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Differential mRNA expression in peripheral blood is associated with oral squamous cell carcinoma: Recent advances and future challenges

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Abstract: Oral squamous cell carcinoma (OSCC) is a malignant tumor triggered by the accumulation of multiple gene mutations in oral epithelial cells. Different OSCC-related biomarkers have been reported in circulation in the peripheral blood that support the occurrence and development of OSCC. Recent advances in high-throughput and highly sensitive detection methods have overcome the limitation of the low concentration of most peripheral blood biomarkers. Hence, blood biomarker detection has become an efficient screening tool for the early diagnosis of OSCC. The growing data available in public cancer and gene databases have provided new foundations for OSCC research. In particular, the identification of OSCC biomarkers using bioinformatic tools has shed new light on the underlying mechanisms as well as on the genetic landscape of OSCC. More recently, mRNA targeting therapies have emerged as valuable anticancer treatment strategies, as they allow for the regulation of the expression of certain functional proteins to reverse genetic abnormalities or induce tissue repair. Thus, mRNA-targeting therapies can be used to regulate the expression of antigens, antibodies, or cellular receptors by immune cells. Particularly, anti-cancer cellular immunotherapy carrying specific mRNAs has attracted significant attention in OSCC treatment. Here, we review the present knowledge on the role of peripheral blood mRNAs in the diagnosis, treatment, development, and prognosis of OSCC. Moreover, we address future research prospects of mRNAs in the peripheral blood in OSCC and the opportunities and challenges that may arise in future clinical therapeutic applications.

Introduction

Oral squamous cell carcinoma (OSCC) is a common malignant tumor with a survival rate of less than 65% after 5 years of resection (Chi *et al.*, 2015). OSCC is caused by gene mutations associated with chewing betel quid, smoking, and drinking. OSCC also results from human papillomavirus infection. To date, several pathogenic proteins have been shown to be associated with OSCC, and the involvement of RNAs, such as micro, circular, and long non-coding RNAs, have also added to the increased complexity of OSCC diagnosis and treatment. Treatments for OSCC have progressed from surgery, radiotherapy, and chemotherapy to immune targeted therapy. For example, lipid nanoemulsion particles loaded with curcumin have shown anticancer properties with effects against OSCC (Liu *et al.*, 2022). Hyperthermia therapy in combination with traditional treatment methods was also shown to represent a new treatment option with enhanced efficacy (Ju *et al.*, 2018). However, hyperthermia therapy is still under investigation and undergoing clinical trials, so it is not widely available. Moreover, a 'magic' approach involves improving malignancy treatment, including regulating the pro-resolution pathway, as its resolvin D2 was shown to reduce the levels of cancer-derived cytokines/chemokines, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-6, chemokine-10 (CXCL10), and monocyte chemoattractant protein-1 (MCP-1), as well as of populations of cancer mediator-induced CD11b$^+$Ly6G myeloid cells and nociception (Song *et al.*, 2020).

Using fluids for early diagnostics has advantages associated with their relatively non-invasive collection

process. Particularly, mRNA in the blood, semen, urine, and saliva has been established to represent a new resource that can replace traditional disease diagnostic methods (Faria et al., 2010). Changes in mRNA expression in the peripheral blood can promote OSCC occurrence and development, and thus can be used for early diagnosis of OSCC (Faria et al., 2010; Srivastava and Ghosh, 2015). Indeed, programmed cell death receptor 1 (PD1), arginase 1 (ARG1), ornithine decarboxylase (ODC), prospero homeobox 1 (PROX1), and other coding genes were identified in patients with OSCC and associated with tumor occurrence and progression via the epithelial–mesenchymal transition (EMT), phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT), and Notch signaling pathways. The Notch signaling pathway enhances the proliferative signal via neural proliferation, and its activity is inhibited by the Numb protein to promote neural differentiation (Musfee et al., 2020; Wang et al., 2021). In addition, the role of inflammation and the tumor microenvironment (TME) have also been investigated in the context of OSCC. Ubiquitin-conjugating enzyme E2C (UBE2C) was related to the pathogenesis and TME of OSCC, which might be a potential diagnostic and therapeutic biomarker (Huang et al., 2021). Particularly, the chronic states of leukoplakia and oral submucous fibrosis gradually develop into OSCC (Nissinen et al., 2016).

Nevertheless, although many studies have identified differentially expressed genes in OSCC, neither research aided by public databases nor experimental evidence has led to actual improvements in OSCC survival rates. Therefore, specific biomarkers for tumor diagnosis are still warranted, and patient-individual differences must be considered. Many scientists have focused on the relationship between bloodborne mRNA levels and OSCC; however, specific diagnostic molecular blood markers have not yet been identified for the inchoate stage diagnosis of OSCC. Therefore, we have reviewed the present knowledge on the role of peripheral blood mRNA biomarkers, identified using bioinformatic tools, in the diagnosis, treatment, and prognosis of OSCC. In addition, we address the opportunities and challenges that may arise in the future application of mRNA biomarkers for OSCC clinical treatment.

Peripheral Blood mRNA and OSCC

Diagnosis
Tissue and saliva mRNA and peripheral blood gene expression in patients with OSCC are currently being investigated to identify biomarkers of OSCC (Bu et al., 2015). In 2010, a study group analyzed the peripheral blood of 27 patients with OSCC and 25 healthy controls. They revealed that peripheral blood plays an important role in the diagnosis and treatment of OSCC. Annexin A1 (ANXA1), which was reported to be downregulated at the mRNA level in OSCC, was the first blood-based biomarker identified and represents a complementary non-invasive approach for diagnosing OSCC because it is present in the blood at significantly low levels, depending on the age, sex, and tumor anatomy of each patient (Faria et al., 2010).

Lymphocytes are the main components of peripheral blood in patients with tumors. The ANXA1 pathogenic mechanism remains unclear but may refer to the "eat me" signals in tumor cells (Faria et al., 2010). Serum levels of IL-17F, which is secreted by peripheral blood mononuclear cells during tumor development, can be used for OSCC diagnosis (Ding et al., 2015). Moreover, the number of $CD3^+$ and $CD4^+$ T cells, which are critical components of the immune system, affects IL-17F and the IL-17F:vascular endothelial growth factor (VEGF) ratio (Hu et al., 2016). Tumor biomarkers in the blood or other body fluids as well as in tissues represent signs of either a normal or abnormal process and/or a specific situation or disease; thus, examining several biomarkers together can promote more accurate results and reliable cancer diagnosis (Hu et al., 2016). In addition, low c-myc expression results in poor prognosis of OSCC (Vora et al., 2007). ARG1 and ODC levels were found to be increased in the peripheral blood of patients with head and neck squamous cell carcinoma (HNSCC) by immunocytochemistry, semiquantitative reverse transcription and quantitative real-time polymerase chain reactions, and the expression of ARG1, ARG2, and ODC was markedly increased in tumor tissues as compared with adjacent tissues (Srivastava and Ghosh, 2015; Zhang et al., 2013).

Prognosis
The disease-free survival rate of patients with reduced post-operative cytokeratin (CK) 17 mRNA expression was found to be significantly increased compared with that of patients with elevated CK17; thus, CK17 is a candidate tumor marker of OSCC, and, of the CK-family, it might be the most suitable marker for diagnosis of OSCC due to its significant upregulation and the strong overexpression in peripheral blood (Kitamura et al., 2017). Indeed, it was reported that the overexpression of CK17 and CK20 is significantly associated with metastases in neck lymph nodes and that CK19 is significantly overexpressed in T3 and T4 OSCC in stages III and IV patients and in poorly differentiated OSCC. Nevertheless, determined by relative quantification, the mean value of CK17 overexpression was significantly higher than that of the other CKs and significantly associated with T1 and T2 OSCC as well as with stages I and II patients (Toyoshima et al., 2007). *VEGF* levels were reported to be enriched in OSCC cases as compared with healthy controls (Nabavi et al., 2019). Serum CK20 is relevant for the prognosis of OSCC, as it indicates disseminated tumor cells in the peripheral blood of postoperative patients with OSCC (Kitamura et al., 2017). In addition, Fas apoptotic inhibitory molecule 2 (FAIM2) was found to be downregulated in OSCC tissues, with KRT13, FAIM2, and CYP2W1 expression in OSCC showing some tendency to be associated with some clinical-pathological parameters and survival (Hartanto et al., 2015). TaqMan low-density array data showed that low-density arrays of selected blood genes can indeed be used to provide insights into cancer progression pathways, and FAIM2 may represent an early biomarker for monitoring tobacco-induced head and neck squamous cell (HNSC) neoplasms (Hasan et al., 2020).

Metastasis

OSCC markers identified from tissues have paved the way for the research on additional biomarkers in the peripheral blood. The crosstalk between myeloid-derived suppressor cells (MDSCs) and cancer cells promotes the malignant processes of OSCC and the immune-suppressive properties of MDSCs, which provides an opportunity for the targeted treatment of tumor-associated immunosuppressive cells (Pang *et al.*, 2020). Analysis of immunocytes in the peripheral blood showed that high IL-18 levels were associated with more CD19$^+$ B cells, where serum IL-37 seemed to be associated with reduced percentage of CD3$^+$CD8$^+$ T cells, indicating that their balance could change the adaptive immune response. We first revealed the different functions of the IL-18/IL-37 ratio in serum and tumor tissues (Ding *et al.*, 2020). High IL-18 expression in cancer tissues of low lymph node metastasis and tumor stages, which is not consistent with the pro-cancerogenic role of serum, and the IL-18:IL-37 ratio can affect B cells (CD19$^+$) and T cells (CD3$^+$ and CD8$^+$) in OSCC, suggesting it to be an efficient biomarker of OSCC. In OSCCs, the transforming growth factors (TGF)-β1/IL-17A stimulate neutrophils and notably induce various cell processes, including migration, proliferation, invasion, stemness, and EMT (Yu *et al.*, 2021). Moreover, IL-17A can promote angiogenesis in several tumors, including OSCC (Ding *et al.*, 2015). Prognostic factors for OSCC include carbonic anhydrase IX that is a potential target for treatment; therefore, it should not be ignored (Eckert *et al.*, 2019).

In just a few years, microarray chip technology, transcription analysis, RNA sequencing, and other high-throughput genetic detection technologies have become affordable and increasingly accurate. In tumors, bioinformatic analysis is performed to identify differential expression and functional enrichment of unknown genes; thus, RNA sequencing has been applied to predict relevant clinical outcomes and is poised to be increasingly used for personalized diagnosis and treatment. In addition, the RankComp algorithm revealed six pairs of relative expression orderings, which have important implications for gene signatures in OSCC (Ou and Wu, 2021). Thus, more tumor biomarkers in the peripheral blood of OSCC patients will be detected in the future by bioinformation analysis. Relevant tumor markers in the peripheral blood of OSCC patients as shown in Fig. 1 and Table 1.

Immune response and escape

Tumor development is associated with gene mutations in local sites and throughout circulation, such as blood circulation and immune response. Owing to the complex processes between local tissues and body circulation, genes found in tissues may be indicators for the development of OSCC in circulation. Recently, PD1 in peripheral blood has been a hot topic; new biomarkers have been found and previous biomarkers have been tested in different studies; such studies aim to identify genes to guide blood analyses. Among them, the immune response plays a major role in the development of OSCC. Immune tolerance is mediated
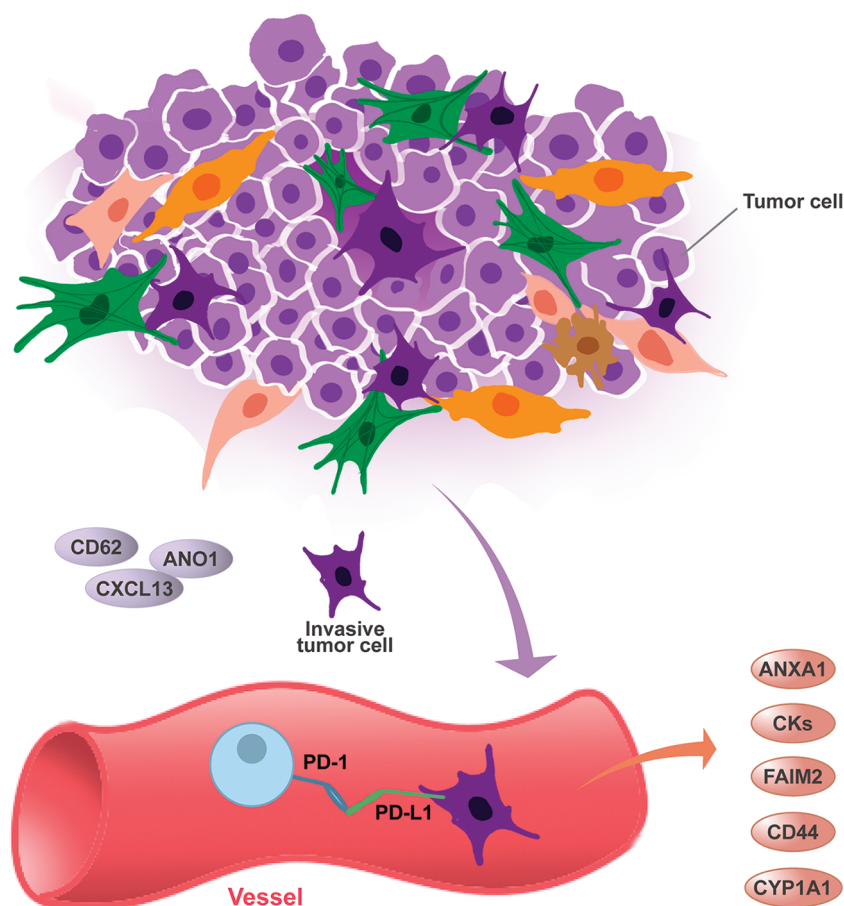

FIGURE 1. The pathological mechanisms of biomarkers and OSCC blood.

TABLE 1

Common Molecular markers and related functions associated with OSCC in peripheral blood

mRNA Gene	Characteristics	Function
ANXA1 Faria et al. (2010)	Low expression in peripheral blood of patients with OSCC; first mRNA tumor marker found in blood.	The mechanism is unclear and may involve an "eat me" signal affecting immune evasion.
CTC Heikenwalder and Lorentzen (2019)	CTCs are a general term for all kinds of tumor cells present in peripheral blood for spontaneous or diagnostic operations, CTCS are shed from solid tumor lesions, enter the peripheral blood circulation, and can develop into metastases under certain conditions.	CTCs has important clinical value for early screening, auxiliary diagnosis, evaluation of metastasis, and determination of malignant degree, progression, and pathological grade of OSCC and as a relatively reliable tumor detection indicator.
CKs Toyoshima et al. (2007)	CK17 has high expression but cannot be detected in peripheral blood, especially in early tumors. *CK17* is significantly upregulated and strongly overexpressed, and might be the most suitable marker for diagnosis of OSCC of the CK-family. Disseminated tumor cells can be detected in peripheral blood during recurrence. *CK19* in peripheral blood is associated with progress and CK20 is related to recurrence of OSCC.	Detecting CK family genes to predict the recurrence of OSCC. Early dissemination of tumor cells to other sites may be an intrinsic rule and an important biological characteristic of OSCC.
TSC1,2 Srivastava and Ghosh (2015)	Analysis of matched peripheral blood from 50 patients showed LOH at both TSC loci. First report to describe LOH at both TSC loci in OSCC. TSC genes are targets of epigenetic inactivation in oral cancer.	High methylation of the TSC gene leads to low expression and LOH, TSC1,2 downregulation in the mTOR signal pathway. Loss of function of these genes may contribute to the constitutive activation of the mTOR signaling pathway, leading to overall cell growth and proliferation.
PD1-PDL1\2\4 Wehrhan et al. (2021)	PD-L1 expression in peripheral blood might be an indicator of the existence of metastatic disease (N+) in OSCC.	Soluble PD1 was found to inhibit dendritic cell activation and to increase the expression of immunosuppressive mediators such as IDO and IL-10. These results outline the complexity of and highlight unsolved questions in PD/PD-L signaling.
CA49 Torres López et al. (2018)	CA49 expression in OSCC tissue samples differed significantly from that in blood samples.	The highest CA9 gene expression was found in a stage T3 OSCC sample.
miR-21, MUC1 Payehghadr et al. (2018)	Increased expression of MUC1 biomarkers in serum are related to tumor size and staging.	Expression analyses of markers.
CXCL13, CXCL10 Li et al. (2019), Pandruvada et al. (2010)	Inflammatory cytokines such as IL-1β have been shown to induce CXCL13 production in differentiated osteoblasts, and human osteoblasts have been shown to express functional CXCR3 and five receptors.	High levels of CXCL13 production by OSCC cells could modulate osteoclast activity and gene expression in the tumor-bone environment.
IL Ding et al. (2020)	IL-6 in serum is a potential biomarker for oral cancers. IL-17 is secreted by peripheral blood mononuclear cells during tumor development. High IL-18 expression in cancer tissues of low lymph node metastasis and tumor stages.	In T cell populations from normal donors, expression of IL-4 (but not of its antagonist, IL-4delta2, or IL-10) is associated with high TLR2 and low TLR1.
TNF-α Hong et al. (2009)	TNF-α is one of the key cytokines that regulate the inflammatory processes in tumor promotion.	TNF-α induces Snail stabilization, is dependent on the activation of the NF-κB pathway, and results in increased cell invasion and migration in OSCC cells.
ARG1 Srivastava and Ghosh (2015)	ARG1 is increased in the peripheral blood of patients with HNSC.	In growing tumors, TAM are referred to as M2-like macrophages, which are cells that display immunosuppressive and tumorigenic functions and express the enzyme arginase 1 (Arg1).
ERK3 Rai et al. (2004)	Overexpression of the gene may be indicative of increased risk of developing oral cancers.	*ERK3* overexpression of the transcript in oral cancer tissues and corresponding peripheral blood of patients as compared to the peripheral blood of normal healthy individuals.
CEA Honarmand et al. (2016)	Increasing the serum levels of CEA provides prognostic information about the course of the disease.	*CEA* mRNA can be considered as a biomarker for detection of the disease in early stages.
VEGF Khani et al. (2019), Gorenjak et al. (2019)	The differences in its expression in the blood of patients with OSCC were explored via qPCR.	*VEGF* mRNA and miR-494 can play a major role in diagnosing OSCC.

by inhibitory signaling pathways, so-called immune checkpoints, of which PD1 is one of the most relevant pathways. PD1 overexpression can indicate a decreased immune response, which can be due to T-cell exhaustion and the promotion of immune tolerance (Wehrhan et al., 2021). Indeed, PD-L1 and PD-L2 are ligands of PD1; the interaction between them represents the majority of local immunosuppression mediation in oral cancer (Wehrhan et al., 2021). PD-L1 binds to PD1, a cell surface receptor expressed by activated T cells and macrophages, and induces apoptosis or the formation of regulatory T cells in these immune cells. Thus, PD-L1 overexpression leads to immunosuppression (Weber et al., 2017). Targeting the PD1/PD-L1 pathway with specific antibodies leads to significantly improved overall survival and quality of life (Weber et al., 2017). The association between lymph node metastases and high PD-L1 expression in the peripheral blood of patients with OSCC indicates that the PD-L1 expression of immune cells might be of high biological relevance.

Many tissue biomarkers may be explored in peripheral blood in the future. However, the blood contains tumor regulation factors, mediators, or inflammation factors of other tumor types. Integrin may represent a potential candidate biomarker for oral cancer. The absence of NOD-like receptor family pyrin-domain-containing protein 3 (NLRP3) inflammasomes enhances the anti-tumor effect of fluorouracil, which allows integrin-signaling for regulating different functions, including cell migration, invasion, proliferation, and movement; thus, integrin may represent a potential candidate biomarker for oral cancer (Russell et al., 2013).

Pathway analysis
To date, only a few studies have explored the different pathways involved in OSCC development within local tumor tissues and circulating peripheral cells. A previous study found that PD-L1 upregulation in the peripheral blood of OSCC patients was associated with poor overall survival (Weber et al., 2019). Zhao et al. (2022) reported that the knockdown of ST2 downregulated constitutive PD-L1 expression, whereas ST2 overexpression enhanced PD-L1 levels via IL-33 signals. Indeed, the IL-33/ST2 mechanism activates the JAK2/STAT3 pathway to directly promote PD-L1 expression, but it also promotes MyD88/NF-κB signals that indirectly further support IFN-γ-induced PD-L1 expression (Zhao et al., 2022). The selectivity of mogamulizumab towards regulatory T cells among the peripheral CCR4$^+$CD8$^+$ cell population may be increased by adjusting the trametinib concentration in the blood, as CD8$^+$ T-cell susceptibility to mogamulizumab is lower than that of regulatory T cells *in vitro* (Ono et al., 2022). In addition, OSCC cells induce MDSC differentiation with an immune suppressive phenotype from peripheral bone marrow cells, enhance the secretion of ARG1, and induce nitric oxide synthase by MDSCs (Pang et al., 2020). The expression of survivin has been reported in OSCC, where it plays a role in reducing apoptotic cell death and increasing the proliferative activity of cancer cells in the peripheral blood. Calixarene 0118 causes the early induction of the

mitogen-activated protein kinase pathway via the immediate response gene *FOS*, a subunit of the activator protein-1 complex, which affects the progression of early tumors (Greer et al., 2022). Glaucocalyxin A (GLA) disrupts redox homeostasis and activates the mitochondrial apoptotic pathway, whereas cell apoptosis is completely prevented by the synthetic peptide Z-VAD(OMe)-FMK and N-acetylcysteine (Wang et al., 2022). Simultaneously, the expression state of ChaC glutathione-specific gamma-glutamylcyclotransferase 1 (CHAC1) significantly affects GLA-mediated apoptosis.

Target and clinical usage
Liquid biopsies (LBs) are currently regarded as clinically essential and non-invasive diagnostic tools for OSCC because they could provide precision and individualized medicine to design tailored treatments (Adeola et al., 2022). Indeed, some peripheral blood serum biomarkers were reported to be related to tumor size and staging, thereby representing valuable tools for early OSCC diagnosis (Payehghadr et al., 2018). Hence, peripheral blood gene expression profiling may be used for the early detection of pre-malignant lesions. In particular, genes in the peripheral blood may be used as surrogates for providing insights into cancer progression pathways and, possibly, as early biomarkers for monitoring tobacco-induced head and neck squamous cell carcinomas (HNSCC), including OSCC (Hasan et al., 2020). A previous study revealed similar alterations in tissue samples and the peripheral blood of tobacco-consuming HNSCC patients concerning polycyclic aromatic hydrocarbon-metabolizing enzymes and associated transcription factors, signaling molecules, DNA repair genes, oncogenes, EMT pathway genes, and cell adhesion/motility genes (Hasan et al., 2020).

As most clinical biochemical analyses involve the use of blood plasma or serum, LBs have several advantages including easy accessibility, reliability, reproducibility, and the possibility of early detection of diseases (Hasan et al., 2020). In addition, LBs may be clinically useful, as they provide a more representative picture of circulating tumor cells (CTCs), circulating tumor DNA and RNA, and tumor-derived exosomes obtained among all types of body fluids (Hasan et al., 2020). Cancer cells have an inherent tendency to detach from the tumor and enter the circulating system, which will support their high proliferation and low cohesiveness. In addition, during apoptosis and necrosis, tumor cells shed their genetic materials and protein content into the bloodstream. Similarly, such substances are also secreted during metastasis; thus, they can be exploited as markers. These properties of cancer cells have been used for early diagnosis of tumors, therapeutic guidance, and recurrence monitoring. For example, increased levels of *CD44s* mRNA in the peripheral circulation were related to tumor progression and metastasis. The CD44 expressed on tumor cells binds weakly to CD62 on endothelial cells to mediate the rolling of tumor cells for extravasation, which further leads to metastasis. Signal transduction induced by CD44 generally occurs via the Wnt, PI3K/AKT, and ERK pathways. A recent study on MDA-MB-468 cells showed

that CD44-HA binding initiates cellular signals through c-Jun, which ultimately upregulates apoptosis inhibitors and supports tumor cell survival (Singh *et al.*, 2022a). In addition, CYP1A1 expression in peripheral blood lymphocytes has been reported to be similar to that observed in surgically resected tissue. Notably, an increased *CYP1A1* mRNA expression in peripheral blood lymphocytes was found to be associated with *CYP1A1* polymorphisms, as well as with tumor invasion and metastasis (Singh *et al.*, 2022b).

Although researchers have determined many biomarkers in the peripheral blood, no specific markers have been reported to detect early OSCC. Therefore, mRNA expression in local tissues should be studied to elucidate the relationship with peripheral blood. The expression of *CK13*, *FAIM2*, and cytochrome P450 family 2 subfamily W member 1 (*CYP2W1*) in OSCC is correlated with selected clinicopathological parameters and the survival rate of patients. These genes were downregulated in OSCC samples compared with adjacent samples.

Anti-Cancer Cellular Immunotherapy and OSCC Blood

Checkpoint inhibitor proteins

PD-L1 expression in OSCC may contribute to an immunosuppressive local TME. A systemic state of immune activation (indicated by a low checkpoint expression in the peripheral blood) or a state of immune tolerance (indicated by a high checkpoint expression) can help determine tumor progression in OSCC (Weber *et al.*, 2019). Indeed, a cutoff point (COP) can be determined to allow for the differentiation of OSCC patients from controls based on PD-L1 expression in the tumor tissue or peripheral blood (Weber *et al.*, 2017). Notably, an increased malignant behavior (higher tumor grade, positive nodal status) may be associated with PD-L1-mediated systemic immune tolerance (Weber *et al.*, 2017). Moreover, PD-L1 expression in the peripheral blood can indicate the existence of metastatic disease (N+) in OSCC. The role of the absolute lymphocyte count in peripheral blood as a predictive biomarker has been validated in metastatic melanoma patients treated with ipilimumab (Darvin *et al.*, 2018). Cytometry by time-of-flight-based immune profiling of peripheral blood samples collected from anti-CTLA-4 and anti-PD1-treated melanoma patients showed a distinct set of biomarkers in response to therapy (Darvin *et al.*, 2018). Apart from circulating CD8$^+$ T cells, CD8$^+$ effector memory type-1 T cells have also been reported as predictive biomarkers for ipilimumab-treated stage IV melanoma (Darvin *et al.*, 2018). Interestingly, evaluation of non-small-cell lung cancer patients showed that PD-L1$^+$ CTCs were more frequent than PD-L1$^+$ cells in the TME (83% *vs.* 41%); however, no correlation was observed between PD-L1 expression in tumors and CTCs (Darvin *et al.*, 2018). Therefore, further investigation is warranted to establish PD-L1 expression on CTCs as a cancer-predictive biomarker (Darvin *et al.*, 2018). In patients with advanced-stage OSCC, surgery along with adjuvant radiotherapy remain the preferred treatments, which are performed with or without chemotherapy (Coletta *et al.*, 2020).

Medicine target therapy

Ganoderma lucidum polysaccharides (GLPS) treatment changes tumor cell morphology and granularity, delays migration, decreases colony formation, and impairs sphere formation, thereby leading to non-invasive and less proliferative behavior in OSCC cells (de Camargo *et al.*, 2022). Moreover, as FOXO3a activation inhibits CDK4/6 and cyclin D1 expression and upregulates p27 and Bim expression of OSCC cells, FOXO3 may be a potent therapeutic target for OSCC (Fang *et al.*, 2011). Genomic studies have dissected the genetic characteristics of the immune compartment to characterize cancer subtypes of immune activity and immune failure. This approach is expected to provide information on the immune status of patients with OSCC and guide new therapies to improve immune-therapy responses (Chai *et al.*, 2020). FOXD1-overexpression leads to stronger tumorigenic abilities by promoting the occurrence and development of tumors (Li *et al.*, 2020). Lycorine hydrochloride induces the mitochondrial apoptotic pathway through ROS mediated by the JNK signaling pathway leading to HSC-3 cell apoptosis, showing that lycorine hydrochloride is a potential drug candidate for OSCC therapy (Li *et al.*, 2021). During the process of treating OSCC, anlotinib targets FGFR3 and inhibits tumor cancer cell proliferation and promotes apoptosis by the FGFR3/AKT/mTOR signaling pathway. Berberine has anti-CSC effects as an adjuvant to usual chemotherapy to improve treatment effects in oral cancer (Lin *et al.*, 2017). Anthocyanin limits the feasibility, transfer, and invasion in OSCC cells. Various genes, including *APP*, *EHMT1*, *ACACB*, *PCNA*, *PLAU*, *FST*, *HMGA2*, *LAMC2*, and *SPP1*, were associated with patient survival based on data from The Cancer Genome Atlas (TCGA) (Dai *et al.*, 2020; Yadav *et al.*, 2021). Downregulation of ANO1 by cinobufagin showed the potential anticancer effect of cinobufagin in OSCC tissues. Baicalein inhibited the expression of Sp1, p65, and p50 by reduction of their relative mRNA levels, and it reduced NF-κB activity in OSCC cells. Public data, clinical tissues, and transfected cells were screened for the molecular mechanism of indirect targeting between GLA and CHAC1, and the results demonstrated that GLA significantly inhibits cell proliferation and induces apoptosis. In addition, GLA-upregulated CHAC1 expression participates in ATF4/CHOP signaling during endoplasmic reticulum stress (Wang *et al.*, 2022). High CHAC1 expression also indicates positive survival, as CHAC1 alternatively regulates the number of tumor cells (Wang *et al.*, 2022). GLA has potential tumor therapeutic effects that trigger the ROS-mediated ATF4/CHOP/CHAC1 axis in patients with tumors (Wang *et al.*, 2022). Therefore, HPRT1 is also a potential prognostic biomarker and therapeutic target in OSCC (Wu *et al.*, 2022).

Autophagy

Autophagy is a mechanism by which cellular material is delivered to lysosomes for degradation, allowing basal turnover of cell components and providing energy and macromolecular precursors. Autophagy plays opposing, context-dependent roles in cancer; thus, interventions to both stimulate and inhibit autophagy have been proposed as cancer therapies. In a control group, it was found to suppress

tumor expression by the digestion of mutated proteins and impacts further cell mutations and cancer processes (Khan *et al.,* 2020). Lack of nutrients and oxygen (methanolic stress) in the cell environment leads to autophagy, which is typically upregulated in cancer cells and provides the amino acids, free fatty acids, and glucose necessary for tumor survival and proliferation (Khan *et al.,* 2020). Low differentiation and p38 activation are associated with poor prognosis in OSCC. Resveratrol may prevent drug resistance as it leads to cell cycle arrest and, subsequently, to apoptosis and autophagy of cancer cells (Cheng *et al.,* 2022). Genipin deters cell proliferation and stimulates apoptosis, and it was recently shown to induce OSCC cell autophagy via PI3K/AKT/mTOR pathway inhibition (Wei *et al.,* 2020). Interferon-gamma-induced autophagy is a cellular protective process and blocking autophagy flux can promote interferon-gamma-mediated OSCC cell apoptosis. Thus, the combination of interferon-gamma and autophagy inhibitors represents a novel strategy for OSCC therapy (Zhou *et al.,* 2021).

Conclusions

As a local tumor of the head and neck, OSCC has great metastasis and invasiveness potential. Therefore, mRNA diagnostic markers identified in the blood of patients with OSCC are ideal for its early diagnosis and treatment. The occurrence and development of early OSCC, as well as the malignant transformation of potentially malignant disorders, are complex processes that rely on host immunity, TME, and inflammation. Cancer-associated inflammation is considered the seventh marker of cancer, with cancer cells producing pro-inflammatory mediators. Moreover, tumor occurrence and development are related to genetic aberrations in oncogenes, tumor suppressors, and other elements of the TME. Hurdles in the use of biomarkers include developing a means of finding reliable biomarkers and realizing their precise clinical applications. In the future, more biomarkers and their mechanisms will be explored for the treatment of cancer. Currently, data available in the TCGA and GEO public databases greatly contribute to a better understanding of the etiological nature of OSCC. Moreover, body fluids hold considerable research value for the early diagnosis of OSCC. However, how regulation of peripheral blood-circulating biomarkers will affect the outcome of the primary tumor and metastasis remains to be clarified.

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