SYSTEMATIC REVIEW

Diagnostic and prognostic role of cancer stem cell biomarkers in oral squamous cell carcinoma; A Systematic Review

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Abstract

Objective: To evaluate the role of cancer stem cell biomarkers in diagnosis and prognosis of OSCC patients.

Methods: The search strategy was entered into PubMed NLM, EBSCO CINAHL, EBSCO Dentistry & Oral Sciences Source, Wiley Cochrane Library, and Scopus. The full text eligible studies (n=7) were assessed for their quality using the JBI Critical Appraisal Checklist to evaluates the methodological quality of the studies based on possibility of bias in its design, conduct, and analysis. Selected studies were further analysed based on different parameters such as publication year, sample size, and outcomes.

Results: A total of 432 studies were identified through the search strategy. A total of 306 records were removed before screening either because of duplication or marked ineligible by the automation tools. The screened records were 126 out of which 104 were removed as they were not conducted on OSCC. Twenty-two reports were sought for retrieval, however, we could not find the full text of 3 studies and12 studies were excluded because the biomarkers were not associated with cancer stem cells. The most common cancer stem cell biomarkers associated with OSCC were MCT1,VEGF-A, GD15, HIF1 α, Ki67, Hsp 70, Cyclin D1, and CD44.

Conclusions: Various stem cell biomarkers have been found to have diagnostic and prognostic role in oral squamous cell carcinoma such as Cyclin D1, VEGF-A, GD15, and CD44. They can be used to predict the overall survival rate, local progression-free survival rate, and distant metastasis-free survival rate in Head and Neck cancer patients

Keywords: Neoplasms, Carcinoma, Squamous Cell, Prognosis, Ki-67 Antigen, Head and Neck, Neoplastic, Stem Cells, Larynx, Nasopharynx, Dentistry.

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Introduction

The head and neck squamous cell carcinoma (HNSCC) include the cancer of the oral cavity, oro-, nasopharynx, and larynx and accounts for approximately 900,000 cases and 400,000 deaths annually worldwide.[1,2] The burden of morbidity and mortality continues to rise as the tobacco-induced cancer epidemic accelerates especially in low and middle-income countries.[3] Oral squamous cell carcinoma (OSCC) accounts for about 90% of all cancers found in the oral cavity.[4,5] The most prominent risk-factor for oral cancer is long-term consumption of tobacco and alcohol followed by human papillomavirus (HPV) and Epstein- Barr virus (EBV) infections.[6]

Oral cancers are aggressive in nature and considered difficult to treat because of their higher loco-regional recurrence, metastasis to lymph nodes, and a higher degree of resistance to radio and chemotherapy (RCT). The conventional therapy including surgery to treat OSCC often leads to functional and aesthetic consequences[7,8] hence indicating the need for better diagnostic and prognostic tools. [9]

The common diagnostic tools to detect oral cancers are an extensive examination of the oral cavity, biochemical investigations, and invasive biopsy.[10] However, advancement in molecular pathology has led to the introduction of various stem cell biomarkers which are associated with the progression of many cancers[9]. Cancer stem cell biomarkers are secreted in the body by the tumour cells and can be of three types; predictive, diagnostic, and prognostic biomarkers. Diagnostic cancer stem cell biomarkers can be used to detect the cancer, whereas, the prognostic cancer stem cell biomarkers are used to evaluate the cancer outcome such as survival rate, line of treatment and overall response to treatment.[11,12]

Cell differentiation is a highly-regulated process and essential for the growth, repair, reproduction, and defence of all living organisms[13]. Stem cells are specialized cells that are capable of

proliferating/differentiating into different types of cells and tissues. The human body is composed of two major types of stem cells, the embryonic mesenchymal stem cell (EMSCs) with self-renewal capability and adult stem cells with limited self-renewal capability hence they are at a greater risk of malignant transformation.[14] Researchers have been working over the past few decades to understand the biology of the OSCC and cancer stem cells (CSCs) so that the therapeutic interventions can be made most effective. The cancer stem cells can play a vital role in the early diagnosis of OSCC and prevent major functional and aesthetic disabilities in patients and can improve the overall survival rate.

The objective of this review was to evaluate the role of cancer stem cell biomarkers in the diagnosis and prognosis of OSCC patients.

Methods

The study design used was systematic review of literature using a specific search strategy described below. The study was conducted at the Aga Khan University Hospital. The study duration was 4 months.

The studies conducted on the diagnostic and prognostic stem cell biomarkers in head and neck cancers were selected. Included were Randomized controlled trials (RCT), retrospective studies, case-control studies, and qualitative research. Opinion pieces, articles, magazines, newspapers, commentaries, editorials, and systematic reviews were excluded in our search. The studies conducted in the English language and those published from 2012 onward were included in the review. Our outcome of interest was studies reporting the diagnostic and predictive significance of cancer stem cell biomarkers. The review was registered with PROSPERO (ID# CRD42022360067), link: https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=360067

For our search strategy PRISMA guidelines were followed and the search dates were August 1st, 2022, till September 30th, 2022. The search terms used were ("Oral cancer" OR tongue OR head OR neck OR mouth) AND ("Carcinoma, Squamous Cell"[Mesh] OR "Squamous cell carcinoma*" OR "Squamous cell*") AND ("Stem cell*" OR "Neoplastic Stem Cells"[Mesh] OR "cancer stem cell*" OR BMI1 OR "salivary biomarker" OR Cytokines OR Diagnosis OR Biomarker OR "Tumour necrosis factor-A" OR "tumour grading" OR "TNM marker" OR "Sentinal lymph node biopsy" OR "Neck dissection") AND (RCT or randomised control trial).

The search strategy was entered into PubMed NLM, EBSCO CINAHL, EBSCO Dentistry & Oral Sciences Source, Wiley Cochrane Library, and Scopus. Only those studies conducted in the past 10 years were included (2012-2022). A total of 431 relevant studies were found out of which 7 were selected.

Two reviewers (TA and AM) independently extracted data from the selected studies on key outcomes addressing the study questions on a data extraction sheet. A third reviewer (RS) was consulted when there were discrepancies between the two reviewers and the extracted data was reassessed or sorted by discussion.

Since it is a systematic review with no human interaction or primary data collection and only review of already documented literature, no major ethical issues were of concern.

The full text eligible studies (n=7) were assessed for their quality using the JBI Critical Appraisal Checklist[22] by two of the co-investigators (TS and FA). The tool evaluates the methodological quality of the studies based on the possibility of bias in its design, conduct, and analysis. The studies were evaluated based on these checklists and they were categorized into low (Score of 1-4), moderate (5-7), and high (8-13) categories (Table-1). All seven studies were included in the final review since both the reviewers categorized them into moderate to high categories.

Results

A total of 432 studies were identified through the search strategy and exported into Endnote X8. A total of 306 records were removed before screening either because of duplication or marked ineligible by the automation tools. The screened records were 126 out of which 104 were removed as they were not conducted on OSCC. Twenty-two reports were sought for retrieval however, we could not find the full text of 3 studies. Twelve studies were excluded because the biomarkers were not associated with cancer stem cells (Figure-1.

Characteristics of the included studies: Out of the seven studies that were included in the review, two were conducted in a lower middle-income country (India),[15,21] two were conducted in an upper middle-income country,China[17,18] and three were conducted in High-income countries[16,19,20,23]. The study design for Zong et al[17], Yang et al[18,] and Patel et al[21] was randomized phase III trial.The retrospective study design was utilized by Stangl et al[16], Dejaco D et al[19], and Leu M et al[20] and Srivastava V K et al[15] used case-control study design. The total number of participants in these studies were 1260. The mean age of the participants was 55.27 years (not

Table-1: Quality Assessment of selected studies through JBI Critical Appraisal Checklist.

Randomized Clinical Trials	**Zhong et al. [17]**	**Yang et al. [18]**	**Patel et al. [21]**
Was true randomization used for assignment of participants to treatment groups?	U / Y*	U	U / Y
Was allocation to treatment groups concealed?	U/ Y	U	Y
Were treatment groups similar at the baseline?	Y	Y / U	Y
Were participants blind to treatment assignment?	U/ Y	U	Y
Were those delivering treatment blind to treatment assignment?	U	U	Y / U
Were outcomes assessors blind to treatment assignment?	Y	Y/ U	Y
Were treatment groups treated identically other than the intervention of interest?	Y	Y	Y
Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analysed?	Y	Y	Y/ N
Were participants analysed in the groups to which they were randomized?	Y	Y	Y
Were outcomes measured in the same way for treatment groups?	Y	Y	Y
Were outcomes measured in a reliable way?	Y	Y	Y
Was appropriate statistical analysis used?	Y	Y	Y
Was the trial design appropriate, and any deviations from the standard RCT design (individual randomization, parallel groups) accounted for in the conduct and analysis of the trial?	Y	Y	Y/ N
Score	9/ 11	9/ 7	12/ 10
Quality	High	High	High
Case-Control Studies			**Srivastava V K et al**
Were the groups comparable other than the presence of disease in cases or the absence of disease in controls?			Y
Were cases and controls matched appropriately?			N
Were the same criteria used for identification of cases and controls?			Y
Was exposure measured in a standard, valid and reliable way?			Y
Was exposure measured in the same way for cases and controls?			Y
Were confounding factors identified?			N
Were strategies to deal with confounding factors stated?			N
Were outcomes assessed in a standard, valid and reliable way for cases and controls?			Y
Was the exposure period of interest long enough to be meaningful?			Y
Was appropriate statistical analysis used?			Y
Score			7
Quality			Moderate
Retrospective		**Dejaco D et al**	**Stangl S et al**
Were the criteria for inclusion in the sample clearly defined?		Y	Y
Were the study subjects and the setting described in detail?		Y	Y
Was the exposure measured in a valid and reliable way?		Y	Y
Were objective, standard criteria used for measurement of the condition?		Y	Y
Were confounding factors identified?		Y	Y
Were strategies to deal with confounding factors stated?		Y	Y
Were the outcomes measured in a valid and reliable way?		Y	Y
Was appropriate statistical analysis used?		Y	Y
Score		8	8
Quality		High	High
Cohort			**Leu M et al**
Were the two groups similar and recruited from the same population?			Y
Were the exposures measured similarly to assign people to both exposed and unexposed groups?			U / Y
Was the exposure measured in a valid and reliable way?			y
Were confounding factors identified?			Y
Were strategies to deal with confounding factors stated?			Y
Were the groups/participants free of the outcome at the start of the study (or at the moment of exposure)?			Y
			Y
Was the follow up time reported and sufficient to be long enough for outcomes to occur?			N/ Y
Was follow up complete, and if not, were the reasons to loss to follow up described and explored?			Y
Were strategies to address incomplete follow up utilized?			Y
Score			9/ 11
Quality			High

Rating score is from 1 (lowest) to 10 (highest); Y= Yes; N=No; U= Unclear. Quality score will be categorized into three groups: Low: 1-4, Moderate: 5-7, and High: 8-12

*= Where rating score is different between the two reviewers (indicated in red).

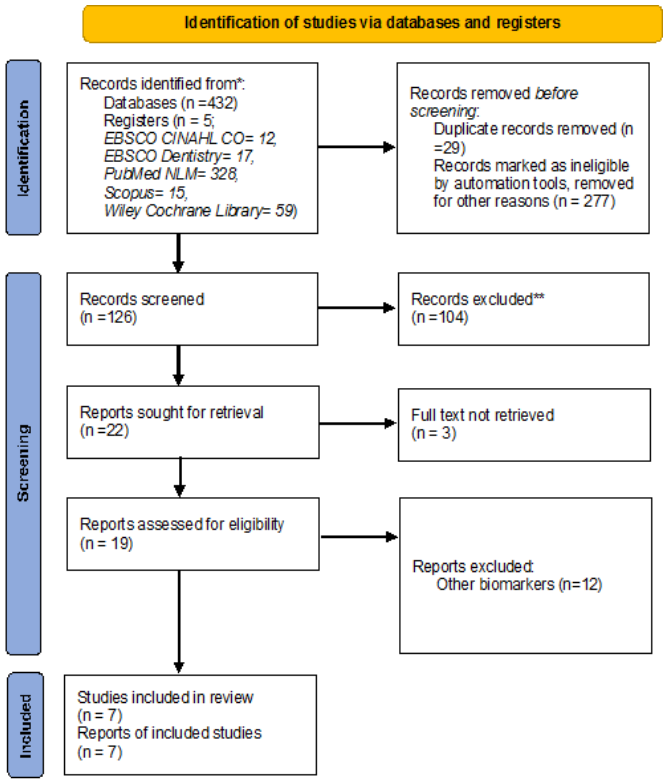

Figure-1: Systematic review study selection approach by PRISMA [23]

endothelial growth factor - A(VEGF-A) levels were significantly elevated in HNSCC patients in their study.[15] The tumours expressing Hsp70 showed significantly lower overall survival rate(OS), local progression-free survival rate (LPFS), and distant metastasis-free survival rate (DMFS) in Stangl's study.[16] Similarly, Zhong et al and Yang et al found that the tumour cells expressing lower cyclin D1 and GDF-15 respectively had better OS, disease-free, and LPFS.[17,18] They also reported that high cyclin D1 in patients with cN2 patients[17], cN+ patient with low GDF-15 and cN- patients with high GDF-15 are better candidates for induction therapy.[18] Dejaco D et al found positive correlation of EGFR, Ki67, and CD44 with the primary tumour-specific growth rates.[19] Leu M et al reported in their retrospective cohort study that MCT1 is a promising biomarker in HNSSC and its high levels indicate poor response to radio-chemotherapy.[20] Patel et al reported in their study that high expression of HIF1 α is a predictor of poor clinical response to cisplatin-radiation therapy in HPV-negative patients with locally advanced HNSSC.[21](Table-2)

Table-2: Characteristics and outcomes of selected studies

Author	Year	Participants	Study design	Stem Cell Biomarker	Outcomes
Srivastava V K et al.[15]	2014	112	Case-control	VEGF-A	Higher in advanced stages (III-IV) of the disease with lymph node involvement
Stangl S et al.[16]	2018	77	Retrospective	Hsp 70	High values had poor prognosis for survival
Zhong et al.[17]	2013	232	Prospective open-label, randomized phase III trial	Cyclin D1	Low levels indicate better overall, progression and disease free, loco-regional and distant metastasis- free survivalHigh cyclin D1 in patients with cN2 patients are better candidates for induction therapy
Yang et al. [18]	2014	230	Prospective randomized Phase- III trial	GDF- 15	Low levels indicate better overall survival and distant metastasis –free survivalcN+ patient with low GDF-15 and cN- patients with high GDF-15 are better candidates for induction therapy
Dejaco D et al [19]	2019	123	Retrospective study	EGFR, Ki67, CD44	Positive correlation with the primary tumor-specific growth rate
Leu M et al [20]	2021	82	Retrospective Cohort	MCT1	High levels indicate poor response to radio- chemotherapy
Patel et al [21].	2020	404	Randomized phase III trial	HIF1α	Low levels are associated with better progression free survival and overall survival to treatment

reported in 3 of the studies) and majority were males (80%).

Outcomes: Srivastava et al found that the serum vascular

Discussion

Tumour biomarkers have been extensively studied in previous researches[24-26]. Almangush et al[24] conducted a systematic review and meta-analysis for prognostic biomarkers SCC of the tongue. The period of the review was 30 years, from 1985-2015. Their initial search was 2579 titles from which 174 studies met the inclusion criteria. These were further shortlisted to 11 studies that focussed on biomarkers of head and neck SCC. In their results, they found cyclin D1 to be a strong prognostic biomarker for the condition while promising results were expected from VEGF A. They did not associate the biomarkers with cancer stem cells. The current study also identified cyclin D1 and VEGF as SCC biomarkers, however, they were associated with cancer stem cells.

Cyclin D1[27] biomarker controls the G1- S phase transition of the cancer stem cell. It upregulates the epithelial-mesenchymal transition (EMT) pathway in epithelial ovarian cancer stem cell-like cells (CSC-LC) which increases the malignancy potential of the condition. The pathway is characterized by the transformation of epithelial cells into EMSCs. Jiao et al[27], in their research, found that increased activity of cyclin D1 increases the invasion and metastasis potential of ovarian cancer. This is in agreement with Zhong et al[17] who found that low levels of cyclin D1 predicted better overall, disease and recurrence-free survival from SCC. They also wanted to determine the relation of cyclin D1 with induction chemotherapy. While surgery is commonly opted as the first line of management for patients with SCC, it is an invasive procedure associated with risks. Induction chemotherapy consists of shrinking the tumour size and overall reducing its malignancy potential to preserve local tissues. The results of Zhong et al[17] showed that patients with advanced stages of SCC and high levels of cyclin D1 had a better overall survival of induction chemotherapy than patients with high levels who underwent surgery. Thus, cyclin D1 levels can be used to select patients for better management and quality of life.

The role of VEGF- A[28] in angiogenesis has been established however, it also has an important role in self-renewal and survival of cancer stem cell-like cells. Srivastava et al[15] found high levels of VEGF- A in patients with SCC with lymph node involvement and in later stages of the condition. They further analysed the effect of vascular endothelial growth factor - A with radio and chemotherapy and found that the patients who responded to treatment had reduced levels of the cancer stem cells biomarker while it was maintained in patients who did not respond to treatment. Hence, it can be concluded that VEGF - A is a useful pre- and post-treatment cancer stem cell-like cells biomarker.

Basheeth and Patil[25] in their literature review with a time span of 20 years (1993-2016) found tumour biomarkers relevant for diagnosis, prognosis, and screening of SCC. They identified several biomarkers for tumour progression including epidermal growth factor receptor (EGFR) and CD44. This is in accordance with the current research as we also found similar results. Epidermal growth factor receptor[29] works along with epithelial growth factor (EGF) and is activated by it.[30] Together they are important for the proliferation, differentiation, and migration of epithelial cells. Epidermal growth factor receptor is responsible for the overall maintenance and function of the cancer stem cells. High levels indicate a poor prognosis as there is a rapid progression of the condition. Epidermal growth factor receptor can be easily tested using immunohistochemistry and according to Basheeth and Patil,[25] it can also be used to assess the treatment responses to SCC.

The biomarker CD44[31] is a physiological receptor for extracellular matrix components and a cofactor for growth factors and cytokines. However, pathologically, CD44 and its isoforms maintain cancer stem cells and promote tumour progression and malignancy potential.[31] This has also been supported by Basheeth and Patil[25] who found that suppression of CD44 and its variants prevent the metastasis of tumours. Given its properties, Dejaco et al[19] also found it useful in measuring the growth rate of the primary tumour, and that higher levels were indicative of highly malignant conditions. However, it was not effective in assessing the involvement of lymph nodes. Adnan Y et al[32] investigated the impact of CSC biomarkers on OS and disease-free survival on Pakistani population and found CD44 to be a predictor for poor OS, associated with advanced American Joint Committee on Cancer stages and T stage tumours in their study although it did not influence the disease-free survival.Other studies conducted in Pakistan[33,34] have evaluated the prognostic role of biomarkers in OSCC; however, the identified biomarkers are not derived from cancer stem cell.

The function of Ki67[35] is unknown, however, its disruption depletes the cancer stem cells niche. Cancer stem cell niches are microenvironments within the neoplastic tissue that preserve cancer stem cells. High levels indicate a rapidly spreading condition with metastasis. While Dejaco et al[19] found it useful to predict the rate of tumour growth, Almangush et al[24] found it to have limited use in the diagnosis of tongue squamous cell carcinoma. Basheeth and Patil[25] stated that its variant, Ki6758,

located in the oral cavity could be useful in the prognosis of SCC.

To our knowledge, this is the first systematic review to recognize MCT 1 as a prognostic biomarker for SCC. Previous studies found MCT 1 in association with glioblastoma cancer stem cell[36] and hepatic adenocarcinoma[37]. They are responsible for the transportation of ions of the cancer stem cells to maintain their viability and function. Thus, any inhibition of the MCT1 can cause the cancer stem cell to degenerate. A study conducted by Leu et al[20] found that high levels of MCT1 show reduced survival rates and high disease progression rates. However, no cut-off points could be found which could differentiate between high and low-risk SCC. MCT1 was also found useful in patients who had undergone radio-chemo therapy as it was effective in measuring a successful response to treatment without surgical resections. [20]

Hypoxia Inducible Factors (HIF)[38] are responsible to maintain the viability of cancer stem cells by increasing the therapeutic resistance of cancer stem cells. There is limited data on HIF and SCC. Almangush et al[24] in their literature search in 2017 found only 4 studies that had used this biomarker for SCC and they did not consider them for further analysis. Patel et al[21], in their study conducted in 2020 found patients with high levels of HIF1-α gave a poor response to treatment with cisplatin radiation and their treatment should be augmented with nimotuzumab for better response. Other than that, they also found that low levels of HIF1-α indicated better progression-free and overall survival of patients.[21] Hence, we concluded that HIF1-α is a better prognostic indicator and is a useful indicator for the management of locally advanced SCC.

The main purpose of Heat Shock Protein (Hsp)[39] is to protect cancer stem cell from drugs, radiation, and, immune system response by maintaining the cells' phenotype. There is limited data available about Hsp and SCC. Our search led to the study conducted by Stangl et al,[16] who found that Hsp was effective in identifying patients with advanced stages of lymph node involvement in SCC thus signifying low overall survival. However, it was not effective in differentiating between primary tumour size or staging of the disease at pre-treatment levels. They concluded that patients with high Hsp 70 and low CD56+ natural killer cells were least likely to benefit from resection surgery and radio chemotherapy, thus labelling them as negative prognostic biomarkers for cancer stem cell.

Growth differentiating factor 15 (GDF-15)[40] is a part of the transforming growth factor family. It enhances the activity of cancer stem cell in SCC by improving their capacity for self-renewal.[40] Depending on the stage of tumour development, it has both agonist and antagonistic effects on tumour growth. Being the former in later stages, overexpression of the biomarker signifies high levels of malignancy.[40] Yang et al[18] found that low levels of GDF-15 with lymph node involvement were associated with better survival rates and better responses to induction chemotherapy. Other than that, patients with high GDF-15 with no lymph node involvement also have better survival rates and response to induction chemotherapy. However, GDF-15 levels were affected by alcohol consumption as patients with negative history of alcohol had higher levels of GDF-15 than those who did. As alcohol was a confounder and no further detail was provided by the authors as they dealt with it, we concluded that GDF-15 may not be the most appropriate biomarker to predict the activity of cancer stem cell for diagnosis and treatment prognosis.

There were some limitations of the present study. There is more literature available on prognosis and treatment response than the diagnosis of the tumour. Diagnostic parameters had to extracted by inferences of tables showing baseline characteristic of the patients. Although there have been several researches in the past which focussed on biomarkers of SCC, this is the first study which associates biomarkers specifically to cancer stem cell of SCC. Other than that, we found a new biomarker, MCT1, which has been previously explored, to be a good biomarker for diagnosis and prognosis of SCC.

Conclusion

The review concluded that various stem cell biomarkers have been found to have diagnostic and prognostic role in oral squamous cell carcinoma such as Cyclin D1, VEGF-A, GD15, and CD44. They can be used to predict the OS, LPFS, and DMFS in Head and Neck cancer patients. Further prospective studies are however, needed to further strengthen their role as cancer stem cell biomarkers in the diagnosis and prognosis of OSCC.

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