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Chapter 4

The Role of Cancer Stem Cells in Head and Neck Squamous Cell Carcinoma and Its Clinical Implications

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Additional information is available at the end of the chapter

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Abstract

Worldwide, more than 550, 000 new cases of head and neck squamous cell carcinoma (HNSCC) are estimated to occur annually, making it the sixth most common human malignancy. Since their discovery in 2007, cancer stem cells (CSCs) in HNSCC have garnered increased interest secondary to their properties of tumorigenicity, differenti- ation, proliferation, and self-renewal. CSCs are intrinsically more resistant to tradi- tional treatments such as radiation and chemotherapy, contributing to potential metastasis and recurrence of HNSCC. This chapter focuses first on normal head and neck stem cells, providing background for the discussion of a number of topics pertaining to the study of HNSCC CSCs including molecular biomarkers and clinical implications. Continued research to elucidate the properties of CSCs will undoubt- edly expand our knowledge surrounding the pathogenesis, metastasis, and relapse of HNSCC. Ultimately, a better understanding of CSC biomarkers, signaling pathways, and mechanisms of resistance will improve therapies and patient outcomes through targeted interventions.

Keywords: Head and neck squamous cell carcinoma, Stem cells, Cancer stem cells, Chemoresistance, Metastasis

1. Introduction

In the United States, over 53, 000 new cases of head and neck squamous cell carcinoma (HNSCC) are estimated to occur each year, with roughly 11, 000 deaths annually [1]. Across the globe, HNSCC has an annual incidence of over 550, 000 cases, making it the sixth most common cancer worldwide. HNSCC accounts for 90% of the malignancies in the head and neck region, affecting the nasal vestibule, nasal cavity, paranasal sinuses, nasopharynx, lips, oral cavity, oropharynx, pharynx, hypopharynx, and larynx. The foundation of treatment for head and neck cancer has been surgery and radiation therapy, while chemotherapy may also

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be employed as an adjunctive treatment. Advancements in surgical technique, radiation strategies and technologies, as well as chemotherapeutic drugs have led to improvements in patient's overall quality of life. However, the prognosis for HNSCC has improved only marginally over the past thirty years, with the five-year survival rate remaining at 50% [2-4]. Even after standard therapy, patients with HNSCC exhibit relatively high rates of local recurrence, regional cervical lymph node recurrence, and to a lesser degree distant metastasis, all of which contribute to significant morbidity and mortality [5]. Often, these recurrences and metastases are more resistant to traditional treatment modalities such as chemoradiation. Unfortunately, primary site recurrence occurs in 10-30% of all patients [6].

Traditional approaches to understanding and treating HNSCC are based on the stochastic model of cancer, where all tumor cells are identical (Figure 1). More recently, the cancer stem cell (CSC) hypothesis has gained increasing traction in explaining tumorigenesis [7]. This theory proposes that cancer maintains a hierarchical order of cells, with only a small subpopulation of CSCs capable of tumor initiation, propagation, and regeneration [8]. Conventional therapies that target rapidly cycling cells are less effective in killing CSCs. CSCs also display increased intrinsic resistance to chemotherapy and radiation therapy. As a result, CSCs are likely to contribute to cancer relapse.

This review aims to provide a succinct yet thorough overview of our current basis for the CSC hypothesis as it pertains to HNSCC. We will start with a brief discussion of the normal epithelium of the head and neck region as well as our current understanding of normal endogenous stem cells of the head and neck region. Evidence for the CSC hypothesis of

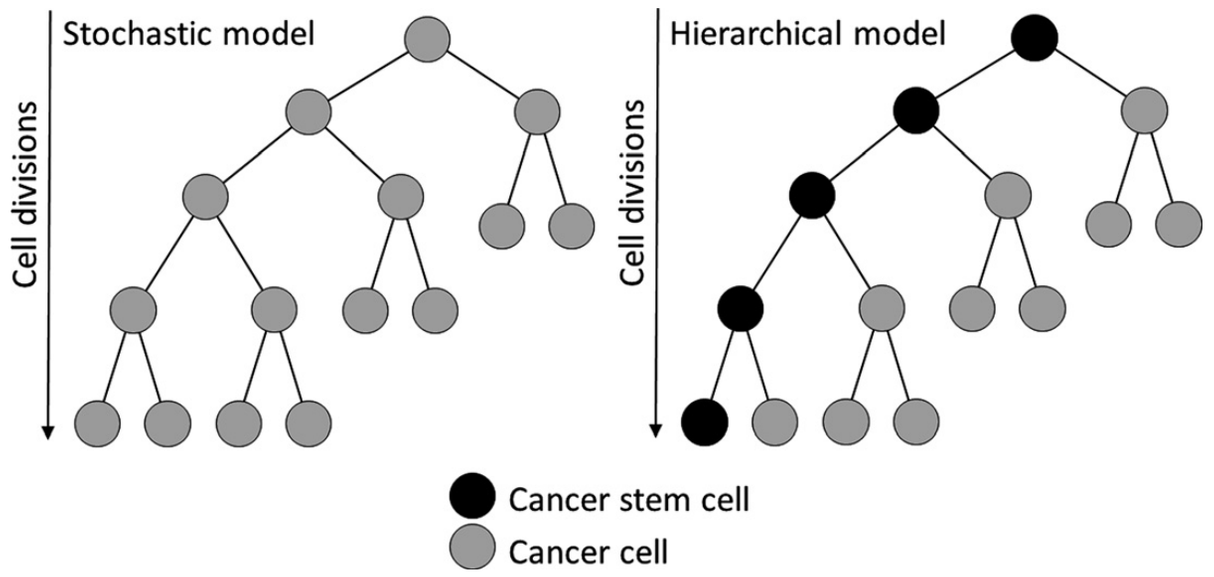


Figure 1. In the stochastic model, all tumor cells have equal abilities to propagate, initiate tumors, and seed metastases. The heterogeneity of tumors in this model is derived from spontaneous phenotypic shifts. The emerging cancer stem cell hypothesis dictates the hierarchical model, in which asymmetric division results in specific and well-defined populations of cancer stem cells and other cancer cells that do not initiate tumors or seed metastases. These cells may consist of populations with decreased proliferative ability (i.e., transit-amplifying cells) or post-mitotic differentiated cells with no further proliferative ability or activity.

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HNSCC will include a discussion of the prospective markers for CSCs in HNSCC, as well as a closer look at the cellular regulation of these CSCs and the clinical implications of these cancer- initiating and propagating cells.

2. Head and neck stem cells in normal tissues

Stem cells are unique in their ability to maintain self-renewal, differentiate into multiple lineage types in the same tissue, and display a high degree of proliferative potential [9]. The somatic stem cell microenvironment or cellular "niche" is vital in maintaining the delicate distinction between self-renewal and uncontrolled proliferation of stem cells [10]. Stem cells and the extracellular matrix secrete factors to maintain the microenvironment, while inhibitory signals from this local microenvironment provide necessary control of proliferation and differentiation to sustain this important subpopulation of cells [11, 12]. Importantly, these quiescent, undifferentiated somatic stem cells also depend on the niche for the transient stimulatory signals necessary for cell division and tissue regeneration [13]. Unlike most of the gastrointestinal tract, which contains a simple epithelial layer to allow for increased absorption, the oral cavity, pharynx, and esophagus are covered by a stratified epithelium that is more similar in structure to other tissues such as skin [14]. The stratified epithelium is composed of multiple layers of cell types oriented in order of increasing differentiation from basal to superficial.

In the normal squamous stratified epithelium, stem cells are located in the basal layer. To replenish the more superficial layers, these stem cells divide asymmetrically to self-renew and produce cells to undergo subsequent differentiation and amplification [11]. Cell division in stratified squamous epithelium results in differentiation, superficial cellular movement, stratification, and ultimately, tissue turnover. In the stratified squamous epithelium, for example, a layer of small, cuboidal, basal stem cells are responsible for cell division and regeneration [15]. Moving superficially, these committed cells further differentiate to increase keratin filament production, flatten, and decrease the size and volume of the nucleus and organelles. The most superficial (corneal or superficial) layers of the oral mucosa demonstrate cell flattening, mem- brane thickening, decreased desmosomes, and eventual sloughing of cells into the oral cavity [16]. The hierarchical normal oral epithelium is renewed approximately every 14–24 days [17]. Increasingly, endogenous oral cavity stem cells are theorized to precede CSCs, as these are the only cells with life span sufficient to accumulate the genetic mutations necessary for malignant transformation [18].

3. Head and neck stem cell markers

In contrast to other tissues or organ systems, relatively few markers have been identified or characterized for normal endogenous stem cells of the head and neck region. To date, most of our knowledge of normal head and neck stem cells is based largely on work on oral epithelial

stem cells (OESCs) and corollaries from the skin and hair follicle, which also maintain a squamous stratified epithelium.

In the 1960s, the first experiments to identify potential stem cells in the oral mucosa utilized pulse-chase experiments with tritiated-thymidine ($_3$H-TdR) to elucidate cell turnover rates in the skin and oral mucosa and identify label-retaining cells in the basal layer [19]. Of the few candidate cell surface markers for OESCs, most are also expressed in other normal oral epithelial basal cells, meaning that much of the research involving OESC markers has involved purification rather than isolation of these cells [20]. Such work is often accomplished based on sorting for cell markers and performing subsequent *in vitro* experiments to test the self-renewal and proliferative properties of these subpopulations. So far, notable candidate oral stem cell markers include keratins K5, 14, 15, and 19, β1- and α6-integrins, integrin $\alpha_6\beta_4$, melanoma chondroitin sulfate proteoglycan (MCSP), $p75_{NGFR}$, B-cell-specific Moloney murine leukemia virus integration site 1 (BMI1), and the p63 transcription factor (Table 1) [20-37].

Aida *et al.* performed telomere analysis of different cell types in normal lingual epithelium to calculate normalized telomere:centromere ratios (NTCRs). Overall, the basal cell group demonstrated the largest NTCR, with a smaller subgroup of these cells maintaining an exceptionally large NTCR, suggesting the presence of stem cells. In general, stem cells are thought to maintain relatively longer telomeres due to a lower telomere turnover rate as well as the potential for telomere upregulation. In addition, samples from older patients contained relatively shorter telomeres, confirming a measurable age-related progression. Finally, immunohistochemistry confirmed the presence of p27, p63, and K19 in the basal layer with relatively scant staining for Ki-67, a well-known marker of cell proliferation [22].

In one study, the magnetic separation of oral human keratinocytes yielded a fraction of α6β +CD71 cells that could regenerate a stratified oral epithelial equivalent *in vitro*. Unlike either of the α6β +CD71+ or or $\alpha_6\beta$ keratinocyte groups, $\alpha_6\beta_+$CD71$_-$ cells also expressed the candidate stem cell markers p63 and Keratin 19 and were negative for two recognized markers of differentiation: cytokeratin 10 or involucrin [23].

Tao *et al.* demonstrate a method of enriching a subpopulation containing both potential stem cells and transit amplifying cells through integrin-β1 adherence to collagen IV. While their subsequent study of p63 expression could not confirm specificity for stem cells in the basal layer, the ΔNp63α and ΔNp63β isoforms may be more specific markers for undifferentiated or immature cells [21].

Through cell lineage mapping, Hogan and coworkers noted a $K14_+K5_+Trp63_+Sox2_+$ subpopulation of long-term stem or progenitor cells located outside the taste buds that are capable of differentiating into both mature taste bud receptor cells as well as keratinocytes. The authors suggest that a similar model may apply to the taste buds of the circumvallate papillae and soft palate, and that their work may prove a model system for future study of these endogenous stem cells [24]. This same group isolated a population of undifferentiated tongue basal cells using Krt5-eGFP transgenic mice that demonstrated self-renewal and differentiation to stratified keratinized epithelial cells *in vitro*.

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Candidate Normal Stem Cell Marker	Site(s) Studied	Function and Significance in Stem Cell Biology	References
Keratin 5	Mouse tongue and soft palate	ntermediate filament protein expressed by basal epithelial cells, only small fraction may actually replicate	[24,29,31]
Keratin 14	Mouse tongue and oral mucosa	Intermediate filament protein expressed by basal epithelial cells, only small fraction of these cells may actually replicate	[24,29,32]
Keratin 15	Human hard palate	Intermediate filament protein, marker of stem cells in hair follicle bulge, expressed in deep tips of palatal epithelial papillae	[33]
Keratin 19	Human hard palate and gingiva	Intermediate filament protein, stem cell marker for cells of hair follicle	[22,23,34,35]
β1-integrin	Cultured human epidermal, buccal, and gingival cells	Important in adhesion to extracellular matrix proteins, increased in cells with higher colony-forming efficiency	[21,36]
α6β4-integrin	Human hard palate and gingiva	Important in adhesion to basement membrane, expressed on α6β4 CD71 basal cells capable of oral epithelium regeneration	[23,33,34]
MCSP	Human hard palate	Cellular surface proteoglycan associated with migration and invasion of melanoma cells, important for maintenance of patterned distribution and clustering of epidermal stem cells	[20,33,37]
p75NGFR	Human esophageal, buccal, gingival, and laryngeal epithelium	Low-affinity neurotrophin receptor in TNF receptor superfamily important in proliferation, cell migration, and tissue regeneration	[25-28]
p63	Rat palate and oral mucosa, human tongue, gingival and buccal epithelium	Transcription factor with multiple isoforms responsible for epidermal stem cell maintenance and regulation	[21,22,24,34]
BMI1	Mouse lingual epithelium	Protein involved with epithelial cell maintenance, proliferation, and tissue regeneration	[29]

Table 1. The function and significance of candidate normal stem cell markers in the head and neck region.

The low-affinity neurotrophin receptor $p75_{NGFR}$ is a member of the tumor necrosis factor receptor superfamily and has effects in cell survival, apoptosis, and intercellular signaling [25]. It has been put forth as a possible stem cell marker for neural crest, mesenchymal, esophageal, oral mucosa [25], and most recently laryngeal epithelium [25, 26]. In addition, $p75_{NGFR}$-positive

basal keratinocytes are able to migrate and initiate regeneration of damaged buccal epithelium [27]. Furthermore, expression of $p75_{NGFR}$ has been shown to be closely related to CSCs in esophageal squamous cell carcinoma as well as laryngeal squamous cell carcinoma [26, 28].

Recent interest in lingual epithelial stem cells has provided some evidence that keratins K5 and K14 may not be specific stem cell markers in this system. Ueno and coworkers utilized immunostaining to reveal that only a small fraction of these keratins K14/K5-positive cells were actually replicating to supply epithelial cells [29]. Rather, this group identified a group of BMI1-positive stem cells that maintain the epithelial cells and can regenerate after irradia- tion-induced tissue injury. Curiously, these potential stem cells were located in the second or third epithelial layer of the interpapillary pit of the filiform papillae. Increasingly, BMI1 is viewed as a candidate marker for CSCs of the head and neck, with the potential for prognostic value based on the location of this intracellular oncoprotein [30].

MCSP, melanoma-associated chondroitin sulfate proteoglycan; NGFR, nerve growth factor receptor; TNF, tumor necrosis factor; BMI1, B-cell-specific Moloney murine leukemia virus integration site 1

4. Origin of head and neck cancer stem cells

In most instances, HNSCC is caused by the accumulation of multiple genetic mutations based on genetic predisposition, which is induced by environmental factors such as tobacco and alcohol abuse or persistent human papilloma virus infection [38]. However, the alterations of multiple molecular and cellular pathways that lead to the development and recurrence of HNSCC are still not well understood. Recently, recurrence and therapeutic resistance of HNSCC has been attributed to a subpopulation of self-sustaining, tumor-initiating CSCs. CSCs are defined by several exclusive features that allow propagation as well as tumor formation and maintenance. These features are: (1) differentiation, giving rise to heterogeneous progeny; (2) self-renewal, which maintains a pool of stem cells which can expand; and (3) homeostatic control, which accounts for tissue specificity [4].

Multiple possible origins for CSCs have been proposed wherein a population of self-renewing cells are formed, leading to tumorigenesis (Figure 2) [39]. In one such scenario, normal stem cells undergo mutations that diminish restraint on replication, thereby creating CSCs that are unresponsive to environmental or intrinsic controls on self-renewal. Another potential source of CSCs are the more differentiated progenitor cells, also known as transit-amplifying cells, which maintain a more limited role in self-renewal yet are far more numerous than stem cells. A third motif of CSC generation explains that well-differentiated, mature cells undergo mutations to dedifferentiate and obtain greater self-renewal potential [5]. There is evidence that these dedifferentiated HNSCC cells may undergo epithelial-mesenchymal transition and invasion, leading to the development of cells with CSC- or mesenchymal characteristics [40].

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5. Initial clues to the concept of head and neck cancer stem cells

The first "leukemia-initiating" CSCs were identified in 1994 by Dick and co-workers through their work with acute myeloid leukemia [41]. In 2003, Al-Hajj *et al.* reported the first CSCs in a solid tumor by separating a tumorigenic subpopulation of breast cancer cells based on the surface cell markers $CD44_{+}/CD24_{-/low}$ [42]. In 2007, a landmark study by Prince *et al.* described a subpopulation of $CD44_{+}$ tumor-initiating cells isolated from HNSCC, although the cell surface markers CD44s and CD44v6 were subsequently described in a majority of normal head and neck tissues as well as HNSCC [43, 44]. Other subpopulations of tumor-initiating cells have since been identified in HNSCC that also fulfill the criteria for CSCs. Furthermore, several of the putative markers of these CSC subpopulations have been linked to cancer recurrence and therapeutic resistance, augmenting the evidence for the CSC hypothesis in HNSCC. Recent interest in the identification of new and improved biomarkers for HNSCC CSCs has spiked due to the prospect of using these tools to improve treatment approaches and overall mortality in this deadly disease.

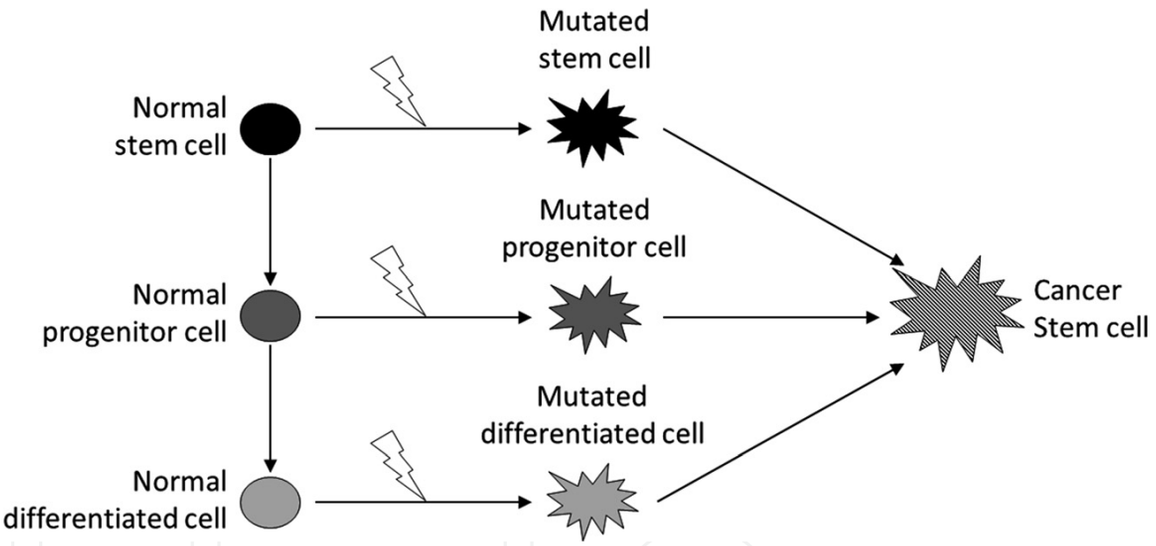


Figure 2. Potential origins for cancer stem cells include normal stem cells, progenitor cells, or fully differentiated cells. To give rise to cancer stem cells, the progenitor and fully differentiated cells acquire mutations to reactivate genes responsible for increased proliferative activity, cell-division, and dedifferentiation.

6. Cancer stem cell assays

The isolation and identification of CSCs is a hefty experimental challenge, as there is no established protocol to verify putative CSCs. Current experimental goals aim to satisfy the CSC criteria of both self-renewal as well as the capacity to develop heterogeneous cell lineages capable of forming tumors identical to the original [45]. Isolation strategies attempt to exploit the unique properties of CSCs that distinguish them from their differentiated progeny. Such

capacities include the efflux of vital dyes by multidrug transporters, enzymatic activity, sphere-forming capacity in low attachment conditions, and the expression of cell surface antigens [46]. There are currently four main strategies for isolation of CSCs: (1) detection of side-population phenotypes by Hoechst 33342 exclusion, (2) sphere-formation assays, (3) assessment of aldehyde dehydrogenase (ALDH) activity, and (4) identification of CSC-specific cell surface markers [45]. To date, the most common modality in identifying HNSCC CSCs relies upon the expression of membrane cell surface antigens present in stem-like cells. As a result, most potential CSC populations are detected by immunohistochemistry or flow cytometry. Many of these antigens were originally put forth as potential targets as a result of their expression in normal stem cells [47, 48]. Herein, we present a review of the most promising putative HNSCC CSC markers: CD44, CD133, and ALDH. We also include a discussion of CD24 and CD10.

Putative Cancer Stem Cell Markers in HNSCC Sites Studied	**Biological Function**	**Significance in HNSCC Stem Cell Biology**	**References**
CD44	Surface glycoprotein involved in cell migration and adhesion	Showed the ability to regenerate tumor *in vivo* but also abundantly expressed in normal squamous epithelia of the head and neck. Associated with Snail (chemoresistance and radioresistance) as well as high coexpression of BMI1 (important for self-renewal and tumorigenesis)	[43,44,49,50,55]
CD133 (Prominin 1)	Transmembrane glycoprotein localized on membrane protrusions and microvilli	Correlated with lymph node metastases and decreased overall survival	[51,52,54,55]
ALDH	Intracellular enzyme most commonly found in the liver. ALDH detoxifies intracellular aldehydes through oxidation	Tumor cells expressing relatively high levels of ALDH have increased tumorigenicity, stem-cell-related genes, drug-resistant genes, and EMT-related genes. Associated with high co-expression of Snail protein, which is an EMT regulator and key factor in self- renewal and tumorigenicity	[47,56,65]

Putative Cancer Stem Cell Markers in HNSCC Sites Studied	Biological Function	Significance in HNSCC Stem Cell Biology	References
CD24	Mucin adhesion molecule for P-selectin and L1 expressed by pre-B lymphocytes and neutrophils during cell development	Associated with increased proliferation and invasion *in vitro* and tumorigenicity *in vivo*, correlates with increased resistance to chemotherapeutic agents	[47,60,62]
CD10	Zinc-dependent metalloendoprotease that cleaves signaling peptides and is found in a wide range of normal tissues	Described as a potential marker for therapeutic resistance and tumor recurrence in HNSCC	[64]

Table 2. The function, significance, and associations of putative cancer stem cell markers in HNSCC.

7. Putative head and neck cancer stem cell markers

7.1. CD44

CD44 is a surface glycoprotein involved in cell migration and adhesion. Prince *et al.* demonstrated the ability of a subpopulation of $CD44_+$ HNSCC cells to regenerate a tumor *in vivo* after transplantation of as few as $5x10_3$ cells into an immunocompromised mouse model [44]. $CD44_+$ cells have been shown to express a high level of BMI1, a prospective normal stem cell bio- marker that also plays a key role in self-renewal and tumorigenesis in malignancy [49, 50]. While CD44 was the first CSC marker established for HNSCC, its role has since come into question. In contrast to earlier studies involving CD44, recent studies have demonstrated the abundant expression of CD44 in most HNSCC tumor cells. In one such study, CD44 was present in 80-100% of tumor cells [43]. However, according to the *hierarchical model*, only a small subset of cells within malignant tissue that are able to generate tumors should stain positive for CSC markers. Furthermore, high percentages of $CD44_+$ cells have been identified in normal squamous epithelia of the head and neck, with up to 60–95% of normal head and neck epithelial cells demonstrating CD44 positivity. Although the role of CD44 in HNSCC has raised some questions, this protein may still prove a valuable role in the identification of CSCs when used in combination with other markers. In the 2009 study by Chen *et al.*, not only did knockdown of Snail expression in $CD44_+ALDH_+$ cells decrease tumor invasion and colony formation, but it also significantly increased sensitivity to chemotherapy and radiotherapy. Specifically, following seven days of Snail siRNA treatment, the $CD44_+ALDH_+$ CSCs exhibited increased sensitivity to cisplatin and etoposide, and radiotherapy. These findings illustrate the potential utility of CSCs as unique cell targets for both chemotherapy and radiation therapy.

7.2. CD133

First described in 1997, CD133 (Prominin 1) is a transmembrane glycoprotein expressed in several normal stem cell populations and malignancies [51]. CD133 is localized to cellular membrane protrusions and microvilli [52]. It is expressed in several solid malignancies including brain, colon, liver, and lung, and has been purported as a unique, specific marker for sarcomas [38]. In 2007, Zhou *et al.* demonstrated the presence of a $CD133_+$ subpopulation in 3.5% of the native cells from a HEp-2 laryngeal cancer cell line. These $CD133_+$ cells continued to proliferate and expand the tumor cell population in sphere formation assays. In cell culture assays, the majority of terminal cells did not express CD133, a finding consistent with the criteria for CSC self-renewal as well as the capacity to give rise to phenotypically unique tumor daughter cells [53]. More recent studies have supported these findings, suggesting the utility of CD133 as a clinically relevant prognostic marker in HNSCC. Canis *et al.* demonstrated an inversely proportional correlation between CD133 expression in primary tumors and overall survival in addition to a positive correlation between CD133 expression and the presence of lymph node metastases [54]. In addition, Yu *et al.* describe an oral cavity squamous cell carcinoma-derived side population of cells with high expression of CD133 and ALDH showed high tumorigenic capacity [55]. Cell viability assays revealed that these side populations of cells were more chemoresistant to cisplatin, fluorouracil, or doxorubicin treatment when compared to the major population of the same cell line. The researchers hypothesized that CD133 may be crucial to modulation of chemosensitivity. They subsequently performed lentiviral-mediated transduction in the side population of cells, which resulted in significant decrease in expression of CD133 mRNA and protein. The silencing of CD133 decreased the percentage of the side population in the cancer cell lines and decreased *in vivo* tumor growth. Furthermore, cisplatin treatment of the CD133 knockdown population diminished cell invasion and clonogenicity, demonstrating the enhanced sensitivity to chemotherapy by targeting CD133. These findings support the role of HNSCC CSCs as novel therapeutic targets in the development of chemotherapeutic drugs.

7.3. ALDH

ALDH is an intracellular enzyme most commonly found in the liver [47]. ALDH detoxifies intracellular aldehydes through oxidation and may play a role in the differentiation of stem cells by oxidizing retinol into retinoic acid. With regard to cancer, high ALDH activity has been linked to subsets of multiple myeloma and acute myeloid leukemia [56]. Prior to its identification with HNSCC, ALDH was labeled a putative CSC marker in both breast and colon cancer. Ginestier *et al.* successfully used high ALDH activity to identify a tumorigenic breast cancer cell fraction capable of self-renewal and generating heterogeneous tumors. In their study, expression of ALDH as detected by immunohistochemistry correlated with a poorer prognosis for breast carcinomas [57].

Expression of ALDH1 in inflammatory breast cancer has been put forth as an independent predictor of early metastasis and decreased survival [58]. In 2009, Chen *et al.* published the first study demonstrating that cells of $ALDH1_+$ lineage have CSC properties and play a role in self- renewal in HNSCC [3]. In a study by Clay *et al.*, HNSCC cells were categorized and isolated

based on either high or low ALDH activity and subsequently implanted into immunocompromised mice. Cells with relatively high levels of ALDH represented a small percentage of cells (1% to 7.8%), but gave rise to tumors from as few as 500 cells in 53% of implantations. In contrast, only 8% of similar implantations with cells expressing low levels of ALDH formed tumor. As a result, ALDH appears to be a relatively selective marker for HNSCC CSCs [56]. In a similar study, 87% of implantations with 1000 $ALDH_+CD44_+$ HNSCC cells generated tumors, compared to only 13% of $ALDH_-CD44_-$ cell implantations, despite utilizing ten times more cells: 10, 000 [59]. $ALDH_+$ cells have also been shown to exhibit higher expression levels of stem cell-related, drug-resistance-associated, and epithelial-mesenchymal-transformation-related (EMT) genes such as Snail [3]. In fact, Snail protein overexpression transformed $ALDH_-$ cells to $ALDH_+$ cells, resulting in increased invasion and tumorigenic properties [65]. Given this association with Snail and EMT, as well as the ability to recapitulate tumors in high percentages after *in vivo* implantation in multiple studies, ALDH may be the most well-established HNSCC CSC marker to date.

7.4. CD24 and CD10

CD24 is a mucin adhesion molecule for P-selectin and L1 expressed by pre-B lymphocytes and neutrophils during cell development [47, 60]. CD24 expression has been shown to increase tumor cell proliferation and further shown to regulate multiple cell properties which contribute to tumor growth and metastasis [60]. It has been correlated with increased spread of breast cancer and has been further identified as a putative CSC marker in pancreatic, ovarian, and colorectal cancers [61, 62]. CD24 has also been associated with tumorigenesis, tumor progression, and malignant transformation of stomach and gallbladder cancers [63]. In a study by Han *et al.* $CD24_+CD44_+$ HNSCC cells were demonstrated to be more proliferative and invasive *in vitro* and more tumorigenic *in vivo.* After implantation in immunodeficient mice, $CD24_+CD44_+$ *cells* formed larger tumors than the $CD24_-CD44_+$ group. $CD24_+CD44_+$ cells were also correlated with slightly increased resistance to chemotherapeutic agents [62]. CD24 is one of the primary surface antigens involved in solid tumors and its role has been established in various human epithelial neoplasias. However, the paucity of research concerning HNSCC precludes its inclu- sion as a CSC biomarker at the present time.

CD10 is a zinc-dependent metalloendoprotease that cleaves signaling peptides and is found in a wide range of normal tissues. It has been described as a potential marker for therapeutic resistance and tumor recurrence in HNSCC [64]. As the field of CSCs remains in its infancy, further investigation regarding the roles of CD24 and CD10 will better elucidate the role of these proteins in HNSCC.

8. Clinical relevance of head and neck cancer stem cells

Today, few studies have evaluated patient HNSCC tumors or tissues and the correlation with clinical data and outcomes. One barrier to the establishment of clinically significant CSC markers in the head and neck region is secondary to the convention of amassing malignancies

from various upper aerodigestive sites with distinctly diverse embryological and biological characteristics [47]. As a result, there is little definitive data with regard to clinical implications of CSCs within HNSCC, the primary exception being prognostic value. Furthermore, no single biomarker for CSC cells in HNSCC has proven absolute in distinguishing this vital subpopulation. The continued study of current prospective CSC markers in HNSCC, combined with the investigation of putative CSC biomarkers from other malignancies, will undoubtedly augment our knowledge and improve our understanding of the pathogenesis of HNSCC. In addition, further knowledge regarding the biomarkers and regulation of normal, native stem cells in the head and neck region will serve as a strong foundation for oncological research. Ultimately, CSCs may prove to be useful diagnostic and prognostic markers for HNSCC, guiding therapy and treatment through personalized approaches and interventions.

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