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REVIEW

Non-Invasive Techniques for Early Detection of Oral Squamous Cell Carcinoma: A Narrative Review

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Abstract: Oral squamous cell carcinoma (OSCC) is the most common malignant tumor of the oral cavity, accounting for the majority of oral cancers, and early detection is crucial for improving patient survival rates and prognosis. Traditional diagnostic methods have limitations, including invasiveness and diagnostic delays, and are insufficient for early detection and distinguishing between similar diseases. In recent years, with the rapid advancement of molecular biology and biotechnology, a variety of emerging non-invasive diagnostic approaches have provided new strategies for early screening and precise diagnosis of OSCC. This review summarizes the cutting-edge technologies in OSCC diagnosis in recent years, including biomarker-based detection (such as microRNA, circRNA, gene methylation, and salivary proteomics), oral microbiome analysis, optical imaging technologies combined with artificial intelligence, and more. These emerging methods not only offer non-invasive or minimally invasive advantages but also enable the detection of potential molecular changes in the early stages of the disease, allowing for early intervention. Despite the challenges in standardization, sensitivity, and specificity optimization that these new technologies face in clinical applications, they undoubtedly offer vast prospects for early detection and personalized treatment of OSCC. This review aims to achieve the following objectives: First, to systematically evaluate the latest research evidence on various emerging non-invasive diagnostic technologies; second, to comprehensively compare their advantages and limitations relative to traditional methods; and finally, to attempt constructing a clinical translation assessment framework for early-stage multimodal diagnostic technologies in OSCC, thereby guiding future translational strategies.

Keywords: OSCC, emerging diagnostic approaches, biomarkers, oral microbiome, optical imaging

Introduction

Oral squamous cell carcinoma (OSCC) is a malignant tumor that typically occurs in the lip vermilion, the junction of the hard and soft palates, or the posterior third of the tongue. It is characterized by varying degrees of differentiation, high invasiveness, and early widespread lymph node metastasis. OSCC predominantly affects individuals aged 40–70 years who have a history of smoking and alcohol consumption.[1] In the United States, OSCC accounts for 3% of cancers in men and 2% in women, with an annual secondary tumor formation rate of 3–7%.[2] OSCC ranks as the predominant form of oral cancer globally, with the disease often presenting without significant symptoms in its early stages, leading to late diagnosis, high incidence, and high mortality rates. Therefore, timely diagnosis of OSCC in its early stages is of crucial importance, as it can effectively prevent disease progression. Traditional diagnostic methods for OSCC include biopsy, endoscopic examination for second primary cancers, and CT scans. With advances in medical science, a number of emerging diagnostic techniques have been developed to provide a more accurate and early detection of OSCC. This review aims to summarize the latest research on emerging diagnostic methods for OSCC, with the goal of facilitating the development of standard diagnostic approaches for OSCC in the future.

Although various research methods have emerged in the diagnosis of oral squamous cell carcinoma, there is currently a lack of systematic integration regarding the technological status, challenges, and translational potential. Therefore, based on the evidence-based review integration theory, this review clearly outlines the technological landscape, identifies core issues, and explores translational prospects to achieve well-defined research objectives. First, this review systematically reviews

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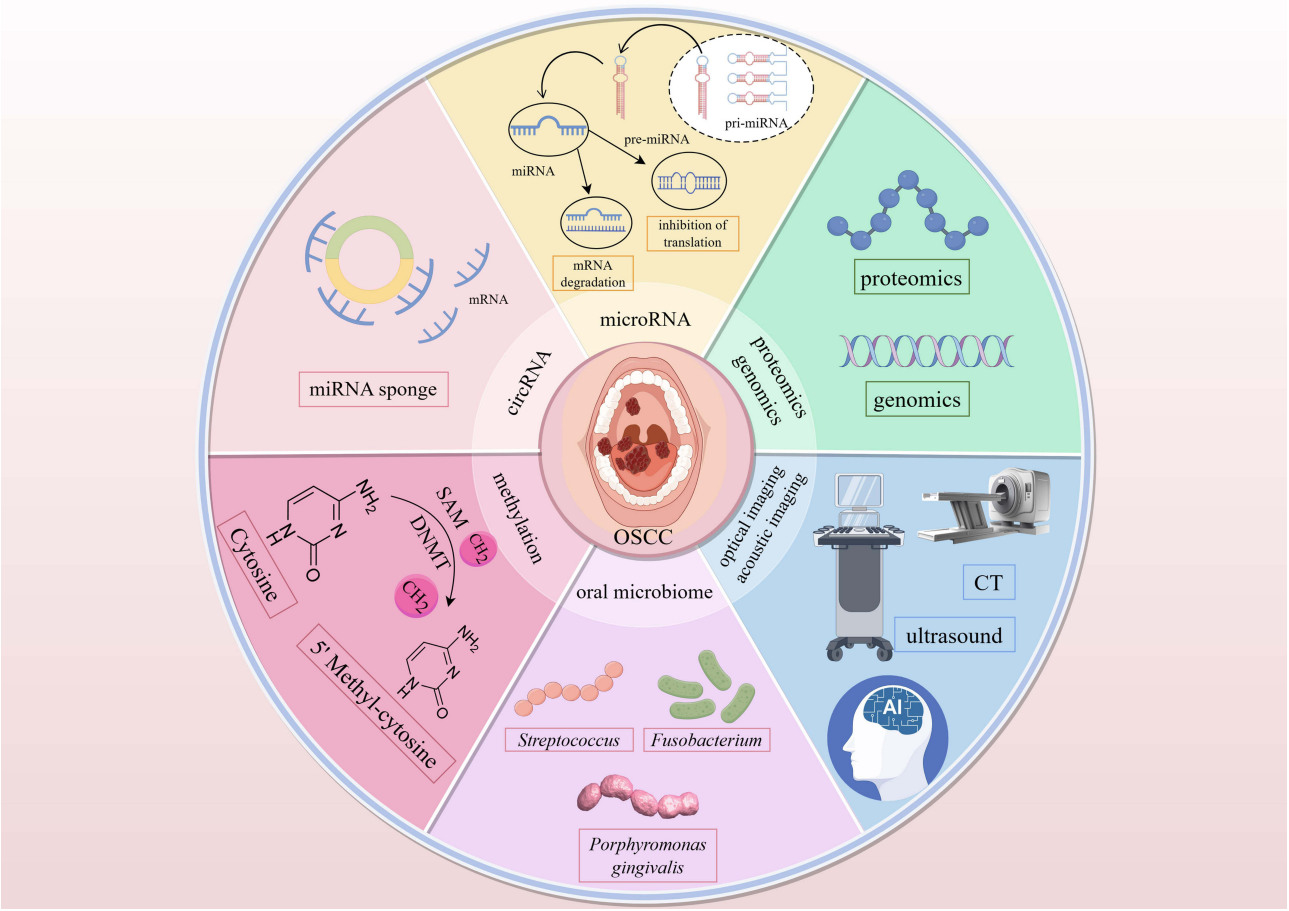
Graphical Abstract



emerging technologies in the field of early diagnosis of oral squamous cell carcinoma in recent years, including biomarker detection (microRNA, circRNA, gene methylation, salivary proteomics), oral microbiome analysis, and optical imaging combined with artificial intelligence diagnosis. It clarifies the core principles and current research status of each technology. Second, it compares and contrasts the advantages and limitations of different emerging diagnostic technologies, providing researchers in related fields with a comprehensive and up-to-date reference on diagnostic technology advancements and clarifying current research hotspots. Finally, we aim to explore the clinical translation potential and application prospects of various emerging technologies, offering theoretical support for advancing the early, precise diagnosis and personalized treatment strategies for oral squamous cell carcinoma.

Specific Diagnosis of OSCC Through Abnormal Expression of microRNA (miRNA)

miRNAs are a class of endogenous non-coding RNAs, typically around 22 nucleotides in length, that exhibit a wide variety of types. miRNAs show complementary sequence interactions with their corresponding mRNAs, enabling them to target mRNA molecules through base pairing. This interaction can lead to mRNA degradation and inhibition of protein translation, thereby affecting gene expression and regulating various cellular processes.[3] Changes in miRNA expression result in alterations in gene profiles that are implicated in diverse biological processes, contributing to the development of various human diseases. miRNAs are highly stable in human body fluids and are less likely to undergo sequence alterations. Circulating miRNAs in body fluids are considered promising biomarkers for disease diagnosis and

prognosis.[4–6] Current literature indicates that during the development of OSCC, the miRNA expression profile in affected oral mucosal cells undergoes specific changes, resulting in a distinctive expression profile associated with the disease. miRNAs, as one of the key factors in OSCC, regulate various cancer-related signaling pathways, including the Wnt, EGFR, PIK3CB and K-ras, Notch, MAPK, and TGF-β signaling pathways, thereby contributing to disease initiation, progression, and metastasis.[7,8] When miRNA expression is dysregulated, such as overexpression or loss of expression, the expression of target genes associated with these processes in OSCC is also specifically affected,[9] further influencing the development of OSCC. Current research has confirmed that detecting abnormal miRNA expression in patients can provide specific diagnostic information for OSCC. This diagnostic method not only allows for distinguishing OSCC from other similar oral diseases but also enhances diagnostic accuracy. miRNAs are widely distributed throughout the human body, found in diverse body fluids, intracellular fluids, saliva, and exosomes, and have significant effects on related biological activities.

Expression Changes of Specific miRNAs in OSCC

A literature review reveals that numerous studies have successfully utilized the abnormal expression of various miRNAs to uncover their role in the clinical diagnosis of OSCC. Nicklas Juel Pedersen et al[10] conducted a miRNome profiling of OSCC and normal oral mucosa (NOM) and identified 567 miRNAs in the OSCC miRNome, with 66 miRNAs showing differential expression between OSCC and NOM. Mayakannan Manikandan et al[11] used microarray analysis to identify 46 miRNAs with differential expression in OSCC and verified the expression of 10 miRNAs via RT-qPCR. They found that let-7a, let-7d, let-7f, and miR-16 were declined in OSCC, whereas miR-29b, miR-142-3p, miR-144, miR-203, and miR-223 were elevated. Additionally, miR-1275 expression varied across tumors, and miR-223 was linked to advanced tumor stages and size. Zhong-Yi Yan et al[12] evaluated a large miRNA profile in OSCC samples and identified seven key miRNAs. Notably, hsa-miR-21, hsa-miR-31, and hsa-miR-338 were notably upregulated, while hsa-miR-125b, hsa-miR-133a, hsa-miR-133b, and hsa-miR-139 were significantly downregulated. Kai-Rui Lu et al[13] examined miR-2355-3p expression utilizing quantitative real-time polymerase chain reaction (qRT-PCR) and conducted a series of experiments comparing the expression in OSCC patients and NOM. They found that miR-2355-3p was elevated in OSCC and could facilitate the radiosensitivity of OSCC cells by targeting SERPINA3. Dan Yang et al[14] collected OSCC tissue samples and adjacent non-cancerous tissue for studying the expression of lncRNA CASC9 and miR-125a-3p. Their analysis revealed that lncRNA CASC9 expression was higher in OSCC tissues, whereas miR-125a-3p expression was lower. Furthermore, there was a negative linkage between the two in OSCC tissues. High expression of lncRNA CASC9 and low expression of miR-125a-3p showed linkage with poor differentiation, advanced TNM stage, lymph node metastasis, and prognosis in OSCC patients. Padmavathi Saravana Murthy et al[15] analyzed patient tissue samples utilizing RT-PCR to examine miRNA-15a-5p and its target gene YAP1. Their findings revealed downregulation of miRNA-15a-5p and overexpression of YAP1 in OSCC patients relative to normal tissues, suggesting its potential as a novel OSCC biomarker. Similarly, Yan Guo et al[16] employed qRT-PCR to assess miR-182-5p expression, identifying its upregulation in OSCC. This miRNA contributed to tumor migration and invasion, correlating with lower differentiation and advanced T and N stages.

Non-Invasive Diagnosis Using miRNA Extracted from Serum, Saliva, and Other Body Fluids

Compared to conventional diagnostic methods, non-invasive diagnostics are relatively easier to apply in clinical settings.[17] Non-invasive diagnostic approaches primarily include serum and saliva testing, among others. Numerous studies have demonstrated the diagnostic value of saliva. Non-invasive diagnostic methods are particularly valuable for groups requiring special care, as well as for large-scale screenings and epidemiological studies. Although biomolecule concentrations, such as hormones and miRNAs, in saliva are extremely low, highly sensitive testing protocols have been developed, making saliva testing a feasible option.[18,19] Additionally, serum and saliva, as alternative diagnostic fluids, offer unique advantages, including ease of collection and the possibility of repeated sampling.[20] Existing literature has highlighted the potential of salivary RNA biomarkers for diagnosing oral cancer and other diseases. Salivary RNA can also aid in oral microbiome profiling and gene expression analysis, offering insights into oral cancers and related conditions.[21] Studies have shown that combining blood and salivary miRNAs for diagnosing OSCC yields high

accuracy,[22] with both fluids individually showing high diagnostic accuracy. Furthermore, oral bacteria, specific gene expression, and the oral microbiome can also serve as diagnostic methods for OSCC. Therefore, saliva has become a promising modality for future research and clinical diagnosis. In terms of diagnostic methods, evaluating miRNA and mRNA expression levels in saliva or serum through PCR screening is considered one of the best approaches for early detection of OSCC. These biomarkers have significant potential for non-invasive early detection of OSCC.[20]

Upon reviewing the literature, numerous studies have leveraged the specific expression changes of serum and salivary miRNAs to diagnose OSCC. Lei Meng et al[23] analyzed serum samples from OSCC patients using PCR and Western blotting, revealing downregulation of miR-379-5p and reduced ROR1 expression. Their findings suggest miR-379-5p negatively modulates ROR1 in OSCC. Naghmeh Bahrami et al[24] extracted RNA and synthesized cDNA, then used RT-PCR to assess the expression levels of miR-24, miR-200, and miR-34 in salivary samples. They found reduced levels of miR-200 and miR-34 in the saliva of OSCC patients relative to healthy controls, while miR-24 was upregulated. In a similar study, Chiara Romani et al[25] employed microarray and RT-qPCR normalization to identify 25 miRNAs with altered expression between OSCC patients and healthy controls, 7 of which were linked to disease-free survival (DFS). Among these, miR-106b-5p, miR-423-5p, and miR-193b-3p were elevated in OSCC saliva, and their combined expression offered strong diagnostic potential. Momen-Heravi F et al[26] identified 13 significantly dysregulated miRNAs from over 700 analyzed, with 11 miRNAs being downregulated and miR-27b notably upregulated in OSCC saliva, including in patients with remission-phase OSCC and oral lichen planus. This miRNA has diagnostic value and can distinguish between OSCC patients and those in remission or with similar diseases. Nayroz Abdel Fattah Tarrad et al[27] analyzed unstimulated saliva samples using qRT-PCR, identifying LINC00657 and miR-106a as potential OSCC diagnostic markers. Their study demonstrated high accuracy in distinguishing OSCC from potentially malignant oral diseases. Maryam Koopaie et al[28] analyzed unstimulated whole saliva from OSCC patients and healthy controls using qRT-PCR, revealing notably lower levels of miR-15a and miR-16-1 in OSCC. Their findings suggest these salivary miRNAs as potential non-invasive biomarkers for early OSCC detection. Similarly, Rocchetti Federica et al[29] examined saliva and plasma samples via real-time PCR, identifying significant downregulation of miR-138 and miR-424 in OSCC patients compared to controls.

Diagnosis Using Exosomal miRNA as Biomarkers

Exosomes, small vesicles measuring around 40–100 nm in size, are secreted by a variety of cell types into the extracellular environment and can be found in a diverse range of bodily fluids.[30] Research has demonstrated that exosomes carry mRNA and miRNA, which, upon release, can be taken up by neighboring or distant cells. The miRNAs within exosomes are critical in modulating processes such as immune response, microenvironment interactions, and tumor progression, contributing to tumor growth, invasion, metastasis, angiogenesis, and drug resistance.[31] Exosomes are particularly implicated in angiogenesis, the formation of new blood vessels from pre-existing ones, which is a crucial mechanism for tumor cells to access the bloodstream from the primary tumor.[32,33] Hypoxia is a common feature of solid tumors, linked to invasiveness and poor prognosis. Angiogenesis is necessary to provide the required oxygen and nutrients for tumor development and metastasis. Research findings unveil that exosomes are pivotal in facilitating tumorigenesis by enhancing angiogenesis. In solid tumors, adequate blood supply marks tumor progression, growth, and metastasis, with tumor cells releasing large amounts of exosomes containing various biomolecules, such as angiogenesis-related molecules. Given the close relationship between exosomes and angiogenesis, tumor progression, and metastasis, tumor-derived exosomes can serve as biomarkers for cancer diagnosis.[34]

Clinically, there have been studies that diagnose and predict the prognosis of OSCC by detecting exosomal miRNA expression, confirming their potential as diagnostic and prognostic biomarkers: Tao He et al[35] isolated exosomes from the plasma samples of 184 OSCC patients and 196 healthy controls before surgery. They also obtained primary OSCC and adjacent non-cancerous tissues from 47 OSCC patients. Through qRT-PCR, they analyzed miR-130a expression and found that exosomal miR-130a levels were higher in OSCC patients relative to healthy controls ($p < 0.0001$). Additionally, elevated miR-130a was detected in OSCC tissues relative to the paired control tissues ($p < 0.0001$). Li Hong He et al[36] used miRNA microarray analysis to identify differentially expressed miRNAs in salivary exosomes from four healthy individuals and four OSCC patients. They further validated miR-24-3p expression employing qRT-PCR, revealing a marked increase in OSCC-derived exosomes relative to controls. These findings demonstrated that exosomal

miR-24-3p may serve as a novel diagnostic biomarker for OSCC. Wei Yan et al[37] found that protein expression of phosphatase and tensin homolog (PTEN) was decreased on chromosome 10 in OSCC patients, but there was no reduction in mRNA expression. Their results showed that miR-130b-3p, a key post-transcriptional regulator, negatively modulated PTEN expression. Further in vitro and in vivo experiments revealed that exosomal miR-130b-3p plays a crucial role in OSCC progression and angiogenesis, shedding light on its potential as both a biomarker and therapeutic target. Ching-Mei Chen et al[38] performed comparative exosomal miRNA profiling between primary tumors and normal oral epithelial cells in OSCC patients. Their findings, validated using qRT-PCR on serum-derived exosomes, indicated that miR-155 and miR-21 were significantly upregulated, while miR-126 was notably downregulated. Both miR-155 and miR-21 exhibit oncogenic properties, inhibiting PTEN and Bcl-6 expression, whereas miR-126 functions as a tumor suppressor by downregulating EGFL7 in OSCC. Ling Li et al[39] conducted miRNA sequencing on exosomes secreted under normoxic and hypoxic OSCC conditions, identifying miR-21 as one of the most highly upregulated miRNAs in hypoxia. Their research suggests that tumor cells in a hypoxic microenvironment release miR-21-enriched exosomes, which are subsequently taken up by normoxic cells, promoting metastatic properties.

Although exosomal miRNAs have not been fully explored to date, they have shown diagnostic potential in clinical settings. Exosomal miRNAs may serve as promising biomarkers with diagnostic potential.

In clinical practice, diagnosing OSCC by detecting miRNA expression not only improves diagnostic accuracy and efficiency but also provides valuable evidence to distinguish OSCC patients from healthy individuals, as well as differentiate between other similar diseases. Furthermore, since miRNAs exhibit specific expression differences across various tissues, developmental stages, and disease states, some miRNAs can be used to determine the degree of differentiation, TNM staging, and prognosis of OSCC, offering significant diagnostic and prognostic value.

Diagnosis of OSCC Using Differential Expression of Circular RNAs (circRNAs)

circRNAs are a type of single-stranded RNA molecules with a covalently closed structure that can be found in a wide range of organisms, including viruses and mammals.[40] It has been demonstrated that circRNAs play important regulatory roles in diverse cancers in humans, generally formed by back-splicing of the covalently linked 3' and 5' ends. These RNAs have been shown to interact with miRNAs, which are co-contributors to cancer development. circRNAs act as miRNA sponges, regulating mRNA expression, and establishing a circRNA-miRNA regulatory axis. In OSCC, the overexpression of different circRNAs has shown both tumor-promoting and tumor-suppressive roles, thus, the differential expression of circRNAs in relevant tissues can serve as a preliminary diagnostic tool for OSCC.

Clinical Use of circRNA in OSCC Diagnosis

Although the potential of circRNAs in tumor-related immune evasion and their associated mechanisms remains inconclusive, extensive research has proven that circRNAs are closely linked to the progression of OSCC by modulating miRNA expression in some cells. Yiyang Chen et al[41] conducted luciferase activity assays to examine the interactions between has_circ_0069313, miR-325-3p, and its downstream target miR-325-3p-Foxp3. They found that has_circ_0069313, an exosomal circRNA, could promote immune evasion by inhibiting the degradation of Foxp3 induced by miR-325-3p. This gene also transferred to T regulatory (Treg) cells, promoting Treg cell function by maintaining Foxp3 levels. Similarly, Ling Gao[42] identified circ-PKD2 as a sponge for miR-204-3p, demonstrating that the downregulation of circ-PKD2 in OSCC is significantly associated with aggressive tumor features. In another study, circ-PVT1 was shown to act as a sponge for miR-106a-5p, with miR-106a-5p binding to its target hexokinase II (HK2) to exert antitumor properties in OSCC by inhibiting HK2. Therefore, in OSCC tissues and cells, circ-PVT1 expression is abnormally high, while miR-106a-5p is downregulated. Clinically, quantifying circ-PVT1, miR-106a-5p, and HK2 levels using methods such as qRT-PCR can serve as diagnostic standards for OSCC.[43] Several studies have also confirmed that circRNAs are involved in glycolysis. For instance, the overexpression of circ-KIAA0907 inhibits the migration, invasion, and glycolysis of OSCC cells while promoting apoptosis and radiosensitivity. Mechanistically, circ-KIAA0907 can absorb miR-96-5p to modulate UNC13C,[44] a finding supported by related studies. Extensive research highlights the interplay between circRNAs and miRNAs, where circRNAs modulate oncogenes and tumor suppressors, impacting OSCC onset and progression. Han L et al[45] explored hsa_circ_0072387 in OSCC, finding its reduced expression in cell

lines and tumor tissues, alongside miR-503-5p upregulation. Functional assays showed that hsa_circ_0072387 overexpression or miR-503-5p silencing inhibited proliferation, migration, invasion, epithelial-mesenchymal transition (EMT), and glycolysis in OSCC cells. Additionally, restoring miR-503-5p expression counteracted the tumor-suppressive effects of hsa_circ_0072387, indicating a regulatory interplay between these molecules. These findings suggest that alterations in hsa_circ_0072387 and miR-503-5p expression could serve as potential biomarkers for OSCC diagnosis and progression monitoring.

Reasons Why circRNA Can Be Considered a Promising Molecular Biomarker

Recently, the significance of circRNAs in disease diagnosis has gradually gained attention, with clear advantages emerging in its diagnostic applications. Due to its covalently closed circular structure, circRNA is far less susceptible to degradation by RNA nucleases compared to linear RNAs, giving it exceptional stability both in vivo and in vitro.[40] This feature allows circRNAs to persist for extended periods in bodily fluids such as blood, saliva, and urine, making them reliable biomarkers for liquid biopsy. Particularly in oncology and neurodegenerative diseases, circRNAs can serve as long-lasting, easily detectable molecules, providing a longer diagnostic window. Moreover, the non-invasive nature of circRNA acquisition offers significant convenience for the diagnosis of OSCC. CircRNAs can be extracted from serum, plasma, and saliva without relying on tumor tissue or other directly sourced cells. Compared to traditional tissue biopsies, liquid biopsy greatly reduces patient discomfort and facilitates regular monitoring, offering substantial advantages in early disease diagnosis, monitoring, and prognostic assessment. Furthermore, circRNAs exhibit high sensitivity and can provide disease-specific diagnoses. Studies have found that circRNA expression notably differs across various cancers, metabolic diseases, cardiovascular diseases, and other conditions, closely correlating with disease progression. Based on their specificity, circRNAs can differentiate between various diseases, and show notable alterations even in the early stages, making them promising markers for early detection. With the advancement of high-throughput sequencing technologies, the detection of circRNAs has become more efficient and cost-effective. Compared to traditional diagnostic methods, high-throughput technologies can quickly and accurately identify disease-related circRNAs at a relatively low cost. This makes circRNA detection widely applicable in clinical practice. In the future, circRNAs are expected to play an increasingly important role in the early detection, monitoring, and treatment of diseases.

Diagnosis of OSCC Through Detection of Cancer Suppressor Gene Methylation and Gene Promoter Methylation Status

In recent years, epigenetic research has shown that DNA methylation is crucial in the initiation and progression of OSCC, particularly through hypermethylation of the promoter regions of tumor suppressor genes, leading to the silencing of their expression and thus promoting carcinogenesis. Therefore, the methylation status of specific genes can serve as potential diagnostic and prognostic biomarkers for OSCC.[46] Yu-Fen Li et al[47] identified biomarkers for OSCC and found that methylation of FLT4 exhibited excellent specificity for detecting OSCC. Using RT-PCR and pyrosequencing to measure methylation levels, they validated the RNA expression and methylation status of FLT4. The median FLT4 expression level in normal tissue samples was 2.14 times higher than in OSCC tissue samples. Similarly, Luca Morandi et al[48] compared 355 CpG sites between OSCC and normal healthy donors as well as the contralateral mucosa. They identified that ZAP70, ITGA4, KIF1A, PARP15, EPHX3, NTM, LRRTM1, FLI1, MIR193, LINC00599, PAX1, and MIR137HG were highly methylated in OSCC patients, while MIR296, TERT, and GP1BB were hypomethylated. The expression levels of ZAP70, GP1BB, H19, EPHX3, and MIR193 fluctuated across different CpG sites. Similarly, Le Chen[49] found that OSCC patients exhibiting CpG island methylation phenotypes had a lower survival rate compared to those without such methylation. CpG sites cg02860732 and cg04342955 have been identified as potential epigenetic biomarkers for the diagnosis of OSCC. Beyond their diagnostic relevance, methylation patterns of specific genes have also been linked to patient survival outcomes. Notably, the expression levels of genes associated with these CpG sites, including AJAP1, SHANK2, FOXA2, MT1A, ZNF570, HOXC4, and HOXB4, demonstrated a notable correlation with OSCC prognosis.[50]

The RECK gene, identified as a novel tumor suppressor gene in recent studies, encodes the RECK protein, a glycoprotein localized on the cell membrane. Research has shown that the expression of RECK and matrix metalloproteinases (MMPs),

which are negatively regulated by RECK, are both abnormal in squamous cell carcinoma. Liu Xizhang et al[51] employed immunohistochemistry to analyze RECK protein expression in OSCC tumor tissues and NOM samples. They found that the high expression rate of RECK in OSCC tissues was evidently lower than in normal tissues, and that RECK expression was related to TNM staging, differentiation, and lymph node metastasis. As a tumor suppressor gene, RECK shows reduced positive expression and low expression rates in OSCC tissues. Clinically, the expression level of RECK protein in oral mucosa can be used for OSCC diagnosis, and RECK gene promoter hypermethylation can also serve as a diagnostic tool. Nguyen Khanh Long et al[52] applied methylation-specific PCR (MSP) to assess the methylation status of the RECK gene in OSCC and NOM tissues. Their analysis revealed hypermethylation of RECK in both primary tumor tissues and adjacent mucosa, while no methylation was detected in healthy control samples. Since RECK hypermethylation suppresses gene expression, leading to lower RECK protein levels, it is considered a frequent epigenetic alteration in OSCC. Given that hypermethylation occurs in both cancerous and adjacent mucosa, assessing the methylation level of the RECK promoter in these tissues may serve as a potential OSCC diagnostic marker. Seiji Baba et al[53] also employed MSP to examine the methylation status of the CHFR gene in 49 primary OSCC cases and six OSCC cell lines. In a subset of 13 cases, adjacent NOM tissues were analyzed, revealing that CHFR hypermethylation was present in some OSCC cases but occurred at low frequencies in adjacent normal tissues. These findings suggest that CHFR gene methylation may represent a potential biomarker for OSCC diagnosis. AAl-Kaabi et al[54] analyzed data on hypermethylation of the p16 (INK4A) and p14 (ARF) gene promoters, finding that hypermethylation of these genes had predictive value for various clinicopathological outcomes and could serve as molecular markers for OSCC prediction. Goot Heah Khor et al[55] conducted a DNA methylation profile analysis to screen for differentially methylated genes in OSCC and identified 33 genes with hypermethylated promoters, which were significantly silenced in OSCC and potentially involved in the carcinogenic mechanisms of oral cancer. Among these, DDAH2 and DUSP1 were highlighted as candidate genes for OSCC, which could serve as potential biomarkers. PSSushma et al[56] compared 50 OSCC biopsy samples and non-malignant controls, identifying a correlation between methylation status and clinical outcomes. Their study suggested that PTEN and p16 downregulation via methylation may drive OSCC progression and aid prognosis, highlighting promoter methylation as a potential biomarker for early detection. Similarly, Keizo Kato et al[57] found p16 and MGMT hypermethylation in both OSCC and adjacent normal mucosa, suggesting it as an early event in OSCC development and a promising diagnostic marker. Muthusamy Viswanathan et al[58] examined the promoter methylation status of several key tumor suppressor genes, including p16, p15, hMLH1, MGMT, and E-cadherin, in OSCC. Their study analyzed 51 p15 gene samples along with 99 samples of other tumor suppressor genes, identifying abnormal methylation patterns in OSCC cases, regardless of tumor stage or anatomical location. In contrast, no methylation abnormalities were detected in normal oral squamous tissues obtained from 25 OSCC. These findings suggest that the detection of abnormal hypermethylation patterns in cancer-related genes could be used for diagnosing high-risk OSCC patients. RCzerninski et al[59] used sodium bisulfite modification of DNA followed by MSP to study the methylation status of two MMR genes, hMLH1 and hMSH2, in 28 OSCC cases. They found high promoter methylation in 14 of the cases. Notably, 100% of patients with multiple oral malignant tumors exhibited hypermethylation of hMLH1 or hMSH2, while only 31.5% of patients with single tumors exhibited the same, suggesting that hypermethylation of MMR genes is closely linked to OSCC and may serve as a diagnostic marker for the disease.

In addition to focusing on the methylation of cancer-related genes, recent studies have also investigated the role of gene promoter methylation in diagnosing OSCC. For example, research has demonstrated that the CpG island region of the TGM-3 gene promoter exhibits notably higher methylation in OSCC tissues compared to normal tissues, with the methylation level closely correlated with tumor stage and grade.[60] Moreover, the promoter regions of p16INK4A and p14ARF genes frequently undergo hypermethylation, which is commonly associated with the loss of cell cycle regulation and further promotes tumorigenesis.[54] Methylation detection of these genes thus offers a promising tool for the early detection of OSCC. Particularly under the influence of environmental factors such as smoking and alcohol consumption, changes in methylation patterns are more pronounced, highlighting that these external factors may promote promoter methylation in OSCC.[61] It is noteworthy that environmental factors like smoking and alcohol consumption can induce DNA methylation alterations, thereby increasing the risk of OSCC development. These factors may influence DNA methylation and the activity of DNA methyltransferases, leading to the silencing of tumor suppressor genes and activation of oncogenes.[62]

Gene promoter methylation represents one of the earliest epigenetic changes in tumorigenesis and can be detected in the early stages of OSCC with high sensitivity. Additionally, the methylation of specific genes, such as p16 and TGM-3, is highly specific to OSCC, enabling the effective differentiation of cancerous tissues from normal tissues. Furthermore, the non-invasive nature of this method and its applicability in large-scale screening offer significant advantages. However, due to individual variability in gene methylation patterns, methylation profiles may differ across patients. Existing methylation biomarkers still require further clinical studies and validation to determine which markers are universal and stable across different populations and clinical stages. At present, the clinical use of methylation for diagnosing OSCC is limited, and research in this area remains relatively scarce. While methylation markers are considered to have diagnostic potential, their practical application is still emerging and will likely develop as time progresses and techniques improve.

Diagnosis of OSCC Through Oral Microbiome Composition Differences

The oral microbiome is a complex ecosystem composed of bacteria, fungi, viruses, and archaea.[63] Research has suggested that certain oral bacterial species, including *Porphyromonas gingivalis, Fusobacterium nucleatum, Streptococcus* species, as well as oral viruses including human papillomavirus (HPV), human herpesvirus 8 (HHV-8), herpes simplex virus 1 (HSV-1), Epstein-Barr virus (EBV), and fungi like *Candida albicans* are linked to OSCC development.[64] Although the critical mechanisms by which the oral microbiome affects OSCC progression are not yet fully understood, clinical applications of microbiome composition differences for OSCC diagnosis are already underway. Studies have confirmed that microbial dysbiosis in the oral environment significantly impacts OSCC development.[65] The underlying mechanisms include stimulating cell proliferation, promoting tumor invasion and angiogenesis, inhibiting apoptosis, inducing chronic inflammation, and generating carcinogenic metabolites.[66] Through oral biopsy samples, data regarding the types, quantities, activities, and distribution of oral microorganisms can be collected. These data show significant differences when compared to those in other similar diseases, as different diseases typically exhibit distinct dominant bacterial species and microbial compositions.[67] The use of the oral microbiome as a diagnostic marker for OSCC has been demonstrated as a promising approach. Furthermore, recent evidence has documented that the oral microbiome can also be utilized to analyze the prognosis and staging of OSCC, thus offering substantial clinical application value.

Diagnosis of OSCC Through Oral Microbiome Composition

The richness of the oral microbiome and the relative abundance of certain bacterial genera have been experimentally shown to serve as potential diagnostic markers for OSCC. For example, Weiwei Heng et al[68] demonstrated that compared to control groups and patients with oral precancerous lesions, patients with OSCC exhibited a significant increase in bacterial diversity in cheek swabs and dental plaque samples, while the bacterial diversity in saliva samples was evidently decreased. Although *Firmicutes* is the most abundant phylum in the bacterial kingdom, the relative abundance of *Firmicutes* was notably diminished in dental plaque and saliva samples from OSCC patients relative to the control group (decreased by 25% and 46%, respectively). In addition, *Bacteroidetes, Fusobacterium, Proteobacteria*, and *Actinobacteria* were notably increased, while TGF-β expression was significantly decreased (by 25% and 32% in cheek mucosa and dental plaque samples). Furthermore, an increased abundance of *Fusobacterium nucleatum, Porphyromonas gingivalis*, and *Prevotella intermedia*, along with a decreased abundance of *Streptococcus, Veillonella*, and *Neisseria*, was observed in OSCC patients.[69] Other studies have revealed the enrichment of inflammation-related bacterial genera in OSCC. Through quantitative insights into microbial ecology combined with linear discriminant analysis and effect size calculations, the microbial composition of oral communities was analyzed. Phylogenetic surveys of the microbial communities showed that OSCC tissues often exhibited lower species richness and diversity, confirming the inflammatory nature of the microbiome associated with OSCC. Additionally, microbial species enrichment at the species level was found to differentiate OSCC from fibrous epithelial polyps.[70] Besides the microbiome composition differences between OSCC patients and control groups, there are also notable differences in the saliva microbiome pre- and post-treatment. Anna I. Mäkinen et al[71] compared microbial profiles and found that, relative to healthy controls, OSCC patients had higher abundances of potential pathogenic bacteria in their saliva microbiomes. Moreover, OSCC

intervention brought about a notable reduction in the α-diversity of the saliva microbiome, with increased variability, a trend that remained evident years after treatment. Another study assessed the oral microbiome of OSCC patients before and after radiation therapy, revealing that during radiation therapy, *Fusobacterium* and *Porphyromonas gingivalis* decreased, while *Streptococcus* increased at radiation doses of 12–16 Gy. Additionally, high abundances of *Fusobacterium* and *Porphyromonas gingivalis* were correlated with poor prognosis.[72] Recent research by Hengyan Zhu et al[73] utilized 16S rRNA V3-V4 amplicon sequencing to characterize the oral microbiome in OSCC patients' oral rinse samples, identifying 45 discriminative bacterial genera. They noted the enrichment of *Fusobacterium, Leptotrichia, Prevotella*, and *Anaerostipes*, while *Streptococcus* and *Granulicatella* were generally less abundant. A substantial amount of research has revealed significant differences in the oral microbiome composition of OSCC patients. Mohammed Muzamil Khan et al[74] reported that, in addition to the increase in *Fusobacterium*, there was a notable increase in *Shivabacter* and *Candida* species, both of which have been associated with cancer. D. L. Mager et al[75] employed DNA-DNA hybridization to assess the content of 40 common oral bacteria and found that *Fusobacterium nucleatum, Porphyromonas gingivalis*, and *Streptococcus mutans* were evidently more abundant in the saliva of OSCC patients, suggesting their potential as diagnostic indicators for OSCC. Purandar Sarkar et al[76] found significant enrichment of *Prevotella, Bacteroides, Pseudomonas, Streptococcus*, and *Noviherbaspirillum* genera in OSCC patients, while *Actinomyces, Serratia, Stenotrophomonas, Clostridium* and *Serratia* genera were significantly reduced. Moreover, metabolic differences caused by the oral microbiota could also serve as diagnostic criteria for OSCC. Aparna et al,[77] utilized 16S rRNA metagenomic analysis, found significant enrichment of *Fusobacterium, Prevotella, Fusobacterium, Bacteroides, Lactobacillus*, and *Bacteroides* in OSCC lesions. The findings of this study indicate increased expression of metabolic pathways related to L-lysine fermentation, pyruvate fermentation, and isoleucine biosynthesis in the microbiota present in OSCC tissues. Mariam Z Kakabadze et al found that *Porphyromonas gingivalis* was linked to destructive processes in the oral cavity, possibly playing a critical role in OSCC development, while *Pseudomonas aeruginosa* may convert nitrite in saliva to nitric oxide, regulating various cancer-associated manifestations.[78] The characteristic microbial communities and differential microbial metabolites in OSCC are closely associated with disease progression. In clinical practice, diagnosing and detecting OSCC based on differences in microbial enrichment or relevant metabolites offers high diagnostic value due to the ease of operation and efficiency of the method.

Staging of OSCC and Differentiation from Other Oral Diseases Based on Oral Microbiome Composition

Recent studies suggest that alterations in the oral microbiome composition may correlate with OSCC progression. The abundance of *Fusobacterium* increases notably as oral cancer progresses from healthy controls (2.98%) to OSCC stage I (4.35%) and stage IV (7.92%). At the genus level, the abundance of *Fusobacterium* increases, while the quantities of *Streptococcus, Haemophilus, Porphyromonas*, and *Actinomyces* decrease as the cancer advances. *Porphyromonas gingivalis, Prevotella intermedia, Streptococcus constellatus, Haemophilus influenzae*, and *Filifactor alocis* have been linked to OSCC, with their abundance progressively increasing from stage I to stage IV. Conversely, the abundance of *Streptococcus mutans, Haemophilus parainfluenzae*, and *Porphyromonas pasteri* correlates negatively with OSCC progression.[79] In addition, Federica Di Spirito et al observed that in OSCC patients, the phyla *Proteobacteria* and *Bacteroidetes* increased, while *Firmicutes* decreased. They also noted an increase in periodontal pathogens and the consistent rise of *Candida*, a commonly studied oral fungus, in OSCC patients.[80] Oral microbiomes not only enable early detection of OSCC, but research also suggests that differences in microbiome composition can distinguish precancerous, early, and late cancer stages. Specific bacterial genera and their abundance in cancerous tissues can provide insights into the development of OSCC. For example, *Capnocytophaga, Fusobacterium*, and *Treponema* genera were enriched in cancer patients, while *Streptococcus* and *Rothia* genera were more abundant in the precancerous group. *Fusobacterium* was particularly linked to early cancer stages, whereas *Capnocytophaga* was evidently linked to advanced cancer stages. Microbial composition-based predictions of cancer progression demonstrated high accuracy, with dense microbial and immune network interactions observed in the precancerous group.[81] Thus, the oral microbiome composition can serve as a reliable basis for determining the stage of OSCC.

When comparing OSCC with other similar oral diseases, significant differences in the oral microbiome composition are observed. For instance, a comparison between OSCC and oral leukoplakia revealed that *Bacteroidetes* and *Solobacterium* genera were evidently more abundant in OSCC patients, while *Streptococcus* abundance was notably lower in the OSCC group than in the leukoplakia group.[82] Alejandro Herreros-Pomares et al employed 16S rRNA gene V3-V4 hypervariable region sequencing to compare and analyze bacterial populations and diversity in the oral microbiomes of homogeneous leukoplakia (HL), proliferative verrucous leukoplakia (PVL), OSCC, and PVL prior to OSCC (PVL-OSCC). They observed that OSCC patients had fewer amplicon sequence variants (ASVs), with *Fusobacterium* making up more than 30% of the microbiome. Penalty regression was used to identify microbial markers that distinguish these diseases. The study found that HL was enriched with *Streptococcus sanguinis, Streptococcus salivarius, Porphyromonas gingivalis, Tannerella forsythia, Pasteurella multocida*, and *Megasphaera micronucleoides*; PVL was enriched with *Prevotella intermedia, Campylobacter rectus, Pneumococcal Streptococcus*, and *Lactobacillus acidophilus*; OSCC was enriched with *Leadbetteri, Fusobacterium nucleatum, Porphyromonas gingivalis, Tannerella forsythia, Streptococcus mutans*, and *Actinomyces naeslundii*; PVL-OSCC was enriched with *Leptotrichia* species, *Fusobacterium nucleatum*, and *Campylobacter jejuni*, and dysbiosis was observed to differentiate oral precancerous lesions from cancerous conditions.[83] By comparing changes in the oral microbiomes of patients with different oral diseases, OSCC can be effectively distinguished from other potentially malignant conditions, thus improving the diagnostic accuracy of OSCC.

In their research on the correlation between dysbiosis in the oral microbiome and gene expression in patients with OSCC, Liuyang Cai et al conducted an analysis of mucosal bacterial communities, host whole-genome transcriptomes, and DNA CpG methylation profiles in both tumor and adjacent normal tissues of OSCC patients. They found a notable increase in the relative abundance of seven bacterial species in the OSCC tumor microenvironment, including *Fusobacterium nucleatum, Leptotrichia buccalis, Oral Streptococcus dysgalactiae, Mesorhizobium loti, Mesorhizobium cartoni, Eubacterium nodatum*, and *Prevotella intermedia*. These tumor-enriched bacteria were found to correlate positively with 206 upregulated host genes, primarily involved in signaling pathways related to cell adhesion, migration, and proliferation. These cellular processes are thought to drive cancer progression and metastasis. Furthermore, through a comprehensive analysis of bacterial transcriptomes and bacterial methylation correlations, the study identified at least 20 dysregulated host genes, whose promoter regions exhibited reversed CpG methylation patterns associated with bacterial pathogen enrichment. This suggests that pathogens may regulate gene expression through epigenetic modifications. Notably, *Fusobacterium nucleatum* was shown to upregulate the *SNAI2* gene (a key transcription factor in EMT), which interacts with the E-cadherin/β-catenin signaling pathway, TNFα/NF-κB pathway, and extracellular matrix remodeling to promote cellular invasion, unveiling a causal relationship between the microbiome and OSCC.[84] Shuwei Zhang et al studied the transcriptomic profile of immortalized human oral epithelial cells exposed to *Fusobacterium nucleatum* infection. A total of 3307 mRNAs (|Log2FC|>1.5) and 522 lncRNAs (|Log2FC|>1) were identified to be differentially expressed compared to uninfected immortalized oral epithelial cells, revealing changes in lncRNAs and potential hub genes in oral epithelial cells following *F. nucleatum* infection.[85] This finding may be related to the development of oral cancer.

The use of the oral microbiome for OSCC diagnosis offers several advantages over traditional diagnostic methods, including in terms of time, accuracy, disease differentiation, and cancer staging. Allan Radaic et al demonstrated through swab sample analysis that microbiome changes may occur in histologically normal areas, including mucosal regions near malignant sites. Additionally, changes in specific microbiome species in oral swab samples preceded tissue-level changes, suggesting that microbiome-based diagnostic methods via oral biopsy could detect OSCC earlier than tissue-based diagnostics, providing an opportunity for early clinical detection.[86] Given the challenge of early detection in OSCC, this approach holds significant potential for early detection of OSCC.

Combined Optical and Acoustic Imaging Systems with AI for Assisted Diagnosis of OSCC

Optical imaging for the diagnosis of OSCC has garnered increasing attention in recent years within the field of oral medicine. Advanced optical imaging technologies can provide clear and accurate early screening and diagnosis of OSCC, which is crucial for improving treatment outcomes and patient survival rates.[87] Recently, studies have combined optical

and acoustic imaging techniques with AI and algorithms, enhancing early disease screening capabilities, improving diagnostic efficiency, reducing medical costs, minimizing human errors, and promoting the development of telemedicine.

Qifan Ma et al[88] extracted radiomics features from pre-treatment CT images and selected the optimal features for OSCC diagnosis through univariate analysis and least absolute shrinkage and selection operator regression. They found that the neural network model exhibited high accuracy, sensitivity, and specificity for OSCC diagnosis. Nisha Chaudhary et al[89] constructed an AI model based on the analysis of histological images of oral cancer and precancerous tissues. They found that high-resolution AI image datasets could be used for diagnosing OSCC and assisting in the classification of OSCC subtypes, facilitating the rapid diagnosis of OSCC and its subtypes. Zeynab Pirayesh et al[90] evaluated the diagnostic performance of AI-based medical image analysis studies and discovered that deep learning models for detecting OSCC in histopathological images demonstrated significantly high accuracy, improving the objectivity and reproducibility of the diagnosis. Jiaxin Yu et al[91] used Lugol's iodine-enhanced Micro-CT imaging to evaluate the resection margin status of OSCC in three-dimensional space. Their findings suggested that Lugol's iodine-enhanced Micro-CT imaging could clearly delineate tumor boundaries in 3D, providing more accurate measurements of mucosal and deep resection margins when comparing imaging and pathological images, which aids in the diagnosis of OSCC through CT imaging. Annarita Fanizzi et al[92] extracted the total tumor volume from pre-treatment CT images of 499 patients in the OPC-Radiomics public dataset and trained an Inception-V3 CNN architecture. Their model achieved an area under the curve (AUC) of 73.50% in independent testing, indicating high accuracy in OSCC diagnosis. Suliman Mohamed Fati et al[93] highlighted the potential of AI to assist doctors and specialists in making accurate diagnoses. Using a hybrid feature set (AlexNet, DWT, LBP, FCH, and GLCM) with an artificial neural network (ANN), they achieved an accuracy of 99.1%, specificity of 99.61%, sensitivity of 99.5%, precision of 99.71%, and an AUC of 99.52%. Mehran Ahmad et al[94] employed various AI techniques to assist clinical doctors. Their study used a support vector machine (SVM) algorithm with a feature fusion of DenseNet201, GLCM, HOG, and LBP. The model achieved an accuracy of 97.00%, precision of 96.77%, sensitivity of 90.90%, specificity of 98.92%, F-1 score of 93.74%, and an AUC of 96.80%.

In studies utilizing machine learning for the assisted diagnosis or prediction of OSCC, SVM and ANN are the most commonly used algorithms. These machine learning techniques demonstrate high specificity and sensitivity, exhibiting strong performance in both diagnostic and prognostic analyses in oral cancer research. Mehak Malhotra et al[95] highlighted that AI-based tools could enable secondary prevention of early-stage OSCC by facilitating early detection and timely treatment.

Although acoustic imaging has not been as widely studied or applied as optical imaging in OSCC diagnosis, some studies have explored the potential of acoustic imaging techniques for OSCC detection. Peter A. Pellionisz et al[96] developed an approach that identifies viscoelastic differences in tissues by mixing two ultrasound beams to generate beat frequencies, thereby differentiating tissue regions. By focusing their imaging plane on multiple axial cross-sections within the tissue, they provided 3D imaging for enhanced contrast between normal and abnormal tissues. Their research ultimately led to the development of a mobile vibrational acoustic imaging system, capable of generating comparative images of normal and abnormal tissues within minutes.

Diagnosis of OSCC Using Proteomic and Genomic Evidence in Saliva

Saliva has been widely recognized for its diagnostic potential, particularly in young individuals, the elderly, and immunocompromised patients, as well as in large-scale screenings and epidemiological studies. Currently, highly sensitive testing procedures are widely available, enabling the quantitative measurement of various hormones and drugs in saliva, even at extremely low concentrations.[18] Recent advancements suggest that known salivary RNA biomarkers can be utilized for the diagnosis of oral cancer and other diseases. Salivary RNA can serve as a tool for identifying oral bacteria and determining the expression of specific genes, offering medically relevant insights into the oral microbiome, oral cancer, and other related conditions. Given its non-invasive nature and diagnostic potential, salivary RNA is increasingly being explored for future research and clinical applications.[21]

Nayroz Abdel Fattah Tarrad et al[27] collected unstimulated saliva samples and employed qRT-PCR to assess the levels of LINC00657 and miR-106a in different groups. Their findings suggested that salivary LINC00657 and miR-106a could

serve as promising diagnostic biomarkers for OSCC. Specifically, LINC00657 was able to distinguish OSCC from potentially malignant oral diseases with high diagnostic accuracy, while low salivary miR-106a levels were associated with malignant tumors. Anum Kazmi et al[97] evaluated salivary MMP-8 levels in patients with oral submucous fibrosis (OSF) and OSCC. Compared to the control group, OSCC and OSF patients exhibited lower expression of MMP-8, indicating an inverse relationship between MMP-8 levels and OSCC/OSF progression. As MMPs play a crucial role in extracellular matrix remodeling, their presence in saliva could serve as a non-invasive biomarker for the early detection of OSCC. Similarly, Zohra Saleem et al[98] analyzed unstimulated saliva samples from healthy individuals, OSF, and OSCC patients utilizing enzyme-linked immunosorbent assay (ELISA) to measure MMP-12 levels. Their findings showed a progressive increase in MMP-12, with OSCC patients exhibiting the highest expression. This suggests salivary MMP-12 as a potential non-invasive biomarker for early OSF and OSCC diagnosis. Given its high expression across various cancers, MMP-12 has the potential to act as a predictive and prognostic biomarker for multiple malignancies.[99] Yu-Jen Jou et al[100] used NanoLC-MS/MS to analyze OSCC patients (T1–T4) and controls (n=35), validating biomarkers via Western blot and ELISA. Their findings revealed S100A8, hemoglobin delta, and γ-G globulin in T3–T4 OSCC, while S100A7 was elevated in T1–T2 stages. Notably, salivary S100A8 levels increased with OSCC progression, from 3.4% in T1 to 100% in T4, whereas S100A7 was more prevalent in early-stage cases (20.7% of T1 and 11.1% of T2 cases). AUROC curve analysis demonstrated that ELISA-based S100A8 detection exhibited high sensitivity, specificity, and accuracy for OSCC diagnosis, suggesting that salivary S100A8 could be a specific and sensitive biomarker for OSCC detection. Additionally, Yu-Jen Jou et al[100] identified ZNF510 as a potential OSCC-associated salivary biomarker through immunohistochemical analysis of oral tissues, revealing evidently higher ZNF510 levels in OSCC tissues compared to non-OSCC controls. Receiver operating characteristic curve analysis for early-stage (T1+T2) and late-stage (T3+T4) OSCC indicated an area under the curve (AUC) > 0.95, confirming the potential of ZNF510 as a diagnostic biomarker. Proteomic analysis further identified a 24-peptide form of ZNF510, which may assist in the early detection of OSCC.[101] Moreover, OSCC patients exhibited increased levels of transferrin in saliva, which was confirmed using MALDI-TOF/TOF MS, Western blotting, and ELISA. The elevated salivary transferrin levels correlated with tumor size and staging, confirming its potential role as a biomarker for early detection of oral cancer.[102]

Beyond saliva, blood also plays a crucial role in OSCC diagnosis, with combined blood and saliva testing improving detection accuracy for oropharyngeal squamous cell carcinoma.[22] For instance, Shih-Jung Cheng et al[103] observed that the expression of placental growth factor (PlGF) in OSCC specimens was associated with disease progression and prognosis. Blood serum samples were collected, and serum PlGF levels were measured by ELISA. Preoperative OSCC patients had evidently higher serum PlGF levels compared to normal controls, and after surgical resection of the tumor, PlGF levels decreased to levels close to those of normal controls. Using a 19.1 pg/mL threshold, the study reported 80% sensitivity, 56% specificity, and a 78% positive predictive value for tumor recurrence. These results highlight serum PlGF as a potential biomarker for monitoring treatment response, progression, recurrence, and prognosis.

Summary and Future Outlook

OSCC is a common and lethal type of oral cancer, and early detection remains key to improving survival rates.[104] Although traditional diagnostic methods, such as clinical examination, imaging, and tissue biopsy, are widely applied, they still face limitations in terms of efficiency and accuracy. With the advancement of molecular biology technologies, many emerging diagnostic methods have shown great potential, particularly in improving diagnostic sensitivity, specificity, non-invasiveness, and convenience. Currently known diagnostic indicators are summarized in Table 1. Therefore, systematically organizing and comparing the core principles, current applications, advantages, and limitations of these emerging diagnostic technologies, while identifying key breakthroughs for their clinical translation, holds critical theoretical and practical significance for advancing the optimization and upgrading of early-stage oral squamous cell carcinoma diagnosis systems, thereby substantially improving patient survival rates and prognosis.

Current reviews on early diagnosis of OSCC predominantly focus on a single diagnostic tool or category, such as salivary proteomics,[105] genetics, molecular biomarkers of proteins and metabolites,[106] or the microbiome.[107] Few reviews have consolidated emerging diagnostic tools for OSCC. This review addresses this gap by analyzing and comparing these diagnostic tools. This integrated information may one day assist clinicians in diagnosing OSCC at an earlier stage.

Table 1 OSCC Diagnostic Criteria Comparison Table

Diagnostic Indicators	Expression in OSCC	References
let-7a	Downregulated	[11]
let-7d	Downregulated	[11]
let-7f	Downregulated	[11]
miR-16	Downregulated	[11]
miR-29b	Upregulated	[11]
miR-142-3p	Upregulated	[11]
miR-144	Upregulated	[11]
miR-203	Upregulated	[11]
miR-223	Upregulated, associated with advanced tumor stage and size	[11]
miR-1275	Can distinguish different tumors	[11]
hsa-miR-21	Significantly upregulated	[12]
hsa-miR-31	Significantly upregulated	[12]
hsa-miR-338	Significantly upregulated	[12]
hsa-miR-125b	Significantly downregulated	[12]
hsa-miR-133a	Significantly downregulated	[12]
hsa-miR-133b	Significantly downregulated	[12]
hsa-miR-139	Significantly downregulated	[12]
miR-2355-3p	Upregulated	[13]
miR-2355-3p	Targets SERPINA3 and enhances radiosensitivity	[13]
LncRNA CASC9	Upregulated	[14]
miR-125a-3p	Downregulated	[14]
miRNA-15a-5p	Downregulated	[15]
YAP1	Upregulated	[15]
miR-182-5p	Upregulated, associated with OSCC differentiation and T/N stages	[16]
miR-379-5p	Downregulated, negatively regulates ROR1	[23]
miR-200	Downregulated	[24]
miR-34	Downregulated	[24]
miR-24	Upregulated	[24]
miR-106b-5p	Upregulated	[25]
miR-423-5p	Upregulated	[25]
miR-193b-3p	Upregulated	[25]
miRNA-27b	Upregulated, significantly higher in OSCC patients in remission	[26]
miR-15a	Downregulated	[28]
miR-16-1	Downregulated	[28]
miR-138	Significantly downregulated	[29]
miR-424	Significantly downregulated	[29]
miR-130a	Significantly upregulated	[35]
miR-24-3p	Significantly upregulated	[36]
miR-155	Significantly upregulated	[38]
miR-126	Significantly downregulated	[38]
miR-21	Significantly upregulated	[39]
miR-503-5p	Upregulated	[45]
has_circ_0069313	Expression increased, promotes immune escape	[41]
circ-PKD2	Downregulated	[42]
circ-PVT1	Acts as a sponge for miR-106a-5p	[43]
miR-106a-5p	Binds to hexokinase II and inhibits HK2	[43]
circ-KIAA0907	Expression increased	[44]
hsa_circ_0072387	Significantly downregulated	[45]
FLT4RNA	Hypermethylated	[47]
ZAP70, ITGA4, KIF1A, PARP15, EPHX3, NTM, LRRTM1, FLI1, MIR193, LINC00599, PAX1, MIR137HG	Hypermethylated	[48]

(*Continued*)

Table 1 (Continued).

Diagnostic Indicators	Expression in OSCC	References
MIR296, TERT, GP1BB	Hypomethylated	[48]
RECK	Supramethylated	[52]
CHFR	Hypermethylated	[53]
DDAH2, DUSP1	Hypermethylated	[55]
PTEN, p16	Hypermethylated	[56]
p16, p15, hMLH1, MGMT, E-cad gene promoters	Hypermethylated	[58]
Fusobacterium	Increased abundance	[79]
Streptococcus, Haemophilus, Porphyromonas, Actinomyces	Decreased abundance with OSCC progression	[79]
Fusobacterium periodonticum, Micrococcus, Filifactor alocis, Streptococcus constellatus, Haemophilus influenzae	Gradual increase in abundance	[79]
Streptococcus pneumoniae, Haemophilus parainfluenzae, Porphyromonas pasteri	Decreased abundance with OSCC progression	[79]
Firmicutes, Bacteroidetes, Candida	Increased abundance	[80]
Actinobacteria	Decreased abundance	[80]
Capnocytophaga, Fusobacterium, Treponema	Enriched in cancer group	[81]
Streptococcus, Rothia	Enriched in premalignant group	[81]
Capnocytophaga leadbetteri, Fibrobacteria, Gingivalis, Showa spirillum, Salivary Mycoplasma, Nancycoccus	Enriched in OSCC	[83]
Rubella, *Nucleobacter, Leptospira, Catonella morbi, Oral digestion Streptococcus, Ape digestion Streptococcus, Uri-anaerobic Bacteroides*	Significantly increased relative abundance	[84]

Emerging miRNA-based diagnostics focus on small non-coding RNA molecules that play significant roles in tumor initiation and progression. These miRNAs can be detected in various bodily fluids, such as blood and saliva, of cancer patients. Compared to traditional histopathological examinations, miRNA detection offers higher sensitivity and specificity, with the added advantage of being non-invasive, thus enabling earlier diagnosis.[108] CircRNAs, which exhibit high stability in OSCC and persist in bodily fluids for extended periods, also show promise as early diagnostic biomarkers. By evaluating circRNAs from simple blood or saliva samples,[109] it is possible to avoid the invasive procedures required in traditional diagnostic methods, thereby reducing patient discomfort. An imbalance in the oral microbiome has been strongly linked to the onset and progression of multiple oral diseases. Analyzing changes in the oral microbiome can help detect abnormalities in the early stages of cancer, with samples easily collected through non-invasive means such as saliva or oral swabs. This method provides the advantages of non-invasiveness, convenience, and real-time monitoring. Furthermore, DNA methylation is an important mechanism of gene expression modulation and is closely linked to the development of multiple cancers. The diagnostic role of classic tumor suppressor genes such as p53 represents a significant research direction in oral cancer studies. Their immunohistochemical staining patterns may predict the mutational status of p53 in oral epithelial dysplasia, potentially offering additional diagnostic insights that warrant further investigation.[110] In OSCC, specific gene methylation changes have been confirmed as early signs for cancer detection. Optical imaging techniques combined with AI for image analysis offer fast detection speeds, non-invasiveness, and other advantages. Similarly, salivary proteomics provides a convenient method to identify biomarkers for OSCC. As a non-invasive biological sample, saliva can be easily collected and analyzed.

Compared to traditional methods such as histopathological examinations, imaging studies, and tissue biopsies, emerging diagnostic approaches offer significant advantages in multiple aspects. These new methods can detect cancer at earlier stages, overcoming the limitations of traditional diagnostic techniques that typically identify tumors only after they have progressed to a certain extent. Many of these emerging methods utilize bodily fluid samples, such as saliva and blood, which are non-invasive and convenient, avoiding the invasive procedures associated with tissue pathology. Furthermore, these methods are generally more acceptable to patients and demonstrate higher sensitivity and specificity,

offering potential support for personalized treatment. Based on molecular diagnostic results, physicians can develop individualized treatment plans for patients by considering their genetic characteristics and microbial profiles, thereby improving therapeutic outcomes.

Although emerging diagnostic methods have demonstrated tremendous potential for early detection of OSCC, these technologies still face several limitations. In practical applications, certain techniques may be constrained by inherent limitations of the detection technologies themselves. The expression levels of miRNA and circRNA, for example, can be influenced by various external factors, leading to potential inaccuracies in results. Additionally, some emerging diagnostic methods are still in the research phase and lack standardized protocols, particularly regarding sample processing, detection workflows, and result interpretation. The use of different methods across laboratories may result in inconsistent findings. Therefore, further standardization research is required to ensure the comparability of diagnostic results across different laboratories and clinical settings. From an economic perspective, some emerging methods necessitate high-precision equipment and technological support, which could result in higher costs. This limitation restricts their application in resource-limited settings, such as primary healthcare facilities or underserved regions. Moreover, molecular biomarkers like gene methylation are susceptible to sample quality issues, including contamination or degradation, which may impact the accuracy of the results.

Despite these challenges, emerging diagnostic methods hold immense potential for the early detection, accurate diagnosis, and personalized treatment of OSCC. Although they face technical and practical hurdles, ongoing advancements in these technologies are expected to drive the future development of OSCC diagnosis and treatment.

Abbreviations

OSCC, oral squamous cell carcinoma; miRNA, microRNA; qRT-PCR, quantitative real-time polymerase chain reaction; Treg, T regulatory; EMT, epithelial-mesenchymal transition; MMPs, matrix metalloproteinases; MSP, methylation-specific PCR; AI, artificial intelligence; SVM, support vector machine; ANN, artificial neural network; OSF, oral submucous fibrosis; ELISA, enzyme-linked immunosorbent assay; AUC, area under the curve.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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