Review

Immune Microenvironment in Oral Potentially Malignant Disorders and Oral Cancer

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Immune Microenvironment in Oral Potentially Malignant Disorders and Oral Cancer

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Simple Summary: One of the critical challenges in understanding OPMD is the oral cavity's unique environment that combines well-developed innate and adaptive immune responses. In this context, oral keratinocytes interact with immune cells and mediators linked to the pathogenesis of cancer, including increased rates of malignant transformation of oral lesions. The literature shows overwhelming data to support that the immune microenvironment (IME) has a direct role in promoting invasion and malignant transformation of oral keratinocytes. This makes OSCC a unique cancer that arises in a typically inflamed environment and hijacks the same immune response to progress and invade. The mechanisms by which oral cancer pathogenesis and progression are influenced by inflammation are complex and involve inflammatory mediators, immune cells, and other stromal cells in the TIME.

Abstract: Multiple studies have investigated the impact of the tumor immune microenvironment (TIME) on oral squamous cell carcinoma (OSCC), with most focusing on three key cellular components: lymphocytes, macrophages, and neutrophils, as well as the molecular mechanisms underlying inflammation-mediated OSCC invasion. Although the specific roles of each cell type vary depending on their subtypes and the characteristics of OSCC, several consistent patterns have been identified. TIME plays a critical role at every stage of OSCC progression, from tumor initiation and growth to invasion and metastasis. Understanding the communication signals -the language- between tumor cells and the TIME, encoded through various proteins secreted by immune cells, is essential for controlling tumor progression and developing effective treatments for OSCC. This review provides an overview on how TIME influences the progression of the Oral Potentially Malignant Disorders (OPMDs) to OSCC as well as OSCC's invasion, focusing on the contributions of various immune cells within the TIME. Additionally, we discuss recent advances in immunotherapy for OSCC, highlighting strategies to enhance immune responses and improve treatment outcomes.

Keywords: Inflammation; Immune cells; OSCC; OPMDs; Tumor Immune Microenvironment

Epidemiology of Oral Cancer

Oral and pharyngeal cancer are one of the most common cancers in the world ranking as the sixth most common cancer [1], with more than 400,000 new cases annually [2]. Almost two-thirds of oral-pharyngeal cancer cases occurring in developing countries [2]. The incidence rates for oral cancer are higher in some geographical regions (e.g., India, Sri Lanka, Pakistan, France, Hungary, Brazil, Uruguay and Puerto Rico) [3].

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Globally, oral cancer occurs in men more than women [4]. This could be also contributed to the risk factors practiced by men more than females. In some countries such as Sri Lanka, India, Pakistan and Bangladesh, oral cancer is ranking as the most common cancer in men and may contribute up to 25% of all new cases of cancer [1].

About 94% of oral cancers occur in old people above the age of 45 years [5]. Only few cases of oral cancer may affect young patients. The incidence and mortality rates in young adults seem to raise in western countries such as European Union and parts of United States [6,7].

Site of oral cancer is linked strongly to the risk factors. The most common site for oral cancer among Western population is tongue. While the most common site among Asian population is buccal mucosa due to betel nut and tobacco chewing [8]. Oral cancer may occur also on floor of mouth, palate, and gingiva. Lip cancer has distinct incidence as it is rare in non-white populations, however the incidence rates were reported to be highest in white populations in Canada and Australia [1]. In Australia, lip cancer counts more than 50% of all oral cancer cases [9].

Etiology and Risk Factors

Oral cancer has multifactorial etiology. Smoking, chewing tobacco and betel nut, snuff dipping, and alcohol misuse are established risk factors for oral cancer. Sunlight is strongly suggestive risk factor for lip cancer. While other possible risk factors for oral cancer may include viruses, radiation, and immune deficiency [1]. Tobacco and alcohol consumption are considered as the most important etiological factors for oral cancer [10]. Smokers and heavy drinkers have 38 times higher risk of developing oral and pharyngeal cancer more than nonsmokers and non drinkers [11]. The use of topical steroids was reported recently as an additional important risk factor associated with the malignant transformation of OPMDs to OSCCs [12]. On the opposite side, diet rich with fruit and vegetables was found to reduce the risk of developing oral cancer by at least a quarter [13]. This suggests a diet deficient in antioxidants is a predisposing factor towards the development of oral cancer [14,15] as well as for potentially malignant disorders [16].

It was documented that oral cancer risk factors, mainly, alcohol consumption and smoking can strongly influence the tumor growth by negatively impacting the TIME [17,18].

Prognosis

The 5-year survival rate for localized oral cancer tumor is greater than 80%. This survival rate usually drops to 50% in case of regional metastasis, and it drops to less than 30% in case of distant metastasis [19]. Many factors may affect on the prognosis and survival rate. The most important prognostic factor in oral cancer is the presence of cervical lymph node metastasis [20]. On the other side, oral cancer screening programs and early detection of OPMDs and oral cancer improves dramatically the prognosis and reduce mortality from oral cancer [21]. Similarly, the association of OSCC with precursor lesions showed significant improvement of the prognosis with lower mortality compared to OSCCs without association with precursor lesion [22].

Oral Potentially Malignant Disorders (OPMDs)

Oral potentially malignant disorders (OPMDs) are those lesions that have an increased risk of transformation to oral cancer. This term was first used in 2005 and re-affirmed in 2020 by a working group to replace the concept of “precancer” that was first introduced in 1805 by European panel of physicians [23]. OPMDs may appear clinically as white, red, and mixed white and red lesions, also they may appear as plaque/plateau, smooth, corrugated, granular, verrucous, or atrophic lesions with variable size [24,25].

These disorders include Leukoplakia, Proliferative Verrucous Leukoplakia (PVL), Erythroplakia, Oral Submucous Fibrosis (OSF), Oral lichen planus (OLP), Actinic Keratosis (Actinic Cheilitis), Palatal Lesions in Reverse Smokers, Oral Lupus Erythematosus (OLE), and Dyskeratosis Congenita (DC) [23].

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The ratio of progression of OPMDs to cancer vary from 1.4% to 49.5% through out a period ranging from 12 months to 20 years [26]. Squamous cell carcinoma is the most common cancer arising in patients with OPMD.

The first step of the malignant transformation of the OPMDs may occur when the OPMDs' epithelial cells start communicating with the surrounding immune microenvironment by shaping it and making the TIME ready for seeding the tumor cells. Recently, it was shown that OPMDs can promote carcinogenesis by remodeling of the adjacent microenvironment and making it favorable condition for the initiation of oral cancer [27]. This may reflect the importance of early detection of OPMDs and treating it as the first defense line in controlling oral cancer [28].

Tumour Immune Microenvironment-TIME

The tumour immune microenvironment is a complex ecosystem that includes diverse cell types, such as adaptative immune cells (e.g., CD8+ and CD4+ cells, regulatory T cells, among others) [29] and myeloid immune cells (e.g., macrophages, neutrophils, monocytes, mast cells and myeloid-derived suppressor cells, among others) [30].There are also cancer-associated fibroblasts promoting immunomodulatory activities contributing to immune evasion, remodel of the extracellular matrix and tumor progression [31]; as well as the blood vessel endothelial cells providing plasticity, controlling the passage of oxygen, cells, and fluids [32]. Many other markers and changes in the TIME were reported to favor the malignant transformation of OPMDs or even the metastasis of OSCCs, those included: PD-1 PDL-1 [33], MMPs Cortactin FISH and invadopodium [34], TNFα [35] and salivary immune cells and mediators [36]. The TIME may differ according to the intrinsic features of cancer cells, patients, and cellular composition which can promote suppression or progression of the tumor [37]. (Figure 1)

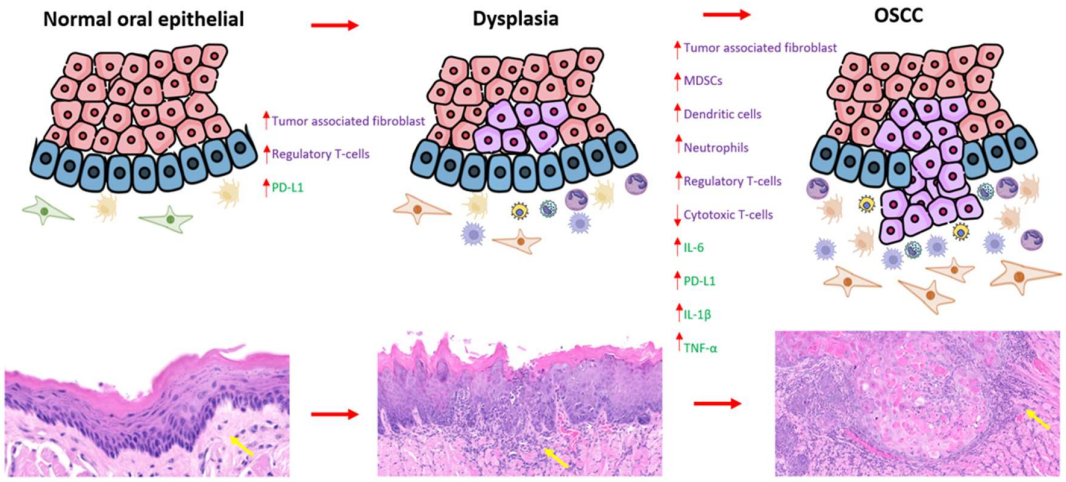

Figure 1. Changes in the TIME (yellow arrow) through progressing from normal epithelium, dysplastic epithelium to OSCC.

Tumor Associated Myeloid-Derived Suppressor Cells (MDSCs) and Dendritic Cells (DCs)

MDSCs exert suppressive activity on NK, T and B cells leading to tumor immune evasion. Recent studies explored the TIME and the crosstalk mechanism of DCs and MDSCs contribute to SCC malignant progression [38,39]. Tumor-related MDSCs enhance OSCC progression through cell proliferation, migration and invasion leading patient poor prognosis. Additionally, OSCC cells can induce MDSCs differentiation by upregulating the expression of arginase-1 (Arg-1) and inducible nitric oxide synthase (iNOS) [38]. Clinical data from patients, including biopsies and blood samples,

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have demonstrated the infiltration of DCs and MDSCs in OSCC malignant tumor, some of which lymph node metastasis or premalignant OPMDs. These cells were activated via the TNF-α/NF-κB/CXCR-4 pathway. Furthermore, monocytes- derived MDSCs (M-MDSCs) express higher levels of phosphorated STAT3 (pSTAT3), interleukin-10 (IL-10) and programmed death-ligand 1 (PD-L1) compared to polymorphonuclear MDSCs (PMN-MDSCs). M-MDSC are effective in suppressing T cells proliferation by inhibiting interferon-gamma (IFN-γ) production and promoting regulatory T cell (Treg) generation via transforming growth factor beta (TGF-β). However, M-MDSCs also promote Th17 cell through cyclooxygenase 2 (COX-2) and oxide synthase (NOS). The interplay between MDSCs and DCs appears to play a crucial role in regulating epithelial cells behavior and promoting tumor progression [39–41]. Cytometry analysis of tumor cell samples from lymph nodes of OSCC patients revealed high PD-L1 expression in dendritic cells (DCs). This was associated with reduced T-cell efficacy, contributing to poor prognosis [42]

In addition, clinical studies, using mouse models provide valuable insights into the role of the TIME and serve as guides for developing future therapeutic combination approaches in OSCC. TIME analysis in mouse tumor models has revealed an increase in CD155 + PD-L1 + T cells and effector memory MDSCs during the middle and late stages of tumor progression. Blockade of TIGIT/CD155 and PD-1/PD-L1 signalling pathways in these models significantly inhibited tumor growth, enhanced the proportions of effector T cells, and increased cytokine secretion [43].

The use of small molecules and inhibitors further enhances the understanding of the MDSCs' role in the OSCC. For instance, in OSCC models induced by chemical carcinogens (mimicking tabacco-associated OSCC) or MOC2 injection, inhibitors targeting PI3K (associated with granulocytes) and CXCR1/2, (i.e., a myeloid chemokine receptor inhibitor) effectively reduced tumor evasiveness and burden [44]. Moreover, SX-682-mediated MDSC inhibition has been shown to enhance the efficacy of NK cell transfer therapy [45].

In a mouse xenograft model, the CCL2/CCR2 axis was found to play a critical role in mediating crosstalk between cancer cell and stromal components within the TIME, underscoring its significance in OCSS progression [46].

Inflammatory states can lead to cancer cell proliferation and invasion. Combining a high-fat diet (HFD) and oral cancerogenic induced by 4NQO, Peng J, et al. 2021 showed an increase of invasive OSCC, compared to regular diet non-obese mice and changes on the TIME promoting an expansion of the MDSCs recruited through the CCL9/CCR1 pathway, only for HFD mice. In addition, tumor tissue of obese patients showed a positive correlation between an increase in MDSCs and local adipocytes in OSCC [47].

Tumor Associated Mast Cells

Mast cells (MCs) infiltrate the TIME, exhibiting both anti-tumor and pro-tumor activities depending on the type of stimulus, and play a regulatory role in tumor progression across various cancer types. They regulate tumor progression in different tumor types. Single-cell RNA sequencing and fluorescence in-situ hybridization were used to develop an MC-associated risk signature model based on immune infiltration in tissue sections from patients with HNSCC. This model identified nine MC-related prognostic genes: KIT, RAB32, CATSPER1, SMYD3, LINC00996, SOCS1, AP2M1, LAT and HSP90B1. High expression of RAB32, CATSPER1, SMYD3, AP2M1 and HSP90B1 were associated with poor prognosis, while high levels of the other genes were correlated with improved prognosis. This model could serve as a tool for patients' stratification and the identification of immunotherapeutic strategies [48].

The influence of MCs on OSCC progression is well-documented, although the molecular mediators underlying this relationship have only recently been elucidated. MCs can modulate tumor progression either directly or indirectly, depending on their localization withing the tumor or at a distance from the TIME. This interaction can result in contrasting effects, such as reduced tumor cells invasion or enhances tumor cell proliferation. in proliferation or invasion reduction of the tumor cells. For instance, co-culture with CC chemokine ligand 2 (CCL2) has been shown to increase tumor

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proliferation. Conversely, indirect stimulation by MCs has been reported to reduce tumor cell invasion [49].

Tumor-Infiltrating Lymphocytes (TILs)

The influence of TILs on OSCC are consistent, with several studies agreeing that CD8+, CD45RO+, CD57+ TILs, and CD4+ central memory T cells (CD45RO+/CCR7+) are all correlated with improved prognosis. As in other tumours, CD8+ cytotoxic T lymphocytes (CTL) are thought to improve survival by directly killing malignant cells expressing tumour associated peptides on class I major histocompatibility molecules (MHC I). Given these roles, certain studies have aimed to understand TIL recruitment and OSCC defences. The knockdown of CXCL14 in OSCC lines (MOC1 and MOC2) injected into immunocompetent mice slows tumour growth in a TIL dependent fashion, suggesting that CXCL14 may have a protective role. Furthermore, metastatic OSCCs express less CXCL14 than the primary tumours, supporting the notion that CXCL14 are implicated in TIL recruitment and tumor suppression [50].

In contrast, transforming growth factor-β (TGF-β) is elevated in most OSCCs as a product of the tumour or the surrounding TIME. As a well-established immunosuppressive cytokine, TGF-β can inhibit the cytotoxic capabilities of CD8+ cells. Inhibition of TGF-β in a CTL OSCC co-culture conversely improves cytotoxicity and reduces OSCC growth. As such, direct or indirect control of TGF-β expression may be a primary mechanism by which OSCCs suppress CTL function [51].

However, directly improving the function of CD8 or CD4 TILs to combat tumour growth may prove premature. Specifically, regulatory T cell ablation in 4-NQO induced OSCC in murine models upregulates effector T cell activity/proliferation but also promotes OSCC incidence and burden. This increase in malignancy is likely TIL dependent, as subsequent depletion of CD4 and CD8 antibodies abrogates these consequences. [52].

Tumor-Associated Macrophages

Tumor-associated macrophages (TAMs) are the predominant leukocytic component of the OSCC TIME, primarily residing in the tumour adjacent stroma rather than OSCC nests. The majority of TAMs are monocyte-derived, though tissue-resident macrophages have also been identified [53]. These monocyte-derived macrophages are attracted by a number of chemotactic cues produced by the tumor, including CCL2/MCP1 (monocyte chemotactic protein-1), CCL3/MIP-1α (macrophage inflammatory protein-1alpha), CCL4//MIP-1β (macrophage inflammatory protein-1beta), and CCL5/RANTES (regulated on activation, normal T-cell expressed and secreted).

While literature on TILs is fairly consistent, the influence of macrophages on OSCC is somewhat less unclear. TAMs have been shown to either promote or inhibit OSCC proliferation, invasion, and migration under different circumstances. These contrasting functions have been explained through a M1 and M2 model of macrophages, which suggests that macrophages can be polarised to an anti-tumor M1 (classically activated macrophages) or pro-tumor M2 (alternatively activated macrophages) phenotype.

M1 polarisation is thought to be induced by bacterial PAMPs or interferon gamma (IFN-γ) from Th1 cells. The primary way M1 cells are thought to be anti-tumor is through their improved antigen presentation capabilities, which are a result of higher MHC II expression and lysosomal activity. This improves CD4+ T cell activation, triggering tumour specific CTL expansion and limiting OSCC growth. Patient data supports this, as M1 polarised TAM abundance is strongly correlated with CTL activation. M1 cells also produce ROS/RNS, leading to direct cancer cytotoxicity, as well as proinflammatory cytokines, including IL-12, IL-23, TNFα, to recruit neighbouring immune cells.

In contrast, Th2-derived anti-inflammatory cytokines such as IL-4, IL-10, IL-13 are thought to polarise M2 macrophages. OSCC TAMs are primarily M2, and several pathways by which OSCCs promote this polarisation have been identified. For instance, OSCCs from the Cal-27 and SCC-9 lines overexpress the miR-29a-3p. This microRNA is then encapsulated in exosomes and capable of suppressing macrophage SOCS1 expression, activating STAT6 signalling which promotes M2

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polarisation. [54]. Furthermore, Cal-27 and SCC25 release exosomes with CMTM6 on the surface that can activate the ERK1/2 signalling in macrophages to induce M2 polarization [54]. OSCCs have also been determined to overexpress CCR7 to promote macrophage recruitment and M2 polarisation, though the precise mechanism is still unclear. [55]. Once induced, M2 cells produce a wide range of immunosuppressive effects including Foxp3+Treg induction and production of soluble cytokines/receptors including IL-1 receptor antagonist, IL-10 and TGF-β. M2 cells also express abundant surface PD-L1 and secrete Arg1 to inhibit T cell development. This combined immunosuppressive environment indirectly aids OSCC growth. M2 cells have also been recognized to be capable of directly facilitating OSCC growth. For instance, TGF-β triggers the TβRII/Samd3 axis in OSCCs, inducing VEGF expression. TAMs can also directly produce other growth factors (ex. epidermal growth factor, fibroblast growth factor, platelet-derived growth) in addition to matrix metalloproteinases (MMPs), promoting invasion. Consistent with these results, reconstitution of xenograft OSCC models with M2 macrophages promotes tumour progression, vascularization, and recurrence after therapy.

However, some evidence suggests that M1 macrophages may in fact promote tumor growth and invasion [56]. For instance, exosomes from Cal-27 and SCC25, the same cell lines suggested by Tang et al. to induce M2 polarisation [54], were later suggested by You et al. to trigger M1 polarisation instead [57]. These M1 cells released IL6, increasing OSCC malignancy and epithelial mesenchymal transition markers. IL6 could also stimulate the Jak/Stat3 axis, promoting the formation of exosomes with THSB1 that could trigger more M1 polarisation. In SCC25, conditioned media from M1 macrophages have been shown to improve OSCC development in vitro and in vivo by triggering the ErbB2/phosphoinositide 3-kinase (PI3K)/AKT/ERK pathway. Another possible explanation for the observed pro-tumor role of M1 macrophages is their ability to produce TNF-α, which several studies have identified to be a strong promoter of OSCC growth and invasion.

Tumor Associated Neutrophils (TANs)

More recently, interest in the role of tumor associated neutrophils (TANs) on OSCCs have grown. Neutrophil infiltration in OSCC is generally associated with poor clinical outcomes; accordingly, a neutrophil to lymphocyte ratio (NLR) has been identified as a reliable prognostic marker for shorter overall survival [58]. In most cancers, neutrophil recruitment to the TIME is mediated by several inflammatory cytokines including TNFα, IFNγ, GM-CSF, and IL-8. OSCCs can exploit these pathways to trigger neutrophil recruitment in a number of ways. For instance, VAP1 overexpression in OSCCs can act in an autocrine manner to trigger NFκB, eventually leading to IL-8 secretion. [59].

Once present, TANs are hypothesised to be polarised to either an N1 or N2 phenotype, similar to that of macrophages. This polarisation is dependent on the cytokines present at the local microenvironment, with IFN-β inducing the N1 phenotype while TGF-β induces the N2 phenotype. N1 TANs are typically considered to have an antitumor effect, activating both the innate and adaptive system. Notably, these TANs are an effective APC which express co-stimulatory molecules upon stimulation with GM-CSF or IFN-γ. They also facilitate cytotoxicity directly (through the production of apoptotic mediators and ROS) and indirectly via antibody-dependent cellular cytotoxicity (ADCC). In contrast, N2 TANs are generally protumor and can promote tumour growth through elastase secretion while releasing MMP8, MMP9, and VEGF to facilitate metastasis and angiogenesis. Furthermore, N2 TANs can secrete arginase, suppressing T cell activation, and release IL-18, inhibiting NK cell activation. [60].

As the OSCC TIME is elevated in IL-1β, IL-6, and TGF-β, it is thought that N2 neutrophils play the predominant role in OSCC development. However, as with M1 TAMs, N1 TANs may have a larger role than conventionally established. Co-culturing neutrophils and OSCC increases TNFα secretion from neutrophils, a hallmark of N1 TANs. TNFα stimulation can induce MMP secretion and invadopodia formation in a PI3K dependent manner, upregulating invasion. With TNFα expression

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in the lamina propria also higher in patients with progressing OSCCs, N1 neutrophils may in fact promote tumour formation under certain conditions. [61]

Cancer Associated Fibroblast (CAF)

Fibroblasts are integral components of the tumor immuno microenvironment (TIME) and exhibit a complex duality in their functional roles that can significantly influence tumor progression. Numerous studies have demonstrated that fibroblasts can exert tumor-suppressive effects through various mechanisms. Conversely, other research indicates that fibroblasts can undergo a transformation into cancer-associated fibroblasts (CAFs) in response to signals from the tumor. In this activated state, CAFs can promote tumor growth. This dynamic shift in fibroblast identity underscores their complex role in cancer biology, highlighting the importance of understanding their behavior in the TME for developing effective therapeutic strategies. [62]

Fibroblasts represent one of the most abundant cell types within the stroma. These cells are instrumental in the synthesis and remodeling of various extracellular matrix (ECM) components, which are essential for the maintenance of tissue homeostasis and functionality. [63] They contribute to the production of several extracellular matrix (ECM) proteins, including fibrillar collagens (types I, III, and V), proteoglycans, fibronectin, glycosaminoglycans, and various other glycoproteins and fibrillar elements. Collectively, these substances establish a three-dimensional framework that generates osmotic-active scaffolds within the stromal interstitial tissue. [63,64]

Cancer-associated fibroblasts (CAFs) can originate from a variety of sources, including local fibroblasts, endothelial cells, hematopoietic stem cells, preadipocytes, and tumor epithelial cells through the process of epithelial-mesenchymal transition. These Cancer-associated fibroblasts (CAFs) act as "factories" that generate a diverse array of proteins, signaling molecules, cytokines, and growth factors. They enhance tumor angiogenesis and promote tumor invasion, thereby contributing to tumor progression and metastasis. [65,66] They can be identified both in vitro and in vivo through a panel of marker proteins/genes such as PDGFR, SMA, fibroblast-associated protein (FAP), and fibroblast-specific protein 1 (FSP1). [67]

Cancer-associated fibroblasts (CAFs) are a key component of the tumor stroma in many cases of oral squamous cell carcinoma (OSCC) [68]. Their presence is associated with resistance to immunotherapy and correlates with poorer disease-free survival outcomes in patients [69,70]. Additionally, CAFs have been detected in potentially malignant lesions as well as in histologically normal mucosa adjacent to OSCC (HNMOSCC). This indicates that CAFs may influence the tumor microenvironment and contribute to the progression of disease, suggesting their potential role in early tumorigenic changes. [71,72]

Immunohistochemical staining of alpha-smooth muscle actin (αSMA) was utilized as a marker for cancer-associated fibroblasts (CAFs), and Kellerman's scoring criteria were applied to evaluate their frequency in oral squamous cell carcinoma (OSCC). Datar et al. demonstrated that the frequency scores of αSMA-positive CAFs in OSCC ranged from 1 to 4. Notably, approximately 40% of OSCC cases, exhibited a CAF score of 3, indicating that 41-60% of the stromal area was occupied by CAFs. In contrast, among cases of histologically normal mucosa adjacent to oral squamous cell carcinoma (HNMOSCC), 13.3% showed no expression of CAFs, while the remaining cases exhibited a CAF score of 1. The presence of cancer-associated fibroblasts (CAFs) in histologically normal mucosa adjacent to oral squamous cell carcinoma (HNMOSCC) supports the hypothesis that CAFs contribute to field cancerization and may facilitate the development of second primary tumors. [73]

Enhancing Tumor Treatment Through Immune Modulation and Combination Therapies

Head and neck cancer treatment usually involves multiple approaches. For oral cavity cancer, surgery is typically followed by chemoradiotherapy (CRT). In cases where comorbidities restrict the use of standard cytotoxic chemotherapy, the EGFR monoclonal antibody cetuximab is often combined with radiation therapy. Beyond conventional treatments, PD-L1 and immune checkpoint inhibitors represent advanced approaches in the management of OSCC. High level of PD-L1

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expression within tumor contributes to immune evasion, fostering resistance to treatment. This overexpression is also associated with increased tumor recurrence and reduced disease-specific survival in OSCC [74]. The FDA has approved the immune checkpoint inhibitors pembrolizumab and nivolumab for the treatment of recurrent or metastatic HNSCC. Both pembrolizumab and nivolumab target the PD-1 receptor on T cells, blocking its interaction with PD-L1 and PD-L2 on tumor cells, thereby enhancing immune-mediated tumor destruction. Understanding PD-1/PD-L1 levels is crucial when combining chemotherapy with immunotherapy, as these biomarkers influence treatment efficacy and guide therapeutic strategies. The effectiveness of PD-L1 inhibitors in OSCC may be limited, particularly in cases where high PD-L1 expression originates directly from cancer cells rather than immune cells [75] This finding underscores the need for more personalized treatment approaches, potentially integrating PD-L1 inhibitors with additional therapies to enhance outcomes for patients with locally advanced OSCC.

Recently, a study showed that cisplatin, a chemotherapy drug commonly used in the treatment of OSCC, upregulate PD-L1, and as a result potentially limit cytotoxic T-cell activity [76]. However, curcumin, a well-established anti-cancer and chemo-preventive properties as a phytochemical drug, downregulated PD-L1 and reduced regulatory T-cell populations [77]. Meanwhile, radiotherapy combined with immune checkpoint inhibition inducing PD-L1 expression on immune cells and carcer-associated fibroblast and enhancing the effects of checkpoint inhibitors [78]. Ferroptosis is a newly recognized mechanism of regulated cell death. A high FP score correlated with a low gene copy number burden and high immune checkpoint expressions, suggesting that targeting ferroptosis pathways could enhance immunotherapy and chemotherapy responses in OSCC [79].

There are some promising breakthrough methods being developed in OSCC treatment. Zhan XK, et al. in 2023 developed a biohybrid system based on *Spirulina platensis* (SP) and the STING agonist ADU-S100. This system enhanced immune cell infiltration and alleviated hypoxia in the OSCC TME, which improved the effectiveness of PD-1 therapy. Their findings suggest a novel approach for enhancing immunotherapy outcomes by targeting the TME [80]. Tumor-associated macrophages (TAMs) are key player in the tumor microenvironment and significantly influence treatment response. Research explored the immunosuppressive effects of PLIN2 (adipose differentiation-related protein) in OSCC, specifically TAMs. PLIN2 belongs to the perilipin family and is a marker of lipid droplets. High levels of PLIN2 in TAMs correlated with immune suppression and a poorer prognosis, suggesting that targeting PLIN2 could help restore immune balance in the TME [81].

Kondo Y, et al. investigated in 2021 the immunosuppressive role of TGF-beta in OSCC. They found that TGF-beta inhibits cytotoxic T lymphocyte (CTL) function by promoting the expansion of Tregs and cancer-associated fibroblasts. Their work supports the use of TGF-beta inhibitors in combination with immune checkpoint inhibitors to restore CTL function in OSCC [82].

Emerging therapeutic strategies and combination therapies are showing great promise in enhancing cancer treatment outcomes. Su W et al. proposed a novel approach that integrates TME-reactive oxygen species/pH dual-responsive nano prodrugs and oncolytic herpes simplex virus (HSV-1) therapy [83]. This combination effectively reshapes the tumor microenvironment, boosting immune response and transforming “cold” tumors into “hot” ones, thereby enhancing the efficacy of immune checkpoint inhibitors. Similarly, Wu C et al. explored a new combination therapy in a Phase II clinical trial for esophageal squamous cell carcinoma (ESCC), evaluating a neoadjuvant regimen of PD-1 blockade, nanoparticle albumin-bound paclitaxel (nab-paclitaxel), and S-1. Their study provided valuable insights into drug resistance mechanisms and demonstrated the potential of spatial proteomics to guide personalized cancer treatment, highlighting the promise of these emerging therapeutic approaches [84].

Integrating photodynamic and photothermal therapies with immune modulation in oral cancer is showing promising outcome by not only directly destroying cancer cell but also enhancing immune response. These approaches utilize advanced nanomaterials and photosensitizers to generate reactive oxygen species and heat upon light exposure, effectively killing tumor cells. This process induces

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immunogenic cell death, releasing tumor antigens and stimulating the immune system, which boost the infiltration of cytotoxic T cells into the tumor microenvironment. Here are some important breakthroughs that have significantly advanced the treatment. Shi et al. 2023 developed a bacterial nanomedicine from *Porphyromonas gingivalis* (nmPg), using protoporphyrin IX as a photosensitizer to enhance PDT and activate immune responses against OSCC [85]. Adding doxorubicin (DOX) showed synergistic effects, significantly inhibiting tumor growth and metastasis. While Zhou JY, et al. developed in 2022 the nanocomposite TiO2@Ru@siRNA, combining a ruthenium-based photosensitizer with siRNA targeting HIF-1α. It triggers dual photodynamic effects, damaging cancer cells and reducing hypoxia, while activating T-cells and lowering immunosuppressive factors, leading to significant tumor inhibition in animal models [86]. Zhang L, et al. in 2023 used graphdiyne oxide in PDT to increase the stiffness of OSCC cells, making them more vulnerable to CD8+ T-cells [87]. Also, Liu JC, et al. developed in 2024 the carrier-free nanocomplex NPsR, combining Chlorin e6 (Ce6) and the BRD4 inhibitor JQ1. This combination induces immunogenic cell death, reduces PD-L1 expression, and enhances immune response. The addition of RGD peptide facilitates targeted delivery, boosting PDT efficacy and overcoming resistance [88].

These studies highlight the potential of integrating photodynamic therapy (PDT) and photothermal therapy (PTT) with immune modulation, chemotherapy, and advanced drug delivery systems for OSCC treatment. By addressing tumor heterogeneity, invasiveness, and immune evasion, these strategies offer promising avenues to enhance therapeutic efficacy, reduce recurrence, and improve overall patient outcomes in managing OSCC.

Conclusion

One of the critical challenges in understanding OPMD is the oral cavity's unique environment that combines well-developed innate and adaptive immune responses. In this context, oral keratinocytes interact with immune cells and mediators linked to the pathogenesis of cancers, including increased rates of malignant transformation of oral lesions. The literature shows overwhelming data to support that the immune microenvironment (IME) has a direct role in promoting invasion and malignant transformation of oral keratinocytes. This makes OSCC an unique cancer that arises in a typically inflamed environment and that hijacks that same immune response to progress and invade.

The mechanisms by which oral cancer pathogenesis and progression are influenced by inflammation are complex and involve inflammatory mediators, immune cells, and other stromal cells in the TIME. Further research is needed to translate these novel findings in new diagnostic, prognostic and therapeutic options for OSCC

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