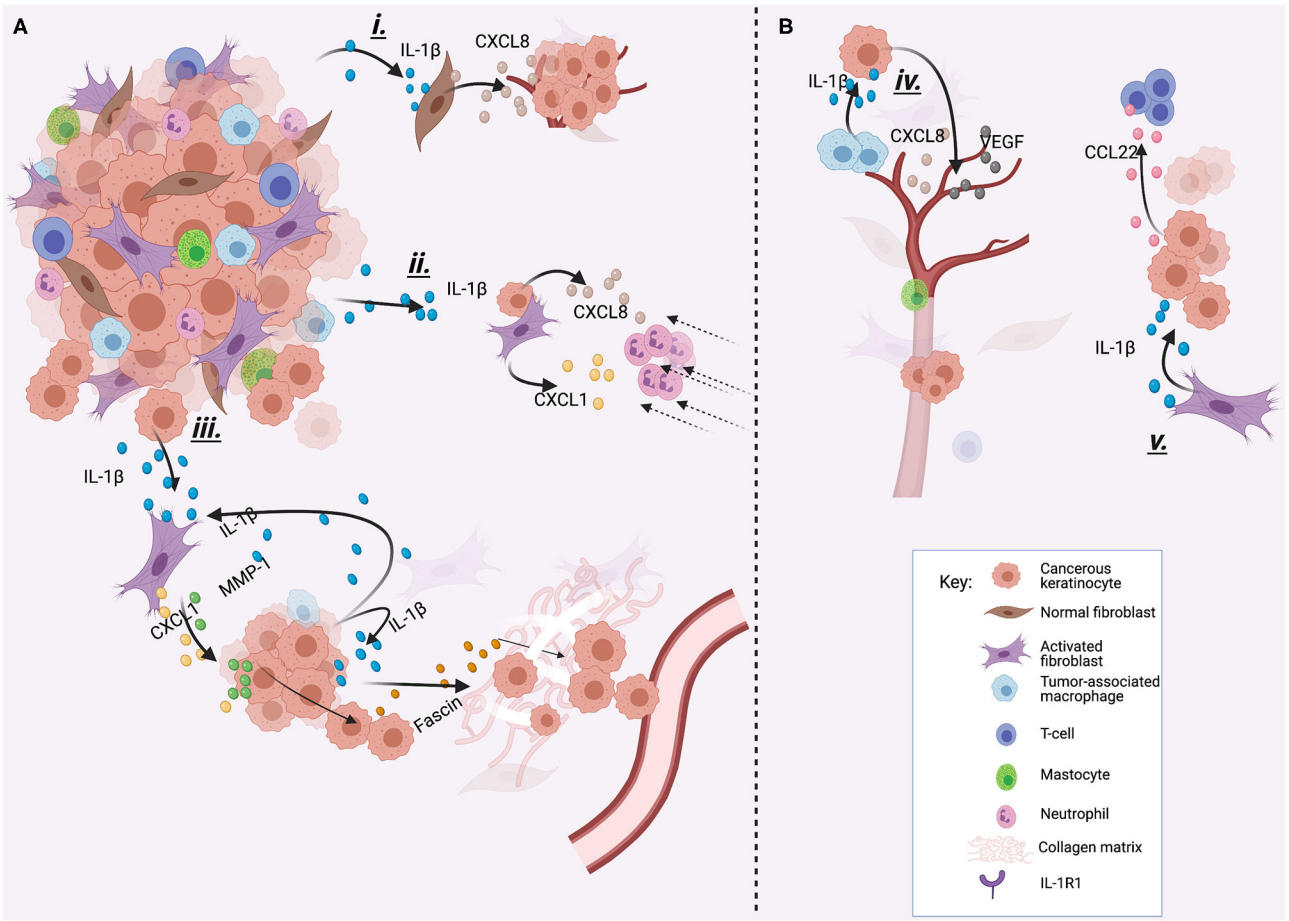

FIGURE 3 | Different examples of how IL-1βcan promote cancer progression. IL-1β can be produced by tumor cells **(A)** or by other cells of the TME **(B)**. (i) IL-1β produced by tumor cells stimulates CXCL8 release from normal oral fibroblasts, which increases tumor growth. (ii) IL-1β stimulates CXCL1 and CXCL8 production by CAFs and tumor cells which attracts neutrophils to the TME, and neutrophil accumulation has been related with poor outcome in HNSCC. (iii) IL-1β released by tumor cells induces CXCL-1 and MMP-1 secretion by CAFs. This induces invasion and migration of cancer cells. Also, secreted IL-1β can act in a paracrine way inducing fascine release, which helps in the degradation of the extracellular matrix and invasion. (iv) TAMs produce IL-1β to stimulate cancerous cells to produce VEGF and CXCL8, inducing angiogenesis. (v) IL-1β produced by CAFs induces the release of CCL22 by tumor cells, which recruits T regulatory cells, who have been associated with worse prognosis. This image was created with Biorender.

main functions of icIL-1RA are not related to IL-1R1 binding. IL-1R1 is located on the membrane surface, thus, icIL-1RA would require secretion into the extracellular space for it to block IL-1R1, although IL-1R1 intranuclear localization in oral dysplastic and cancer cells has been recently described [65]. It has been proposed that the main function of icIL-1RA is to counteract the intranuclear action of IL-1α. icIL-1RA may act intracellularly by binding to other cytoplasmic proteins in order to interfere with the downstream cascade. In fact, icIL-1RA has been reported to interact with the third component of the COP9 signalosome (CSN3) inhibiting CSN-associated kinases [91]. The signalosome (CSN) is found in the cytoplasm and nucleus of all mammalian cells, and among other functions, it has kinase activity that induce phosphorylation of proteins involved in signal transduction. When interacting with CSN3, icIL-1RA1 inhibits phosphorylation of p53, c-Jun and IκB thereby inhibiting IL-1α-mediated IL-6 and CXCL8 transcription. These inhibitory actions may also affect the p38 MAPK signal transduction pathway, as transfected keratinocytes with icIL-1RA1 showed no detectable phosphorylated p38 MAPK when stimulated with IL-1α [91]. Similar icIL-1RA1 inhibitory mechanisms have also been reported in intestinal epithelial cells [89]. Böcker et al. [140] also reported inhibition of IL-1-induced CXCL8 expression by icIL-1RA1, but did not specify the mechanism underlying this inhibition. It has been proposed that the role of CSN3 is to bring icIL-1RA1 close to a kinase in order to inhibit its action. So, icIL-1RA1 would block an upstream kinase in the p38 MAPK or NF-κB pathways, indirectly inhibiting p38 MAPK or NF-κB phosphorylation and consequently, its downstream products, such as IL-6 and CXCL8 [91].

It can be hypothesized then, that downregulation of icIL-1RA in HNSCC could lead to de-regulated expression of pro-inflammatory cytokines related to cancer development by allowing the un-controlled activation of IL-1α and

NF-κB (**Figure 3**). This speculation is based on the finding that OSCC constitutively express higher levels of IL-1α and NF-κB than healthy controls [8, 100, 118, 141] and that levels of IL-6 and CXCL8 are elevated in OSCC [142, 143]. In addition, IL-1α can interact directly with nuclear DNA an induce malignant transformation [40]. Thus, if endogenous levels of IL-1α are constitutively overexpressed, and its mains inhibitor (icIL-1RA) is downregulated, there could be more chances for icIL-1α to induce malignant transformation. In agreement with this hypothesis, a recent study comparing nuclear and cytoplasmic IL-1α expression in OSCC showed that the high expression of nuclear IL-1α in combination with EGFR, was associated with perineural invasion and high risk of recurrence and worse progression-free survival, compared to OSCC expressing none or moderate nuclear IL-1α in combination with EGFR [144]. These studies suggests that uncontrolled nuclear IL-1α activity might be of clinical importance.

There is some controversy about the role of IL-1RA in HNSCC. Shiiba et al. [80] reported an increase of IL-1RA (T3/T4) compared to early OSCC cases (T1/T2), suggesting that IL-1RA expression could increase tumor progression. Similar observations have been reported by other authors in gastric [145] and cervical carcinomas [146], reporting a more aggressive behavior from IL-1RA expressing tumors. A possible explanation to this could be that over time, endogenous IL-1 antagonism (which is likely to be beneficial in antagonizing disease progression) changes the tumor phenotype to one that is less susceptible to IL-1 inhibition. Thus, the disease progresses, and IL-1RA levels remain high [71]. Also, most of the aforementioned studies measured sIL-1RA, so the increase in sIL-1RA may be due to a decrease in icIL-1RA levels. Nevertheless, these contradictory functions of IL-1RA in different cancers only highlights the multiple functions that IL-1RA displays in relation to the specific tissue, cell type or the microenvironment in which it is present.

IL-1R1

A number of different polymorphisms of the *IL1R1* gene have been related with a reduced (rs956730) or increased (rs3917225) risk for developing HNSCC [147]. IL-1R1 is overexpressed by oral dysplastic and OSCC cells compared to normal oral keratinocytes [65], which seems to provide phenotypic advantages. IL-1R1 promotes oral cancer growth and metastasis by upregulating CXCR4 (a chemokine receptor involved in tumor progression, angiogenesis and metastasis) after IL-1β stimulation, and IL-1R1 inhibition with recombinant IL-1RA has shown to reverse these effects [66]. IL-1R1 is also constitutively expressed by normal oral fibroblasts [11]. This is of importance as HNSCC cells are able to secrete IL-1β which stimulates other cells of the TME (such as fibroblasts) to generate chemokines and other inflammatory molecules creating an inflammatory TME with cancer promoting properties [11].

TRANSLATIONAL POTENTIAL

Utility as a Saliva Biomarker

The discovery that saliva contains molecules that are able to translate the presence or activity of local or systemic diseases has opened a new diagnostic field known as salivary diagnostics [148]. The use of saliva as a diagnostic method is very practical, as saliva can be collected in an easy, non-invasive way. As there is evidence suggesting that IL-1α, IL-1β, and IL-1RA are involved in the pathogenesis of HNSCC and can be detected in the saliva of cancer patients [149–151], different studies have explored their possible use as diagnostic or prognostic biomarkers for this cancer.

IL-1β is overexpressed in the saliva of oral cancer patients compared to oral leukoplakia and control patients [152, 153] and IL-1β salivary levels have been shown to discriminate between OSCC subjects and controls [154–156], but not between oral potentially malignant disorder (OPMD) patients and healthy subjects [155]. The reported AUC of salivary IL-1β to differentiate between OSCC and control individuals varies between 0.729 and 0.7724 [154, 155] but increases to 0.901 when considering only late stage OSCC [155]. Also, the discriminatory power of salivary IL-1β increases when used with other markers, such as CXCL8, SAT1 and DUSP1 [156]. In the study by Singh et al. salivary IL-1β failed to distinguish between post-treatment OSCC individuals and healthy subjects, suggesting a normalization of IL-1β salivary levels after tumor removal [155]. In agreement, a study in which the authors analyzed the expression of 50 cytokines (including IL-1β, IL-1 α, and IL-1RA) in the saliva of 16 OSCC patients before and after surgical intervention, showed a significant decrease in salivary IL-1β levels after tumor resection. No significant changes in other cytokine levels were reported [150]. Similar results were also reported elsewhere [157]. IL-1α expression is also reported to be increased in the saliva of tongue SCC (TSCC) patients compared to controls and has also been associated with tumor growth pattern [9, 158]. Patients presenting with endophytic TSCC exhibited significantly higher IL-1α levels compared to exophytic TSCC, which correlated with a decreased survival rate in the group of endophytic tumors [158].

IL-1RA can also be detected in the saliva and its expression is reported to be significantly decreased in the saliva of OPMD and OSCC patients compared to healthy controls [151]. Opposite to salivary IL-1RA, plasma circulating IL-1RA levels have been shown to be increased and OSCC patients and correlated with tumor size, but were not related with different outcome measures [159]. By itself, salivary IL-1RA displayed a poor performance in diagnosing OSCC, but in combination with other proteins (SLC3A2 and S100A2), it was able to distinguish between individuals with OSCC from healthy controls and OPMD patients, with AUC of 0.89 and 0.87 respectively [151].

The use of salivary IL-1α, IL-1β, and IL-1RA as HNSCC biomarkers is promising, but as there are many local and systemic diseases that can give rise to elevated salivary IL-1 or decreased IL-1RA levels (e.g., periodontal disease, oral lichen planus, Sögren's syndrome) [94, 160, 161], which increases the likelihood of false positives, more clinical studies are needed before translating this into clinical practice.

Therapeutics

Many beneficial functions of IL-1 inhibition have been described in different cancers, thus, targeting IL-1 has been proposed as a possible therapy for IL-1 expressing tumors, such as melanoma, gastric and breast cancers, among others [162]. In gastric

cancer, recombinant IL-1RA inhibited tubule formation [163] and reduced proliferation and migration of endothelial cells *in vitro* in a dose-dependent manner [164]. Similar results have been reported in breast cancer. A murine experimental breast cancer model showed that treatment with anakinra (a recombinant form of IL-1RA with FDA approval for the treatment of rheumatoid arthritis and cryopyrin-associated periodic syndromes) reduced the size and number of bone metastases as well as tumor angiogenesis [165]. Taken together, these data suggest that rIL-1RA could be a beneficial alternative for the inhibition of tumor-dependent angiogenesis, probably by reducing the production of VEGF, CXCL8, endothelin-1, IL-1β and hepatocyte growth factor (HGF) [164–166].

Anakinra has been used in clinical trials for the treatment of some cancers. The first study to use anakinra as a cancer treatment was a phase II clinical trial of pre-multiple myeloma. Anakinra in combination with dexamethasone was found to increase the progression-free survival as well as overall survival in patients at high risk of progressing to multiple myeloma, by targeting the IL-1/IL-6 pathway [167]. In refractory metastatic colorectal cancer, anakinra in combination with fluorouracil (an anti-metabolite) and bevacizumab (anti-EGF monoclonal antibody) showed good efficacy with low toxicity. Currently, there are several clinical trials where recombinant IL-1RA is being tested either as a monotherapy or in combination for the treatment of different cancers, including multiple myeloma, prostate, breast, pancreatic, and colorectal cancers (https://clinicaltrials.gov/ct2/results?cond=Cancer&term=termIL1RA+OR+Anakinra&cntry=&state=&city=&dist=). Nevertheless, care must be taken when considering IL-1RA therapy for HNSCC treatment, as recombinant IL-1RA is likely reduce the innate immunity response in already ill patients, which in theory, could worsens the disease. Thus, IL-1RA replacement therapy may be only appropriate for IL-1 producing tumors [162].

Exogenous IL-1RA (i.e., anakinra) corresponds to the secreted isoform of IL-1RA, which is present in very low levels in oral keratinocytes, as oral keratinocytes constitutively express icIL-1RA1. It is not entirely clear how exogenous IL-1RA works. It is thought that exogenous IL-1RA acts in a similar manner to sIL-1RA, by blocking IL-1R1 on the cell membrane. Nevertheless, exogenous IL-1RA has been shown to be incorporated into the cytoplasm of cardiac myocytes during ischemia, mimicking the intracellular form of IL-1RA, at least in terms of intracellular distribution [168]. Whether exogenous IL-1RA can replace icIL-1RA (which expression is lost during the malignant transformation process of oral keratinocytes and OSCC) functions is not known.

Despite the data supporting an oncogenic role of the IL-1/IL-1R1 axis in HNSCC, there is a lack of studies that have explored the use of IL-1 inhibition for HNSCC treatment. A recent study [115] showed that exogenous IL-1RA can inhibit the growth of Cal27 cells (a tongue squamous adenocarcinoma cell line) *in vitro*, but more importantly, can potentially interrupt the oral carcinogenesis process *in vivo*. Submucosal injections of IL-1RA into the tongue of mice during 4NQO-induced oral carcinogenesis interrupted the malignant transformation process. This was done presumably by downregulating genes that were upregulated during the 4NQO-induced carcinogenesis process, such as the oncogene Myc and COX-2 [115]. In addition, anakinra has been shown to overcome erlotinib (an EGFR inhibitor) resistance in a HNSCC mouse xenograft cancer model, suggesting its use as a possible strategy to overcome EGFR inhibitor resistance for HNSCC treatment [169]. Although these initial data are promising, there are no clinical trials that have assessed anakinra for HNSCC treatment and more research in this area is warranted.

CONCLUSION

There is compelling evidence that the IL-1 signaling pathway is deregulated in HNSCC, with overexpression of agonistic molecules and downregulation of inhibitory factors. This results in a dysregulated signaling pathway that mediates the development of a pro-inflammatory microenvironment prone to tumor development, progression and metastasis. Oral carcinogenesis is a multistep process which includes three phases: initiation, promotion, and progression. The available evidence suggests IL-1 signaling to influence the promotion and progression phases of the malignant transformation process. The increased presence of IL-1 in HNSCC support the idea that salivary IL-1 could be of use as a screening tool for the early detection of cancer, probably as part of a biomarker panel rather than as a single marker. Early data shows promise, although more rigorous studies are needed before this can be translated into clinical practice. IL-1 inhibition is already being tested as a possible treatment alternative for different cancers, such as myeloma, breast, pancreatic and colorectal cancers, and there are *in vivo* animal studies showing promising results for HNSCC treatment. However, there is still a long way to go before this can be applied in a clinical setting.

AUTHOR CONTRIBUTIONS

All three authors contributed equally to the writing of this review. The concept and outline were agreed upon by all. SN wrote the initial draft and constructed the figures. All authors agree on the final version for submission.

REFERENCES

1. Kuper H, Adami HO, Trichopoulos D. Infections as a major preventable cause of human cancer. *J Intern Med.* (2000) 248:171–83. doi: 10.1046/j.1365-2796.2000.00742.x

2. Apte RN, Dotan S, Elkabets M, White MR, Reich E, Carmi Y, et al. The involvement of IL-1 in tumorigenesis, tumor invasiveness, metastasis and tumor-host interactions. *Cancer Metastasis Rev.* (2006) 25:387–408. doi: 10.1007/s10555-006-9004-4

3. Niklander SE. Inflammatory mediators in oral cancer: pathogenic mechanisms and diagnostic potential. *Front Oral Health*. (2021) 2. doi: 10.3389/froh.2021.642238
4. Portier M, Zhang XG, Ursule E, Lees D, Jourdan M, Bataille R, et al. Cytokine gene expression in human multiple myeloma. *Br J Haematol.* (1993) 85:514–20. doi: 10.1111/j.1365-2141.1993.tb03341.x
5. Voronov E, Dinarello CA, Apte RN. Interleukin-1alpha as an intracellular alarmin in cancer biology. *Semin Immunol.* (2018) 38:3–14. doi: 10.1016/j.smim.2018.10.006
6. Mantovani A, Barajon I, Garlanda C. IL-1 and IL-1 regulatory pathways in cancer progression and therapy. *Immunol Rev.* (2018) 281:57–61. doi: 10.1111/imr.12614
7. Elaraj DM, Weinreich DM, Varghese S, Puhlmann M, Hewitt SM, Carroll NM, et al. The role of interleukin 1 in growth and metastasis of human cancer xenografts. *Clin Cancer Res.* (2006) 12:1088–96. doi: 10.1158/1078-0432.CCR-05-1603
8. Rao SK, Pavicevic Z, Du Z, Kim JG, Fan M, Jiao Y, et al. Pro-inflammatory genes as biomarkers and therapeutic targets in oral squamous cell carcinoma. *J Biol Chem.* (2010) 285:32512–21. doi: 10.1074/jbc.M110.150490
9. Goertzen C, Mahdi H, Laliberte C, Meirson T, Eymael D, Gil-Henn H, et al. Oral inflammation promotes oral squamous cell carcinoma invasion. *Oncotarget.* (2018) 9:29047–63. doi: 10.18632/oncotarget.25540
10. Al-Sahaf S, Hendawi NB, Ollington B, Bolt R, Ottewell PD, Hunter KD, et al. Increased abundance of tumour-associated neutrophils in hpv-negative compared to HPV-positive oropharyngeal squamous cell carcinoma is mediated by IL-1R Signalling. *Front Oral Health*. (2021) 2. doi: 10.3389/froh.2021.604565
11. Al-Sahaf S, Hunter KD, Bolt R, Ottewell PD, Murdoch C. The IL-1/IL-1R axis induces greater fibroblast-derived chemokine release in human papillomavirus-negative compared to positive oropharyngeal cancer. *Int J Cancer.* (2019) 144:334–44. doi: 10.1002/ijc.31852
12. Alves AM, Diel LF, Lamers ML. Macrophages and prognosis of oral squamous cell carcinoma: A systematic review. *J Oral Pathol Med.* (2018) 47:460–7. doi: 10.1111/jop.12643
13. Trellakis S, Bruderek K, Dumitru CA, Gholaman H, Gu X, Bankfalvi A, et al. Polymorphonuclear granulocytes in human head and neck cancer: enhanced inflammatory activity, modulation by cancer cells and expansion in advanced disease. *Int J Cancer.* (2011) 129:2183–93. doi: 10.1002/ijc.25892
14. Ward MJ, Thirdborough SM, Mellows T, Riley C, Harris S, Suchak K, et al. Tumour-infiltrating lymphocytes predict for outcome in HPV-positive oropharyngeal cancer. *Br J Cancer.* (2014) 110:489–500. doi: 10.1038/bjc.2013.639
15. Garlanda C, Dinarello CA, Mantovani A. The interleukin-1 family: back to the future. *Immunity.* (2013) 39:1003–18. doi: 10.1016/j.immuni.2013.11.010
16. Dinarello CA. Introduction to the interleukin-1 family of cytokines and receptors: Drivers of innate inflammation and acquired immunity. *Immunol Rev.* (2018) 281:5–7. doi: 10.1111/imr.12624
17. Mantovani A, Dinarello CA, Molgora M, Garlanda C. Interleukin-1 and related cytokines in the regulation of inflammation and immunity. *Immunity.* (2019) 50:778–95. doi: 10.1016/j.immuni.2019.03.012
18. Palomo J, Dietrich D, Martin P, Palmer G, Gabay C. The interleukin (IL)-1 cytokine family–Balance between agonists and antagonists in inflammatory diseases. *Cytokine.* (2015) 76:25–37. doi: 10.1016/j.cyto.2015.06.017
19. Dower SK, Urdal DL. The interleukin-1 receptor. *Immunol Today.* (1987) 8:46–51. doi: 10.1016/0167-5699(87)90238-6
20. Zheng Y, Humphry M, Maguire JJ, Bennett MR, Clarke MC. Intracellular interleukin-1 receptor 2 binding prevents cleavage and activity of interleukin-1alpha, controlling necrosis-induced sterile inflammation. *Immunity.* (2013) 38:285–95. doi: 10.1016/j.immuni.2013.01.008
21. Kobayashi Y, Yamamoto K, Saido T, Kawasaki H, Oppenheim JJ, Matsushima K. Identification of calcium-activated neutral protease as a processing enzyme of human interleukin 1 alpha. *Proc Natl Acad Sci U S A.* (1990) 87:5548–52. doi: 10.1073/pnas.87.14.5548
22. Gayle RB, Poindexter K, Cosman D, Dower SK, Gillis S, Hopp T, et al. Identification of regions in interleukin-1 alpha important for activity. *J Biological Chemistry*. (1993) 268:22105–11. doi: 10.1016/S0021-9258(20)80654-1
23. Labriola-Tompkins E, Chandran C, Varnell TA, Madison VS, Ju G. Structure-function analysis of human IL-1 alpha: identification of residues required for binding to the human type I IL-1 receptor. *Protein Eng.* (1993) 6:535–9. doi: 10.1093/protein/6.5.535
24. Kim B, Lee Y, Kim E, Kwak A, Ryoo S, Bae SH, et al. The Interleukin-1alpha Precursor is biologically active and is likely a key alarmin in the IL-1 family of cytokines. *Front Immunol.* (2013) 4:391. doi: 10.3389/fimmu.2013.00391
25. Dinarello CA. Interleukin-1, interleukin-1 receptors and interleukin-1 receptor antagonist. *Intern Rev Immunol.* (1998) 16:457–99. doi: 10.3109/08830189809043005
26. Dinarello CA, Thompson RC. Blocking IL-I: interleukin I receptor antagonistin vivo andin vitro. *Immunol Today.* (1991) 12:404–10. doi: 10.1016/0167-5699(91)90142-G
27. Hammerberg C, Arend WP, Fisher GJ, Chan LS, Berger AE, Haskill JS, et al. Interleukin-1 receptor antagonist in normal and psoriatic epidermis. *J Clin Invest.* (1992) 90:571–83. doi: 10.1172/JCI115896
28. Maier JA, Statuto M, Ragnotti G. Endogenous interleukin 1 alpha must be transported to the nucleus to exert its activity in human endothelial cells. *Mol Cell Biol.* (1994) 14:1845–51. doi: 10.1128/mcb.14.3.1845-1851.1994
29. Di Paolo NC, Shayakhmetov DM. Interleukin 1alpha and the inflammatory process. *Nat Immunol.* (2016) 17:906–13. doi: 10.1038/ni.3503
30. Maier JA, Voulalas P, Roeder D, Maciag T. Extension of the life-span of human endothelial cells by an interleukin-1 alpha antisense oligomer. *Science (New York, NY).* (1990) 249:1570–4. doi: 10.1126/science.2218499
31. Mariotti M, Castiglioni S, Bernardini D, Maier JA. Interleukin 1 alpha is a marker of endothelial cellular senescent. *Immunity & ageing: I & A.* (2006) 3:4. doi: 10.1186/1742-4933-3-4
32. Acosta JC, Banito A, Wuestefeld T, Georgilis A, Janich P, Morton JP, et al. A complex secretory program orchestrated by the inflammasome controls paracrine senescence. *Nat Cell Biol.* (2013) 15:978–90. doi: 10.1038/ncb2784
33. Corradi A, Franzi AT, Rubartelli A. Synthesis and secretion of interleukin-1 alpha and interleukin-1 receptor antagonist during differentiation of cultured keratinocytes. *Exp Cell Res.* (1995) 217:355–62. doi: 10.1006/excr.1995.1097
34. Cohen I, Rider P, Vornov E, Tomas M, Tudor C, Wegner M, et al. IL-1alpha is a DNA damage sensor linking genotoxic stress signaling to sterile inflammation and innate immunity. *Sci Rep.* (2015) 5:14756. doi: 10.1038/srep14756
35. Kawaguchi Y, Hara M, Wright TM. Endogenous IL-1alpha from systemic sclerosis fibroblasts induces IL-6 and PDGF-A. *J Clin Invest.* (1999) 103:1253–60. doi: 10.1172/JCI4304
36. Wolf JS, Chen Z, Dong G, Sunwoo JB, Bancroft CC, Capo DE, et al. IL (Interleukin)-1 promotes nuclear factor- B and AP-1-induced IL-8 expression, cell survival, and proliferation in head and neck squamous cell carcinomas. *Clinical Cancer Research.* (2001) 7:1812–20.
37. Orjaloa AV, Bhaumika D, Genglera BK, Scotta GK, Campisi J. Cell surface-bound IL-1 is an upstream regulator of the senescence-associated IL-6/IL-8 cytokine network. *PNAS.* (2009) 106:17031–6. doi: 10.1073/pnas.0905299106
38. Werman A, Werman-Venkert R, White R, Lee JK, Werman B, Krelin Y, et al. The precursor form of IL-1alpha is an intracrine proinflammatory activator of transcription. *Proc Natl Acad Sci U S A.* (2004) 101:2434–9. doi: 10.1073/pnas.0308705101
39. Wessendorf JH, Garfinkel S, Zhan X, Brown S, Maciag T. Identification of a nuclear localization sequence within the structure of the human interleukin-1 alpha precursor. *J Biol Chem.* (1993) 268:22100–4. doi: 10.1016/S0021-9258(20)80653-X
40. Stevenson FT, Turck J, Locksley RM, Lovett DH. The N-terminal propiece of interleukin 1 is a transforming nuclear oncoprotein. *Cell Biology.* (1997) 94:508–13. doi: 10.1073/pnas.94.2.508
41. Buryskova M, Pospisek M, Grothey A, Simmet T, Burysek L. Intracellular interleukin-1alpha functionally interacts with histone acetyltransferase complexes. *J Biol Chem.* (2004) 279:4017–26. doi: 10.1074/jbc.M306342200
42. McMahon GA, Garfinkel S, Prudovsky I, Hu X, Maciag T. Intracellular precursor interleukin (IL)-1α, but not mature IL-1α, is able to regulate human endothelial cell migration *in vitro*. *J Biol Chem.* (1997) 272:28202–5. doi: 10.1074/jbc.272.45.28202

43. Palmer G, Trolliet S, Talabot-Ayer D, Mezin F, Magne D, Gabay C. Pre-interleukin-1alpha expression reduces cell growth and increases interleukin-6 production in SaOS-2 osteosarcoma cells: Differential inhibitory effect of interleukin-1 receptor antagonist (icIL-1Ra1). *Cytokine.* (2005) 31:153–60. doi: 10.1016/j.cyto.2005.03.007
44. Dinarello CA. Biologic basis for interleukin-1 in disease. *Blood.* (1996) 87:2095–147. doi: 10.1182/blood.V87.6.2095.bloodjournal8762095
45. Rider P, Carmi Y, Guttman O, Braiman A, Cohen I, Voronov E, et al. IL-1alpha and IL-1beta recruit different myeloid cells and promote different stages of sterile inflammation. *J Immunol.* (2011) 187:4835–43. doi: 10.4049/jimmunol.1102048
46. Kostura MJ, Tocci MJ, Limjuco G, Chin J, Cameron P, Hillman AG, et al. Identification of a monocyte specific pre-interleukin 1 beta convertase activity. *Proc Natl Acad Sci U S A.* (1989) 86:5227–31. doi: 10.1073/pnas.86.14.5227
47. Joosten LA, Netea MG, Dinarello CA. Interleukin-1beta in innate inflammation, autophagy and immunity. *Semin Immunol.* (2013) 25:416–24. doi: 10.1016/j.smim.2013.10.018
48. Dinarello CA, Ikejima T, Warner SJ, Orencole SF, Lonnemann G, Cannon JG, et al. Interleukin 1 induces interleukin 1. I Induction of circulating interleukin 1 in rabbits in vivo and in human mononuclear cells in vitro. *J Immunol.* (1987) 139:1902–10.
49. Dinarello CA. Immunological and inflammatory functions of the interleukin-1 family. *Annu Rev Immunol.* (2009) 27:519–50. doi: 10.1146/annurev.immunol.021908.132612
50. Jobling SA, Auron PE, Gurka G, Webb AC, McDonald B, Rosenwasser LJ, et al. Biological activity and receptor binding of human prointerleukin-1 beta and subpeptides. *J Biol Chem.* (1988) 263:16372–8. doi: 10.1016/S0021-9258(18)37603-8
51. Dinarello CA. Interleukin-1 and interleukin-1 antagonism. *Blood.* (1991) 77:1627–52. doi: 10.1182/blood.V77.8.1627.bloodjournal7781627
52. Luheshi NM, Rothwell NJ, Brough D. Dual functionality of interleukin-1 family cytokines: implications for anti-interleukin-1 therapy. *Br J Pharmacol.* (2009) 157:1318–29. doi: 10.1111/j.1476-5381.2009.00331.x
53. Voronov E, Shouval DS, Krelin Y, Cagnano E, Benharroch D, Iwakura Y, et al. IL-1 is required for tumor invasiveness and angiogenesis. *Proc Natl Acad Sci U S A.* (2003) 100:2645–50. doi: 10.1073/pnas.0437939100
54. Niklander S, Bordagaray MJ, Fernández A, Hernández M. Vascular endothelial growth factor: a translational view in oral non-communicable diseases. *Biomolecules.* (2021) 11:85. doi: 10.3390/biom11010085
55. Rigante D, Frediani B, Cantarini L. A Comprehensive overview of the hereditary periodic fever syndromes. *Clinical Rev Allergy Immunol.* (2016) 54:446–53. doi: 10.1007/s12016-016-8537-8
56. Cohen J. The immunopathogenesis of sepsis. *Nature.* (2002) 420:885–91. doi: 10.1038/nature01326
57. Giacomelli R, Ruscitti P, Alvaro S, Ciccia F, Liakouli V, Di Benedetto P, et al. IL-1beta at the crossroad between rheumatoid arthritis and type 2 diabetes: may we kill two birds with one stone? *Expert Rev Clin Immunol.* (2016) 1–7. doi: 10.1586/1744666X.2016.1168293
58. Kim RY, Pinkerton JW, Gibson PG, Cooper MA, Horvat JC, Hansbro PM. Inflammasomes in COPD and neutrophilic asthma. *Thorax.* (2015) 70:1199–201. doi: 10.1136/thoraxjnl-2014-206736
59. Mosley B, Urdal DL, Prickett KS, Larsen A, Cosman D, Conlon PJ, et al. The interleukin-1 receptor binds the human interleukin-1 alpha precursor but not the interleukin-1 beta precursor. *J Biol Chem.* (1987) 262:2941–4. doi: 10.1016/S0021-9258(18)61450-4
60. Greenfeder SA, Nunes P, Kwee L, Labow M, Chizzonite RA, Ju G. Molecular cloning and characterization of a second subunit of the interleukin 1 receptor complex. *J Biol Chem.* (1995) 270:13757–65. doi: 10.1074/jbc.270.23.13757
61. Dinarello CA. Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunol Rev.* (2018) 281:8–27. doi: 10.1111/imr.12621
62. Cullinan EB, Kwee L, Nunes P, Shuster DJ, Ju G, McIntyre KW, et al. IL-1 receptor accessory protein is an essential component of the IL-1 receptor. *J Immunol.* (1998) 161:5614–20.
63. Gallis B, Prickett KS, Jackson J, Slack J, Schooley K, Sims JE, et al. IL-1 induces rapid phosphorylation of the IL-1 receptor. *J Immunol.* (1989) 143:3235–40.
64. Weber A, Wasiliew P, Kracht M. Interleukin-1 (IL-1) pathway. *Sci Signal.* (2010) 3:cm1. doi: 10.1126/scisignal.3105cm1
65. Niklander SE, Crane HL, Darda L, Lambert DW, Hunter KD. The role of icIL-1RA in keratinocyte senescence and development of the senescence-associated secretory phenotype. *J Cell Sci.* (2021) 134. doi: 10.1242/jcs.252080
66. Sun Y, Zhu D, Wang G, Wang D, Zhou H, Liu X, et al. Pro-inflammatory cytokine IL-1β up-regulates CXC chemokine receptor 4 *via* Notch and ERK signaling pathways in tongue squamous cell carcinoma. *PLoS ONE.* (2015) 10:e0132677. doi: 10.1371/journal.pone.0132677
67. Colotta F, Dower SK, Sims JE, Mantovani A. The type II 'decoy' receptor: a novel regulatory pathway for interleukin 1. *Immunol Today.* (1994) 15:562–6. doi: 10.1016/0167-5699(94)90217-8
68. Colotta F, Re F, Muzio M, Bertini R, Polentarutti N, Sironi M, et al. Interleukin-1 type II receptor: a decoy target for IL-1 that is regulated by IL-4. *Science (New York, NY).* (1993) 261:472–5. doi: 10.1126/science.8332913
69. Kuhn PH, Marjaux E, Imhof A, De Strooper B, Haass C, Lichtenthaler SF. Regulated intramembrane proteolysis of the interleukin-1 receptor II by alpha-, beta-, and gamma-secretase. *J Biol Chem.* (2007) 282:11982–95. doi: 10.1074/jbc.M700356200
70. Arend WP, Joslin FG, Thompson RC, Hannum CH. An IL-1 inhibitor from human monocytes. Production and characterization of biologic properties. *J Immunol.* (1989) 143:1851–8.
71. Kurzrock R, Hickish T, Wyrwicz L, Saunders M, Wu Q, Stecher M, et al. Interleukin-1 receptor antagonist levels predict favorable outcome after bermekimab, a first-in-class true human interleukin-1alpha antibody, in a phase III randomized study of advanced colorectal cancer. *Oncoimmunology.* (2019) 8:1551651. doi: 10.1080/2162402X.2018.1551651
72. Hannum CH, Wilcox CJ, Arend WP, Joslin FG, Dripps DJ, Heimdal PL, et al. Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. *Nature.* (1990) 343:336–43. doi: 10.1038/343336a0
73. Arend WP, Welgus HG, Thompson RC, Eisenberg SP. Biological properties of recombinant human monocyte-derived interleukin 1 receptor antagonist. *J Clin Invest.* (1990) 85:1694–7. doi: 10.1172/JCI114622
74. Arend WP, Joslin FG, Massoni RJ. Effects of immune complexes on production by human monocytes of interleukin 1 or an interleukin 1 inhibitor. *J Immunol.* (1985) 134:3868–75.
75. Haskill S, Martin G, Van Le L, Morris J, Peace A, Bigler CF, et al. cDNA cloning of an intracellular form of the human interleukin 1 receptor antagonist associated with epithelium. *Cell Biology.* (1991) 88:3681–5. doi: 10.1073/pnas.88.9.3681
76. Muzio M, Polentarutti N, Sironi M, Poli G, De Gioia L, Introna M, et al. Cloning and characterization of a new isoform of the interleukin 1 receptor antagonist. *J Exp Med.* (1995) 182:623–8. doi: 10.1084/jem.182.2.623
77. Malyak M, Guthridge JM, Hance KR, Dower SK, Freed JH, Arend WP. Characterization of a low molecular weight isoform of IL-1 receptor antagonist. *J Immunol.* (1998) 161:1997–2003.
78. Malyak M, Smith MF, Abel AA, Hance KR, Arend WP. The differential production of three forms of IL-1 receptor antagonist by human neutrophils and monocytes. *J Immunol.* (1998) 161:2004–10.
79. La E, Rundhaug JE, Fischer SM. Role of intracellular interleukin-1 receptor antagonist in skin carcinogenesis. *Mol Carcinog.* (2001) 30:218–23. doi: 10.1002/mc.1031
80. Shiiba M, Saito K, Yamagami H, Nakashima D, Higo M, Kasamatsu A, et al. Interleukin-1 receptor antagonist (IL1RN) is associated with suppression of early carcinogenic events in human oral malignancies. *Int J Oncol.* (2015) 46:1978–84. doi: 10.3892/ijo.2015.2917
81. Lee JK, Kim SH, Lewis EC, Azam T, Reznikov LL, Dinarello CA. Differences in signaling pathways by IL-1beta and IL-18. *Proc Natl Acad Sci U S A.* (2004) 101:8815–20. doi: 10.1073/pnas.0402800101
82. Naik SM, Cannon G, Burbach GJ, Singh SR, Swerlick RA, Wilcox JN, et al. Human keratinocytes constitutively express interleukin-18 and secrete biologically active interleukin-18 after treatment with pro-inflammatory mediators and dinitrochlorobenzene. *J Invest Dermatol.* (1999) 113:766–72. doi: 10.1046/j.1523-1747.1999.00750.x
83. Carriere V, Roussel L, Ortega N, Lacorre DA, Americh L, Aguilar L, et al. IL-33, the IL-1-like cytokine ligand for ST2 receptor, is a chromatin-associated nuclear factor in vivo. *Proc Natl Acad Sci U S A.* (2007) 104:282–7. doi: 10.1073/pnas.0606854104

84. Onoufriadis A, Simpson MA, Pink AE, Di Meglio P, Smith CH, Pullabhatla V, et al. Mutations in IL36RN/IL1F5 are associated with the severe episodic inflammatory skin disease known as generalized pustular psoriasis. *Am J Hum Genet.* (2011) 89:432–7. doi: 10.1016/j.ajhg.2011.07.022
85. Mora J, Schlemmer A, Wittig I, Richter F, Putyrski M, Frank AC, et al. Interleukin-38 is released from apoptotic cells to limit inflammatory macrophage responses. *J Mol Cell Biol.* (2016) 8:426–38. doi: 10.1093/jmcb/mjw006
86. Boutet MA, Najm A, Bart G, Brion R, Touchais S, Trichet V, et al. IL-38 overexpression induces anti-inflammatory effects in mice arthritis models and in human macrophages in vitro. *Ann Rheum Dis.* (2017) 76:1304–12. doi: 10.1136/annrheumdis-2016-210630
87. Perrier S, Kherratia B, Deschaumes C, Ughetto S, Kemeny L. IL-1ra and IL-1 production in human oral mucosal epithelial cells in culture: differential modulation by TGF-~1 and IL-4. *Clin Exp Immunol.* (2002) 127:53–9. doi: 10.1046/j.1365-2249.2002.01685.x
88. Mizutani H, Black R, Kupper TS. Human keratinocytes produce but do not process pro-interleukin-1 (IL-1) beta. Different strategies of IL-1 production and processing in monocytes and keratinocytes. *J Clin Invest.* (1991) 87:1066–71. doi: 10.1172/JCI115067
89. Garat C, Arend WP. Intracellular IL-1Ra type 1 inhibits IL-1-induced IL-6 and IL-8 production in Caco-2 intestinal epithelial cells through inhibition of p38 mitogen-activated protein kinase and NF-kappaB pathways. *Cytokine.* (2003) 23:31–40. doi: 10.1016/S1043-4666(03)00182-0
90. Phillips WG, Feldmann M, Breathnach SM, Brennan FM. Modulation of the IL-1 cytokine network in keratinocytes by intracellular IL-la and IL-1 receptor antagonist. *Clin Exp Immunol.* (1995) 101:177–82. doi: 10.1111/j.1365-2249.1995.tb02295.x
91. Banda NK, Guthridge C, Sheppard D, Cairns KS, Muggli M, Bech-Otschir D, et al. Intracellular IL-1 receptor antagonist type 1 inhibits IL-1-induced cytokine production in keratinocytes through binding to the third component of the COP9 signalosome. *J Immunol.* (2005) 174:3608–16. doi: 10.4049/jimmunol.174.6.3608
92. Groves RW, Giri J, Sims J, Dower SK, Kupper TS. Inducible expression of type 2 IL-1 receptors by cultured human keratinocytes. Implications for IL-1-mediated processes in epidermis. *J Immunol.* (1995) 154:4065–72.
93. Kondo M, Yamato M, Takagi R, Namiki H, Okano T. The regulation of epithelial cell proliferation and growth by IL-1 receptor antagonist. *Biomaterials.* (2013) 34:121–9. doi: 10.1016/j.biomaterials.2012.09.036
94. Dubost JJ, Perrier S, Afane M, Viallard JL, Roux-Lombard P, Baudet-Pommel M, et al. IL-1 receptor antagonist in saliva; characterization in normal saliva and reduced concentration in Sjogren's syndrome (SS). *Clin Exp Immunol.* (1996) 106:237–42. doi: 10.1046/j.1365-2249.1996.d01-824.x
95. Sharpless NE, Sherr CJ. Forging a signature of in vivo senescence. *Nat Rev Cancer.* (2015) 15:397–408. doi: 10.1038/nrc3960
96. Davalos AR, Coppe JP, Campisi J, Desprez PY. Senescent cells as a source of inflammatory factors for tumor progression. *Cancer Metastasis Rev.* (2010) 29:273–83. doi: 10.1007/s10555-010-9220-9
97. Woods RV, Adler-Storthz K, Dayman GL, Francis GM, Grimm EA. Interleukin-1 regulates interleukin-6 secretion in human oral squamous cell carcinoma in vitro: possible influence of p53 but not human papillomavirus E6/E. *Cancer Res.* (1998) 58:3142–9.
98. Lee CH, Chang JS, Syu SH, Wong TS, Chan JY, Tang YC, et al. IL-1beta promotes malignant transformation and tumor aggressiveness in oral cancer. *J Cell Physiol.* (2015) 230:875–84. doi: 10.1002/jcp.24816
99. von Biberstein SE, Spiro JD, Lindquist R, Kreutzer DL. Interleukin-1 receptor antagonist in head and neck squamous cell carcinoma. *Arch Otolaryngol Head Neck Surg.* (1996) 122:751–9. doi: 10.1001/archotol.1996.01890190047012
100. Leon X, Bothe C, Garcia J, Parreno M, Alcolea S, Quer M, et al. Expression of IL-1alpha correlates with distant metastasis in patients with head and neck squamous cell carcinoma. *Oncotarget.* (2015) 6:37398–409. doi: 10.18632/oncotarget.6054
101. Coppe JP, Patil CK, Rodier F, Sun Y, Munoz DP, Goldstein J, et al. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biol.* (2008) 6:2853–68. doi: 10.1371/journal.pbio.0060301
102. Sparmann A, Bar-Sagi D. Ras-induced interleukin-8 expression plays a critical role in tumor growth and angiogenesis. *Cancer Cell.* (2004) 6:447–58. doi: 10.1016/j.ccr.2004.09.028
103. Ancrile B, Lim KH, Counter CM. Oncogenic Ras-induced secretion of IL6 is required for tumorigenesis. *Genes Dev.* (2007) 21:1714–9. doi: 10.1101/gad.1549407
104. Loaiza N, Demaria M. Cellular senescence and tumor promotion: Is aging the key? *Biochim Biophys Acta.* (2016) 1865:155–67. doi: 10.1016/j.bbcan.2016.01.007
105. Yang X, Cheng H, Chen J, Wang R, Saleh A, Si H, et al. Head and neck cancers promote an inflammatory transcriptome through coactivation of classic and alternative NF-κB pathways. *Cancer Immunol Res.* (2019) 7:1760–74. doi: 10.1158/2326-6066.CIR-18-0832
106. Zhang J, Peng B. NF-kappaB promotes iNOS and VEGF expression in salivary gland adenoid cystic carcinoma cells and enhances endothelial cell motility in vitro. *Cell Prolif.* (2009) 42:150–61. doi: 10.1111/j.1365-2184.2009.00588.x
107. Julien S, Puig I, Caretti E, Bonaventure J, Nelles L, van Roy F, et al. Activation of NF-kappaB by Akt upregulates Snail expression and induces epithelium mesenchyme transition. *Oncogene.* (2007) 26:7445–56. doi: 10.1038/sj.onc.1210546
108. Nakayama H, Ikebe T, Beppu M, Shirasuna K. High expression levels of nuclear factor kappaB, IkappaB kinase alpha and Akt kinase in squamous cell carcinoma of the oral cavity. *Cancer.* (2001) 92:3037–44. doi: 10.1002/1097-0142(20011215)92:12<3037::AID-CNCR10171>3.0.CO;2-%23
109. Jimi E, Kokabu S, Matsubara T, Nakatomi C, Matsuo K, Watanabe S. NF-κB acts as a multifunctional modulator in bone invasion by oral squamous cell carcinoma. *Oral Science International.* (2016) 13:1–6. doi: 10.1016/S1348-8643(15)00038-5
110. Tanaka T, Nakayama H, Yoshitake Y, Irie A, Nagata M, Kawahara K, et al. Selective inhibition of nuclear factor-κB by nuclear factor-κB essential modulator-binding domain peptide suppresses the metastasis of highly metastatic oral squamous cell carcinoma. *Cancer Sci.* (2012) 103:455–63. doi: 10.1111/j.1349-7006.2011.02174.x
111. Duffey DC, Chen Z, Dong G, Ondrey FG, Wolf JS, Brown K, et al. Expression of a dominant-negative mutant inhibitor-kappaBalpha of nuclear factor-kappaB in human head and neck squamous cell carcinoma inhibits survival, proinflammatory cytokine expression, and tumor growth in vivo. *Cancer Res.* (1999) 59:3468–74.
112. Wu D, Wu P, Zhao L, Huang L, Zhang Z, Zhao S, et al. NF-κB Expression and outcomes in solid tumors: A systematic review and meta-analysis. *Medicine (Baltimore).* (2015) 94:e1687. doi: 10.1097/MD.0000000000001687
113. Alam M, Kashyap T, Pramanik KK, Singh AK, Nagini S, Mishra R. The elevated activation of NFκB and AP-1 is correlated with differential regulation of Bcl-2 and associated with oral squamous cell carcinoma progression and resistance. *Clin Oral Investig.* (2017) 21:2721–31. doi: 10.1007/s00784-017-2074-6
114. Lakhanpal M, Yadav DS, Devi TR, Singh LC, Singh KJ, Latha SP, et al. Association of interleukin-1beta−511 C/T polymorphism with tobacco-associated cancer in northeast India: a study on oral and gastric cancer. *Cancer Genet.* (2014) 207:1–11. doi: 10.1016/j.cancergen.2014.01.002
115. Wu T, Hong Y, Jia L, Wu J, Xia J, Wang J, et al. Modulation of IL-1beta reprogrammes the tumor microenvironment to interrupt oral carcinogenesis. *Sci Rep.* (2016) 6:20208. doi: 10.1038/srep20208
116. Hazuda DJ, Strickler J, Kueppers F, Simon PL, Young PR. Processing of precursor interleukin 1 beta and inflammatory disease. *J Biol Chem.* (1990) 265:6318–22. doi: 10.1016/S0021-9258(19)39328-7
117. He KF, Zhang L, Huang CF, Ma SR, Wang YF, Wang WM, et al. CD163+ tumor-associated macrophages correlated with poor prognosis and cancer stem cells in oral squamous cell carcinoma. *Biomed Res Int.* (2014) 2014:838632. doi: 10.1155/2014/838632
118. Bae JY, Kim EK, Yang DH, Zhang X, Park YJ, Lee DY, et al. Reciprocal interaction between carcinoma-associated fibroblasts and squamous carcinoma cells through interleukin-1alpha induces cancer progression. *Neoplasia.* (2014) 16:928–38. doi: 10.1016/j.neo.2014.09.003
119. Huang YH, Chang CY, Kuo YZ, Fang WY, Kao HY, Tsai ST, et al. Cancer-associated fibroblast-derived interleukin-1β activates protumor C-C motif

chemokine ligand 22 signaling in head and neck cancer. *Cancer Sci.* (2019) 110:2783–93. doi: 10.1111/cas.14135
120. Liss C, Fekete MJ, Hasina R, Lam CD, Lingen MW. Paracrine angiogenic loop between head-and-neck squamous-cell carcinomas and macrophages. *Int J Cancer.* (2001) 93:781–5. doi: 10.1002/ijc.1407
121. Hakelius M, Koskela A, Reyhani V, Ivarsson M, Grenman R, Rubin K, et al. Interleukin-1-mediated effects of normal oral keratinocytes and head and neck squamous carcinoma cells on extracellular matrix related gene expression in fibroblasts. *Oral Oncol.* (2012) 48:1236–41. doi: 10.1016/j.oraloncology.2012.06.013
122. Glogauer JE, Sun CX, Bradley G, Magalhaes MA. Neutrophils Increase Oral Squamous Cell Carcinoma Invasion through an Invadopodia-Dependent Pathway. *Cancer Immunol Res.* (2015) 3:1218–26. doi: 10.1158/2326-6066.CIR-15-0017
123. Wei LY, Lee JJ, Yeh CY, Yang CJ, Kok SH, Ko JY, et al. Reciprocal activation of cancer-associated fibroblasts and oral squamous carcinoma cells through CXCL1. *Oral Oncol.* (2019) 88:115–23. doi: 10.1016/j.oraloncology.2018.11.002
124. Alcolea S, Antón R, Camacho M, Soler M, Alfranca A, Avilés-Jurado FX, et al. Interaction between head and neck squamous cell carcinoma cells and fibroblasts in the biosynthesis of PGE2. *J Lipid Res.* (2012) 53:630–42. doi: 10.1194/jlr.M019695
125. Morita Y, Morita N, Hata K, Nakanishi M, Kimoto N, Omata T, et al. Cyclooxygenase-2 expression is associated with vascular endothelial growth factor-c and lymph node metastasis in human oral tongue cancer. *Oral Surg Oral Med Oral Pathol Oral Radiol.* (2014) 117:502–10. doi: 10.1016/j.oooo.2013.12.410
126. St John MA, Dohadwala M, Luo J, Wang G, Lee G, Shih H, et al. Proinflammatory mediators upregulate snail in head and neck squamous cell carcinoma. *Clin Cancer Res.* (2009) 15:6018–27. doi: 10.1158/1078-0432.CCR-09-0011
127. Yang B, Jia L, Guo Q, Ren H, Hu Y, Xie T. Clinicopathological and prognostic significance of cyclooxygenase-2 expression in head and neck cancer: A meta-analysis. *Oncotarget.* (2016) 7:47265–77. doi: 10.18632/oncotarget.10059
128. Lee MK, Park JH, Gi SH, Hwang YS. IL-1β Induces Fascin Expression and Increases Cancer Invasion. *Anticancer Res.* (2018) 38:6127–32. doi: 10.21873/anticanres.12964
129. Dong GW, Do NY, Lim SC. Relation between proinflammatory mediators and epithelial-mesenchymal transition in head and neck squamous cell carcinoma. *Exp Ther Med.* (2010) 1:885–91. doi: 10.3892/etm.2010.124
130. St John MA. Inflammatory mediators drive metastasis and drug resistance in head and neck squamous cell carcinoma. *Laryngoscope.* (2015) 125 Suppl 3:S1–11. doi: 10.1002/lary.24998
131. Lee CH, Wong TS, Chan JY, Lu SC, Lin P, Cheng AJ, et al. Epigenetic regulation of the X-linked tumour suppressors BEX1 and LDOC1 in oral squamous cell carcinoma. *J Pathol.* (2013) 230:298–309. doi: 10.1002/path.4173
132. Alevizos I, Mahadevappa M, Zhang X, Ohyama H, Kohno Y, Posner M, et al. Oral cancer in vivo gene expression profiling assisted by laser capture microdissection and microarray analysis. *Oncogene.* (2001) 20:6196–204. doi: 10.1038/sj.onc.1204685
133. Choi P, Chen C. Genetic expression profiles and biologic pathway alterations in head and neck squamous cell carcinoma. *Cancer.* (2005) 104:1113–28. doi: 10.1002/cncr.21293
134. Cromer A, Carles A, Millon R, Ganguli G, Chalmel F, Lemaire F, et al. Identification of genes associated with tumorigenesis and metastatic potential of hypopharyngeal cancer by microarray analysis. *Oncogene.* (2004) 23:2484–98. doi: 10.1038/sj.onc.1207345
135. Leethanakul C, Patel V, Gillespie J, Shillitoe E, Kellman RM, Ensley JF, et al. Gene expression profiles in squamous cell carcinomas of the oral cavity: use of laser capture microdissection for the construction and analysis of stage-specific cDNA libraries. *Oral Oncol.* (2000) 36:474–83. doi: 10.1016/S1368-8375(00)00039-7
136. Schmalbach CE, Chepeha DB, Giordano TJ, Rubin MA, Teknos TN, Bradford CR, et al. Molecular profiling and the identification of genes associated with metastatic oral cavity/pharynx squamous cell carcinoma. *Arch Otolaryngol Head Neck Surg.* (2004) 130:295–302. doi: 10.1001/archotol.130.3.295
137. Whipple ME, Mendez E, Farwell DG, Agoff SN, Chen C. A genomic predictor of oral squamous cell carcinoma. *Laryngoscope.* (2004) 114:1346–54. doi: 10.1097/00005537-200408000-00006
138. Lallemant B, Evrard A, Combescure C, Chapuis H, Chambon G, Raynal C, et al. Clinical relevance of nine transcriptional molecular markers for the diagnosis of head and neck squamous cell carcinoma in tissue and saliva rinse. *BMC Cancer.* (2009) 9:370. doi: 10.1186/1471-2407-9-370
139. Koike H, Uzawa K, Nakashima D, Shimada K, Kato Y, Higo M, et al. Identification of differentially expressed proteins in oral squamous cell carcinoma using a global proteomic approach. *Int J Oncol.* (2005) 27:59–67. doi: 10.3892/ijo.27.1.59
140. Böcker U, Damiao A, Holt L, Soo Han D, Jobin C, Panja A, et al. Differential expression of interleukin 1 receptor antagonist isoforms in human intestinal epithelial cells. *Gastroenterology.* (1998) 115:1426–38. doi: 10.1016/S0016-5085(98)70021-6
141. Bates AM, Gomez Hernandez MP, Lanzel EA, Qian F, Brogden KA. Matrix metalloproteinase (MMP) and immunosuppressive biomarker profiles of seven head and neck squamous cell carcinoma (HNSCC) cell lines. *Transl Cancer Res.* (2018) 7:533–42. doi: 10.21037/tcr.2018.05.09
142. Tsai MS, Chen WC, Lu CH, Chen MF. The prognosis of head and neck squamous cell carcinoma related to immunosuppressive tumor microenvironment regulated by IL-6 signaling. *Oral Oncol.* (2019) 91:47–55. doi: 10.1016/j.oraloncology.2019.02.027
143. Gaba FI, Sheth CC, Veses V. Salivary biomarkers and their efficacies as diagnostic tools for Oral Squamous Cell Carcinoma: Systematic review and meta-analysis. *J Oral Pathol Med.* (2018) 50:299–307. doi: 10.1111/jop.12791
144. Rajan A, Gibson-Corley KN, Choi AB, Ofori-Amanfo GK, Ten Eyck P, Espinosa-Cotton M, et al. Impact of nuclear interleukin-1 alpha and EGFR expression on recurrence and survival outcomes in oral squamous cell carcinomas. *J Oncol.* (2019) 2019:5859680. doi: 10.1155/2019/5859680
145. Iizuka N, Hazama S, Hirose K, Abe T, Tokuda N, Fukumoto T, et al. Interleukin-1 receptor antagonist mRNA expression and the progression of gastric carcinoma. *Cancer Lett.* (1999) 179–84. doi: 10.1016/S0304-3835(99)00162-7
146. Fujiwaki R, Iida K, Nakayama K, Kanasaki H, Hata K, Katabuchi H, et al. Clinical significance of interleukin-1 receptor antagonist in patients with cervical carcinoma. *Gynecol Oncol.* (2003) 89:77–83. doi: 10.1016/S0090-8258(02)00154-3
147. Tian Y, Sun Y, Wu J, Xiong Z, Niu F, Li H, et al. Impact of IL1R1 polymorphisms on the risk of head and neck cancer in Chinese Han population. *Gene.* (2020) 757:144927. doi: 10.1016/j.gene.2020.144927
148. Bonne NJ, Wong DT. Salivary biomarker development using genomic, proteomic and metabolomic approaches. *Genome Med.* (2012) 4:82. doi: 10.1186/gm383
149. SahebJamee M, Eslami M, AtarbashiMoghadam F, Sarafnejad A. Salivary concentration of TNFα, IL1α, IL6, and IL8 in oral squamous cell carcinoma. *Med Oral Patol Oral Cir Bucal.* (2008) 13:E292–5.
150. Kamatani T, Shiogama S, Yoshihama Y, Kondo S, Shirota T, Shintani S. Interleukin-1 beta in unstimulated whole saliva is a potential biomarker for oral squamous cell carcinoma. *Cytokine.* (2013) 64:497–502. doi: 10.1016/j.cyto.2013.08.011
151. Shan J, Sun Z, Yang J, Xu J, Shi W, Wu Y, et al. Discovery and preclinical validation of proteomic biomarkers in saliva for early detection of oral squamous cell carcinomas. *Oral Dis.* (2019) 25:97–107. doi: 10.1111/odi.12971
152. Brailo V, Vucicevic-Boras V, Lukac J, Biocina-Lukenda D, Zilic-Alajbeg I, Milenovic A, et al. Salivary and serum interleukin 1 beta, interleukin 6 and tumor necrosis factor alpha in patients with leukoplakia and oral cancer. *Med Oral Patol Oral Cir Bucal.* (2012) 17:e10–5. doi: 10.4317/medoral.17323
153. Arellano-Garcia ME, Hu S, Wang J, Henson B, Zhou H, Chia D, et al. Multiplexed immunobead-based assay for detection of oral cancer protein biomarkers in saliva. *Oral Dis.* (2008) 14:705–12. doi: 10.1111/j.1601-0825.2008.01488.x
154. Lee LT, Wong YK, Hsiao HY, Wang YW, Chan MY, Chang KW. Evaluation of saliva and plasma cytokine biomarkers in patients with

oral squamous cell carcinoma. *Int J Oral Maxillofac Surg.* (2018) 47:699–707. doi: 10.1016/j.ijom.2017.09.016
155. Singh P, Verma JK, Singh JK. Validation of salivary markers, IL-1β, IL-8 and Lgals3bp for detection of oral squamous cell carcinoma in an Indian population. *Sci Rep.* (2020) 10:7365. doi: 10.1038/s41598-020-64494-3
156. Brinkmann O, Kastratovic DA, Dimitrijevic MV, Konstantinovic VS, Jelovac DB, Antic J, et al. Oral squamous cell carcinoma detection by salivary biomarkers in a Serbian population. *Oral Oncol.* (2011) 47:51–5. doi: 10.1016/j.oraloncology.2010.10.009
157. Val M, Sidoti Pinto GA, Manini L, Gandolfo S, Pentenero M. Variations of salivary concentration of cytokines and chemokines in presence of oral squamous cell carcinoma. A case-crossover longitudinal prospective study. *Cytokine.* (2019) 120:62–5. doi: 10.1016/j.cyto.2019.04.009
158. Korostoff A, Reder L, Masood R, Sinha UK. The role of salivary cytokine biomarkers in tongue cancer invasion and mortality. *Oral Oncol.* (2011) 47:282–7. doi: 10.1016/j.oraloncology.2011.02.006
159. Boldrup L, Coates P, Gu X, Wang L, Fåhraeus R, Wilms T., et al. Low potential of circulating interleukin 1 receptor antagonist as a prediction marker for squamous cell carcinoma of the head and neck. *J Oral Pathol Med.* (2021) doi: 10.1111/jop.13187
160. Tobón-Arroyave SI, Jaramillo-González PE, Isaza-Guzmán DM. Correlation between salivary IL-1beta levels and periodontal clinical status. *Arch Oral Biol.* (2008) 53:346–52. doi: 10.1016/j.archoralbio.2007.11.005
161. Rhodus NL, Cheng B, Bowles W, Myers S, Miller L, Ondrey F. Proinflammatory cytokine levels in saliva before and after treatment of (erosive) oral lichen planus with dexamethasone. *Oral Dis.* (2006) 12:112–6. doi: 10.1111/j.1601-0825.2005.01165.x
162. Dinarello CA. Why not treat human cancer with interleukin-1 blockade? *Cancer Metastasis Rev.* (2010) 29:317–29. doi: 10.1007/s10555-010-9229-0
163. La E, Fischer SM. Transcriptional regulation of intracellular IL-1 receptor antagonist gene by IL-1 in primary mouse keratinocytes. *J Immunol.* (2001) 166:6149–55. doi: 10.4049/jimmunol.166.10.6149
164. Gong Z, Ma J, Su H, Guo T, Cai H, Chen Q, et al. Interleukin-1 receptor antagonist inhibits angiogenesis in gastric cancer. *Int J Clin Oncol.* (2018) 23:659–70. doi: 10.1007/s10147-018-1242-2
165. Holen I, Lefley DV, Francis SE, Rennicks S, Bradbury S, Coleman RE, et al. IL-1 drives breast cancer growth and bone metastasis *in vivo*, *Oncotarget*. (2016) 7:75571–84. doi: 10.18632/oncotarget.12289
166. Ma J, Sun X, Guo T, Su H, Chen Q, Gong Z, et al. Interleukin-1 receptor antagonist inhibits angiogenesis *via* blockage IL-1alpha/PI3K/NF-kappabeta pathway in human colon cancer cell. *Cancer Manag Res.* (2017) 9:481–93. doi: 10.2147/CMAR.S147699
167. Lust JA, Lacy MQ, Zeldenrust SR, Witzig TE, Moon-Tasson LL, Dinarello CA, et al. Reduction in C-reactive protein indicates successful targeting of the IL-1/IL-6 axis resulting in improved survival in early stage multiple myeloma. *Am J Hematol.* (2016) 91:571–4. doi: 10.1002/ajh.24352
168. Abbate A, Salloum FN, Vecile E, Das A, Hoke NN, Straino S, et al. Anakinra, a recombinant human interleukin-1 receptor antagonist, inhibits apoptosis in experimental acute myocardial infarction. *Circulation.* (2008) 117:2670–83. doi: 10.1161/CIRCULATIONAHA.107.740233
169. Stanam A, Gibson-Corley KN, Love-Homan L, Ihejirika N, Simons AL. Interleukin-1 blockade overcomes erlotinib resistance in head and neck squamous cell carcinoma. *Oncotarget.* (2016) 7:76087–100. doi: 10.18632/oncotarget.12590

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership