Review Article

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Role of cancer stem cells in head-and-neck squamous cell carcinoma – A systematic review

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Abstract:

Targeting cancer stem cell (CSC) subpopulation within the tumor remains an obstacle for specific therapy in head-and-neck squamous cell carcinoma (HNSCC). Few studies in the literature describe a panel of stem cell makers, however a distinct panel has not been put forth. This systematic review aims to enhance the knowledge of additional markers to accurately relate their expression to tumorigenesis, metastasis, and therapy resistance. Databases, including *PubMed, Google Scholar, Ebsco,* and *Science Direct,* were searched from 2010 to 2017 using various combinations of the following keywords: "Stem cell markers in HNSCC" and "chemoresistance and radioresistence in HNSCC." Original experimental studies (both *in vitro* and *in vivo*) published in English considering stem cell markers in HNSCC, were considered and included. We excluded articles on tumors other than HNSCC, reviews, editorial letters, book chapters, opinions, and abstracts from the analyses. Forty-two articles were included, in which 13 types of stem cell markers were identified. The most commonly expressed CSC markers were CD44, aldehyde dehydrogenase, and CD133, which were responsible for tumorigenesis, self-renewal, and therapy resistance, whereas NANOG, SOX-2, and OCT-4 were involved in metastasis and invasion. Identification of an accurate panel of CSC markers is the need of the hour as nonspecificity of the current markers poses a problem. Further studies with a large sample size would help validate the role of these CSC markers in HNSCC. These CSC proteins can be developed as therapeutic targets for HNSCC therapy, making future treatment modality more specific and effective.

Keywords:

Aldehyde dehydrogenase, cancer stem cells, CD133, CD44, NANOG, OCT-4, SOX-2, targeted therapy

Introduction

Although CSCs form a very small proportion of the tumor cell population, they play a significant role in determining outcomes. Generally, CSCs refer to the cancer cells capable of self-renewal and differentiation, which makes them resistant to radiotherapy and chemotherapy.[1] CSCs have stem features like that of normal cells (NSCs) such as self-renewal, high proliferation abilities, high migration capacity, and drug resistance.[2] In disease progression, tumor initiation and metastasis, and treatment resistance in head-and-neck squamous cell carcinoma (HNSCC), CSCs play a vital role.[3] Even in a small spectrum of cells, existing in a tissue, CSCs can be clearly separated from other cells.[4]

At present, development of new therapeutic planning is hindered because of lack of suitable and reliable markers for identification of CSCs. Although the role of CSCs in the

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renewal and initiation of tumors has been discovered,[5] the association between CSCs and metastasis is yet to be rooted out. In stem cells and CSCs, similar features are found, such as activation of DNA-repair machinery and expression of drug transporter ABC. Understanding the biology of cancer stem cells (CSCs) with difference in biologic behaviors from NSCs will help in the identification of molecular targets for targeted therapy.[1-5] Furthermore, it has been reported that patients with high expression of CD44, aldehyde dehydrogenase (ALDH) 1, and SOX2 had worse prognosis.[6-10] The prognostic value of CSCs in HNSCC remains controversial. CSC therapy-resistance is because of adaption, quiescence, survival, damaged DNA repairing, and detoxification/multidrug resistance. Because these traits can be shared by CSCs from different malignancies, it is essential to know the nature of the underlying biology of these driving mechanisms.[8,11-14] A larger area of cells that proliferate symmetrically, with both daughter cells showing the stem cell phenotype, and CSCs vividly differ from adult cells. These cells have increased replicative potential resulting from mutations in stem/progenitor. CSCs and NSCs in adult somatic tissues show common features of slow cycling and self-renewal. It is still doubtful whether or not CSCs are fully dependent on the niche, like NSCs that vary in different types of tumors.[5,10,15]

Despite all studies and analyses done on CSCs, no single biomarker has been found to define the CSC population for HNSCC, exactly. A set of biomarkers are needed to accurately define this population for identification and targeted therapy. Targeting CSCs in HNSCC for chemoresistance, radioresistence, and immune evasion mechanism remains a cornerstone for novel adjunct therapies. The aim of the current chemotherapy and radiation treatment for HNSCC is to debulk the tumor, whereas the CSC hypothesis shows that the elimination of CSCs is the only way to treat cancer with high efficacy. This systematic review aims to identify a panel of existing HNSCC CSC markers that are involved in cancer progression, metastasis, treatment resistance, and prognosis. The review also explores the resistance mechanism of CSCs in HNSCC and outlines the differences between CSCs and NSCs.

Materials and Methods

Key question

A key question was constructed according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA). The question was *Can a specific panel of CSC markers be identified that play a major role in tumorigenesis, metastasis, and therapy resistance.* This systemic review was reported according to the PRISMA checklist (www.prisma.statement.com).[16] Patient, Intervention, Comparison, and Outcomes method as applicable in relation to the topic of the review is as follows:

- Patients: Individuals with HNSCC
- Intervention: Stem cell markers
- Comparison: Intercomparison between various stem cell makers for HNSCC
- Outcomes: Correlation of stem cell markers with tumorigenesis, metastasis, and therapy resistance.

Study design

A systematic review was done on studies which used the appropriate panel of surface antigens having stem cell-like property which play important role in tumorigenesis, metastasis, and resistance to therapy in HNSCC.

Inclusion criteria

- Full-length English articles were considered that focused on surface antigen having stem cell-like property with their role in the biological behavior of tumor
- Articles that emphasize the role of CSC in resistance to the therapy in HNSCC.

Exclusion criteria

- Tumors other than HNSCC
- Articles other than original research, such as reviews, letters, personal opinions, book chapters, and conference abstracts were excluded
- Insufficient information or results not individualized for HNSCC were excluded.

Data sources and strategy of search

PubMed, Google Scholar, Ebsco, Scopus, and ScienceDirect databases were used to search for appropriate articles using keywords (stem cell markers in HNSCC and chemoresistance and radioresistence in HNSCC) [Table 1]. The search included all articles published up to March 2017, across all databases, with no time restrictions. In addition, the reference lists of selected articles were checked for additional relevant study which could have been missed during electronic search.

Data collection process

The data collection was done in the following three steps:

1. Evaluation of collected articles
2. Shortlisting of articles which included CSC antigen
3. Evaluation of methodology and assessment of results.

Included studies and information recorded were as follows:

- Author, year of publication, and countries
- Study characteristics (type of surface antigen of CSCs).

Assessing risk of bias

The Cochrane Collaboration tool (Higgins JPT, Green

S *et al*., 2011) was applied to assess the risk of bias for randomized controlled trials. Bias was evaluated as a judgment (high, low, or unclear) for individual elements from seven domains. Risk of bias was assessed for each included study from six aspects namely (1) random sequence generation (selection bias), (2) allocation concealment (selection bias), (3) blinding of outcome assessment (detection bias), (4) incomplete outcome data (attrition bias), (5) selective reporting (reporting bias), and (6) other bias. Risk of bias was rated by two independent researchers (PS and DA). Disagreements were discussed and resolved by a third researcher (SVS).

Synthesis of results

The results of individual studies were summarized and the most appropriate surface antigen for CSCs was analyzed and grouped; pathways leading to therapy resistance were analyzed and summarized. Summarization of individual points of interests across the selected studies was carried out [Figure 1].

Results

Search results

On searching with the above-mentioned keywords, 427 search results were identified. However, these included review articles, short communications, and journal publications. Among those, 200 articles were identified as potentially relevant. The title and abstract of the articles were reviewed. Seventy-five articles that fit the inclusion criteria were included. The selected articles were further reviewed by two researchers (RSR and DA) for their reliability. In case of any disagreement, consultation from a third reviewer (SVS) was employed. Among the 75 articles, 33 articles were excluded.

Study results

A total of 42 articles were selected based on the reviewer's decision.[4,6-9,11,12,17-51]

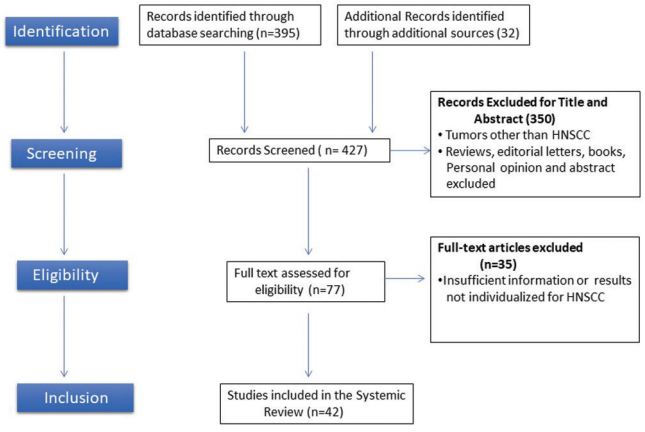

Figure 1: PRISMA flow diagram of studies selection

The selected articles included original research articles that used different biomarkers as a tool to elicit different genes which played an important role in tumorigenesis, metastasis, and radio and chemoresistance. The list of the selected articles is given in Table 2. A total of 15 biomarkers were identified in these studies among which the most commonly expressed markers were CD44, ALDH, and CD133. Twenty-five studies investigated CD44, while ten studies analyzed ALDH and six studies CD133. Seven studies combined the investigations on CD44 and ALDH, whereas three studies demonstrated the combined role of CD44 and CD133. Eight articles (Lee *et al*., Upadhyay *et al*., Shiina *et al*., Habu *et al*., Huang *et al*., Qiao *et al*., Koo *et al* and Tsai *et al*.) suggested the role of OCT 4, NANOG, and SOX2 as CSC markers in therapy resistance (both radio and chemotherapy) and reported that targeting these markers would lead to the inhibition of HNSCC. Table 3 shows the most common CSC markers involved in tumorigenesis, metastasis, and therapy resistance.[24,32,33,36,39,40,45,49] Table 4 shows the difference between NSCs and CSCs.[52,53]

The risk of bias of the original studies included is shown in Figure 2. Only one study was of high risk of bias.[51]

A number of studies concluded that CSCs are virtually resistant to radiotherapy and chemotherapy through different mechanisms and lead to tumor relapse after therapy. Table 5 enlists the causes for radioresistence and chemoresistance.[15,54-56]

Discussion

HNSCC is a common malignancy, being the eighth and thirteenth most common malignancy in the world for males and females, respectively.[1] Despite advancement in treatments, late-stage diagnosis results in poor prognosis and recurrence with metastases to locoregional lymph nodes. CSCs constitute a pool of self-sustaining cells with the ability to cause the heterogeneous lineages of cancer cells that compound the tumor. The term CSCs do not suggest their origin but the functional properties of the cells. CSCs possess significant resistance to current treatment modalities, such as chemotherapy and radiotherapy because of their abilities of self-renewal and regeneration.[1] For the establishment of prognostic biomarkers and target-specific drugs, recognition of accurate CSCs marker is necessitated.

CSCs show a tendency to be radio and chemoresistant; they also remain inactive for long periods of time and can evade conventional therapy. Even after the completion of treatment, these cells retain the capacity to become active and proliferate, tending to the establishment of distant metastasis and local recurrences. Conventional therapy is successful in scattering or debulking the

Table 1: Methodology employed for the review

Statement of the objective	Method/methodology	Resources utilized	Resources utilized
To analyze and critically evaluate the research article that focuses on panel of surface antigens having CSCs like property and their role in tumorigenesis, metastasis, biological behavior, and treatment resistance in HNSCC	Collection of research articles followed by critically evaluating them which focused on surface antigen having CSCs like property and reviewed for proper panel of antigens which are making tumor resistance to therapy and their overall prognosis	PubMed, Google Scholar, EBSCO, Scopus, Science Direct, e-Journals	("neoplastic stem cells"[MeSH Terms] OR ("neoplastic"[All Fields] AND "stem"[All Fields] AND "cells"[All Fields]) OR "neoplastic stem cells"[All Fields] OR ("cancer"[All Fields] AND "stem"[All Fields] AND "cell"[All Fields]) OR "cancer stem cell"[All Fields]) AND ("Markers"[Journal] OR "markers"[All Fields])

CSCs: Cancer stem cells, HNSCC: Head-and-neck squamous cell carcinoma

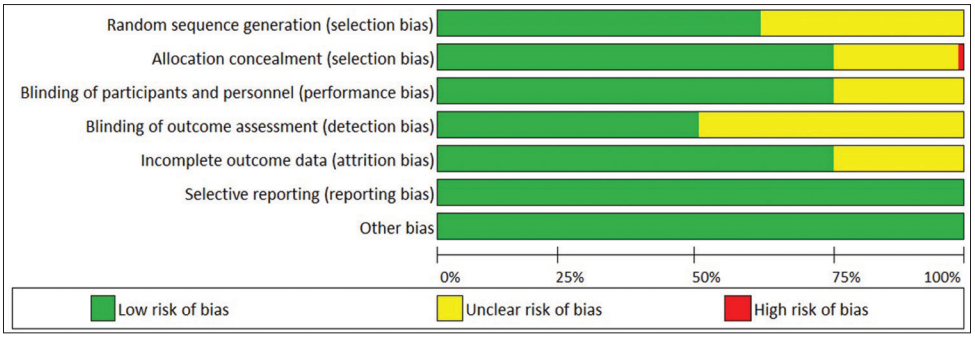

Figure 2: Assessment of risk of bias

tumor; chemotherapeutic drugs have shown a high rate of success [Figure 3]. It is suggested that slow-growing CSCs attempt to escape from conventional therapies, but in the passage of time, these cells are activated and regenerate tumors relatively with high recurrence rates.[1,8,9] Numerous studies have been done on CSCs, but none have identified an accurate marker or a panel of markers that can predict tumorigenesis, metastasis, and treatment resistance. To date, the most common modality in identifying CSCs in head and neck cancer relies on the expression of membrane cell surface antigens present in stem like cells [Figure 4].

CD44 is a well-known marker for CSC identification, and belongs to a large cell surface glycoprotein. It is thought to be involved in tumor progression and metastasis through its role as a regulator of growth, survival, differentiation, and migration. Many researchers have shown that subpopulations of CD44 containing cells which are emerging from both primary tissues and cell lines have higher potential for tumor sphere formation, differentiation, proliferation, migration, invasion, and resistance to chemotherapeutics.[11] The frequency of CD44-positive cells correlates with tumorigenesis, aggressive tumors, and higher rates of recurrence following radiotherapy. Higher CD44 expression is associated with poor prognosis and recurrence. CD44 v3 immunoexpression and CD44 v3+/CD24- immuno-phenotypes could give prognostic information associated with unfavorable clinical outcomes in the case of HNSCC.[7,11,23,25,34,44] Mohanta *et al*. concluded that CD44 and CD147 together improve the prognostic efficacy of tumor differentiation, imparting the properties of increased self-renewal, migration, and invasion. A study conducted by Mannelli *et al*. showed CD44-positive cells with highest clonogenic capacity. Okamoto *et al*. found that HNSCC-CD44+ cells showed high expression levels of chemo-resistant genes (ABCB1, ABCG2, CYP2C8, and TERT) and indicated that CD44-positive cells were more resistant to chemotherapeutic agents compared to CD44-negative cells. Researchers found that the high expression of CD44 was correlated with a greater tendency for locoregional or distant metastasis and resistance to radio/chemotherapy. Grau *et al*. suggested that CD44 can interplay with estimated glomerular filtration rate which activates C-MET, focal adhesion kinase, and AKT signalling pathways that result in upregulation of reduced glutathione which in turn might lead to increase in radio-resistance.[47,51,57,58]

ALDH1 is a member of the ALDH family of cytosolic isoenzymes, which are highly expressed in many NSCs and CSCs. The penta-span transmembrane glycoprotein has been identified as an evident CSC marker in majority of carcinomas such as skin, liver, brain, lungs, prostate, and colorectal cancers. This glycoprotein has the ability to induce spheroid formation, self-renewal, tumor formation, increased invasion capabilities, and resistance to chemotherapeutic treatment in HNSCC cell lines and primary tissue samples.

Kurth *et al*. found that inhibition of ALDH1A3-positive HNSCC cells might improve therapeutic response to radiotherapy as these cells may contribute to tumor relapse after irradiation. According to Chen *et al*., higher

Table 2: Summary of selected articles

Author	Year	Technique	CSC markers	Description of receptors and cellular localization	Expression of biomarker	Methodology	Conclusion
Krishnamurthy and Nör[17]	2012	*In vitro* and *in vivo*	ALDH CD44	ALDH cytoplasm Integrin-β1 Membrane	High High	Flowcytometry	Expressions of these markers are associated with CSCs
Song *et al*.[11]	2010		CD29 CD44 BMi1	Integrin-β1 membrane Integrin-β1 membrane Polycomb protein cytoplasm	Low High High	Flowcytometry, IHC, WB, RT-PCR, MIA	Higher expression of CD44 is associated with tumorigenesis and Bmi1 associated with metastasis
Harper *et al*.[18]	2010	*In vitro*	CD44	Hyaluronan receptor, adhesion protein membrane	High	IHC, Idu pulse chase	Higher expression of CD44 cells has stem cell property showing greater resistance to apoptosis
Davis *et al*.[19]	2010	*In vitro* and *in vivo*	CD44	Hyaluronan receptor, adhesion protein membrane	High	Flowcytometry, Bioluminescent NT Imaging, Boyden chambers	*In vitro* CSC do not have sufficient ability to invade basement membrane in comparison to in vivo
Pries *et al*.[20]	2008	*In vitro*	CD44	Hyaluronan receptor, adhesion protein membrane	High	Flow cytometry	CD44+ tumor stem cells may play a key role in the establishment of permanent HNSCC cell lines
Sun *et al*.[21]	2010	*In vitro*	CD44	Hyaluronan receptor, adhesion protein membrane	High	RT-PCR, CFA, FACS	Higher expression of stem cell markers was detected in SP than in MP cells
Häyry *et al*.[6]	2010	*In vitro*	Bmi1 c-myc Snail	-	High	TMA, IHC	Negative Bmi1 immunoexpression might serve as a marker of poor prognosis in oral tongue carcinoma patients
Chen *et al*.[22]	2011	*In vitro* and *in vivo*	ALDH1 Sox2 NANOG Oct3/4 α-SMA Vimentin E-Cadherin	ALDH cytoplasm Transcription factor cytoplasm Transcription factor cytoplasm	High High High High High High Low	Clone formation assay, Immuno- fluorescence, FACS, QRT-PCR, Matrigel invasion assay	Higher colony-forming ability was seen in cells with ALDH1 expression
Joshua *et al*.[7]	2012	*In vivo*	Lin-CD44+	Hyaluronan receptor, adhesion protein membrane	High	Flowcytometry, IHC	Higher CD44 expression is associated with poor prognostic factors and recurrence
Kokko *et al*.[23]	2011	*In vitro*	CD44	Hyaluronan receptor, adhesion protein Membrane	High	IHC	CD44 overexpression associated with 5 year survival was found statistically significant in oro-and hypopharynx but not in oral cavity
Mărgăritescu *et al*.[8]	2011	*In vitro*	CD44 CD133 CD117	Hyaluronan receptor, adhesion protein Membrane	High High High	IHC	CD44 has limited utility in identifying oral CSCs, while CD117 and CD133 expression appears to be limited more in identifying mesenchymal stem cells

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Table 2: Contd...

Author	Year	Technique	CSC markers	Description of receptors and cellular localization	Expression of biomarker	Methodology	Conclusion
Tsai *et al*.[24]	2011	*In vivo* and *In vitro*	NANOG Oct4 Bmi1 CD117 CD133 ABCG2		High High High High High High	IHC, FACS, RT-PCR, WB, MTTA	Oral cancer Stemness markers (Oct4 and NANOG) overexpression may promote the OSCC's recurrence to resist cisplatin
Tan[25]	2012	*In vitro*	CD44s	Hyaluronan receptor, adhesion protein Membrane	High	Immunohisto-chemistry	High expression of CD44s indicates better prognosis
Liu *et al*.[26]	2013	*In vitro*	ALDH1 CD133	ALDH, cytoplasm Prominin-1 Membrane	High High	IHC	Predictors for malignant transformation
Freier *et al*.[27]	2013	*In vitro*	CCND1 ZNF217	-	High Low	TMA, FISH	Different molecular pathways are specific to localization
Noto *et al*.[28]	2013	*In vivo* and *In vitro*	CD44 SSE4	Stage specific embryonic antigen 4	High High	Flow cytometry, FACS, SFA, IHC	CD44+SSE4 cells exhibits the characteristics of CSc in OSCC and provide a target for the development of more effective therapies
Pozzi *et al*.[29]	2015	*In vitro*	CSC enriched cells	-	High	SFA, CVA, ICC, RT-PCR, NNMT enzyme activity	CSCs may represent a promising target for an anticancer therapy
Athanassiou-Papaefthymiou *et al*.[30]	2014	*In vitro*	CD44v1,2 CD44v4,6	Hyaluronan receptor, adhesion protein Membrane	High High	RT-PCR, FACS	High CD44v6 expression in advanced metastatic HNSCC
Khammanivong *et al*.[31]	2014	*In vitro*	CD44 Bmi1	Hyaluronan receptor, adhesion protein membrane Polycomb protein Cytoplasm	High High	QRT-PCR, Flow cytometry	Inhibition of BMP signaling potentiates the long-term survival of HNSCC CSCs which is mediated by SMURF1 so targeting SMURF1 and restoring Bmi1 signaling may offer a new therapeutic approach to promote differentiation and reduction of CSC populations leading to reduced drug resistance and disease recurrence
Koo *et al*.[32]	2015	*In vitro* and *in vivo*	Oct4	-	High	IHC	Oct4 may be a critical regulator of HNSC CSCs and its targeting may be potentially valuable in the treatment of HNSC CSCs
Qiao *et al*.[9]	2014	*In vivo* and *in vivo*	OCT4 SOX2	-	High High	IHC	Oct4+Sox2 + profile may contribute to the malignant transformation of oral mucosa
Huang *et al*.[33]	2014	*In vitro*	ALDH1 CD44 OCT4 SOX2	-	High High High High	IHC	ALDH1, CD44, OCT4 and SOX2 are closely related in TSCC, and the expression of SOX2 can be used as a prognostic indicator of TSCC

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Table 2: Contd...

Author	Year	Technique	CSC markers	Description of receptors and cellular localization	Expression of biomarker	Methodology	Conclusion
Todoroki *et al*.[34]	2016	*In vitro*	CD44v3 CD24	-	High High	CGA, SFA, RT-PCR IHC	CD44v3 immunoexpression and CD44v3+/ CD24- immunophenotypes could give prognostic information associated with unfavorable clinical outcomes
Leinung *et al*.[35]	2015	*In vitro*	ALDH1A1 CD44 EGFR	- Hyaluronan receptor, adhesion protein Membrane		IHC, flow cytometry	Neither ALDH1A1 nor CD44, alone or combined, were sufficient to determine the CSC population in HNSCC but ALDH1A1 was shown to be a possible prognostic marker for worse survival
Habu *et al*.[36]	2015	*In vitro*	Oct3/4 NANOG	-	High High	Flowcytometry, RT-PCR, IHC	Expression of Oct3/4 can be considered a potential predictor for selecting patients at high risk of developing DNM
He *et al*.[12]	2015	*In vitro*	Bmi1	-	High	IHC, CMAIA, SACFA, FACS, DLRA	Bmi1-mediated migration and invasion of TSCC is related to cancer stem-like cells
Yanamoto *et al*.[37]	2014	*In vitro*	CD44v6 ABCG2	-	High High	IHC	Local recurrence with NAC treated patient is associated with cancer stem like cells
Chinn *et al*.[38]	2015		CD44/ ALDH+CD44/ ALDH–	Hyaluronan receptor, adhesion protein Membrane/ ALDH Cytoplasm	High Low	Flowcytometry WHA, BLI	CD44high/ALDH+cells compared to CD44low/ ALDH have capacity of tumorigenesis and greater rate of tumor growth
Valiyaveedan *et al*.[39]	2015	*In vivo* and *In vitro*	CD44 ABCG2 NOTCH1 CD133 OCT-4	Hyaluronan receptor, adhesion protein membrane Prominin-1 membrane Transcription factor cytoplasm	High High High High	PCR, IHC, SFA, WHA	The treatment naïve and recurrent cohorts, increased CSCs, as indicated by CD44/BMI1/ABCG2 signified poor prognosis
Wilson *et al*.[4]	2016	*In vitro*	CD44 NOTCH1 c-MET ALDH1A3	Hyaluronan receptor, adhesion protein Membrane	High	Flow cytometry IHC	Expression of these CSCs markers were significant after irradiation
Shiina *et al*.[40]	2015	*In vitro*	CD44 ALDH NANOG Oct Sox2 KLF4	Hyaluronan receptor, adhesion protein membrane ALDH cytoplasm Transcription factor cytoplasm Transcription factor cytoplasm Transcription factor cytoplasm Transcription factor	High High High High High High	FACS, QPCR	Selective oncogenes are responsible for the survival of tumor cells and chemoresistance to HNSCC

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Table 2: Contd...

Author	Year	Technique	CSC markers	Description of receptors and cellular localization	Expression of biomarker	Methodology	Conclusion
Kurth *et al*.[41]	2015	*In vitro* and *in vivo*	ALDH	-	High	Immunofluorescence, flow cytometry, WB	ALDH1A3+HNSCC cells may contribute to tumor relapse after irradiation, and inhibition of this cell population might improve therapeutic response to radiotherapy
Rudy *et al*.[42]	2016	*In vivo*	Wnt pathway	-		CAM Assay, Q-PCR, WNT974 treatment assay	WNT974 may have a role in future HNSCC therapy
Johansson *et al*.[43]	2016	*In vitro*	CD4 CDH1 CDH2 FOXC2 TWIST1 VIM FN1	Hyaluronan receptor, adhesion protein Membrane	High Low High High High High	QRT-PCR, Boyden chamber assay, flow cytometry	CD44high/EGFR low phenotype are associated with EMT with radioresistence
Ghuwalewala *et al*.[44]	2016	*In vitro*	CD44 CD24 OCT4 SOX2 NANOG CMYCoct4sox2	Hyaluronan receptor, adhesion protein membrane Cell adhesion protein Transcription factor cytoplasm Transcription factor cytoplasm Transcription factor cytoplasm Transcription factor cytoplasm	High Low High	MACS, flow cytometry, RT-PCR, WB, Immuno-fluorescence, IHC, TMA, SFA, MWHA	CD44 high - CD24 low cell population displays increased CSC and EMT property
Upadhyay *et al*.[45]	2016		Notch 1	-		Q-RT-PCR, IHC, MTTA, WHA, OFA, AIGA	The basis for therapeutic targeting of NOTCH1 in tongue cancer
Grau *et al*.[46]	2016		CD44 HLA1 Pancyto-keratin p-EGFR	Hyaluronan receptor, adhesion protein Membrane		IHC	There is an enrichment of cells with stem-like markers in relapsed tumors when compared with the primary tumor so this finding should be considered when developing treatment strategies
de Moraes *et al*.[47]	2017	*In vitro*	CD24 CD44 CD133 ALDH1 CD29 Ki-67	Hyaluronan receptor, adhesion protein membrane	High High High High High High	IHC	The expression of putative stem cell markers in oral cavity and oropharynx squamous cell carcinoma, with participation of CD44-positive cells in association with poor survival outcome
Liebig *et al*.[48]	2017	*In vitro*	Hedgehog signaling pathway (Hh)	-	High	CF assay	Over expressed Hh associates with poor prognosis

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Table 2: Contd...

Author	Year	Technique	CSC markers	Description of receptors and cellular localization	Expression of biomarker	Methodology	Conclusion
Lee *et al*.[49]	2017	*In vivo* and *In vitro*	CD133	Prominin-1 Membrane	High and ectopic	Cloning of CD133, WB, CVT, CFA Microarray	CD133 has a functional role in regulating stem cell properties in HNSCCs by promoting colony formation, ALDH activity, and increased expression of CSC markers such as OCT4 and NANOG and inhibition of tumor growth cisplatin
Saghravanian *et al*.[50]	2017	*In vitro*	CD44 p63	Hyaluronan receptor, adhesion protein Membrane	High High	IHC	Understanding initiating mechanisms and pathogenesis of OSCC by using these markers could result in novel therapeutic target in cancer treatment
Mohanta *et al*.[51]	2017	*In vitro*	CD44 CD147	Hyaluronan receptor, adhesion protein Membrane	High High	IHC, Flow cytometry, CFA, RT-PCR, WHA, MIA	CD44 and CD147 together improve the prognostic efficacy of tumor differentiation, imparting properties of increased self-renewal, migration, and invasion

CSCs: Cancer stem cells, HNSCC: Head-and-neck squamous cell carcinoma, OSCC: Oral squamous cell carcinomas, IHC: Immunohistochemistry, RT-PCR: Reverse transcription-polymerase chain reaction, QRT-PCR: Quantitative RT-PCR, BMi1: B lymphoma Mo-MLV insertion region 1 homolog, ALDH1: Aldehyde dehydrogenase 1, EMT: Epithelial-mesenchymal transition

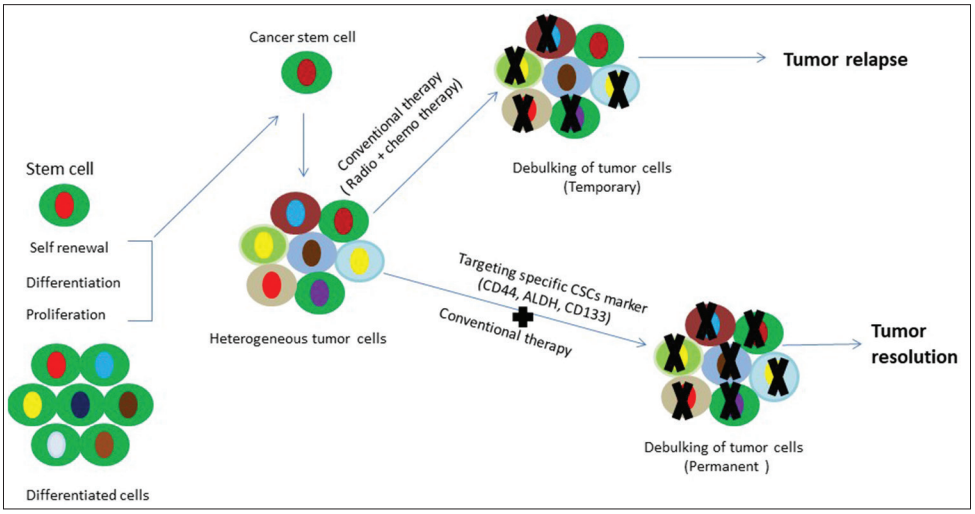

Figure 3: Schematic diagram showing conventional therapy and targeted therapy

colony-forming ability was seen in cells with ALDH expression. There is a significant overlap in the ALDH and CD44 populations, with 50.6%–74.4% of ALDH1 cells expressing CD44. Huang *et al*. showed that ALDH, CD44, OCT4, and Sox2 are closely related in tongue squamous cell carcinoma and the expression of Sox2 can be used as a prognostic indicator. In contrast, a study by Leinung *et al*. suggested that neither ALDH nor CD44, alone or combined, was sufficient to determine the CSC population in HNSCC, but ALDH was shown to be a possible prognostic marker for poor survival. Chinn *et al*. concluded that CD44 high/ALDH-positive cells compared to CD44low/ALDH-negative cells have the capacity of tumorigenesis and greater rate of tumor growth.

Liu *et al*. conducted a study to evaluate the expression of ALDH1 and CD133 in oral lichen planus and OSCC. They found clinically significant value for ALDH positivity (48%) who developed oral cancer ($P < 0.001$). Similarly, 59.4% of patients having CD133 positivity developed oral cancer ($P < 0.001$). A multivariate

analysis revealed that ALDH1 and CD133 expression was associated with 4.17-fold and 2.86-fold increased risk of oral cancer, respectively, and they concluded these markers can be considered as predictors to identify high risk of developing oral cancer.[26] Habu *et al*. evaluated the expression of Oct3/4 and NANOG in HNSCC cell lines. Oct3/4 showed a sensitivity of 82.0% and 61.5%, respectively. The author concluded that Oct3/4 and NANOG could represent probable CSC markers in HNSCC, and Oct3/4 could be considered as a potential predictor for distant nodal metastasis.[36]

Table 3: List of most common cancer stem cell markers[1,5]

Number	CSC markers	Different role in HNSCC
1	CD44	Self renewal, metastasis
2	ALDH	Tumorigenicity, chemoresistance
3	CD133	Tumorigenicity, self renewal, EMT
4	Oct-4	Metastasis, tumor invasion
5	NANOG	Regulates pluripotency and tumorigenesis of cancer stem cells
6	Sox-2	Tumorigenesis, metastasis
7	C-Met	Tumorigenesis, metastasis
9	TWIST	EMT
10	E-Cadherin	EMT, invasion
11	Bmi1	Cellular proliferation
12	Snail	EMT

CSC: Cancer stem cells, HNSCC: Head and neck squamous cell carcinoma, EMT: Epithelial-mesenchymal transition, ALDH: Aldehyde dehydrogenases, BMi1: B lymphoma Mo-MLV insertion region 1 homolog

CD133, another important stem cell marker, was found to be associated with either CD44 or ALDH. Lee *et al*. demonstrated that ectopic overexpression of CD133 significantly promotes properties of stemness in KB cell lines. Furthermore, CD133 promotes chemoresistance by arresting transition of the cell cycle and reducing apoptosis, which results in inhibition of tumor growth in fluorouracil- or cisplatin-injected mouse tumor model. They also reported that elevated levels of CD133 may lead to HNSCC chemo-resistance through increased stemness and cell cycle arrest. Many researchers have stated that carcinoma cell lines show enhanced clonogenicity in CD133-positive cells that are responsible for an EMT phenotype, tumor sphere formation, self-renewal, proliferation, tumorigenicity, and multilinear differentiation.[49,59]

The present review also highlights the significant role of CSCs marker expression in HNSCC radiosensitivity. Radioresistant tumors show upregulation of stem cell markers such as CD44, ALDH, CD133, Oct-4, Sox2, NANOG, and BMI1, which are indicative of stemness and self-renewal. Ghuwalewala *et al*. concluded that CD44 high-CD24 low cell population displays increased CSC and EMT property.[44] In their study, cell lines of OSCC showed increased expression of Sox2, NANOG, and Oct-4. NANOG has been shown to be a therapeutic target controlling CSC self-renewal in HNSCC. Overexpression of Oct-4 might promote tumor-initiating properties in OSCC by mediating EMT.

Table 4: Comparison between normal stem cells and cancer stem cells[52,53]

Property	Stem cells	CSCs
Self-sufficiency for growth signals	Within normal limit	Increased
Insensitivity to antigrowth signals	Balanced	Increased
Evasion of apoptosis		Increased
Limitless ability to replicate	Controlled	Uncontrolled
Sustained angiogenesis	Controlled	Uncontrolled
Tissue invasion and metastasis	Absent	Present
Balance between growth and antigrowth signals	Balance	Imbalance
Balance between the proliferation signal and anti-proliferation signal	Proper	Improper
Degree of dependence on the stem cell niche	Balance	Imbalance
Cell cycle	Regular	Irregular
DNA damage repair	Prompt	Delayed or interrupted

CSCs: Cancer stem cells

Table 5: Causes of radioresistance and chemoresistance of cancer stem cells[15,54-56]

Causes	Radio resistance	Chemoresistance
1	Alterations in EGFR, PI3K/AKT, and RAS Pathways	ABC transporters and multidrug resistance
2	Deregulation of TP53 associated intrinsic apoptosis	Detoxification enzyme involving in chemotherapy resistance
3	Hypoxia induced neovascularization	Inactivation of apoptosis
4	EMT	Due to aldehyde dehydrogenase activity
5	Involvement of miRNAs - Upregulated - miR-16, miR-29b, miR-1254, and miR-150, miR-210, miR-381, miR-296-5p.miR-31	Enhanced DNA repair mechanisms
	Downregulated miR-205, miR-324-3p, miR-93-3p, miR-203 and miR-4501	Changes in drug target interaction

miRNAs: Micro RNAs, EGFR: Estimated glomerular filtration rate, ABC: ATP-binding cassette, EMT: Epithelial-mesenchymal transition

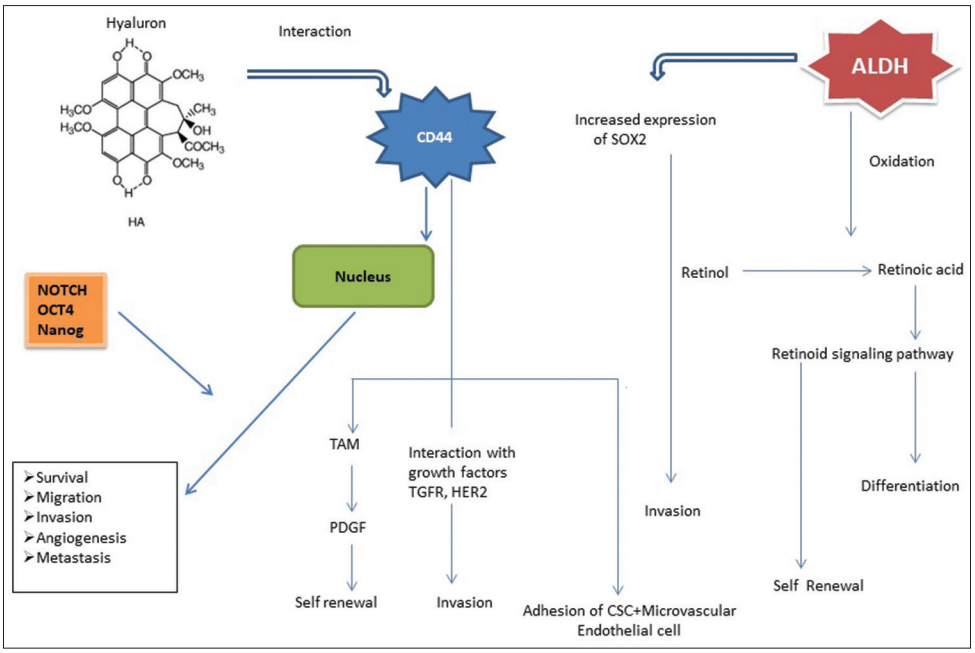

Figure 4: Role of stem cell proteins in cancer

Shiina *et al*. found that NANOG, Oct4, Sox2, and KLF-4 were upregulated in the CD44 v3 high and ALDH1 high cell population isolated from HNSCC and by adding 200 k Da-HA, it significantly decreased the ability of cisplatin to induce tumor cell death. This suggested both a decrease in tumor cell death and an increase in tumor cell survival, leading to the enhancement of chemoresistance. CSCs exhibit chemoresistance related to the ABC transporter expressed in these cells.[40] Notch is a signaling pathway which plays a role in both CSC maintenance and chemo-resistance. The Notch pathway is involved in the processes of tumor progression and metastases including tumor initiation, as well as self-renewal of CSCs.[4,39,45,60] Upadhyay *et al*. pointed out that Notch activation is governed by gamma secretase inhibitor, or shRNA-mediated knockdown of Notch could lead to decreased capacity of spheroid formation, transformation, survival, and migration of HNSCC cells and concluded that targeting Notch signalling pathway may lead to better therapeutic outcome in tongue cancer.[39,44,45] Another major pathway is WNT/beta signaling pathway which maintains the self-renewal property of NSC and CSCs. The mechanism of chemoresistance through this pathway is still not completely understood, and varies among cell lines and tumor types. One potential mechanism is through the upregulation of ABC (ATP-binding cassette) pumps.[42,48]

In addition to CSC biomarkers, microenvironmental factors, such as niche-specific properties, also represent potential therapeutic targets to allow the eradication of HNSCC cells. A niche is a microenvironment that supports CSC survival and growth, and niches may also represent potential therapeutic targets that need further research in HNSCC. Recent evidence suggests that HNSCC CSCs reside in perivascular niches which can represent a potential target, and therapeutic strategies exploiting the mutual dependence of CSCs and endothelial cells can reduce the rate of metastasis and recurrence in HNSCC.[10]

Few researchers have discussed differences in the biological behavior of NSCs and cancer cell. Genomic integrity is characteristic of NSCs, whereas in some tumorigenic cells, either loss of genomic integrity or scarcity of it makes them different from NSCs. Cancer cells have the capacity to access and maintain extragenetic mutations. In NSCs, the acquisition of differentiation is generally associated with loss of self-renewal capacity and overall reduction in cell proliferation. Tissue-specific stem cells give rise to limited number of differentiated cell types that are generally specific to one type of tissue. Interconversion among tumorigenic cancer cells is a significant mechanism which explains how these cells can become phenotypically heterogeneous and resist therapy.[60-62] Self-renewal and slow cycling are characteristics features shared by both NSCs in adult somatic tissues and CSCs.

Self-sufficiency for growth signals, insensitivity to antigrowth signals, evasion of apoptosis, limitless ability to replicate, sustained angiogenesis, tissue invasion, and metastasis make NSC different from CSCs. Identification and a better understanding of reliable molecular markers are needed to characterize CSCs in HNSCC. CSC markers themselves can serve as potential targets for anticancer

therapy. Targeting CSC-specific markers and their molecular pathways may help in developing novel CSC diagnostics and therapeutic approaches. Along with conventional chemotherapy and radiotherapy, targeted therapy against the stem cell markers identified is an interesting prospect to research. Few limitations of this review were that original research articles published in language other than English were not considered. The selected articles were a combination of both *in vivo* and *in vitro* studies, and difference in expression of markers based on *in vivo* and *in vitro* issue is debatable.

Conclusion

Causes of failure of conventional therapy in HNSCC is a critical factor for local recurrence and distant metastasis. The research provides a new conceptual planning for anti-cancer therapy by identifying a sub-population of cells that possesses high tumorigenic potential. Currently, there is no single biomarker to define the CSC population accurately for HNSCC. A promising target panel of CSC markers identified through this systematic review were CD44, ALDH, and CD133, which were responsible for tumorigenesis, self-renewal, and therapy resistance, whereas NANOG, SOX-2, and OCT-4 were involved in metastasis and invasion.

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Conflicts of interest
There are no conflicts of interest.

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