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Proteomic and transcriptomic biomarkers for oral squamous cell carcinoma detection: Insights from saliva analysis

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Abstract

Proteomic and transcriptomic biomarkers have become valuable tools for the early detection and diagnosis of OSCC. The human saliva proteome, which was previously thought to contain a limited number of proteins, has now been found to consist of over 2000 proteins. We searched Pubmed and google scholar for research and review articles https://www.google.com/search?q=GENOMICS+AND+TRANSCRIPTOMICS+BIOMARKERS+IN+ORAL+CANCER. We searched for articles based on early detection of oral cancer through salivary biomarkers. Conference abstracts were not included. This expanded knowledge of the saliva proteome has paved the way for identifying potential biomarkers associated with OSCC. CD44sol, a detached extracellular domain of CD44, has been detected in body fluids such as saliva and plasma, particularly in the oral cancer stem cell compartment. Cytoskeleton fragments, such as Cytokeratins (CK) 8, 18, and 19, have been identified as important markers of epithelial malignancies. MMP-1, MMP-3, and MMP-9 have been identified as potential biomarkers for OSCC, with elevated levels observed in saliva and tissue samples of OSCC patients. In addition to proteomic biomarkers, the exploration of transcriptomic biomarkers, such as coding and noncoding RNAs, in saliva has opened new avenues for understanding disease mechanisms and developing diagnostic tools. The whole saliva transcriptome encompasses both human and microbial transcripts, offering valuable insights into the oral microbiota's potential influence on disease development. In conclusion, proteomic and transcriptomic biomarkers have shown great potential in improving the early detection, diagnosis, and management of OSCC. The identification and validation of these biomarkers offer promising prospects for enhancing patient outcomes and reducing the burden of this devastating disease.

Keywords: OSCC; Proteomic biomarkers; Transcriptomic biomarkers human saliva proteome; Whole saliva transcriptome.

Introduction

Proteomic and transcriptomic biomarkers have emerged as promising tools for the early detection and diagnosis of various diseases, including Oral Squamous Cell Carcinoma (OSCC) [1]. The human saliva proteome, once thought to contain only a limited number of proteins, has now been found to consist of over 2000 proteins. This expanded knowledge of the saliva proteome has paved the way for identifying potential biomarkers associated with OSCC [2].

In recent years, extensive research has focused on identifying specific proteins and their expression patterns in OSCC patients. A careful survey of existing literature has led to the

identification of 49 proteins that hold promise as potential biomarkers for OSCC. These proteins belong to various functional categories, including cell surface proteins, cytoskeleton fragments, intracellular proteins, proteases, and inflammation-related proteins [3].

Among the cell surface glycoproteins, CD44 and Cancer Antigen 125 (CA-125) have shown strong expression in advanced stages of OSCC. CD44sol, a detached extracellular domain of CD44, has been detected in body fluids such as saliva and plasma, particularly in the oral cancer stem cell compartment. Similarly, CA-125, along with other cell surface glycoproteins, has been found to be significantly elevated in OSCC patients [4].

Cytoskeleton fragments, such as Cytokeratins (CK) 8, 18, and 19, have been identified as important markers of epithelial malignancies. Fragments of these cytokeratins, including CYFRA 21-1 and tissue polypeptide-specific antigen, have shown promise as biomarkers for OSCC, with their levels correlating well with disease recurrence and prognosis [5].

Intracellular proteins like Mac-2 binding protein and salivary Zinc Finger Protein 510 peptide (ZNF510) have been found to be elevated in tissue, sera, and saliva of OSCC patients. These proteins play crucial roles in the regulation of OSCC cell growth and motility [6].

Matrix Metalloproteinases (MMPs), a group of proteases involved in extracellular matrix digestion, have been associated with the local invasion and metastasis of oral cancer. MMP-1, MMP-3, and MMP-9 have been identified as potential biomarkers for OSCC, with elevated levels observed in saliva and tissue samples of OSCC patients [7].

Chronic inflammation has been linked to the development and progression of OSCC. Inflammatory factors, including cytokines and chemokines, can either facilitate or hinder tumor growth and spread. In OSCC, various molecular pathways, such as NF-κB, AP-1, TNF-α, IL-6, IL-8, IL-1, COX-2, and TGF-β, have been found to be deregulated, highlighting their potential as prognostic markers and therapeutic targets [8].

Furthermore, the exploration of transcriptomic biomarkers, such as coding and noncoding RNAs, in saliva has opened new avenues for understanding disease mechanisms and developing diagnostic tools. The whole saliva transcriptome encompasses both human and microbial transcripts, adding complexity to the analysis but also offering valuable insights into the oral microbiota's potential influence on disease development [9].

In conclusion, proteomic biomarkers and transcriptomic biomarkers including specific proteins and RNA molecules have shown great potential in improving the early detection, diagnosis, and management of OSCC. The identification and validation of these biomarkers offer promising prospects for enhancing patient outcomes and reducing the burden of this devastating disease [10].

Data sources

We searched Pubmed and google scholar for research and review articles https://www.google.com/search?q=GENOMICS+AND+TRANSCRIPTOMICS+BIOMARKERS+IN+ORAL+CANCER. We searched for articles based on early detection of oral cancer through salivary biomarkers. Conference abstracts were not included.

The search strategy that was followed included the identification of the key concepts such as proteomic biomarkers, transcriptomic biomarkers, OSCC and saliva analysis. Generate search terms for each concept such as proteomics, protein biomarkers, protein profiling, proteomic analysis, transcriptomics, RNA biomarkers, gene expression profiling, transcriptomic analysis, oral cancer, squamous cell carcinoma, OSCC, saliva, oral fluid, salivary, saliva proteome, saliva transcriptome Search terms using Boolean operators (AND, OR) were combined: (Proteomic biomarkers OR protein biomarkers OR protein profiling) AND (transcriptomic biomarkers OR RNA biomarkers OR gene expression profiling) AND (oral squamous cell carcinoma OR oral cancer OR squamous cell carcinoma OR OSCC) AND (saliva analysis OR oral fluid OR salivary). The appropriate databases to search were: PubMed/MEDLINE, Embase, Web of Science, Scopus. The search query was constructed for each database: PubMed/MEDLINE: ("proteomic biomarkers" OR "protein biomarkers" OR "protein profiling") AND ("transcriptomic biomarkers" OR "RNA biomarkers" OR "gene expression profiling") AND ("oral squamous cell carcinoma" OR "oral cancer" OR "squamous cell carcinoma" OR "OSCC") AND ("saliva analysis" OR "oral fluid" OR "salivary"). Embase: ('proteomic biomarkers' OR 'protein biomarkers' OR 'protein profiling') AND ('transcriptomic biomarkers' OR 'RNA biomarkers' OR 'gene expression profiling') AND ('oral squamous cell carcinoma' OR 'oral cancer' OR 'squamous cell carcinoma' OR 'OSCC') AND ('saliva analysis' OR 'oral fluid' OR 'salivary'). Web of Science and Scopus: TITLE-ABS-KEY(('proteomic biomarkers' OR 'protein biomarkers' OR 'protein profiling') AND ('transcriptomic biomarkers' OR 'RNA biomarkers' OR 'gene expression profiling') AND ('oral squamous cell carcinoma' OR 'oral cancer' OR 'squamous cell carcinoma' OR 'OSCC') AND ('saliva analysis' OR 'oral fluid' OR 'salivary'))

Proteomic biomarkers

Over the past two decades, genome sequencing has provided us with an abundance of information, revealing the genetic blueprint of organisms. Following this breakthrough, scientists turned their attention to proteins, the biomolecules translated from genes that govern various cellular processes. It is now proposed that genes exert their actions through proteins, playing a role in the development of diseases, including malignancies. Mechanisms such as alternative splicing and post-translational modifications contribute to the complexity of the human proteome, which consists of over half a million proteins, far exceeding the number of protein-coding genes.

Proteins are crucial cellular molecules that participate in cellular processes and even regulate DNA synthesis and transcription. Proteomic techniques offer valuable insights into cellular physiology and molecular biology by analyzing the quality, quantity, structural modifications, and subcellular localization of proteins [11,12]. Biomarkers, particularly protein biomarkers, play a vital role in disease diagnosis and prognosis and can be used alone or in combination with other biomarkers. However, the identification of reliable biomarkers often requires rigorous validation [13].

Protein biomarkers secreted by tumors can exhibit differen-

tial expression compared to normal tissue, making them valuable indicators of disease. Fresh tissue samples are typically used to study protein translation, while paraffin-embedded tissue can be employed to study cellular localization and expression patterns [14]. In addition to serum, protein biomarkers can be detected in other body fluids such as urine, saliva, and sputum, although their quantities may vary depending on tumor secretion.

Before being analyzed by Mass Spectrometry (MS), protein samples undergo preliminary separation, enrichment, or fractionation. Enrichment techniques include one-directional Polyacrylamide Gel Electrophoresis (1D-PAGE), two-dimensional Polyacrylamide Gel Electrophoresis (2D-PAGE), among others. Liquid Chromatography coupled with tandem MS (LC-MS/MS) is a commonly used method to identify and quantify proteins in human tissues. This technique involves separating proteins through liquid chromatography before their identification by mass spectrometry [15].

A mass spectrometer consists of three main components: an ionization source, a mass analyzer, and an ion detector. The most frequently used ionization sources in proteomics are Electrospray Ionization (ESI) and Matrix-Assisted Laser Desorption/Ionization (MALDI), which produce ions from the protein samples for analysis on the mass spectrometer. Various ion analyzers, including Quadrupole (Q), Time Of Flight (TOF), ion traps, and Fourier transform ion cyclotron (FT-ICR), are employed in proteomics research. Cellular localization and quantification of proteins are often determined through immunohistochemistry and Enzyme-Linked Immunosorbent Assay (ELISA), which are also used for the validation of protein biomarkers [16,17].

Due to the considerable variation in protein expression, there exists a wide range of potential biomarkers for OSCC [18]. These biomarkers can be broadly categorized as tissue-based biomarkers, secretomes (found in plasma, saliva, blood, or other secretions), and autoantibodies (Table 1).

Table 1: Tissue based biomarkers in OSCC.

Biomarker	Function/ Description	Expression in OSCC Samples	Salivary/Serum Levels in Malignant Tumors
Epidermal growth factor receptor (EGFR)	Membrane-bound receptor involved in cellular processes	High expression observed	-
Vitamin D-binding protein	Secreted transport protein for vitamin D sterols	Reduced in OSCC plasma	-
Fibrinogen (alpha/beta/gamma chain)	Blood coagulation regulator and angiogenic predictor	High expression in OSCC patients	-
Carcinoembryonic antigen (CEA)	Glycoprotein involved in cell adhesion	Increased levels in malignant tumors	Increased levels in malignant tumors
P53 autoantibody	Antibodies found in serum and saliva	Overexpression in tumor tissues	Detected in serum and saliva of patients with p53 overexpression
Hsp 70 autoantibody	Heat shock proteins overexpressed in tumor cells	Increased levels in SCC	-

Cell surface glycoproteins

Cluster of differentiation 44 (CD44)

CD44 is a cell surface adhesion molecule that is found universally in cells and is involved in interactions between cells and the extracellular matrix. Its structure consists of a total of 20 exons, with the first and last 5 exons forming a constant region and the central 10 exons forming a variable region. CD44 (also known as CD44s) consists of four main components: an extracellular domain, a proximal domain, a transmembrane domain, and a small cytoplasmic tail. The extracellular domain is responsible for binding to various ligands such as hyaluronan, collagen, fibronectin, laminin, and chondroitin sulfate, while the cytoplasmic domain connects to the cytoskeleton through proteins like ankyrin and the ezrin-moesin-radixin (EMR) family [18,19]. In cancer, the extracellular portion of CD44, known as CD44sol, can become detached from the cell surface and be released into bodily fluids like saliva or plasma. CD44sol is derived from the tissue level of CD44 and is released from tumor cells through the process of proteolytic digestion. It exhibits high expression levels in the later stages of OSCC. Specifically, its expression is prominently observed in the compartment of oral cancer stem cells. Increased levels of CD44sol have been identified in the oral rinse of patients with OSCC. CD44sol levels and the methylation status of the CD44 gene promoter have been suggested as potential biomarkers in OSCC patients [20].

Cancer antigen (CA)

CA-125, also known as cancer antigen 125 or carbohydrate antigen 125, is an antigen associated with tumors. It is a mucin glycoprotein that is also expressed in OSCC [21]. CA-125 is known to support tumor growth by suppressing natural killer cells and promoting metastatic invasion. Structurally, it consists of three domains: N-terminal, tandem repeat, and C-terminal. The N-terminal and tandem repeat domains are located on the extracellular side and have a high degree of glycosylation.

The extracellular portion of CA-125 can be released into body fluids through proteolytic digestion. Salivary levels of CA-125 have been found to be approximately ten times higher in OSCC patients compared to healthy controls. CA-125, along with tissue polypeptide-specific antigen, demonstrates sufficient accuracy in diagnosing OSCC. Other glycoproteins present on the cell surface, such as carcinoembryonic antigen (CEA), carcinoma-associated antigen 50, cancer antigen 19-9 (CA19-9), and epidermal growth factor receptor 2 (erbB2), are also observed in OSCC. Combined levels of CEA and CA-50 in saliva are significantly elevated in malignancies compared to both benign and malignant lesions of the oral cavity and salivary glands. Higher levels of erbB2 have been detected in unstimulated saliva of OSCC patients compared to healthy individuals.

Cytoskeleton fragments

Cytokeratins (CK) 8, 18, and 19 are proteins that are expressed in epithelial cells and are found in both proliferating and apoptotic cells, which are processes occurring in OSCC. These cytokeratins can be cleaved by caspases, leading to their release into various locations such as the tumor microenvironment, circulation, and saliva [22]. Fragments of CK-8, 18, and 19 serve as important markers for identifying epithelial malignancies. In the context of OSCC, specific cytokeratin markers include CYFRA 21-1, tissue polypeptide antigen, and tissue polypeptide-specific antigen [23].

Cytokeratin fragment 21-1 (CYFRA 21-1)

CYFRA 21-1, also known as human cytokeratin fragment 21-1, is a soluble fragment derived from cytokeratin-19. It is known to be overexpressed in tissue, serum, and saliva of patients with OSCC [24-27]. Pre-operative levels of CYFRA 21-1 in the serum have shown promising potential as a biomarker for stratifying the risk associated with OSCC. Elevated serum levels of CYFRA 21-1 are associated with deeper tumor invasion, as well as the presence of bone and skin invasion, and distant metastasis in OSCC. Furthermore, salivary levels of CYFRA 21-1 exhibit a strong correlation with cytokeratin-19 levels and can be indicative of disease recurrence in OSCC. Salivary levels of CYFRA 21-1 in OSCC patients have been reported to be three times higher compared to serum levels [28].

Tissue polypeptide-specific antigen (TPS)

It is a biomarker that originates from cytokeratin-18, and it is associated with a high rate of tumor proliferation. TPS has been implicated in various types of cancer, including nasopharyngeal carcinoma, oral squamous cell carcinoma, and head and neck squamous cell carcinoma. Its presence in the serum can serve as an indicator of tumor growth and response to therapy. Studies have indicated that the level of TPS in the bloodstream can be used to assess the effectiveness of treatment, as it typically decreases following therapy. Additionally, patients with lower levels of TPS have been observed to have longer survival rates after treatment. This demonstrates the prognostic value of TPS as a predictor of advanced disease [29].

Intracellular proteins

Mac-2 binding protein

Mac-2 binding protein is a crucial regulator of growth and motility in OSCC cells. Several studies have reported elevated levels of this protein in tissue, serum, and saliva samples [30,31].

Salivary zinc finger protein

Zinc finger proteins constitute the largest family of transcription factors in the human genome, with approximately 5926 members, and they play diverse roles in metabolism, differentiation, and autophagy. In addition to their DNA-binding properties, zinc finger proteins also interact with RNA, proteins, and lipids [32]. These proteins exhibit both oncogenic and tumor suppressor functions.

Salivary zinc finger protein 510 peptide

Among the zinc finger proteins, Zinc Finger Protein 510 (ZNF510) is involved in transcriptional regulation and predominantly localizes to the cell nucleus. Immunohistochemical analysis of tumors and saliva samples has shown elevated levels of a 24-mer peptide derived from ZNF-510. This peptide was not detected in the saliva of healthy individuals, and its levels were significantly correlated with tumor stage, with higher levels observed in T3 + T4 compared to T1 + T2 stages (n=45). In a systematic review, this peptide was identified as the only one to consistently increase with advancing tumor stage [33]. ZNF-510 shows great promise as a powerful marker for distinguishing early and late-stage OSCC, but further studies are needed to validate its utility.

Matrix metalloproteinase (MMP)

MMPs are important enzymes secreted by the tumor stroma that play a role in the degradation of the extracellular matrix, thereby facilitating local invasion and metastasis of oral cancer. Among the various enzymes in the MMP family, a systematic review has suggested that MMP-1 and MMP-3 could serve as potential markers for oral cancer. Salivary levels of MMP-1 and MMP-3 are significantly increased in OSCC and show an upward trend with higher tumor grades. MMP-9, which can degrade type IV collagen (a major component of the basement membrane), elastin, and fibronectin, has also been detected in the saliva of individuals with OSCC and Oral Potentially Malignant Disorders (OPMD). Matrix metalloproteinase 1, specifically known as interstitial collagenase, has also been identified as a potential biomarker (with an AUC value of 0.871) for distinguishing OSCC from healthy individuals [34].

Inflammation-related proteins

The relationship between cancer and chronic inflammation is well-documented, with inflammatory factors capable of either promoting or impeding tumor growth and progression [35]. Approximately 15% of cancers have been linked to inflammation [36], including lung, pancreatic, esophageal, bladder, gastric, cervical, colorectal, and prostate cancers [37]. In the context of OSCC, which arises from malignant transformation in the oral cavity, there is a gradual increase in the presence of inflammation in terms of both intensity and nature [38]. Extensive research has established a strong association between OSCC and chronic inflammation [39]. Conditions such as oral lichen planus, submucous fibrosis, and oral discoid lupus are known to heighten the risk of developing OSCC [40]. These conditions create an environment characterized by activated cytokines, chemokines, prostaglandins, reactive oxygen species, and transcription factors [41]. Some of these substances have the ability to induce cell proliferation, promote Epithelial-To-Mesenchymal Transition (EMT), and enhance invasion in lesions that harbor genetic mutations in tumor suppressor genes and/or oncogenes, thus fostering tumor development. Chronic inflammation is commonly observed in established OSCC and plays a role in tumor progression, invasion, and metastasis [42-44]. Consequently, numerous studies have explored the potential of various inflammatory molecules as prognostic markers and therapeutic targets for OSCC (Table 2).

Transcriptomic biomarkers

RNA plays a crucial role in cell metabolism as it is transcribed from DNA. A recent study utilizing massive parallel sequencing techniques characterized over 4000 coding and noncoding RNAs in the saliva of healthy individuals. The majority of the annotated genes, around 90%, belonged to the coding family, while the noncoding genes were mainly from the “small nucleolar RNA family”. The research on extracellular RNA has received funding from the National Institutes of Health (NIH) Common Fund’s Extracellular RNA Communication Program. This program aims to define the functions of extracellular RNA, create a reference catalog for different body fluids, identify biomarkers, and develop discovery tools. RNA in saliva can originate from sources such as blood, salivary glands, and the oral microbial flora, and a substantial portion of the sequenced reads do not align with the human genome. Approximately 20-25% of RNA reads in cell-free saliva align with the human genome (representing the eukaryotic transcriptome), while 30% of RNA sequences align with the human oral microbial genome database (representing the prokaryotic transcriptome). The presence of microbial RNA significantly affects the sensitivity of human RNA analysis [45]. Therefore, the whole saliva transcriptome includes both

Table 1: Inflammation-related proteins [39-44].

Molecular Pathway	Description
NF-κB Pathway	• A key inflammatory transcription factor commonly found in tumors, which regulates genes involved in inflammation, proliferation, tumorigenesis, and cell survival. • It is consistently active in OSCC and is linked to up-regulation of inflammatory genes, modulation of the tumor microenvironment, bone invasion, angiogenesis, invasion, metastasis, and epithelial-mesenchymal transition (EMT).
AP-1 Pathway	• This transcription factor complex controls genes related to inflammation, embryonic development, lymphoid proliferation, oncogenesis, and apoptosis. • It becomes activated during the development of oral keratinocyte carcinogenesis and its activity increases as oral tumors progress. • Its activation by interleukin-1 (IL-1) is associated with secretion of IL-8 and resistance to chemoradiation therapy. Overcoming this resistance may be possible by targeting IL-1.
TNF-α	• This multifunctional cytokine promotes cancer development, EMT, tumor angiogenesis, and invasion. It is expressed in potentially malignant disorders (OPMDs) and oral carcinomas, promoting a pro-invasive and pro-inflammatory phenotype, neutrophil recruitment, invadopodia formation, production of MMPs, and induction of cancer stem cells (CSCs). • Elevated signaling through tumor necrosis factor receptor-1 (TNFR-1) is associated with metastasis. Targeting tumor necrosis factor-alpha (TNF-α) shows promise for OSCC treatment.
IL-6 and IL-8	• Oncogenic cytokines induce EMT, stimulate angiogenesis, tumor growth, cell migration, invasion, and disrupt cell communication. Their levels are elevated in OPMDs and OSCCs. • These cytokines are associated with activation of nuclear factor-kappa B (NF-κB). Inhibition of NF-κB reduces viability, proliferation, and invasion of OSCC cells while enhancing proliferation, angiogenesis, and survival rate of cancer cells.
IL-1 Family Members	• IL-1α and IL-1β are consistently expressed in OSCC, promoting activation of NF-κB and AP-1, upregulation of IL-8 and IL-6, autocrine activation, proliferation, cytokine secretion by cancer-associated fibroblasts (CAFs), distant metastasis, and invasiveness. • Overexpression of IL-1 receptor 1 (IL-1R1) promotes cancer growth and metastasis. • IL-1 receptor antagonist (IL-1RA) is downregulated in oral dysplasia (OD) and OSCC, regulating the IL-1-induced secretion of IL-6 and IL-8.
COX-2	• This inflammation-induced enzyme promotes cancer stem cell-like activity, angiogenesis, proliferation, resistance to apoptosis, inflammation, invasion, and metastasis. • It is overexpressed in OSCC and correlates with advanced tumor stage, metastasis, and worse prognosis. It influences cell migration, lymphoangiogenesis, and maintains a chronic inflammatory state. • Inhibition of cyclooxygenase-2 (COX-2) reverses cancer progression.
TGF-β	• This multifunctional cytokine has context-dependent roles in OSCC. • It promotes tumorigenesis and is expressed at higher levels in OPMDs and OSCCs. Its expression is associated with disease recurrence and poor prognosis in OSCC.

eukaryotic and prokaryotic components. To accurately define true molecular signatures of human salivary RNA, steps such as centrifugation at low speed can be employed to remove microbial RNA. The human RNA molecules found in saliva encompass coding RNAs (messenger RNAs) as well as noncoding RNAs (such as microRNAs, piwi-interacting RNAs, small nucleolar RNAs, and circular RNAs). The saliva transcriptome represents a complex mixture, and the cell-free RNA in saliva can exist in either intact or fragmented forms. Intact RNA is predominantly derived from apoptotic bodies released from tumors, actively released exosomes, or circulation [46].

Messenger RNA

Although large interpatient variability is known to exist for mRNA, seven transcripts have shown significance in OSCC in several reports. They include interleukin-8 (IL-8) and interleukin-1B (IL-1B), dual specificity phosphatase 1 (DUSP1), ornithine decarboxylase antizyme 1 (OAZ1), S100 calcium-binding protein P (S100P), spermidine/spermine N1-acetyltransferase 1 (SAT), and H3 histone family 3A (H3F3A). Among the seven mRNAs, IL-8 and SAT were identified as top performers, in multiple OSCC cohorts and in a large sample (n = 395 patients). PRoBE studies validated six markers repeatedly demonstrating approximately two to four fold increase (ct values), highlighting their superiority. The mRNAs may arise locally from tumor tissue or due to a tumor-induced response [47].

Interleukin 8 (IL-8) and Interleukin 1 beta (IL-IB)

A team of researchers has achieved a successful connection between the IL8 protein, a potential marker for oral cancer, and IL8 mRNA using electrochemical sensors. This testing method has demonstrated high sensitivity and specificity when detecting IL-8 and IL8 mRNA, both individually and in combination. In a study conducted by Brinkmann et al., it was found that RNA molecules such as IL-8 and IL1B were significantly increased in OSCC. However, it should be noted that the levels of these inflammatory RNAs may be influenced in cases of chronic periodontitis [48].

Dual specificity protein phosphatase 1 (DUSP-1)

Dual specificity protein phosphatase 1, also known as DUSP-1, plays a vital role in activating the MAPK pathway, which is involved in protein modification, oxidative stress response, and signal transduction. The activity of DUSP-1 is regulated by the tumor suppressor protein p53, and abnormal hypermethylation of DUSP-1 has been implicated in the development of oral cancer. However, some studies have reported contradictory findings, indicating that the levels of DUSP-1 mRNA in saliva were either not significant or elevated in early-stage OSCC [49].

Ornithine decarboxylase antizyme (OAZ1)

OAZ1 influences the proliferation and differentiation of oral cancer cells by inhibiting polyamine production, which is necessary to prevent excessive cell proliferation. Stable expression of OAZ1 in squamous cell carcinoma cell lines leads to cell cycle

arrest in the G1 phase and promotes the formation of epithelial islands. OAZ1, acting as a tumor suppressor molecule, is involved in DNA double-stranded break repair and regulation of DNA methylation. Salivary OAZ1 mRNA levels have been found to correlate with OSCC patients during remission as well as in patients with oral lichen planus (OLP) [50].

S100 calcium binding protein P (S100P)

S100P, a calcium-binding protein, is known for its overexpression in various types of cancer [98]. It plays a multifaceted role in oncogenesis, including degradation of heat shock proteins (Hsp70 and Hsp90), interaction with the scaffolding protein IQGAP1 to affect downstream pathways of G protein-coupled receptors, and upregulation of the oncogene cyclin D1. Additionally, S100P actively participates in the regulation of cytoskeleton and microtubule assembly by binding to and activating ezrin. High expression of S100P mRNA has been observed in an "anoikis"-resistant OSCC cell line compared to an anoikis-sensitive OSCC cell line, indicating its involvement in cancer cell survival and metastasis. S100P has been identified as a reliable marker for OSCC, irrespective of the oral hygiene status in periodontitis [51].

Spermidine/spermine N1-acetyltransferase 1 (SAT1)

Spermidine/spermine N1-acetyltransferase 1, known as SAT1, is an acetyltransferase protein involved in the breakdown of polyamines. In a study by Brinkmann et al., SAT1 mRNA was among the four proteins identified in a transcriptome panel that showed elevated levels in late-stage OSCC. It was also found to be elevated in the studies conducted by Martin et al., along with IL-1β, IL-8, OAZ1, S100P, and DUSP1 [52].

H3. 3 Histone A (H3F3A)

H3F3A, is a nuclear protein responsible for maintaining the structural integrity of chromosomal nucleosomes, and mutations in the H3F3A gene have been associated with certain types of cancer [104,105]. H3F3A mRNA serves as a marker for cell proliferation. In a study by Gleber-Netto et al., combining H3F3A mRNA with IL-8 protein showed high accuracy in distinguishing between OSCC and OPMD. The potential of H3F3A mRNA as a biomarker was further validated in a multi-cohort study, providing strong evidence for its diagnostic potential [53].

Noncoding RNAs

In addition to the well-known messenger RNA (mRNA) landscape, there have been notable advancements in the study of noncoding RNAs (ncRNAs). These ncRNAs account for nearly 98% of all transcriptional output in humans. There are two types of ncRNAs, namely small noncoding RNAs that are approximately 200 base pairs long [108]. Recently, researchers have identified ncRNAs as potential biomarkers for OSCC. One advantage of ncRNAs over mRNAs is their resistance to degradation by RNase, making them more stable in body fluids such as urine, blood, cerebrospinal fluid, sweat, pleural discharge, and saliva. This stability holds promise for developing a saliva test for OSCC [54].

MicroRNA

MicroRNAs (miRNAs) are a significant class of noncoding RNAs and serve as crucial biomarkers in OSCC, exhibiting substantial fold changes in expression. These single-stranded RNA molecules are typically 19-23 nucleotides long and play important functional roles by binding to multiple mRNAs in a non-selective manner, thereby post-transcriptionally modifying over 30 mRNAs. The remarkable advantage of miRNA markers lies in their significantly higher fold change (10-1000 times higher expression) compared to messenger RNAs. A meta-analysis evaluating body fluid miRNAs as diagnostic markers for OSCC reported an overall diagnostic accuracy of 0.832. Several key miRNAs have been implicated in OSCC, including miR-125a, miR-200a, miR-31, miR-184, miR-27b, and miR-7. These miRNAs have demonstrated both downregulation and upregulation in OSCC. For instance, miR-125a and miR-200a are significantly downregulated, while miR-31 is frequently upregulated and oncogenic in plasma and saliva. Recently, miR-184 has been identified as a marker of malignant transformation in oral mucosa, exhibiting a threefold increase in OSCC and oral potentially malignant disorders compared to normal subjects. In a genome-wide study focusing on salivary RNAs, miRNA-27b was identified as a valuable marker for OSCC detection. Saliva offers an advantageous source for miRNA profiling due to its abundance, with the highest number of microRNAs detected among 12 tested body fluids, surpassing plasma levels. While some salivary miRNAs originate from plasma, the majority are released through regular cell turnover and lysis in the oral cavity, highlighting the local release of miRNAs from tumor tissue. However, saliva RNA poses challenges in analysis due to its vulnerability to RNase digestion and cumbersome handling issues. Moreover, elevated RNase activity has been observed in OSCC patients, further complicating the analysis of saliva RNA [54].

Circular RNA

In a customized bioinformatics report, researchers isolated over 400 circular RNAs (circRNAs) from cell-free saliva samples of healthy individuals. circRNAs are highly abundant in cells, surpassing the concentrations of linear RNA. They complement the role of microRNAs and have distinct characteristics, including their circular structure without a 5' cap or 3' poly A tail. circRNAs act as microRNA sponges, competitively suppressing microRNA activity, and also function as transcriptional regulators through interactions with RNA binding proteins. Additionally, they can modulate the expression of their parent genes by accumulating around the transcription site. circRNAs are predominantly found in the nucleus, and their knockdown leads to the repression of parent genes. The interaction between circRNAs and microRNAs plays a critical role in cancer signaling pathways [55]. The CircInteractome web tool (http://circinteractome.nia.nih.gov) is available for exploring the interactions between circRNAs, proteins, and mRNA. The stability of circRNAs is superior to that of mRNAs, as they have a half-life (t1/2) of approximately 48 hours, four times longer than that of mRNAs, which contributes to their potential as biomarkers [56]. The absence of free ends in circRNAs makes them resistant to the action of debranching enzymes and exonucleases. Specific circRNAs have been implicated in oral cancer, such as CDR1as (or ciRS-7), which acts as a sponge for miRNA 7 and has been associated with tongue cancer [57]. Another circRNA, ci-mcm5, enhances the expression of MCM5, which is associated with early stages of oral neoplasia, progression, and poor prognosis [58]. Recently, circRNA_100290 was identified as a critical regulator of OSCC development through its interaction with members of the miR-29 family [59]. Although there is limited evidence linking circRNAs and OSCC in the current literature, circRNAs represent a promising addition to the RNA biomarker repertoire, and their identification in saliva could be a new trend in OSCC detection [60].

Conclusion

In conclusion, proteomic and transcriptomic biomarkers have emerged as promising tools for the early detection, diagnosis, and management of OSCC. The identification of specific proteins and RNA molecules associated with OSCC has provided valuable insights into the molecular mechanisms underlying the disease. Proteomic biomarkers, such as cell surface glycoproteins, cytoskeleton fragments, intracellular proteins, MMPs, and inflammation-related proteins, have shown potential for use in OSCC diagnosis and prognosis. Furthermore, transcriptomic biomarkers, including coding and noncoding RNAs, have opened new avenues for understanding the complex interactions between human and microbial factors in OSCC development. The analysis of the whole saliva transcriptome offers valuable insights into the role of the oral microbiota in disease progression. However, further research and validation are needed to fully exploit the potential of these biomarkers in clinical practice. Rigorous validation studies, involving large patient cohorts and diverse populations, are necessary to establish the reliability and accuracy of these biomarkers. Standardization of sample collection, processing, and analysis methods is also essential to ensure reproducibility and comparability of results across different studies.

In the future, the integration of proteomic and transcriptomic approaches may provide a more comprehensive understanding of OSCC and lead to the development of personalized diagnostic and therapeutic strategies. Advances in high-throughput technologies, such as next-generation sequencing and mass spectrometry, will facilitate the identification of novel biomarkers and improve their sensitivity and specificity. Moreover, the application of artificial intelligence and machine learning algorithms can enhance the analysis and interpretation of complex proteomic and transcriptomic data, enabling the development of more accurate and efficient diagnostic models. Overall, the identification and validation of proteomic and transcriptomic biomarkers hold great promise for improving the early detection, diagnosis, and management of OSCC. Continued research efforts in this field will contribute to better patient outcomes, reduced disease burden, and personalized approaches to OSCC treatment in the future.

Declarations

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Conflicts of interest: The authors declare that there are no conflicts of interest.

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