REVIEW

Clinical and Prognostic Significance of Cancer Stem Cell Markers in Oral Squamous Cell Carcinoma- A Scoping Review

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Abstract

Introduction: This scoping review was done to study the clinical and prognostic significance of cancer stem cell markers in oral squamous cell carcinoma, in the literature published from 2000 to 2022.

Materials and Methods: PubMed, EMBASE and Scopus databases were thoroughly searched using various combinations of keywords and Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines were followed for analysis.

Results: Database search yielded a total of 79 articles, out of which 30 articles were selected based on the inclusion and exclusion criteria. Out of 2132 total samples, 1920 specimen were oral squamous cell carcinoma (OSCC), while the remaining samples included 176 cases of oral epithelial dysplasia and 36 samples of normal oral mucosa. Out of 30 selected articles, immunohistochemistry (IHC) was used in 25 studies. Among those, IHC alone was used in 20 studies, while in the other 5 IHC was used along with other techniques. The most commonly studies cancer stem cell marker was CD44 (66.67%) followed by CD133 (16.67%). ALDH1 and SOX2 were studied in 23.33%, OCT4 in 20%, CD24 in 16.67%, BMI-1 and NANOG in 10%, p63 and CD29 in 6.67% respectively.

Conclusion: Among the cancer stem cell markers CD44, CD133, ALDH1, OCT-4, CD24, NANOG, and Bmi-1, showed positivity in OSCC, while SOX2 showed both positive and negative expressions in OSCC. Even though IHC seems to be the most common techniques used, special molecular techniques such as quantitative real-time PCR and immunofluroscence provides more accurate results.

Keywords: cancer stem cell, immunohistochemistry, oral squamous cell carcinoma

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Introduction

Head and neck squamous cell carcinoma (HNSCC) are the most common histological type of head and neck cancer, which is the sixth leading cancer by incidence worldwide, resulting in more than 200,000 deaths annually. Although treatments for head and neck squamous cell carcinoma have been progressing rapidly, the overall survival of patients is relatively low because of the existence of regional and distant metastases at the time of diagnosis. More seriously, the five-year survival rate of HNSCC on the whole is lower than 50%. Accumulated evidence suggests that cancer stem cells (CSCs) might play an important role in the progression and prognosis of cancers, including HNSCC.[1]

In the past few years, cancer stem cells (CSC) hypothesis has attracted much attention with respect to tumor initiation, progression and in understanding the fundamental biology of molecular signatures responsible for aggressive behavior of the tumor. The hypothesis emphasizes that a small subset of cancer cells with stem-cell characteristics (known as CSCs), possess unlimited proliferative potential and are responsible for tumor formation with phenotypically heterogeneous cell population. This stem cell-like cancer cell population is distinguished from other tumor cells by the expression of stemness related markers such as CD133, CD44, CD24, CD271, Octamer-binding transcription factor 4 (Oct-4), Sex determining region Y-box-2 (Sox-2), Nanog homeobox (NANOG), Aldehyde dehydrogenase-1 (ALDH-

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1), ATP-binding cassette sub-family G member-2 (ABCG2) and Polycomb Group Ring Finger Protein (Bmi-1).[2]

In oral cancer, as in other tumors, the high rate of mortality (40–50%) is attributed to therapy resistance and the resulting incidences of local recurrence (30%). Current treatment protocols target the dividing cancer cells, leading to regression of the tumor bulk. However, the treatment protocols are often unable to eliminate the critical cancer stem cells population that are protected by specific resistance mechanisms. An understanding of markers that specify the cancer stem cells-driven mechanisms of resistance, survival and tumor recurrence could provide new directions for accurate prognostication.[3] Although the association between the expression of CSC markers and survival of OSCC has been investigated, reports evaluating prognostic value of CSCs in oral squamous cell carcinoma is still scarce. This scoping review was done to assess the clinical and prognostic significance of cancer stem cell markers in oral squamous cell carcinoma.

Materials and Methods

Protocol:

PubMed, EMBASE and SCOPUS databases were thoroughly searched using combination of keywords: Oral Squamous Cell Carcinoma, Cancer stem cells, CD133, CD44, CD24, CD271, podoplanin, ALDH1, ABCG2, Oct-4, Sox-2, Bmi-1 and NANOG. The search was merged in reference manager software. The retrieved records were reviewed systematically and any discrepancy was resolved by mutual consensus. The inclusion was restricted to articles in English language. While conducting this analysis, the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines were followed.

Search strategy:

All the literature searches were done in the advanced search field with search strategy. Literature searches, based on the relationship between CSC markers expression and clinical and prognostic outcomes were thoroughly performed from the data bases. The search strategy was based on the following main keywords: (neoplasm or cancer or tumor or malignancy or malignant or carcinoma) and (oral cancer, squamous cell carcinoma) and (neoplastic stem cell or neoplastic colony forming unit or tumor stem cell or tumor-initiating cell or cancer stem cell or CSC) and (biomarker or marker), prognosis, prognostic, diagnosis or screening or detection.

Case-control or cohort studies published on oral squamous cell carcinoma, studies evaluating the association between CSC markers expression and overall survival (OS), disease-free survival (DFS)/ relapse-free survival (RFS) and cancer-specific survival (CSS) and/or clinicopathological features of oral squamous cell carcinoma and expression of CSC markers detected by immunohistochemistry (IHC), Flow cytometry analysis, Quantitative reverse transcription PCR (qRT-PCR) and immunofluorescence in primary tumor tissues were included.

Book chapters, letters, conference abstracts and abstracts without full text, Studies that were not related to the topic of interest (eg; when the studies investigated other solid tumors or other diseases), studies with lack of sufficient and useful data and studies with increased risk of bias were excluded.

Study selection and data extraction:

All search records were transferred to Endnote software to remove the duplicate files. The eligible studies were identified after the independent screening of the titles and abstracts based on the inclusion criteria. For each of the included articles, the following descriptive data were collected: the name of first author, country and year of conduction of the study, detection method, age, sex, sample size, CSC marker assessed, median or mean follow-up times, clinicopathological parameters, cut-off value and related survival data. The primary outcome was the relationship between the CSC markers expression and OS in OSCC patients. Other outcomes of interest were relationship between the CSC markers expression and important clinicopathological parameters of OSCC.

Risk of Bias and Quality Assessment of Studies:

The publications were critically appraised separately by three authors in accordance with the Joanna Briggs Institute Reviewer's Manual of 2017 (https://joannabriggs.org/).

Results

Database search yielded a total of 79 articles, out of which 30 articles were selected according to the inclusion and exclusion criteria. Table 1 shows the details of the collected articles. Out of the 2132 total samples, 1920 specimen were oral squamous cell carcinoma. The remaining samples include 176 cases of oral epithelial dysplasia and 36 samples of normal oral mucosa. Out of 1920 OSCC cases, 76% were males and 24% were females.

Out of 30 articles, immunohistochemistry (IHC) were used in 25 studies. Among those, in 20 studies IHC alone were used, while in the other 5 studies IHC were used along with NANOSTRING mRNA analysis, insitu hybridization, flow cytometry analysis, qPCR and immunofluorescence. Cancer stem cell markers used in these studies were Bmi-1, c-myc, Snail, USP22, CD44, ALDH1, SOX2, CD24, OCT4, NANOG, SALL4, STAT3, p63, CD147, CD133, CD29 (integrin-β1), SLC3A2, Notch1, BCL11B, CXCR-4, PKC-δ and L1CAM.

Cancer stem cell markers such as CD44 was analysed in majority of the studies (66.67%), followed by CD133 (26.67%). ALDH1 and SOX2 were studied in 23.33%, OCT4 in 20%, CD24 in 16.67%, BMI-1 and NANOG in 10%, p63 and CD29 in 6.67% of the studies respectively. Stem cell markers such as C-MYC, USP22, tumor protein 53, SALL4, STAT3, CD147, SLC3A2, Notch1, TGF-B, BCL11B, CXCR-4, PKC-δ, L1CAM were studied individually or with other stem cell markers.

Discussion

Cancer stem cells (CSCs) are a subset of cells within cancer that display stemness characteristics, including the ability to divide asymmetrically. This self-renewal capacity of CSCs results in the production of heterogeneous population of cancer cells.[14] Solid tumors, such as HNSCC, are histologically heterogeneous and contain various types of cells, including tumor cells, stromal cells, and inflammatory cells. Until recently, the significance of the subpopulation of cancer cells that mediate the development and growth of tumors was poorly

Table 1: Summary of articles included in the scoping review

Sl. No:	Author and Year	Total Number of Samples	Technique Used	CSC Markers Studied
1.	Häyry V et al, 2010	73	IHC	Bmi-1, c-myc, Snail and proliferation marker Ki-67 expression
2.	Piao S et al, 2012	319	IHC	USP22
3.	Athanassiou-Papaefthymiou M et al 2014	10 cell lines	Real Time RT-PCR, Immunofluorescence	CD44 and its variants
4.	He KF et al, 2014	65	IHC	TAM markers CD68 and CD163 as well as the cancer stem cell (CSC) markers ALDH1, CD44, and SOX2
5.	Kang YH et al, 2015	57	IHC	transcription factor NANOG, cancer stem cell marker CD44, and mutant tumor protein 53
6.	Todoroki K et al, 2016	50	IHC	CD44v3 and CD24
7.	Baillie R et al, 2016[4]	10	IHC, NANOSTRING mRNA analysis, Insitu hybridization	OCT4, NANOG, SOX2, SALL4, STAT3, CD44, p63
8.	Mohanta S et al, 2017[5]	53	IHC, Flow cytometry analysis	CD44 and CD147
9.	de Moraes FP et al, 2017	83	IHC	CD24, CD44, CD133, ALDH1, CD29 (integrin-β1) and Ki-67
10.	Saghravanian N et al, 2017[6]	45	IHC	p63 and CD44
11.	Singh A et al, 2018	50	IHC	CD133 and Oct-4
12.	Boxberg M et al, 2018	108	IHC	CD44
13.	Linge A et al, 2019	92	IHC	p16 status, the CSC markers CD44 and SLC3A2 and hypoxia-associated gene signatures CD44
14.	Garcia AS et al, 2019[7]	91	IHC	podoplanin and CD44v6
15.	de Vicente JC et al, 2019[8]	180	IHC	SOX2
16.	Rizzo D et al, 2020	69	IHC	CD44, CD133, Oct-4, Nanog, and Sox-2
17.	Mirhashemi M et al, 2020[9]	60	Quantitative reverse transcription PCR	CD24 and CD44
18.	Ma Z et al, 2020	6	Flow cytometry analysis, IHC	CD133+
19.	Ghazi N et al, 2020	60	qRT-PCR	SOX2 and OCT4
20.	Luna EC et al, 2020[10]	30	IHC	CD133
21.	Rao RS et al, 2020	80	IHC IRS score	ALDH1, Bmi1, and OCT4
22.	de Freitas Filho SA et al, 2021	63	IHC	ALDH1 and Notch1
23.	Ghazi N et al, 2021[11]	55	IHC	TGF-B and CD44
24.	Jakob M et al, 2021	180	IHC	ALDH1, BCL11B, BMI-1, and CD44
25.	Shaik MV et al, 2021	30	Immunofluorescence	CD133, CD44, OCT4, and SOX2
26.	Al-Magsoosi MJ et al, 2021	10	IHC, qPCR and immunofluorescence	CD24, CD44 and CD29
27.	CaspaGokulan R et al, 2021[12]	51	IHC, Immunofluorescence	CXCR-4, PKC-δ and CD133
28.	Hendawy H et al, 2021	44	IHC	CD44 and ALDH1
29.	Adnan Y et al, 2022	100	IHC	CD44, CD133, L1CAM, and SOX2
30.	Vipparthi K et al, 2022[13]	8	Flow cytometry	CD44, CD24 and ALDH

understood. The identification of small cell subpopulations with high tumorigenicity in various solid tumors has implicated tumor stem cells in the tumorigenesis theory. Identification and isolation of cancer stem cells constitute a major experimental challenge. Cell surface markers, such as CD44, CD24, CD29 (integrin-β1), CD90, CD133, epithelial-specific antigen (ESA), and the expression of the detoxifying enzyme aldehyde dehydrogenase 1 (ALDH1), have been used to isolate and enrich CSCs from various tumors.[15]

Cancer stem cell markers such as Bmi-1, USP22, CD44, Oct-4, Integrin- β1, CD133, Notch1 and SOX2 are associated with recurrence in OSCC. 15-22 The absence of Bmi-1 protein in the tumour cells was associated with a higher risk of recurrence.[23] Oral-derived HNSCC expressed highest CD44v4 and v6 levels corresponded with staging, showing also an increasing tendency with recurrence and metastasis.[24] Expression of Oct-4 and integrin-β1 are linked to recurrent tumors and a higher incidence of distant metastasis in HNSCC. Expression of markers OCT4 and SOX2 in metastatic stage tissue revealed their involvement in the recurrence of cancer cells.[22]

CSC markers such as USP22, CD44, CD24, Oct-4, Integrin-β1, CD133, ALDH1, Bmi-1 and Notch1) are associated with lymph node metastasis and therapy resistance.[25-27] Increased CD24 levels enhance tumor growth and metastasis. ALDH1 expression showed increased significance in cases having OSCC with lymph node metastasis compared to non-metastatic cases. Highest level of CD44v6 expression was detected in advanced metastatic HNSCC suggesting a link between CD44v6 expression and HNSCC metastasis, while highest CD44v4 was detected in a stage IV HNSCC refractory to chemotherapy which developed recurrence. There are subpopulations of cells with increased ALDH1 activity rendering them resistant to oxidative damage caused by conventional therapies. This could possibly explain the reason for the correlation of increased ALDH1 activity with poor clinical prognosis as recurrence rates tend to be higher.[26] Expression of CD44, CD24 and CD29 correlates with the increased growth rate and resistance to radiotherapy in head and neck squamous cell carcinoma.[25] Some studies have suggested CD133 as a potential prognostic marker and therapeutic target.[28]

CD44 is a cell surface glycoprotein that acts as a receptor for hyaluronic acid and as an adhesion molecule. This cell surface protein plays a role in tumor cell invasion, metastasis, and angiogenesis by interacting with certain matrix metalloproteinases. CD44 was the first CSC marker described in a solid malignancy, and a high frequency of CD44 positive cells in HNSCC strongly correlates with recurrence and tumor aggressiveness.[17] CD44v, a variant of CD44 showed differential expression in HNSCC which may be a representative for the morphological changes inherent during tumor progression in HNSCC.[24] Five-year disease-free survival rates tended to be lower in patients who were CD44-positive.[5]

CD133 (prominin-1) is found in epithelial cells and associated with many solid tumours, including those related to prostate carcinoma, thyroid carcinoma, hepatoma, renal tumours, and oral cancer. CD133 is negatively correlated with the survival in OSCC patients. CD133 expression gradually decreases with cell differentiation, which is likely linked to changes in cancer metabolism.[28] Some studies showed that elevated levels of CD133 lead to OSCC invasiveness and metastasis, associated with the up-regulation of embryonic and stemness markers.[22]

Aldehyde dehydrogenase 1 (ALDH1) is a cytosolic isoform of ALDH, and high expression of ALDH1 is a predictor of poor clinical outcome in many cancers.[29] Tamatani et al noted that ALDH1 is significantly associated with increasing histologic grades and lymph node metastasis. Studies showed that, strong immunoexpression of ALDH1 may help to predict a worse prognosis in the overall survival of patients with oral cancer.[21] Some other studies showed about the greater invasive capacity of ALDH1 positive cells and may be an important indication that CSCs frequently undergo EMT when compared to ALDH1 negative population of cells and thus explain the larger tumor size, advanced stage, presence of metastatic deposits in lymph nodes with high ALDH1 expression.[30]

SRY related HMG-box gene 2 (SOX2) is a transcription factor modulating the expression of several genes essential for the maintenance of the embryonic stem cell phenotype. In cancer, SOX2 protein expression has been linked with a worse prognosis as it promotes drug resistance, metastasis, survival, and proliferation. For OSCC, SOX2 expression is a controversial marker considering that some studies have reported SOX2 to be linked to lymph node metastasis and poor survival, while others have found increased SOX2 expression to improve prognosis.[31] SOX2 as a transcription factor in pluripotency and self-renewal of embryonic stem cells plays a key role in the survival of malignant squamous cell against apoptosis.[32]

Octamer-binding protein 4 is a homeodomain transcription factor belonging to the Pit-Oct-Unc family. The Oct4 plays a major role in the self-renewal of embryonic stem cells and maintains their pluripotency through interaction with other transcription factors. OCT4 is reported to maintain the survival of CSCLCs partly by inhibiting apoptosis through the OCT4/TCL1/AKT1 pathway.[22]

CD24 is a 27-amino-acid single-chain protein that is O- and N-glycosylated and is bound to the extracellular matrix and the extracellular membrane by a glycosylphosphatidylinositol anchor.[33] The downregulation of CD24 inhibits proliferation and induces apoptosis in tumor cells, whereas increased CD24 levels enhance tumor growth and metastasis.[15] NANOG is an early transcription factor and pluripotent marker that maintains the self-renewal of embryonic and mesenchymal stem cells. Recent studies revealed that NANOG is highly detected in poorly differentiated carcinomas and late-stage tumors. Moreover, a positive relationship between enhanced NANOG expression and lymph node metastasis of carcinoma was reported.[17] Some studies have shown that NANOG cytoplasmic expression to be a strong prognostic predictor in OPSCC and was the only prognostic marker retaining its significance at Cox multivariate analysis in the entire series.[34]

Bmi-1 is an essential constituent of the polycomb repressive complex 1, a key epigenetic regulator. Through chromatin and histone modifications (for example, methylation), it controls the cell cycle and self-renewal of tissue stem cells.[23] It plays an

important role in carcinogenesis and stem cell renewal through chromatin and histone modification and thereby influence the major tumor suppressor genes such as Rb and p53.[26] Bmi-1 is overexpressed in OSCC cells when compared with normal mucosa and is thought to influence cell proliferation and survival in oral carcinogenesis.[23] Some studies showed that loss of Bmi-1 immunoexpression seems to correlate with clinical outcome of oral tongue SCC.[26]

Among the cancer stem cell markers CD44, CD133, ALDH1, OCT-4, CD24, NANOG, and Bmi-1, showed positivity with OSCC, while SOX2 showed both positivity and negativity with OSCC. Immunofluorescence staining of tissue samples of OSCCs were used to establish the tissue localization pattern for cells expressing various stem cell markers. Flow cytometry were used to quantitatively check for the same maker. Flow cytometric data clearly demonstrated the expression pattern of receptors, and intracellular markers in OSCC.[22] MicroRNA (mRNA extraction from each sample for qualitative real-time polymerase chain reaction (qRT-PCR) were used to study the expression of markers. Quantitative real-time PCR was used to quantitate the level of expression of markers.[32] Some study showed real time quantitative RT-PCR was successful in detecting variant forms of CD44. Reactivity by immunocytochemistry did not pick up any variant forms for cell line, indicating that both Fluorescence Activated Cell sorting (FACS) and real time RT-PCR have higher sensitivity. Fluorescent immunocytochemistry seems most valuable in providing an insight for the potential biological functions of CD44 as extrapolated from their pattern of cellular distribution.[24]

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