

## The Concept of Cancer Stem Cell in Oral Squamous Cell Carcinoma

Gokul Sridharan

Gokul Sridharan, Department of Oral Pathology and Microbiology, YMT Dental College and Hospital, Kharghar, Navi Mumbai-410210, Maharashtra, India

Correspondence to: Gokul Sridharan, MDS, Department of Oral Pathology and Microbiology, YMT Dental College and Hospital, Kharghar, Navi Mumbai-410210, Maharashtra, India

Email: [dr\\_gokuls@gmail.com](mailto:dr_gokuls@gmail.com)

Telephone: +919022792310

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### ABSTRACT

Oral squamous cell carcinoma (OSCC) is an epithelial malignancy commonly affecting developing countries. The disease progression is characterized by a complex molecular mechanism resulting in accumulated genetic mutations causing uncontrolled cell proliferation. These genetic alterations are primarily evident in somatic stem cells and recently, it has been demonstrated that a subset of these cells referred to as cancer stem cells play an important role in cancer progression. Cancer stem cells are a small subset of cells which were found to be highly tumorigenic with capability of self renewal and behavior akin to tumor progenitor cells. The origin of cancer stem cells could be from normal somatic stem cells, dedifferentiated tumor cells, fusion of tumor epithelial cells with hematopoietic stem cells or due to the process of epithelial-mesenchymal transition. Identification of cancer stem cells could be done by markers such as CD44, CD133, E-cadherin; Wnt signaling pathway, Oct-4 and ABCG2. Cancer stem cells are known to be responsible for tumor recurrence, metastasis and resistance to chemotherapy. The concept of cancer stem cells and its association with OSCC is relatively new and debatable and detailed study is necessary for its better understanding. This editorial highlights the relevant findings on cancer stem cells, its origin, identification and its role in OSCC.

**Key words:** Cancer stem cell; Oral squamous cell carcinoma; Potentially malignant disorders; Targeted therapy; Molecular markers

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### INTRODUCTION

Ever since the term cancer was coined by Celsus in 47 AD there has been an exponential rise in the quest to understand the entire process of carcinogenesis for the benefit of mankind. Much of the research is focused on early diagnostic and therapeutic implications of cancer. Since cancer is a disorder of cell growth and behavior, its ultimate cause has to be decided at the cellular and sub-cellular level. Improved technological advancements have enabled researchers to identify numerous cancer associated genes over the past two decades. Based on these research findings and the prior knowledge on carcinogenesis, seven fundamental changes in cell physiology are described to determine the malignant behavior of a cell<sup>[1]</sup>. One of the recent concepts of understanding the genetic makeup of a cancer cell is the description of cancer stem cell in invasion, metastasis as well as resistance to treatment.

A physiological stem cell has the ability to differentiate into some or all of the cell types required to maintain homeostasis within a particular tissue, organ system or an entire organism<sup>[2]</sup>. According to developmental status, stem cells can be classified as embryonic or adult stem cells. Adult stem cells play a role in tissue homeostasis and regeneration as well as in the biology of several diseases including cancer<sup>[3]</sup>. The stem cells in the oral epithelium are distributed primarily in the basal and suprabasal layer which are identifiable with the aid of several markers. The gold standard for the identification of adult stem cells is genetic inducible fate mapping and transplantation<sup>[4]</sup>. This technique involves placing a permanent genetic mark on a putative stem cell *in vivo* such that the cell will be labeled and tracked as it is genetically passed from one progeny to another. The ability of the cell to self renew and undergo

differentiation can be measured by this method. Transplantation assays help in testing the ability of the cell to reform an entire tissue after transplantation<sup>[2]</sup>. Some of the important characteristics that define stem cells include the capacity for self renewal by which they perpetuate themselves and differentiation that gives rise to all types of mature cells and leading to organogenesis<sup>[5]</sup>. Identification of physiological stem cells and understanding its properties is a prerequisite to discuss the concepts of cancer stem cell.

Cancer stem cells (CSCs) as defined by American Association of cancer research, are a subset of cells with the capability of self renewal and differentiation into the heterogeneous lineages that constitute the tumor mass<sup>[6]</sup>. The idea that cancer can originate from a small population of cells with stem cell like properties was proposed some 150 years back by Francesco Durante in 1874<sup>[7]</sup>. The first evidence of role of cancer stem cell was documented in human acute myeloid leukemia by Bonet and Dick (1997)<sup>[8]</sup>. Al-Hajj *et al*<sup>[9]</sup> (2003) was the first to identify CSC in solid tumors wherein it was found that few cells isolated from breast cancer were capable of initiating a tumor. Since then CSC's were isolated from tumors of brain<sup>[10]</sup>, lung<sup>[11]</sup>, prostate<sup>[12]</sup>, pancreas<sup>[13]</sup>, liver<sup>[14]</sup>, head and neck and melanoma<sup>[15]</sup>.

In head and neck tumors, cancer stem cell isolation was first performed by Prince *et al*<sup>[16]</sup> (2007) through the expression of CD44 by few subpopulation of cells. Few other studies attempted to isolate CSC's from oral squamous carcinomas via the expression of stem cell markers like CD44, CD133, CD117, OCT-4 and Aldehyde dehydrogenase<sup>[17,18]</sup>.

Oral squamous cell carcinoma (OSCC) is a malignant tumor of multifactorial etiology with tobacco being one of the important risk factor. It is one of the common epithelial malignancies that may arise either de novo or more commonly from pre-existing diseases which are collectively referred to as potentially malignant disorders<sup>[19]</sup>.

## CANCER STEM CELLS

The properties which symbolizes cancer stem cells are self renewing ability to differentiate into diverse phenotype; ability to initiate tumors from minute numbers and high chemoresistance<sup>[20]</sup>. The chemoresistance, tumorigenic and metastatic activities as well as in their glycosylation patterns helps in differentiating them from normal adult stem cells<sup>[21]</sup>.

The role of CSC is explained by the hierarchical theory of tumor development which hypothesizes that tumorigenicity resides in a small subpopulation of cells composing the tumor that retain the capacity of stemness. Based on the observations of this study model, two types of CSCs can be distinguished: one is stationary CSCs which remain incorporated in the epithelia, are unable to spread and are responsible for resistance to chemo- and radiotherapy and serve to increase tumor volume while the other being movable CSCs which are capable of migrating, are localized at host-tumor interface and responsible for the ability to metastasize locoregionally and/ or remotely<sup>[22]</sup>. The CSC hypothesis has two main components; one being the cellular origin of the tumor and the second component is the responsibility of CSC for maintaining tumor growth<sup>[5]</sup>.

The most plausible concept surrounding the origin of CSC is that they are derived from the normal adult epithelial stem cells which are capable of self renewing and also has a long life thereby maintaining the mutagenicity over a period of time<sup>[23]</sup>. This may be applicable to OSCC, which requires accumulation of sufficient mutations over a prolonged period and affects only those cells which have a long life and hence stem cells are involved. Also similar characteristics of stem

cells and CSC suggest the origin of the latter from the former. Other postulates regarding origin of CSC includes progenitor cells, which in presence of additional mutations would lead to transformation and re-acquire the self renewal potential of stem cells<sup>[24]</sup>. The fusion of hematopoietic stem cells with a differentiated epithelial cell or with mutated epithelial somatic cell may result in attainment of stem cell property by these cells<sup>[5]</sup>. This hypothesis is untested in OSCC and it is poorly understood on how a mutated tumor cell might de-differentiate into a stem cell. One possible explanation might be the continuous presence of carcinogenic stimulus that may bring about this effect which needs to be proved. Another possible mode of CSC acquisition is through epithelial-mesenchymal transition (EMT) which is the ability of the epithelial cell to acquire mesenchymal characteristics under the influence of specific environmental stimuli. Also CSC may result from neosis which is a type of cell division in cancer whereby DNA damage can generate senescent multinucleated cells which further give rise to cells with stem cell like characteristics<sup>[25]</sup>.

The second component is the ability of CSC to maintain tumor growth and its role in invasion and metastasis. This specificity of CSC can be explained mainly by epithelial-mesenchymal transition, stem cell niche and side population cells. EMT is a process that allows a polarized epithelial cell to assume a mesenchymal cell phenotype which is characterized by enhanced motility and invasiveness<sup>[26]</sup>. This is a crucial process in cancer progression, providing cancer cells with the ability to escape from the primary site, invade stromal tissues and migrate to distant regions of the body. The loss of E-cadherin and gain of N-cadherin expression known as cadherin switching is a major hallmark of EMT<sup>[27]</sup>. EMT is an important factor in conferring stem cell phenotype and the various regulators of this function are Twist<sup>[28]</sup>, Snail<sup>[29]</sup>, miR-200a<sup>[30]</sup>, HIF-1 $\alpha$ <sup>[31]</sup>, inflammation mediators through TGF- $\beta$ , PGE2 and IL-1 $\alpha$ <sup>[32]</sup>. These findings suggest the role of EMT in acquisition of stem cell like properties thereby aiding in invasion and metastatic ability of head and neck carcinomas. On the contrary, tumor microenvironment is generally involved in the invasion and metastatic potential of OSCC and this occurs irrespective of whether the tumor cells acquire stem cell phenotype or otherwise. Hence, it should be considered that though acquisition of stem cell property may play a role, it is not the sole process by which cancer spreads.

Tumor cells with or without stem cell like characteristics depend on their immediate environment or niche for their survival and function. Non-epithelial stromal cells, inflammatory cells and vasculature have been proposed as key components of the niche. In head and neck tumors, majority of stem cells are found in the vicinity of a perivascular niche<sup>[33]</sup>. Soluble factors like VEGF secreted by endothelial cells may induce the proliferation and enhance the survival of head and neck cancer stem cells and also protect these cells from anoikis<sup>[34]</sup>. Angiogenesis is a known mechanism of tumor growth which is utilized by the invading tumor cell for its survival. Based on the understanding of the EMT and tumor microenvironment, it would be prudent to attempt targeted therapy against these factors which may be beneficial in oral cancer treatment.

A major challenge in our understanding of cancer stem cell is related to its identification and expression in tumor tissue. Currently, there are no specific markers available for cancer stem cells and most of the markers tried are found to have overlapping expression with normal stem cells. Some of the identification methods evaluated are in-vitro clinical trials done to identify keratinocytes (normal and malignant)<sup>[35]</sup>. Identification of long surviving cells in oral mucosa

can be done by using surface markers and flow cytometry. Surface markers include Oct-4 expression, a transcription factor of POU family that is expressed in undifferentiated pluripotent cells<sup>[18]</sup>, CD 133, a glycosylated protein<sup>[36]</sup>, CD44, a cell surface glycoprotein<sup>[16]</sup>; ALDH enzymes<sup>[37]</sup>; E-cadherin and side population cells using a vital DNA dye Hoeschts33342<sup>[26]</sup>. A significant progress in this regard is the development of orosphere assay which helps to generate cancer stem cell from primary lesions or from cancer stem cell lines<sup>[33]</sup>.

Therapeutic implications revolve around the possibility of CSCs being resistant to chemo- and radiotherapy and thereby identify therapy targeted at these cells. Various mechanisms are hypothesized by which these cells acquire the resistance. The DNA protection and damage repair pathways may lead to momentous chemo- and radioresistance. Increased free radical scavenging and reduce reactive oxygen species levels might potentially play a role in CSC's resistance<sup>[23]</sup>. Oral CSCs express high ATP binding cassette (ABC) transporters that can actively efflux drugs and shield them from adverse effects of chemotherapeutic insult. These cells also possess a unique mechanism to resist cell death including modified anti-apoptotic machinery, increased pump activity and decreased cell division<sup>[6]</sup>. Hypoxia inducing factor are found to be overexpressed in CSC which may be responsible for some aspect of radiation resistance in head and neck squamous cell carcinoma<sup>[38]</sup>. The resistance of CSC to various therapeutic agents might be of significance in tumor recurrence. Although surgery is the mainstay in the treatment of OSCC, use of chemo and radiotherapy to eliminate dormant tumor cells is a usual practice. The presence of CSC may hinder complete removal of tumor cells and lead to recurrence. Thus targeted therapy aimed at CSCs are being tried, the treatment protocol combining debulking of tumor followed by cancer stem cell targeted therapy. Among the various drugs, salinomycin has been found to be a selective inhibitor of breast cancer stem cell<sup>[39]</sup>. Metformin, an anti-diabetic drug was found to be effective against CSC and is currently under trial<sup>[20]</sup>. Several dietary products such as resveratrol are also found to directly or indirectly affect the property of CSCs<sup>[40]</sup>.

## FUTURE IMPLICATIONS

This editorial provides a general overview of cancer stem cells. Cancer stem cells as applicable to oral squamous cell carcinoma needs further validation for better understanding and its applications in clinical settings. An important aspect is the reliability of identifying and characterizing CSC based on the cell surface markers. Considerable overlap in the expression patterns of these markers between normal stem cells and CSC hinders their usefulness and reliability. Contradictory literature findings have suggested the unreliability of CD44, CD117 and CD133 for CSC identification.

Much of OSCC arise from potentially malignant disorders which includes leukoplakia, erythroplakia and oral submucous fibrosis that are present in the oral cavity for a prolonged period and their constant exposure to carcinogens result in the accumulation of sufficient genotypic and phenotypic alterations. The existence of cancer stem cell with regard to these lesions needs to be elucidated and if they are actually present, their exact role on intitation and progression of OSCC needs to be identified.

Tumor microenvironment is inclusive of various factors such as stromal cells, angiogenesis, hypoxia inducible factor, inflammation and is responsible of invasion and metastatic ability of tumor cells. These are also suggested to aid the existence of CSC. Modification of the tumor microenvironment can be considered as an important therapeutic aid to nullify the action of cancer stem

cells. This is important as the tumor cells despite its nature utilize its microenvironment for survival and progression.

Targeted therapy aimed at elimination of CSC with respect to OSCC has not yet been studied. Whether these therapies are specific for cancer stem like cells or they act on other tumor cells and normal stem cells requires confirmation. It may be only possible to utilize this as an adjuvant therapy to the more conventional therapies.

## CONCLUSION

Technological advancements have led to identification of more and more molecular and cellular alterations in various forms of cancer with efforts continuing in this direction. While it may help in improving our understanding of carcinogenesis, its applicability in treatment of cancers is of paramount importance. Research should aim at identification of any of these markers as a potential therapeutic aid for better treatment results, prognosis and reduced morbidity and mortality.

## CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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**Peer reviewers:** Pablo Varela-Centelles, Profesor Asociado, Departamento de Estomatología. Facultad de Medicina y Odontología, Universidad de Santiago de Compostela, Entrerrios s/n, 15782 Santiago de Compostela. A Coruña. Spain; Nazanin Mortazavi, Department of Oral Medicine, School of Dentistry, Babol University of Medical Sciences, Babol, PO BOX 47135-853, Iran.