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Cancer Stem Cells – A Theranostic Approach

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Abstract

Cancer stem cells, or CSCs, are subpopulations of cancer cells that have traits in common with normal stem cells, such as the capacity to self-renew and differentiate into many lineages, which can promote tumor growth and heterogeneity. The role of CSCs in metastasis, drug resistance, and cancer outbreaks is sufficiently understood. Thus, the regulation of CSCs may offer an effective therapeutic strategy that prevents the growth and invasion of tumors. Despite the fact that targeting CSCs is important for treating cancer, not many studies have thoroughly examined the characteristics of oral CSCs. It has been demonstrated that oral CSCs can accelerate the development of oral cancer by activating or inhibiting a number of cellular and molecular pathways (including the calcium regulatory system, his tone changes, and microRNA network). Therefore, gaining further knowledge about the characteristics and behaviors of oral malignancies will aid in the development of novel therapeutic approaches. This study examines the current state of knowledge regarding CSC markers, including SOX2, NANOG, ALDH1, OCT4, phosphorylated STAT3, CD133, CD24, CD44, and Musashi-1. It places special emphasis on the markers' applicability and validity in the research on cancer stem cells related to oral cavity squamous cell carcinoma, as well as how the markers' widespread deployment may allow them to be categorized into a CSC hierarchy.

Keywords: Cancer Stem Cells, Tumorigenesis, Relapse, Metastasis, miRNA.

Introduction

Tumor stem cells (SCs) are a pluripotent subpopulation of cells that has the ability to self-renew, initiate new tumors, differentiate, migrate, and spread. The idea and characteristics of cancer stem cells Tumor-initiating cells, or CSCs, are the tiny subset of cells that make up a tumor bulk and are considered to be essential to the tumor population. Although the idea of CSCs was first put forth in the 1800s, it wasn't until 1994 that Dick and associates were able to identify leukemia stem cells for the first time, providing compelling evidence in support of the tumor heterogeneity theory.

Normal Stem Cells Vs Cancer Stem Cells

While adult stem cells are unipotent but can regain totipotent properties under in vitro conditions, resulting in the induced pluripotent stem cells (iPSCs), embryonic stem cells are totipotent. A illustrates the various sources of normal SCs, their biological properties of indefinite division through self-renewal, and generation of differentiated cells under appropriate conditions. In B, after accumulating genetic changes brought on by carcinogens, adult epithelial stem cells have the potential to convert malignantly, producing CSCs. These CSCs continue to have the basic characteristics that allow them to self-renew and produce differentiated (cancer) cells, which promote the growth and spread of cancer.

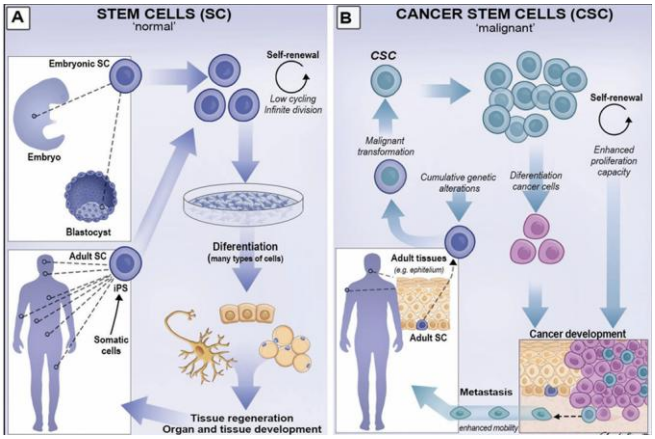
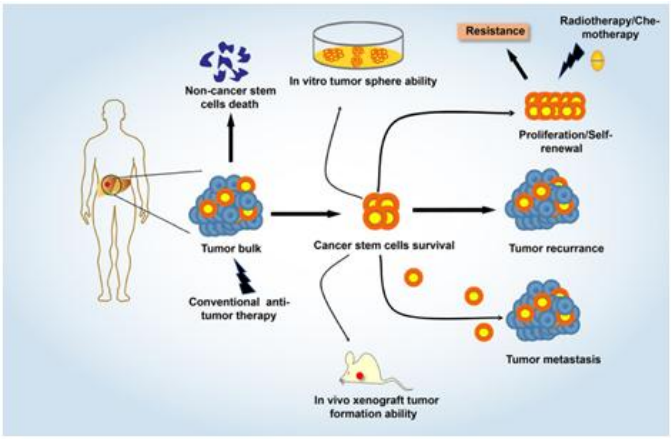


Diagram showing cancer stem cells (B) and normal stem cells (A)

Characteristics of Cancer Stem Cells

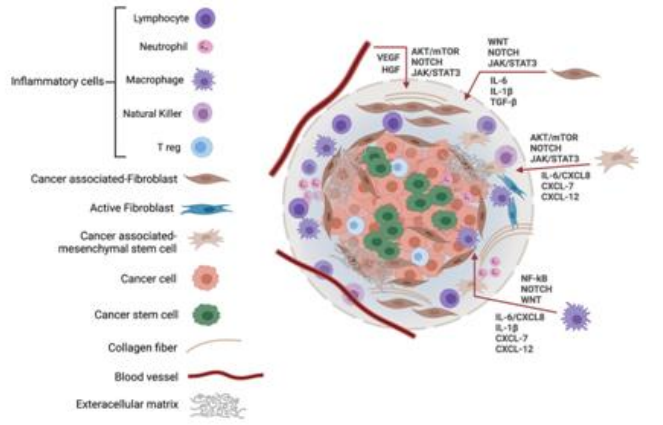
The realization that not all tumor cells are created equal is the basis of the CSC idea. CSCs' potent tumorigenicity in xeno transplantation in vivo is a crucial characteristic.



CSC has the capacity to create tumor spheroids in vitro, which is an indication of the CSCs' capacity for self-renewal and proliferation. A tiny quantity of cancer stem-like cells can cause tumors to grow in mice when they live in vivo. Moreover, CSCs possess innate drug resistance, dormant properties, and the capacity to initiate distant cancer metastases.

The cancer stem cell microenvironment

Through adhesion molecules and paracrine factors, CSCs interact with altered cells as well as other stromal cells within the tumor microenvironment. These micro environmental interactions stimulate angiogenesis, encourage the development of CSCs, draw in stromal and immune cells, and facilitate the invasion and spread of tumors.



Together with blood vessels and extracellular matrix, the tumor tissue microenvironment is made up of a range of cells, including tumor cells, cancer stem cells, inflammatory cells, and cancer-associated fibroblasts. CSCs generate cytokines and growth factors, including as VEGF, CXCL8, and IL-6, in response to hypoxic stress and matrix, and use EGFR, NOTCH, WNT, and other signaling cascades to control their own growth.

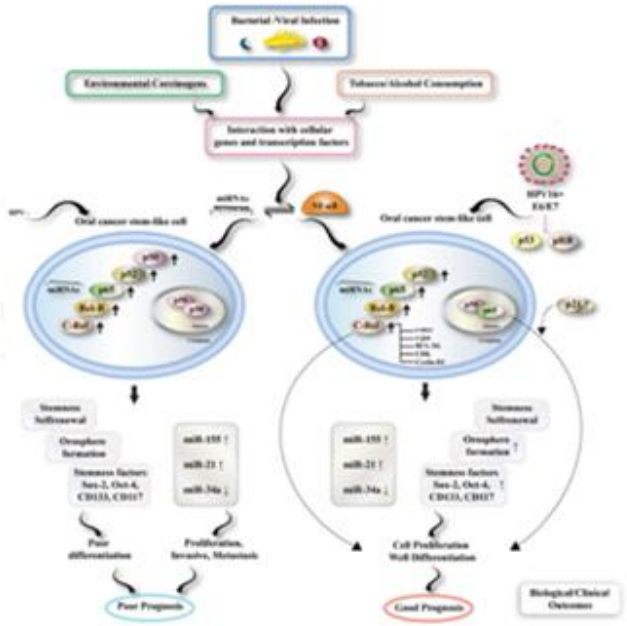
VEGF, vascular endothelial growth factor; HGF, hepatocyte growth factor; mTOR, mammalian target of rapamycin; IL, interleukin; CXCL, CXC-motif chemokine ligand; JAK, Janus kinases; STAT, signal transducer and activator of transcription.

Inflammatory molecules

According to studies, cancer and cancer stem cells from a variety of malignancies, including leukemia, ovarian, breast, glioma, pancreatic, prostate, and colon cancers, frequently activate the NF-κB pathway. Furthermore, miRNAs continue to be the other important modulatory molecule involved in the carcinogenesis process. Additionally, they could operate as tumor inhibitor genes or oncogenes, effectively interacting with NF-κB and other molecules.

Bano and colleagues distinguished cancer stem-like SP cells from HPV-positive OSCC cell lines and the primary tumors, generating oro-spheres that exhibited Sox-2, Oct4, CD117, and CD133 expression, stemness markers. The cells that were infected with HPV16 showed selective over-expression of the viral oncogenes E6/E7, differentially up-regulated expression of NF-kB proteins, and increased numbers of orospheres. They also over expressed c-Rel and selectively activated p65, which hetero-dimerized with p50 to demonstrate increased DNA binding activities.

Moreover, HPV +ve cancer stem cells over expressing HPV16 oncogene E6, which controls sustaining stemness, have shown selective over expressed miR-21 and miR-155 and down-regulated miR-34a. While HPV infections including participation p65 with de-regulated production of particular miRNAs resulted in the comprehensive differentiation of tumors and more acceptable prognosis, HPV-ve CSCs only show weak differentiations, p50 homo-dimerization, and the poorest prognosis.



The diagram illustrating how NF-κB and miRNA affect OCSCs and regulation in the presence or absence of HPV16 infection, as well as how their interactions with other gene products affect prognosis and determine

whether treatment results in an acceptable or poor outcome

Signaling Pathways Utilized by CSCs in HNSCC

The regulation of normal stem cell self-renewal and differentiation involves multiple processes. The regulation of organ system patterning and differentiation has been linked to the NOTCH and Sonic Hedgehog (SHH) pathways. Secreted glycoprotein SHH binds to the transmembrane receptor Patched 1 (PTCH1), which releases Smoothened (SMO) and sets off a cascade of intracellular events that cause the transcription factor GLI1 (glioma-associated oncogene homolog 1) to translocate into the nucleus. There, it triggers transcription of genes linked to proliferation, such as GLI1, PTCH1, WNT1, forkhead box protein M1 (FOXM1), and CCND1.

Furthermore, SHH-induced signals have a negative correlation with E-cadherin and a positive correlation with the expression of Snail and MMP9, indicating that SHH signaling may play a significant role in HNSCC invasion and metastasis via promoting EMT.

Genetic changes such as loss-of-function mutations in PTCH1, gain-of-function mutations in SMO (T241M, L412F, S533N, W535L, and R562Q), and amplification of the GLI1 or GLI2 genes abnormally stimulate SHH signaling cascades in tumor cells. SHH signaling has been demonstrated to be active in a number of CSCs, including HNSCC, gastric cancer, liver cancer, and breast cancer.

It's interesting to note that in HNSCC patients, higher levels of GLI1 are associated with lymph node metastases, recurrence, and the worst prognosis. Findings of the SHH pathway, particularly GLI1 and SHH, in HNSCC may indicate potential targets for the development of anticancer therapies in the future.

NOTCH1 is another significant participant associated with CSCs in HNSCC. The NOTCH protein family comprises heterodimeric transmembrane receptors that are divided into three domains: intracellular, transmembrane, and extracellular. The intracellular domain moves into the nucleus and uses the CBF1, Suppressor of Hairless, Lag1 (CSL) transcription factor family to control transcription. Target genes involved in cellular differentiation such as HERP, HEY, and HES are activated transcriptionally by this complex.

Key cell cycle regulators cyclin D1, cyclin A, p21, and p27 are among the other NOTCH targets. Several forms of malignant lesions have been shown to express NOTCH receptors abnormally. Numerous stem cell functions in HNSCC, including cell proliferation, differentiation, survival, and self-renewal, depend on NOTCH signaling.

Regarding anti-cancer treatments, γ secretase inhibitors that inhibit NOTCH1 decrease tumor development and prevent CSC function in a variety of tumor types, such as gliomas, brain tumors, and breast cancer. On the other hand, elevated expression of NOTCH1 and JAG1, a NOTCH 1 ligand, is linked to a worse prognosis in HNSCC patients, but high levels of NOTCH1 correspond with higher resistance to cisplatin in HNSCC patients.

Furthermore, by specifically targeting CSCs in HNSCC, inhibition of NOTCH1 delays carcinogenesis, decreases CSC self-renewal and maintenance, and enhances the effectiveness of cisplatin and 5-fluorouracil. Grilli et al., on the other hand, have found a favourable correlation between NOTCH1 expression and non-recurrent disease, longer OS rates, and improved prognosis in patients with HNSCC.

One of the signaling cascades that regulates CSC maintenance and differentiation in HNSCC is the

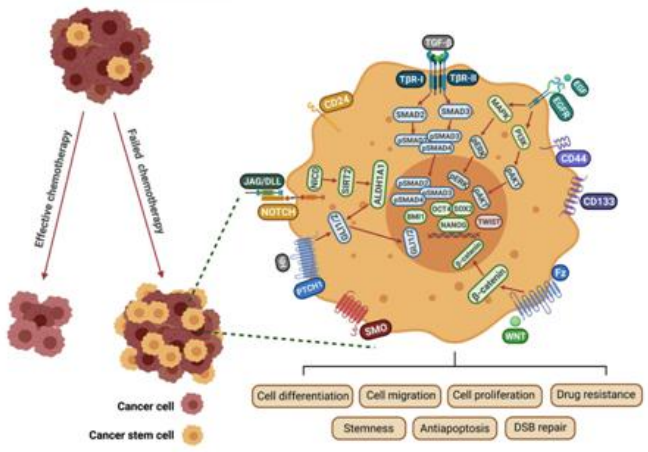
epidermal growth factor receptor pathway. In order to control cell growth, survival, differentiation, angiogenesis, and invasion, EGFR is a transmembrane receptor tyrosine kinase that is activated by a variety of ligands, including epidermal growth factor (EGF) and transforming growth factor-α (TGF-α). This activation of EGFR then leads to the activation of downstream signaling cascades, including PI3K/AKT, MEK-ERK, and phospholipase C signalling.

In patients with HNSCC, over expression of EGFR is linked to therapy resistance and subpar clinical results. A growing body of research suggests that EGFR is essential for the establishment of HNSCC stemness. In HNSCC cells, it has been demonstrated that CD44 interacts with EGFR to stimulate cell migration, proliferation, cisplatin resistance, and apoptosis inhibition; however, these results should be verified in tissues obtained from HNSCC patients. Furthermore, through a PI3K-dependent mechanism, EGFR induces glycolysis, EMT, and CSC characteristics, which in turn causes HNSCC metastasis.

According to a recent study, in a human tongue SCC cell line, EGFR activation causes SOX2 phosphorylation at Y277, blocking ubiquitination and the autophagic clearance of SOX2. Furthermore, in a human papillomavirus-16 (HPV-16)-positive cell line, EGFR overexpression is positively connected with a higher functional proportion of ALDH high CSCs; the underlying mechanism, however, is still unknown. Additionally essential for the self-renewal and differentiation of stem cells is WNT signaling.

Canonical and non-canonical WNT signaling pathways are distinguished by the involvement of β-catenin. When WNT protein binds to the Frizzled (Fz) seven transmembrane receptor and the coreceptor lipoprotein receptor-related protein 5 or 6 (LRP 5/6) to create a functional complex, the WNT/β catenin signaling cascade is triggered. Thus, in order to stimulate transcription of downstream targets including cyclin D1 (CCND1), cyclooxygenase 2 (COX2), bone morphogenetic protein 4 (BMP4), matrix metalloproteinases 7 (MMP7), and C-MYC, β-catenin uncouples from the degradation complex and translocates into the nucleus.

WNT/β-catenin signaling has been shown to be essential for preserving the CSC phenotype in a number of cancer types. A possible mechanism could be that β-catenin interacts with CD44, and that WNT-induced β-catenin suppression reduces OCT4 and CD44 expression in HNSCC cells. Additionally, it has been observed that via upregulating the expression of SOX2, WNT pathway activation increases the rate of CSC proliferation and encourages stemness and sphere formation in HNSCC cell cultures. Furthermore, via controlling DNA damage repair in HNSCC, β-catenin is a key mediator of cisplatin resistance.



Mechanisms underlying treatment resistance in HNSCC generated by CSC.

Fz stands for frizzled; EGF and EGFR stand for epidermal growth factor and receptor, respectively. Double-strand break (DSB), delta-like ligand (DLL), and jagged ligand (JAG) NICD, or the NOTCH protein's

intracellular domain; SMO, or smoothened; PI3K, phosphoinositide 3-kinases; MAPK, mitogen-activated protein kinase; ALDH1, Aldehyde dehydrogenase 1 family; TGF-β, transforming growth factor; TβR, transforming growth factor β receptor; PATCH1, protein patched homolog 1; OCT4, Octamer-binding transcription factor 4; SOX2, Sex determining region Y-box 2; BMI1, B lymphoma Mo-MLV insertion region 1 homolog; NANOG, Nanoghomeobox.

Surface Markers Used For the Identification

Currently, in vitro tumorsphere development and in vivo limiting-dilution tumorigenicity experiments in immunocompromised mice are the gold standards for identifying CSCs.

Numerous cell surface markers, such as CD133, CD44, CD90, CD34, ALDH1, EpCAM, and others, can be utilized to identify CSC-rich subtypes in a variety of solid tumor and hematological malignancy types, as studies have shown.

Even if CSCs do not express these markers precisely, it is nevertheless possible to enrich CSC subgroups in vitro by combining one or more markers. For example, leukemia stem cells can potentially be separated using CD34, CD38, and IL3Rα together. Additionally, one of the best-characterized biomarkers for CSC isolation is CD133, a pentaspan membrane glycoprotein. The first application of CD133 as a GSC (glioblastoma stem cell) marker occurred. Only the CD133+ subpopulation of primordial GSCs can sustain cancer and generate heterogeneity; CD133− cells cannot. Recent research has revealed that CD133 co-expression in tumor cells enhances the CSC phenotype, as does co-expression of other CSC markers such integrin α6 and ALDH.

There are two more prevalent surface markers of CSCs: CD44 and ALDH1. To identify cancer cells having stemness traits, they can be utilized either by themselves or in conjunction with other markers. A multitude of solid CSCs have been isolated using the combination of CD44+CD24− and ALDH1+, particularly for the enrichment of oral squamous cell carcinoma stem cells and breast CSCs.

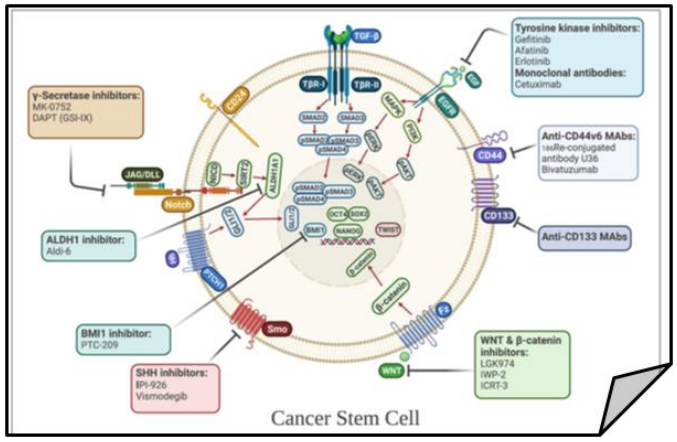
Furthermore, several more potent CSC markers have been discovered in subsequent investigations. For instance, in syngeneic mice models of medulloblastoma and human glioblastoma, SSEA-1 (stage-specific embryonic antigen) was found to be a CSC marker. These CSC indicators help with cancer diagnosis, prognosis evaluation, and detection in addition to identifying and enriching CSC subgroups. Here, we've compiled a list of frequent surface markers for CSCs found in a range of solid and hematological cancers.

Marker	Detected in healthy tissue	Expression in cancer stem cells
CD133	Expressed in various cell types and tissue sites, especially proliferating cells	Breast, colon, brain, liver, lung, melanoma, ovarian, pancreatic, and prostate
CD44	Broadly on multiple tissues	Bladder, breast, colon, brain, gastric, head and neck, leukemia, liver, ovarian, pancreatic, and prostate
CD90	T cells; neurons	Breast, brain, liver, and lung
CD34	Hematopoietic and endothelial progenitors	Hematopoietic malignancies
CD24	Broadly on B cells; neuroblasts	Breast, colon, liver, ovarian, and pancreatic
CD38	Multiple stages of B and T cells	Negative on leukemia stem cell
CD71	Broadly on multiple tissues	Negative on gastric stem cell
CD15/SSEA-1	Myeloid cells; adult neural stem/progenitor cells	Brain and melanoma
CD54/ICAM1	Endothelial cells; pneumocytes; lymphoid cells	Gastric, liver, and esophageal
CD166/ALCAM	Membranous expression in various tissue	Colon, lung, melanoma, and prostate
CD177	Bone marrow, intestine, and lymphoid tissue	Lung, leukemia, and ovarian
ALDH1A1	Broadly on multiple tissues	Bladder, breast, colon, brain, gastric, head and neck, lung, pancreatic, and prostate
ABCG2	Broadly on multiple tissues	Brain, head and neck, lung, melanoma, osteosarcoma, and prostate
ABCB5	Keratinocyte progenitors	Melanoma
EpCAM	Pan-epithelial marker	Breast, colon, lung, and pancreatic
LGR5	Broadly on multiple tissues	Breast, colon, gastric, and head and neck
BMI-1	Broadly on multiple tissues	Breast, brain, head and neck, leukemia, pancreatic, and prostate
Integrin α6	Broadly on multiple tissues	Breast, prostate, and brain
CXCR4	Broadly on multiple tissues	Renal, breast, brain, and pancreatic
Nestin	Nerve cells; neural stem cell	Melanoma, brain, osteosarcoma, ovarian, and prostate

Embryonic surface antigen specific to SSEA-1 stage 1. ALCAM-activated leukocyte cell adhesion molecule, ICAM1 intercellular cell adhesion molecule-1, EpCAM, an epithelial cell adhesion molecule, is a member of subfamily B of the ATP binding cassette subfamily B, or ABCB5. G-protein-coupled receptor 5 is contained in the leucine-rich repeat LGR5. CXCR4 Chemokine receptor with C-X-C motif.

Targeting and Challenges in HNSCC Cancer Stem Cell-Directed Therapy

Early research from clinical trials has shown minimal success with monotherapy compared to conventional medicines, despite the advancements in targeted therapy for HNSCC. Therefore, in order to counteract chemoresistance, stop tumor relapse, and prevent metastasis, novel treatment approaches that specifically target CSCs are being developed to be utilized in conjunction with traditional non-targeted therapies.



Therapeutics targeting cancer stem cells in HNSCC. A few anti-CSC medications that are presently being studied clinically. Targeting CSC-associated surface markers and signaling pathways, such as developmental pathways, that control the upkeep and survival of CSCs is one of their modes of action.

Targeting Self-Renewal Pathways

Today, inhibiting the major self-renewal signaling cascades mediated by EGFR, NOTCH, WNT, and SHH is one of the most promising approaches to target HNSCC CSCs. When EGFR is blocked with gefitinib, c-MYC and NANOG levels are decreased. These two factors are necessary for reprogramming induced pluripotent stem cells. Afatinib, a second-generation tyrosine kinase inhibitor, inhibits the development and proliferation of tumor spheres, induces radiosensitization, and down regulates CD44 and OCT4 to decrease the self-renewal and invasive characteristics of HNSCC CSCs in culture.

Since NOTCH, WNT, and SHH signaling are crucial for the maintenance of CSC in HNSCC, treating recurrent or metastatic HNSCC may benefit from targeting these pathways. The inhibitor of β-catenin responsive transcription (ICRT-3) halts the cell cycle and reduces the motility of HNSCC cells in relation to WNT signaling.

SHH inhibitors have a similar pattern; EGFR inhibitor-resistant HNSCC cells treated with the SHH inhibitor IPI-926, according to one study, limit tumor development and prevent tumor recurrence in patient-derived HNSCC xenografts. Furthermore, in HNSCC cells, the SHH pathway inhibitor vismodegib increases radiation-induced DNA damage and reduces GLI1 and Survivin expression.

Targeting Metabolic and Cell Surface Markers

It's possible that the markers utilized to distinguish and enhance CSCs could be exploited as targets for HNSCC treatment.

Therapeutic target	Compound	Mechanism	Model
Nanog	Silencing	Suppresses tumorigenic and CSCs-like abilities	In vitro
Grp78	Silencing	Inhibits tumor growth and stem cell regulatory proteins i.e., slug and Oct-4	In vitro
CD44	Silencing	Decreases migration, EMT, and reduces the expression of snail, vimentin, N-cadherin and slug	In vitro
Inhibiting translation elongation	SVC112	Increases the progression of cell-cycle slows and delay DNA repair following radiation. Improves colony and sphere formation	In vitro
Let-7d/CDC34 axis	Niclosamide	Induces cell cycle arrest in G1 phase	In vitro, in vivo
ST4	MEDI0641	Decreases the CSC fraction, and tumor regression	In vivo
cMET/FZD8	PF-2341066	Decreases tumor initiation, sphere formation, and metastatic spread	In vivo
CD44v6	Anti-CD44v6 antibody BIWA-IRDye800CW and -Indium-111	Detection of tumor regions and invasive zones	In vivo
CD44	Radionuclide ^{186}Re-cmAb (U36)	Dose-limiting myelotoxicity, reduction in tumor size	Human
ALDH1	Alda-89, Aldi-6	In combination with cisplatin improves apoptosis and decreases tumor growth	In vitro, in vivo
Porcupine (PORCN) (Wnt signaling)	LGK974	High response in HNSCC with Notch loss of function mutation	In vitro
FGF	BGJ398	Reduces ALDH^{high}CD44^{high}, sensitization to cisplatin	In vitro
Bmi1/AP-1	PTC-209	Cisplatin plus PTC-209 potently eradicates Bmi1 + CSCs and suppresses progression of tumor	In vitro

Conclusion

CSCs are among the key participants in the development and spread of cancer. Numerous studies have shown that these subpopulations of cancer cells are linked to various cancer characteristics, including metastasis, tumorigenicity, and recurrence. CSCs are hence referred

to be the cancer's root cause. Furthermore, one of the promising and evasive therapy strategies that intended to improve efficacy and specificity for eliminating tumors and lowering systemic or off-target toxicity would be to target CSCs. As a result, one of the busy and quickly expanding topics would be research into the further description and tailored therapies for head and neck CSCs. Considering that CSCs influence cellular and molecular targets and pathways (such as microRNAs, histone changes, and calcium controls) in order to fulfill their tumorigenic activities. Thus, a deeper comprehension of the functions of CSCs may present special chances for the creation of novel therapeutic platforms that specifically target CSCs in the management of a variety of malignancies.

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