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Cancer stem cells in head and neck cancer: A Mini Review

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Abstract

Head and neck cancer (HNC) is a multifaceted and genomically complex disease and rapidly emerging preclinical and clinical studies have provided a broader landscape of signaling. It is being realized that intra-tumor heterogeneity, genetic and epigenetic mutations considerably challenge wide ranging therapeutics and patients frequently develop locoregional recurrences, second primary tumours and distant metastases. Using high-throughput technologies, it has been revealed that existence of different subpopulations of cells within tumor mass with different phenotypic and functional properties with distinct tumour-initiating potential is responsible to HNC resistance. In light of accumulating evidence reported in recent years, it is now known that different intracellular proteins and cell surface markers have been used to study CSCs. This review provides an overview of CSC biomarkers in HNC treatment and their potential as therapeutic targets in improving the diagnosis, prognosis and treatment of HNC patients for new therapeutic strategies with information about estimation of prognosis and treatment decision. Further studies regarding biomarkers are necessary to determine the specific role of CSCs in HNC which could be useful in development of new therapeutic strategies to eliminate CSCs and maximize clinical outcome. Furthermore, CD44 still need more research in HNC once the studies show contradictions. Studies using lineage tracing and deep sequencing will provide a comprehensive understanding of CSC model and extent to which it is accountable for resistance against therapeutics and carcinogenesis.

Key words: Cancer stem cells, head and neck neoplasms, biomarkers.

Head and neck cancer

Head and neck cancer (HNC) is a heterogeneous disease and broadly categorized into carcinomas arising from the mucosal epithelia of the head and neck region, cell types of thyroid and salivary glands (1-4).

There are several risk factors leading to HNC development including tobacco consumption, alcohol consumption, human papillomavirus (HPV) infection, chronic inflammation, radiation, diet, genetic predisposition and occupational exposure to dry cleaning agent perchloroethylene, pesticides, man-made mineral vitreous fibers (MMMF), polycyclic aromatic hydrocarbons and others (5-10).

The choice of HNC treatment depends on the clinical and histopathology parameters of tumor (TNM stage, type and size of tumor), side effects, and overall health. Several therapies and treatment protocols are currently being used to treat HNC including surgery, radiotherapy and chemotherapy that may be used in combination or single (11-14). Regarding to chemotherapy treatment the main drugs utilized are cisplatin, docetaxel, gemcitabine, cetuximabe, methotrexate, platinol and 5-fluorouracil in combination or single treatment (15-19).

Although different approaches have been evaluated to chemotherapy treatment, the prognosis of HNC is still poor with overall survival rate less than 50% within the first 2 years after treatment. Patients have presented resistance, tumor recurrence and several toxicities to chemotherapy treatment as such as mucositis, dysphagia, dermatitis, permanent xerostomia, altered swallowing function, sensorineural hearing loss and others (20-23). The most interest area of biomarkers is associated to cancer stem cells (CSCs), a group of cells that are present in tumor that have been studied due to its ability to cause recurrence of tumor and resistance against molecular therapeutics (24-25).

Cancer stem cells and head and neck cancer biomarkers

The biology of CSCs has provided many answers about the biological development of cancer. Studies have confirmed that tumors have a small population of CSCs which induce drug resistant phenotype mainly through rewiring of intracellular signaling cascades (Figure 1). Probably the failure of treatments, high rates of recurrence and metastasis may be due to inability of therapeutic interventions to effectively eliminate population of tumor stem cells. Furthermore, it is suggested that more aggressive tumors contain a large amount of CSCs (26- 29).

CSCs are defined as cells that have ability for tumor growth and self-renewal. Cell culture studies and information obtained from xenografted mice indicated that HNC-CSCs can acquire characteristics of HNC (30-31). They are recognized by surface biomarkers or molecules involved in the metabolism of specific signaling pathways which has showed high power to isolate CSCs populations from tumors (28-29, 32-36).

Recent studies have demonstrated that HNC tumors contain CD44, CD 133 and ALDH1 expressing CSCs subpopulation, which are main biomarkers for identification of CSCs of HNC tumors (31,37-40).

After the discovery until today several studies in HNC related to CSCs have been performed to facilitate

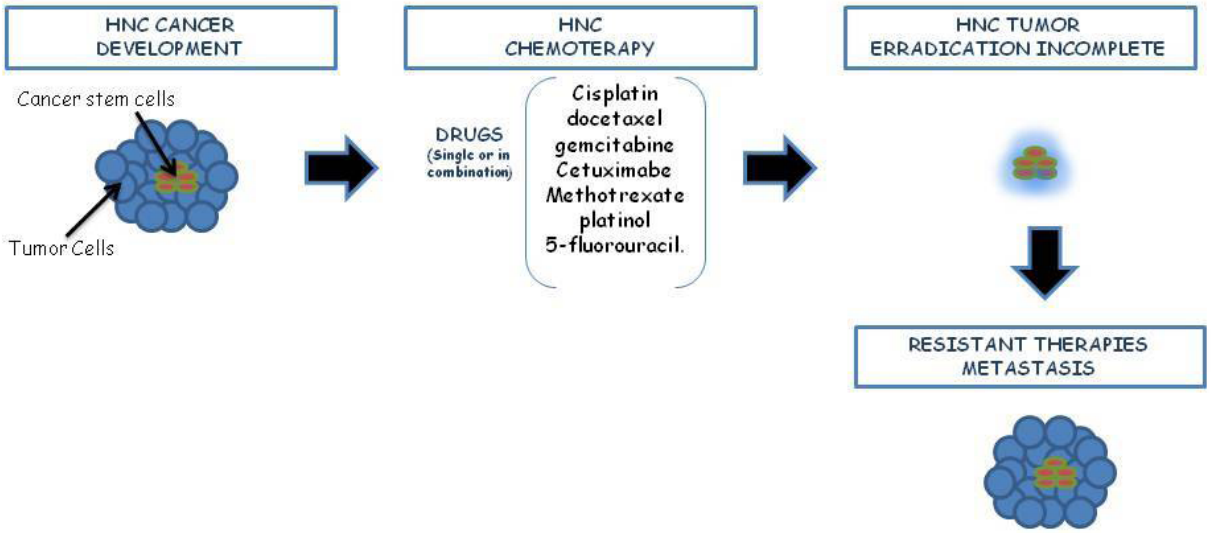

Figure 1. HNC chemotherapy treatment and presence of CSCs- Resistant and metastasis.

the development of new therapeutic agents which can improve clinical outcome (31, 41-54).

CD44 CSCs biomarker

CD44 was first CSCs biomarker discovered in HNC cells, and reportedly, a minor population of CD44+ cancer cells, had ability of self-renewal and differentiation and formed new tumors in vivo which were therapeutically resistant (31).

CD44, a transmembrane large cell surface glycoprotein that acts as a receptor of hyaluronic acid and is involved in cell adhesion and migration (55). There are direct pieces of evidence highlighting its frequent overexpression and notable ability of self-renewal, differentiation and tumor formation in vivo, metastasis and chemo resistance (31,41- 42,44- 45, 47, 52- 53).

The most recent study evaluated 7 cell lines of larynx, tongue, gingiva and oral cavity cancer (Hep-2, PE/CA-PJ49, PE/CA-PJ34, PE/CA-PJ46, Ca 9-22, HSC-2, HSC-3) and 5 primary tissue specimens of HNC. Researchers identified CSCs population and confirmed increased expression of CD44 biomarker in this subpopulation compared to parental cells in all tested cell lines. Furthermore they performed in vivo experiments to investigate tumor formation of CSC-enriched populations concluding that the tumor size developed in mice with CSC-enriched populations had an increased ability to form tumor in vivo when compared to the parental cell line (52).

Yanamoto et al (2014) (47) evaluated 89 tumors of oral tongue squamous cell carcinoma patients and confirmed that increased expression of CD44v6, a variant isoform of CD44, is associated to invasion lymph node metastasis and local recurrence. Another study in 2014 evaluated a total of 10 HNC cell lines of various clinical outcomes (true vocal corde – University of Michigan (UM)-SCC10A; lymph node - UM-SCC10B; lymph node - UM-SCC22B ; epiglottis - UM-SCC11A; Supraglottic - UM-SCC11B; Floor of the mouth - UM-SCC14A; Floor of the mouth - UM-SCC14B; Hypopharynx - UM-SCC22A; Lateral tongue - UM-SCC47; Lateral tongue - UM-SCC103; base of tongue - UM-SCC6) confirmed that highest level of CD44v6 was present in advanced metastatic HNC while CD44v4, another variant of CD44, was associated with tumorigenesis and highly expressed in stage IV HNC refractory to chemotherapy which developed recurrence (44).

It has been convincingly revealed that $CD44^{+}$ population demonstrated stronger tumor forming capacity as compared to $CD44^{-}$ cells (42). CD44 and SSEA-4 expressing cells preferentially expressed some stemness genes and were therapeutically more resistant to 5-fluorouracil (5-FU) and cisplatin (41) and, the study of Han et al., (2013) that evaluated a submaxillary salivary gland cancer cell line ((ATCC®HTB-41) showing that CD44 is highly expressed in this cell line and the population presents stemness characteristics of self-renewal and differentiation (45).

In contrast studies have showed that CD44 in CSC and non-CSC forming cells in HNC presenting similar expression (46, 54). It has been suggest that CD44 cannot be utilized as a CSCs biomarker because CSCs themselves hold the ability to be heterogenous due to genetic alterations and hence cannot be used as a selective biomarker of cell adhesion and migration and can assume relevant potential as a biomarker if combined with another parameters, such as proliferative activity (56).

Data showed that expression of CD44 can be associated with chemotherapy response to cancer treatment being a useful criterion for response to treatment. (57) However further studies in different populations of HNC patients and in different subtypes of HNC with different therapeutic combinations will be helpful in a deeper comprehension of cancer treatment.

CD133 CSCs biomarker

CD133 is a cell-surface glycoprotein comprising five trans-membrane (58). CD133 is noted to be frequently overexpressed in HNC cells presenting slow progression and worse prognosis in patients with HNC-that indicate a survival mechanism of tumor. It has an invasive capability, higher potential for clonogenicity and resistance to anti-tumor treatments (43, 45, 48, 49, 52- 54). Zhang et al (2010) evaluated two human tongue cancer cell lines (SCC-016 and UPCI: SCC-076) and bucal

cancer cell line (UPCI: SCC-29B). $CD133^+$ cancer cells were present in all of the cell lines in a small subpopulation and demonstrated considerably enhanced tumorigenic potential as compared to CD133- subpopulations (47). Yu et al., (2015) reported that CD133 expressing cells had a drug resistant phenotype (48). Mannelli et al (2015) evaluated 29 tumors of HNC and the association of CD133 with CSCs and Wei et al (2014) confirmed the human laryngeal carcinoma Hep-2 cell line possess CD133+ population with power of cell proliferation and in vitro invasion and resistance to treatment (49; 53).

Data clearly suggested that expression of CD133 associated with poor clinical response to chemotherapy and chemo resistance of CD133 in different types of cancer, including HNC patients treated with cisplatin, 5-fluorouracil or doxorubicin chemotherapy. (59-61).

ALDH CSCs biomarker

Aldehyde dehydrogenase (ALDH) is a group of isozymes involved in cell differentiation, detoxification and drug resistance by intracellular oxidation of the aldehydes. ALDH1A1 has been associated to CSCs population of HNC and worse prognosis, metastasis development and resistance to treatment being an independent predictor of overall survival with increased invasion capacity (50-51).

A meta-analysis assessing the association of ALDH expression in HNC performed in 2014 confirmed that increased expression of ALDH1 is associated to ability of tumor differentiation, involvement of lymph node metastasis and low level of survival (50). Qian et al., (2014) evaluated the expression of ALDH1A1 in eighty-one HNC patients in advance stage and confirmed that increased expression of ALDHA1 was associated to poor prognosis and formation of metastasis (51).

Literature data about HNSCC treatment and ALDH biomarker show that cisplatin do not eliminate CSCs that were identified by detecting the biomarker ALDH (ALDH high) and CD44 (CD44 high) promoting self-renewal and survival of CSCs in *vitro* which causes treatment failure. (62-63).

Final Considerations

Despite the different research groups have detected CD44, CD133 and ALDH1 biomarkers in HNC cells related to CSCs and resistance to HNC treatment it is needed more investigation about the molecular mechanisms by which CSCs act in HNC tumor and how their confer resistance to chemotherapy treatment.

CSCs small population have been identified through of CD44, CD 133 and ALDH1A1 cell surface biomarkers in HNC tumor and they may be responsible to ability to reproduce malignant tumors indefinitely, regulation of tumor growth, metastasis, and HNC treatment resistance. There are a few studies that evaluated HNSCC chemotherapy and identification of CSCs biomarkers in treatment outcome. These studies show resistance of chemoterapy treatment in HNSCC when there are CSCs detected trough of CD44, CD133 and ALDH1 treatment. Further studies regarding biomarkers and HNC chemotherapy treatment are needed to determine the specific role of CSCs in HNC which could lead to the development and application of new therapeutic strategies to eliminate CSCs within the tumor thus better improving the therapeutic outcome of HNC. Furthermore, CD44 still need more research in HNC once the studies show contradictions.

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Other articles in this theme issue include references (64-75).

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