Understanding cancer stem cells in head and neck cancer: An insight from oral medicine point of view

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Abstract:
Cancer stem cells are altered stem cells that give rise to tumors. Head and neck cancer accounts for the significant amount of cancers that occur in the world. There is pointing evidence that cancer stem cells contribute to the origin of head and neck cancers. Understanding the cancer stem cell biology, identifying them and targeting these cells are highly important to effectively manage head and neck cancers. The present article discusses the importance of the role of cancer stem cells in head and neck cancers from an oral medicine point of view.

Key words:
Cancer stem cells, head and neck cancer, identification, oral squamous cell carcinoma, targets

Introduction

Stem cells are cells capable of self-renewal. They are capable of differentiation and produce differentiated daughter cells in large numbers. Mutation in genes that regulate self-renewal property of stem cells can lead to the development of cancer. These unregulated groups of stem cells proliferate endlessly leading to cancer.[1] The present article gives insight into cancer stem cells (CSCs) of head and neck cancer from an oral medicine point of view.

Cancer Stem Cells of Head and Neck Cancer

Head and neck squamous cell carcinoma (HNSCC) is the sixth most commonly occurring tumor in the world.[2] Cancer stem cell is a cell that resides within the tumor mass, capable of self-renewal and has the ability to produce a wide variety of cancer cells that constitute the tumor.[3] Cancer stem cells may be one of the reasons for the failure of current therapies against cancer, because current management strategies fail to target CSC.[4] They are responsible for tumor growth, metastasis, and for the development of anti-apoptosis mechanism.[5] Cancer stem cells have the capacity to survive for a longer time, exhibit resistance to toxic substances, express telomerase resulting in chromosomal instability, have the ability to grow independently and possess high capacity for migration. They also exhibit mechanism such as active transport (multidrug resistance transporter 1 [MDR1] and ATP-binding cassette sub-family G member 2 [ABCG2]) of chemotherapeutic substances out of the membranes of cells leading to resistance to anticancer drugs. CSCs are classically in G0 phase of cell cycle and hence exhibit chemoresistance to anticancer drugs which rely on cell cycle.[6,7]

In cancer stem cell model theory, the normal stem cells accumulate genetic and epigenetic mutations leading to genotypic and phenotypic alterations. Through the symmetric division, these tumorigenic stem cells give rise to identical stem cells leading to aberrations in the self-renewal process resulting in clonal expansion. Signaling pathways involved in this process are Wnt, Hedgehog, Notch, and Oct-4. This tumor initiating mechanism is also aided by mediators such as matrix metalloproteinase-9, laminin, tenascin, and inflammation (due to radiation, microbes, chemicals).[8]

An important perspective is to identify and kill these CSCs for an efficient management of these tumors. Cancer stem cells in head and neck was first isolated by Prince et al. by identifying them through the expression of CD44.[9] CD44+ and CD24+ cells are the cancer stem cells found in HNSCC. These cells exhibit self-renewal, differentiation and clonogenicity. Also, they are found to be resistant to anticancer drugs such as cisplatin and gemcitabine. CD24+ cells exhibit more resistance to anticancer drugs. CD44+ is responsible for self-renewal, tumorigenesis, tumor progression and metastasis, whereas CD24+ is responsible for tumorigenesis, tumor aggressiveness, metastasis, and tumor progression.[10] Patients with increased CD44+ experience high rate of treatment failures.[11] CD44+ cells possess the ability to avoid the body’s immune system detection.[12] Aldehyde dehydrogenase (ALDH)}
activity is also a specific marker of CSCs in HNSCC. Immunophenotypic studies indicate that oral squamous cell carcinoma (OSCC) patients with CD24 and CD44 double positive cells show low survival rate due to their aggressive behavior and worst prognosis.[9]

HNSCC cancer stem cells can also be identified by cell markers such as expression of CD133 (prominin-1).[3,4] The cells expressing the marker CD133 is found to be resistant to paclitaxel.[5] These stem cells are usually located in the perivascular niches in the invasive front.[6] Notch 1 signaling pathway mutation, epithelial-mesenchymal transition (EMT) is probably involved in the etiology of origin of cancer stem cells.[7] Patients with Twist 1 and Bmi-1 (B-cell specific Moloney murine leukemia virus insertion site 1) tend to have worst prognosis as evidenced in nasopharyngeal carcinomas.[8,9] Bmi-1 has a role in regulating the self-renewal property of stem cells. Bmi-1 gene is thought to be responsible for head and neck cancers.[10] Over expression of Bmi-1 results in downregulation of E-cadherin which results in enhanced motility, invasiveness of cancer cells and accelerates nodal metastasis.[11] Also over expression of Hypoxia inducible factor-1α (HIF-1α) and TrkB induces EMT and leads to metastasis and increased invasiveness in HNSCC. Signal transducer and activator of transcription 3 (Stat3), protein kinase B (Akt) and extracellular signal-regulated kinases signaling are activated by factors secreted by tumor cells and promote endothelial cells. In turn endothelial cell secreted factors such as interleukin-6 (IL-6) and IL-8 (CXCL8) promote and protect the tumor stem cells by upregulating Bmi-1 expression.[12] Interruptions of this cross-talk between endothelial and tumor cells can open new avenues in the management of HNSCC. Primitive cancer stem cells (pCSC) may get converted to endothelial cells to generate vascular supply for the growing tumor. Vascular endothelial derived growth factor receptor-2 is the molecular marker for such pCSC.[4]

Stem cell markers relevant to HNSCC include Oct-3/4, Sox-2, Nanog, c-Met.[11,12] Identification can also be done based on the ability of the cells to eliminate Hoechst 33342. Side population cells are tumor cells with stem cell phenotype. They are found in HNSCC where they express high levels of ABCG2, Bmi-1, CD24, and Oct-4.[13]

Cancer stem cells are maintained by Oct-4, Notch, Wnt/Catenin, bone morphogenic protein (BMP), sonic Hedgehog signaling pathway and Musashi-1. Cancer stem cells are located and maintained effectively in the hypoxic areas of the tumors. The actions of hypoxia on stem cells are facilitated by HIF.[14]

Cancer stem cells are thought to be resistant to conventional management techniques such as chemotherapy and radiotherapy. Treatment measures targeted toward cancer stem cells may prevent recurrences of oral cancer and also improve survival rates in these groups of oral cancer patients [Figure 1]. There may be <1 CSC for every 2500 cells of HNSCC tumor. Another important point to note is the self-renewal property of stem cells requires molecular pathways such as Wnt, Notch and Hedgehog and these pathways are necessary to give rise to the tumor. Wnt signaling pathway has an important role in embryogenesis and cancer development. Cancer stem cells may arise from a normal stem cell or from the fusion of hemopoietic stem cell with differentiated or mutated epithelial cell. It may also arise from dedifferentiation of matured cell, EMT, and neosis.[15] They can also arise from progenitor cells by genetic or epigenetic mutations.[13,15]

EMT is involved in the formation of CSCs and plays an important role in the development of metastasis. EMT is a process, where cancer cell with epithelial characteristics changes to that of the mesenchymal characteristic by changes in cell polarity and adhesion.[16] EMT is associated with loss of E-cadherin and gain of N-cadherin, which in turn is connected to the progression of tumors. CSC can also be identified through side populations and capability to form tumor spheres. High levels of ALDH, c-MET, Twist, and Snail are associated with increased metastasis. EMT is regulated by Twist, Snail, miR-200a, HIF-1α, transforming growth factor (TGF), prostaglandin E2, and IL-1. Slug plays an important role in HNSCC through the promotion of EMT and cell migration.[17] CSC markers CD44, CD24, Oct-4, integrin-β1, and HIFs are related to poor prognosis on radiotherapy. Side population cells exhibiting increased expression of ATP-binding cassette transporter proteins show resistance to chemotherapy. Side population cells have increased quantities of MDR1 and ABCG2 transporter.[18] More research should be done to develop new novel drugs to target CSCs. This will contribute to the efficient management of HNSC, decreased recurrence rate and metastasis and increased survival rate.[19] It is observed that CSC of HNSCC can be made sensitive to chemotherapy through knockdown of Bmi-1 and CD44.[20] It has been found that aldehyde dehydrogenase 1 (ALDH1+) CSCs possess high self-renewal and radioresistance.[19]

Cancer stem cell markers CD133, CD44, glucose regulated protein 78, Nanog, pathways such as Wnt, Nanog, Hedgehog should be targeted to develop novel therapies. CSC vascular niches in which CSCs interact with angiogenesis should be targeted, for example iCaspase-9. Coaxing differentiation of CSC to the normal cell by using BMP-4 can be attempted.[21]

Cancer stem cells are often found in the invasive fronts of HNSCC close to blood vessels (within a radius of 100 μm). Cancer stem cells in HNSCC can be identified by their sphere-forming capacity in low attachment conditions. Micro-RNA such as Let7, micro-RNA-200c is observed to regulate the tumorigenicity of CSC. Head and neck CSCs cultured in either in a matrix-based assay like soft agar or in ultra-low-attachment plates are observed to grow in suspension and generate tumor spheres. This assay called as “Orosphere assay” is suited to study the pathobiology of head and neck cancer stem cells. Orosphere is a nonadherent spheroid colony of at least 25 cells sorted from HNSCC [Figure 2].[13,22,23] CD24+ /CD44+ cells from HNSCC cell lines are found to possess ability to create large tumors in mice in addition of retaining stemness characteristics such as self-renewal and differentiation. CD44+ cells express high levels of BMI1 gene which is responsible for self-renewal and tumorigenesis. This CD44+ is found to be abundantly expressed in head and neck cancers. CD29 may also be present in HNSCC cell lines. Higher levels of Nanog genes are also expressed by CD44+ and CD24+ cells. It should be remembered that Nanog genes are
Shanbhag: Cancer stem cells in head and neck cancer

Figure 1: Controlling head and neck cancers through targeting cancer stem cells

Figure 2: Orosphere formation is characteristic of cancer stem cells

associated with stemness of human embryonic stem cells. CD24 + is also positively expressed in salivary gland tumors of stage II/IV. It is observed that the intensity of ALDH and CD24 immunostaining correlates with severity of oral epithelial dysplasia and CD24 is efficient in identification of malignant tissues from normal tissues.

Krüppel-like factor 4 (Klf4) inhibits p53 gene and activates the p21 gene. p53 promotes proliferation and p21 is known to be a gene associated with pluripotency and is involved in the conversion of normal somatic cells to stem cells. Overexpression of Klf4 has been observed in OSCC and is correlated to decreased survival and increased tumorigenicity. Lgr5 (Leucine-rich repeat-containing G-protein coupled receptor 5)/GPR49 (G-protein coupled receptor 49) is a tumor suppressor gene which has a role in establishing the expansion of stem cells in the niche.

In Salivary gland tumor such as adenoid cystic carcinoma, CSCs can be identified via potential markers such as ALDH, CD133, and CD24. Epithelial growth factor which promotes self-renewal of CSCs has been found to be upregulated in adenoid cystic carcinoma. c-Kit (CD117) which has a role in maintaining the stem cell properties has been found in adenoid cystic carcinoma. CSCs are possessing anchorage independent growth with the capacity to form spheroids in the laboratory, have also been detected in mucoepidermoid carcinoma.

Oral cancer stem cells also show increased expression of nestin, CK 19 (cytokeratin-19), and CD117 (c-kit). Also, decreased expression of involucrin and CK13 (cytokeratin-13) which are necessary for differentiation is observed in these types of cells. CD133+ CSCs exhibit increased clonogenicity, increased tumorigenicity and tumor invasiveness. Also, they are largely resistant to standard chemotherapy. TGF-β signaling may be involved in imparting the self-renewal capacity to nonstem cells. Blocking TGF-β1 signaling restrains dedifferentiation of mature cells especially the one which are hypoxia mediated, decreases clonogenicity and self-renewal of CSCs.

Basal stem cells of the oral epithelium, when infected by HPV-16 and 18 (Human Papillomavirus Type 16 and 18) have been found to express oncogenic proteins E6 and E7. This E6 and E7 acts on proteins such as p53, Notch-1, p16, cyclin-D1, EGFR (Epidermal Growth Factor Receptor), and activates Wnt signaling pathway which may lead to dedifferentiation of oral cancer cells to CSCs leading to genesis of tumor forming CSC in HPV-16 positive oropharyngeal carcinoma.

HPV-positive oropharyngeal squamous cell carcinoma expressing high amounts of CD98 has been found to be correlates with worst prognosis of overall survival.

Nicotine increases ALDH1 cells, self-renewal, tumor sphere forming ability, increased expression of stemness related genes, modifies EMT, and contributes to the genesis of CSC. Also, it is found to increase the expression of ABCG2 transporter in side population cells, leading to efflux of doxorubicin and mitoxanthrone. The CD44+ expression is found to be significantly higher in smokers. Arecoline stimulates β-catenin expression, and β-catenin levels are more in CSCs than normal.
oral epithelial cells and also arecoline upregulates vimentin which in turn is involved in EMT. Higher expression of BCL and CFLAR gene may be seen leading to decreased apoptosis. Low expression of autophagy genes Beclin-1 is observed in tobacco related OSCC. Autophagy maintains CSCs and promotes chemoresistance. CD44+ and CD133+ oral cancer cells exhibit resistance to chemoradiotherapy. CSC also exhibit increased drug efflux capacity and decreased apoptosis.[29]

Invasiveness and tumorigenicity of OSCC cells are correlated with increased expression of Sox-2. The gene Sox-2 is essential for maintaining the stemness of CSCs. Decreasing the expression of Sox-2 can decrease invasiveness and EMT features. Targeting Sox-2 has potential to control tumorigenesis and anti-apoptotic genes, increase the survival of OSCC patients, decrease drug resistance and increase the sensitivity of the tumor to combined chemoradiotherapy (radiation and cisplatin).[30] ALDH positive CSCs with high tumorigenic capacity, the ability to self-renew, and differentiate to a wide variety of cells have been detected in HNSCC cell lines.[31]

Salinomycin and metformin are found to act against CSCs. Stem cell niche (the tumor microenvironment) should be targeted because they contribute to the survival of CSCs.[32] Macrophages are associated with maintenance of stemness of CSCs and in facilitating the formation of the microvasculature that provides nutrients for the tumor. Fibroblasts and endothelial cells support invasiveness, angiogenesis and promotes stemness of CSCs.[33] Differences in self-renewal property of normal and cancer stem cells should be identified, and those differences should be targeted in cancer therapies so that normal stem cells are spared.[34]

Endothelial cell initiated signaling induce Bmi-1 expression and self-renewal of stem cells and destroying these endothelial cell will have a detrimental effect on CSCs. Vascular endothelial growth factor secreted by endothelial cells activates PI3K-Akt signaling, resulting in a proliferation of HNSCC CSCs. Endothelial cell secreted factors protects CSCs from anoikis.[35] Histone deacetylases are overexpressed in head and neck carcinomas and responsible for poor prognosis and chemoresistance. It has been successfully targeted in SCID mouse xenograft model using suberoylanilide hydroxamic acid (SAHA) through downregulation of Nanog. SAHA reverses cisplatin chemoresistance and formation of tumor sphere; inhibits cell proliferation, tumor growth, and metastasis.[36]

RhoC oncogene has been found to promote tumor metastasis. Inhibition of RhoC using shRNA in HNSCC cell lines is found to reduce CSCs markers such as ALDH and CD44; downregulate stem cell transcription factors such as sox-2, Oct-3/4, and Nanog; and decrease tumor sphere formation.[37] So developing chemotherapeutic drugs targeting RhoC oncogene is a good idea. Oct-4 may confer apoptotic resistance to CSCs on exposure to chemotherapeutic drugs and it can be used as a marker to detect chemoresistant CSCs.[38] Downregulation of enzyme nicotinamide n-methyltransferase inhibits cell growth and tumorigenicity in oral cancer cell lines, and this fact can be used as a therapeutic target to treat HNSCC.[39]

HIFs contribute to the maintenance of CSCs by promoting cell proliferation with less DNA damage. HIFs contribute to the tumor promoting microenvironment by promoting differentiation and proliferation of tumor macrophages, endothelial cells, and T-cell. So targeting HIFs should be considered to eliminate CSCs.[40] Curcumin has been observed to upregulate miR-145 (tumor suppressor micro-RNA) resulting in inhibition of SOX9/ADAM 17 axis and IL-6/Stat3 signaling pathway thereby inhibiting tumor initiating cells of head and neck cancer.[41] Fractionated radiotherapy is promising in the management of CSCs. Administration of hypoxic cell sensitizers to the hypoxic volume of the tumor containing CSC seems to be effective.[42]

Conclusion

Recent research reveals how etiologic factors give rise to CSCs that eventually culminates in the origin of HNSCC. Various etiologies including HPV infections can cause normal stem cells to get converted to cancer stem cells. HNSCC CSCs are predominantly identified by surface glycoprotein CD44. Discovering new novel HNSCC CSC specific markers have benefits of early detection of head and cancer stem cells and helps not only to initiate timely effective treatment with better prognosis, but also has the potential to detect and prevent metastasis. Current therapies are unable to effectively treat HNSCC as they have little or no effect on CSCs. Hence, future cancer therapy should be targeted toward CSCs through various modes such as by interfering with the signaling pathways and genes responsible for the origin of CSCs, targeting the tumor microenvironment, preventing the dedifferentiation of somatic cells, controlling inflammation, and EMT, etc. More research on cancer stem cell biology, nanotechnology, and genomics will eventually help us to understand the different aspects of these cells. It can be concluded that identifying and targeting cancer stem cells are highly important in the management of head and neck cancers.

References

Shanbhag: Cancer stem cells in head and neck cancer


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