

MINI-REVIEW

Cancer Stem Cells in Head and Neck Squamous Cell Carcinoma: A Review

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Abstract

Research indicates that a small population of cancer cells is highly tumorigenic, endowed with the capacity for self-renewal, and has the ability to differentiate into cells that constitute the bulk of tumors. These cells are considered the “drivers” of the tumorigenic process in some tumor types, and have been named cancer stem cells (CSC). Epithelial-mesenchymal transition (EMT) appears to be involved in the process leading to the acquisition of stemness by epithelial tumor cells. Through this process, cells acquire an invasive phenotype that may contribute to tumor recurrence and metastasis. CSC have been identified in human head and neck squamous cell carcinomas (HNSCC) using markers such as CD133 and CD44 expression, and aldehyde dehydrogenase (ALDH) activity. Head and neck cancer stem cells reside primarily in perivascular niches in the invasive fronts where endothelial-cell initiated events contribute to their survival and function. Clinically, CSC enrichment has been shown to be enhanced in recurrent disease, treatment failure and metastasis. CSC represent a novel target of study given their slow growth and innate mechanisms conferring treatment resistance. Further understanding of their unique phenotype may reveal potential molecular targets to improve therapeutic and survival outcomes in patients with HNSCC. Here, we discuss the state-of-the-knowledge on the pathobiology of cancer stem cells, with a focus on the impact of these cells on head and neck tumor progression, metastasis and recurrence due to treatment failure.

Keywords: Cancer stem cells - epithelial-mesenchymal transition - head and neck squamous cell carcinoma

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Introduction

Worldwide, head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer, affecting over 400,000 patients, and leading to over 200,000 deaths annually (Jemal et al., 2011). In the last 30 years, progress in the treatment of head and neck cancer has improved the quality of life of patients via the use of innovative surgical and endoscopic techniques that are aimed at the preservation of organ function, mainly in laryngeal tumors (Allegra et al., 2012). The 5-year survival rate for these patients has remained in the range 50-60% for the last three decades (Carvalho et al., 2005). The main causes of death remain the recurrence of locoregional disease that is unresponsive to conventional treatments and distant metastases (Allegra et al., 2012).

It is becoming increasingly evident that an improvement in the survival of head and neck cancer (HNC) requires improved understanding of tumorigenesis, metastasis and recurrence.

Recent studies on the pathobiology of HNSCC have led to the discovery of a small population of cancer cells that is highly tumorigenic, capable of self-renewal,

and behave as tumor progenitor cells. Such behavior is consistent with the features of cancer stem cells (CSC) (Prince et al., 2007). Targeted elimination of these CSC has been considered a new conceptual framework for HNC treatment.

Cancer Stem Cells

The term “cancer stem cells” is defined by the American Association for Cancer Research Workshop on Cancer Stem Cells as a cell within a tumor that possesses the capacity to self renew and to generate heterogeneous linkages of cancer cells that comprise the tumor (Clarke et al., 2006). Cancer stem cells are functionally defined as a subset of tumor cells that exhibit the ability of self-renewal and multipotency, serving as progenitor cancer cells (Bonnet et al., 1997).

The basic characteristics that distinguish CSCs are: *i*) promotion of tumorigenesis when they are transplanted into immunosuppressed mice; *ii*) possession of specific cell-surface markers that are not expressed by noncancer stem cells; *iii*) tumors that arise from CSCs include both tumorigenic and nontumorigenic cells (heterogeneity);

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and *iv*) capacity for self-renewal in seriated transplants over several generations (Singh et al., 2004; Collins et al. 2005; Fang et al., 2005; Dalerba et al., 2007; Hermann et al., 2007; Eramo et al., 2008).

These characteristics are derived from the intrinsic properties of CSCs, which reside in their ability to duplicate, differentiate, and control homeostasis. This cell subpopulation has been identified in several solid tumors, including head and neck cancer, and it shows certain characteristics that give it the ability to maintain the tumor population, metastasize, and be resistant to chemoradiotherapy (Allegra et al., 2012).

Development and Cancer Stem cells

In development, a highly orchestrated and hierarchical process is observed in which a stem cell progressively loses multipotency giving rise to restricted progenitor cells, which in turn differentiate into the cells that constitute the bulk of tissue or organ. In cancer, the cell of origin is the cell that receives the first oncogenic hit(s). A candidate cell of origin is the stem cell, which has the inherent potential of self-renewal and longevity, and therefore is more susceptible to acquired genetic or epigenetic changes that result in transformation (Zhang et al., 2012). On the other hand, it is not clear if cancer stem cells originate solely from the transformation of normal stem cells. CSCs may also arise from restricted progenitors or differentiated cells that have acquired self-renewal properties as a consequence of genetic or epigenetic alterations (Visvader et al., 2008).

Origin of Cancer Stem Cells

Two main hypotheses exist regarding the origin of CSCs: *i*) origin from a somatic tissue cell that undergoes genetic mutations, becomes cancerous, and acquires stem characteristics; and *ii*) derivation from embryonic stem or adult cells as a result of genetic mutations. The mode of onset may depend on the location of the origin of the tumor (Allegra et al., 2012).

The present theory of formation of head and neck cancer can be summarised as follows: Caused by a questionably genetic disposition during a chronic inflammation caused by permanent tobacco and alcohol abuse, mechanic irritation or viral infection, a spontaneous accumulation of various genetic alterations develops leading to a manifestation of a malignant phenotype. Then clonal divergence and selection lead to a formation of a carcinoma (Braakhuis et al., 2002; 2005).

If one carries over the afore-mentioned to the present assumption of the genesis of cancer stem cells, certain parallelisms can be verified, whereby the formation of cancer stem cells is not conclusively clarified for any tumour entity. Currently three hypotheses are discussed (Wollenberg et al., 2011).

i) Source of “normal” stem cells with cancerous phenotype; *ii*) Source from differentiated cells with oncogenic mutation (Return to self-regeneration and multipotency); *iii*) Fusion of stem cells with tumour cells (much discussed with the formation of bone metastases).

The Cancer Stem Cell Hypothesis

The idea that cancer can originate from a small population of cells with stem cell properties was proposed about 150 years ago by Francesco Durante in 1874. In “Nesso fisio-patologico tra la struttura dei nei materni e la genesi di alcuni tumori maligni [Nessus pathophysiological between the flaw structure of the mother and the genesis of some malignant tumors],” Durante explains why some aberrant epithelial or connective elements that remained inert for a long time take up highly tumultuous and abnormal activities. His theory was revived and popularized by the German pathologist Cohnheim, who lived during the same period (1839-1884) (Cohnheim et al., 1875). The theory was revisited 90 years later by Till and McCulloch, and later by Pierce et al (1960) (Till et al., 1961).

Reya et al in 2001 redefined Durante’s scientific theory as “...a strict parallelism can be made between normal stem cells and cancer stem cells: tumors often originate from the transformation of normal stem cells, similar signals can adjust the self-regeneration in normal stem and in tumor cells, and tumor cells may include ‘cancer stem cells,’ rare cells with an indefinite regenerative potential that leads to tumorigenesis (Reya et al., 2001).”

In 1997, Bonnet et al. (1997) were the first to isolate “cancer stem cells” in samples of acute myeloid leukemia. In 2003 Al-Hajj et al. (2003) first identified and isolated a population of cancer stem cells from breast cancer, showing that only a subset of them, which exhibited expression of the surface markers CD44+/CD24-/low, had tumorigenic capacity.

In head and neck tumors, Prince et al. (2007) first identified and isolated a cellular subpopulation expressing the surface marker CD44 that exhibited stem-like characteristics and was capable of reproducing when a tumor was implanted in immunosuppressed mice.

The CSC theory is based on our understanding of embryological development and stem cell-derived organogenesis, where a few specific cells are capable of asymmetric division leading to the generation of diverse progenitor cells responsible for the creation of complex and heterogeneous organs (Reya et al., 2001). The fundamental concept underlying the cancer stem cell hypothesis is that not all tumour cells in a cancer are equal (Krishnamurthy et al., 2012). The CSC theory explains that there exists a hierarchy of cells, where CSC are capable of unregulated asymmetric division, which is responsible for self-renewal and generation of a diverse population of differentiated progenitor cells that ultimately make up a heterogeneous tumor (Reya et al., 2001). The bulk of the tumor tissue, however, is composed of rapidly proliferating cells, called transit-amplifying cells and post-mitotic differentiated cells, which do not contribute to tumor initiation (Krishnamurthy et al., 2012).

The following are key features of cancer stem cell hypothesis (Prince et al., 2008): *i*) Only a small fraction of the cancer cells within a tumor have tumorigenic potential when transplanted into immunodeficient mice; *ii*) The cancer stem cell sub-population can be separated from the other cells by distinctive surface markers; *iii*)

Tumors resulting from the cancer stem cells contain the mixed tumorigenic and non-tumorigenic cells of the original tumor; *iv*) The cancer stem cell sub-population can be serially transplanted through multiple generations, indicating that it is a self-renewing population.

The Stochastic and Hierarchic Model of Tumor Expansion

Currently two models describing growth behaviour are being discussed and compared.

The Stochastic model was proposed by Nowel et al in 1976. According to this theory, tumors originate from a single cell, and tumor progression is derived from a more aggressive subpopulation selected within an original clone over time (Nowell et al., 1976). In other words, there would be one “mother cancel cell”, which stochastically proliferates by entry into the cell cycle, whereby each cell within the tumour possesses the same ability to promote tumour growth. Therefore each cell is equally potent to initiate a tumour (Wollenberg et al., 2011). The concept of multistep progression foresaw the stochastic accumulation of numerous genetic mutations underlying the process of neoplastic transformation of solid tumors; it also justified the transition from precancerous to invasive carcinoma as a consequence of the progressive accumulation of genetic mutations, which ultimately determines the origin of a predominant clone and results in a selective advantage over other changed cell populations (Garozzo et al., 1999; Califano et al., 2000; Allegra et al., 2006; Allegra et al., 2009) (Figure 1).

On the contrary to the above is the hierarchial theory which hypothesizes that the tumor originates from embryonic stem cells or somatic cells (present in all tissues) undergoing mutations. These changed stem cells give rise to stem cells that are further altered. Unlike the previous theory, in the hierarchical model, during cell division, one of the two daughter cells retains the ability to replicate, whereas the other loses this capacity and differentiates. Differentiated CSCs represent the majority of the tumor; further mutations that alter the characteristics of the parent cells may intervene during the process of CSC duplication, giving rise to cells that are functionally different. Unlike the stochastic model, the hierarchical model considers that tumorigenicity resides in a small subpopulation of cells composing the tumor that retain the capacity of stemness (Allegra et al., 2012).

Therefore, a tumor can be compared to an aberrant organ that is maintained in a manner similar to that of normal tissues. This body contains a small proportion of CSCs that feed tumor growth, give it the ability to resist radio- and chemotherapy, and promote local or distant

metastasis. The remaining cellular components of the tumor represent the tumor mass formed by aberrantly differentiated cells that have lost the ability to replicate (Prince et al., 2007).

During tumor progression, the CSC population can perform several tasks. Thus, the following CSC subpopulations can be distinguished: stationary CSCs, which remain incorporated in the epithelia, are not able to spread, are responsible for resistance to chemo- and radiotherapy, and serve to increase tumor volume; and movable CSCs, which are capable of migrating, are localized at the host–tumor interface, and are responsible for the ability to metastasize locoregionally and/or remotely. These specificities of CSCs give rise to two phenomena: niches and the epithelial–mesenchymal transition (EMT) process (Allegra et al., 2012) (Figure 2).

Epithelial Mesenchymal Transition

Epithelial-mesenchymal transition (EMT) is the process that allows a polarized epithelial cell to assume a mesenchymal cell phenotype, which is characterized by enhanced motility and invasiveness (Kalluri et al., 2003). The EMT process is a fundamental stage of embryogenesis (Mani et al., 2008; Morel et al., 2008). It also plays critical role in fibrosis (Kalluri et al., 2003) and cancer (Thiery et al., 2002; Shook et al., 2003; Kalluri et al., 2009; Thiery et al 2009). During EMT, epithelial cells break cell–cell and cell–matrix connections and migrate elsewhere (Radisky et al., 2008). During tumor progression, some CSCs undergo EMT and acquire the ability to infiltrate surrounding tissues and metastasize (Thiery et al., 2002). EMT occurs when the cells are dissociated from each other, lose the expression of epithelial markers and earn the expression of mesenchymal markers, and change their polarization and cytoskeletal structure to establish new cell–matrix interactions (Iwatsuki et al., 2010) (Key features of EMT are summarized in Table 1).

A critical step in EMT is the loss of cell polarity. Three protein complexes (Par, Crumbs, Scribble) participate in establishing and maintaining apico-basal polarity in epithelial cells (Moreno-Bueno et al., 2008). Snail alters epithelial cell polarity by repressing the transcription of Crumbs3 and abolishing the localization of both Par and Crumbs complexes at the junctions (Whiteman et al., 2008). Another hallmark of EMT is the loss of E-cadherin, which appears to be correlated with tumor progression. The loss of E-cadherin is considered a crucial step in the progression of papilloma to invasive carcinoma (Perl et

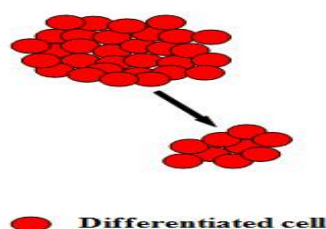


Figure 1. The Stochastic Model

Table 1. Characteristics of Normal Epithelial and Mesenchymal Cells

	Epithelial cell	Mesenchymal cell
Morphological characteristic	Cobblestone Polarized	Elongated Non-polarized
Behavioural characteristic	Non-motile Non-invasive	Migratory Invasive
Molecular markers	Ecadherin Desmoplakin Cytokeratin	Vimentin N cadherin Snail

al., 1998). It is regulated by a number of transcription factors such as Snail (Batlle et al., 2000), Twist (Yang et al., 2004) and ZEB1 (Sánchez-Tilló et al., 2010). The transcription factor Snail controls EMT by repressing E-cadherin expression (Cano et al., 2000). Increased Twist expression is found in metastatic breast cancer and is required for EMT and breast cancer metastasis (Yang et al., 2004). Tumors undergoing EMT acquire resistance to chemotherapy (Yang et al., 2006; Cheng et al., 2007; Li et al 2009).

Twist mediates EMT in breast cancer cells and enhances resistance to paclitaxel (Cheng et al., 2007). Colorectal cancer-derived epithelial cell lines expressing EMT markers exhibit mesenchymal morphology and resistance to oxaliplatin (Yang et al., 2006). This data show that the acquisition of a mesenchymal phenotype correlates with increased invasiveness of tumor cells, leading to recurrence/metastasis and poor clinical prognosis.

EMT is involved in the acquisition of cancer stem cell properties. In nasopharyngeal carcinomas, miR200a regulates EMT and induction of stem-like characteristics by targeting E-cadherin repressor ZEB2 via β -catenin signalling, thus induces stem-like traits (Xia et al., 2010).

In head and neck cancer, Twist1 induces Bmi-1 (B-cell specific Moloney murine leukemia virus insertion site 1), which in turn downregulates E-cadherin. Bmi-1 has an essential role in the regulation of self-renewal of stem cells (Park et al., 2004; Valk-lingbeek et al., 2004; Spivakov et al., 2007; Widschwendter et al., 2007). Patients with high Twist1 and Bmi-1 tend have worst prognosis (Yang et al., 2010).

It has been reported that hypoxia or overexpression of HIF-1 α induces EMT and metastasis in head and neck cancer cells. HIF-1 α regulates the expression of Twist by binding to the hypoxia-response element (HRE). Notably, siRNA-mediated repression of Twist in hypoxia

or HIF1-a overexpression reversed EMT and metastasis. Co-expression of HIF-1 α , Twist and Snail in human head and neck tumors correlates with metastasis and poor prognosis (Yang et al., 2008) (Figure 3)

Stem Cell Niche

Physiological stem cells and cancer stem cells depend on their immediate environment or niche for their survival and function (Borovski et al., 2011). The cellular and non-cellular components of the niche provide cues that regulate proliferative and self renewal signals, thereby helping cancer stem cells maintain their undifferentiated state (Kuhn et al., 2010). Non-epithelial stromal cells, inflammatory cells and the vasculature have been proposed as key components of the niche that support and sustain cancer stem cells (Fuchs et al., 2004). It has been postulated that a niche should show the capacity to take up and maintain newly introduced stem cells upon depletion (Morrison et al., 2008).

In head and neck tumors, the vast majority of the stem cells are found within a 100 microns radius of a blood vessel (Krishnamurthy et al., 2010). Using the SCID mouse model of human tumor angiogenesis (Nör et al., 2001), it was observed that specific ablation of tumor associated endothelial cells with an inducible Caspase-9 results in the decrease in the fraction of head and neck cancer stem cells (Krishnamurthy et al., 2010). It is becoming increasingly evident that the molecular crosstalk between HNSCC and endothelial cells is mutually relevant (Neiva et al., 2009; Zhang et al., 2010). Tumor cell-secreted factors activate Stat3, AKT and ERK signaling and enhance the survival and angiogenic potential of endothelial cells (Zhang et al., 2010). Whereas endothelial cell-secreted factors (e.g. IL-6, CXCL8) enhance the migration of tumor cells and protect them against anoikis (Neiva et al., 2009). Notably, endothelial cell-secreted factors promote the survival and self-renewal of cancer stem cells in HNSCC via upregulation of Bmi-1 expression (Krishnamurthy et al., 2010).

These studies demonstrate the existence of a functionally relevant perivascular niche in head and neck cancer, and suggest that targeted disruption of the crosstalk between endothelial cells and cancer stem cells might be beneficial for the treatment of head and neck cancer patients.

Methods of Identification of Cancer Stem Cells

Identification and isolation of cancer stem cells constitute a major experimental challenge. Researchers attempt to isolate these cells by identifying properties that distinguish stem cells from their differentiated progeny and from stromal cells. The methods used for the identification and isolation of tumor stem cell populations apply the same techniques used to identify normal stem cells from their differentiated progeny. Cancer stem cells can be identified via surface markers, determination of ALDH activity, ability to efflux vital dyes, and ability to form tumor spheres *in vitro*.

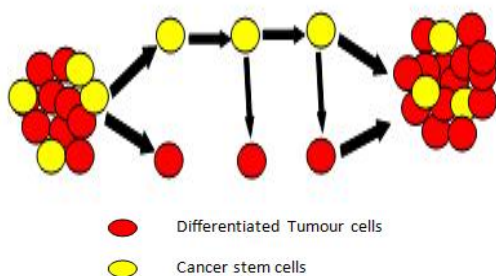


Figure 2. The Hierarchic Model

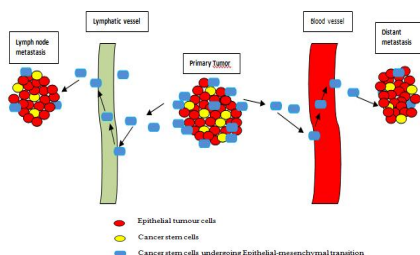


Figure 3. The Role of EMT in the Metastatic Spread of HNSCC

Surface antigens

CD133: CD133 is a transmembrane glycoprotein characterized by its tendency to localize to cellular protrusions. CD133 is a protein commonly expressed in hematopoietic stem cells, endothelial progenitor cells, and various normal tissue stem cells. CD133 was first described as a CSC marker in leukemia and glioblastoma (Bonnet et al., 1997; Zhou et al., 2007). In some HNSCC cell lines (e.g., hep-2), CD133+ cells were found to have increased clonality when compared with CD133- cells (Zhou et al., 2007). Oral cancer stem-like cells from cell lines and primary tumors were found to have an increased expression of CD133, and displayed increased migration and tumorigenicity as compared with controls. Correlation of Oct-4, Nanog, and CD133 status showed a poorer prognosis for oral cancer patients with increased CD133 expression (Chiou et al., 2008). Recently, CD133+ cells were found to possess increased clonogenicity, invasiveness, and tumorigenicity as compared with CD133- cells, along with resistance to paclitaxel (Zhang et al., 2010).

CD44: CD44 is a surface glycoprotein that is involved in cell migration and adhesion. It is a known receptor of hyaluronic acid and interacts with other "ligands," such as matrix metalloproteases (Kajita et al., 2001; Isacke et al., 2002). Prince et al first demonstrated that CD44 expression could be used to isolate a subpopulation with increased tumorigenicity in head and neck tumors (Prince et al., 2007). Several independent studies also confirm that CD44 either alone or in combination has the properties of a cancer stem cell marker and being a tumor initiator (Baumann et al., 2010; Chikamatsu et al., 2012). Certain forms of CD44 (i.e., v3, v6, v10) are associated with tumor progression and metastatic spread of HNSCC (Wang et al., 2009).

CD24: CD24 is a mucin adhesion molecule expressed by pre-B lymphocytes and neutrophils. Functionally, CD24 promotes metastasis, as it has been identified as a ligand of P-selectin, an adhesion receptor found on activated endothelial cells and platelets. Lim and Oh showed that the cytoplasmic expression of CD24 was associated with adenocarcinoma of the colon, stomach, bladder, and ovaries, whereas there is no evidence of this activity in head and neck cancer (Lim et al., 2005).

ALDH activity: ALDH is an intracellular enzyme that is present normally in the liver. Its best-known functions are the retinol conversion to retinoic acid and the oxidation of toxic aldehyde metabolites, such as those formed during the alcohol metabolism and certain chemotherapeutic drugs (eg, cyclophosphamide and cisplatin) (Bosron et al., 1988; Thomasson et al., 1991; Visus et al., 2007). In cancer, ALDH+ cells were identified in the breast and the brain (Ginestier et al., 2007; Rasper et al., 2010). In these tumors, ALDH+ cells were characterized as highly tumorigenic cells that can self-renew, which are hallmarks of cancer stem cells. In HNSCC, ALDH enriches for cancer stem cells and is involved in epithelial-to-mesenchymal transition (EMT) (Chen et al., 2009).

Side Populations: Another strategy used to identify highly tumorigenic cellular subpopulations is based on the

ability of these cells to efflux a fluorescent dye that binds to DNA. The cell populations isolated using this method are called side populations (Zhang et al., 2009). The dye used to isolate side populations is Hoechst 33342. Cells that are able to expel the dye, similar to certain chemotherapeutic drugs, express a group of transmembrane transporters, such as multidrug resistance transporter 1. They are involved in resistance to chemotherapy because of their ability to efflux the drug from the cell and prevent the action of the chemotherapeutic agent (Hirschmann-Jax et al., 2004).

Formation of tumor spheres: CSCs grown in culture conditions without serum retain an undifferentiated state. The addition of growth factors guides them toward proliferation and formation of cell aggregates that are termed tumor spheres (Allegra et al., 2012). Okamoto et al reported that CSCs isolated from cell lines from carcinoma of the oral cavity were highly capable of forming spheres and expressed high levels of CD44 (Okamoto et al., 2009). Chiou et al. (2008) studied two cell lines and primary tumors of the oral cavity and showed that the isolated CSCs had a high capacity to form tumor spheres and expressed high levels of CD133 (Chiou et al., 2008). However, in a study on 43 primary tumors of the head and neck, Lim et al. (2011) reported that only 6% (3/43) of the primary tumors formed spheres (Lim et al., 2011).

Tumorigenesis

Cancer is defined by unregulated cell division and growth. CSC are believed to represent a mechanism for tumorigenesis and potentially offer a novel area of study for developing more effective treatments for HNSCC. HNSCC CSC were first described by Prince and colleagues in 2007 based on CD44 expression (Prince et al., 2007). In their experiments, they demonstrated enhanced tumorigenicity in the CD44high subpopulation (as few as 5,000 cells) as compared to CD44low cells, even when injecting $>1 \times 10^6$ cells. The resulting tumors, derived from the CD44 high injections, demonstrated renewal of CD44 high cells, indicating self-renewal, and regeneration of a heterogeneous tumor, thus meeting the definition of a CSC. Similar experiments using the enzymatic marker ALDH were also able to demonstrate that cells with ALDH+ expression had greater rates of tumorigenesis in mouse flank and neck injections (Chen et al., 2009).

Gene expression signatures in ALDH/CD44-sorted HNSCC cells demonstrated BMI-1, a known CSC marker, to be differentially overexpressed, and when knocked down, demonstrated reduced tumorigenesis (Krishnamurthy et al., 2010). Sun and Wang looked at CMET+ cells compared to CMET- and found increased tumorigenicity in a flank injections and found higher percentage of implantation in CMET+ cells compared to CD44+ cells and slightly lower than ALDH+ cells (Sun et al., 2011). In addition, Zhang et al looked at HNSCC cell lines and oral cavity primary tumors identified the presence of SP cells. *In vitro* and *in vivo* analysis demonstrated SP cells had greater clonal expansion and greater tumorigenicity relative to non-SP cells (Zhang et al., 2009).

Analysis of this data, collectively, lends support to the concept that HNSCC follows the cancer stem cell hypothesis, where subpopulations of cancer cells have significantly higher tumorigenic potential than others.

Metastasis

In HNSCC, understanding the cellular mechanisms of invasion and metastasis is critical to developing new diagnostics and therapeutic modalities. CSC offer a unique mechanism for metastasis given their ability for tumor growth at the primary site, but also at the distant sites. Davies et al has shown that HNSCC CD44^{high} cells have greater migration, invasion and metastatic potential compared to CD44^{low} cells (Davis et al., 2010). Gene expression studies comparing ALDH⁺ cells and ALDH⁻ cells demonstrated elevated levels of the metastatic and epithelial–mesenchymal transition (EMT) biomarkers CMET, TWIST, and SNAIL (Chen et al., 2011; Sun et al., 2011). Zhang et al demonstrated that Side population cells have also been associated with metastasis (Zhang et al., 2009). Collectively, these findings support CSC as important mediator and potential target in HNSCC metastasis. However despite these associations, the evidence and mechanisms of CSC mediated metastasis remains scant.

Recurrence and Resistance to Therapy

Despite an increasing amount of research investigating the mechanisms responsible for treatment failure and resistance in HNSCC, outcomes remain largely unchanged.

CSC have been shown to be especially resilient to toxic insult in a variety of malignancies, and may represent critical mediators of chemo- and radio-resistance within the diverse cellular population of a tumor. CSC possess unique mechanisms to resist cell death, including modified anti-apoptotic machinery, increased pump activity, and decreased cell division (Clarke et al., 2006). In HNSCC, a higher percentage of CD44⁺ cells in a patient’s primary tumor has been shown to be associated with higher rates of treatment failure, while cells expressing the putative CSC markers CD44, CD24, Oct4, and integrin-β1 were associated with poor outcomes following radiotherapy (Joshua et al., 2012; Koukourakis et al., 2012). CSC, as defined by CD44 expression, have a greater resistance to pro-apoptotic stimuli (TNF-α and anti-Fas antibody) and a greater capacity for resistance to chemotherapeutic agents compared to non-CSC (Chikamatsu et al., 2012; Okamoto et al., 2009).

With regard to SP cells, Zhang et al. (2009) demonstrated they possess qualities necessary for chemoresistance, with elevated expression of ABC transporter proteins (Zhang et al., 2009). Mani et al. (2008) established a relationship between these transformed cells and CSC; after induction of EMT in breast cancer cells, via activation of Snail/ Twist, cells adopted stem-like properties of growth and tumorigenicity (Mani et al., 2008). In addition, just as hypoxia maintains the pluripotency of embryonic stem cells, a similar process may be involved with promotion of

the CSC phenotype and its anti-apoptotic characteristics; hypoxia often represents low blood flow, which limits the distribution of chemotherapeutic drugs, and causes increased resistance to radiation, which requires sufficient oxygen tension to produce oxygen free radicals for cytotoxicity. Hypoxia inducible factors (HIFs) are over expressed in CSC and may be responsible for some aspect of radiation-resistance in HNSCC (Vlashi et al., 2009).

The enhanced mechanisms of CSC to endure and adapt to toxic insults may help explain treatment failures and poor outcomes in HNSCC, and a more sophisticated understanding of their unique survival machinery may illuminate points of vulnerability and lead to novel CSC-specific targets.

Therapeutic Implications

Taking studies on other cancer entities and the model of hierarchic tumour initiation into account the following order of events must be adopted (Figure 4).

The heterogenic tumour will be treated with conventional means. During the surgical intervention residual cancer cells remain in the incisional margin, in the neighbourhood of the tumour and in the adjacent tissue space; those will be treated postoperatively or primarily with combined or primary radiotherapy. The heterogenic tumour’s differentiated tumour cells will be destroyed. The considerably chemo- and radio-resistant cancer stem cells remain; because of their stem cell characteristics they are able to generate a tumour, which histologically matches the tissue from where it originated from. This model further emphasizes the immense implication of safe margins during surgical measures and demonstrates that the objective of future therapies must be the development

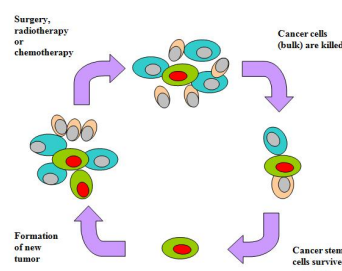


Figure 4. Circle of Therapy Resistance Induced by CSC

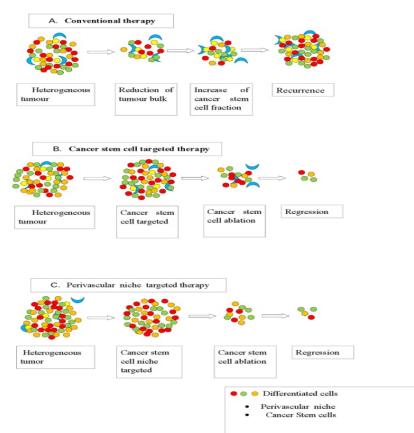


Figure 5. Possible Implications of the Cancer Stem Cell Hypothesis for Therapy

of specific drugs against the cancer stem cells of a tumour, which remain after the removal of the tumour bulk via conventional therapy treatments.

In Figure 5, we propose a hypothetical model for the response of HNSCC to different therapeutic strategies. HNSCC is represented as a complex tissue where the cancer stem cells 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 de-bulking the tumor. However, it is proposed that slow-growing cancer stem cells evade conventional therapies, and, with the passage of time, these cells are activated and regenerate tumors locally or at distant sites (Figure 3A). This might help to explain the relatively high recurrence rates in patients with HNSCC. In contrast, targeting the cancer stem cells either directly (Figure 3B) or via their niche (Figure 3C) could lead to a more definitive response, since the cancer stem cells are the putative drivers of recurrence and metastatic spread. An emerging concept is the combined use of conventional chemotherapy and cancer stem-cell-targeted therapy. This drug combination is appealing, since such strategy could potentially allow for tumor debulking (with conventional drugs) and prevention of recurrence/metastases (cancer-stem-cell-targeted drugs).

Conclusion

The discovery of a small subpopulation of cells that possess exquisitely high tumorigenic potential, provides a new conceptual target for cancer therapy. Further work to better understand the CSC-specific molecular pathways will be critical in understanding the mechanism of tumorigenesis, metastasis, recurrence due to treatment failure with the ultimate goal of developing novel CSC diagnostics and therapeutic targets.

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