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Review Article

Pathology Section

Understanding Cancer Stem Cells in Head and Neck Squamous Cell Carcinoma: A Critical Update in Development of New Approaches

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ABSTRACT

Cancer Stem Cells (CSCs) have an important role in Head and Neck Squamous Cell Carcinoma (HNSCC). HNSCC is considered as one of the major health problems throughout the world. These self-renewing cells are responsible for resistance to conventional anti-cancer therapy. CSCs have the ability of cancer recurrence; metastasis and can form a heterogeneous tumour. The idea of 'CSCs' has led the scientific community to a new era in the field of research and possibly effective treatment modalities for cancer in the future. This paper aims primarily to review the recent advancements made in the use of stem cells in the treatment of cancer. Secondly, this review presents a discussion on the consideration of CSCs being the backbone in the development of cancer and, precisely the role played by the CSCs in carcinogenesis and its outcome leading to development of possible cancer treatment in the future.

Medical databases including Pubmed Central, Google Scholar, Scopus, Copernicus, Science Direct, etc., were used to find all relevant articles related to HNSCC and its relation with CSCs, various mechanisms and therapeutic approaches. Various therapeutic approaches have been employed for the management of HNSCC such as surgical method, chemotherapies and radiotherapies and combinations or formulations of different drugs, but in most cases complete cure has been a failure. This is mostly because the CSCs escape such therapies. However, recent developments have led to the use of targeted therapy such as targeting cell surface markers or signalling pathways, targeting micro environment or blocking epithelial mesenchymal transition, immunotherapy and other approaches as well, leading to the complete eradication of CSCs in HNSCC successfully. Thus, this review presents a better understanding of CSCs and its mechanism of action for development of new treatment modalities.

Keywords: Cancer cells, Radiotherapies, Targeted therapy, Therapeutic, Tumour

INTRODUCTION

Head and neck squamous cell carcinoma can be considered as solid tumours with heterogeneous content and usually have its origin in the epithelial tissue of the oral cavity, pharynx and larynx. A peculiar type of cancer cells has been found to be present in HNSCC and other tumours, similar in behaviour to tumour progenitor cells but having characteristic features consistent with CSCs [1,2]. The CSCs are defined as the cells in the tumour growth having a tumour initiating potential. There are three important characteristics of a normal stem cell:

1. Capacity to self-renew;
2. Ability to strictly control stem cell numbers;
3. Ability to divide and differentiate for generation of all functional properties of that tissue [3].

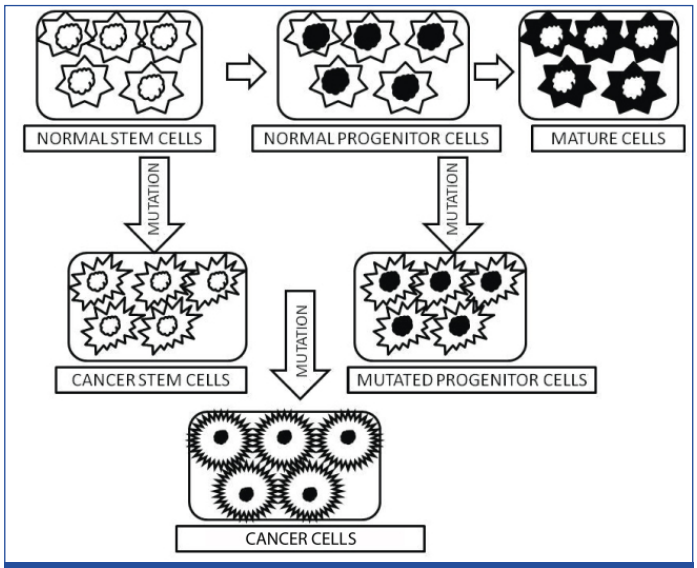
As compared to normal stem cells, the CSCs are known to have no control on the cell numbers. A very small number of CSCs are responsible for the growth of the tumour cells. The identification of the cell type capable of sustaining the neoplastic growth is one of the fundamental problems in cancer. It is evident that most of the cancers are clones and each cancer cell represents the progeny of one cell, but the cell type (CSCs) possessing the tumour-initiating cell function and the methods to recognise them is still unclear [4].

Recently, the CSCs were also shown to be present in solid tumours such as breast cancer and brain tumours [5,6]. The CSCs not only have self-renewal capability but also can generate a wide spectrum of progeny like normal stem cells [7]. Therefore, newer therapeutic approaches are likely to be developed through the identification of CSCs in HNSCC that regulate the growth, metastasis, and treatment resistance of tumour.

CSCs Origin

Two important factors are considered to recognise the origin of the CSCs [Table/Fig-1]:

1. Cells are cancerous depending upon a number of mutations



[Table/Fig-1]: Schematic diagram depicting the origin of cancer stem cell (CSC). Normal stem cells produce normal progenitor cells which undergoes genetic mutation to be transformed into cancer cells.

[8];

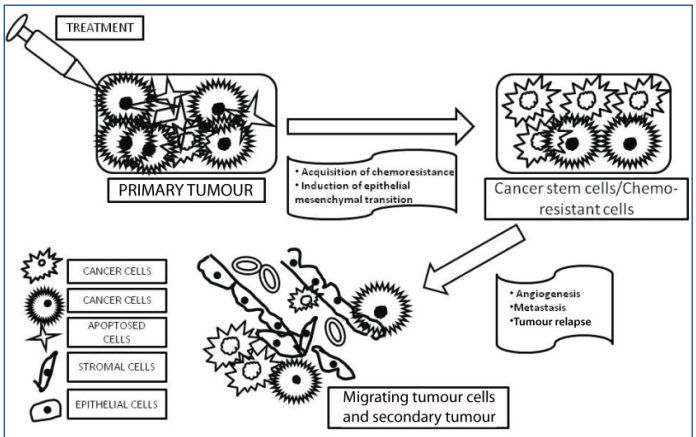
2. Absence of genetic constraints on both self-renewal and proliferation capabilities of CSCs [9];

Thus, CSCs can be obtained from either the self-renewing normal stem cells or from the progenitor cells with the ability of self-renewal due to mutations [10].

Outcomes for Cancer Treatment

There are three possibilities of cancer stem cells playing a role in tumourigenesis [Table/Fig-2]:

1. Development of the primary tumour due to mutation of normal stem cells or progenitor cells into CSCs.
2. Chemotherapy destroys most of the primary tumour cells but not CSCs which may become refractory; hence, may lead to recurrence of tumour.



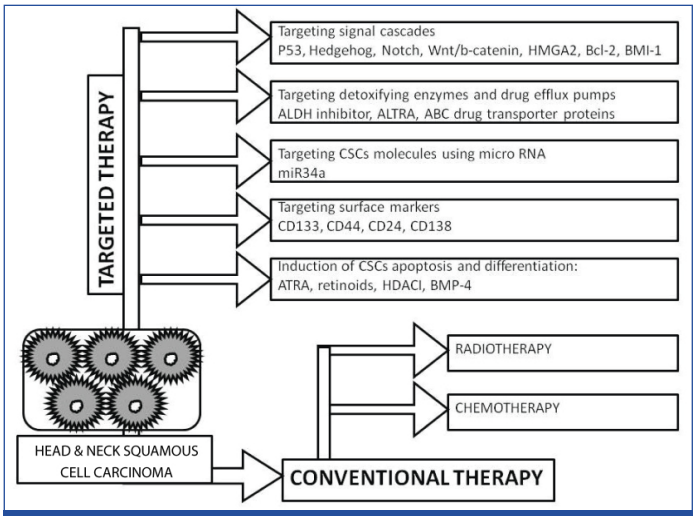
[Table/Fig-2]: Pictorial representation showing the outcomes of cancer treatment. Primary tumour cells undergoing chemotherapy can eventually lead to the development of chemo-resistant cancer stem cells leading to recurrence of metastasis.

3. CSCs may migrate from the primary tumour to distal sites leading to metastasis [11].

The CSCs are comparatively quiescent than other cancer cells. The absence of activated hyperproliferation signals such as tyrosine kinase makes the CSCs resistant to the toxic anticancer drugs that usually target the rapidly dividing cells.

CSCs Therapy

CSCs therapy includes cancer therapy by targeting the population of CSCs utilising several methods [Table/Fig-3]. Chemotherapy and radiotherapy are conventional therapy that are toxic to the healthy tissues and are not always able to kill CSCs, leading to multiple malignancies [12]. Moreover, by the process of Epithelial Mesenchymal Transition (EMT) in embryogenesis, CSCs can also promote metastatic characteristics of tumours [13-15]. Hence, CSCs therapy has become an important focus of cancer research to eliminate malignancies completely. There are various approaches that can target CSCs, most importantly the underlying cause that maintains it and can be utilised for new therapeutic strategies for head and neck cancer treatment. These approaches can be summarised as follows:



[Table/Fig-3]: Diagrammatic representation of the current cancer therapies for head and neck squamous cell carcinoma targeting the population of cancer stem cells (CSC).

1. Targeting signalling pathways: The normal stem cells share common signalling pathways with CSCs like Hedgehog (Hh), Notch, Wnt/b-catenin, or High Mobility Group AT-hook 2 (HMGA2), B-cell lymphoma 2 (Bcl 2), B lymphoma Mo-MLV insertion region 1 homolog (BMI-1), and others that undergo aberrant activation or dysregulation to give rise to CSCs [16-18]. Amongst these, BMI-1 is considered as one of the important stem cell-related genes involved in the regulation of carcinogenesis in head and neck cancers. It was reported that the chemosensitivity of CSCs in HNSCC improved after knockdown of BMI-1 and CD44. Furthermore, the sensitivity of HNSCC cells increased to cisplatin on knockdown of CD44; thus, accounting for CSCs in response to chemotherapy [19]. This suggests that those anticancer drugs having the formulations of various inhibitors of such signalling pathways can be an answer to target the CSCs. However, the drawback of using such inhibitors is their adverse effect on the normal stem cells. Therefore, a combination of other CSCs-targeting therapies can also be used in anticancer drug formulations in order to improve their specificity.

2. Targeting detoxifying enzymes and drug efflux pumps: Certain CSCs are rich in drug detoxifying enzymes like Aldehyde Dehydrogenases (ALDH)-1. The importance of ALDH1 is its specificity as CSCs marker for the identification of highly tumourigenic cells present in HNSCC [20]. It has been shown that ALDH1+ CD44+ cells in HNSCC cells promote tumour propagation by resistance to radiotherapy and maintaining CSCs like properties [21]. Studies have also shown that the use of specific ALDH inhibitor Diethylamino-Benzaldehyde (DEAB) or All-Trans Retinoic Acid (ATRA) caused inhibition of ALDH1 activity in breast CSCs, resulting in reduced aggressiveness. Thus, the sensitivity of breast CSCs was increased towards chemotherapeutic drugs [22]. It was demonstrated earlier that the survival fraction of CSCs was decreased by UCN-01 (a checkpoint kinase inhibitor) in combination with ATRA with irradiation, thus suggesting for a powerful radio-sensitising strategy in HNSCC [23]. Moreover, the ATP Binding Cassette (ABC) drug transporter proteins common for both normal and CSCs are efflux pumps and protect the cells from xenotoxins. These pumps offer multiple drug resistance to the CSCs. It also has been reported that in lung cancer cells the use of pharmacological molecules in vitro and in vivo to target the ABC drug transporter proteins has led to increased sensitivity of CSCs to chemotherapy and radiotherapy [24].

3. Targeting CSCs molecules using micro-RNA: CSC markers can directly target certain micro-RNAs for their down-regulation. For example, miR-34a is one such micro-RNA which is a key negative regulator of CD44+ prostate cancer cells. This suggests the use of miR-34a as novel therapeutic agent against prostate CSCs [25].

4. Induction of CSCs apoptosis and differentiation: The resistance to chemotherapy and radiotherapy can be produced by anti-apoptotic proteins in CSCs such as Interleukin-4 (IL-4) produced by CD133+ colon carcinoma cells [26]. Experiments have shown that the sensitivity of CD133+ colon carcinoma cells to chemotherapeutic drugs (oxaliplatin and 5-fluorouracil)

increased successfully by the use of IL-4 neutralising antibody and IL4 antagonists to target IL-4. Inducing the terminal differentiation of CSCs by agents like ATRA and retinoids, Histone Deacetylase Inhibitors (HDAI) and Bone Morphogenetic Protein 4 (BMP 4) can also be an approach to inhibit the self-renewal of CSCs instead of killing CSCs [27-29].

The origin of tumour may be accompanied by a deorganisation of the normal cellular structure of the tissue/organ and/or an increased proliferative potential known as anaplasia [30]. A number of morphological characteristics are usually considered to define anaplasia, including the following:

a. Abnormal nuclear morphology;
b. Loss of polarity;
c. Mitosis;
d. Pleomorphism;
e. Stromal alterations.

Characteristics of CSCs

1. Resistance to chemotherapy: There are various factors that contribute to drug resistance in cancers for e.g., glutathione and its enzyme structure like topoisomerase II, O6-methylguanine-DNA-methyltransferase, dihydrofolate reductase, metallothioneins, and ABC transporter proteins which are encoded by the multidrug resistant gene, the multidrug resistant protein, and the breast cancer resistant protein1 [31,32]. Therefore, it becomes crucial to investigate relationship between CSCs and these factors.

2. Resistance to irradiation: The ability of cancerous cells to survive and cause tumour recurrence suggests the property of radio-resistance of CSCs [33].

3. Invasion/metastatic activity: The ability of the malignant tumour cells to invade and disseminate into normal tissue help them to metastasise into other tissues. CSCs have high invasion activity as evident from the fact that even surgical operations cannot remove some of the infiltrating cancer cells and causes recurrence. This is also supported by the higher expression of CD44 and chemokines. Chemokine (C-X-C motif) receptor 4 (CXCR4) mediates cell migration in CD133+ cancer cells [34,35].

Preparation of CSCs

1. Cell surface markers: A useful way to separate CSCs from many types of tumours is by cell surface markers, such as CD133. Generally, CD133 molecule (a transmembrane pentaspan protein) is used as a marker of normal haematopoietic stem cells and organ-specific stem cells [36]. The expression of CSC markers in cancers differs in patterns, histological types and degrees of differentiation. The benefit of using such markers is the isolation of CSCs by Fluorescence-Activated Cell Sorting (FACS) and analysis of their biological characteristics for therapeutic purposes.

2. Sphere formation assay: The sphere forming methods are used by CSC researchers to concentrate CSCs in culture. However, it is better to use a monolayer culture method to characterise CSCs. The monolayer-cultured CSCs provide the advantage of being expanded as a homogeneous population [37].

3. Aldehyde dehydrogenase activity: ALDH is a detoxifying enzyme which blocks alkylating agents by oxidising intracellular aldehydes to carboxylic acids. It has been shown earlier that ALDH increases in Tumour Stem Cells (TSCs) [38,39]. Increasing evidence is available which reports that ALDH is expressed strongly by many types of CSCs and can be purified from tumours and cancer cell lines [40-42].

4. Side population: Studies have shown the presence of TSCs in both Side Population (SP) and non-SP and that stem cell marker are not expressed by SP cells [43,44]. It has been also demonstrated that only SP cells and not the non-SP cells possess the property of self-renewal in culture resistance to anti-cancer drugs including mitoxantrone, and formation of tumours when transplanted in vivo [45-50].

5. Niche for CSCs: The regulation of TSCs involves both intrinsic mechanism and extracellular signals which are derived from specialised microenvironment "niche". This is proved by the fact that the ablation of such niche results in loss of TSCs. It appears that such niche is also required by CSCs for tumourigenesis. A premetastatic niche is formed by the bone-marrow derived progenitors before the arrival of cancer cells in the tumour specific premetastatic sites. This is evident from the fact that the ablation of such niche prevents tumour metastasis [51]. Experiments involving specific ablation of endothelial cells associated with tumour using inducible caspase-9 in Severe Combined Immunodeficiency (SCID) mouse model of human tumour angiogenesis showed that there is a decrease in fraction of head and neck CSCs [52]. Similarly, it has been also demonstrated that targeting the CSC niche produced more durable response in HNSCC; thus, developing a new concept of using both conventional chemotherapy and CSCs targeted therapy [53].

Signalling Pathways Involved in CSCs Maintenance

Genetic alterations cause maintenance of TSCs, amplification of precursors, or transformation of differentiated cells to CSCs.

1. p53 pathway: Studies have shown that loss of p53 function promotes accelerated cell proliferation and malignant transformation [54]. The p21 cyclin-dependent kinase (cdk) inhibitor which is considered as the effector of p53 regulates the progression of cells through the G1 cell-cycle phase. However, the use of p21 gene itself as an oncogenic target in human cancers has not been demonstrated.

2. Activation of receptor tyrosine kinase pathway: Signalling pathways of Receptor Tyrosine Kinases (RTKs) that play a role in maintenance of TSCs and amplification of precursors such as Platelet-Derived Growth Factor Receptor (PDGF), Epidermal Growth Factor receptor (EGF), fibroblast growth factor receptor, and insulin-like growth factor 1 receptor are frequently mutated in tumours [55].

3. Notch signalling pathway: Receptors play an important role in biological functions such as cell proliferation, differentiation, survival, and tumourigenesis [56]. Evidences are available which suggest that apart from maintaining the multi-potentiality of neural stem cells the notch activation plays a role in tumourigenesis.

4. Wnt signalling pathway: There are diverse developmental processes like cell proliferation and fate decisions that involves coordination of Wnt family of secreted proteins [57-59].

5. Hedgehog signalling pathway: The processes like proliferation, development, and tumourigenesis also involve Hh signalling. Studies have shown the involvement of ectopic activation of Hh signalling in tumour formation in the central nervous system (CNS), thus suggesting an important role of Hh signalling in brain tumourigenesis [60-62].

6. Stemness in tumour cells: The adult stem cells have a relatively long half-life and can suffer prolonged exposure to genotoxic stress during tumour development. This may cause accumulation of initial mutations leading to cancer [63].

7. Biomarkers in CSCs: There exists a difference in the expression of CSCs markers in cancers depending on its pattern, histological types and degrees of differentiation. Such markers are beneficial in the isolation of CSCs and analysis of their biological characteristics for effective therapeutic purposes.

8. CD133: Studies have shown the use of CD133 as a surface marker of CSCs in solid primary tumours like medullo-blastomas

and glioblastomas, as well as cancers of epithelial tissues. CD133 molecule (a transmembrane pentaspan protein) is also considered as a universal marker of normal haematopoietic stem cells and organ-specific stem cells [64].

9. CD44: Several functions are attributed to the CD44 protein such as cell adhesion, motility, proliferation, drug resistance, and cell survival [65,66]. Experiments have also shown the role of CD44 in lymphocyte homing, wound healing, and cell migration, cancer cell growth and metastasis [67,68]. An immune-deficient mouse model was used in one of the first studies on CSCs in HNSCC. This study demonstrated that those CD44+ cancer cells that are responsible for formation of new tumours in vivo along with the ability of self-renewal and differentiation constitute only 10% of the cells in a HNSCC primary tumour. However, some of HNSCC expressing CD44s (standard form) and CD44 v6 (alternative splice variant), particularly in laryngeal cancers are associated with a poorer disease-free survival [69].

10. CD24: Recent studies have reported the role of CD24 in the developmental hedgehog pathway that is often active in CSCs [70].

11. CD138: Tumour cells that lack CD138 are capable of clonogenic growth and are relatively drug resistant. Such functional properties have also been attributed to CSCs in several human cancers [71].

Type of cancer	CD molecule
Prostate cancer	CD147+ [72]
Classic Hodgkin's lymphoma	CD20+ [73]
Non-Hodgkin's lymphoma	CD47+ [74]
Hepatocellular carcinoma	CD90+ [75]
Osteosarcoma	CD117+ [76]
Acute myeloid leukaemia	CD32+ or CD25+ or both [77], CD34+ CD38– [78]
Multiple myeloma	CD138– CD20+/ CD27+ [79]

[Table/Fig-4]: List of CD molecules related to various cancer types [72-79].

12. Other CD molecules: Additionally, a list of other CD molecules in CSCs in various types of cancer is provided in the [Table/Fig-4] [72-79].

CONCLUSION

A better understanding of the resistance mechanisms of CSCs in HNSCC is required involving future studies for improving therapy and possibly preventing tumour spread or recurrence. The important roles of CSCs in progression of cancer can be evaluated by complementary in vitro approaches. Investigation of CSCs gives the idea of making novel targets for cancer that can be helpful in drug resistance and may combat the tumour cell metastasis [80]. When the patients are given therapy to cancer, great care must be taken as cancer stem cells are similar to normal stem cells. New and improved experimental approaches such as in vitro assay systems, will allow scientists to evaluate the relation between the different cell types within a tumour and their microenvironment.

REFERENCES

[1] Prince ME, Sivanandan R, Kaczorowski A, Wolf GT, Kaplan MJ, Dalerba P, et al. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. Proc Natl Acad Sci USA. 2007;104(3):973-78.
[2] Wicha MS, Liu S, Dontu G. Cancer stem cells: an old idea- -a paradigm shift. Cancer Res. 2006;66:1883-90.
[3] Bixby S, Kruger GM, Mosher JT, Joseph NM, Morrison SJ. Cell intrinsic differences between stem cells from different regions of the peripheral nervous system regulate the generation of neural diversity. Neuron. 2002;35(4):643-56.
[4] Hope KJ, Jin L, Dick JE. Human acute myeloid leukemia stem cells. Arch Med Res. 2003;34(6):507-14.
[5] Dick JE. Breast cancer stem cells revealed. Proc Natl Acad Sci USA. 2003;100:3547-49.
[6] Galderisi U, Cipollaro M, Giordano A. Stem cells and brain cancer. Cell Death Differ. 2006;13:05-11.
[7] Al-Hajj M, Wicha MS, Ito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumourigenic breast cancer cells. Proc Natl Acad Sci USA. 2003;100(7):3983-88.
[8] Kopelovich L, Herbert BS. Heritable one-hit events defining cancer prevention? Cell Cycle. 2013;12:2553-57.
[9] Morrison SJ, Qian D, Jerabek L, Thiel BA, Park IK, Ford PS, et al. A genetic determinant that specifically regulates the frequency of haematopoietic stem cells. J Immunol. 2002;168:635-42.
[10] Al-Hajj M, Becker MW, Wicha M, Weissman I, Clarke MF. Therapeutic implications of cancer stem cells. Curr Opin Genet Dev. 2004;14(1):43-47.
[11] Jordan CT, Guzman ML, Noble M. Cancer stem cells. N Engl J Med. 2006;355(12):1253-61.
[12] Winquist RJ, Boucher DM, Wood M, Furey BF. Targeting cancer stem cells for more effective therapies: Taking out cancer's locomotive engine. Biochem Pharmacol. 2009;78:326-34.
[13] Chen C, Wei Y, Hummel M, Hoffmann TK, Gross M, Kaufmann AM, et al. Evidence for epithelial-mesenchymal transition in cancer stem cells of head and neck squamous cell carcinoma. PLoS One. 2011;6(1):e16466.
[14] Davis SJ, Divi V, Owen JH, Bradford CR, Carey TE, Papagerakis S, et al. Metastatic potential of cancer stem cells in head and neck squamous cell carcinoma. Arch Otolaryngol Head Neck Surg. 2010;136(12):1260-66.
[15] Sun S, Wang Z. Head neck squamous cell carcinoma c-Met+ cells display cancer stem cell properties and are responsible for cisplatin-resistance and metastasis. Int J Cancer. 2011;129(10):2337-48.
[16] Maugeri-Saccà M, Zeuner A, De Maria R. Therapeutic targeting of cancer stem cells. Front Oncol. 2011;1:10.
[17] Muller JM, Chevrier L, Cochaud S, Meunier AC, Chadeneau C. Hedgehog, Notch and Wnt developmental pathways as targets for anti-cancer drugs. Drug Discov Today: Disease Mechanism. 2007;4:285-91.
[18] Merchant AA, Matsui W. Targeting Hedgehog-a cancer stem cell pathway. Clin Cancer Res. 2010;16(12):3130-40.
[19] Chen YC, Chang CJ, Hsu HS, Chen YW, Tai LK, Tseng LM, et al. Inhibition of tumourigenicity and enhancement of radio-chemosensitivity in head and neck squamous cell cancer-derived ALDH1-positive cells by knockdown of Bmi-1. Oral Oncol. 2010;46(3):158-65.
[20] Chen YC, Chen YW, Hsu HS, Tseng LM, Huang PI, Lu KH, et al. Aldehyde dehydrogenase 1 is a putative marker for cancer stem cells in head and neck squamous cancer. Biochem Biophy Res Commun. 2009;385(3):307-13.
[21] Visus C, Ito D, Amoscato A, Maciejewska-Franczak M, Abdelsalem A, Dhir R, et al. Identification of human aldehyde dehydrogenase 1 family member A1 as a novel CD8+ T-cell-defined tumour antigen in squamous cell carcinoma of the head and neck. Cancer Res. 2007;67(21):10538-45.
[22] Croker AK, Allan AL. Inhibition of aldehyde dehydrogenase (ALDH) activity reduces chemotherapy and radiation resistance of stem-like ALDHhiCD44+ human breast cancer cells. Breast Cancer Res Treat. 2012;133(1):75-87.
[23] Bertrand G, Maalouf M, Boivin A, Battiston-Montagne P, Beuve M, Levy A, et al. Targeting head and neck cancer stem cells to overcome resistance to photon and carbon ion radiation. Stem Cell Rev. 2014;10(1):114-26.
[24] Xia X, Yang J, Li F, Li Y, Zhou X, Dai Y, et al. Image-based chemical screening identifies drug efflux inhibitors in lung cancer cells. Cancer Res. 2010;70(19):7723-33.
[25] Liu C, Kelnar K, Liu B, Chen X, Calhoun-Davis T, Li H, et al. The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44. Nat Med. 2011;17(2):211-15.
[26] Todaro M, Alea MP, Di Stefano AB, Cammareri P, Vermeulen L, Iovino F, et al. Colon cancer stem cells dictate tumour growth and resist cell death by production of interleukin-4. Cell Stem Cell. 2007;1(4):389-402.
[27] Massard C, Deutsch E, Soria JC. Tumour stem cell-targeted treatment: elimination or differentiation. Ann Oncol. 2006;17(11):1620-24.
[28] Butler LM, Zhou X, Xu WS, Scher HI, Rifkind RA, Marks PA, et al. The histone deacetylase inhibitor SAHA arrests cancer cell growth, up-regulates thioredoxin-binding protein-2, and down-regulates thioredoxin. Proc Natl Acad Sci USA. 2002;99(18):11700-05.
[29] Lombardo Y, Scopelliti A, Cammareri P, Todaro M, Iovino F, Gulotta G, et al. Bone morphogenetic protein 4 induces differentiation of colorectal cancer stem cells and increases their response to chemotherapy in mice. Gastroenterology. 2011;140(1):297-309.
[30] Stricker TP, Kumar V. Neoplasia. Robbins and Cotran Pathologic Basis of Disease. 8th ed. Philadelphia: Saunders/Elsevier; 2010. pp. 259-330.
[31] Gottesman MM, Fojo T, Bates SE. Multidrug resistance in cancer: role of ATP-dependent transporters. Nat Rev Cancer. 2002;2(1):48–58.
[32] Szakacs G, Paterson JK, Ludwig JA, Booth-Genthe C, Gottesman MM. Multidrug resistance in cancer. Nat Rev Drug Discov. 2006;5:219-34.
[33] Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, et al. Glioma stem cells promote radio-resistance by preferential activation of the DNA damage response. Nature. 2006;444(7120):756-60.
[34] Hermann PC, Huber SL, Herrler T, Aicher A, Ellwart JW, Guba M, et al. Distinct populations of cancer stem cells determine tumour growth and metastatic activity in human pancreatic cancer. Cell Stem Cell. 2007;5(3):313-23.
[35] Liu G, Yuan X, Zeng Z, Tunici P, Ng H, Abdulkadir IR, et al. Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. Mol Cancer. 2006;5:67-78.
[36] Karbanová J, Missol-Kolka E, Fonseca AV, Lorra C, Janich P, Hollerová H, et al.

The stem cell marker cd133 (prominin-1) is expressed in various human glandular epithelia. J Histochem Cytochem. 2008;56(11):977-93.
[37] Pollard SM, Yoshikawa K, Clarke ID, Danovi D, Striker S, Russell R, et al. Glioma stem cell lines expanded in adherent culture have tumour-specific phenotypes and are suitable for chemical and genetic screens. Cell Stem Cell. 2009;4(6):568-80.
[38] Tomita H, Tanaka K, Tanaka T, Hara A. Aldehyde dehydrogenase 1A1 in stem cells and cancer. Oncotarget. 2016;7(10):11018-32.
[39] Cai J, Cheng A, Luo Y, Lu C, Mattson MP, Rao MS, et al. Membrane properties of rat embryonic multipotent neural stem cells. J Neurochem. 2004;88(1):212-26.
[40] Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, et al. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Cell. 2007;1(5):555-67.
[41] Korkaya H, Paulson A, Iovino F, Wicha MS. HER2 regulates the mammary stem/progenitor cell population driving tumourigenesis and invasion. Oncogene. 2008;27(47):6120-30.
[42] Pearce DJ, Taussig D, Simpson C, Allen K, Rohatiner AZ, Lister TA. Characterization of cells with a high aldehyde dehydrogenase activity from cord blood and acute myeloid leukaemia samples. Stem Cells. 2005;23(6):752-60.
[43] Mitsutake N, Iwao A, Nagai K, Namba H, Ohtsuru A, Saenko V. Characterization of side population in thyroid cancer cell lines: cancer stem-like cells are enriched partly but not exclusively. Endocrinology. 2007;148(4):1797-803.
[44] Morita Y, Ema H, Yamazaki S, Nakauchi H. Non-side-population haematopoietic stem cells in mouse bone marrow. Blood. 2006;108(8):2850-56.
[45] Haraguchi N, Utsunomiya T, Inoue H, Tanaka F, Mimori K, Barnard GF, et al. Characterization of a side population of cancer cells from human gastrointestinal system. Stem Cell. 2006;24(3):506-13.
[46] Hirschmann-Jax C, Foster AE, Wulf GG, Nuchtern JG, Jax TW, Gobel U, et al. A distinct "side population" of cells with high drug efflux capacity in human tumour cells. Proc Natl Acad Sci USA. 2004;101(39):14228-33.
[47] Kondo T, Setoguchi T, Taga T. Persistence of a small subpopulation of cancer stem-like cells in the C6 glioma cell line. Proc Natl Acad Sci USA. 2004;65(14):781-86.
[48] Patrawala L, Calhoun T, Schneider-Broussard R, Zhou J, Claypool K, Tang DG. Side population is enriched in tumourigenic, stem-like cancer cells, whereas ABCG2+ and ABCG2- cancer cells are similarly tumourigenic. Cancer Res. 2005;65(14):6207-19.
[49] Ponti D, Costa A, Zaffaroni N, Pratesi G, Petrangolini G, Coradini D, et al. Isolation and in vitro propagation of tumourigenic breast cancer cells with stem/progenitor cell properties. Cancer Res. 2005;65(13):5506-11.
[50] Szotek PP, Pieretti-Vanmarcke R, Masiakos PT, Dinulescu DM, Connolly D, Foster R, et al. Ovarian cancer side population defines cells with stem cell-like characteristics and Mullerian Inhibiting Substance responsiveness. Proc Natl Acad Sci USA. 2006;103(30):11154-59.
[51] Kaplan RN, Riba RD, Zacharoulis S, Bramley AH, Vincent L, Costa C, et al. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature. 2005;438(7069):820-27.
[52] Krishnamurthy S, Dong Z, Vodopyanov D, Imai A, Helman JI, Prince ME, et al. Endothelial cell-initiated signalling promotes the survival and self-renewal of cancer stem cells. Cancer Res. 2010;70(23):9969-78.
[53] Krishnamurthy S, Nör JE. Head and neck cancer stem cells. J Dent Res. 2012;91:334-40.
[54] Toledo F, Wahl GM. Regulating the p53 pathway: in vitro hypotheses, in vivo veritas. Nat Rev Cancer. 2006;6(12):909-23.
[55] Schubbert S, Shannon K, Bollag G. Hyperactive Ras in developmental disorders and cancer. Nat Rev Cancer 2007;7(4):295-308.
[56] Radtke F, Raj K. The role of Notch in tumourigenesis: oncogene or tumour suppressor? Nat Rev Cancer. 2003;3(10):756-67.
[57] Logan CY, Nusse R. The Wnt signalling pathway in development and disease. Annu Rev Cell Dev Biol. 2004;20:781-810.
[58] Moon RT, Kohn AD, De Ferrari GV, Kaykas A. WNT and beta-catenin signalling: diseases and therapies. Nat Rev Genet. 2004;5(9):691-701.
[59] Reya T, Clevers H. Wnt signalling in stem cells and cancer. Nature. 2005;434(7035):843-50.
[60] Pasca di Magliano M, Hebrok M. Hedgehog signalling in cancer formation and maintenance. Nat Rev Cancer 2003;3(12):903-911.
[61] Ruiz i Altaba A, Palma V, Dahmane N. Hedgehog-Gli signalling and the growth of the brain. Nat Rev Neurosci. 2002;3(1):24-33.
[62] Ruiz i Altaba A, Sanchez P, Dahmane N. Gli and hedgehog in cancer: tumours, embryos and stem cells. Nat Rev Cancer. 2002;2(5):361-72.
[63] Lobo NA, Shimono Y, Qian D, Clarke MF. The biology of cancer stem cells. Annu Rev Cell Dev Biol. 2007;23:675-99.
[64] Kusumbe AP, Mali AM, Bapat SA. CD133-expressing stem cells associated with ovarian metastases establish an endothelial hierarchy and contribute to tumour vasculature. Stem Cells. 2009;27(3):498-508.
[65] Marhaba R, Zoller M. CD44 in cancer progression: adhesion, migration and growth regulation. J Mol Histol. 2004;35(3):211-31.
[66] Afify A, Purnell P, Nguyen L. Role of CD44s and CD44v6 on human breast cancer cell adhesion, migration, and invasion. Exp Mol Pathol. 2009;86(2):95–100.
[67] Ponta H, Sherman L, Herrlich PA. CD44: from adhesion molecules to signalling regulators. Nat Rev. 2003;4(1):33-45.
[68] Ishimoto T, Oshima H, Oshima M. CD44 (+) slow-cycling tumour cell expansion is triggered by cooperative actions of Wnt and prostaglandin E(2) in gastric tumourigenesis. Cancer Sci. 2009;101(3):673-78.
[69] Staibano S, Merolla F, Testa D, Iovine R, Mascolo M, Guarino V, et al. OPN/CD44v6 overexpression in laryngeal dysplasia and correlation with clinical outcome. Br J Cancer. 2007;97:1545-51.
[70] Keysar S, Jimeno A. More than markers: biological significance of cancer stem cell defining-molecules. Mol Cancer Ther. 2010;9(9):2450-57.
[71] Matsui W, Huff CA, Wang Q, Malehorn MT, Barber J, Tanhehco Y, et al. Characterization of clonogenic multiple myeloma cells. Blood. 2004;103(6):2332-36.
[72] Hao JL, Cozzi PJ, Khatri A, Power CA, Li Y. CD147/EMMPRIN and CD44 are potential therapeutic targets for metastatic prostate cancer. Current Cancer Drug Targets. 2010;10(3):287-93.
[73] Oki Y, Younes A. Does rituximab have a place in treating classic hodgkin lymphoma? Curr Haematol Malig Rep. 2010;5(3):135-39.
[74] Chao MP, Alizadeh AA, Tang C, Mylebust JH, Varghese B, Gill S, et al. Anti-CD47 antibody synergizes with rituximab to promote phagocytosis and eradicate non-Hodgkin lymphoma. Cell. 2010;142(5):699-713.
[75] Lingala S, Cui YY, Chen X, Ruebner BH, Qian XF, Zern MA, et al. Immuno histochemical staining of cancer stem cell markers in hepatocellular carcinoma. Exp Mol Pathol. 2010;89(1):27–35.
[76] Adhikari AS, Agarwal N, Wood BM, Porretta C, Ruiz B, Pochampally RR, et al. CD117 and Stro-1 identify osteosarcoma tumour-initiating cells associated with metastasis and drug resistance. Cancer Res. 2010;70(11):4602-12.
[77] Saito Y, Kitamura H, Hijikata A, Tomizawa-Murasawa M, Tanaka S, Takegi S, et al. Identification of therapeutic targets for quiescent, chemotherapy- resistant human leukaemia stem cells. Sci Transl Med. 2010;2:17-19.
[78] Natasha YF, Markus HF. ABCB5 gene amplification in human leukaemia cells. Leuk Res. 2009;33:1303-05.
[79] Matsui W, Wang Q, Barber JP, Brennam S, Smith BD, Borrello I, et al. Clonogenic multiple myeloma progenitors, stem cell properties, and drug resistance. Cancer Res. 2008;68(1):190-97.
[80] Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CHM, Jones D L et al. Cancer stem cells-Perspectives on current status and future directions: AACR Workshop on Cancer Stem Cells. Cancer Research. 2006;66(19):9339-44

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