

Cancer stem cells: An insight

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Abstract

Cancer stem cells (CSCs) have the ability to self-renew and are present in most tissues including breast, brain, lung, head and neck, prostates, testis, ovary, esophagus, colon and liver. Their origin is yet to be discovered though a series of hypotheses have been proposed in this regard. CSCs play a role in not only the creation of cancer but also its evolution, metastasis and recurrence. CSCs have an important role in cancer therapy and the resistance toward chemotherapeutic agents. This article reviews the characteristics of CSCs in terms of origin, methods of isolation and cancer therapy.

Keywords: Cancer stem cells, cancer stem cell markers, head and neck squamous cell carcinoma, metastasis, stem cell niche

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Received: 31.08.2017, **Accepted:** 21.10.2017

INTRODUCTION

Cancer stem cells (CSCs) were first identified by John Dick in acute myeloid leukemia in the late 1990s. As a stem cell biologist, Dick proved important role of CSCs. Since the beginning of 21st century, there is an intense focus on cancer research.

CSCs are cancer cells that possess characteristics associated with normal stem cells, specifically the ability to give rise to all cell types found in a particular cancer sample. CSCs are therefore tumorigenic (tumor-forming), perhaps in contrast to other nontumorigenic cancer cells. CSCs may generate tumors through the stem cell processes of self-renewal and differentiation into multiple cell types. Such cells are proposed to persist in tumors as a distinct population and cause relapse and metastasis by giving rise to new tumors. Therefore, development of specific therapies targeted at CSCs holds hope for improvement of survival and quality of life of cancer patients, especially for patients with metastatic disease.^[1,2]

CSCs are located in the invasive fronts of head and neck squamous cell carcinomas (HNSCCs) close to blood vessels (perivascular niche). Endothelial cell-initiated signaling events are critical for the survival and self-renewal of these stem cells. Markers such as aldehyde dehydrogenase (ALDH), CD133 and CD44 have been successfully used to identify highly tumorigenic CSCs in HNSCC.^[2,3]

Here, we provide a review in the field of CSCs with a focus on head and neck cancers.

ORIGIN OF CANCER STEM CELLS

The fundamental concept underlying the CSC hypothesis is that not all tumor cells in a cancer are equal.^[3] The CSC hypothesis is fundamentally based on the application of stem cell concepts derived from embryogenesis to understanding of the tumorigenic process.

The following are key features of the CSC hypothesis:

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How to cite this article: Moharil RB, Dive A, Khandekar S, Bodhade A. Cancer stem cells: An insight. J Oral Maxillofac Pathol 2017;21:463.

Access this article online

Quick Response Code:



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10.4103/jomfp.JOMFP_132_16

(1) Only a small fraction of the cancer cells within a tumor have tumorigenic potential when transplanted into immunodeficient mice; (2) the CSC subpopulation can be separated from the other cancer cells by distinctive surface markers; (3) tumors resulting from the CSCs contain the mixed tumorigenic and nontumorigenic cells of the original tumor and (4) the CSC subpopulation can be serially transplanted through multiple generations, indicating that it is a self-renewing population” (Prince and Ailles, 2008). Therefore, CSCs are capable of self-renewal and differentiating into other distinctive cells that make up the tumor mass.^[3]

Various types of stem cells give rise to progenitor cell which have the ability to further divide into specialized or differentiated cells that carry out the specific functions of the body. It is controversial as to whether CSCs arise from stem cells, progenitor cells or differentiated cells present in adult tissue. The issue is currently under debate and the numbers of theories of origin of stem cells are presented.^[3]

HYPOTHESIS NUMBER 1: CANCER CELLS ARISE FROM STEM CELLS

In this, cancer cells could utilize the existing stem cell regulatory pathways to promote their self-renewal. The ability to self-renew gives stem cells long lifespan relative to those of mature, differentiated cells. It has therefore been hypothesized that the limited lifespan of a mature cell makes it less likely to live long enough to undergo the multiple mutations necessary for tumor formation and metastasis.^[4,5]

HYPOTHESIS NUMBER 2: CANCER CELLS ARISE FROM PROGENITOR CELLS

The number of progenitor cells is more abundant in adult tissue than the stem cells and they have partial capacity for self-renewal. Their abundance relative to stem cells in adult tissue forms the basis of hypothesis suggesting progenitor cells as a source of CSC.^[4,6]

HYPOTHESIS NUMBER 3: CANCER CELLS ARISE FROM DIFFERENTIATED CELLS

Another school of researchers have suggested that cancer cells could arise from mature, differentiated cells that somehow dedifferentiate to become more stem cell like. In this scenario, the requisite oncogenic (cancer-causing) genetic mutations would need to drive the dedifferentiation process as well as the subsequent self-renewal of the proliferating cells. This model leaves open the possibility that a relatively large population of cells

in the tissue could have tumorigenic potential; a small subset of these would actually initiate the tumor. Specific mechanisms to select which cells would dedifferentiate have not been proposed. However, if a tissue contains a sufficient population of differentiated cells, the laws of probability indicate that a small portion of them could, in principle, undergo the sequence of events necessary for de-differentiation. Induction of Epithelial–Mesenchymal Transition (EMT) in differentiated human epithelial cells leads to the acquisition stem cell-like phenotype and formation of CSCs. The role of EMT in carcinomas including HNSCCs has now been well established^[4,7] [Figure 1].

There are currently two accepted models for cancer development as follows:^[8]

- The stochastic model suggests that every cancer cell is able to initiate new tumor growth equally
- The alternate hypothesis is that every tumor contains a rare population of cells termed CSCs or cancer-initiating cells [Figure 2].

STEM CELL NICHE

Physiological stem cells and CSCs depend on their immediate microenvironment or niche for their survival and function (Borovski *et al.*, 2011). The cellular and noncellular components of the niche provide signals that regulate proliferative and self-renewal signals, thereby helping CSCs to maintain their undifferentiated state (Kuhn and Tuan, 2010). Nonepithelial stromal cells, inflammatory cells and the vasculature have been proposed as key components of the niche that support and sustain CSCs (Fuchs *et al.*, 2004). This raised the hypothesis that one could suppress the survival of CSCs by disrupting the interactions with their supportive niche. Indeed, it has been recently suggested that the hematopoietic stem cell

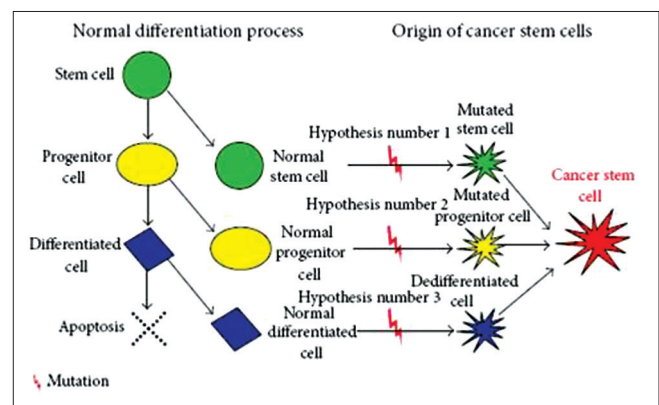


Figure 1: Hypothesis suggesting origin of cancer stem cells. (Source: Amit Shah *et al.* The Evolving Concepts of Cancer Stem Cells in Head and Neck Squamous Cell Carcinoma. Scientific World Journal 2014)

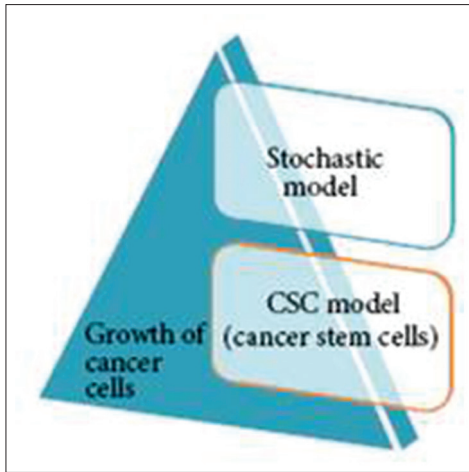


Figure 2: Models for cancer development. (Routray and N. Mohanty. Cancer Stem Cells Accountability in Progression of Head and Neck Squamous Cell Carcinoma: The Most Recent Trends!Molecular Biology 2014)

niche is a potential therapeutic target for metastatic bone tumors (Shiozawa *et al.*, 2011).

In HNSCCs, the majorities of the stem cells are localized close to blood vessels and depend on interactions with components of the niche for their survival (Krishnamurthy *et al.*, 2010). In addition to providing nutrients and oxygen to cells, endothelial cells secrete factors that promote the self-renewal and survival of head and neck CSCs [Figure 3].^[3]

METHODS OF IDENTIFICATION OF CANCER STEM CELLS

CSC expresses specific markers that vary considerably depending on tumor type or tissue of origin. There is no universal marker for CSCs.

The most commonly applied methods for the identification of CSCs are as follows.^[8]

1. Xenotransplantation assays, the gold-standard for identification of CSCs, are used to assess the tumorigenicity and self-renewing potential of the putative CSC population
2. CD44, the most well-recognized CSC marker, is a large cell surface glycoprotein that is involved in cell adhesion and migration
3. ALDH (an intracellular enzyme normally present in the liver) activity is known to enrich hematopoietic stem cells and recently has been revealed to enrich cells with increased stem-like properties in solid malignancies.

PANEL OF MARKERS FOR CSCS ARE AS FOLLOWS

The panel of markers for CSCs is shown in Table 1.^[8,9]

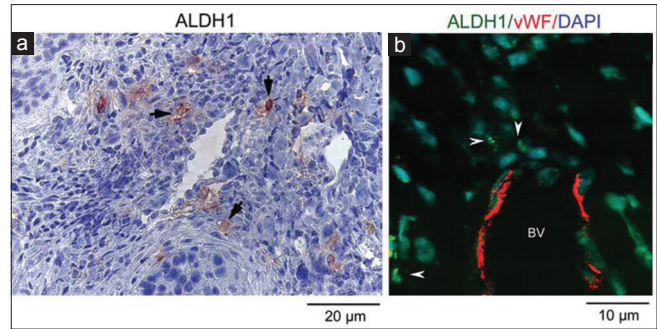


Figure 3: Photomicrographs of a primary head and neck squamous cell carcinoma showing aldehyde dehydrogenase + cells close to blood vessels. (a) Light photomicrograph after immunohistochemistry for aldehyde dehydrogenase 1 (red staining; black arrows). (b) Confocal microscopy after immunohistochemistry for aldehyde dehydrogenase 1 (green) to identify putative cancer stem cells (white arrowheads), von Willebrand Factor (red) to identify blood vessels and DAPI (blue) to identify the cell nuclei. (Source: S. Krishnamurthy and J.E. Nor. Head and Neck Cancer Stem Cells J Dent Res. 2012)

Table 1: Panel of markers for cancer stem cells

Tumor	CSC Marker	References
Breast	CD44+, CD24, ALDH+	Al Haji <i>et al.</i> 2003, Ginestier <i>et al.</i> 2007
Lukemia	CD34+, CD38-	Bonnet & Dick 1997
Head and Neck	CD44+	Prince <i>et al.</i> 2007
Lung	CD 133+	Eramo <i>et al.</i> 2008
Prostate	CD 133+, CD 44+, α 2 β 1	Collins <i>et al.</i> 2005

SIGNALING PATHWAYS INVOLVED IN MAINTAINING CANCER STEM CELLS IN HNSCC

Wnt/ β -Catenin

An important pathway involved in maintaining the stem cell niche and tumorigenesis is Wnt/ β -catenin signaling, normally involved in regulating pluripotency in embryonic and somatic stem cells and intimately involved in tissue homeostasis. There are three different pathways involved in Wnt signaling: the canonical Wnt/ β -catenin cascade, the noncanonical planar cell polarity pathway and the Wnt/Ca²⁺ pathway. The canonical pathway is the one implicated in tumorigenesis. Wnt receptor complexes are bound by Wnt ligand, leading to intrinsic kinase activity of the APC complex to inhibit β -catenin. Specifically, Wnt stabilizes β -catenin and enhances ABCB1/MDR-1 transcription, which is multidrug resistance genes expressed in stem cells. Furthermore, β -catenin accumulates and translocates to the nucleus to bind the N terminus of LEF/TCF transcription factors. Binding subsequently causes activation of *Myc*, *AXIN2* and *CYCLIND1* genes. Wnt, through the activation of β -catenin, also plays a role in metastasis and EMT. When this pathway becomes dysregulated, neoplastic proliferation occurs. Inhibiting these pathways is another potential target of therapeutic agents.^[10]

Epithelial–mesenchymal transition

EMT plays a significant role in normal development and wound healing by inducing cellular changes leading to breakdown of cell-to-cell interactions. Breaking cell communications is a critical step in transforming adherent, stationary epithelial cells into migratory cells. Based on its role in normal tissues, researchers have studied and proven how EMT transforms an epithelial cell into a cancer cell capable of migration, leading to increased metastasis and progression of solid tumors.

Brabletz *et al.* hypothesized that there were two types of CSCs: stationary CSCs (sCSCs) and migrating CSCs (mCSCs). sCSCs are embedded in the epithelia and are nonmobile, while mCSCs mediate tumor cell metastasis. They proposed that mCSCs were derived from sCSCs who underwent EMT. In other words, EMT was an essential component involved in promoting metastasis from the CSCs protected within the niche. In EMT, E-cadherin and β -catenin are downregulated while vimentin, fibronectin and N-cadherin are upregulated. Mani *et al.* demonstrated this upregulation in mammary epithelial and breast cancer cells.^[10]

Hypoxia-inducible factors

Hypoxic stress is one important factor in the niche. Numerous studies have demonstrated that tumor hypoxia is an independent factor leading to increased metastatic potential, malignancy and resistance to treatment. Hypoxia is thought to contribute to the malignant transformation of cells by increasing expression of drug-resistant genes, facilitating tumor invasion and metastasis, reducing expression of DNA repair genes and decreasing genomic stability. It was also shown that tumor hypoxia may regulate cell differentiation, supporting the theory that it may facilitate the maintenance of CSCs. The primary mediator of hypoxia-induced cell signaling is hypoxia-inducible factor-1 (HIF-1), which is often correlated with tumor development and decreased patient survival. HIF proteins play a critical role in angiogenesis, facilitating the growth of vasculature within tumors.^[10]

CANCER STEM CELLS RESPONSIBILITY IN PROGRESSION OF ORAL SQUAMOUS CELL CARCINOMA

Oral mucosa consists of a number of distinct layers and has self-renewing capacity. Mostly the basal layer cells are in the process of preparing for cell division, which later remain or move to a suprabasal position and become committed to terminal differentiation and stratification. The first hypothesis recommended that the CSCs in oral

squamous cell carcinoma (OSCC) could be from local basal layer-derive adult Stem Cell (SC) or progenitor, which accumulate additional genetic alterations with time, within the tumor. The other proposed mechanism suggested the origin of CSCs to be either putative nonepithelial stem cell sources in the oral mucosa (which include vessel wall-derived cells, blood-derived stem cells, muscle-derived stem cells and adipose-derived stem cell) or due to cell fusion between a hematopoietic stem cell and a mutated oral keratinocyte or can also originate through dedifferentiation of mature cells.^[5,11]

After understanding the possible role of CSCs in HNSCC, Oliveira *et al.* tried to find the possible influences of these CSCs in OSCC using CD44 and CD24. They suggested in their result that the absence of immunoexpression of these two investigated markers can be used in combination with other clinicopathologic information to improve the assessment of prognosis in OSCC.^[8,12]

Costea *et al.* in their hypothesis suggested a potential involvement of the stromal microenvironment in OSCC progression. As already known, the activated fibroblasts (myofibroblasts) inside the tumor stroma stimulate the transformed keratinocytes, thus influencing stem cell division patterns and with further genetic alterations of these keratinocytes leads to evolution of more invasive clones.^[8,13]

OSCC is found to rely on hypoxia cellular response system for tumor progression. Focal hypoxia which is found in OSCC may also be due to quantitative and qualitative alterations in tumor vasculature, leading to local reduction of oxygen availability.^[8,14]

CANCER STEM CELLS IN DISEASE PROGRESSION AND METASTASIS

Metastasis is a complex, multistep process that involves a specific sequence of events; namely, cancer cells must escape from the original tumor, migrate through the blood or lymph to a new site, adhere to the new site, move from the circulation into the local tissue, form micrometastases, develop a blood supply and grow to form macroscopic and clinically relevant metastases. It has been suggested that a small, and most likely specialized, subset of cancer cells drives the spread of disease to distant organs. Some researchers have proposed that these unique cells may be CSCs.^[4]

A simplified “unifying hypotheses” on origin and role of CSCs in carcinogenesis is explained schematically [Figure 4].

THERAPEUTIC IMPLICATIONS

Conventional therapies become less effective when tumors progress from an organ-confined disease into locally invasive and metastatic cancers. This is due to genetic abnormalities causing overexpression of oncogenic signaling pathways as well as downregulation of tumor suppressor gene products such as p53, PTEN or Rb in cancer cells. Furthermore, CSCs' quiescent state makes them resistant to standard chemotherapy. Chemotherapeutic agents normally act on rapidly dividing cells that are actively synthesizing DNA. In comparison to other cells within the tumor, because CSCs are not rapidly synthesizing DNA, they are relatively protected from the toxicity of chemotherapy, leading to resistance. CSCs are thought to be one of the main factors causing relapse and locally advanced and metastatic cancers. Therefore, based on the identification of the above cell surface and functional markers, researchers started developing targeted therapies to attack these cells normally resistant to conventional therapy.^[10]

Hypothetical model for the response of HNSCC to different therapeutic strategies has been proposed.

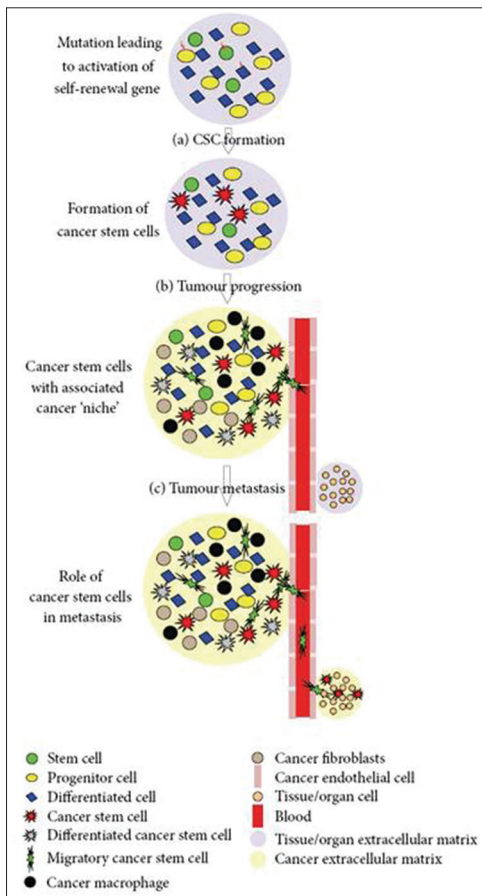


Figure 4: "Unifying Hypothesis" on origin of cancer stem cell, its role in tumor progression and metastasis. (Source: Amit Shah *et al.* The Evolving Concepts of Cancer Stem Cells in Head and Neck Squamous Cell Carcinoma. Scientific World Journal 2014)

HNSCC is represented as a complex tissue where the CSCs constitute a relatively small number of cells that are capable of undergoing self-renewal and differentiating into a complex and heterogeneous tumor. Conventional chemotherapeutic drugs are successful in debulking the tumor. However, it is proposed that slow-growing CSCs evade conventional therapies, and with the passage of time, these cells are activated and regenerate tumors locally or at distant sites. This might help explain the relatively high recurrence rates in patients with HNSCC. In contrast, targeting the CSCs either directly or *via* their niche could lead to a more definitive response, since the CSCs are the putative drivers of recurrence and metastatic spread. An emerging concept is the combined use of conventional chemotherapy and CSC targeted therapy^[3] [Figure 5].

CHALLENGES IN HEAD AND NECK CANCER STEM CELL RESEARCH

One of the biggest challenges in CSC research has been the development of methods for culture, expansion and

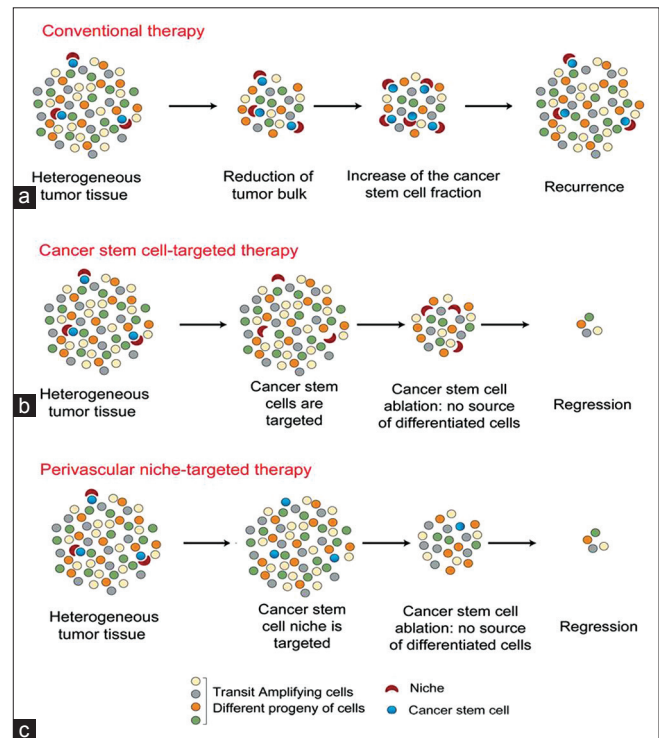


Figure 5: Possible implications of the cancer stem cell hypothesis for therapy. (a) Conventional chemotherapy targets primarily the highly proliferative cells that constitute the bulk of the tumor. With suitable microenvironments, the cancer stem cells proliferate and the tumor recurs. (b) Direct cancer stem cell targeting or (c) indirect cancer stem cell targeting via disruption of their perivascular niche can potentially eliminate cancer stem cells. Ablation of the stem cells may inhibit the regeneration of the tumor and ultimately result in tumor regression. (Source: S. Krishnamurthy and J.E. Nor. Head and Neck Cancer Stem Cells J Dent Res. 2012)

analyses of undifferentiated cancer cells *in vitro*. The method of enriching for CSCs by sphere generation under low attachment culture conditions has been proposed and used in various cancer models such as breast, neural and prostate (Dontu *et al.*, 2003; Pastrana *et al.*, 2011)^[15] and adapted for HNSCC (Krishnamurthy *et al.*, 2010).

Despite intense research in the area of CSC biology in recent years, the understanding of the impact of CSCs on the pathobiology of HNSCC is still not clear.^[3] One of the reasons for this is the need to perform studies with primary HNSCC specimens, which are difficult to obtain.

There is still controversy over the existence of CSCs in cell lines (Locke *et al.*, 2005; Harper *et al.*, 2007).^[16] In addition, the expansion of CSCs is frequently performed *in vivo*, which is time-consuming and expensive. However, existing *in vitro* methods offer limited capacity for expansion of CSCs in an undifferentiated state.^[3]

CONCLUSION

The discovery of CSCs has heralded an exciting era in our understanding of HNSCC with significant implications for diagnosis, prognosis, treatment and ultimately patient outcomes. Currently, there is no single biomarker to define the CSC population accurately for HNSCC. Indeed, it seems a set of markers will be required to more narrowly define this population to achieve the best chance of developing targeted identification and treatment.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Atena M, Reza AM, Mehran GA. Review on the biology of cancer stem cells. *Stem Cell Discov* 2014;4:83-9.
2. Mitra D, Malkoski SP, Wang XJ. Cancer stem cells in head and neck cancer. *Cancers (Basel)* 2011;3:415-27.
3. Krishnamurthy S, Nor JE. Head and neck cancer stem cells. *J Dent Res* 2012;91:334-40.
4. Shah A, Patel S, Pathak J, Swain N, Kumar S. The evolving concepts of cancer stem cells in head and neck squamous cell carcinoma. *ScientificWorldJournal* 2014;2014:842491.
5. Allan AL, Vantghem SA, Tuck AB, Chambers AF. Tumor dormancy and cancer stem cells: Implications for the biology and treatment of breast cancer metastasis. *Breast Dis* 2006;26:87-98.
6. Li F, Tiede B, Massagué J, Kang Y. Beyond tumorigenesis: Cancer stem cells in metastasis. *Cell Res* 2007;17:3-14.
7. Biddle A, Mackenzie IC. Cancer stem cells and EMT in carcinoma. *Cancer Metastasis Rev* 2012;31:285-93.
8. Routray S, Mohanty N. Cancer stem cells accountability in progression of head and neck squamous cell carcinoma: The most recent trends! *Mol Biol Int* 2014;2014:375325.
9. Tabarestani S, Ghafouri-Fard S. Cancer stem cells and response to therapy. *Asian Pac J Cancer Prev* 2012;13:5951-8.
10. Brianna N, Harris B, Sinha UK. Cancer stem review of the literature and the implications in head and neck cancer *J Cancer Res Cells Updates* 2013;2:186-93.
11. Zhou ZT, Jiang WW. Cancer stem cell model in oral squamous cell carcinoma. *Curr Stem Cell Res Ther* 2008;3:17-20.
12. Oliveira LR, Oliveira-Costa JP, Araujo IM, Soave DF, Zanetti JS, Soares FA, *et al.* Cancer stem cell immunophenotypes in oral squamous cell carcinoma. *J Oral Pathol Med* 2011;40:135-42.
13. Costea DE, Tsinkalovsky O, Vintermyr OK, Johannessen AC, Mackenzie IC. Cancer stem cells-new and potentially important targets for the therapy of oral squamous cell carcinoma. *Oral Dis* 2006;12:443-54.
14. dos Santos M, Mercante AM, Louro ID, Gonçalves AJ, de Carvalho MB, da Silva EH, *et al.* HIF1-alpha expression predicts survival of patients with squamous cell carcinoma of the oral cavity. *PLoS One* 2012;7:e45228.
15. Pastrana E, Silva-Vargas V, Doetsch F. Eyes wide open: A critical review of sphere-formation as an assay for stem cells. *Cell Stem Cell* 2011;8:486-98.
16. Harper LJ, Piper K, Common J, Fortune F, Mackenzie IC. Stem cell patterns in cell lines derived from head and neck squamous cell carcinoma. *J Oral Pathol Med* 2007;36:594-603.