

Targeting head and neck tumoral stem cells: From biological aspects to therapeutic perspectives

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Abstract

Head and neck squamous cell cancer (HNSCC) is the sixth most common cancer in the world. Effective therapeutic modalities such as surgery, radiation, chemotherapy and combinations of each are used in the management of the disease. In most cases, treatment fails to obtain total cancer cure. In recent years, it appears that one of the key determinants of treatment failure may be the presence of cancer stem cells (CSCs) that escape currently available therapies. CSCs form a small portion of the total tumor burden but may play a disproportionately important role in determining outcomes. CSCs have stem features such as self-renewal, high migration capacity, drug resistance, high proliferation abilities. A large body of evidence points to the fact that CSCs are particularly resistant to radiotherapy and chemotherapy. In HNSCC, CSCs have been increasingly shown to have an integral role in tumor initiation, disease progression, metastasis and treatment resistance. In the light of such observations, the present review summarizes biological characteristics of CSCs in HNSCC, outlines targeted strategies for the successful eradication of CSCs in HNSCC including targeting the self-renewal controlling pathways, blocking epithelial mesenchymal transition, niche targeting, immunotherapy approaches and highlights the need to better understand CSCs biology for new treatments modalities.

Key words: Biology; Head and neck neoplasms; Oral cancer; Neoplastic stem cells; Molecular targeted therapy; Radiation therapy; Chemotherapy

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Core tip: The cancer stem cells (CSCs) theory offers an insight into why currently available therapies for head and neck cancer fail so often. Eradication of cancers may

require the targeting and elimination of CSCs, especially for head and neck squamous cell cancer (HNSCC). This represents a challenge because many pathways, such as those involved in self-renewal, are shared by CSCs and their normal counterparts and might lead to major toxicities. Developing radio sensitizing strategies is investigated and appears to eliminate CSCs. Overcoming chemo resistance, radio resistance and immune evasion mechanisms of CSCs remains a cornerstone of novel adjuvant therapies specifically targeting CSCs in HNSCC.

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INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) remains a major health problem throughout the world, with an estimated 500000 new cases diagnosed yearly^[1]. HNSCC refers to a group of cancers that originate in the epithelium of the oral cavity, pharynx and larynx. Currently, therapeutic strategies for HNSCC include surgery, radiotherapy, chemotherapy, concurrent chemoradiation and monoclonal antibodies. Despite progress in the field of oncology, the overall 5-year survival rate of HNSCC is below 50%, unchanged in the last 30 years^[2]. Local recurrence affects about 60% of patients and metastases develop in 20% of cases. Locoregional failure is linked to unfavorable outcome^[3,4]. A new, more strategic approach is needed for the treatment of recurrent head and neck squamous cell carcinoma, as most cases cannot be cured with current therapeutic modalities. The presence of a peculiar subpopulation of cells has been identified in several tumors, including HNSCC: This small population of cancer cells possesses the capability to self-renewal, is highly tumorigenic, and behaves as tumor progenitor cells. Such characteristics are consistent with the features of cancer stem cells (CSCs)^[5,6]. The role of these cells in HNSCC progression and metastasis is a significant point to be further emphasized on for eliminating the disease. Indeed, in addition to their ability for self-renewal, differentiation, and regeneration, CSCs possess significant resistance to radiochemotherapy^[7,8]. Furthermore, by being able to do epithelial mesenchymal transition (EMT), which is a key step in embryogenesis, CSCs might facilitate the metastatic characteristics of tumors^[9-11]. Therefore, targeted elimination of these CSCs could define new therapeutic strategies for head and neck cancer treatment. If the most common method for identifying CSCs relies on the expression of specific cell surface antigens that enrich for cells with CSC properties, their detection within the total tumor

bulk remains a challenge. Indeed, the development of new CSC targeting therapeutic strategies is currently obstructed by the lack of trustworthy markers for the identification of CSCs^[12-14]. Besides, molecular mechanisms at the basis of CSCs origin are yet not fully understood. Nonetheless, targeting self-renewal pathways in CSCs, such as the Wnt, Notch, and Hedgehog pathways, or specific CSC markers, such as CD133, CXCR1, and CD44 may offer therapeutic benefits to head and neck cancer therapy^[13]. In addition to CSC biomarkers, micro environmental factors, such as niche-specific properties constitute obvious potential targets in order to eradicate high-risk HNSCC cells; to abolish the crosstalk between endothelial cells and CSCs in a targeted manner might be relevant for the treatment of head and neck cancer patients^[15]. This review discusses the properties of head and neck tumoral stem cells, outlines initial targeted therapeutic strategies against them, and presents challenges for the future (Figure 1).

CSCS IN HSNCC: IDENTIFICATION, CHARACTERIZATION AND PROPERTIES

Role of stem cell molecular markers

HNSCC are solid tumors with heterogeneous content. Indeed, into the tumor, not all cells possess the capacity for self-renewal and unlimited growth. In tumor architecture, it is widely agreed that CSCs are held accountable for tumor growth whereas differentiated cells usually contribute to the tumor bulk^[5]. CSC populations are defined by four key features: Only a small portion of intratumoral cancer cells can form a new tumor in an *in vivo* xenograft assay, particular cell surface markers allow to identify CSC populations from non-CSC populations, the ability to generate endless copies of themselves through self-renewal, and the potential to give rise to differentiated non-stem cell cancer progeny^[16]. As all chemotherapy regimens often damage normal, rapidly dividing cells, CSC-like populations, with low turnover and infrequent cell cycling, may escape treatment^[17]. Thus, there is an urgent need for early detection of CSCs in the tumor cell population. Identification of CSCs based on increased expression of certain markers in cancerous tissue is the basis of the target therapy which is described later in this review. It is more clear that the development of novel therapeutic strategies will come about through identification of HNSCC CSC populations that regulate tumor growth, metastasis, and treatment resistance. Thanks to the development of immunofluorescence tools, it is possible to more easily isolate CSCs using their surface proteins. The main molecular markers implicated in HNSCC CSC detection are summarized in Table 1.

Role of the CD44 marker

One of the first studies of CSCs in HNSCC using an immunodeficient mouse as model demonstrated that a minor population of CD44+ cancer cells, which account

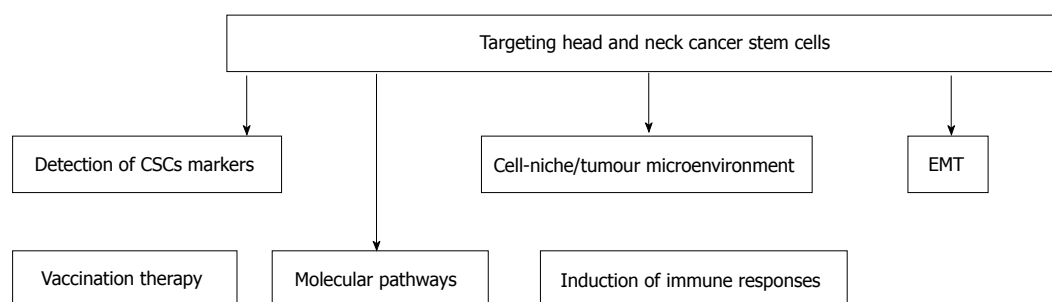


Figure 1 Therapeutic perspectives through a multistrategic approach. CSCs: Cancer stem cells; EMT: Epithelial mesenchymal transition.

Table 1 Main molecular markers implicated in head and neck squamous cell cancer cancer stem cell detection

Ref.	Stem cell marker	Cancer cell lines studied
Prince <i>et al</i> ^[5]	CD44, BMI 1	HNSCC generated in immunodeficient mouse model
Wei <i>et al</i> ^[25]	CD133	HNSCC cell lines (hep-2)
Chen <i>et al</i> ^[21]	ALDH 1	Immunodeficient mouse model
Krishnamurthy <i>et al</i> ^[15]	ALDH	Head and neck squamous cell carcinoma

HNSCC: Head and neck squamous cell cancer; ALDH: Aldehyde dehydrogenase.

for less than 10% of the cells in a HNSCC primary tumor could give rise to new tumors *in vivo* and displayed the ability of self-renewal and differentiation. The CD44 protein is a cell surface glycoprotein that is responsible for cell adhesion, migration and homing. It is a receptor for hyaluronic acid and can also interact with other ligands such as collagen species and matrix metalloproteases^[5]. Takahashi *et al*^[18] demonstrated that cell-cell dissociation and actin remodeling in tumor necrosis factor-induced EMT were mediated by specific interaction between CD44 and hyaluronan; another result was an enhanced motility. CD44+CD24-CSCs play a critical role in tumor progression and metastasis^[19]. Some of HNSCC with CD44s (standard form) and CD44 v6 (alternative splice variant) expressions are associated with a poorer disease-free survival, in laryngeal cancers particularly^[20]. Also, high levels of nuclear BMI-1 were found in CD44+CD24- cells of the tumor population. BMI-1 is a stem cell-related gene involved in the mechanisms of carcinogenesis in head and neck cancers^[5]. By simultaneous evaluating both CD44 and BMI-1, it could lead to precise characterization of the CSC population within the tumor cellular architecture.

Aldehyde dehydrogenase activity

Aldehyde dehydrogenase (ALDH) has also been considered to be a marker for identifying HNSCC CSCs. The ALDH family, of which ALDH1 is a member, is a family of cytosolic isoenzymes, which are highly expressed in many stem and progenitor cells. These enzymes are responsible for oxidizing intracellular aldehydes and contribute to the oxidation of retinol to retinoic acid, in stem cell differentiation notably; moreover, ALDH1 is involved in the resistance of progenitor

cells to chemotherapeutic agents. Many studies have proved the role of ALDH1+ cells in tumorigenesis, metastasis and chemo resistance in HNSCC. For instance, Chen *et al*^[21] showed that ALDH1+ CD44+ cells resist radiotherapy and maintain CSC-like properties in HNSCC cells which allow them to promote tumor propagation^[22]. Recently, Krishnamurthy *et al*^[15] found that the combined use of ALDH1 and CD44 is more relevant for identifying CSC-like populations as it is more selective than any other marker used alone. It is clear that only one marker is not sufficient to identify a pure CSC population in HSNCC. The best chance of developing targeted identification and treatment goes through a panel of markers with a more narrowly definition of CSCs.

Other markers and role of side population cells

Several studies evidenced the abilities of CD133+ stem-like cells: They possess higher clonogenicity, higher tumorigenic potential and are more invasive, in comparison with CD133- cells. CD133+ cells play a crucial role in the resistance to standard chemotherapy with paclitaxel^[23]. CD133 antigen also known as prominin-1 is a glycoprotein that is encoded by the *PROM1* gene. It is a member of pentaspan transmembrane glycoproteins (5-transmembrane, 5-TM), which specifically localize to cellular protrusions. If it was initially considered as a marker for hematopoietic stem cells^[24], it has been then identified as a CSC marker in several cancers and particularly in the laryngeal cancer, using the Hep-2 cell line. Indeed, in an *in vivo* study, CD133+ cells sorted from the Hep-2 cell line had higher tumorigenic potential than CD133- cells^[25]. Higher CD133 levels are found in CD44+ cancer stem-like cells in comparison with CD44- cells in HNSCC, which support the putative

Table 2 Main determinants of cancer stem cell radioresistance

Molecular determinants of radioresistance	Mechanism
Intrinsic determinants	Enhanced DNA repair capability Protection from oxidative DNA damage Activation of the cell survival pathways (PI3K/Akt, WNT/ β -catenin, notch)
Extrinsic determinants	Expression of drug efflux pumps Hypoxic environment

role of CD133+ as a CSC marker. Using CD133 might serve to identify head and neck cancer patients that are resistant to conventional chemotherapy^[26]. Furthermore, side population cells have shown to express stem cell properties when isolated from cancer samples. Their identification does not rely on the relative binding of antibodies but is based on their ability to efflux a fluorescent dye that binds to DNA^[27,28]. Side population cells are more tumorigenic, chemoresistant and have displayed self-renewal *in vivo*. Besides, side population cells show a more aggressive schema of tumour growth (*in vitro*)^[29]. New strategies to target these cells need to be designed. Above all, further research on the exact role of side population cells and their implication in tumourigenesis is required as the exact mechanisms are not yet fully understood.

MOLECULAR STRATEGIES TO TARGET CSCS IN HNSCC

CSCs and therapeutic resistance

CSCs have important implications regarding cancer treatment and may lead to new perspectives on therapeutic strategies with a rethink of actual treatment paradigm. Indeed, indiscriminate cytoreduction is the aim of current chemotherapy and radiation treatment for HNSCC whereas the CSC hypothesis suggests that the elimination of CSCs is the only way to treat cancer effectively. Thus, significant reductions in the tumor volume are not enough to prevent tumor recurrence in HNSCC. Moreover, evidence suggests that CSCs have inherent drug and radiation resistance, rendering most conventional therapies ineffective. Radio resistance of CSCs has been attributed to their self-renewal capacity, DNA repair capacity, free-radical scavenging, upregulation of cell cycle control mechanisms and specific interactions with the stromal microenvironment. Chemotherapy resistance is frequently related to accelerated drug transport and to drug metabolism^[30,31]. Bmi-1 and CD44 knockdowns have led to an improvement of CSCs chemosensitivity in HNSCC. In particular, knockdown of CD44 increased the sensitivity of HNSCC cells to cisplatin, underlying the crucial of CSCs in the response to chemotherapy^[32]. Concerning Bmi-1, a stem-cell-related gene, which participates in the self-renewal of hematopoietic and neuronal stem cells, and has been implicated in the tumorigenesis of various malignancies the experiment showed that that knockdown

of Bmi-1 increased the effectiveness of radiotherapy and resulted in inhibition of tumor growth in nude mice transplanted with ALDH1+ CSCs^[33]. Moreover, Chen *et al*^[32] focused on the Snail superfamily of zinc-finger transcription factors, implicated in the regulation of EMT during embryonic development. The importance of SNAIL1 in the growth of cancer cells and their metastatic potential has been shown in various malignancies^[34]. Chen *et al*^[32] found that the endogenous co-expression of ALDH1+ and Snail resulted in decreased ALDH1 expression, inhibition of CSC-like properties, and decreased tumorigenesis in ALDH1+ CD44+ cells. By regulating the EMT, Snail is a key factor in maintaining CSC properties, and could be used as a therapeutic measure for the treatment of HNSCC. Besides, Snail small interfering RNA could reduce resistance to chemo radiotherapy in ALDH1+ cells^[32]. Ultimately, the expression of drug efflux pumps by CSCs, another mechanism of chemo resistance remains to be explored in HNSCC. A better understanding of resistance mechanisms in HNSCC CSCs will require future studies and constitutes a prerequisite for improving therapy and possibly preventing tumor spread or recurrence. The main determinants of CSC radioresistance are summarized in Table 2.

Targeting stem cell niches

Beyond intrinsic factors, the unique CSC microenvironment could play a crucial role in the radio resistance of CSCs. Indeed, it has been showed that stromal environment and CSC niche play a vital role in the behavior of cancer cells. As the vast majority of the stem cells are found within a 100 μ m-radius of a blood vessel in HNSCC, the existence of a perivascular niche was suggested. Using the SCID mouse model of human tumor angiogenesis, it was observed that specific ablation of tumor-associated endothelial cells with an inducible Caspase-9 result in the decrease of the fraction of head and neck CSCs^[15]. Thus, targeting the stem cell niche directly can weaken the source of nutrition and change the essential signals needed by CSCs to proliferate. Therapeutic strategies as suggested by Tang *et al*^[35] included targeting candidate CSCs and their microenvironment 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 towards target therapy in HNSCC. Similarly,

Krishnamurthy and al showed that targeting CSCs either directly or *via* their niche could lead to a more durable response in HNSCC, hence the emergence of a new concept using both conventional chemotherapy and CSC-targeted therapy^[36]. The niche provides the soil for CSC self-renewal and maintenance, stimulating essential signaling pathways in CSCs and leading to secretion of factors that promote angiogenesis and long-term growth of CSCs. Hence, the role of targeting "vascular niche" in treatment of HNSCC cannot be neglected. The use of anti-angiogenic agents, such as bevacizumab, could be a therapeutic strategy in HNSCC; if it mediates CSC depletion in gliomas it could prove useful in reducing the proportion of HNSCC CSCs. Exploiting the functional interdependence of CSCs and vascular endothelial cannot be neglected in order to reduce the rate of HNSCC recurrence and metastasis^[37-46].

EMT and molecular pathways

EMT is the process that allows a polarized epithelial cell to assume a mesenchymal cell phenotype, which is characterized by enhanced motility and invasiveness. The crosstalk between HNSCC cells and other cells of the tumor microenvironment could lead to EMT, which enhances the motility of carcinoma cells and endows them with stem cell properties. The invasive phenotype of cells that have undergone EMT allows them to penetrate the lymphatic and/or angiogenic vasculature. Blocking the crosstalk between tumor and stromal cells, and thus inhibiting EMT might be a therapeutic strategy in HNSCC. The activation of the EMT program has been shown in HNSCC populations thanks to microarray analysis; moreover, in these cells, the molecular characterization of gene expression also allowed to show the activation of Wnt/beta-catenin signaling pathway, usually involved in the maintenance of pluripotency, differentiation and proliferation. Inhibitors of this pathway are in clinical trials in several cancers^[47-49]. Numerous molecules targeting the Wnt pathway are either in the discovery stage or early phase 1 trials directed variously against Wnt/Receptor interactions and cytosolic and nuclear signaling^[50,51]. Furthermore, others implicated molecular pathways are still under investigation in HNSCC, including the promising JAK/STAT pathway. In HNSCC-CD44+ALDH1+ transplanted immunodeficient mice, an inhibitor of STAT3 combined with radiotherapy significantly suppressed tumorigenesis and improved the survival rate^[52]. Other drugs have been formulated to target other pathways in CSC formation such as Notch or Hedgehog but the ability of these drugs to selectively target CSCs while preserving normal stem cells remains a challenge. In nasopharyngeal carcinomas, targeting beta-catenin signaling pathway through E-cadherin repressor ZEB2 by using miR200a, allowed to induce stem-like traits, including CD133+ side population, sphere formation capacity, increased Oct4 and ALDH expression in tumor spheres, and tumorigenicity *in vivo*^[53]. TrkB, a 145-KDa receptor tyrosine kinase is supposed to be both invol-

ved in EMT and invasion process of cancer cells in HNSCC. Studies showed that downregulation of TrkB, suppressed tumor growth^[54]. Ultimately, recent studies have reported the role of hypoxia or overexpression of HIF-1 α in the induction of EMT and metastasis in head and neck cancer cells. HIF-1- α regulates the expression of Twist by binding to the hypoxia-response element. Co-expression of HIF-1- α , Twist in human head and neck tumors correlates with metastasis and poor prognosis^[55]. It is undeniable that EMT is a central process in the acquisition of stem-like properties and ultimately contributes to local invasion and metastatic spread frequently observed in patients with head and neck cancer.

IMMUNOTHERAPEUTIC APPROACHES TARGETING HEAD AND NECK TUMORAL STEM CELLS

CSC-induced immune responses

Beyond chemo resistance and radio resistance, emerging CSC targeted therapies in HNSCC have to overcome another major hindrance: Immune-escape-mechanisms of CSC. Indeed, current immunotherapy is mainly based on antigens presented to effector T cells by dendritic cells. Or, generally, these antigens are selected and derived from bulk tumor cells; they are not derived of CSCs that may not express immunogenic differentiation antigens^[38]. CSCs also may be defective in antigen presentation due to the downregulation of human leukocyte antigen (HLA) surface expression^[39]. Therefore, in a heterogeneous tumor entity, CSCs may lead to a treatment failure and disease progression, escaping from the attack of current immunotherapy. Concerning HNSCC, a better knowledge of the crosstalk between CSCs and the immune system is crucial in order to develop specific targeted therapies, the immunogenicity of HNSCC-CSCs having been observed recently. Recently, a CD8 defined T-cell epitope of ALDH1 was identified as a potential target^[22]. Among reported CSCs markers, ALDH1 is the most specific CSC marker used to identify highly tumorigenic cells present in HNSCC^[21]. ALDH1 has been recognized as an antigen-source eliciting a humoral immune response in HNSCC. *Visus et al*^[22] showed that ALDH1A1 peptide was an HLA-A2-restricted, naturally presented, CD8+ T cell-defined tumor-antigen. ALDH1 peptide-specific CD8+ T cells could only recognize HLA-A2+ HNSCC cell lines overexpressing ALDH1 but not a human fibroblast cell line. Moreover, the data presented by Liao *et al*^[40] have shown that the host immune system is able to recognize and distinguish CSCs with ALDH1 phenotype from non-CSC cells. In addition to ALDH1, other cancer antigens were found to be preferentially expressed in CSCs: Cyclin A1 was reported in leukemic stem cells of acute myeloid leukemia whereas DNAJB8 was identified as novel cancer antigen in renal CSCs^[56,57]. This specific expression of cancer antigens may enable us to target

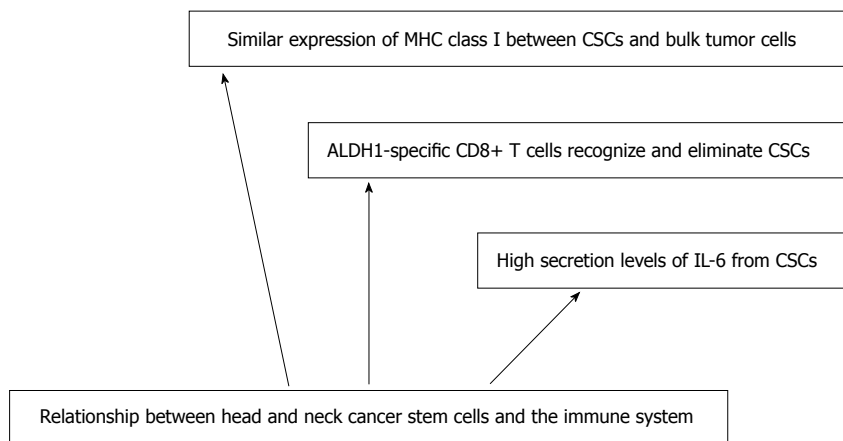


Figure 2 Immunotherapeutic approaches. CSCs: Cancer stem cells; ALDH: Aldehyde dehydrogenase.

CSCs specifically. Moreover, development of ALDH1A1 peptide-based vaccines for therapy represents a novel area for future research in HNSCC.

ALDH1A1: A potential target for vaccination therapy

Another attractive approach to target CSCs is to develop antitumor T-cell vaccines. Studies on vaccination against antigen ALDH1A1+ of CSCs have been performed and have achieved significant progress. Visus *et al*^[41] have demonstrated the ability *in vivo* of generated ALDH1A1-specific cytotoxic T lymphocytes to eliminate ALDH (bright) cells present in HLA-A2+ HNSCC carcinoma cell lines. They also found antitumor activity by adoptive immunotherapy with ALDH1A1-specific cytotoxic T lymphocytes *in vivo*. The elimination of ALDH (bright) cells thanks to ALDH1A1-specific CD8+ T cells could inhibit tumor growth and metastases^[41]. Ning *et al*^[42] investigated immunogenicity induced by murine ALDH (high) CSC used as a source of antigen to prime derived-cells as a vaccine for malignant squamous cell carcinoma in immunocompetent mice used as hosts. High immunogenicity was found among ALDH (high) CSCs with a most effective role as an antigen source in comparison with unselected tumor cells. A high level of IgG produced by splenocytes subjected to CSC-tumor-lysate-pulsed derived-cells and the binding of the antibody from CSC-vaccinated murine hosts to CSCs which resulted in the CSCs lysis *via* complement-dependent cytotoxicity have been observed. Studies showed that cytotoxic T lymphocytes generated from peripheral blood mononuclear cells or splenocytes harvested from CSC-vaccinated hosts had the ability to kill CSCs *in vitro*^[42]. Consistent with the findings of Ning group, Duarte *et al*^[43] first demonstrated an ALDH (high) CSC-based vaccine could drastically reduce both tumor volume and occurrence in a rat colon carcinoma syngeneic model: 50% of the CSC-based vaccinated animals became resistant to tumor development and a 99.5% reduction in tumor volume compared to the control group occurred. Beyond the fact that these studies provide a greater view of the immune biology of

CSCs, vaccination with CSCs has proved to be effective in killing head and neck CSCs specifically, reducing tumor volume and preventing tumor recurrence.

Immune suppressive role of CSCs

Immunotherapeutic approaches for HNSCC are complicated due to the deep immune suppression induced by this disease. Mechanisms such as increased apoptosis of tumor-specific CD8+ T-cells and increased tumor-infiltrating T regulatory cells in peripheral blood and at the tumor site have been demonstrated^[58]. Krishnamurthy *et al*^[15] showed that the location of CSCs was in close proximity to blood vessels. Clinically, patients with recurrent HNSCC showed an increased concentration of IL-6 in serum in comparison with patients with primary HNSCC^[44]. Elevated IL-6 levels could independently predict tumor recurrence, poor survival, and tumor metastasis^[45]. Yu *et al*^[44] demonstrated that secretion levels of IL-6 from CSCs were crucial to maintain the self-renewal and tumorigenic properties of CSCs in HNSCC. On the one hand, CSCs can be recognized and inhibited in their outgrowth by the immune system and on the other hand, CSCs can promote tumor progression either by immunoediting for CSCs that are more suitable to survive in an immunocompetent host or by establishing conditions that facilitate tumor outgrowth within the tumor immune-microenvironment. Tumor associated macrophages may play a critical role in tumor progression by interacting with the tumor microenvironment and tregs are thought to promote tumor progression^[59]. In a study concerning primary human gliomas, the distribution of TAM at the invasive tumor front was correlated with the presence of CD133+ glioma CSCs. Tumor associated macrophages could significantly enhance the invasive capability of glioma stem cells through paracrine production of TGF-B1^[60]. The role of tumor associated macrophages in the regulation of CSCs drug resistance has been identified by Jinsuhi *et al*^[61] They found a large amount of tumor associated macrophages in CD44+ ALDH+ colon tumor and CD133+ ALDH+ lung cancer cells: Those

macrophages allow activating Sonic Hedgehog pathways in CSCs in cooperation with IL-6. Targeting tumor associated macrophages by inhibiting either the myeloid cell receptors colony-stimulating factor-1 receptor or chemokine receptor improves chemotherapeutic efficacy, inhibits metastasis and increases antitumor T cell responses in pancreatic ductal adenocarcinoma^[62]. All these findings validate the interplay between CSCs and the tumor immune microenvironment. Therefore, specific targeting of head and neck tumoral stem cells by immunotherapeutic approaches may lead to more efficacious and lasting therapeutic results in the future. Nonetheless, it seems necessary to address several points before immunotherapeutic approaches targeting CSCs can be brought into clinical trials. These include the effective isolation of CSCs from bulk tumor mass to measure potential immunotherapeutic effects on CSC, to determine the antigen-profile presented on CSCs specifically to identify specific CSC targets as well as the induction and enhancement of antigen processing and presentation of CSC epitopes. A lot of work remains to be done to get a better understanding of the immune suppressive role of CSCs in HNSCC. The various immunotherapeutic approaches are displayed in Figure 2.

CONCLUSION

The CSC theory provides new opening for the treatment of HNSCC. This theory also helps to explain why currently available therapies for head and neck cancer so often fail. Eradication of cancers may require the targeting and elimination of CSCs, especially for HNSCC and thus, there is an urgent need to alter the current paradigm in drug development. Efforts are still advocated to determine specific markers and methods to specifically target these cells, towards a more specific tumor treatment. To date, no antibody selectively targeting CSC has been described in HNSCC yet, but candidates are under investigation. For instance, CD44v6 antibodies either radiolabeled or coupled with a cytotoxic drug entered phase I clinical testing in patients with HNSCC. In a phase I dose escalation study, the treatment with a radiolabeled antibody showed promising anti-tumor effects^[63]. Clearly, huge variety of approaches to eradicate CSCs is being explored, and particularly *in vitro* assays; there still remains the issue of how to avoid unwanted toxicity *in vivo*. Developing radio sensitizing strategies is also being investigated and appears to eliminate CSCs. Overcoming chemo resistance, radio resistance and immune evasion mechanisms of CSCs remains a cornerstone of novel adjuvant therapies specifically targeting CSCs in HNSCC. Bertrand *et al.*^[64] demonstrated that the combination of UCN-01 (a checkpoint kinase inhibitor) and ATRA (all-trans retinoic acid) with irradiation decreased the survival fraction of CSCs and could be used as a powerful radio sensitizing strategy in HNSCC. Furthermore, advances in nanotechnology could allow a better understanding of the regulatory mechanisms that govern CSC biology *in*

vivo.

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