

## Laryngeal cancer stem cells

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### Abstract

*Laryngeal squamous cell carcinoma (LSCC) is one of the most commonly diagnosed malignancies in the head and neck region with an increased incidence rate worldwide. Cancer stem cells (CSCs) are a group of cells with eternal life or infinite self-renewal ability, which have high migrating, infiltrative, and metastatic abilities. Though CSCs only account for a small proportion in tumors, the high resistance to traditional therapy exempts them from therapy killing and thus they can reconstruct tumors. Our current knowledge, about CSCs in the LSCC, largely depends on head and neck studies with a lack of systematic data about the evidences of CSCs in tumorigenesis of LSCC.*

*Certainly, the combination of therapies aimed at debulking the tumour (e.g. surgery, conventional chemotherapy, radiotherapy) together with targeted therapies aimed at the elimination of the CSCs might have a positive impact on the long-term outcome of patients with laryngeal cancer (LC) in the future and may cast a new light on the cancer treatment.*

**Keywords:** *Laryngeal Carcinoma; Cancer Stem Cells.*

### Introduction

Laryngeal squamous cell carcinoma (LSCC), originating from laryngeal epithelial tissue, is one of the most commonly diagnosed malignancies in the head and neck region with an increased incidence rate in middle-aged and elderly men, worldwide [1-3]. For early stage and localized LSCC, surgery, radiation, chemotherapy, and combination therapy are among the routine therapeutic techniques, however, radio-chemotherapy, the only therapeutic strategy for advanced and metastasized cases, has only limited effectiveness in treatment of late stage cancers. Despite considerable improvements in the laryngeal carcinoma treatment, which improved the quality of patients' life, the survival

rates remained unchanged during more than three decades [4]. Therefore, novel and further efforts are required for complete understanding of mechanisms underlying laryngeal carcinogenesis and for development of more accurate and effective diagnostic, prognostic and therapeutic applications against laryngeal cancer (LC) [2].

### Identification and characterization

Experimental evidence for the existence of CSCs was obtained in different tumor types (such as myeloblastic leukemia, breast cancer, brain, prostate, colon, lung). These studies consist in the dissociation of human tumors into individual cells, maintained in suspension and then separated according to markers present on their

surface, and then transplanted into NOD/SCID (nonobese diabetic/severe combined immunodeficient) mice. CSCs are identified based on their ability to replicate the tumorigenesis even when transplanted in low numbers in experimental models. For example, a cell of 105 cells of acute myeloid leukemia expresses the surface marker CD34+ and CD38+ and it is able to give rise to the histological heterogeneity of the disease into SCID animals [5-8].

Since the first descriptions of the CSCs, few article for laryngeal cancer, as well as few review for head and neck cancer, have been published. Given the lack of systematic data on CSCs in the LC, our current knowledge largely depends on data inferred from head and neck studies. The reported identification of a population of stem-like cells in head and neck cancer was in 2007 [9].

Isolation and identification of CSCs properties constitute a major experimental challenge. These properties include the efflux of vital dyes by multidrug transporters, enzymatic functions, the sphere-forming capacity in low attachment conditions, and the expression of cell-surface antigens. Two different approaches to identify CSCs within tumors have been proposed: the first one tracks specific surface markers that are selectively expressed on CSCs but not on the bulk of tumor cells; the second approach exploits some functional characteristics, such as a unique pattern of staining with certain dyes, to identify stem-like cells. In particular this latter method is used for the detection by dual-wavelength flow cytometry of the so-called side population (SP) on the basis of the ability of these cells to efflux the fluorescent dye Hoechst 33342 [10]. The SP cells seem to be an enriched source of stem cells [11], represent only a small fraction of the whole cell population and given their ability to efflux drugs, they represent the multi-drug resistant cell fraction within tumors [12, 13].

Currently, the most authoritative method for sorting CSCs is by first identifying the specific markers of CSCs. Cell separation has allowed us to identify and isolate specific CSCs in different types of human cancer, through specific combinations of cell surface markers and transplantation into immunodeficient mice. Stem cell populations with specific markers are the only cell populations that are able to give rise to tumours within *in vivo* mouse models that recapitulate their original phenotype [14]. Such markers would have important potential as predictors for local tumor control. CD133 and CD44 expression, and aldehyde dehydrogenase (ALDH) activity are the CSCs markers that have been identified in LSCC.

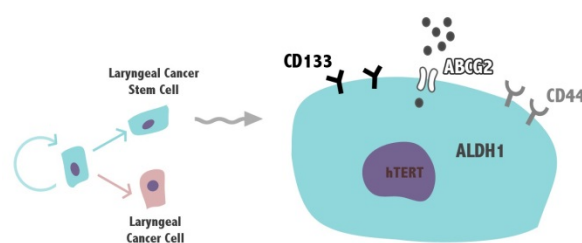
The study of CSCs in LC is still in the primary stage. Prince et al. have reported that CD44+ cancer cells are detected in the primary laryngeal carcinoma.

Though CD44+ cancer cells only account for less than 10%, they have very high tumor-formation ability *in vitro*. Some experimental results have indicated that CD133 is one of the markers for laryngeal carcinoma stem cells [9,15,16].

The role of the markers in LC progression is not clear yet and above all no markers have been defined yet as the most adequate for the characterization of the niches of tumor stem cells. Likely, several combinations of these markers would allow a more appropriate characterization of CSCs in LC. It should be also ascertained those most closely related to prognosis and to therapeutic treatment resistance. So, it could be possible to delineate a more correct stratification of patients at risk, speculating also to directly interfere with the activity of these molecules, as is the case in other malignancies, in order to establish more personalized therapeutic strategies.

Therefore, the study and identification of cell surface markers is central, to their ability to be used in the future to distinguish healthy tissue from diseased tissue. The molecular characterization of the purified stem cells, through the analysis of the protein genetic profile, will place the foundation for studies that could elucidate the molecular and cellular mechanisms that regulate self-renewal and differentiation, in homeostasis and in cancer. Although laryngeal CSCs research is only in the initial stage, there have been several investigations providing insights into the true identification and characterization of larynx CSCs (Fig. 1).

Luzar et al. provided the initial evidence for the existence of CSCs in LSCC through demonstration of increased human telomerase reverse transcriptase (hTERT) expression in LSCC specimens [17]. hTERT was demonstrated to induce stemness characteristics and promote metastasis and recurrence in distinct cancer types [18]. Therefore, detection of hTERT overexpression might be evaluated as the first clue for involvement of CSLCs in the process of laryngeal carcinogenesis.



**Figure 1**

Laryngeal CSCs are characterized by their stem cell-like properties including self-renewal and they carry distinct surface markers [1].

After identification of CD133, an apical plasma membrane protein with a molecular weight of 117 kDa, as a surface marker for isolation of stem cells from distinct tissues and tumors [19, 20], Zhou et al. analyzed the expression status of CD133 in Hep2 cells, a well characterized cell line used in LSCC research, and isolated CD133 positive cells to investigate their in vitro proliferation and differentiation ability [21]. CD133 positive cells constitute only a small population within the tumor. They have the potential to induce tumor formation in animal models even when injected as few as 100 cells [22,23]. CD133 enriched cell populations have been also demonstrated to have increased potential for self-renewal and multi-lineage differentiating ability in vivo [2]. Zhou et al. explored CD133 expression in Hep-2 cell population through immunocytochemistry and flow cytometry analysis, and found that less than <5% of cells in the Hep-2 cells expressed CD133. Moreover, immuno-magnetic separation of CD133 positive cells and their in vitro analysis demonstrated their capacity for self-renewal, increased proliferation, and multilineage differentiation [21]. This finding pointed CD133 as a promising marker for cancer stem cells in LSCC [1].

CSCs possess ability to reconstitute the cellular heterogeneity found in the original tumor. The plasticity potential of malignant cells has been also described in epithelial tumours as a mechanism that allows the epithelial cells to transdifferentiate into mesenchymal cells through the process EMT (epithelial-mesenchymal transition). CSCs in LSCC also have a plasticity potential to bidirectional switch between the EMT and epithelial phenotypes, through both EMT and the reverse process of MET (mesenchymal-epithelial transition) [24-26].

The analysis of the existing literature suggests a crosstalk between LSCC cells and other cells of the tumour microenvironment results in EMT. The invasive phenotype of cells that have undergone EMT allows them to penetrate the lymphatic and/or angiogenic vasculature.

## Discussion

In recent years, comprehensive treatment measures such as laryngeal surgery, radiotherapy, chemotherapy, and gene therapy have gained a higher 5-year survival rate for patients with LC, but 30% to 40% of them still died of tumor recurrence or metastasis [27]. Hence, there is an urgent need to explore the mechanism of origin, invasion, as well as metastasis of the in order to design new treatment methods.

During the past decade, a stem-cell-like subset of cancer cells has been identified in many malignancies. These cells, referred to as cancer stem cells (CSCs), are of particular interest because they are believed to be the clonogenic core of the tumor and therefore represent the

cell population that drives growth and progression [28, 29].

Although there have been important improvements recently on cancer diagnosis and therapy, LSCC still remains to be one of the leading causes of cancer deaths among men. For especially late stage LSCC cases, treatment strategies commonly fail to give positive clinical outcome, which necessitates understanding the underlying mechanisms of LSCC carcinogenesis and developing novel therapy tools based on the enlightened pathogenic processes [3].

Although CD133 is proposed as an effective surface molecule for isolation of laryngeal CSLCs, additional biomarkers are needed for true identification and characterization of CSLCs, which will provide the opportunity to determine appropriate gene and miRNA expression signatures associated with these cells through utilization of oligonucleotide microarray technologies, qRT-PCR, and similar methods. Analysis of specific signaling pathways involved in acquisition and maintenance of CSLC properties implicated in pathologic processes will let the discovery of new drugs for treating LSCC and development of novel therapeutic strategies with the help of further studies needed in this field [1]. The elimination or inhibition of these CSCs can be considered a new conceptual framework for cancer treatment [3].

## Conclusions

Laryngeal cancer stem cells appear to play a major role in tumour recurrence and metastatic spread, common causes of the high morbidity and death.

Certainly, the combination of therapies aimed at debulking the tumour (e.g. surgery, conventional chemotherapy, radiotherapy) together with targeted therapies aimed at the elimination of the CSCs and EMT might have a positive impact on the long-term outcome of patients with laryngeal cancer (LC) in the future and may cast a new light on the cancer treatment.

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