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REVIEW ARTICLE

Biological biomarkers of oral cancer

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Abstract

The oral squamous cell carcinoma (OSCC) 5 year survival rate of 41% has marginally improved in the last few years, with less than a 1% improvement per year from 2005 to 2017, with higher survival rates when detected at early stages. Based on histopathological grading of oral dysplasia, it is estimated that severe dysplasia has a malignant transformation rate of 7%–50%. Despite these numbers, oral dysplasia grading does not reliably predict its clinical behavior. Thus, more accurate markers predicting oral dysplasia progression to cancer would enable better targeting of these lesions for closer follow-up, especially in the early stages of the disease. In this context, molecular biomarkers derived from genetics, proteins, and metabolites play key roles in clinical oncology. These molecular signatures can help predict the likelihood of OSCC development and/or progression and have the potential to detect the disease at an early stage and, support treatment decision-making and predict treatment responsiveness. Also, identifying reliable biomarkers for OSCC detection that can be obtained non-invasively would enhance management of OSCC. This review will discuss biomarkers for OSCC that have emerged from different biological areas, including genomics, transcriptomics, proteomics, metabolomics, immunomics, and microbiomics.

KEYWORDS
biomarkers, genomics, human papilloma virus (HPV), immunomics, metabolomics, microbiomics, OPMD, oral potentially malignant disorders, oral squamous cell carcinoma, OSCC, proteomics

1 | INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most common oral cancer type, accounting for about 90% of all oral cancer cases.[1-3] The OSCC 5 years survival rate of 41% has marginally improved in the last few years, with less than a 1% improvement per year from 2005 to 2017.[4,5] Conversely, when OSCC is detected in the early stages, the survival rates increase to >85%, highlighting the importance of

early detection and need for early biomarkers. Unfortunately, almost half of OSCC cases worldwide are diagnosed at later stages.[6,7] Primary risk factors for OSCC include tobacco use and heavy alcohol consumption[8] and the presence of oral potentially malignant disorders (OPMDs).[9-11] OPMDs are a group of lesions that "carry a risk of cancer development in the oral cavity, whether in a clinically definable precursor lesion or in clinically normal mucosa",[12] with oral leukoplakia being the most frequent OPMD.[13] Based on histopathological grading of oral dysplasia, it is estimated that severe dysplasia has a malignant transformation rate of 7%–50%, followed by moderate dysplasia (3%–15%) and mild dysplasia (<5%).[10] Despite these numbers, oral dysplasia grading does not reliably predict its clinical behavior and is by nature imprecise, with a high intra- and inter-observer variability in diagnosis,[14,15] making it currently impossible to predict accurately which dysplastic lesions will progress to OSCC.[16] Thus, more accurate markers predicting oral dysplasia progression to cancer would enable better targeting of these lesions for closer follow-up, especially in the early stages of the disease.[16]

In this context, molecular biomarkers derived from genetics, proteins, metabolites, autoantibodies and microbiome play key roles in clinical oncology (Figure 1). These molecular signatures can help predict the likelihood of OSCC development and/or progression and have the potential to detect the disease at an early stage, and support treatment decision-making and predict treatment responsiveness.[17] Also, identifying reliable biomarkers for OSCC detection that can be obtained noninvasively would enhance management of OSCC. This review will discuss biomarkers for OSCC that have emerged from different biological areas, including genomics, transcriptomics, proteomics, metabolomics, immunomics and microbiomics and their tissue/cell or biofluid of origin.

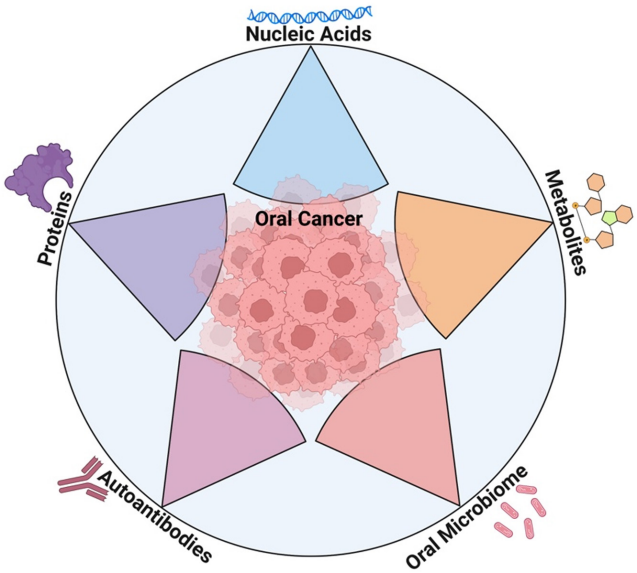

FIGURE 1 Biological biomarkers of oral cancer.

2 | NUCLEIC ACID-BASED BIOMARKERS

2.1 | Genomics

The growing development of molecular technology has made it possible to use nucleic acid molecules as potential noninvasive diagnostic biomarkers, as genetic materials can be amplified from trace amounts, enabling highly specific detection via the pairing of complementary nucleotides.[18] In fact, genetic materials-based diagnostics has become the gold standard for many diseases and it was fundamental for effective coronavirus disease 2019 (COVID-19) disease control during the 2020 pandemic.[18,19]

Besides standard cellular genetic materials, it is now possible to detect and isolate cancer stem cells (CSCs) and circulating tumor cells (CTCs) from a background of normal cells in blood[20–24]; Exosomes, extracellular vesicles released by normal and tumor cells into the bloodstream, which can contain tumor-specific proteins and nucleic acids[21,22,25]; or even cell-free nucleic acids, fragmented nucleic acids released into the bloodstream through apoptosis or necrosis, which includes cell-free DNA (cfDNA) and cell-free RNA (cfRNA). In patients with cancer, cfDNA that is released from tumor cells is often referred to as circulating tumor DNA (ctDNA).[20–22,26] Table 1 summarizes the recent studies in the literature.

Both cfDNA in plasma[27] and ctDNA in saliva[28] have shown promise as DNA biomarkers for human papillomavirus (HPV)-positive OSCC patients. DNA biomarkers play an important role in OSCC detection and they can be used to detect both HPV-positive and HPV-negative OSCC. For HPV-positive OSCC, the HPV DNA can be extracted from liquid biopsies, such as saliva and plasma. HPV ctDNA from oral rinses can be used to detect oropharyngeal cancers including OSCC with a 94% specificity and 78% sensitivity.[29] In addition to the ctDNA in saliva, HPV cell free DNA (cfDNA) in the plasma can be used to detect OSCC with a specificity of 100% and sensitivity of 72%.[30] Therefore, ctDNA and cfDNA have been used to diagnose the disease, predict prognosis, and monitor treatment outcomes.

Comparing HPV-positive with HPV-negative gene mutations in OSCC cases, Gillison et al.[31] reports increased frequencies on phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), zinc finger protein 750 (ZNF750), fibroblast growth factor receptor 3 (FGFR3), castor zinc finger 1 (CASZ1), phosphatase and tensin homolog (PTEN), CYLD lysine 63 deubiquitinase (CYLD) and DEAD-box helicase 3 X-linked (DDX3X) genes in HPV-positive cases, whereas HPV-negative OSCC cases presented increased frequencies on tumor protein p53 (TP53), FAT atypical cadherin 1 (FAT1), cyclin dependent kinase inhibitor 2A (CDKN2A), notch receptor 1 (NOTCH1), caspase 9 (CASP8), HRas proto-oncogene, and GTPase (HRAS) genes. Shi et al.[32] also validates increased TP53 gene mutations in 75%–85% of HPV-negative head and neck squamous cell carcinomas (HNSCC), including OSCCs compared to controls.

TABLE 1 Nucleic acid-based biomarkers of OSCC.

Author/Year	Sources of biomarkers	Type of markers	Method	Sample size	Potential Biomarkers	Expression in OSCC
Genomic biomarkers for HPV-positive OSCC						
Campo et al.[157]	Plasma	HPV cfDNA	Systematic review and Meta-analysis	457	cfHPV DNA	Increased in OSCC compared to control
Tang et al.[158]	Saliva	HPV DNA	Nested PCR	650	HPV16	Increased in OSCC compared to control
Rosenthal et al.[29]	Saliva	HPV DNA	qPCR and Cobas® HPV Test	45	fHPV16, p16INK4a	Increased in OSCC compared to control
Rettig et al.[159]	Plasma	HPV ctDNA	Droplet Digital PCR	110	ctHPV DNA16, 18, 31, 33, and 35	Increased in OSCC compared to control
Cao et al.[160]	Plasma	HPV ctDNA	Droplet Digital PCR	34	HPV ctDNA	Increased in OSCC compared to control
Haring et al.[161]	Plasma	HPV ctDNA	Droplet Digital PCR	12	ctHPV16 DNA	Increased in OSCC compared to control
Veyer et al.[162]	Plasma	HPV ctDNA	Droplet Digital PCR	66	ctHPV16 DNA	Increased in OSCC compared to control
Reder et al.[163]	Plasma	HPV cfDNA	qPCR	50	HPV oncogenes E6 and E7	Increased in OSCC compared to control
Mazurek et al.[30]	Plasma	HPV cfDNA	qPCR	263	cfHPV-DNA	Increased in OSCC compared to control
Chera et al.[164]	Plasma	HPV ctDNA	Digital droplet PCR	218	ctHPV16 DNA	Increased in OSCC compared to control
Lee et al.[165]	Plasma	HPV ctDNA	RT-qPCR	55	ctHPV16 DNA	Increased in OSCC compared to control
Simoens et al.[166]	Tissue	HPV DNA	RT-PCR	99	E6/E7 + P16(INK4a)	30.9% prevalence in OSCC
Gillison et al.[31]	Tissue	Gene Mutations	Whole genome sequencing	484	PIK3CA, ZNF750, FGFR3, CASZ1, PTEN, CYLD, and DDX3X	Increased gene mutation frequency in HPV-positive OSCC, compared to HPV-negative OSCC
					TP53, FAT1, CDKN2A, NOTCH1, CASP8, and HRAS	Increased gene mutation frequency in HPV-negative OSCC, compared to HPV-positive OSCC
Genomic biomarkers for HPV-negative OSCC						
Yang et al.[167]	Saliva	ctDNA mutations	Review article	274	TP53, CDKN2A, PIK3CA, FAT1, and NOTCH1	Increased in OSCC compared to control
Puttipanyalears et al.[168]	Saliva	Gene methylation	RT-PCR	24	Thyroid Releasing Hormone gene cg01009664	Increased in OSCC compared to control
D'Cruz et al.[169]	Oral Rinse	Gene mutations	PCR	15	TP53	Two identified mutations; 67% of the patients had codon 72 polymorphisms
Shanmugam et al.[170]	Oral Rinse	Gene expression	Digital Droplet PCR	121	TP53, CDKN2A, FAT1, CASP8, NOTCH1, HRAS and PIK3CA	87.6% of the samples presented at least 1 mutation on the genes
Wise-Draper et al.[33]	Plasma	Gene expression	ELISA	36	DEK	Decreased in poor prognosis compared to control
Schneider et al.[171]	Tissue	Gene expression	TCGA Database Analysis	499	GRP78/BiP	Increased gene expression in OSCC compared to control and associated with poor patient survival

TABLE 1 (Continued)

Author/Year	Sources of biomarkers	Type of markers	Method	Sample size	Potential Biomarkers	Expression in OSCC
Sato et al.[172]	Tissue	Protein expression	Immunohistochemistry	32	Casein kinase 1ε (cK-1ε) and CD44	Downregulated in OSCC compared to control
					Differentiated embryonic chondrocyte gene 1 (DEC1)	Upregulated in OSCC compared to control
Wilde et al.[173]	Tissue	Protein expression	Immunohistochemistry	297	p16	Increased in OSCC compared to control
Shieu et al.[174]	Tissue	Single-Nucleotide Polymorphisms	RT-qPCR	568	Lysine methyltransferase 2C (KMT2C) SNPs rs4725443 and rs6943984	TC or TC + CC genotype of rs4725443 > TT genotype.
Shi et al.[175]	Tissue	Gene expression	TCGA and GEO databases	520	SEC61G	Increased in OSCC compared to control
Rapado-González et al.[176]	Tissues, blood and saliva	cfDNA	qPCR	34	ALU60	Increased in OSCC patients compared to control, but not statistically significant
Burcher et al.[177]	Tissue/Blood	DNA Damage Repair gene mutation	ELISA	170	BRCA1, BRCA2, ATM, PALB2, ARID1A and CDK12	Increased in OSCC compared to control
Shi et al.[32]	Cell line derived from an oral cancer-induced mouse model	Gene Mutation	Whole-exome sequencing (WES),	N/A	TP53, Fat1, Notch1, Kmt2d, Fat3, and Fat4	TP53 mutations have 75%–85% prevalence in OSCC
Arora et al.[178]	Databases and in vitro	Gene expression	Bioinformatics	545	TFRC and NCBP2	Increased in OSCC compared to control; NCBP2 depletion reduced OSCC cell proliferation, migration, and invasion
Yang et al.[179]	Databases	Gene dysregulation	Bioinformatics	335	SPP1, FN1, CXCL8, BIRC5, PLAUR, and AURKA	Upregulated in OSCC compared to Control
					TEX101, DSG2, SCG5, ADA, BOC, SCARA5, FST, SOCS1, and STC2	Can be utilized to predict prognosis of OSCC patients
Transcriptomic biomarkers						
Dioguardi et al.[180]	Tissue	MicroRNAs	Systematic review and Meta analysis	708	miR-21	Upregulated in OSCC compared to control; Aggregated Hazard Ratio 1.29
Xie & Wu[181]	Tissue	MicroRNA	Systematic Review and Meta analysis	777	miR-21	Upregulated in OSCC compared to control; Aggregated Hazard Ratio 1.93;

(Continues)

TABLE 1 (Continued)

Author/Year	Sources of biomarkers	Type of markers	Method	Sample size	Potential Biomarkers	Expression in OSCC
Niklander et al.[182]	Tissue, In vivo and In vitro	MicroRNAs	Systematic Review	N/D	miR-21, mir-146a, miR-181b, miR-184, miR-345	Increased in OSCC compared to control and oncogenic
					miR-375	Downregulated and tumor suppressor
Palaia et al.[34]	N/D	MicroRNA	Systematic Review	3102	miR-16-let-7b, miR-21, miR-24, miR-24-3p, miR-27a-3p, miR-27b, miR-31, miR-92b, miR-136, miR-147, miR-148a, miR-150-5p, miR-155, miR-181a, miR181b, miR-184, miR-187, miR-191, miR-196a, miR-196b, miR-200b-2p, miR-210, miR-220a, miR-223, miR-323-5p, miR-412-3p, miR-423-5p, miR-483-5p, miR-494, miR-503, miR-512-3p, miR-626, miR-632, miR-646, miR-668, miR-887, miR-1250, miR-3262, miR-3651, miR-5100	Upregulated in OSCC compared to control
					miR-let-7d, miR-9, miR-29a, miR-30a-5p, miR-99a, miR-125a, miR-139-5p, miR-145, miR-186, mir-200a, miR-223, miR-223-3p, miR-320a, miR-338-3p, miR-758, miR-769-5p	Downregulated in OSCC compared to control
Troiano et al.[183]	Blood, Serum, and Plasma	MicroRNA	Systematic Review	1586	miR-21, miR-455-5p, miR-155-5p, miR-372, miR-373, miR-29b, miR-1246, miR-196a, and miR-181	Upregulated in OSCC compared to control
					miR-204, miR-101, miR-32, miR-20a, miR-16, miR-17, and miR-125b	Downregulated in OSCC compared to control
Scholtz et al.[36]	Saliva	MicroRNAs	RT-qPCR	87	miR-345 miR-31-5p, and miR-424-3p miR-21 miR-184 miR-191	Upregulated in OSCC compared to control
Shen et al.[184]	Tissue	MicroRNA	RT-qPCR	70 for miR and 50 for target genes	miR-21-5p	Upregulated in OSCC compared to control
					ADH7 gene	Downregulated in OSCC compared to control

TABLE 1 (Continued)

Author/Year	Sources of biomarkers	Type of markers	Method	Sample size	Potential Biomarkers	Expression in OSCC
Robison et al.[185]	Tissue	MicroRNAs	RT-qPCR	16	miR-155, miR-196a, miR-375, and miR-221	Upregulated in OSCC compared to control; Gender bias toward lymphatic invasion in lesions presenting around the perineal and abdominal regions
Shan et al.[186]	Tissue	Long Noncoding RNA	RT-PCR	368	M6A-related lncRNAs HMOX1, NFE2L2, NOS2, NOS3, and TP53	Downregulated in OSCC compared to control; Oxidative Stress in Oral Cancer
Rajthala et al.[187]	Tissue and OSCC-derived cancer-associated fibroblasts	MicroRNAs	In Situ Hybridization and miRNA Semi-Quantification.	50 tissues +18 OSCC-derived cancer-associated fibroblasts	miR-138	Downregulated in OSCC compared to control
Qin et al[188]	Tissue and In vitro	MicroRNAs	RT-qPCR and Western blot.	60 tissues +6 cell lines	miR-32-5p	Upregulated in OSCC compared to control
Jia et al. (2021)[37]	Tissue, In vivo PDX and In vitro	Circulating RNA	RT-qPCR	100	circFAT1, circ_0000231, circ_0001742, circ_0000264, circ_0002837, circ_0007976	Increased in OSCC compared to Control. CircFAT1 promotes cancer stemness and immune evasion by promoting STAT3 activation.
Extracellular Vesicles-Omics						
Saito et al.[189]	N/A	Oncogene	Review	N/A	NANOG and SOX	Increased in OSCC compared to control
Benecke et al.[39]	Plasma	Extracellular vesicles markers	Flow Cytometry	21	CD9, CD63, CD81 and TSG101	Increased in OSCC compared to control
Zhuang et al.[40]	In vitro *and* In vivo	Exosomal MicroRNAs	Exosomal miRNAs sequencing	N/A	miR-1246 and miR-205	Upregulated in OSCC compared to control
Wu et al.[41]	In vitro *and* In vivo	MicroRNAs	RT-qPCR	N/A	Cancer stem cell small extracellular vesicles, M2-tumor-associated macrophages	Increased in OSCC compared to control
Cancer Stem Cells and Circulating Tumor Cell markers						
Fukumoto et al.[24]	N/A	Cancer Stem Cells	Review	N/A	OCT4, NANOG, and SOX2	No specific markers for OSCC CSC other than those of general embryonic stem cells
Varun et al.[45]	N/A	Cancer Stem Cells	Review	N/A	OCT4, SOX2, NANOG, ALDH1, CD44, CD24, CD133 and Musashi-1	Display CSC characteristics
Rodini et al.[42]	N/A	Cancer Stem Cells	Review	N/A	CD44 and ALDH1	Display CSC characteristics; Majority of OSCC CSC isolations performed with CD44 marker

(Continues)

TABLE 1 (Continued)

Author/Year	Sources of biomarkers	Type of markers	Method	Sample size	Potential Biomarkers	Expression in OSCC
Baillie et al.[190]	N/A	Cancer Stem Cells	Review	N/A	OCT4, NANOG, SOX2, STAT3, CD44, CD24, CD133, Musashi-1, ALDH1, PRR, ATR1 and ATR2	Display CSC characteristics
Philouze et al.[191]	Tissue	Cancer Stem Cells	Immunohistochemistry	28	CD44, gamma-H2AX, and p-ATM	Display CSC characteristics
Ma et al.[46]	Tissue	Cancer Stem Cells	Magnetic-activated cell sorting	6	CD133, NANOG, SOX2, ALDH1A1, and OCT4	Display CSC characteristics; CD133 is negatively correlated with OSCC patients' survival
Curtin et al.[55]	N/A	Circulating Tumor Cells	Systematic review	N/A	N/A	CTCs does not appear to be related to tumor differentiation or size; CTCs may be prognostic for both disease-free survival and overall survival
Qayyumi et al.[52]	Blood	Circulating Tumor Cells	Immuno-magnetic beads separation	192	N/A	Progressively increased counts of CTC cells as OSCC progresses from stage I to IV. CTC detection Sensitivity - 94.32%, CTC detection specificity - 98%, and CTC detection accuracy - 95.17%
Wang et al.[192]	Blood	Circulating Tumor Cells	Flow cytometry	53	N/A	CTC counts were significantly reduced within 2–4 weeks of chemoradiation
Morgan et al.[53]	Blood	Circulating Tumor Cells	Surface-enhanced Raman scattering nanoparticle-based separation	125	N/A	Higher CTC counts associated with survival. CTC count of 675 defined as threshold between OSCC recurrence and distant disease, with sensitivity of 69%, and specificity of 68%.
Chang et al.[54]	Blood	Circulating Tumor Cells and circulating Cancer Stem Cells	Flow cytometry	34	N/A	Overall survival associated with higher CTC counts; Higher CSC ratio predicted disease progression within the first 3 months of chemotherapy.
Fanelli et al.[193]	Blood	Circulating Tumor Cells	Filtration and immunocytochemistry	53	TGF-β Receptor I	Expression correlated with poor progression-free survival

2.2 | Transcriptomics

In addition to genetic mutations, changes in gene expression levels and profiles, also known as the transcriptome, have also served as biomarkers for OSCC.[33] For example, the DEK proto-oncogene (DEK), a known oncogene, was shown to modulate the chromatin structure and remodel proteins. HPV-negative status and advanced tumor stage in HNSCC patients were found in conjunction with a downregulation in plasma DEK oncogene levels.[33] Besides changes in genetic expression, epigenetic modifications, such as alterations in DNA methylation patterns, can also be used as biomarkers for OSCC. Palaia et al.[34] reported that the differentially methylated CpG site, cg01009664, of the thyrotropin-releasing hormone (TRH) gene had a sensitivity of 82.61% and specificity of 92.59%, as assessed via bioinformatic approaches, and demonstrated the potential of epigenetic modifications as biomarkers for OSCC diagnosis.

RNA biomarkers, including microRNAs (miR) are known to serve an oncogenic or suppressor functions for their target genes under certain conditions,[35] and their up- or down-regulation can used for prediction of OSCC prognosis. For example, miR-345 and miR-31-5p are upregulated in OSCC patients.[36] By influencing mRNA translation and transcript degradation, microRNA expression can further influence its target gene expression through a common axis. Therefore, microRNA upregulation and downregulation has been used to make predictions about good or poor prognosis of OSCC.[34] Recently, Jia et al.[37] determined that circular RNA for FAT1 (circFAT1,) is specifically expressed in OSCC, but not in normal adjacent epithelial tissues. CircFAT1 was significantly increased in human OSCC with lymph node metastasis compared with human OSCC without lymph node metastasis. Importantly, they found that circFAT1 promotes cancer stemness and immune evasion through enhancing signal transducer and activator of transcription 3 (STAT3) activation, suggesting that circFAT1 is not only a biomarker for OSCC but also an important therapeutic target.

2.3 | Multi-omics of extracellular vesicles

Extracellular vesicles (EVs)—vesicles released by cells to communicate with other cells[38]—have also been demonstrated to be a powerful source of biomarkers for OSCC detection. For instance, Benecke et al.[39] report significantly elevated levels of tumor-derived extracellular vesicles positive for PanEV makers (tetraspanins CD9, CD63, and CD81) in OSCC patients compared to healthy controls. Additionally, EVs carry nucleic acids and proteins as their cargo, which may be useful as biomarkers.[38] MiRs carried in EVs are altered in OSCC patients and have been used to detect OSCC.[40,41]

2.4 | Cancer stem cells (CSC) and circulating tumor cells (CTC)

Cancer stem cells (CSCs) are a subset of cancer cells that have characteristics of stem cells, such as self-renewal and asymmetrical cell division, which can produce heterogeneous populations of cancer cells. Further, CSCs show greater malignant potential, such as higher anti-apoptosis activity, invasiveness, metastatic potential, chemo-resistance, and survivability compared to other subsets of cancer cells.[24]

In OSCC, it has been reported that CSCs play important roles in the development and progression of the disease.[24,42-44] Yet, no specific markers defining CSCs in OSCC have been found to date, and thus, the majority of CSC isolated from oral cancers have mainly been based on the P-glycoprotein 1 (CD44) marker, which is also a marker for breast CSC, or other generic embryonic stem cell markers, such as octamer-binding transcription factor 4 (OCT4), nanog homeobox (NANOG), and SRY-box transcription factor 2 (SOX2).[24,45] In addition, Ma et al.[46] isolated OSCC cells positive and negative for prominin-1 (CD133) and reports that CD133-positive cells presented higher growth rate, self-renewal, cisplatin resistance in vitro, and stronger tumourigenic potential in vivo compared to those negative for CD133.

Circulating tumor cells (CTCs) are cells that actively or passively detach themselves from a primary tumor and pass through the bloodstream. Various spontaneous or iatrogenic factors are implicated in this process and, thus CTCs showcase tumor heterogeneity without the need for an invasive tissue biopsy.[47-49] Since CTCs can be quickly eliminated by several different processes, such as immune attacks, shear stress, cell death due to loss of contact with the extracellular matrix or neighboring cells (anoikis), oxidative stress and the lack of cytokines and growth factors, they undergo a series of adaptations in order to survive. These adaptations include losing the expression of epithelial cellular adhesion molecule (EpCAM), keratins, and E-cadherin and upregulating matrix metalloproteinase (MMP) activity, which enables these cells to navigate through the local extracellular matrix and enter the microvasculature.[50,51]

In OSCC, Qayyumi et al.[52] demonstrate progressive increased counts of CTC cells as OSCC progresses from stage I to IV. Remarkably, the authors also demonstrate that CTC cells have a very high detection sensitivity and specificity (94% and 98%, respectively), leading to an overall detection accuracy of 95%. Counterintuitively, Morgan et al.[53] and Chang et al.[54] report higher CTC counts were associated with overall survival. In this context, Curtin et al.[55] performed a systematic review of the literature and found that the presence of CTCs does not appear to be related to tumor differentiation or size. Additionally, the authors point out that specific CTC results for oral cancer patients were either inconsistent or mixed with data from other anatomical sites and pathologies within the head and neck. Given the increasing evidence suggesting that CTCs have diagnostic and prognostic potential as biomarkers for OSCC, there is a clear need for studies that can elucidate the relevance of CTCs in OSCC.[55]

3 | PROTEIN-BASED BIOMARKERS FOR OSCC

Over 2200 different proteins have been cataloged in saliva, which is close to the amount of proteins found in plasma (over 2600), thus

making both fluids potential sources of biomarkers for OSCC.[56-58] Table 2 summarizes the recent studies in the literature.

The majority of the studies presented here (68%) used saliva as the source of biomarkers, possibly due to saliva sampling being fast, noninvasive, and well tolerated, besides being a safe procedure for healthcare providers.[59] Additionally, saliva is a very promising source of protein OSCC biomarkers given that it is in direct contact with the cancerous lesions and the oral mucosa.[60]

A minority of studies (26%) focused on blood-based biopsies. Gautam et al.[61] identified 16 potential biomarkers for OSCC, using high-throughput screening methods, including Fibrinogen alpha and Beta Chains, Fibronectin-1, and Serum amyloid A-1, which were increased in the OSCC group up to 5.36-fold. One major difference, however, is the greater sensitivity that a saliva-based approach has compared to a serum-based approach. Zheng et al.[62] examined both saliva and serum carcinoembryonic antigen (CEA) and N-α-acetyltransferase 10 protein (Naa10p) expression individually. The sensitivity and specificity of CEA and Naa10p in saliva were 80.2%, 81.7% and 81.1%, 83.3%, respectively, while the sensitivity and specificity of CEA and Naa10p in serum were 68.9%, 73.3% and 70.8%, 75.0% respectively. The combined detection of CEA and Naa10p in saliva led to the greatest sensitivity and specificity (92.5% and 85.0%, respectively). Similarly, Rajkumar et al.[63] demonstrated 3-fold higher levels of cytokeratin-19 fragment CYFRA 21-1 in saliva compared to serum levels. Noteworthy, though, is that in their systematic review, Ali et al. (2020) found non-conclusive evidence for increased CYFRA 21-1 in saliva samples, due to the presence of biases and limitations in the studies evaluated.

A minority of studies (20%) used more invasive biopsy methods (i.e., tissue biopsy) and determined that CD44 was differentially expressed in OSCC and OPMD, versus healthy tissues, with significantly increased levels in OSCC, followed by OPMD, compared to controls.[64] Similarly, other studies[65-67] identified glucose transporters, especially Glucose transporter 1 (GLUT-1) and 3 (GLUT-3), as significantly increased in OSCC compared to controls.

A third of the studies (36%) demonstrated that inflammatory cytokines, such as interleukin 6 (IL-6), interleukin 8 (IL-8), tumor necrosis factor alpha (TNF-α), and interleukin 1 beta (IL-1β) are significantly increased in OSCC compared to controls. Regarding OPMDs, most of these studies indicated a significantly progressive increase in the inflammatory cytokines, with OSCC levels being significantly higher than those in OPMD, and both were significantly higher than controls (OSCC > OPMD > Controls). Two reports[68,69] contradict these studies. Khyani et al.[68] indicated no significant differences between OSCC and OPMD, with both being significantly higher than controls, whereas Schiegnitz et al.[69] indicated no significant difference between OPMD and controls, and both were significantly lower than OSCC. These differences, however, could be due to the use of a higher detection limit for these particular reports compared to the rest of the studies, thus, reducing the ability to distinguish OPMD from OSCC and controls. The systematic reviews, however, do not agree with each other in this regard. Arroyo et al.[70] reported that both OSCC and OPMD levels were significantly elevated compared to controls, but not significantly different between each other (i.e., OSCC and OPMD > Controls), whereas Ferrari et al.[71] reported that all studies demonstrated that OPMD levels were significantly higher than controls, but significantly lower than OSCC (OSCC > OPMD > Control), with an effective power of discrimination among the samples—Area under the curve (AUC) ranging from 0.70 to 0.99. Given these findings, additional systematic review studies are needed to determine whether OPMD are a different group compared to OSCC and controls.

4 | METABOLITE-BASED BIOMARKERS FOR OSCC

In addition to protein- and proteomic-based studies, recent studies also include the detection and analysis of the metabolomic biomarkers associated with oral cancer. Nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry (MS) combined with liquid chromatography (LC), gas chromatography (GC), capillary electrophoresis (CE), or ultra-high-performance liquid chromatography (UHPLC) are often utilized for detection of small molecules and metabolomic investigations.[72,73] Table 3 summarizes the recent studies in the literature.

4.1 | Salivary metabolomic biomarkers

Salivaomics is a broad collection of technologies used to investigate the different types of molecules found in saliva. Several investigators have proposed the use of salivary metabolomics to differentiate precancerous from malignant OSCC to help improve the diagnosis and prognosis of OSCC. For instance, Ishikawa et al.[74] identified 3-methylhistidine as a significant prognostic factor, which is consistent with Cadoni's study,[75] which reported serum 3-methylhistidine as a biomarker for predicting head and neck cancer. Tantray et al.[76] identified 15 signature salivary metabolites that could differentiate OSCC from OPMD and controls. Recently, Patil & More[77] conducted a systemic review of 10 publications on the use of salivary metabolomics for diagnosing oral cancer and they found that 1-methylhistidine is also one of the metabolic biomarkers for oral cancer. Additionally, they concluded that the salivary biomarkers found were a result of perturbations in pathways involved in the metabolism of amino acids, proteins, carbohydrates, and nucleic acids throughout multistage carcinogenesis developments in oral cancer. Furthermore, de Sá Alves et al.[78] concluded that the malate–aspartate shuttle, the beta-alanine metabolism pathway, and the Warburg effect were three important altered metabolic pathways identified in OSCC.

Multiple studies reported different metabolic profiles in saliva samples from OSCC cases. Supawat et al.'s study[79] determined that trimethylamine *N*-oxide (TMAO) and glycine were significantly higher in oral cancer patients when compared with saliva samples taken from normal subjects. Consistent with Supawat

TABLE 2 Protein-based biomarkers for OSCC.

Author/Year	Source of biomarkers	Method	Sample size	Proteins identified	Findings
Riccardi et al.[57]	Saliva	Systematic Review	N/D	IL-1α, IL-1β, IL-6, IL-8, IL-1Ra, IL-10, TNFα, VEGF-α, MMP1, MMP2, MMP3, MMP9, AATα, HAPβ, C3, hemopexin, serotransferrin, transthyretin, fibrinogen β, resistin and proline-rich proteins (a, b and g)	Increased in OSCC compared to Control.
Pillai et al.[116]	Saliva and Serum	Systematic review	N/D	EGFR, Vitamin D-binding protein, Fibrinogen, CEA,	Increased in OSCC compared to Control.
Arroyo et al.[70]	Saliva	Systematic review and meta-analysis	986	CEA, CRP, CYFRA-21-1, Her-2/neu, erbB-2, IL-1α, IL-1β, IL-6, IL-8, TNF α and Naa10p	Increased in OSCC and OPMD compared to Control for CEA and CYFRA21-1 only
Ferrari et al.[71]	Saliva	Systematic review	948	IL-6, IL-8, IL-17, IL-1β, TNF-α, IFN-γ, MIP-1β, GRO, VEGF and IP-10	OSCC > OPMD > Control
AlAli et al.[194]	Saliva	Systematic review	775	CYFRA 21-1 and MMP-9	Non-conclusive evidence due to the presence of biases and limitations in the studies evaluated
Dikova et al.[195]	Saliva	Multiplex ELISA	157	IL-1α, IL-6, IL-8, TNF-α, HCC-1, MCP-1 and PF-4	OSCC > OPMD > Control
Sivadasan et al.[196]	Saliva	ELISA	67	CD44	OSCC > OPMD > Control
Ameena et al.[197]	Saliva	ELISA	90	TNF-α	OSCC > OPMD > Control
Deepthi et al.[198]	Saliva	ELISA	90	TNF-α	OSCC > OPMD > Control
Zheng et al.[62]	Saliva and Serum	ELISA	202	CEA, Naa10p	OSCC > OPMD > Control Salivary detection had the greatest sensitivity and specificity compared to Serum
Lee et al.[199]	Saliva	Multiplex ELISA	65	IL-6, IL-8, IL-1β, TNF-α, IFN-γ, MIP-1β, Eotaxin and GRO	Increased in OSCC compared to Control
Abbas et al.[200]	Saliva	ELISA	50	IL-17	Increased in OSCC compared to Control
Seyedmajidi et al.[201]	Saliva and Serum	ELISA	40	CD44	Increased in OSCC compared to Control, although not statistically significant
Awasthi et al.[202]	Saliva	ELISA	64	CYFRA 21-1 and LDH	Increased in OSCC and OPMD compared to Control
				Amylase	Increased in Control compared to OSCC and OPMD
Khyani et al.[68]	Saliva	ELISA	105	IL-6 and IL-8	Increased in OSCC and OPMD compared to Control
Peisker et al.[203]	Saliva	ELISA	60	MMP-9	Increased in OSCC compared to Control
Yu et al.[204]	Saliva	LC–MS	478	MMP1, KNG1, ANXA2 and HSPA5	Increased in OSCC compared to OPMD and Control
Polz-Dacewicz et al.[205]	Saliva	ELISA	118	IL-10, TNF-α, TGF-β and VEGF	Increased in OSCC compared to Control
Gleber-Netto et al.[206]	Saliva	ELISA	180	IL-1β and IL-8	OSCC > OPMD > Control
Dineshkumar et al.[207]	Saliva	ELISA	300	IL-6	OSCC > OPMD > Control

(Continues)

TABLE 2 (Continued)

Author/Year	Source of biomarkers	Method	Sample size	Proteins identified	Findings
Ghallab & Shaker[208]	Saliva and Serum	ELISA	45	Chemerin and MMP-9	OSCC > OPMD and Control
Rajkumar et al.[63]	Saliva and Serum	ELISA	200	CYFRA 21-1	OSCC > OPMD > Control Salivary CYFRA 21-1 levels were three-fold higher when compared to serum levels
Aziz et al.[209]	Saliva	Multiplex ELISA	63	IL-10 and IL-13	Increased in OSCC compared to Control
Gautam et al.[61]	Plasma	nanoLC-MS/MS	28	CRP, Fibrinogen alpha and Beta Chains, Fibronectin-1, Serum amyloid A-1, C4b-binding protein beta chain	Increased in OSCC compared to Control up to 5.36-fold
				Catalase, Flavin reductase, Carbonic anhydrase 1 and 2, SOD1, Purine nucleoside phosphorylase, APOA4, Desmoplakin, Desmoglein-1, Lumican	Decreased in OSCC compared to Control up to 4.28-fold
Zhang et al.[210]	Serum	Protein Microarray Assay	50	GDF15, MCSF, I309, MMP-3, CTACK, AXL	Increased in OSCC compared to Control
Schiegnitz et al.[69]	Serum	ELISA	205	IL-6 and IL-8	Increased in OSCC compared to OPMD and Control
Xu et al.[66]	Serum, Tissue	Western blot	68	GLUT-1	Increased in OSCC compared to Control
Ramos-García et al.[211]	Tissue	Systematic review and meta-analysis	1210	p53	OSCC > OPMD > Control
Ramos-García & González-Moles[212]	Tissue	Systematic review and meta-analysis	2746	β-Catenin	Increased aberrant expression in OSCC compared to control Aberrant Expression - Aggregated Hazard Ratio 1.77 Membrane loss – Aggregated Hazard Ratio 2.29
Botha et al.[67]	Tissue and cell lines	Systematic review	N/D	GLUT-1, GLUT-3,	Increased in OSCC compared to Control
				GLUT-2, GLUT-4, GLUT-8, GLUT-13, SGLT-1 and SGLT-2	Not significantly difference
Upadhaya et al.[213]	Tissue	Immunohistochemistry	40	ZO-1 and E-cad	Control > OPMD > OSCC
Ghazi et al.[64]	Tissue	Immunohistochemistry	55	CD44	OSCC > OPMD > Control
Zhang et al.[214]	Tissue	Immunohistochemistry	178	p53, Ki-67, P16, β-catenin, c-jun, c-met, IMP-3, COX-2, PDPN, CA9	Increased in OSCC compared to Control
Gissi et al.[215]	Tissue	Immunohistochemistry	77	p53 and Ki-67	Increased in OSCC compared to Control
Mumtaz et al.[216]	Cell line/Tissue	NanoLC-MS/MS	14	Transferrin receptor, THBS2, LGALS3BP and DNAJB11	Increased in OSCC compared to Control

Abbreviations: AXL, receptor tyrosine kinase; CA9, carbonic anhydrase 9; CEA, Carcinoembryonic Antigen; CEA, Carcinoembryonic Antigen; COX-2, cyclooxygenase-2; CTACK, cutaneous T cell-attracting chemokine; CYFRA 21, cytokeratin-19 fragment; MCP-1, monocyte chemoattractant protein-1; MIP-1β, Macrophage Inflammatory Protein-1 beta; MMP-1, Matrix metallopeptidase 1; MMP-3, Matrix metallopeptidase 3; MMP-9, Matrix metallopeptidase 9; N/D, not disclosed; Naa10p, N-α-acetyltransferase 10 protein; Naa10p, N-α-acetyltransferase 10 protein; OPMD, Oral Potentially Malignant Disorders; OSCC, Oral Squamous Cell Carcinoma; PDPN, podoplanin; PF-4, platelet factor-4; VEGF, Vascular endothelial growth factors.

TABLE 3 Metabolite-based biomarkers for OSCC.

Author/Year	Source of biomarkers	Method	Sample size	Metabolites	Findings
Rodríguez-Molinero et al.[217]	Saliva	Systematic review	3883	L-Fucose	Increased levels in OSCC compared to Control;
				Glycine, proline, citrulline, ornithine, 1-octen-3-ol, hexanoic acid, E-2octenal, heptanoic acid, octanoic acid, E-2-nonenal, nonanoic acid, 2,4-decadienal, 9-undecenoic acid, 3-Heptanone, 1,3-Butanediol, 1,2-Pentanediol, 1-Hexadecanol, Putrescine, cadaverine, thymidine, adenosine, 5-aminopentoate, hippuric acid, phosphocholine, glucose, serine, adrenic acid, Choline, BBCA, urea, 3-hydroxybutyric acid, Pipecolate, Sadenosylmethionine,	Significantly different levels in OSCC compared to Control; There is still a need for more studies, with a larger sample size.
				Ethanol, 2-Pentanone, phenol, Hexadecanoic acid, Undecane, 1-octanol, Butyrolactone, benzyl alcohol	Decreased levels in OSCC compared to Control
Grootveld et al.[87]	Saliva	Review	N/A	1-methylhistidine, 2-oxoarginine and γ-aminobutyryl-lysine l-homocysteate, polyamines (amino acid metabolism); 2-phosphoglycerate (carbohydrate metabolism); pseudouridine (nucleotide biosynthesis pathway); 4-nitroquinoline-1-oxide, ubiquinone and reduced glutathione (oxidative stress pathway); choline, S-adenosylmethionine and methionine (quaternary amine metabolism); BCAAs (TCA cycle, BCAA degradation); urea (urea cycle); and the ketone bodies 3-D-hydroxybutyrate and hydroxy-isovalerate (lipid metabolism)	Perturbations pathways involved in the metabolism of amino acids, proteins, carbohydrates and nucleic acids throughout multistage carcinogenesis developments
Panneerselvam et al.[88]	Saliva	Review	N/A	N/D	Establishing standard operating procedures for the use of saliva samples is mandatory. An effective screening system should be developed by combining conventional and modern technologies.
Patil & More[77]	Saliva	Review	N/A	Glutathione, polyamines, branched chain amino acids, S-adenosylmethionine, pipecolate, choline, glycine, proline, inositol 1,3,4-triphosphate, indole-3-acetate and ethanolamine phosphate, urea, 3-hydroxybutyric acid, pseudouridine, D-glycerate- 2- phosphate, 4-nitroquinoline- 1- oxide, ubiquinone and estradiol valerate	Increased in OSCC compared to Control; Carcinogenesis causes disturbances in the metabolism of carbohydrates, proteins, amino acids and nucleic acids
Vitório et al.[104]	Saliva, serum, plasma and tissue	Review	N/A	Alanine, choline, leucine,isoleucine, glycyl-leucine, glutamic acid, 120.0801 m/z, phenylalanine, alpha-aminobutyric acid, serine, indole-3-acetate, ethanolamine phosphate, s-adenosylmethionine, pipecolate, choline, betaine, pipecolinic acid, propionylcholine, lactic acid, acetone, acetate, putrescine, aspartic acid, glutamate, proline, aspartic acid	Increased in OSCC compared to Control
				Ornithine, o-hydroxybenzoate, ribose-5-phosphate, l-carnitine, acetylphenylalanine, sphinganine, phytosphingosine, s-carboxymethyl-l-cysteine, phenylalanine, valine, l-leucine, glutamine, 6-hydroxynicotinic acid	Decreased in OSCC compared to Control

(Continues)

TABLE 3 (Continued)

Author/Year	Source of biomarkers	Method	Sample size	Metabolites	Findings
Ishikawa et al.[74]	Saliva	CE-TOF-MS	72	Proline, carnitine, 5-hydroxylysine, 3-methylhistidine, adenosine, inosine, and *N*-acetylglucosamine Multivariate analysis: 3-methylhistidine and 5-hydroxylysine	Only 3-methylhistidine found to be a significant prognostic factor
Tantray et al.[76]	Saliva	GC–MS	90	Decanedioic acid, 2-methyloctacosane, octane, 3,5-dimethyl, pentadecane, eicosane, hentriacontane, 5, 5-diethylpentadecane, nonadecane, oxalic acid, 6-phenylundecanea, l-proline, 2-furancarboxamide, 2-isopropyl-5-methyl-1-heptanol, pentanoic acid, and docosane	Increased in OSCC compared to OPMD and Control
Supawat et al.[79]	Saliva	NMR	25	Tyrosine, tryptophan, unk1, unk3, trimethylamine N-oxide and glycine	Increased in OSCC compared to Control
de Sá Alves et al.[78]	Saliva	GC–MS	68	Malic acid, methionine, maltose, protocatechuic acid, inosine, pantothenic acid, dihydroxyacetone phosphate, hydroxyphenylatic acid, galacturonic acid, indole-3-acetic acid, uracil, isocitric acid, ribose-5-phosphate, o-phospho serine, lactitol, gluconic acid, hippuric acid, 3-hydroxypropionic acid and spermidine	Increased in OSCC compared to Control
				Lactose, catechol, 2-ketoadipic acid, leucine, urea, maleic acid, palmitic acid, ornithine, margaric acid, sucrose, octadecanol, threitol, acetoacetic acid, methionine sulfone, phosphoric acid, elaidic acid, mannose, sorbitol, citric acid, and 3-aminopropanoic acid	Decreased in OSCC compared to Control
Song et al.[83]	Saliva	CPSI-MS	373	Putrescine, cadaverine, thymidine, adenosine and 5-aminopentoate	Increased in OSCC compared to Control
				Hippuric acid, phosphocholine, glucose, serine and adrenic acid	Decreased in OSCC compared to Control
Ishikawa et al.[80]	Saliva	CE-TOF-MS	60	Trimethylamine N-oxide, putrescine, creatinine, 5-aminovalerate, pipecolate, *N*-acetylputrescine, gamma-butyrobetaine, indole-3-acetate, N1-acetylspermine, 2′-deoxyinosine, ethanolamine phosphate and *N*-acetylglucosamine	Increased in OSCC compared to OPMD
				N-acetylhistidine and o-acetylcarnitine	Decreased in OSCC compared to OPMD
Ishikawa et al.[81]	Saliva	CE-TOF-MS	48	Ornithine, carnitine, arginine, o-hydroxybenzoate, *N*-acetylglucosamine-1-phosphate, and ribose 5-phosphate	Decreased in OSCC compared to OPMD
Shigeyama et al.[218]	Saliva	GC–MS	74	Ethanol, 2-pentanone, phenol, hexadecanoic acid, disappeared undecane, 1-octanol, butyrolactone and benzyl alcohol and newly produced 3-heptanone, 1,3-butanediol, 1,2-pentanediol and 1-hexadecanol	Decreased in OSCC compared to Control
Sridharan et al.[219]	Saliva	UPLC-QTOFMS	61	D-glycerate-2- phosphate, estrone-3-glucuronide, 4-nitroquinoline-1-oxide, sphinganine-1 phosphate, 1-methyl histidine, inositol 1,3,4-triphosphate, d-glycerate-2-phosphate, 2-oxoarginine, norcocaine nitroxide, pseudouridine, galactosphingosine, and ubiquinone	Increased in OSCC compared to OPMD

TABLE 3 (Continued)

Author/Year	Source of biomarkers	Method	Sample size	Metabolites	Findings
Taware et al.[220]	Saliva	HS-SPME-GC–MS	59	1,4-dichlorobenzene, 1,2-decanediol, 2,5-Bis1,1-dimethylethylphenol, propanoic acid (ethyl ester), E-3-decen-2-ol, acetic acid, propanoic acid, ethyl acetate, 2,4-dimethyl-1-heptene, 1-chloro-2-propanol, 1-chloro-2-butanol, 2-propenoic acid, 2,3,3-trimethylpentane, ethanol, and 1,2,3,4-tetrachlorobutane	Increased in OSCC compared to Control
Mikkonen et al.[84]	Saliva	NMR	75	Fucose, glycine, methanol, proline and 1,2-propanediol.	Increased in HNSCC compared to Control, except proline
Lohavanichbutr et al.[85]	Saliva	NMR and LC–MS/MS	194	Glycine, proline, ornithine and citrulline	Decreased in OSCC compared to Control
Ohshima et al.[86]	Saliva	CE-TOF-MS	43	Choline, p-hydroxyphenylacetic acid and 2-hydroxy-4-methylvaleric acid, valine, 3-phenyllactic acid, leucine, hexanoic acid, octanoic acid, terephthalic acid, γ-butyrobetaine and 3-(4-hydroxyphenyl)propionic acid, isoleucine, tryptophan, 3-phenylpropionic acid, 2-hydroxyvaleric acid, butyric acid, cadaverine, 2-oxoisovaleric acid, N6,N6,N6-trimethyllysine, taurine, glycolic acid, 3-hydroxybutyric acid, heptanoic acid, urea and alanine	Increased in OSCC compared to Control, except Urea
Kamarajan et al.[102]	Saliva, Plasma and Tissue	UPLC-MS/MS for profiling; GC–MS and PCR for validation	Tissue – 103; Saliva – 75; Plasma - 14	Glutamine and glutaminase	Confirmed involvement of glutamate and glutaminolysis. Exogenous glutamine induced stemness via glutaminase, whereas inhibiting glutaminase suppressed stemness in vitro and tumorigenesis in vivo
Ishikawa et al.[82]	Saliva and tissue	CE-TOF-MS	68	3PG, pipecolate, spermidine, Met, SAM, 2AB, Trp, Val, hypoxanthine, Gly-Gly, trimethylamine, N-oxide, guanine, guanosine, taurine, choline, cadaverine, Thr.	Increased in OSCC compared to Control
Zuo et al.[93]	Serum	UHPLC-Q-Orbitrap HRMS	103	Succinic acid, arginine, 9-decanoylcarnitine, asparagine-valine, glutamine, hypoxanthine, sphingosine, and palmitoyl ethanolamide	Increased in OSCC compared to Control
				Hexanoylcarnitine, orotic acid, uric acid, vanillyl mandelic acid, ethyl acetate, and thromboxane B2	Decreased in OSCC compared to Control
Tsai et al.[100]	Plasma, urine, and tissue	NMR	110	Creatine, creatine phosphate, glycine, and tyramine	Downregulated in OSCC (Plasma)
				Aspartate, butyrate, carnitine, glutamate, glutathione, glycine, glycolate, guanosine, and sucrose	Upregulated in OSCC (Tissue)
				Alanine, choline, glucose, isoleucine, lactate, leucine, myo-inositol, O-acetylcholine, oxypurinol, phenylalanine, pyruvate, succinate, tyrosine, valine, and xanthine	Downregulated in OSCC compared to Control (Tissue)
Wu et al.[92]	Serum	UHPLC-QE-MS	Discovery: 60 Validation: 77	Serine and lactic acid	Increased in OSCC compared to Control; enhanced diagnostic efficacy when combined

(Continues)

TABLE 3 (Continued)

Author/Year	Source of biomarkers	Method	Sample size	Metabolites	Findings
Li et al.[91]	Plasma	UHPLC/Q-Orbitrap HRMS	194	Decanoylcarnitine, cholic acid, cysteine, uridine, taurine, glutamate, citric acid and lyso-phosphatydilcholine	Decreased in OSCC compared to Control and OPMD.
Sridharan et al.[94]	Serum	Q-TOF-MS	71	Estradiol-17-beta-3-sulfate, L-carnitine, 5-methylthioadenosine, 8-hydroxyadenine, 2-methylcitric acid, putrescine, and estrone-3-sulfate, 5,6-dihydrouridine, 4-hydroxypenbutolol glucuronide, 8-hydroxyadenine, and putrescine	Increased in OSCC and OPMD compared to Control
Zhang et al.[221]	Tissue	GC–MS	40	Nicotinamide *N*-methyltransferase	Increased in OSCC and fibroblast-like cells compared to Control, but absent in tumor-infiltrating lymphocytes
Yang et al.[95]	Tissue	GC–MS	180	Glutamate, aspartic acid, and proline	Increased in OSCC compared to Control
Paul et al.[99]	Tissue	NMR	180	1,3-Dihydroxyacetone, 2-oxoglutarate, 4-aminobutyrate, acetate, adenine, alanine, asparagine, aspartate, betaine, carnitine, choline, creatine, ethanol, fumarate, glucose, glutamate, glutamine, glycine, guanidoacetate, histidine, homocysteine, inosine, isoleucine, isopropanol, lactate, leucine, lysine, methanol, methionine, o-acetylcarnitine, o-phosphocholine, phenylalanine, serine, taurine, threonine, tyrosine, uracil, valine, myo-inositol, sn-glycero-3-phosphocholine, linoleic acid, MUFA, SFA, triglyceride, total fatty acids, and free fatty acids	Upregulated in OSCC compared to Control, except glucose
Yoshimura et al.[101]	Tissue	IHC	22	Glucose-6-phosphate and lactic acid	Upregulated in OPMD and OSCC
Musharraf et al.[97]	Tissue	GC–MS	51	(6E)-2,6-Dimethyl-2,6-octadiene, 2-Methyl-4-keto-pentan-2-ol, 4-Hydroxybenzaldehyde, cis-*p*-Menthan-3-one, geraniol formate, and stearic acid	Increased in OSCC compared to Control
				Glycine, threonine, glutamine, lysine, proline, alanine, glutamic acid, leucine, serine, 3-heptanol, ethylene glycol, melibiose, and urea	Decreased in OSCC compared to Control
Ogawa et al.[98]	Tissue	CE-TOF-MS	64	Lactate, Fum, Mal, Glu, Gly, Asp, Pro, Cys, Hyp, creatinine, putrescine, AMP, GTP, GDP, GMP	Increased in OSCC compared to Control
				Glucose, 3PG, 2PG, creatine, adenylate and guanylate energy charge	Decreased in OSCC compared to Control
Chen et al.[222]	Cell line	GC–MS	N/A	Glyoxylate and dicarboxylate, fructose, malate, serine, alanine, sorbose, and glutamate.	Glyoxylate and dicarboxylate increased in OSCC compared to Control

TABLE 3 (Continued)

Author/Year	Source of biomarkers	Method	Sample size	Metabolites	Findings
Tripathi et al.[223]	Cell line	NMR	N/A	Acetate, alanine, aspartate, AXP, choline, creatine, fumarate, glutamate, glutamine, glutathione, glycerophosphocholine, glycine, histidine, isoleucine, lactate, leucine, lysine, myo-inositol, *N*-acetyl-aspartate, phenylalanine, phosphocholine, phosphocreatine, proline, pyruvate, taurine, threonine, tryptophan, tyrosine, UDP-sugars, valine	Alterations of phosphatidylcholine/lysophosphatidylcholine and phosphocholine/glycerophosphocholine ratios, and elevated arachidonic acid in HNSCC; Dysregulation in multiple metabolic events, including Warburg effect, oxidative phosphorylation, energy metabolism, TCA cycle anaplerotic flux, glutaminolysis, hexosamine pathway, osmo-regulatory and antioxidant mechanism

Abbreviations: N/D, not disclosed; N/A, not available.

et al.'s study, Ishikawa et al.[80-82] determined that trimethylamine *N*-oxide was significantly higher in the OSCC group than in the OPMD group. Using different identification platforms, trimethylamine *N*-oxide, choline, cadaverine, proline, glutamine, lactate, fucose, and glycine were determined to be consistently different between OSCC and controls.[79,82-86] Song et al.[83] used a combination of conductive polymer spray ionization mass spectrometry (CPSI-MS) and machine learning (ML) analysis and found this approach as a feasible tool for accurate, automated diagnosis of OSCC in clinical practice.

Recently, Grootveld et al.[87] and Panneerselvam et al.[88] conducted a summative assessment of the latest progress on applications dedicated to the diagnostic and prognostic monitoring of oral cancer, especially focused on salivary metabolomic analysis, and suggested that optimal screening programs should involve a combination of both conventional and newly developed technologies. For example, several ML-based data processing and analysis strategies include oral cancer identification, automated disease progression staging, and the application of image processing to distinguish between cancerous and precancerous cells.[89,90]

4.2 | Plasma/serum metabolomic biomarkers

Multiple studies have shown differential metabolic profiles in the plasma and serum of OSCC patients. In a recent study, Li et al.[91] demonstrated that the biomarkers associated with OSCC were closely related to cholic acid metabolism and amino acid metabolism. Additionally, Wu et al.[92] determined that serine and lactic acid gradually increased in benign and malignant salivary gland tumors. Zou et al.[93] used ultra-high-performance liquid chromatography-high resolution mass spectrometry serum metabolomic analysis to demonstrate that succinic acid changes (low levels), hypoxanthine changes (high levels), and tumor grade provided the highest predictive accuracy of patients with OSCC. Further, Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis showed that the imbalance in the amino acid and purine metabolic pathway may affect the prognosis of OSCC. Further, integrative analysis of metabolomic and transcriptomic data can identify prognostic biomarkers associated with OSCC. Using quadrupole time of flight-liquid chromatography-mass spectrometry, Sridharan et al.[94] identified a significant upregulation of putrescine, 8-hydroxyadenine, and 5,6-dihydrouridine in OSCC compared to OPMD, indicating that these metabolites play a potential role in predicting the malignant transformation of OPMD.

4.3 | Tumor tissue metabolomic biomarkers

Oral cancer tissue biopsy is an invasive approach and remains the current clinical gold standard for detection and diagnosis of oral cancer. Tumor tissue metabolomics is used to identify significant metabolic alterations in tumors compared to normal tissues.

Recently, Yang et al.[95] summarized findings from a systemic review of oral biopsies, sample types, and detection techniques applied to oral cancer detection, and concluded that tissue biopsies provide increased diagnostic value compared to liquid biopsies. Kasiappan et al.[96] summarized the published metabolomic data for head and neck cancer and identified significant metabolites that differentiate head and neck cancer from normal controls based on tissue, serum, saliva, cell lines, and urine. Additionally, the authors also discussed the various tools used in metabolomics to identify important metabolites from these sample types. Multiple studies have confirmed that OSCC tissues undergo significant changes in metabolic pathways, including glycolysis, amino acid metabolism, and the pentose phosphate pathway.[72,82,95,97-101] Using Ultra performance liquid chromatography in tandem mass spectrometer (UPLC-MS/MS)-profiling and gas chromatography mass spectrometry (GC–MS)-validation studies, Kamarajan et al.[102] demonstrated that highly active glutaminolysis was involved in primary and metastatic HNSCC tissues; this was marked by high glutamate and low glutamine levels in human head and neck cancer tissue, saliva, and plasma compared to controls. Further, Ishikawa et al.[82] also showed that the glycolysis-Embden–Meyerhof–Parnas (EMP) pathway, tricarboxylic acid cycle (TCA) cycle, and glutathione pathway were aligned with cancer metabolic changes and could be potential discriminant biomarkers, which is consistent with the previous studies by Ogawa and colleagues.[98] Additionally, increased glucose consumption and rise in lactate levels with a concomitant decrease in the levels of glycolysis intermediates in OSCC is possibly the result of the Warburg effect.[82,98-101] These studies suggest that glucose metabolism may be more important for survival and proliferation, whereas glutamine/glutamate metabolism may be essential for subsequent aggressive transitions, including metastasis.[102] Furthermore, Shin et al.[103] highlighted that several publications suggest that the oral and gut microbiome contribute to the etiology of different types of cancers due to their ability to alter the community composition and induce inflammatory reactions, DNA damage, apoptosis, and altered metabolism. Thus, when considering cancer-associated metabolomics, the influence of the microbiota and its repertoire of metabolites should also be considered, since the microbiota are profoundly abundant in the human body and on cancerous tissues. Recently, Vitorio et al.[104] summarized the metabolic landscape of OSCC and evaluated the studies focused on metabolomic analysis and metabolomic biomarker signatures identified so far in saliva, serum, plasma, urine, and tissue, and concluded that validation and optimization are still required to translate these findings into clinical applications.

5 | TUMOR-ASSOCIATED AUTOANTIBODIES–BIOMARKERS FOR OSCC

The discovery of novel potential biomarkers for a disease may shed new light on potentially novel pathophysiological mechanisms. This is particularly true for the field of immunology and the study of antibodies as they are at the center of the human immune defense system against infectious diseases yet are also key components in autoimmune diseases and carcinogenesis.[105,106] Particularly in carcinogenesis, tumors must evade and subvert the host immune response in order to escape immuno-elimination and thereby grow and proliferate. Furthermore, tumors also exploit the immune system together with other host factors to promote angiogenesis to further support their growth.[106] This whole process leads to the production of many abnormal substances that are no longer recognized as the host's own, and this leads to the production of what it is known as tumor-associated autoantibodies.[107]

Autoantibodies are regular Immunoglobulin M (IgM) antibodies, produced by B cells that react with the host's own molecules, such as host proteins, nucleic acids, carbohydrates, lipids, or a combination of these.[108] Although the majority of these antibodies are polyreactive with a moderate affinity, some of these autoantibodies can be highly specific for a particular antigen in one specific cell type in the body,[108] making them promising biomarkers in cancer diagnosis.[109]

For OSCC, in particular, salivary autoantibodies may be more clinically applicable compared to other molecules due to the antibodies having higher specificity, stability, and abundance in saliva, and reagents and platforms required for antibody detection are well-established and readily accessible.[107,110–114] Table 4 summarizes the recent studies in the literature.

Several antigens can elicit OSCC-associated autoantibodies, including metalloproteinase 1 (MMP1) and 3 (MMP3), and Sperm protein 17 (SP17), and these autoantibodies can be uniquely and significantly increased in OSCC compared to controls. Hsueh et al.,[110] demonstrated that a panel of 10 autoantibodies were significantly increased in the OSCC group compared to the control group. The authors also demonstrated that a panel with 4 of these autoantibodies (anti-MMP3, anti-peroxiredoxin 2 (PRDX2), anti- secreted protein acidic and cysteine rich (SPARC), and anti-heat shock protein family A member 5 (HSPA5) was enough to achieve a sensitivity of 63.8% for detection of early-stage OSCC. Tseng et al.,[115] further developed the idea of a ML-based risk prediction model to detect OSCC and found that a panel of 8 autoantibodies improved prediction performance by 13.9% (from 0.698 to 0.795). These studies demonstrate the potential for the use of autoantibodies as OSCC biomarkers, however, further investigations are needed to validate their use.

Other autoantibodies, such as anti-p53, and anti-Survivin, are elevated in more than 50% of tumor types, thus they have become known as “universal” tumor autoantibodies.[105] In a systematic review, Pillai et al.[116] reported increased levels of anti-p53 and anti-Hsp70 autoantibodies in OSSC patients, compared to controls. Similarly, Wu et al.[114] found increased levels of anti-p53, anti-Survivin, anti-heat shock protein 60 (Hsp60), and anti-ribosomal protein lateral stalk subunit P0 (RPLP0) autoantibodies in OSCC, compared to controls. However, upon progression, in the late-stage OSCC group the levels were not significantly different when compared to controls, suggesting that OSCC may subvert the autoantibody processes over time.

TABLE 4 OSCC-associated autoantibodies as biomarkers for OSCC

Author/Year	Source of Biomarkers	Method	Sample size	Antigens Identified	Findings
Pillai et al.[116]	Saliva and Serum	Systematic Review	N/D	p53 and Hsp70	Increased in OSCC compared to Control
Chu et al.[119]	Saliva	LC–MS/MS	30	CPPED1, GLUD1, LMAN2, PTGR1, RAB13, RAC1, UQCRC2 and p53	Increased in OSCC compared to Control, especially in early stage OSCC.
Hsueh et al.[110]	Saliva	Multiplexed Immunoassay	300	ANXA2, CA2, ISG15, KNG1, MMP1, MMP3, PRDX2, SPARC, and HSPA5	Increased in OSCC compared to Control, especially in early stage OSCC ANXA2, KNG1, and MMP1 IgM were significantly higher in relapsed OSCC group, compared to primary OSCC group
Tseng et al.[115]	Saliva	LC-MRM-MS	337	ANXA2, CA2, ISG15, KNG1, MMP1, MMP3, PRDX2, SPARC, and HSPA5	Increased in OSCC compared to Control
Wu et al.[114]	Saliva	Multiplexed immunoassay	348	p53, survivin, Hsp60, and RPLP0	Increased in OSCC compared to Control, especially in early stage OSCC; Late stage OSCC group not significant different compared to Control
Liu et al.[117]	Plasma	ELISA	193	CD47	Increased in OSCC compared to Control; Anti-CD47 autoantibody plasma induces apoptosis via p-AKT (in vitro)
Lin et al.[224]	Serum	Immunoassay	4	p53	Increased in OSCC compared to Control
Schutt et al.[225]	Tissue and Serum	IHC and Elisa	59	Sperm Protein 17 (SP17)	Increased in OSCC compared to Control
Liu et al.[118]	In vitro	ELISA	N/A	ATP-binding cassette subfamily C member 3 (ABCC3)	Plasma containing the autoantibody inducted apoptosis and cell cycle arrest

Abbreviation: N/D, not disclosed.

Interestingly, autoantibodies may also have some antitumor capabilities. Liu et al.[117,118] demonstrated that plasma containing higher levels of autoantibodies positive for integrin-associated protein (CD47) or Adenosine triphosphate-binding cassette subfamily C member 3 (ABCC3) induced apoptosis and cell cycle arrest in 5 different OSCC cell lines, compared to plasma negative for these antigens. In their earlier work, Liu et al.[118] showed that anti-ABCC3 immunoglobulin G (IgG) significantly induced apoptosis and cell cycle arrest in the CAL27 cell line, while no significant effects were found for the SCC15 cell line, even though ABCC3 was expressed in both cell lines. The authors hypothesized that the ABCC3 structure might be different between the two cell lines, resulting in different responses to the autoantibodies. On the other hand, in their more recent work, Liu et al.,[117] demonstrated that plasma containing anti-CD47 autoantibodies induced cell apoptosis and inhibited the invasion of all three OSCC cell lines (CAL27, SCC25, and SCC9 cell lines) via p-AKT suppression.

To demonstrate the feasibility of using autoantibodies to diagnose OSCC, Chu et al.[119] showed that the sensitivity of 8 autoantibodies for OSCC, ranged from 16% to 62%, with an area under the curve (AUC) ranging from 0.656 to 0.796. A panel containing 4 autoantibodies (anti- lectin, mannose binding 2 (LMAN2), anti-prostaglandin reductase 1 (PTGR1), anti-ras-related protein 13 (RAB13), and anti- ubiquinol-cytochrome c reductase core protein 2 (UQCRC2) presented a sensitivity of 76% and AUC of 0.863.

6 | ORAL MICROBIOME-BASED BIOMARKERS FOR OSCC

The oral cavity contains up to 1000 microbial species, comprised of bacterial, fungal, viral, archaeal, and protozoan species, which are known as the oral microbiome. These species interact among themselves and with their host, thus forming symbiotic interactions known as the oralome.[8,120] Even though the oral microbiome is known to be resilient, insults or changes to the microbiome, such as those due to tobacco and alcohol use, can shift the oralome to an unbalanced state of host–microbe interactions, in part characterized by dysbiosis, which can promote diseases in the host, including OSCC.[8,120] Recent cohort studies have demonstrated that poor oral hygiene increases the risk and decreases the survival rates of patients with head-and-neck cancer (HNC),[121,122] while epidemiological data shows that the odds ratio of OSCC are up to 4.6-fold higher in patients with severe periodontitis compared to controls,[123-126] suggesting that oral microbial dysbiosis may play an important role in HNC and OSCC pathogenesis. In fact, early association studies by Nagy and colleagues found that many oral pathogens including *Porphyromonas* spp. and *Fusobacterium* spp. are enriched in OSCC tissues as compared with adjacent heathy ones.[127,128] Interestingly, in a mouse model of oral tumorigenesis, co-infection by the two anaerobic periodontal pathogens *Porphyromonas gingivalis* and *Fusobacterium nucleatum* significantly enhanced the severity of tongue tumors, concomitant with increased STAT3 activation and

increased IL-6 levels in tongue epithelium.[129] Further, anaerobic and facultative bacteria can colonize and grow in tumors.[8,130] For instance, Abed et al.,[131] reported that *F. nucleatum* was detected in CT26 colon tumors 2 h after tail vein injection in vivo, and bacterial proliferation was observed inside of the tumors 24 h and 72 h post-injection.

Given its nature and the recent evidence linking the oral microbiome to cancer, specifically OSCC,[8] the oral microbiome has an immense potential to be a diagnostic biomarker for OSCC. Table 5 summarizes the recent studies in the literature.

Specifically for OSCC, a systematic review with 2809 patients indicated an association between oral microbial dysbiosis and OSCC.[132] Moreover, the majority of these studies (75%) indicated a significant increase in Fusobacteria, especially *F. nucleatum* species, and *P. gingivalis* abundance in OSCC. These bacteria are known to induce the production of inflammatory cytokines, cellular proliferation, migration, and invasion, and inhibition apoptosis, through host cell genomic alterations in OSCC.[133] Additionally, two studies reported significantly larger tumors and larger numbers of lesions in mice infected with *F. nucleatum*, *P gingivalis* and *Treponema denticola* compared to controls.[134,135]

Despite numerous studies on the oral microbiome and OSCC, the focus of these studies has traditionally been on bacterial dysbiosis.[120] Yet, OSCC has also been linked to dysbiosis in the oral mycobiome and virome. Specific changes in OSCC mycobiome have been identified by several studies, such as the enrichment of *Candida*, *Gibberella* and *Hannaella* genera, as well the decrease of *Malassezia* and *Aspergillus* genera in OSCC compared to healthy controls.[136] Moreover, several studies have demonstrated that *Candida albicans* is able to induce carcinogenesis through induction of pro-inflammatory T-helper 17 cells, as well as induction of IL-6 and IL-8 cytokines by oral cells.[136-138] Yet, due to the relatively low prevalence of individual fungal species and the lack of well-characterized reference genomes, further studies are needed on the relationship between mycobial dysbiosis and OSCC.[136,138]

Several viruses has been identified in OSCC tissues, including *Epstein–Barr Virus* (EBV),[139] *Human papillomavirus* (HPV),[140,141] *herpes simplex virus* (HSV)[142] and *Human cytomegalovirus* (HCMV).[143] EBV prevalence is between 48.18%–50.2%, depending on the specimen type (i.e., paraffin-embeded or fresh frozen, respectively).[139,144] Further, She et al.[139] meta-analyzed 13 case–control studies and found that EBV infection is statistically associated with increased risk of OSCC (OR 5.03%–95% CI; 1.80–14.01) compared to controls. These data highlight that EBV infection may be a high-risk factor for OSCC. Although HPV status has been used in clinical settings to categorize OSCC patients, HPV's carcinogenic role in OSCC is still debatable.[145] HPV-positive OSCC has been significantly associated with younger patients with no history of smoking and drinking.[146] Moreover, a distinct oral microbial composition has been reported for HPV–positive OSCC-namely an enrichment in *Lactobacillus*, *Gemella*, *Leuconostoc* and *Weeksellaceae* genera in HPV-Positive OSCC compared with HPV-negative tissues–suggesting that HPV presence may influence the oral microbiome composition toward dysbiosis.[8,147] However, the lack of molecular evidence,[145] heterogeneity due to geographic location and detection methods,[146] and its low prevalence (3%–6%),[140,141,148] with no significant association with OSCC[148] has raised questions whether HPV does in fact drive OSCC carcinogenesis.[145] HSV and HCMV prevalence in OSCC, are 5%[142] and 3%,[143] respectively, which seems to indicate that these viruses may not be high-risk factors for OSCC development, but may instead play a minor role in the disease, since they are known to act as mutagens in other tissues.[149] Nonetheless, more studies are needed to further understand the role of these viruses in OSCC pathogenesis.

Several mechanisms have been proposed to explain the carcinogenic potential of the oral microbiome, including promoting epithelial barrier dysfunction, chronic inflammation, and epigenetic modulation.[8] Kamarajan et al.,[134] for instance, demonstrated that bacteria found in periodontal disease, including *Treponema denticola* promote cancer aggressivity via toll-like receptor 2 (TLR2)/myeloid differentiation primary response 88(MyD88) triggered activation of integrin/FAK signaling. It is intriguing to note that *F. nucleatum* has also been linked to colorectal cancer (CRC), breast cancer and pancreatic cancer.[150-152] In CRC, *F. nucleatum* promotes colorectal carcinogenesis via the adhesin FadA by modulating E-cadherin/β-catenin signaling,[153] especially. via induction of the Wnt/β-catenin modulator Annexin A1.[154] Furthermore, *F. nucleatum* induces autophagy activation and apoptosis inhibition that is dependent on toll-like receptor 4 (TLR4)/MyD88 signaling.[150] It remains to be investigated if similar pathways in OSCC are modulated by *F. nucleatum*.

7 | CURRENT CHALLENGES AND FUTURE PERSPECTIVES

Advancements in genomics, proteomics, metabolomics, immunomics, and microbiomics, as shown in this review, have expanded the understanding of OSCC tumorigenesis and progression, and also the discovery of biomarkers that facilitate the diagnosis and prognosis of the disease. However, tumorigenesis complexity, including synergistic genomic, transcriptomic, proteomic and metabolomic alterations, coupled with an oral microbiome dysbiosis, and patient heterogeneity make it unlikely for a single diagnostic biomarker to be effective for cancer diagnosis. In fact, panels integrating multiple omics may be required for a comprehensive patient evaluation and would enhance the panel reliability, sensitivity and specificity.[8,120,155] Additionally, methodological standardization and reproducible validation of oral cancer biomarkers are still required, since multiple methodologies were used to obtain these biomarkers and contradicting results can still be found in the literature.

As future perspectives, we expect a rise in the use of liquid biopsies, especially the use of saliva for OPMD and OSCC diagnosis and prognosis, as saliva has been widely investigated as a source

TABLE 5 Oral microbiome-based biomarker for OSCC

Authors/Year	Source of biomarker	Method	Sample size	Microbiome	Findings
Katirachi et al.[140]	N/D	Systematic Review and meta-analysis	5007	*Human papillomavirus*	6% (95% CI; 3%–10%) HPV prevalence in OSCC
Peter et al.[226]	Swabs, Saliva, Tissue, Oral rinse and Tissue scraping	Systematic review and meta-analysis	970	*Fusobacterium, Peptostreptococcus, and Parvimonas*	Enriched in OSCC compared to Control
				Haemophilus and Granulicatella	Decreased in OSCC compared to Control
Mauceri et al.[227]	Saliva	Systematic review	1335	*Porphyromonas gingivalis, Fusobacterium nucleatum, Neisseria flavescens, Fusobacterium periodonticum, Prevotella intermedia and Campylobacter spp,*	Enriched in OSCC compared to Control, but it was not possible to profile a specific microbiota associated with OSCC due to the great heterogeneity of the studies
Mun et al.[132]	Saliva, Tissue, Oral rinse and Oral swab	Systematic review	2809	*Fusobacteria*, *Firmicutes*, and *Bacteroidetes*	Enriched in OSCC compared to Control; All the studies identified microbial dysbiosis to be associated with OSCC
Melo et al.[141]	Tissue	Systematic review	383	*Human papillomavirus*	4.4% of the patients were HPV positive; None of the studies found had a control group
Lafuente Ibañez de Mendoza et al.[228]	In vitro and In vivo	Systematic review	N/A	*Porphyromonas gingivalis*	Enriched in OSCC compared to Control; Bacterium involved in epithelial-mesenchymal transition of malignant epithelial cells, neoplastic cell growth, proliferation and invasion
Ramos et al.[229]	Saliva, Tissue, Oral rinse and Oral swab	Systematic review	859	*Fusobacterium nucleatum subsp. Polymorphum and Pseudomonas aeruginosa*	Enriched in OSCC compared to Control; Corroborated dysbiosis in OSCC. Enrichment of taxa associated with inflammation and production of acetaldehyde
She et al.[139]	Tissue	Systematic review	1119	*Epstein–Barr virus*	Positive association with an increased risk of OSCC
Vyhnalova et al.[136]	N/D	Review article	N/A	*Candida albicans*, *Candida etchellsii*, *Candida famata*, *Gibberella*, *Hannaella*, *Rhodotorula*, *mucilaginosa*	Enriched in OSCC compared to Control
				Aspergillus tamarii, *Alternaria*, *Cladosporium*, *Halotolerans*, *Emericella*, *Malassezia restricta*, *Pichia anomala*, *Trametes*	Decreased in OSCC compared to Control
Radaic et al.[8]	Saliva and tissues	Review article	N/A	*Treponema denticola, Porphyromonas gingivalis, Fusobacteria Nucleatum, Tannarella Forsythia, Lactobacillus* spp., *Capnocytophaga gingivalis, Prevotella melaninogenica, Streptococcus mitis. Fusobacteria genera*	Enriched in OSCC compared to Control
				Streptococcus, Capnocytiphaga, Neisseria, Haemophillus and Aggreggatibacter	Decreased in OSCC compared to Control

(Continues)

TABLE 5 (Continued)

Authors/Year	Source of biomarker	Method	Sample size	Microbiome	Findings
Radaic & Kapila[120]	N/D	Review article	N/A	*Candida mycotype*, *Treponema denticola*, *Porphyromonas gingivalis*, *Fusobacteria Nucleatum*, *Tannarella Forsythia Capnocytophaga gingivalis*, *Prevotella melaninogenica*, *Streptococcus mitis*	Enriched in OSCC compared to Control
				Malassezia mycotype, *Streptococcus*, *Neisseria*, *Haemophillus and Aggreggatibacter*	Decreased in OSCC compared to Control
Chattopadhyay et al.[133]	N/D	Review article	N/A	*P. melaninogenica*, *Capnocytophaga gingivalis*, *Lactobacillus vaginalis*, *L. gasseri: L. johnsonii*, *L. fermentum*, *L. salivarius*, *L. rhamnosus*, *Fusobacterium nucleatum*, *F. periodonticum*, *Streptococcus vestibularis*, *S. mitis*, *S. salivarius*, *Prevotella oris*, *and Rothia mucilaginosa*	Enriched in OSCC compared to Control
				Aggregatibacter, *Lautropia*, *Haemophillus*, *Neisseria*, *Leptotrichia*, *P. jejuni: P. melaninogenica*, *and Prevotella pallens*	Decreased in OSCC compared to Control
Perera et al.[128]	Saliva, swab, and tissues	Review article	885	*Fusobacterium*, *Porphyromonas*, *Actinomyces*, *Propionibacterium* spp., *Candida albicans*, *Porphyromonas gingivalis*, *Streptococcus anginosus*, *Capnocytophaga gingivalis*, *Prevotella melaninogenica*, *Streptococcus mitis*, *Micrococcus luteus*, *Prevotella melaninogenica*, *Exiguobacterium oxidotolerans*, *Fusobacterium naviforme*, *Staphylococcus aureus*, *Veillonella parvula*, *Bacteroides fragilis*, *Ralstonia insidiosa*, *Fusobacterium naviforme*, *Peptostreptococcus micros*, *Clavibacter michiganensis subsp. tessellarius*, *Capnocytophaga* sp. *oral strain S3*, *Prevotella* sp. *oral clone BE073*, *Parvimonas sp. oral taxon 110*, *Eubacterium infirmum*, *Eubacterium brachy*, *Gemella haemolysans*, *Gemella morbillorum*, *Gemella sanguinis*, *Johnsonella ignava*, *Streptococcus parasanguinis I Peptostreptococcus stomatis*, *Streptococcus gordonii and Streptococcus salivarius*	Taxa associated with OSCC
Yang et al.[230]	Saliva and whole blood	16S Sequencing	428	*Lachnoanaerobaculum*, *Kingella*, *Parvimonas*	Enriched in OSCC compared to Control and correlated to genes in regulation of oncogenic and angiogenic responses
Gopinath et al.[231]	Whole mouth fluid and Oral Swab	16S Sequencing	94	*Enterobacteriae*, *Neisseria*, *Streptococcus and Fusobacteria*, *Prevotella*, *Treponema*, *Sphingomonas*, *Meiothermus*, *and Mycoplasma*	Enriched in OSCC compared to Control; Tumor surfaces elevated abundances of Porphyromonas, Enterobacteriae, Neisseria, Streptococcus and Fusobacteria, whereas Prevotella, Treponema, Sphingomonas, Meiothermus and Mycoplasma genera were significantly more abundant in deep tissue.

TABLE 5 (Continued)

Authors/Year	Source of biomarker	Method	Sample size	Microbiome	Findings
Gopinath et al.[232]	Whole mouth fluid	16S Sequencing	74	*Porphyromonas*	Correlated to OSCC
				Megasphaera, unclassified Enterobacteria, Salmonella and Prevotella	Correlated to OPMD
				Streptococcus, Rothia and Fusobacterium	Correlated to Control
Ganly et al.[233]	Oral rinse	16S Sequencing	38	*Fusobacterium, Prevotella, Alloprevotella*	Enriched in OSCC compared to Control
				Streptococcus	Decreased in OSCC compared to Control
Yang et al.[234]	Oral rinse	16S Sequencing	248	Fusobacterium periodonticum, Parvimonas micra, Streptococcus constellatus, Haemophilus influenza, and Filifactor alocis (in contrast to decrease of Streptococcus mitis, Haemophilus parainfluenzae, Porphyromonas pasteri, Veillonella parvula)	Enriched in OSCC compared to Control; Higher complexity of oral microbiota communities in stage 4 patients
Hsiao et al.[235]	Saliva	16S Sequencing	289	*Prevotella tannerae, Fusobacterium nucleatum, and Prevotella intermedia*	Enriched in OSCC compared to Control
				Streptococcus tigurinus	Decreased compared to Control
Lee et al.[236]	Saliva	16S Sequencing	376	*Bacillus, Enterococcus, Parvimonas, Peptostreptococcus, and Slackia*	Enriched in OSCC compared to Control
Al-Hebshi et al.[237]	Swabs and tissues	16S Sequencing	20 OSCC biopsies and 20 swabs	*F. nucleatum and P. aeruginosa*	Enriched in OSCC compared to Control
Wang et al.[238]	Saliva and oral swab	16S Sequencing	55	*Porphyromonas* and *Solobacterium* g	Enriched in OSCC compared to Control
				Haemophilus, Corynebacterium, Cellulosimicrobium, and Campylobacter	Decreased in OPMD compared to Control
Hu et al.[239]	Non-stimulated saliva	16S Sequencing	35	*Haemophilus, and Bacillus*	*Bacillus* enriched in OSCC compared to Control, while *Haemophilus* Enriched in OPMD compared to Control
				Streptococcus	Decreased in OPMD and OSCC
Yost et al.[240]	Oral swab	Metatranscriptome sequencing	15	Genera Fusobacteria, Selenomonas, Capnocytophaga, Dialister, and Johnsonella (genus Bacillus; species Porphyromonas catoniae, Kingella denitricans, Capnocytophaga gingivalis, among others, were associated with healthy, tumor-matching sites)	Enriched in OSCC compared to Control
Zhao et al.[241]	Oral swab	16S Sequencing	80	*Fusobacterium, Dialister, Peptostreptococcus, Filifactor, Peptococcus, Catonella and Parvimonas*	Enriched in OSCC compared to Control

(Continues)

TABLE 5 (Continued)

Authors/Year	Source of biomarker	Method	Sample size	Microbiome	Findings
Nie et al.[242]	Tissue	16S Sequencing	305	*Fusobacterium*, *Prevotella*, *Porphyromonas*, *Campylobacter*, *Aggregatibacter*, *Lautropia*, *Asteroleplasma*, *Parvimonas*, *Peptostreptococcus*, *Pyramidobacter*, *Roseburia*, *and Propionibacterium*	Enriched in OSCC compared to Control. The microbiome was highly correlated with tumor clinicopathological features, with several genera
de Abreu et al.[148]	Tissue	Nested PCR	90	*Human papillomavirus*	3.3% of the OSCC patients were positive for HPV. All cases were HPV-16
Nieminen et al.[243]	Tissue	Immunohistochemistry	149	*Treponema denticola*	Dentilisin present in OSCC and the majority of orodigestive tumor samples
Perera et al.[244]	Tissue	ITS2 sequencing	52	C. albicans, C. etchellsii, and Hannaella luteola–like species	Enriched in OSCC compared to Control
				A Hanseniaspora uvarum–like species, Malassezia spp., Aspergillus tamarii, Cladosporium halotolerans, and Alternaria alternata	Decreased in OSCC compared to Control
Listyarifah et al.[245]	Tissue	Immunohistochemistry	60	*Treponema Denticola*	Dentilisin present in 95% of OSCC tumor samples and 40% were immunopositive for dentilisin.
Shin et al.[246]	Tissue	16S Sequencing	72	*Fusobacteria*, *Firmicutes*, *Actinobacteria*, *Proteobacteria and Streptococcus*	Fusobacterium enriched in OSCC compared to Control
				Streptococcus	Decreased in OSCC compared to Control
Kikuchi et al.[144]	Tissue	PCR	233	*Epstein–Barr virus*	50.2% of OSCC; 66.7% of severe dysplasia; 43.1% of mild dysplasia were positive for EBV.
Mokhtari & Beiraghdar[142]	Tissue	PCR	60	*Herpes simplex-1*	5% of the OSCC patients were potivite for HSV
Saravani et al.[143]	Tissue	qPCR	48	*Human Cytomegalovirus*	6.3% of the OSCC patients were positive for HCMV
Harrandah et al.[135]	In vivo	ELISA and Western blot	N/A	*Fusobacteria*	Mice infected with bacteria developed significantly larger and more numerous lesions compared to those not infected.
Kamarajan et al.[134]	In vivo and in vitro	Western blot	N/A	*Treponema denticola*, *Fusobacterium nucleatum*, and *Prophyromonas gingivalis*	Periodontal pathogens promote cancer aggressivity in mice via TLR2/MyD88 triggered activation of integrin/FAK signaling
Gallimidi et al.[129]	In vivo and in vitro	Immunohistochemistry	N/A	*Fusobacterium nucleatum*, and *Prophyromonas gingivalis*	Periodontal pathogens promote cancer aggressivity in mice; Increased STAT3 and IL-6 levels in infected mice compared to control

of OSCC biomarkers in the majority (over 50%) of the studies analyzed. Additionally, saliva use may improve the ability to monitor OPMD and OSCC in patients over time, given its simple and safe collection protocols and ability for collection of larger volumes of samples.[155,156]

Together with liquid-based biopsies, multi-omic panels, such as those containing genomic, transcriptomic, proteomic, metabolomic, immunomic and microbiomic biomarkers may help us to diagnose early lesions or even prevent the onset of OPMDs and OSCC.

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CONFLICT OF INTEREST STATEMENT

David T. Wong has equity in Liquid Diagnostics LLC and RNAmeTRIX Inc.

DATA AVAILABILITY STATEMENT

Not applicable; All materials/information are provided in the manuscript.

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