Review Article

Cancer stem cells: A comprehensive review on identification and therapeutic implications

BR Varun[1], P Jayanthi[2], Pratibha Ramani[3]

[1]Department of Oral and Maxillofacial Pathology, PMS College of Dental Sciences and Research, Thiruvananthapuram, [2]Department of Oral and Maxillofacial Pathology, Azeezia College of Dental Sciences and Research, Kollam, Kerala, [3]Department of Oral and Maxillofacial Pathology, Saveetha Dental College, Chennai, Tamil Nadu, India

Abstract

Cancer stem cells (CSCs) are distinct subpopulations of tumor cells that possess the ability for perpetual self-renewal and proliferation. They produce downstream progenitor cells and cancer cells that drive tumor growth. Studies of many cancer types including oral squamous cell carcinoma (OSCC) have identified CSCs using specific markers, but it is still unclear as to where in the stem cell hierarchy these markers fall. This is compounded further by the presence of multiple CSC subtypes within OSCC, making investigation reliant on the use of multiple markers. This review paper focuses on the current knowledge in CSC markers including OCT4, SOX2, NANOG, aldehyde dehydrogenase 1, CD44, CD24, CD133 and Musashi-1, highlighting their use and validity in OSCC CSC research.

Keywords: Cancer stem cells, markers, self-renewal

Address for correspondence: Dr. P Jayanthi, Department of Oral and Maxillofacial Pathology, Azeezia College of Dental Sciences and Research, Kollam, Kerala, India.
E-mail: drjayanthip@gmail.com
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INTRODUCTION

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. Hence, CSCs are considered to be highly tumorigenic when compared to other cancer cells and are believed to be responsible for biological characteristics of cancer such as rapid growth, invasion and metastasis.[1] CSCs were first identified by a stem cell biologist, John Dick in the year 1997 in acute myeloid leukemia.[2]

Oral squamous cell carcinoma (OSCC) is the most common oral cancer, accounting for more than 90% of the cases in that location. The overall survival rate of OSCC has remained <50% for more than a decade, despite advances in diagnosis and therapy.[3] Surgery with adjuvant radiotherapy (RT) and occasional chemotherapy (CT) is the mainstay of treatment for OSCC. CSCs in OSCC are resistant to RT and chemotherapeutic agents such as cisplatin and 5-fluorouracil. CSCs are predominantly in the inactive Go phase and thus, avoid destruction by RT and CT that usually target actively dividing cancer cells. These posttreatment cancer cell populations may contribute to locoregional recurrence and distant metastasis.[4]

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IDENTIFICATION AND CHARACTERIZATION OF CANCER STEM CELLS

CSCs are identified using specific markers that vary depending on the tumor type or tissue of origin. No single marker has been shown to unequivocally identify CSCs, and it is likely that CSCs exist in an overlapping hierarchy of cell population subsets. Consequently, combinations of various markers have been used for the characterization of CSCs. The list of potential markers of CSCs in OSCC includes CD133, Musashi-1, CD44 and CD24 (in combination with CD44). The enzymatic activity of aldehyde dehydrogenase (ALDH), efflux of vital dyes by multidrug transporters, colony and sphere-forming assays utilizing specific culture conditions and the expression of embryonic stem cell markers have also been used for the identification of CSCs.[5]

Embryonic stem cells markers

CSCs in OSCC express the same proteins that regulate the embryonic stem cells (ESCs). OCT4, NANOG and SOX2 are considered to be the master regulators for self-renewal and maintenance of the stem cell population in the undifferentiated state. Fu *et al*. demonstrated that immunohistochemical expression of OCT4 and SOX2 is significantly higher in tumor-adjacent tissue compared to both normal tissue and the tumor, whereas, NANOG is highly expressed in both tumor and peritumoral tissue, compared to the normal tissue.[6]

NANOG

NANOG is a transcription factor that is widely known as a marker for primitiveness or "stemness." In murine cell lines, NANOG is involved in functionally blocking the differentiation and thus maintaining the pluripotency of ESCs. NANOG is upregulated in different types of cancers and plays a role in tumor transformation, tumorigenicity and metastasis in OSCC and, therefore, has been associated with poor prognosis.[7] NANOG is found to be overexpressed in the CSC population compared to the parental tumor population in OSCC cell lines.[8] Similarly, in lip SCC, NANOG is expressed by three distinct putative CSC subpopulations, both within the tumor nests and the peritumoral stroma.[9]

OCT4

OCT4 is a transcription factor that plays a critical role in early embryogenesis and maintenance of ESC pluripotency. It has been suggested that OCT4 may promote tumor initiation by regulating the process of epithelial–mesenchymal transition (EMT).[10] The expression of OCT4 has been used to define the CSC population in OSCC in conjunction with other CSC markers. In moderately differentiated buccal mucosal SCC, expression of OCT4 has been demonstrated in a distinct subpopulation of CSCs within the tumor nests, the peritumoral stroma and the microvessels within the peritumoral stroma.[11] Increased expression of OCT4 in OSCC has been associated with early stage of the disease, and better prognosis, and a molecular mechanism explaining this association has yet to be elucidated.[6]

SOX2

The SOX2 protein is a SRY-related HMG box transcription factor involved in multiple signal transduction pathways that control cell proliferation, migration, invasion, stemness, tumorigenesis and anti-apoptosis. SOX2 is known to complex with OCT4, and in murine cell lines has been shown to control the expression of the NANOG gene.[12] In SCC of the buccal mucosa, SOX2 is expressed within the tumor nests, the peritumoral stroma and the microvessels within the peritumoral stroma, similar to OCT4.[11] Fu *et al*. showed that SOX2 expression in OSCC correlated with small tumor size and early tumor stage, and better disease-free survival.[6] Chou *et al*. demonstrated that overexpression of SOX2 enhances the invasiveness and xenotransplantation tumorigenicity in OSCC cells. The study also showed that silencing SOX2 suppresses the expression of drug resistance and anti-apoptotic genes.[13]

Oral squamous cell carcinoma cancer stem cell markers

CD44

CD44 is a cell surface hyaluronan receptor protein having contrasting roles in both cell migration and adhesion. It has been widely used as a CSC marker in epithelial malignancies including OSCC, where higher expression of CD44 is noted in CSCs compared to parental cells.[8] Overexpression of CD44 has been associated with decreased overall survival, increased locoregional recurrence and increased resistance to RT in OSCC, thus exhibiting many of the characteristics of CSCs.[14] Expression of the variant isoform CD44 v6 has also been found to be significantly associated with regional nodal metastasis, pattern of invasion, depth of invasion, perineural invasion and local recurrence in multiple solid tumors including OSCC.[15] Kosunen *et al*. showed that irregular staining of CD44 in tumor cells is associated with poor tumor differentiation and advanced stage, whereas Braumüller *et al*. demonstrated that there was no prognostic significance of CD44 v6 expression in OSCC. These differences may, in part, be explained by the expression of CD44 v6 by CSCs, as well as inflammatory cells within the tumor stroma.[16,17]

Quintanilla *et al*. suggested that CD44 may be considered as a marker of partially differentiated cells as the expression

of CD44 is lost during induced cellular reprogramming to the undifferentiated state. This may indicate a progressive gain of CD44 expression as CSCs progress to a more differentiated phenotype, and this implies that CD44 is in fact a relatively mature marker, likely downstream of the true CSC population. Interestingly, downregulation of CD44 also leads to reduced expression of OCT4, suggesting that CD44 has a functional role in maintaining stem cell properties.[18]

CD24

CD24 is a cell surface glycoprotein involved in cell adhesion and metastasis and has been identified in wide variety of cancer cells. Ghuwalewala *et al*. showed that cells expressing high levels of CD44 and low levels of CD24 exhibited both CSC properties and characteristics of EMT.[19] In OSCC cell lines, CD44 v3+/CD24− population demonstrated higher sphere-forming capacity, higher drug resistance and expressed higher mRNA levels of CSC-related genes.[20]

CD133

CD133 is a transmembrane protein that plays a key role in the organization of plasma membrane topology. Overexpression of CD133 is often used as a CSC marker in many solid tumors including OSCC. CD133+ cells have also been found to coexpress CD44, and the CD133+/CD44+ immunophenotype has been found to correlate significantly with poorer overall survival, supporting the idea that cells expressing these proteins have a more aggressive phenotype.[14] The expression of CD133 in oral epithelium increases from normal epithelium, through dysplasia, to carcinoma and CD133+ oral leukoplakia has been shown to be more than three times as likely to progress to OSCC compared to CD133− lesions.[21] In several cell lines, CD133+ cells have been found to overexpress ESC markers, including OCT4 and NANOG, and also display CSC characteristics such as tumorsphere formation, tumorigenicity and chemoresistance.[22]

Musashi-1

Musashi-1, a translational regulator, has been considered a marker of CSCs. The expression of Musashi-1 has been associated with higher stage and poorly differentiated status of OCSCC, and is significantly correlated with CD133, suggesting a functional role for these two proteins in oral carcinogenesis.[21]

Aldehyde dehydrogenase 1

ALDH is a cytosolic enzyme responsible for catalyzing the pyridine nucleotide-dependent oxidation of aldehydes to carboxylic acids. ALDH has been used as a CSC marker in OSCC, with ALDH+ cells demonstrating plasticity with the ability to form tumorspheres in serum-free media.[23] Although there are many isoforms of ALDH, ALDH1 appears to be of particular importance. ALDH1 is likely to play a role in malignant transformation of oral leukoplakia to OSCC given that ALDH1+ leukoplakia is more than three times more likely to develop OSCC.[24] Overexpression of ALDH1 is also found to be correlated with nodal metastasis.[25] Zou *et al*. demonstrated that ALDH+ subpopulation expresses many known CSC-related genes that are not seen in the ALDH− population.[23] In a study by Chen *et al*., cells expressing ALDH1 coexpressed snail, an EMT marker and also displayed evidence of radioresistance. Knockdown of snail resulted in decreased expression of ALDH1 and inhibited CSC properties, with resultant decreased tumorigenicity.[26]

THERAPEUTIC IMPLICATIONS

Studies have shown that the stromal environment and CSC niche play a key role in the behavior of cancer cells and, hence, targeting the stem cell niche directly can weaken the source of nutrition and change the essential signals needed by CSCs to proliferate. Tang *et al*. suggested that targeting CSCs and their microenvironmental niche, which contributes to self-renewal of these cells along with the reactive oxygen species status of these cells and tweaking their intracellular milieu to facilitate apoptotic death signals over proliferative effects may facilitate a new prospective toward target therapy in cancers.[27]

Krishnamurthy and Nör proposed a hypothetical model for the response of HNSCC to different therapeutic strategies. They suggested an emerging concept of combining the use of conventional CT and CSC-targeted therapy.[28]

Hypoxia has been understood to play a key role in tumor progression and hypoxic tumor microenvironment, in turn, has control over the CSCs. Therefore, when the antiangiogenic agents are administered in combination with CSC-targeted drugs, more effective results are attained in cancer therapy, along with inhibiting hypoxia-inducible factors.[29]

CONCLUSION

CSCs serve as crucial elements in the growth of a tumor mass by providing selfrenewal capacity. These cells are considered to play a key role in recurrence, metastasis and radioresistance in OSCC. With regard to these aspects, the survival rate of patients with OSCC may be associated with the increased expression of CSC markers. Currently, there is no single biomarker available for accurate isolation

and characterization of CSCs in OSCC. Identification of reliable markers is required to characterize CSCs in OSCC as this could ensure the clinical effectiveness of future targeted treatments, possibly resulting in a more effective outcome.

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Conflicts of interest

There are no conflicts of interest.

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