Molecular pathways of oral cancer that predict prognosis and survival: A systematic review

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Abstract:

Several genes and pathways associated with oral squamous cell carcinoma (OSCC) are significant in terms of early detection and prognosis. The objective of this literature review is to evaluate the current research on molecular pathways and genes involved in oral cancer. Articles on the genes involved in oral cancer pathways were evaluated to identify potential biomarkers that can predict survival. In total, 36 articles were retrieved from internet databases, including EBSCO Host, Google Scholar, PubMed, and Science Direct, using the keywords "biomarker of oral cancer," "pathways of oral cancer," "genes involved in oral cancer," and "oral cancer pathways." A total of 36 studies related to OSCC were chosen. Most of the studies used cell lines, while others used archival tissues, few studies followed up the cases. Three major interlinked pathways found were the nuclear factor kappa B (NF-kB), PI3K-AKT, and Wnt pathways. The commonly mutated genes were cyclin D1 (CCND1), Rb, p53, FLJ10540, and TC21. The NF-kB, PI3K-AKT, and Wnt pathways are most frequently involved in the molecular pathogenesis of oral cancer. However, the CCND1, Rb, p53, FLJ10540, and TC21 genes were found to be more accurate in determining patients' overall survival. Polymerase chain reaction, immunohistochemistry, and immunoblotting were the commonly used detection methods.

Keywords:

Biomarkers, molecular pathways, prognosis, survival, targeted therapy

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Introduction

Cancer is a multistep process in which cell mutations accumulate and lead to initiation, progression, sustenance of proliferation, signaling, evasion of growth suppressors, avoidance of cell death, continual replication, angiogenesis, invasion, and metastasis. Cancer involves the mutation of several genes resulting in various pathway alterations.[1-5] Among the various head and neck cancer subtypes, approximately 10% comprise oral squamous cell carcinoma (OSCC).[6,7] It is necessary to elicit the genes and the pathways involved in oral cancer progression. Recent studies have examined the oncogenic signaling pathways, such as glycogen synthase kinase 3 (GSK-3β), c-Myc, AKT, β-catenin, p53, and nuclear factor kappa B (NF-kB), and suggested their roles in oral cancer progression. This systematic review aims to identify the most commonly involved genes and pathways in OSCC.

Methodology

A systematic review was conducted as per the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) [Table 1]. The key question was "which are the most commonly involved genes and pathways in OSCC?"

Study design

A systematic review was conducted by considering all the published original research articles that emphasized the genes and associated pathways involved in head and neck cancer. We also included studies that evaluated the genes that can predict oral cancer prognosis.

Inclusion criteria

Articles that evaluated genes and pathways in OSCC were selected.

Exclusion criteria

Review articles, theses, personal judgments, book chapters, powerpoint presentations, and studies that did not validate their results were excluded. Studies on saliva and serum were also excluded as we concentrated on articles pertaining to patient tissues.

Information sources and search strategy

An automated online search of databases, such as EBSCO host, Google Scholar, PubMed, and NCBI, was performed using a combination of phrases and keywords such as "biomarker of oral cancer," "pathways of oral cancer," "genes involved in oral cancer," and "oral cancer pathways." The relevant articles were also cross-referenced to retrieve additional articles.

Study selection

We set the search strategy per the guidelines given by the STROBE. The initial selection was done by reviewing the titles followed by the abstracts. Articles that were believed to be suitable were re-evaluated by two authors (RSR and DA). Disagreements on the articles were resolved by consulting a third author (SVS).

Data collection process

The overall data collection involved the following information: authors, journals in which the article was published, year of publication, research focus, methodologies employed, results obtained, conclusions drawn, and future scope of research in the given field.

Synthesis of results

We summarized the results of the individual studies and listed the pathways involved as well as the survival rate of the patients. Genes involved in these pathways were also analyzed.

Results

Study selection

The articles were critically evaluated based on STROBE [Figure 1], and 36 of these were considered for the review.[8-43]

Study characteristics

Of the 36 selected studies, seven studies were published in 2000–2010 [Figure 2].[8-10,12,14,15,17,20-27,29-37,39-43]

Synthesis of results

Of the 36 studies [Table 3], 34 studies emphasized the genes that regulated pathways in oral cancer progression:[8-12,14-20,22-43]

Twenty-one studies focused on genes and the associated pathways that explained the initiation, proliferation, and progression of OSCC.[10,11,14,16,18-20,23-31,34,35,37,39,43]

The pathways that were found to be commonly associated with OSCC included the PI3K-AKT pathway (seven studies)[10,11,13,14,18,29,39] and the NF-kB pathway (four studies).[8,20,26,39]

Three studies focused on genes associated with patient survival rate, including cyclin D1 (CCND1), Rb and p53, FLJ10540, and TC21.[17,19,28] Three studies showed the involvement of transforming growth factor-beta pathways in OSCC.[26,31,44]

The most commonly used techniques were polymerase chain reaction (PCR), immunoblotting assay, and immunohistochemistry (IHC). Other techniques employed were microarray assays, migration and Matrigel invasion assays, ELISA, transwell migration assays, flow cytometry, and luciferase assays.

Figure 1: Study design

Table 1: Methodology employed
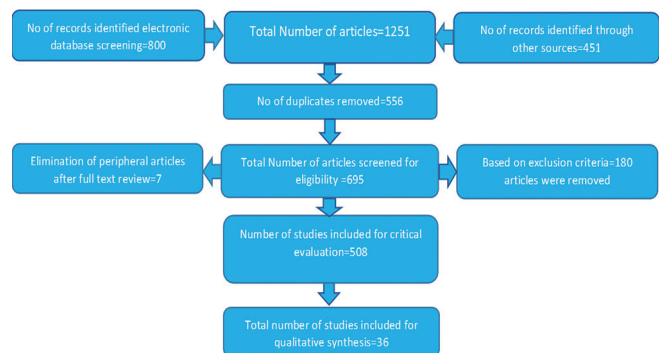
Method/methodology	Resources utilized
Comprehensive review of original research articles related to biomarkers involved in pathways of oral cancer, assessment of the methodology used, and identification of the potential biomarkers which can predict survival	e-journals, published journals, Scopus, HELINET, EBSCO host, Google Scholar, PubMed
	Reporting as per STROBE analysis

STROBE: STrengthening the Reporting of OBservational studies in Epidemiology

Discussion

The potential pathways involved in the pathogenesis of oral cancer [Figure 3] and the potential biomarkers that predict patient survival must be explored to improve patient's quality of life.

Genes and pathways involved in molecular pathogenesis influencing patient prognosis

There are 16 studies listed in Table 2 that show the involvement of various pathways and genes that predict patient's survival. The NF-kB, PI3K-AKT, and Wnt pathways and the CCND1, Rb, p53, FLJ10540, and TC21 genes are commonly mutated and can predict the patient's survival rate. The related studies and their summarized findings are described below:

Rb and p53 pathways indicate poor survival in OSCC.[19] CCND1 IHC expression dictates the overall survival rate of patients in OSCC.[17] FLJ10540 expression contributes to a reduced patient survival rate.[11,44] TC21 expression serves as a poor prognostic indicator in OSCC.[28] An increased NF-kB pathway showed a low survival rate in head and neck squamous cell carcinoma (HNSCC).[38] Phosphorylated AKT, epidermal growth factor receptor

Figure 2: Articles publication frequency by different countries year wise (36 selected articles)

variant III (EGFRvIII), and disease stage determine the survival of the patients.[10] In the present review, the most common pathways associated with OSCC patient's survival were the PI3K-protein kinase B (PKB)/AKT and NF-kB pathways.[8,10,38] It is important to identify these pathways, since their therapeutic interruption could possibly increase patient survival rates.

Cyclin D 1

CCND1 is a protein that regulates the cell cycle and is encoded by the CCND1 gene on chromosome 11q13. In a study conducted by Hanken *et al*., 2014,[17] CCND1 amplification and overexpression were associated with Ki-67 expression in HNSCC. There was overexpression of CCND1 that was associated with an increased relapse rate and lymph node metastasis, which dictated poor patient survival (79%).[17] In the phosphorylation of pRB, a major regulator of the G1/S transition of cell cycle CDK4/6-CCND complex is involved. The pRB phosphorylation results in the release of bound E2F transcription factors, premature transition into S-phase, and a resultant nondiploidy of DNA. In numerous epithelial malignancies, amplification of CCND1 locus, 11q13, has been reported. Approximately 30%–50% of OSCC tumors show amplification or increased protein expression.[45-48] CCND1 can be targeted through the inhibition of cyclin-dependent kinase 4.

Rb and p53 pathway

The Rb protein plays a key role in cell cycle regulation as its phosphorylation will release the brakes on the cell cycle with uncontrolled proliferation. Jayasurya *et al*., 2005[19] conducted a study on Rb and p53 pathways. CCND1, Rb, p16, CDK4, P21, Bcl-2, p53, and the proliferating cell nuclear antigen were analyzed in OSCC by IHC. Their results showed that the Rb and p53 pathways are interconnected. The Rb protein increased
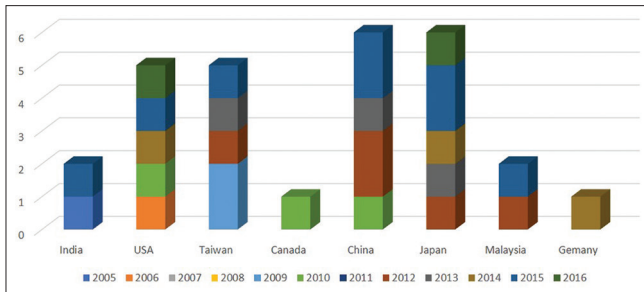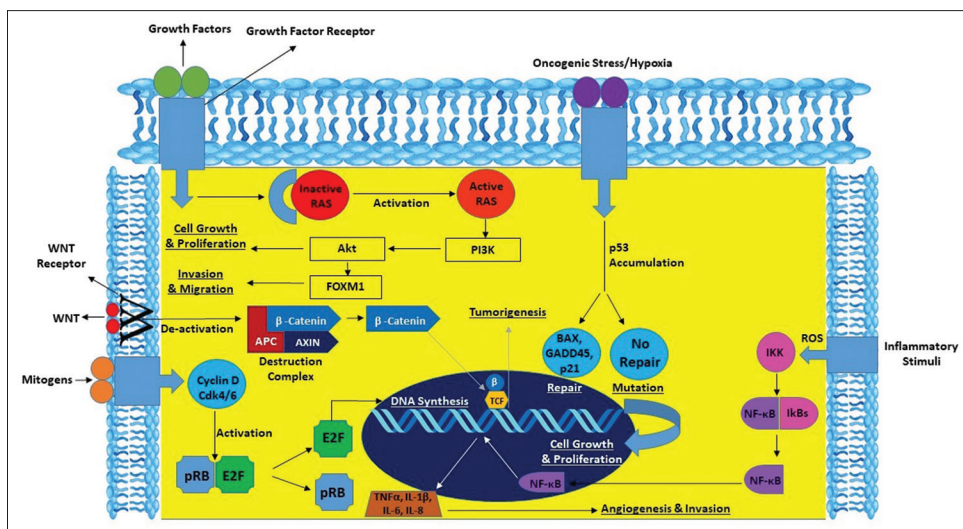

Figure 3: Interplay of genes and pathways in oral cancer

Table 2: Genes and pathways indicating the patient survival rate

Author and years	Gene/pathway	Gene expression	Survival rate
Jayasurya *et al*., 2005[19]	Rb and p53	Increased	Poor
Ravindran *et al*., 2015[31]	β-catenin, Oct-4, and Nanog	Increased	Poor
Lee *et al*., 2015[26]	IL-β	Increased	Poor
Hanken *et al*., 2014[17]	CCND1	Increased	Poor
Shimizu *et al*., 2012[34]	Annexin 10	Increased	Poor
Chen *et al*., 2012[12]	SLP-2	Increased	Poor
Macha *et al*., 2010[28]	TC21	Increased	Poor
Anbo *et al*., 2013[8]	NF-kB	Increased	Poor
Yan *et al*., 2010[38]	NF-kB	Increased	Poor
Wei *et al*., 2013[37]	TAZ	Increased	Poor
Chang *et al*., 2013[10]	AKT, EGFRvIII	Increased	Poor
Li *et al*., 2012[27]	GOLPH 3 mRNA	Decreased	Good
Fukumoto *et al*., 2014[14]	WWP2	Decreased	Poor
Monteiro *et al*., 2014[51]	EMMPRIN	Increased	Poor
Ohyama *et al*., 2013[30]	TGF-β and EGF pathway gene	Decreased	Poor
Chen *et al*., 2015[44]	Aurora-A, FLJ10540, MMP-7, MMP-10	Increased	Poor

IL-β: Interleukin beta, CCND1: Cyclin D1, NF-kB: Nuclear factor kappa B, EGFRvIII: Epidermal growth factor receptor variant III, MMP: Matrix metalloproteinase, TGF-β: Transforming growth factor-beta, SLP-2: Stomatin-like protein-2

CCND1 and reduced p16 expression and showed a worse prognosis. The relative risk was found to be 2.92 for the Rb gene and 1.57 for p53. The Rb proteins, such as CCND1 were associated with the overall survival and remission of the patients compared to the p53 proteins. Overexpression of CCND1 and underexpression of p16 indicate poor survival.[19] Rb and p53 are the two key pathways that are frequently involved in the progression of OSCC. Some studies found that nearly 90% of cases involved one or both pathways, as well as abnormalities in either the Rb gene or the p53 gene. Some of the data suggest that these pathways are universally targeted in carcinogenesis and oral cancer.[19]

FLJ10450

Matrix metalloproteinase-2 (MMP-2) plays an essential role in tumor development and its expression correlates with metastasis in various cancers including OSCC. A study conducted by Chen *et al*., 2009[11] stated that FLJ10540 and FOXM1 upregulate the expression of MMP-2 and FLJ10540 plays a role in OSCC progression and metastasis. Increased expression of FLJ10540 in OSCC correlates with poor survival of the patients.[11] Reduction in the expression of FLJ10540 through siRNA inhibits the invasion and migration of the OSCC. Analysis showed a positive correlation of FLJ10540, FOXM1, and MMP-2 with aggressive behavior of OSCC. The findings suggest that FLJ10540 is an important prognostic factor besides a new therapeutic target in the FLJ10540/FOXM1/MMP-2 pathway for OSCC management.[11]

TC21

TC21 belongs to the Ras family, which is involved in various cell functions, including cellular proliferation, apoptosis, differentiation, vesicle trafficking, cytoskeletal organization, and nuclear transport. TC21 protein interacts with both Erk2 and PI3-K indicating the involvement of these pathways in TC21-mediated signaling in oral cancer cells. In the study conducted by Macha *et al*., 2010,[28] there was gradual increase of TC21 expression from normal tissue to leukoplakia and malignancy that was clinically significant (97.1%). They observed an increased TC21 expression in the early stages of OSCC (T1 + T2) than in the advanced stages (T3 + T4). Moreover, interaction of TC21 with Erk2, 14-3-3 σ, and PI3-K was also observed. Its interaction with Erk2, 14-3-3 σ, and PI3-K suggest that TC21 is a signaling pathway for oral cancer.[28] TC21 bypasses the inhibitory function of domain-negative Ras. TC21 is involved in both growth and morphologic transformation, suggesting that it plays major role in cellular growth through signaling pathways. Interestingly, it initiates the AKT, serine/threonine kinase pathways, which cause cell proliferation, survival, and transformation through various effectors, such as Bad, mTOR, and GSK-3β.[28] It is suggested that TC21 expression is an early event in carcinogenesis, and it coincides with poor prognosis of OSCC.

Nuclear factor kappa B pathway

NF-kB is involved in cell survival, cancer progression, inflammation, and immune response. Increase in NF-kB raises the Interleukin-6, which in turn increases the expression of signal transducer and activator of transcription. Enzyme cycloxygenase-2 (COX-2) is the target molecule of NF-kB, and it synthesizes prostaglandins from arachidonic acid. Overexpression of COX-2 induces cancer progression by increasing vascularity and cellular proliferation and the evasion of apoptosis processes.[38] Further metastasis releases vascular endothelial growth factors and MMP, which are inhibited by pyrrolidine dithiocarbonate. NF-kB

Table 3: Studies scrutinized for biomarker and gene expression

Year	Author	Country	SCC Samples	Biomarker/ Pathway	Methods	Gene expression	Conclusion
2005	Jayasurya *et al*.[19]	India	348 OSCC patient tissue	Rb gene p53 gene	IHC	Downregulation of p16 and upregulation of CCND1	Rb pathway comparatively more important than p53 pathway in oral cancer prognosis
							Overexpression of CCND1 and underexpression of p16 are associated with a decreased patient survival rate
2006	Gibson *et al*.[16]	USA	Cell line	Bcl-2 TNFRSF5	a. Effect on differentiation b. Effect of detachment c. Effect of Rz279 c. Effect of BCL-2 Gene expression by immunoblotting	Increased BCL-2 gene expression	Effect of different apoptotic stimuli on normal cells and oral cancer cells can be explained by expression of pro-apoptotic genes
2009	Chen *et al*.[11]	Taiwan	256 OSCC tissues, Cell lines	FLJ10540/ FOXM1/ MMP-2	Immunoblot analysis, gelatin zymography	Upregulated FLJ10540, FOXM1, MMP-2	FLJ10540 upregulation cause increased progression of OSCC with poor patient survival
2009	Chi *et al*.[13]	Taiwan	3 pairs of resected OSCC specimens	977 proteins were quantified	MALDI-TOF-MS IHC, transwell migration assay, Western blot analysis, cell proliferation assay	IFN signaling path-way was significant	UCRP overexpressed in OSCC lesions at different sites within the oral cavity
2010	Henson and Gollin[18]	USA	Cell lines	FGFR3, KLF13	PCR Western blot assay, cell proliferative assay, transfection	Overexpression of FGFR3, KLF13	KLF13 and FGFR3 expression causes OSCC
2010	Yan *et al*.[38]	China	30 SCCHN tissue and cell lines	NF-kB	IHC Western blot assay NK-kB luciferase reporter assay Electromotility shift assay Immunofluorescence Cell migration assays	Increased expression of NF-kB	NF-kB significantly correlated with increased SCCHN metastasis Inhibition of NF-kB expression reduces hematologic and lymphatic metastasis of tumors
2010	Macha *et al*.[28]	India	120 leukoplakia, 83 OSCC, 30 nonmalignant patients	TC21	IHC, Western blot analysis, co-immunoprecipitation	Overexpression of TC21	TC21 interacts with Erk2, PI3-K, 14-3-3f, and 14-3-3r proteins It is an early diagnostic marker of OSCC with poor prognosis
2012	Chen *et al*.[12]	China	180 patients and cell lines	SLP-2	PCR, Western blot, IHC	Overexpression of SLP-2	Overexpression of SLP-2 in TSCC cell lines, and overexpression of SLP-2 in TSCC tissues compared to adjacent noncancerous tissue
2012	Li *et al*.[27]	China	179 TSCC tissue, cell lines	GOLPH 3	RT-PCR Western blotting Immunohistochemistry	GOLPH 3 mRNA were upregulated in TSCC	Upregulated GOLPH 3 correlated with poor prognosis and reduced survival of the patients with cN0 oral tongue cancer
2012	Shimizu *et al*.[34]	Japan	100 OSCC tissue, cell lines	Annexin A10	qRT-PCR and Immunoblotting analysis Transfection with shRNA plasmid Cellular proliferation assays Flow cytometry Immunohistochemistry	Annexin A10 upregulated	ANXA10 overexpressed in OSCC-associated tumor progression by promoting cell-cycle progression in the G1 phase through activation of ERK/MAPK pathways

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Table 3: Contd...

Year	Author	Country	SCC Samples	Biomarker/ Pathway	Methods	Gene expression	Conclusion
2012	Vincent-Chong *et al*.[36]	Malaysia	27 OSCC patient tissue	ISG15, Nestin, and WNT11	qRT-PCR	Overexpressed ISG15, Nestin, and WNT11	Several genomic copy number alterations showed correlation with ISG15, Nestin, and WNT11
2013	Anbo *et al*.[8]	Japan	2 cell lines	NK-Kb	RT-PCR, luciferase reporter assay, colony-forming assay	NK-kB overexpressed	Hypoxia plays a role in NF-kB signaling pathway in OSCC. The NF-kB pathway can be used as a therapeutic target
2013	Jian *et al*.[20]	Taiwan	OSCC tissue	Gα12	Microarray assay (OSCC tissue) qRT-PCR IHC and Western blot assay (OSCC tissue) Migration and Matrigel Invasion assay (OC-3, CGHNC9 cell lines) ELISA (IL-6 and IL-8) Microarray assay (OSCC tissue) Culture media (HSC3, SCC25, OC3, and CGHNC9 cell lines) Transwell invasion assay (cell lines)	Elevated expression of Gα12	Gα12 is a potential biomarker for therapeutic purpose. Since Gα12 causes migration and invasiveness of oral carcinoma
2013	Ohyama *et al*.[30]	UK	Cell lines	MALT-1	Microarray analysis RT-PCR Gene ontology and pathway analysis Wound-healing assay	MALT-1 down-regulated in OSCC	MALT1 acts as a tumor suppressor and the loss of expression stimulates progression, invasion and metastasis of oral cancer and promoting EMT pathway
2013	Chang *et al*.[10]	Taiwan	108 HNC tissue	EGFRvIII	IHC, DNA sequencing	Increase in EGFRvIII and weight EGFR	Data suggest that pAKT and EGFRvIII could be used as prognostic markers
2013	Yoshida *et al*.[40]	Japan	OSCC tissue=12, dysplasia=36 and, normal mucosa=12 cell lines	Notch-1	RT-PCR, Western blotting, Immunofluorescence	Increased expression of Notch-1	Notch-1 expression involved in increased proliferation of tumor cells with TNF-α dependent invasion
2013	Wei *et al*.[37]	China	52 TSCC specimen, Cell lines	Transcriptional coactivator with PDZ binding motif (TAZ)	Cell immunofluorescence, Real-time RT-PCR, Western blotting analysis, immunohistochemistry	Increased TAZ in TSCC	Clinical pathological correlation with TAZ indicates poor survival. It plays a crucial role in the tumourigenesis of TSCC
2014	Jiang *et al*. [21]	China	9 samples of OSCC	NNMT, BAX, MMP-9, VEGF	Microarray data analysis, gene otology, differential expression of gene analysis, protein-protein interaction network analysis	NNMT, BAX, MMP-9, VEGF were upregulated	A group of genes and pathways are involved in the progression of oral cancer

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Table 3: Contd...

Year	Author	Country	SCC Samples	Biomarker/ Pathway	Methods	Gene expression	Conclusion
2014	Yen *et al*.[39]	Taiwan	40 tissue samples, cell lines	MicroRNA-99a and IGF1R	Quantification of miRNA qRT-PCR Lentiviral-infected cancer cell lines Transwell assay Cell proliferation Migration and invasion assay Lung colonization assay Immunoblot analysis Gene expression analysis Immunoblot assay Western blot assay Luciferase reporter assay Drug treatment Cell culture and Immunoblot assay (using cancer cell lines)	MicroRNA-99a downregulated and insulin-like growth factor I receptor upregulated in OSCC	miR-99a is frequently downregulated and functions as a tumor suppressor in OSCC cells and miR-99a regulates IGF1R within a reciprocal regulation suggesting that miR-99a can target IGF1R in cancer therapy
2014	Fukumoto *et al*.[14]	Japan	104 Cell lines	WWP2	PCR Western blot IHC Migration assay Cellular growth	WWP2 overexpressed	WWP2 promotes OSCC through PTEN/PI3K/AKT pathway
2014	Hanken *et al*.[17]	Berlin	546 HNSCC patients	CCND1	IHC FISH	CCND1 expression increased in HNSCC	Ki-67 expression was significantly associated with CCND1 amplification and CCND1 overexpression in HNSCC
2015	Lee *et al*.[26]	Taiwan	32+40 OSCC patients who received curative surgery	IL-1β	qPCR, IL-1b ELISA, cell proliferation assays, cytokine antibody array analysis, inflammation CBA analysis, tube formation assay, morphology assay, cell migration assays	IL-1β expression increased in OSCC	The study showed the close association of IL-1β, carcinogen exposure, and pathogenesis of OSCC
2014	Natarajan *et al*.[29]	India	57 OSCC tissues	S100A4	Immunohistochemistry	S100A4 overexpressed in OSCC	Overexpression of S100A4 significantly correlated with lymph node involvement, clinical stage, pattern of invasion and metastasis
2014	Zhang *et al*.[42]	USA	Cell lines	TKT	Western blot assay Knockdown of TKT or AK2 expression in UM1 and UM2 cancer cell lines Knockdown of ASCT2 Cell proliferation assay (siTKT and siAK2) Glucose assay Lactase assay Glutamine assay siTKT and siAK2	TKT overexpressed in the oral cancer cells	Knockdown of TKT or AK2 significantly inhibits cancer cells Due to deficiencies in the PPP caused by siRNA (TKT, UM1, and UM2) cells, it showed increased uptake of glucose and glutamine, and production of lactate

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Table 3: Contd...

Year	Author	Country	SCC Samples	Biomarker/ Pathway	Methods	Gene expression	Conclusion
2015	Fukumoto *et al*.[15]	Japan	36 OSCC tissue with cell lines	miRNA-26a/b	qRT-PCR Cell proliferation, migration and invasion assay XTT assay Matrigel assay RT-PCR Plasmid construction and dual luciferase reporter assay Western blot and qRT PCR Pathway regulation	miRNA-26a/b significantly downregulated in OSCC	miR-26a/b were shown as tumor suppressors in OSCC miR-26a/b directly regulated TMEM184B in OSCC TMEM184B was upregulated in OSCC, causing migration and invasion of cells and functions as an oncogene
2015	Li *et al*.[41]	Taiwan	40 OSCC patient tissue	FLT4, KDR, and TFPI2	Pyrosequencing methylation assay, and qRT-PCR, pyrosequencing methylation assay, quantitative RT-PCR Illumina golden gate methylation array Methylation array profiling	FLT4, KDR, and TFPI2 methylation indicates early and advanced stages of OSCC	FLT4, KDR, and TFPI2 are early diagnostic markers of buccal OSCC
2015	Kong *et al*.[23]	Malaysia	87 OSCC and 61 normal tissues	TWIST and ZEB2	Tissue microarray, IHC	TWIST and ZEB2 increased expression in OSCC	TWIST and ZEB2 co-expression seen in OSCC patients with poor survival
2015	Koyama *et al*.[24]	Japan	OSCC cell lines-8, OSCC tissue 109	ANGPTL3	qRT-PCR Immunoblotting analysis IHC qRT-PCR Western blotting Cellular growth Cell-cycle analysis Tumorigenesis and tumor growth in vivo	ANGPTL3 Overexpressed in OSCC	ANGPTL3 expression contributes to activation of the MAPK signaling pathway via ERK pathway, which cause tumor progression
2015	Ravindran *et al*.[31]	India	60 patient tissue cell lines	β-catenin, Oct-4, and Nanog	Immunohistochemistry, immunofluorescence, Western blot and co-immunoprecipitation	Positive correlation between B-catenin, Oct-4, and Nanog	β-catenin and Oct-4 are independent biomarkers, and their interaction will be the crucial event in oral carcinoma. Nanog, β-catenin, and Oct-4 were well-correlated with stage, grade, lymph node metastasis and prognosis of OSCC
2015	Ren *et al*.[32]	China	Cell lines	miR-139	MTT assay, flow cytometry	Increased expression of miRNA on cancer cell	miRNA-139 causes apoptosis of cancer cells by inducing the AKT pathway
2015	Severino *et al*.[33]	Brazil	18 OSCC	MicroRNA (miR-21, miR-203, and miR-205)	Differential gene expression analysis, RT-PCR	Increased expression of miR-21, miR-203, and miR-205	miR-21, miR-203, and miR-205 are correlated with molecular invasion. MiR-31 and miR-130b known to inhibit cancer progression. It suggests that microRNA is used as a diagnostic tool for detection of cancer metastasis

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Table 3: Contd...

Year	Author	Country	SCC Samples	Biomarker/ Pathway	Methods	Gene expression	Conclusion
2016	Sushma *et al*.[35]	India	50 OSCC	PTEN and p16	Bisulfite conversion of DNA samples, MSP	Reduced expression of PTEN and p16 in OSCC	PTEN and p16 methylation causes cancer progression
2016	Kimura *et al*.[22]	Japan	100 patients OSCC tissues With cell lines	ARNT2	Immunoblot analysis, IHC, plasmid construction, and transfection of ARNT2 overexpression vector, proliferation assay, immunofluorescence, glucose cellular uptake assay	ARNT2 upregulated in OSCC	Through VHL-HIF1-α-GLUT-1 axis, ARNT2 shows downregulation in OSCC patients. It is a potent biomarker for proliferation, and a prognostic indicator as well as a therapeutic target of OSCC
2016	Languino *et al*.[25]	USA	Not mentioned	TGF-β	Immunoblotting	TGF-β upregulated in HNSCC	Exomes from fibroblasts in OSCC patients contain TβRII. Transfer of fibroblast exosomes increases TGF-β signaling and if devoid of TβRII, it remains unresponsive to TGF-β ligand, even in the absence of exosome transfer
2016	Zhang *et al*.[43]	China	89 TSCC patient's tissue	Notch, Smad3 Jagged I Slug	RT-PCR Immunofluorescence assay Western blot analysis	Notch, Smad3 Jagged I Slug mRNA expression was higher in TSCC	Notch1 and Smad3 correlate with clinical stage, but Jagged1 does not clinically correlate Notch1, Smad3, and Jagged1 Slug expression is higher in lymph node metastasis cases To block the TGF-β1-Smad3-Jagged1-Notch1 Slug pathway, blocking of Jagged1 or HEY1 is required
2016	Bornstein *et al*.[9]	USA	235 HNSCC	IL-10 and integrin	TCGA HNSCC database	IL-10 and integrin was upregulated	IL-10 and integrin pathways are involved in cancer progression

SCC: Squamous cell carcinoma, OSCC: Oral SCC, IHC: Immunohistochemistry, CCND1: Cyclin D1, MMP: Matrix metalloproteinase, MALDI-TOF-MS: Matrix-assisted laser desorption/ionization-Time of flight-Mass spectrometry, UCRP: Ubiquitin cross-reactive protein, FGFR3: Fibroblast growth factor receptor 3, PCR: Polymerase chain reaction, SCCHN: SCC of the head and neck, RT-PCR: Reverse transcription polymerase chain reaction, qRT-PCR: Quantitative reverse transcription polymerase chain reaction, NPC: Nasopharyngeal carcinoma, MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, SLP-2: Stomatin-like protein-2, TSCC: Tongue SCC, IL: Interleukin, HNC: Head and neck cancer, HNSCC: Head and neck SCC, EGFRvIII: Epidermal growth factor receptor variant III, TNF-α: Tumor necrosis factor alpha, NNMT: N-methyltransferase, VEGF: Vascular endothelial growth factor, IGF1R: Insulin-like growth factor I receptor, FISH: Fluorescence in situ hybridization, CBA: Cytometric bead array, TKT: Transketolase, PPP: Pentose phosphate pathway, MSP: Methylation-specific polymerase chain reaction, TGF-β: Transforming growth factor-beta, TCGA: The cancer genome atlas, IFN: Interferon, NF-kB: Nuclear factor kappa B, ELISA: Enzyme-linked immunosorbent assay, MALT-1: Mucosa-associated lymphoid tissue 1, EMT: Epithelial-mesenchymal transition, MAPK: Mitogen-activated protein kinases, ERK: Extracellular signal-regulated kinase, PDZ: Post synaptic density protein (PSD95), Drosophila disc large tumor suppressor (Dlg1), and zonula occludens-1 protein , BAX: BCL2-Associated X Protein, XTT: Sodium 3,3'-[1(phenylamino)carbonyl]-3,4-tetrazolium]-3is(4-methoxy-6-nitro) benzene sulfonic acid hydrate, PTEN: Phosphatase and tensin homolog

contributes to tumor hematologic and lymphatic metastasis.[38] A similar study conducted by Anbo *et al*., 2013[8] observed that under hypoxic conditions, NF-kB is activated and tumor metastasis and growth is increased.[8] Similarly, the knockdown of NF-kB expression inhibited tumor cell proliferation. This indicates that an increase in NF-kB may result in reduced patient survival.

PI3K-protein kinase B/AKT pathway
The activated PKB/AKT pathway initiates various cellular functions, including growth, angiogenesis, metabolism, survival, proliferation, protein synthesis, apoptosis, and transcription.[49,50] The study conducted by Sushma *et al*., 2016[35] showed that methylation of

PTEN and p16 is involved in the progression of OSCC, but promoter hypermethylation leads to inactivation of the PTEN and p16 genes, which activates AKT phosphorylation. Similarly, the study conducted by Fukumoto *et al*., 2014[14] showed an increase in WWP2 expression, which in turn reduced the expression of PTEN. Further, expression or upregulation of PI3K-PKB/AKT pathway induces tumor growth and results in poor prognosis.

Wnt signaling pathway
The study conducted by Ravindran *et al*., 2015[31] reported involvement of the Wnt/β-catenin pathway in the transformation of potentially malignant disorders to the

cancerous stage in the oral cavity.[31] The Wnt/β-catenin pathway plays a major role in the carcinogenesis, and there is a significant correlation between metastatic grading and lymph node metastasis ($P \leq 0.001$ for overall survival and <0.039 for disease-free survival).[31] The intracellular expression of β-catenin showed poor prognosis in overall survival of the patient. Moreover, β-catenin along with Nanog and Oct-4 expression showed a worse prognosis than β-catenin alone. The Wnt signaling pathway is often mutated, and Wnt/β-catenin pathway can be a potential target for anticancer therapy.

This review shows that among the various biomarkers and pathways, the most commonly involved in OSCC are Rb, p53, TC21, FLJ10540, CCND1, PI3K-AKT, NF-kB, and Wnt pathways. Only one study by Monteiro *et al.*, 2014 showed increased EMMPRIN expression to correlate with poor prognosis and survival.[51] The Rb, p53, and PI3K/AKT pathways were associated with overall patient survival rate.

The present review considered the evaluation of biomarkers and pathways in tissues as it closely relates to their activity in oral cancer and eliminates false positives/negatives compared to serum/saliva. Evaluation of biomarker expression in the saliva/serum results in wide variability in the levels of potential OSCC salivary biomarkers in both noncancerous individuals and OSCC patients.[52] There is a need for further validation of OSCC serum/salivary biomarkers with individuals who have either a chronic oral inflammatory disease or other types of cancers but do not have OSCC. Following meticulous standardization, a comparative study of the expression of oral cancer biomarkers and pathways with correlation of their expression in tissues versus saliva/serum would be an interesting prospect to investigate. The present systematic review highlights the importance of therapeutic targeting of biomarkers and pathways involved in OSCC that could help improve prognosis.

Commonly employed techniques
PCR, Western blot analysis, IHC, microarray, gel electrophoresis, cell proliferation assays, immunofluorescence, proliferative assays, ELISA, and cell cycle assays are the most commonly used techniques. Among these, the most preferred technique was PCR as it is highly sensitive and specific. However, techniques such as IHC and Western blot analysis were frequently used to validate the results. Microarray assays were employed to validate large numbers of gene expression. Based on the analysis, PCR seems to be a reliable technique in determining the expression of proteins and upregulation of genes. A real-time PCR is advantageous over a semi-quantitative PCR as we can monitor the progress of a PCR reaction in real time. At the same time,

a relatively small amount of PCR product (DNA, cDNA or RNA) can be quantified.

Conclusion

Although extensive studies have been conducted on these biomarkers and pathways using advanced technology, early diagnosis and treatment remain to be a pitfall. The findings of the present review emphasize the need for research in the domain of targeted cancer therapy. Therefore, it is essential to develop novel therapeutic mechanisms that can alter or inhibit these biomarkers and pathways.

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Conflicts of interest
There are no conflicts of interest.

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