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Abstract

Head and neck squamous cell carcinoma (HNSCC) is the most common form of head and neck cancer. Annually, more than half a million individuals are diagnosed with this devastating disease, with increasing incidence in Europe and Southeast Asia. The diagnosis of HNSCC often occurs in late stages of the disease and is characterized by manifestation of a high-grade primary tumor and/or lymph node metastasis, precluding timely management of this deadly cancer. Recently, HNSCC cancer stem cells have emerged as an important factor for cancer initiation and maintenance of tumor bulk. Like normal stem cells, cancer stem cells can undergo self-renewal and differentiation. This unique trait allows for maintenance of the cancer stem cell pool and facilitates differentiation into heterogeneous neoplastic progeny when necessary. Recent studies have suggested coexistence of different cancer stem cell populations within a tumor mass, where the tumor initiation and metastasis properties of these cancer stem cells can be uncoupled. Cancer stem cells also possess resistant phenotypes that evade standard chemotherapy and radiotherapy, resulting in tumor relapse. Therefore, understanding distinctive pathways relating to cancer stem cells will provide insight into early diagnosis and treatment of HNSCC. In this review, we highlight current advances in identifying cancer stem cells, detail the interactions of these cells with the immune system within the tumor niche, and discuss the potential use of immunotherapy in managing HNSCC.

Keywords: flow cytometry, tumor microenvironment, squamous cell carcinoma of the head and neck, neoplastic stem cells, tumor immunology, metastasis

Introduction

Head and neck cancers cause severe disfigurement, speech impairment, and difficulty in breathing and swallowing. The most common form is head and neck squamous cell carcinoma (HNSCC), which arises from the epithelial lining of the inner moist surfaces of pharynx, larynx, oral, and nasal cavities. While overall incidence and mortality of HNSCC in the United States has steadily declined over the past 2 decades, it remains a global health burden, particularly in Europe and Southeast Asia (Simard et al. 2014).

Chronic tobacco use and alcohol use are the main etiologies associated with HNSCC, although chronic human papillomavirus infection association with oropharyngeal cancer has been reported in individuals who are not alcohol or tobacco users (Sathish et al. 2014). Despite significant efforts to prevent and treat HNSCC, the mortality rate remains high due to late diagnosis of the disease and delayed administration of chemotherapy and radiotherapy. Therefore, understanding the basic biology of HNSCC formation and progression is necessary to improve diagnostics and/or treatment plans for HNSCC patients.

Over the past decade, the concept of cancer stem cells in tumor initiation and maintenance received significant attention. It is now known that not all tumor cells are equal; a small subpopulation of cancer cells can behave primitively, like stem cells, with the ability to self-renew and differentiate (Kreso and Dick 2014). Because of their slow cycling nature, cancer stem cells are particularly resistant to standard chemotherapy and radiotherapy. Cancer stem cells can re-create entire heterogeneous populations of a tumor posttreatment, causing tumor relapse. Importantly, tumors with high numbers of cancer stem cells are more aggressive and reflect a poorer prognosis. Therefore, studying cancer stem cells in HNSCC may provide new insights into management of this disease. In the first part of the review, we discuss the latest reports on identification and characterization of different cancer stem cell populations in HNSCC, with a specific focus on current limitations and recent advancements of cancer stem cell detection. The second part of the review emphasizes interactions of cancer stem cells within the tumor microenvironment and provides insights into tumor immunology pertaining to cancer stem cells.

Identification and Characterization of HNSCC Cancer Stem Cells

To date, flow cytometry/fluorescence-activated cell sorting (FACS) is the most widely employed method to identify and isolate cancer stem cells from various tumor types. Cell surface antigens on HNSCC cancer stem cells allow for the use of fluorochrome-conjugated antibodies to identify these cells based on individual or a combination of markers. Among these,

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CD44 is well characterized and was one of the first markers used to identify HNSCC cancer stem cells (Prince et al. 2007). CD44+ cells isolated from HNSCC express high levels of nuclear Bmi-1, a key epigenetic regulator that controls cell cycle progression of stem cells (Prince et al. 2007). Furthermore, these CD44+ cells possess the capacity to self-renew and differentiate, as demonstrated by serial passaging in vivo to form heterogeneous tumor populations (Prince et al. 2007). A combination of CD44 with other markers, such as the cell adhesion molecule CD24, are more reliable in isolating HNSCC cancer stem cells when compared with CD44 alone (Han et al. 2014).

Several new cell surface antigens have recently been identified as potential markers for HNSCC cancer stem cells. When HNSCC cell lines are treated with cisplatin or radiation, resistant clones are found to express high levels of CD10 (Fukusumi et al. 2014). Through peptidase activation, CD10 generates peptides to support proliferation of stem and progenitor cells. CD10+ cells isolated from HNSCC display enhanced sphere formation in vitro and tumor formation in vivo, as well as increased expression of the stem cell marker Oct3/4 (Fukusumi et al. 2014). Moreover, elevated CD10 has been reported in tumors of refractory HNSCC patients and corresponds to local recurrence, distant metastases, and a higher histologic tumor grade (Piattelli et al. 2006). A recent study used sphere culture to enrich HNSCC cancer stem cells for plasma membrane proteomics (Yan et al. 2013). When compared with matched adherent cells, CD166 (a transmembrane glycoprotein that mediates cell-cell adhesion) had significantly higher expression in spheroid cells. At low cell density, $CD166^{hi}$ cancer cells formed larger tumors than $CD166^{lo}$ cells in nude mice xenograft models and were able to repopulate the heterogeneous tumor population, suggesting cancer stem cell behavior (Yan et al. 2013). Importantly, $CD166^{hi}$ cells are localized to the tumor invasive front of HNSCC, a typical locale for cancer stem cells (Yan et al. 2013).

Apart from cell surface antigens, HNSCC cancer stem cells have been isolated on the basis of functional activities of aldehyde dehydrogenase (ALDH) and ATP-dependent ABC transporters. ALDH is a large family of enzymes that catalyze oxidation of aldehydes to carboxylic acids (Marcato et al. 2011). The ALDH1A1 isoform is commonly thought to be responsible for enhanced ALDH activity in cancer stem cells, including HNSCC (Yang et al. 2014). Side population (SP) cells are a subset of cancer stem cells defined by their ability to efflux Hoechst DNA binding dye and chemotherapeutic drugs via ABC transporters. SP cells have been described in various HNSCC lines and display cancer stem cell properties in vitro and in vivo (Zhang et al. 2009; Tabor et al. 2011; Yanamoto et al. 2011). Interestingly, activation of epidermal growth factor receptor (EGFR), a receptor tyrosine kinase often overexpressed in HNSCC, has been shown to increase SP cells in HNSCC lines. This phenotype can be reversed by addition of EGFR inhibitor (Chen et al. 2006). In another study, elevated Wnt/β-catenin signaling was found in SP cells isolated from HNSCC lines (Song et al. 2010). Targeting these signal transduction pathways may allow for the elimination of SP cells in HNSCC, although this will require a more complete understanding of how EGFR and Wnt signaling modulates SP cell survival and expansion.

While all cancer stem cells are endowed with the ability to initiate tumors, only a subset of cancer stem cells can initiate metastasis. A recent study identified 2 distinct populations of cancer stem cells in HNSCC: $CD44^{hi}/ESA^{hi}$ cancer stem cells are proliferative with an epithelial phenotype, while $CD44^{hi}/ESA^{low}$ cancer stem cells are migratory with a mesenchymal phenotype (Biddle et al. 2011). Intriguingly, among these populations, only cancer stem cells that are also $ALDH1^{hi}$ can revert from mesenchymal to epithelial phenotype to reestablish new epithelial metastasis (Biddle et al. 2011). Similarly, work from our laboratory shows that tumor initiation and metastatic potential of cancer stem cells can be uncoupled. By targeting combinatorial mutations of $Kras^{G12D}$ and $Smad4^{-/-}$ in keratin K15+ stem cells, we generated transgenic mice that developed multilineage tumors (White et al. 2013). K15+ stem cells are located in the bulge area of hair follicles as well as in the bottom rete ridge of the tongue epithelium (Bose et al. 2013). Upon serial passage of these tumors, 2 distinct cancer stem cells (CD34+/CD49f+ and SP cells) were expanded. While both cancer stem cell populations were capable of tumor initiation in vivo, only the SP cells were highly metastatic to the lungs, and expansion of SP cells was attributed to miR-9 overexpression (Fig. 1). We also discovered that SP cells displayed a strong epithelial-to-mesenchymal transition phenotype (White et al. 2013). All together, these studies provide evidence that cancer stem cells possess different tumor initiation and metastasis properties, and they further stress the importance of distinguishing different cancer stem cell populations based on their functional phenotypes.

Current Limitations and Recent Advances in HNSCC Cancer Stem Cell Identification

Current methods of cancer stem cell identification and isolation have some limitations. First, while FACS analysis is a robust tool to identify cancer stem cells, extrinsic factors (e.g., machine calibration, use of proper controls, and compensation/gating protocols) and intrinsic factors (e.g., cell confluency, specific splice variants/isoforms, and clonal variations) can significantly affect the accuracy and reproducibility of results. Second, in the context of cell surface antigens, expression does not necessarily correspond to cancer stem cell behavior or clinical manifestation, as was shown in CD44+ cells in HNSCC (Chen et al. 2014). Finally, stemlike characteristics in cancer cells can be lost or regained depending on interactions with tumor stroma (Chaffer et al. 2011). For instance, without factors from tumor stroma, adherent cells in culture may lose cancer stem cell phenotypes that would otherwise be detectable in animal tumor models with an intact tumor stroma. Therefore, investigating cultured cancer stem cells using immunostaining or FACS is severely limited. A more physiologically relevant context to study cancer stem cells, such as live cell imaging, in tumor tissue is needed.

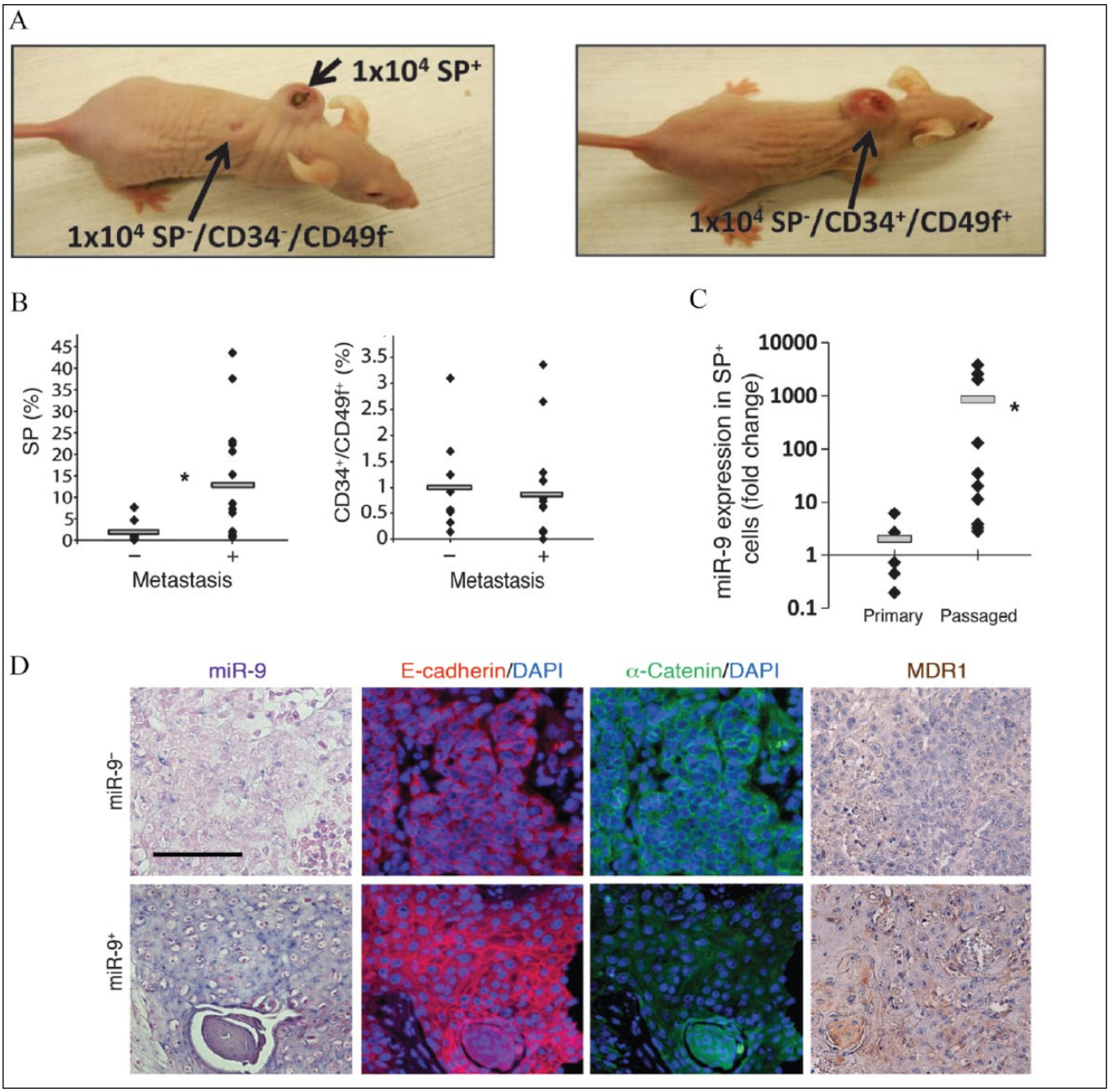

Figure 1. Coexistence of different cancer stem cell populations in tumor. (**A**) Two distinct cancer stem cells (CD34+/CD49f+ and side population [SP]) isolated from serially passaged K15.Kras^{G12D}.Smad4$^{-/-}$ tumors can reinitiate larger tumor formation when compared with the noncancer stem cell counterparts. (**B**) Metastatic tumors have a higher percentage of SP cells (left panel). In contrast, there is no difference between CD34+/CD49f+ cancer stem cells in metastatic and nonmetastatic tumors (right panel). (**C**) SP cells from passaged tumors have increased miR-9 expression when compared with primary tumors. (**D**) SP cells overexpressing miR-9 displayed EMT phenotype, as demonstrated by gain of E-cadherin and loss of α-catenin.

An innovative approach to identify cancer stem cells was recently described to overcome these limitations. A lentiviral-based reporter system was generated to distinguish cancer stem cells based on stem cell transcriptional regulators Nanog, Sox2, and Oct4 (Tang et al. 2015). Specifically, a Nanog promoter was designed to drive 6 tandem repeats of Sox2 and Oct4 regulatory elements (term SORE6), which in turn activated the expression of a fluorescent protein marker (Fig. 2). SORE6+ cells behave like cancer stem cells with the ability to self-renew and differentiate into heterogeneous populations. Furthermore, these cells have an increased sphere-forming capacity, can initiate metastasis in nude mice xenograft models, and are resistant to chemotherapeutic drugs (Tang et al. 2015). In the context of HNSCC, increased expression of Nanog, Sox2, and Oct4 was confirmed in a population of nasopharyngeal carcinoma cells and was specifically localized to the invasive front of the tumor. The expression level also correlated with high tumor grade and poor survival (Luo et al. 2013). Furthermore, expression of

Sox2 and Oct4 as mediated by Lin28B/Let7 was recently shown to promote "stemness" in HNSCC (Chien et al. 2015). These studies suggest the potential use of SORE6 constructs to identify HNSCC cancer stem cells. The SORE6 construct is versatile enough to detect cancer stem cells using intravital or live tissue imaging. A recent study reported the successful culture of an organotypic HNSCC sliced tumor (Gerlach et al. 2014). Tumor slices were viable and could maintain their morphology for up to 6 d in vitro (Fig. 3), thus providing ample time for analysis, such as evaluating SORE6+ cancer stem cells in their natural tumor microenvironment.

Another cutting-edge technology that will significantly affect the study of cancer stem cells is mass cytometry (CyTOF; Han et al. 2015). CyTOF couples the power of flow cytometry (i.e., the ability to analyze a single cell at high speed) with mass spectrometry (i.e., the ability to label cells with >100 stable heavy metal isotopes) to distinguish cancer stem cells using multiparametric analyses. Importantly, the use of unique heavy metal isotopes rather than fluorochromes eliminates the need for manual compensation associated with spectral overlap and autofluorescence, thereby reducing variability of results (Leipold and Maecker 2012). The characterization of sorted cancer stem cells from a phenotypic and functional perspective can now be achieved at the single-cell level. For instance, a recent study used CyTOF to identify leukemia stem cells and detected a myriad of phosphoproteins specifically activated in leukemia stem cells harboring a FLT3 mutation (Han et al. 2015). The full potential of this technology is just beginning to emerge and will allow us to simultaneously profile HNSCC cancer stem cells from phenotypic and functional approaches, as well as study drug interactions and responsiveness in cancer stem cells.

Tumor Immune Network and Relationship with Cancer Stem Cells in HNSCC

Primary tumors grow within a complex network of host stromal tissue consisting of epithelial cells, vascular and lymphatic vessels, cytokines and chemokines, and infiltrating immune cells (Fridman et al. 2012). Immune cell types and populations within tumors vary from patient to patient. These cells include macrophages, dendritic cells, mast cells, natural killer (NK) cells, naïve and memory lymphocytes, B cells, effector T cells (including T-helper cells [Th1, Th2, Th17], T-regulatory [Treg] cells, and T-follicular helper cells), and cytotoxic T cells (Fridman et al. 2012).

These cells, represented in Figure 4, play an essential role in tumor promotion and suppression. Cytotoxic T lymphocytes (CTLs) and CD8+ effector T cells release perforin and granzyme leading to tumor cell apoptosis. Activated CTLs can migrate to peripheral tissues to directly kill tumor cells either by contact-mediated cytotoxicity or by indirect secretion of cytokines (Ahlers and Belyakov 2010). The difficulty in targeting T cells is described as immune tolerance. Causes for immune tolerance include anergy, exhaustion, and suppression, which occur by incomplete activation, chronic antigen exposure, and T-cell inhibition by other immune cells, such as Treg and stromal cells (Lesokhin et al. 2015; Liotta et al. 2015).

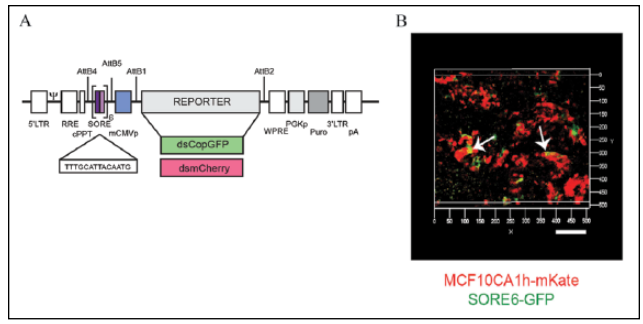

Figure 2. The SORE6 reporter system identifies cancer stem cells based on functional properties. (**A**) SORE6 lentiviral reporter system allows identification of putative cancer stem cells based on expression of Nanog, Oct2, and Sox4. (**B**) The lentiviral construct can be used to identify cancer stem cells in tumor section. Figures reprinted with permission from Tang et al. (2015).

A naïve T cell requires activation through interaction of a T-cell receptor and an antigen presented by the major histocompatibility complex (MHC) molecules on antigen-presenting cells in addition to a costimulatory signaling (Lafferty and Cunningham 1975). In laryngeal squamous cell carcinoma, there is evidence of MHC class I downregulation allowing for immune evasion. This finding was associated with low CTLs, increased lymph node metastasis, and reduced survival in 50% of HNSCCs (Ogino et al. 2006). It has been reported that normal hematopoietic stem cells downregulate MHC class I, suggesting that low MHC class I expression favors stem cells, including cancer stem cells in HNSCC (Le Blanc et al. 2003). In cancer stem cells, the antigen-presenting machinery for MHC class I and class II, such as transporter associated with antigen processing, low molecular weight protein, and β-microglobulin, are all downregulated when compared with noncancer stem cells (Di Tomaso et al. 2010).

NK cells and macrophages are major effector cells for innate immunity. NKG2D is an activating receptor on NK cells and CD8+ T cells. NKG2D binds to MHC class I chain-related proteins. Overexpression of NKG2D ligands in cancer cells leads to tumor rejection in mice and humans. There is also a correlation between NKG2D overexpression and T-cell infiltrates. This observation can be attributed to an upregulation of NKG2D ligands in response to DNA damage and stress in cancer cells (Soriani et al. 2009). HNSCC stem cells in the primary tumor were shown to be more susceptible to NK-targeted killing as compared with their differentiated counterparts, depending on ligand expression (Tseng et al. 2010; Tallerico et al. 2013). A higher number of NK cells in HNSCC patients is correlated with longer overall survival and better outcomes; this has been shown in esophageal, breast, gastric, colorectal, and metastatic carcinoma, whereas the lack of MHC class I and NKG2D ligand expression results in resistance to immune-mediated killing (Taghavi et al. 2015). Overall, it has been shown that NK cells are effective against cancer stem cells in HNSCC. It has yet to be demonstrated whether NK cells are downregulated or inefficient in more aggressive HNSCCs.

Tumor-associated macrophages (TAMs) are major inflammatory components of the tumor microenvironment (Chanmee

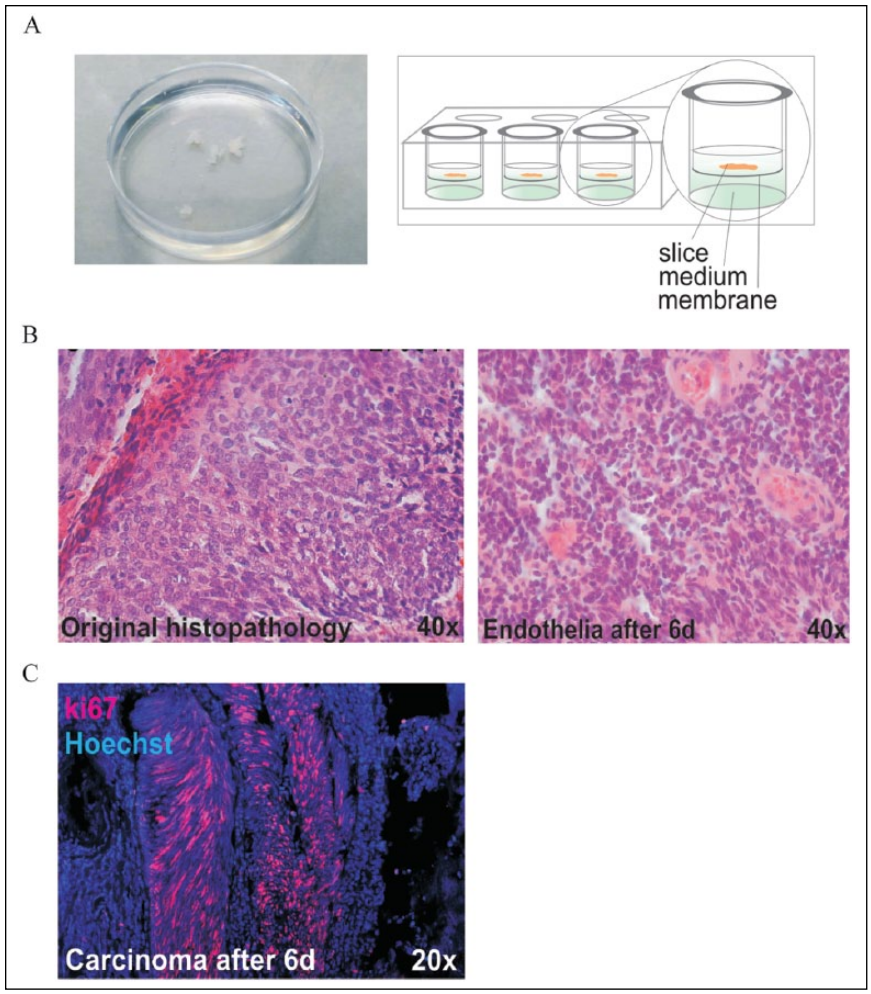

Figure 3. Organotypic slice culture of head and neck squamous cell carcinoma. (**A**) Left panel shows organotypic head and neck squamous cell carcinoma tumor slices after sectioning with a tissue chopper at 350-µm thickness. Right panel is a drawing depicting the suspension of tumor tissue slices on membrane culture insert. The air-media interface allows oxygen and nutrient exchange to prolong viability of tissue in culture. (**B**) Comparable morphology of primary tumor (left) and tissue slices (right) after 6 d of in vitro culture. (**C**) High proliferation is maintained in cancer cells after 6 d of in vitro culture. Figures reprinted with permission from Gerlach et al. (2014).

et al. 2014). TAMs are polarized into M1 and M2 macrophages by the influence of tumor cells and microenvironment. M1 phenotypic macrophages kill pathogens and promote activation of CTLs (Coffelt et al. 2009). M2 phenotypic macrophages stimulate a CD4+ and Treg T-cell response, which are immunosuppressive and promote angiogenesis and tissue remodeling. M2 TAMs are responsible for the active secretion of TGF-β, IL-6, IL-10, and endothelial growth factor (EGF), which prime the microenvironment for tumor growth (Qian and Pollard 2010). While high M2 TAMs have been associated with a worse prognosis, other studies have shown M1 phenotypic TAMs are correlated with a good prognosis and promote antitumor functions (Yang et al. 2015).

Cytokines produced by tumor and host stroma determine the primary tumor microenvironment. Cancer stem cells primarily reside in the invasive tumor front, an area essential for growth, signal transduction, and cell fate determination (Borovski et al. 2011; Hildebrand et al. 2014). Tumor cells recruit immunosuppressive cells, such as M2 polarized TAMs, by secreting colony-stimulating factor, TGF-β, and vascular EGF, as well as chemokine ligands 2, 3, 4, 5, and 8 (Coffelt et al. 2009). Specifically, IL-1, IL-6, and IL-8 produced by monocytes and macrophages are recruited to the tumor and are involved in driving cancer stem cell renewal (Korkaya et al. 2011). These cytokines activate STAT3/NF-κB signaling pathways, which in turn stimulate additional cytokine production and macrophage recruitment (Korkaya et al. 2011). Mice with xenograft CAL27 human HNSCC line treated with inhibitors to signaling molecules, such as MAPK-38 and mTOR, have shown a significant downstream reduction in tumor-promoting cytokine expression, resulting in reduced lymphogenesis and angiogenesis (Leelahavanichkul et al. 2014; Dillenburg-Pilla et al. 2015). These studies suggest that antibody blockade of cytokine receptors and signaling molecules may be an effective method for cancer stem cell depletion.

Recently, lethally irradiated NOD SCID gamma mice humanized with cord blood hematopoietic stem cells (known as XactMice) were developed (Morton et al. 2015). This model enables study of HNSCC in a microenvironment educated by human cytokines, T and B cells, and stroma. This mouse model re-creates the human tumor microenvironment in a way that the other current animal models are lacking. The stroma has been shown to promote progression of HNSCC and is a key component in the tumor microenvironment. Tumors and other sites of inflammation actively recruit stromal cells by expression of IL-6 (Rattigan et al. 2010). Tumor cells are directly influenced by stromal cells to increase angiogenesis, induce immunosuppression, and inhibit apoptosis (Liotta et al. 2015). Importantly, human HNSCC cells xenografted into recipient animals have enhanced tumor growth when coinjected with stromal cells (Kansy et al. 2014).

Cancer Stem Cell Niche and Metastasis in HNSCC

Genome sequencing has suggested that cancer cells in the primary tumor can accumulate mutations vital to becoming metastatic (Yachida et al. 2010). A recent study showed that the incidence of mouse oral cancer metastasis is genetically

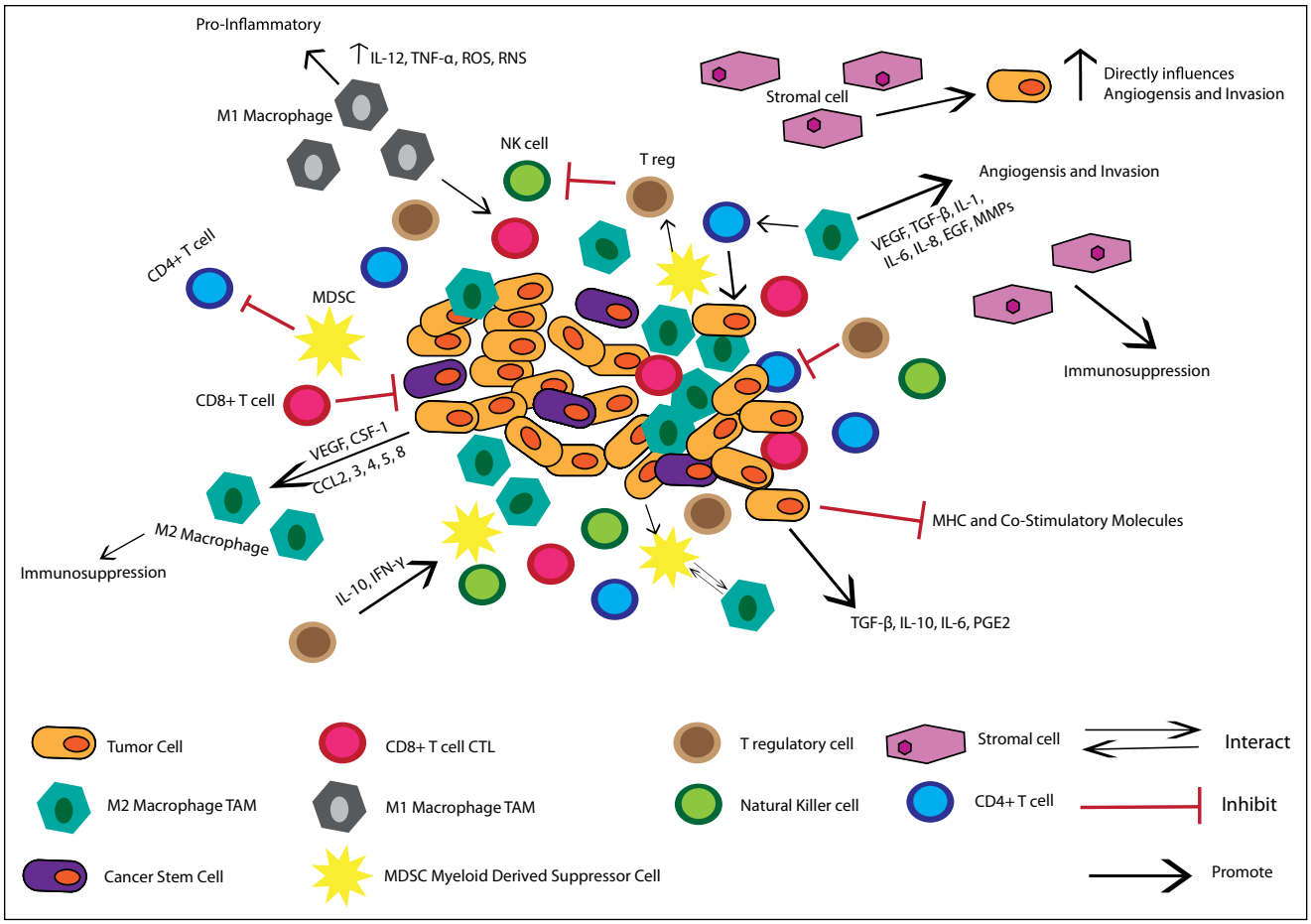

Figure 4. The proinflammatory and immunosuppressive mechanisms of the tumor microenvironment allow for tumor growth and suppression of the immune system. Cancerous cells are able to escape the immune system by loss of functions of major histocompatibility complex (MHC) class I and costimulatory molecules and production of immunosuppressive molecules such as TGF-β, PGE2, IL-6, and IL-10. CD4+ T cells are able to promote Th17 and other inflammatory molecules, whereas Tregs inhibit CD4+, CD8+, and NK cells. CD8+ T cells are effective killers of cancer stem cells. M2 tumor-associated macrophages (TAMs) induce immunosuppressive CD4+ T cells and Tregs and promote tumor growth by release of cytokines (EGF, TGFβ, IL-1, IL-6), angiogenesis (TGFβ, VEGF, IL-1, IL-6, IL-8), and invasion (MMPs, IL-1, TNFα). M1 TAMs promote tumor killing by activating CD8+ T cells and secreting IL-12, TNFα, reactive oxygen species, and reactive nitrogen species. Myeloid-derived suppressor cells induce Tregs, secrete IL-10, and inhibit CD4+ and CD8+ T cells. Stromal cells are involved in downregulation of immune function and in modulation of tumor cells to increase proliferation, angiogenesis, invasion, and metastasis. CTL, cytotoxic T lymphocyte; NK, natural killer.

conserved in human data sets, allowing for the potential use of novel genetic biomarkers derived from animal models to predict metastasis (Chalivendra et al. 2015). Metastases derived from the primary tumor are resistant to chemotherapy and have the capacity for long-term self-renewal (Kreso and Dick 2014). The characteristics and stem markers of metastatic stem cells suggest that they may originate from cancer stem cells found in the primary tumor.

The tumor microenvironment plays a pivotal role in allowing and directing cancer stem cells to seed distal organs. Cancer cell dissemination is actively influenced by paracrine factors, such as vascular EGF, EGF, matrix metalloproteinases, and M2 TAMs (Oskarsson et al. 2014). For example, migrating tumor cells secrete EGF to create a gradient effect, which guides additional cancer cells and macrophages to the site of metastasis (Noy and Pollard 2014). EGFR is overexpressed in >95% of HNSCC patients, and increased phosphorylation of downstream kinases correlates with poor patient outcome, lymph node metastasis, and poor tumor differentiation (Sok et al. 2006). Furthermore, it was shown that macrophages line the vasculature and direct migration of cancer cells to distant organs hospitable for cancer stem cell seeding (Schreiber et al. 2011). Interestingly, a recent study based on a nude mouse model xenografted with HNSCC human cell lines showed that primary tumor growth occurs concurrently with metastasis to cervical lymph nodes and that these processes are dependent on tumor-host interactions (Oskarsson et al. 2014). Thus, cancer stem cells from primary and metastatic tumor niche designate the locations, stromal cell types, signaling efficacy, and extracellular matrix integrity required to establish the metastatic site (Oskarsson et al. 2014).

Targeting of Cancer Stem Cells with Immunotherapy in HNSCC

Stem cells have become the target for novel treatment strategies due to their ability to withstand chemotherapy, high rate of

reoccurrence, and role in metastasis (Davis et al. 2010). ALDH is a specific marker for cancer stem cells and has been the target of in vitro primed CD8+ T cells. Elimination of $ALDH^{hi}$ cancer stem cells results in tumor regression, prevention of metastasis, and prolonged life in immunodeficient mice xenografted with HNSCC (Marcato et al. 2011). Recently, generation of anti-CD3/anti-CD133 antibodies bound to cytokine-induced killer cells was shown to target $CD133^{hi}$ cancer stem cells (Huang et al. 2013; Li et al. 2015). To target the surrounding stroma of the tumor and to increase T-cell proliferation, stroma cells cocultured with T cells were treated with an indoleamine 2,3-dioxygenase inhibitor (methyl-L-tryptophan) and resulted in a downregulation of immune-regulatory effects on T-cell proliferation (Kansy et al. 2014). These studies suggest the potential of generating cancer stem cell–activated T cells in vitro, which are able to directly target and eliminate tumors in vivo, as well as eliminate a potent T-cell inhibitor, thereby allowing for an increased T cell–mediated response.

The development of cancer vaccines that target cancer stem cells through dendritic and other professional antigen-presenting cells has been promising. Dendritic cells pulsed with cancer stem cells, such as $ALDH^{hi}$, are more effective as an antigen source in inducing a protective antitumor immune response than whole lysate or nontumor stem cells (Ning et al. 2012). Primed cancer stem cells express the necessary costimulatory molecules—CD80, CD86, and CD40—to produce a significant Th1 response in vitro (Li et al. 2015). Other dendritic cell targeting is achieved with neurospheres enriched in cancer stem cells; these dendritic cells provide a higher level of protection against specific tumor types. The above studies highlight the importance of cancer stem cells as potential therapeutic targets for treating solid tumors. Future investigations focusing on the potential cancer vaccination of HNSCC cancer stem cells will expedite its translation to the forefront of adjuvant therapy.

Conclusions

Given the multifaceted roles of cancer stem cells in initiation, progression, metastasis, and relapse of HNSCC, significant efforts have been expended to identify cancer stem cells from cell lines and tumors derived from patient samples and mouse models of HNSCC. Current identification methods have limitations that need to be addressed. Sophisticated systems, such as CyTOF, can distinguish cancer stem cell populations based on global phenotypic and functional changes to reveal molecular signatures unique to these cells. No doubt, these molecular profiles will allow us to distinguish patient-specific cancer stem cell subsets and pinpoint these cell populations in the earlier stage of HNSCC for timely diagnosis and treatment. Incorporating real-time imaging to study cancer stem cells in live HNSCC tissue will allow us to further understand the molecular interactions between tumor and stroma that contribute to survival and acquired metastatic potential of cancer stem cells. During HNSCC formation, the immune system actively modulates the tumor and its surrounding microenvironment by expression of immunologic markers. These specific markers will provide prognostic insights and help predict responsiveness to tumor immunotherapy. Finally, it is of profound importance to understand the biology of cancer stem cells and their interactions within the complex tumor niche to design an effective combination of therapies targeting HNSCC cancer stem cells.

Author Contributions

L.K. Dionne, E.R. Driver, contributed to data acquisition and interpretation, drafted and critically revised the manuscript; X.J. Wang, contributed to data acquisition and interpretation, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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