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Review Article

Cancer stem cells in head and neck cancer

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Abstract: Cancer stem cells (CSCs) are a unique population of cells found within tumors that are able to self-renew, restore the original heterogeneity of a tumor following treatment, and show increased tumorigenic potential when compared to other cancer cells. It is thought that they are responsible for the recurrence of tumors as well as the resistance to treatment that is seen clinically. CSCs are known to be involved in head and neck cancer (HNCs) specifically, as evidence for their existence can be found in head and neck squamous cell carcinoma (HNSCC), mucoepidermoid carcinoma (MEC), and adenoid cystic carcinoma (ACC), among others. Here, findings from various approaches to identifying and targeting CSCs and their downstream effectors in HNC are summarized, with an emphasis on recent advancements. Prognostic and therapeutic markers are discussed for each specific type of HNC, and novel treatment strategies and current clinical trials involving CSCs are detailed as well. The information provided here is intended to further the research on this important topic and lead to clinical impact in the battle against HNC.

Keywords: Head and neck cancer, cancer stem cells, head and neck squamous cell carcinoma, mucoepidermoid carcinoma, adenoid cystic carcinoma

Introduction

The cancer stem cell (CSC) hypothesis states that tumors contain a small proportion of cells that possess the ability to self-renew and to generate the nontumorigenic cells that make up the bulk of a tumor [1]. The first notable functional experiments concerning CSCs were performed in 1994 when researchers found that tumorigenic acute myeloid leukemia cells were a subpopulation cells positive for Cluster Domain (CD) 34, a transmembrane glycoprotein associated with hematopoietic stem cells, and negative for CD38, a marker of differentiation [2]. Today, it is known that CSCs are responsible for the most significant aspects of tumor growth and also have the intrinsic ability to resist chemotherapy and radiation, and therefore are also responsible for the local recurrence of cancer following treatment [3]. They are slow-growing, long-living, and may even be responsible for distant metastasis by translocating through the tissue and establishing a tumor of a similar makeup at a secondary site [3]. Importantly, CSCs can be enriched by traditional regimens of chemotherapy and radiotherapy, effectively selecting for a subset of cells that are even more difficult to treat [4, 5]. Therefore, it is of utmost importance to find ways to target CSCs specifically in order to decrease the likelihood that they will survive treatment and lead to recurrence of the cancer.

Head and neck cancers (HNC) can arise in a number of anatomic subsites including the oral cavity, pharynx, larynx, salivary glands, nasal cavity, and paranasal sinuses, among others, and are responsible for approximately 60,000 new cancer cases in the United States each year [6]. Head and neck squamous cell carcinoma (HNSCC) is by far the most common type of HNC as it represents more than 90% of HNC cases [7]. Nasopharyngeal carcinoma (NPC), an epithelial malignancy that arises in the nasopharynx, shares many characteristics with HNSCC despite having epidemiological and histological differences [8]. The cancers that make up the other 10% of HNC are aggressive and reflect unfavorable clinical outcomes as well. Mucoepidermoid carcinoma (MEC) is the most common salivary gland malignancy, accounting

for 10-15% of all tumors of the salivary gland and 30-35% of all malignant salivary tumors [9]. Its histology shows a glandular neoplasm that reflects a mixture of epidermoid, mucous, and intermediate cells with characteristics of oncocytoid, columnar, and clear cells [10]. While low-grade MEC tumors have a more favorable prognosis, the 5-year survival for high-grade tumors is at 22.5% [11]. Adenoid cystic carcinoma (ACC) is a cancer often found in the salivary glands, especially the parotid gland and submandibular salivary glands, that can arise less frequently in other structures as well [12]. Histologically, it is classified into 3 main categories based on architectural pattern: tubular, cribriform, and solid; the cribriform pattern is the most distinct, and the tubular and solid patterns have the potential to be confused with other diagnoses [12]. ACC of the head and neck has a 5-year survival reported at 68% [13]. Lastly, sinonasal undifferentiated carcinoma (SNUC) is a rare neoplasm derived from Schneiderian epithelium of the nasal cavity and paranasal sinuses [14]. It is difficult to diagnose due to its similarities to other cancers with poorly differentiated morphology and possible neuroendocrine differentiation that occur at the same anatomical site, such as lymphoepithelial carcinoma and olfactory neuroblastoma [15]. The survival rate has been reported to be approximately 56% in multiple studies [15, 16]. While not exhaustive in their representation of non-squamous HNC, these examples demonstrate that non-squamous HNCs are diseases with poor prognoses that require more research and analysis. This also supports the notion that the terms HNC and HNSCC should not be used interchangeably.

There is ample evidence to support the existence of CSCs in HNC, both of squamous and non-squamous origin. For HNSCC, it was shown that purified cells positive for CD44, a cell surface protein known to have a pivotal role in CSCs, could reproduce the heterogeneity found in the original tumor [17, 18]. Further, CD44+ cells were also more likely to form lung metastases in mice, likely due to their increased migratory and invasive abilities [19]. Side population (SP) cells are a subset of progenitor cells that are able to extrude the dye Hoescht 33342 and exhibit CSC-like characteristics such as tumorigenic potential, stem-like gene expression, and chemoresistance [20]. Song et al. showed that SP cells, are found in higher concentrations in metastatic HNSCC cell lines when compared to low metastatic cell lines [21]. However, the use of SP characteristics to define HNC CSCs is controversial [22]. CSCs have been characterized in MEC, where cells that expressed high levels of both putative stem cell markers aldehyde dehydrogenase (ALDH), an enzyme involved in processes of stemness, and CD44 exhibited increased tumorigenic potential in vivo and increased salisphere formation in vitro [23]. In fact, a common way of identifying CSCs is by high co-expression of ALDH and CD44 [23, 24]. Another CSC marker is c-MET, the receptor of hepatocyte growth factor (HGF), which has been shown to be involved with sphere forming capacity and correlated with other CSC markers in HNSCC [25, 26]. Lastly, the NOTCH pathway has been shown to have important involvement in nearly every property of stemness in many different kinds of cancer [27, 28]. This review examines the current understanding of CSCs in HNC, highlights important recent developments, and provides explanations of therapeutic strategies and clinical trials in hopes of spurring the development of more treatment options for HNC patients.

Methods

Literature search was performed on department database using the keywords: Cancer stem cells, chemotherapy, markers and head and neck malignancy. All results were inspected for appropriate content and resultant/relevant medical literature was utilized in the preparation of this review. A similar search was conducted on clinical trials.gov and the results were inspected for appropriate content. Exclusion criteria included all other malignancies.

Discussion

Markers for cancer stem cells

Markers can serve many purposes for CSC research, including identification, sorting, prognostic, and therapeutic functions. In terms of HNSCC, Yu and Cirillo recently summarized the major CSC markers in HNC and described the evidence for several well-accepted markers of stemness, including CD44, ALDH1, Oct3/4, Nanog, and Sox2, as reliable means of identifying and isolating CSCs [24]. Interestingly, they

considered the evidence for use of CD133, another popular CSC biomarker, as a CSC marker to be controversial and suggested it may be a more useful marker in patients with lymph node metastases [24]. It also must be noted that their work focused less on non-squamous HNC, as it only included analysis of 2 such studies, but they did identify CD44, Oct4, and Nanog as CSC markers in MEC and ALDH1 in eyelid sebaceous carcinoma as markers with prognostic significance [24]. In another study, the oncofetal antigen 5T4 was identified as a CSC marker in HNSCC by Kerk et al. They also found that high expression of the protein correlated with lower overall survival when compared to tumor samples that had low 5T4 expression [29]. Linge et al. identified CSC markers: c-MET and SLC3A2, a subunit of the amino acid transporter CD98, as markers of poor prognosis in HNSCC tumors that were negative for human papillomavirus (HPV) 16 DNA [30]. CD98 itself has separately been identified as a prognostic indicator in HNSCC [31]. High-mobility group AT-hook 2 gene (HMGA2) is a known marker of epithelial to mesenchymal transition (EMT) in many cancers, including HNC [32]. Because CSCs are thought to be involved in EMT, it follows that HMGA2 expression can possibly mark characteristics of CSCs. In NPC, Luo et al. implicated FoxM1, a transcription factor involved in cell proliferation, in cancer progression and enhanced tumorigenicity in NPC, supporting its utility as a stem cell marker in HNC [33]. Liu et al. identified another marker in NPC named far upstream element-binding protein 1 (FBP1), a transcriptional regulator of the oncogene c-Myc, which was positively correlated with poorer survival [34]. It should be noted that NPC is sometimes grouped with HNSCC depending on the specific aspect of the disease being studied.

For MEC, Binmadi et al. showed that immunohistochemical staining of CD44 correlated with higher grade of tumor [35]. Xu et al. found that CD44, CD133, and SRY-Box Transcription Factor 2 (SOX2) had no prognostic significance for palatal MEC when evaluated alone, but they did exhibited prognostic significance when the three markers were analyzed together [36]. For ACC, Wang et al. showed that CD133 expression correlated with a worse prognosis in salivary ACC cases [37]. Panaccione et al. built on this research by showing that ACC cells with stemness properties co-expressed CD133 and the transcription factor SOX10, which pointed SOX10 as a possible CSC marker in ACC [38]. They also showed that these CD133+/SOX10+ cells had NOTCH1 activity, providing further evidence for their role in stemness [38]. c-KIT, the receptor of stem cell factor (SCF), was established as a marker by Phuchareon et al. and, in cases that highly expressed c-KIT mRNA, was shown to correlate with expression of SCF as well as with perineural invasion [39]. SOX2 was shown to be a novel prognostic biomarker in patients with ACC by Dai et al. who correlated its high expression with worse 5-year overall survival and disease-free survival [40]. Finally, SOX2 was also shown to correlate with recurrence in sinonasal undifferentiated carcinoma (SNUC) and squamous cell carcinoma [41]. The use of these markers can help identify novel pathways that are active in HNC CSCs, provide information about patient prognosis, and possibly even identify new therapeutic targets that may lead to improved clinical outcomes.

Pathways downstream of these markers

There are specific pathways that are active in CSCs which must be elucidated in order to show which particular mechanisms are involved in the survival and differentiation of these cells. The markers detailed in the previous section are expanded upon here. In HNSCC, it was shown that CSCs were associated with regulation of EMT markers and phosphorylation of Akt, a regulator of important growth pathways, glycogen synthase kinase 3β (GSK3-β), a kinase involved in metabolism, and mammalian target of rapamycin (mTOR), a protein well known to be involved in many cancers [42]. Expression of integrin β1, a regulator of cell migration, was shown to be related to expression of NOTCH1 in HNSCC [43]. HNSCC SP cells expressed ABCG2, a protein known to be involved in chemoresistance, and also reflected dysregulated signaling in the Wnt/B-catenin pathway, which is normally involved in stem cell regulation and suggests that this pathway could be targeted to eliminate CSCs [21]. In non-squamous HNC, regarding HMGA2, Fehr et al. found that its expression is decreased with expression of the fusion gene CREB-regulated transcriptional coactivator 1 and mastermind-like gene MAML2, denoted as CRTC1-MAML2 [44]. The presence of the CRTC1-MAML2 fusion

in MEC was identified as a favorable prognostic indicator by Fehr et al. and multiple other studies as well [44-46]. The concept of vasculogenic mimicry (VM), the formation of vascular channels that lack endothelial cells, has previously been shown to be linked to CSCs [47]. Wang et al. showed specifically that CD133 correlated with levels of VE-cadherin, matrix metallopeptidase 2 (MMP-2), and MMP-9, all of which are markers of VM [37].

Therapeutic agents which target CSC specific pathways

The overall purpose of identifying markers and downstream pathways is to lead to therapeutic interventions, which are discussed here. Goldie et al. found that FERM containing domain 4A (FRMD4A) caused nuclear accumulation of the transcriptional coactivator-YAP, which is involved in transcriptional activation of cell proliferation, and silencing of FRMD4A decreased growth and metastasis of HNSCC xenografts [48]. Kuo et al. had success in using the livestock antibiotic salinomycin to target CSCs specifically, as was evidenced by the decreased expression of CD44 and B Cell-Specific Moloney Murine Leukemia Virus Integration Site 1 (Bmi-1), another marker of stemness, following treatment [42]. Moon et al. were able to decrease self-renewal, resistance to chemotherapy, and tumorigenesis in vivo by targeting integrin β1 [43]. Inhibition of focal adhesion kinase (FAK), a downstream partner of integrin β1, also resulted in decreased self-renewal and decreased expression of markers of stemness [43]. 5T4, the oncofetal antigen discussed previously, was shown to be another therapeutic target in HNSCC preclinical models. Therapeutic inhibition via the antibody-drug conjugate MEDI0641 caused a reduction in CSCs in vitro, and mice receiving the treatment showed less tumor recurrence compared to controls [29]. McDermott et al. found a way to battle the chemoresistance property of CSCs. By first identifying fibroblast growth factor (FGF) signaling as enriched in CSCs resistant to cisplatin, they then were able to inhibit the fibroblast growth factor receptor (FGFR) to either target CSCs specifically or sensitize CSCs to cisplatin treatment [49]. As discussed above in regards to NPC, FoxM1 was implicated as a novel CSC marker [33]. Interestingly, Jiang et al. had previously found that inhibition of FoxM1 led to apoptosis and suppression of proliferation and angiogenesis in NPC [50]. Taken together, these results indicate that FoxM1 inhibition could be an effective way of targeting CSCs in NPC. Also in NPC, FBP1 was identified as a therapeutic target, and knockdown of Fructose-Bisphosphatase 1 (FBP1) reduced cell proliferation and tumorigenesis in vivo [34].

There has also been success shown in targeting CSCs in other HNCs such as MEC. Nakano et al. showed that Bmi-1 was upregulated in cisplatin-resistant MEC cells [51]. However, this effect was reversed by mTOR inhibition, which suggests a new therapeutic strategy for treating this disease [51]. Another possible therapeutic strategy was proposed by Guimaraes et al. They found that inhibition of histone deacetylases (HDAC), enzymes that have a role in cancer epigenetics, allowed for sensitization of MEC cells to cisplatin treatment via disruption of CSCs by decreasing ALDH expression [52]. Moreover, Andrews et al. found a way to target CSCs via inhibition of mouse double minute protein 2 (MDM2), which is involved in regulation of the tumor suppressor gene p53. By targeting the MDM2-p53 interaction with the MDM2 inhibitor MI-773, they found a decrease in the expression of Bmi-1 and a decrease the proportion of cells expressing high levels of ALDH and CD44 [53].

Targeting of CSCs in ACC has also proved to be useful. Similarly to MEC, decreased ALDH and CD44 expression in ACC cells was caused by treatment with HDAC inhibition, and this treatment also sensitized the cells to cisplatin [54]. As mentioned above, SOX2 was shown to be a prognostic marker for ACC. Shimoda et al., however, found that knockdown of SOX2 showed weaker CSC regulatory effects than knockdown of brachyury, a T box transcription factor, which implicates brachyury as a viable therapeutic target [55]. Moreover, brachyury knockdown was also shown to sensitize ACC to chemotherapy and radiotherapy, providing a similar but distinct therapeutic option [56]. Panaccione et al. showed that knockdown of NOTCH1, SOX10, and their common effector fatty acid binding protein 7 (FABP7) led to suppression of stemness properties and cell death [38]. **Table 1** provides a summary of the prognostic and therapeutic markers or targets detailed in this

Table 1. A summary of prognostic and therapeutic markers in both squamous and non-squamous HNC (downstreams partners are included if identified by the study)

SCC	Marker name	Downstream partners in pathway	Importance: Prognostic vs. Therapeutic	References
HNSCC	ABCG2	B-catenin	Therapeutic	[21]
HNSCC	FRMD4A	YAP, CD44	Both	[48]
HNSCC	Integrin β-1	FAK	Both	[43]
HNSCC	5T4	--	Both	[29]
HNSCC	CD98	--	Prognostic	[31]
HPV-negative HNSCC	c-MET	--	Prognostic	[30]
HPV-negative HNSCC	SLC3A2	--	Prognostic	[30]
HNSCC and SNUC	SOX2	--	Prognostic	[41]
NPC	FoxM1	Nanog, Sox2, Oct4, Cyclin D1, Bcl-2, VEGF	Therapeutic	[33, 50]
NPC	ALDH	Oct4, Bmi-1, Sox2, vimentin	Prognostic	[57]
NPC	FBP1	c-Myc	Both	[34]
Non-SCC				
MEC	CD44	--	Prognostic	[35]
MEC	Bmi-1	mTOR	Therapeutic	[51]
MEC	ALDH	HDAC	Therapeutic	[52]
MEC	HMGA2	CRTC1-MAML2 fusion	Prognostic	[44]
MEC	Bmi-1	MDM2	Therapeutic	[53]
Palatal MEC	CD44/CD133/SOX2	--	Prognostic	[36]
ACC	ALDH/CD44	HDAC	Therapeutic	[54]
ACC	NOTCH1, SOX10, FABP7	B-catenin, STAT3	Therapeutic	[38]
ACC	c-KIT	SCF, ERK1/2	Prognostic	[39]
ACC	SOX2	--	Prognostic	[40]
ACC	Brachyury	Various markers of EMT	Therapeutic	[55, 56]

review. Therapeutic inhibition of CSCs has historically proved to be a difficult task, but recent advances indicate that this is an increasingly viable treatment strategy.

Current clinical trials that targets CSCs in HNC

There are several clinical trials currently in progress or recently completed that target CSC pathways. Interestingly, there are also current trials that involve some proteins that were not previously thought to be involved in CSCs but now have a demonstrated role in CSC pathways. **Table 2** provides a summary of these clinical trials, as well as updates on their current status. Trials were selected for inclusion in the table based on their relevance to the evidence presented in this manuscript. Terminated trials were only included if they were completed recently.

Conclusion

A review of the major pathways involved in CSCs in HNC is presented here. Importantly, a distinction is made between HNSCC and non-squamous HNC, and their relevant pathways are discussed separately since the non-squamous cancers behave very differently from the squamous cancers. Prognostic markers are identified and summarized, and then the pathways active downstream of these markers are discussed. Therapeutic markers are then discussed, and a detailed account of the processes used in successful therapeutic strategies is provided as well. Finally, a summary of current or recent clinical trials is provided. The hope is that this information will aid researchers in the quest for therapeutic intervention in HNC by targeting of CSCs, and that the results of those investigations will lead to increased survival for HNC patients.

Disclosure of conflict of interest

None.

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Table 2. Current clinical trials targeting pathways known to be involved in CSCs (trials were identified based on their relevance to the evidence presented in this review)

SCC	Name	Arms	Phase	Status
HNSCC	NCT04007744	Inhibition of PD-1 via pembrolizumab and Hedgehog signaling with sonidegib	1	Recruiting
HNSCC	NCT01353625	Inhibition of DNA-PK and mTOR via CC-115	1	Active, not recruiting
HNSCC	NCT03740100	Inhibition of P13K and mTOR via bimiralisib	2	Recruiting
HNSCC	NCT03148665	Detection of CD44 via OncAlert for presence of disease	N/A	Active, not recruiting
HNSCC	NCT03422536	Inhibition of c-MET signaling via HGF inhibitor ficlatuzumab	2	Active, not recruiting
HNSCC	NCT02706691	Inhibition of FGFR via infigratinib	2	Completed
HNSCC	NCT03088059	Inhibition of FGFR via rogaritinib	2	Recruiting
HNSCC	NCT03292250	Inhibition of FGFR via nintetanib	2	Unknown
HNSCC	NCT04424641	Inhibition of 5T4 via GEN1044	1/2	Recruiting
Non-SCC				
ACC	NCT03691207	Inhibition of gamma secretase via AL101	2	Recruiting
ACC	NCT03639168	Cisplatin and inhibition of HDAC via chidamide	2	Recruiting
ACC	NCT03422679	Inhibition of NOTCH signaling via CB-103	1/2	Recruiting
ACC	NCT02780310	Inhibition of c-KIT, etc. via lenvatinib	2	Active, not recruiting
ACC	NCT04209660	Inhibition of cKIT, etc. via lenvatinib and inhibition of PD-1 via pembrolizumab	2	Recruiting

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