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ORAL NEOPLASIA (F ALAWI AND A LE, SECTION EDITORS)

Oral Cancer and Cancer Stem Cells: Relevance to Oral Cancer Risk Factors, Premalignant Lesions, and Treatment

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Abstract Cancer stem cells are recognized as the most critical cancer cells. They are responsible for cancer progression, the development of metastasis, and treatment failures. There are a number of well-studied surface proteins and enzymatic processes that can be used to isolate cancer stem cells from the bulk of the other cancer cells. The role of cancer stems cells in premalignant lesions of the oral cancer is poorly understood but slowly evolving. Novel therapies are being developed to more effectively eradicate cancer stem cells and improve patient outcomes. Efforts to improve our understanding of this important subpopulation of cancer cells is vital in directing further studies to advance our ability to prevent patients from developing oral cancer and to providing more effective treatment for those that do.

Keywords Oral cancer · Cancer stem cells · Leukoplakia · Erythroplakia

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Introduction

The identification of a subset of highly tumorigenic cells in head and neck cancer has resulted in a dramatic change in our understanding of cancer development, progression, and therapy. These highly tumorigenic cancer cells are referred to as cancer stem cells (CSC). CSC have been identified in most solid tumors including squamous cell cancer of the head and neck. There is accumulating evidence that CSC are the critical cell population frequently responsible for treatment failures, metastasis, and recurrence [1•, 2•].

After the initial identification of CSC, there was considerable optimism that this discovery would rapidly lead to novel and more effective treatments for cancer. Further studies have revealed that complex molecular mechanisms and interactions with surrounding cells and tissues regulate CSC behavior. Despite these challenges, our understanding of this critical population of cancer cells continues to increase, and we are beginning to see the development of therapies that can specifically target CSC.

Cancer cells must exhibit three key characteristics to be considered CSC: tumorigenicity, self-renewal, and the ability to reproduce tumor heterogeneity. Typically, CSC represent a small subpopulation of all the cancer cells. Exploitation of differences in cell surface protein expression and/or biologically active enzymes allow the CSC to be isolated from other cancer cells for study. Although the ability of individual cancer cells to produce "spheroids" in low attachment growth conditions is now widely utilized to confirm their status as CSC, the gold standard remains implantation of the cells in animal models with demonstration of tumor growth, the recreation of a heterogeneous cancer cell population consistent with the original tumor and the ability to serially passage the cancer stem cells.

Cancer Stem Cells Markers in Oral Cavity Squamous Cell Cancer—An Overview

Cancer stem cells are identified by their differential expression of specific molecular markers or enzymatic reactions in comparison to the other cancer cells. By exploiting the expression differences of CSC specific markers, flow cytometry can be employed to separate the CSC population from the other cancer cells. Isolating CSC from the bulk of cancer cells is the key step in investigating the factors that regulate their biologic behavior [3–5].

Highly tumorigenic cancer cells, the CSC, were first isolated from leukemia and were shown to have the ability to regenerate the primary leukemia [6]. The leukemic CSC were identified by their expression of the cell surface proteins CD34 and CD38 [7]. Al-Hajj et al. reported the first isolation of CSC from a solid tumor. They found that breast cancer cells expressing high levels of CD44 and low levels of CD24 were tumorigenic, able to recreate the heterogeneity of the primary tumor and demonstrated a capacity for unlimited self-renewal [7]. CSC have now been isolated from almost every solid tumor type including those arising in the oral cavity [7–15].

CD44

In 2007, a small subpopulation of highly tumorigenic head and neck squamous cell cancer (HNSCC) cells with a stem cell-like phenotype were isolated using the surface protein CD44 [8]. This was the first report of the isolation of CSC in HNSCC. The identification and collection of cancer cells expressing high levels of CD44 is achieved with flow cytometry utilizing fluorochrome-conjugated anti-CD44 antibodies that bind to epitopes within the conserved region of the CD44 extracellular domain to identify the cells of interest.

Studies have shown that HNSCC cells expressing high levels of CD44 ($CD44^{high}$) have a more primitive morphologic appearance and express higher levels of the stem cell marker BMI-1. BMI1 (B lymphoma Mo-MLV insertion region 1 homologue) regulates self-renewal in a wide range of tissue stem cells and can function as an oncogene through its regulation of p16 and p19, which are cell cycle inhibitor genes [16]. In an immunocompromised mouse model, $CD44^{High}$ HNSCC cells are tumorigenic, reproduce tumor heterogeneity, and can be serially isolated and transplanted indicating their capacity for self-renewal [9]. The $CD44^{high}$ cells have been shown to be highly metastatic [17]. Alternative splicing is the basis for the structural and functional diversity of CD44, and CD44 splice variants seem to be related to tumor progression, invasion, and metastasis. Downstream effects of CD44 binding include invasion and metastasis [18]. $CD44^{high}$ oral cancer CSC populations can be further sub-divided into motile or non-motile phenotypes depending on the levels of epithelial specific antigen expression [19]. By genetic labeling of oral cancer cell lines, it has been shown that $CD44^{high}$ CSC comprise the majority of the invasive oral cancer cells that were resistant to the DNA damaging effects of ionizing radiation [20, 21]. CD44 provides a potential therapeutic target for HNSCC CSC but this requires further investigation.

ALDH

Attempts to identify a more selective marker for HNSCC CSC revealed that aldehyde dehydrogenase (ALDH) can be used to isolate CSC from HNSCC [9, 22]. The ALDH+ CSC are more tumorigenic than $CD44^{high}$ HNSCC CSC with the capacity to produce tumors in an immunocompromised mouse model with as few as 50 cells, a tenfold reduction from the number of $CD44^{High}$cells required [9]. ALDH was initially identified as a normal stem cell marker and then as a cancer stem cell marker in a number of tumors including myeloma, breast cancer, colon cancer, leukemia, and HNSCC [9, 22, 23]. The ALDH enzyme family contains 19 separately encoded genes. Two isoforms, ALDH1A1 and ALDH3A1, are known to play a critical role in adult stem cells [24]. ALDH catalyzes the oxidation of toxic aldehydes which are generated by oxidative stress. As a result, ALDH activity is vital to the regulation of the oxidative stress response including that caused by radiation and chemotherapy. ALDH activity is significantly upregulated during fractioned irradiation and cells with high levels of ALDH expression are more resistant to the effects of radiation [25]. These results suggest that HNSCC CSC, which express high levels of ALDH, will be more likely to survive and maintain their tumorigenic potential after irradiation. ALDH activity is measured by an enzymatic reaction, and ALDH-positive cells are collected using flow cytometry. Although ALDH proves to be an excellent marker for the CSC isolation and characterization, given that it is also expressed in normal adult stem cells, it is not likely to provide a useful therapeutic target.

CD133

CD133 is a transmembrane glycoprotein that is expressed by hematopoietic stem cells, endothelial precursor cells, glial stem cells, and prostate epithelial stem cells [26]. CD133 was originally described as a CSC marker in leukemia and glioblastoma [2]. It has been reported to be a marker of CSCs in the brain, colon, pancreas, prostate, liver, lung, kidney, and esophagus [26]. In 2007, Zhou et al. determined that laryngeal CD133+ cells have a CSC phenotype [27]. Studies in oral cancer have shown that a low percentage of tumor cells are CD133+. These cells are highly tumorigenic and have increased resistance to chemotherapy. Although CD133 may

prove to be a useful CSC marker in HNSCC, other researchers have failed to identify CD133+ cells in oral cancer [28, 29]. Therefore, to understand the role of CD133 in HNSCC CSC would require further investigations.

c-Met

c-Met signaling has been reported as a critical regulator of tumor progression, tissue invasion, and metastasis. Upregulation of c-Met expression in prostate cancer cells induced a stem cell-like phenotype, and c-Met expression has been found to be critical to colon CSC maintenance [30, 31]. c-Met has been demonstrated to be an important factor regulating myogenesis in the developing tongue indicating a role in stem cell regulation in oral tissues [32]. There is evidence that higher levels of c-Met expression results in shorter survival rates in patients with tongue cancer [33]. Together, these results indicate possible implications of c-Met signaling in oral tumorigenesis. The importance of c-Met expression in oral CSCs was confirmed by Lim et al. [34]. They reported that in a c-met knockdown model, the sphere-forming capacity and stem cell markers expression in HNSCC CSC were diminished. Knockdown of c-Met expression also augmented cisplatin sensitivity by decreasing side population cells, inhibited tumor formation in a xenograft mouse model, and increased the survival of the mice [34]. c-Met provides a potential opportunity to target CSC in HNSCC by altering their behavior and increasing their sensitivity to chemotherapy.

Side Population (SP)

Fluorescence activated cell sorting (FACS) is a standard method for identifying CSC which also allows, through exclusion of the vital DNA dye Hoechts 33342, isolation of a subpopulation of cells that efflux the dye via cell membrane ABCG2 transporter pump activity. These cells are referred to as the side population (SP). SP cells can be separated from the non-dyed population of cells, express CSC markers, and have significant tumorigenic potential [35–39]. In HNSCC, SP cells are highly tumorigenic and have been shown to express stem cell markers including BMI-1 and Oct4 [28]. The size of SP population has been found to be small ranging from 0.2 to 10 % of the cancer cell population in HNSCC cell lines and primary tumors [28]. Activity of transporter pump ABCG2 in HNSCC CSC, as is evident in SP cells, suggests inhibiting the activity of these transporters might be an effective strategy to increase the CSC sensitivity to chemotherapy.

Relevance of Cancer Stem Cells to Oral Cavity Cancer

Worldwide, the number of new patients with oral squamous cell cancer is estimated to exceed 300,000 annually [40]. Traditionally, oral cavity cancer was considered a predominantly male illness, affecting six men for every woman. During the past 10 years that male preponderance has decreased markedly to a 2:1 ratio and has shifted to include a much younger patient population [40]. Development of oral cancer has been shown to progress through a series of molecular genetic changes. The mutations and epigenetic changes that result in a malignant phenotype are acquired from the loss of genomic integrity typically in association with exposure to various risk factors. Our understanding of the role of CSC in the development of oral cancer is at the early stages of investigation but holds promise for the development of more effective therapy for premalignant and malignant lesions.

CSCs and Risk Factors for Oral Cancer

Although the cell of origin of CSC in HNSSC remains controversial, there is accumulating evidence that indicates normal stem cells may provide the major source. Evidences indicate that other cancers besides those developed within the gastrointestinal tract (e.g., central nervous system, skin, etc.) also initiate from the normal stem cells [41•, 42–44]. Normal mucosa in the upper aero-digestive tract contains stem cells residing in the basal layer. It has been proposed that transient amplifying (TA) cells, derived from stem cells may be another source for CSC. However, TA cells are slightly more differentiated than stem cells and highly proliferative, but do not live long enough to accumulate the multiple genetic mutations and epigenetic changes required for malignant transformation. Recently, Tang et al. reported that oral papillomas and oral squamous cell cancer arise from the tongue epithelial basal stem cells [44]. There is accumulating data suggesting that carcinogens and other factors previously reported to be associated with head and neck cancer (e.g., smoking, alcohol, human papilloma virus (HPV) infection, and long-term inflammation) have transformative effects on normal stem cells that can lead to a malignant phenotype.

Tobacco and alcohol use are the best known risk factors for the development of HNSCC. These two factors have a synergistic effect and traditionally have been held to be responsible for the majority of the squamous cell carcinomas that develop in the head and neck area. However, these risk factors do not account for as many as 40 % of oral cavity squamous cell cancers. It is apparent that yet unidentified genetic, environmental, infectious, and nutritional factors could influence the risk of developing head and neck cancer [45].

Tobacco use has long been recognized as an independent contributor to the development of many cancers including oral cancer. Overwhelming epidemiologic and scientific evidence confirms that tobacco use, in any form, is a significant risk factor for the development of cancer [46]. A variety of carcinogens are present in tobacco smoke and smokeless tobacco products including polycyclic aromatic hydrocarbons, nicotine, aldehydes, and heavy metals. These compounds lead to the formation of DNA adducts, cause oxidative damage, and disrupt the cell growth cycle, which can contribute to the development of cancer [47].

Oral epithelial cells subjected to long-term nicotine exposure exhibit enhancement of their stem cell like molecular signature as revealed by an increase in ALDH activity in a dose-dependent fashion. Nicotine exposure also increases the proportion of CD133-positive cells. Interestingly, inhibition of the zinc-finger transcription factor, Snail, blocks the nicotine-initiated stem-ness conversion and epithelial to mesenchymal transition in these cells. This suggests a possible therapeutic role for Snail inhibition that would target the CSC population [48].

Heavy alcohol consumption is a recognized risk factor for the development of malignant tumors of the upper aero-digestive tract with a known synergistic effect when used in combination with tobacco. Alcohol and its metabolites, particularly acetaldehyde, have numerous effects on cell biology including activation of the cytochrome P-450 pathway, excess formation of reactive oxygen species with resultant cellular damage and cell cycle deregulation. Acetaldehyde is a major ethanol metabolite which is produced by ALDH. Acetaldehyde interferes with DNA synthesis and repairs machinery which can lead to mutagenic changes and malignant transformation [49, 50]. To date, there has been very limited research investigating the direct link between alcohol ingestion and CSC.

In a recent in vivo study, simulating exposure to ethanol and tobacco a change in the stem cell population and behavior in tongue epithelium was observed. It also reported that the addition of ethanol to tobacco exposure enhanced the stem cell altering effects over tobacco exposure alone [51]. There is an obvious need for further investigation into potential relationships between CSC and alcohol and tobacco consumption.

Human papilloma virus (HPV) is a primary etiologic factor for HNSCC that occurs in the oropharynx, including the tongue base. HPV-related HNSCC is associated with younger age at the time of cancer diagnosis [52]. Interestingly, when compared to smoking related HNSCC, unique pathologic profiles have been reported that are consistent with the changing incidence of HPV-related HNSCC. Patients with HPV-associated HNSCC have a different risk profile, associated with less tobacco and alcohol use, and improved survival compared to patients with HPV-negative HNSCC. Despite the overall better prognosis for patients with HPV-associated HNSCC, approximately 30 % of HPV+ tumors fail to respond to treatment, recur locally, or spread distantly [52, 53].

Recently, HPV infection has been linked to the development of cancer at other sites than the oropharynx, although at a much lower rate [54]. In the head and neck, persistent infection with HPV mainly occurs in the reticular cryptic epithelium in the tonsils and lingual tonsil. HPV genotype 16 is the most common HPV involved in HNSCC development. HPV displays marked tropism for the epithelial basal layer where the adult epithelial stem cells responsible for tissues renewal reside [55, 56]. Viral gene expression in these basal cells is limited to the early viral genes E5, E6, and E7. These genes are known to disrupt the cell cycle via production of proteins altering cell cycle checkpoints. This occurs as a result of the inactivation of tumor suppressor genes including p53 and Rb and by interfering with other key cellular proteins involved in apoptosis and malignant cell transformation [55, 56].

The effects of HPV on CSCs are poorly understood. Patients with HPV+ HNSCC seem to respond better to standard treatment than those with traditional risk factors and have a better overall prognosis [57•, 58]. It was hypothesized that patients with HPV+ HNSCC may respond more favorably to treatment because HPV+ tumors might harbor fewer CSC. Several studies have refuted this hypothesis and revealed that CSC frequency is not consistently lower in HPV+ HNSCC tumors or cell lines [59, 60]. The expression of the CSC marker CD44 was lower in patients with HPV+ HNSCC than in those with HPV- HNSCC [61]. In contrast, there is evidence that HPV16-positive HNSCC is associated with increased ALDH staining [60]. Together, these conflicting results suggest that the CSCs biologic phenotype weights heavier than their absolute number in predicting a favorable response to treatment as seen in HPV+ tumors [59, 60]. Tang et al. determined that CSC from HPV+ and HPV-HNSCC cell lines are equally resistant to cisplatin therapy, in contrast with the difference in treatment outcomes by HPV status [59]. Epidermal growth factor receptor (EGFR) is a member of the ErbB family of receptors, its activation by endogenous ligands results in activation of intracellular tyrosine kinase. Abnormal activation of the EGFR, which is common in head and neck cancer, leads to enhanced proliferation and other tumor-promoting effects [62]. The inverse relation between EGFR levels and HPV status in oral cavity-derived cancer cells may provide the explanation for the better response rates of HPV-associated oropharyngeal cancer [58]. Additional work is needed to better delineate the biological difference between CSC residing in HPV + versus HPV- HNSCC.

It is widely accepted that chronic inflammation predisposes individuals to the development of a variety of cancers. Accumulating evidence implicates oral bacteria as a causative factor in the etiology of oral cancer. Population-based studies report a significantly increased risk of oral and esophageal

cancers in patients with pre-existing tooth loss and/or periodontal disease. These findings strongly suggest that chronic periodontal disease may be a contributing factor to oral carcinogenesis [63]. The local and systemic inflammatory response induced by oral bacteria may also play a role in the activation of tobacco- and alcohol-related carcinogens and have been implicated in the progression of oral cancer [64].

A recent study developed a model of chronic periodontal disease with repeated infection and prolonged exposure of oral cancer cells to porphyromonas gingivalis [65]. This experimental model induced morphological changes in cancer cells towards an elongated shape associated with decreased expression of epithelial cell markers, suggesting acquisition of an epithelial-to-mesenchymal transition phenotype. Prolonged exposure to *P. gingivalis* also promoted the invasive properties of oral cancer cells as well as increasing their resistance to the chemotherapy agent Taxol. Intriguingly, long-term infection with *P. gingivalis* induced an increase in the expression levels of CD44 and CD133 and promoted the tumorigenic properties of cancer cells when compared to non-infected counterparts [65]. Controlling periodontal disease may not only be beneficial for oral cancer prevention (and of other cancers) but might play an important role in HNSCC treatment by decreasing the CSC phenotype.

Cancer Stem Cells and Leukoplakia, Erythroplakia, and Field Cancerization

Oral leukoplakia and erythroplakia are two oral pre-malignant lesions with a propensity for progression to invasive cancer. Leukoplakia is the most common oral premalignant lesion, with a frequency of malignant transformation between 17 and 35 % [66]. Histologic grading of epithelial dysplasia is currently the most important factor for determining its risk of malignant transformation; however, histologic grading has the potential for subjectivity. Currently, there are no effective treatments that prevent malignant transformation. The development of more accurate predictors of malignant transformation and effective therapy for leukoplakia are needed. In a recent study, the utility of the CSC markers ALDH and CD133 in predicting malignant transformation of leukoplakia was assessed. Kaplan–Meier analysis revealed that 48.1 % patients with ALDH1-positivity developed oral cancer compared with only 12.6 % of those with ALDH1-negativity. As for CD133, 59.4 % patients with CD133-positivity developed oral cancer compared with only 16.5 % of those with CD133-negativity [67]. Collectively, these data demonstrated that ALDH1 and CD133 expression can be correlated with malignant transformation in patients with leukoplakia, suggesting that they may serve as predictors to identify leukoplakia with a high risk of oral cancer development [67]. The presence of HNSCC CSC markers in high-risk leukoplakia also suggests a role for pre-malignant progenitors for the CSC-driven progression of leukoplakia to cancer.

Oral erythroplakia is relatively uncommon but has a high reported rate of malignant transformation of 14–67 % [68, 69]. Little is known about the factors that predict malignant conversion in erythroplakia. Evaluation of the HNCSS stem cell markers BMI-1 and ALDH in erythroplakia indicate that they can be used to stratify the risk of developing a malignancy. ALDH1 expression alone was significantly associated with an increased risk of malignant transformation. The combination ALDH1 and BMI-1 expression was a strong indicator for malignant transformation with a positive predictive value of 78.6 % [68]. Similar to leukoplakia, the strong association of two CSC markers with the risk of malignant transformation suggests a potential role for cancer stem cell precursors in the fate of these lesions.

The concept of field cancerization is generally accepted and proposes that normal tissue adjacent to the cancer contains cells with a pre-neoplastic molecular profile which might eventually lead to development of recurrent cancer or second primary tumors [70]. The cellular basis of field cancerization is explained by two competing models. The polyclonal origin theory proposes that mutations occur in cells located at multiple sites due to carcinogen exposure and have the potential to lead to multi-focal carcinomas or multiple independent tumors. In this situation, tumor arising in adjacent fields would be genetically different. In contrast, the monoclonal origin theory theorizes that mutated cells from the initial lesion migrate to separate areas and develop multiple lesions that share a common clonal origin [70, 71]. Currently, our understanding of the inherent properties of the CSCs including tumorigenesis and migration and the detection of CSC-specific markers in the normal mucosa adjacent to cancers strongly suggests that CSC play a role in the field cancerization. Additionally, CSC behavior can support both theories of field cancerization (Fig. 1).

Cancer Stem Cell Directed Therapy

Oral cancer is the most prevalent and aggressive epithelial tumor of the head and neck region with the poorest outcome and high rate of failure to traditional therapies including surgery, chemotherapy and radiotherapy. Significant contributing factors for radiation and chemotherapy resistance relate to CSC properties including a slow cell division rate, capacity for DNA repair, and high expression of drug-efflux pumps. Given that even a single remaining CSC has the ability to recreate a new tumor, targeting and eradicating CSC is key in our efforts to achieve better cure rates. Multiple strategies have been proposed that would target the CSC subpopulation by utilizing their expression of surface proteins, targeted

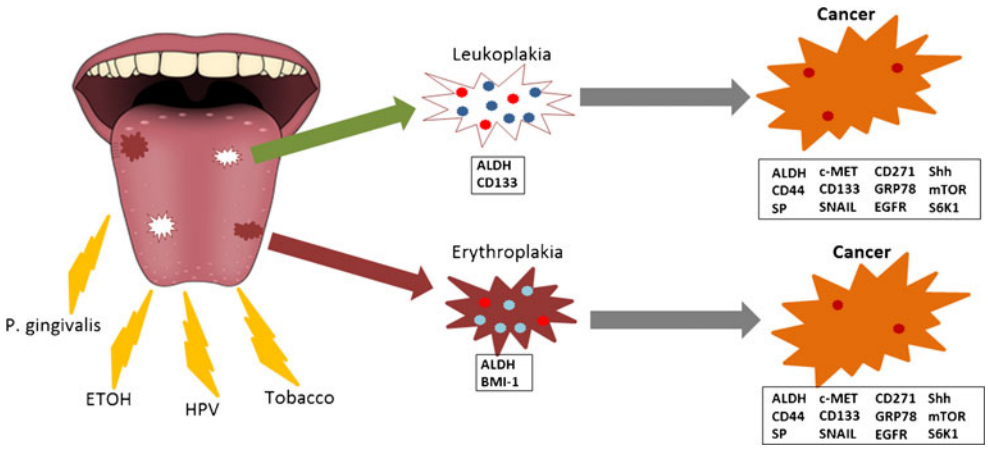

Fig. 1 Cancer stem cells and pre-malignant lesions. Normal stem cells accumulate genetic changes that dysregulate their behavior and lead to a malignant phenotype (represented by the *red cells*). These cells can be identified in leukoplakia and erythroplakia and in true malignant lesions by several know cancer stem cell markers

immunotherapy, and increasing CSC sensitivity to chemotherapy and radiation therapy.

Hyaluronic acid is a ligand for CD44 and its clinical relevance as therapeutic agent to target CD44-positive CSC is of increasing interest. Bourguignon et al. reported that hyaluronic acid induces CD44 interaction with the stem cell transcription factors Nanog, Oct-4, and Sox2. Further investigation is required to determine if targeting this interaction will induce CSC apoptosis and overcome chemotherapy resistance [72]. Anti-CD133 therapies are also being evaluated for their ability to target CSC in HNSCC. Inhibition of CD133 function reduced tumor cell proliferation in two studies, one employed a bacterial toxin conjugated to an anti-human CD133 monoclonal antibody and the other used a single-chain variable fragment targeting CD133 [73, 74]. Recently, Murillo-Sauca et al. reported that inhibition of CD271 decreased tumor formation, suggesting CD271 as a promising target for anti-CSCs therapies in oral cancers [75]. Overall, the most promising anti-CSC therapeutic strategy in HNSCC seems to be directed at leveraging the unique cell surface marker profile of CSC. Significant work remains to be done in identifying the most relevant CSC surface markers to be used as therapeutic targets.

It is accepted that immunotherapy remains a complex but intriguing option for cancer therapy. As one of its key goals is to prime the patient's immune system to recognize cancer cells, anti-cancer vaccines hold significant potential for anti-CSC therapy. Cancer stem cell lysates derived from HNSCC have been used as an antigen source for priming dendritic cells to produce an anti-cancer vaccine. This strategy has demonstrated efficacy in vitro and in animal models [76, 77]. Current studies at the University of Michigan have a goal of developing an autologous CSC-based therapeutic vaccine for clinical use in an adjuvant setting [76]. Using the patient's own immune system to specifically target CSCs would be a very effective addition to current anti-cancer therapies.

Furthermore, sensitizing CSC to chemotherapy and radiation remains an active area of cancer research. Salinomycin has been demonstrated to work in synergy with cisplatin and paclitaxel to increase apoptosis in head and neck CSC [78]. GRP78 has been reported to be an important regulator of protein folding, as well as cell survival and resistance to chemotherapy. Inhibition of GRP78 sensitized HNSCC with a CSC phenotype to chemotherapy and radiation [79]. In another study, application of ATRA (tretinoin), a retinoid derivative involved in terminal differentiation of cells, increased CSC radiosensitivity via inhibition of CPK1/2 [80]. Inhibition of Shh/mTOR/S6K1 pathways also leads to increased CSC radiosensitivity in HNSCC, suggesting therapeutic benefit by targeting these pathways as new options for increasing CSC radiosensitivity [81]. A recent report determined that targeting CSC with an EGFR inhibitor resulted in decreased tumor growth, and increased sensitivity to cisplatin of HNSCC [82]. These investigations, while promising, need further study before entering clinical trials [83•].

Conclusion

Oral cavity-derived cancer remains a deadly disease which even when cured can cause severe functional and esthetic impairment. Despite advances in our understanding, HNSCC behavior in individual patients cannot yet be accurately predicted based on tumor stage, histology, gene, or protein expression/activity. CSC likely play a role in leukoplakia, erythroplakia, and field cancerization. A more detailed evaluation of the factors regulating the behavior of CSC, which represent the critical cancer cells fraction within a given tumor, will be essential to the development of more effective therapies. Further experimentation and clinical trials to assess the efficacy of novel targeted therapies tailored to match individual CSC profile are needed in our era of precision medicine.

Compliance with Ethical Standards

Conflict of Interest Victoria M Prince, Silvana Papagerakis, and Mark E Prince declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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