Review Article

Implications of Stem Cells in Head and Neck Squamous Cell Carcinoma: An Evolving Concept

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ABSTRACT

Head and neck squamous cell carcinoma stands as one of the major cancer deaths over the globe. Surgery, chemotherapy and radiotherapy being the mainstay of management, no satisfactory improvements in post-treatment survival rate of patients have been achieved over past few decades. In recent times, a noble concept of cancer stem cells, known as the 'cancer stem cell hypothesis' has gained a huge popularity due to its ability to explain the causes of pitfalls present in management of cancers. Various cancer stem cell molecular markers have been identified as being particular identity to particular cancer stem cells and they have been shown to possess properties like self-renewal, tumorigenicity and differentiation similar to normal somatic stem cells. This article outlines the introduction to stem cells, identification in head and neck squamous cell carcinoma based on molecular markers like CD44+ and ALDH1+ and the proposed mechanisms of resistance to conventional therapies. Various approaches that are underway to use the 'cancer stem cell hypothesis' in management of cancer by targeting the signaling pathways of tumorigenesis are also enlightened here which is assumed to invite a new era in cancer management.

Keywords: Head and neck squamous cell carcinoma; cancer stem cell hypothesis; molecular markers; signaling pathways; tumorigenesis.

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) including oral squamous cell carcinoma is documented as sixth most common malignancy in the world accounting for estimated 500,000 new cases and 200,000 deaths per year and covers approximately 8-10% of all cancers in south west Asia.[1-7] It comprises 90% of head and neck cancers and originates from the stratified squamous epithelium of the aero digestive tract that include lips, oral cavity, nasal cavity, paranasal sinuses, pharynx and the larynx.[8] As a result of advanced understanding of the disease with respect to its diagnosis and management, there is significant improvement in quality of life of patients but long term survival rates have remained static or have improved marginally over past few decades. And still, the mainstays of management are chemotherapy, surgery and radiation therapy.[1] Five year survival rates for HNSCC after treatment have remained somewhat the same in more than three decades.[9] Thus, it seems a better understanding of the knowledge regarding etiology, pathogenesis, diagnosis and management of HNSCC needs to be developed to correctly determine the loopholes and address them.

Various experiments have successfully shown evidences that growth and development of a tumor and its transition into a cancer might be dependent on a minority of population of cells, also designated

as 'subpopulation,' present in approximately less than 1% of total population of tumor cells. These cells have been referred to as the cancer stem cells (CSCs).[10] The theory proposed that presumes the role of these subpopulation cells in development of cancers has been popularized as the cancer stem cell hypothesis (CSC hypothesis) which mentions that the CSCs are a minor population of cells within the tumor that possess unique and mention worthy properties like: a)Differentiation: giving rise to a heterogeneous progeny, b)Self-renewal: maintaining the intact stem cell pool for propagation and c)Homeostatic control: ensuring proper harmony and regulation between differentiation and self renewal depending on the genetic and environmental factors of each organ tissue and this defines the tissue specificity of CSCs.[11] Further, CSCs have been postulated to arise either from the normal stem cells as a result of gene mutation that render the stem cells cancerous and/or develop from mutated progenitor cells that experience genetic alterations to become differentiated and acquire CSCs like features.[1,12]

After being experimentally defined in hematopoietic malignancy by John Dick and colleagues in 1994,[13] a number of investigations have identified CSCs in solid malignancies including breasts, brain, prostate, lung, colon, pancreas, liver and skin.[1, 14-21] Similarly, Prince et al in 2007 identified a subpopulation of cells in head and neck squamous cell carcinoma by demonstration of membrane CD44+ and nuclear BMI1+.[1] This raises a strong suspicion that eradication of these cancer stem cells can probably be a critical part of any successful anticancer therapy including HNSCC, and may explain why conventional cancer therapies are rarely completely curative.[12] This article reviews the nature of cancer stem cell and its identification as it applies to HNSCC and several possible implications of exploring role of CSCs in HNSCC in relation to diagnosis and therapeutic management of the same.

STEM CELLS AND CANCER STEM CELLS (CSCs): AN OVERVIEW

What are stem cells?

Stem cells are biological cells found in all multi-cellular organisms that can divide (through mitosis) and differentiate into diverse specialized cell types and can self-renew to produce more stem cells. This postulates that adult stem cells form a pool of long living cells that continuously supply undifferentiated cells to their compartments that forms the basis of homeostatic control in adult tissues underlying regular cell turnover.[22] This property is simply represented by the hierarchy that stem cell possess (Figure 1).

What are cancer stem cells (CSCs)?

The cancer stem cell hypothesis postulates that the tumor heterogeneity with regards to initiation, progression, response to therapy, and metastasis is the result of mutations of genes that render a normal somatic cell cancerous or may cause a cancer cell to become stem cell like after experiencing further genetic alterations.[11,23] The cancer stem cell thus born is thought to be able to give rise to additional CSCs and to a multitude of more differentiated and functionally divergent cancer cells, as that of normal somatic tissue stem cells. Another important postulation of this hypothesis states that only a small subset/subpopulation of cancer cells pose the unique property of tumorigenicity (initiation and growth) rather than the bulk of tumor. Thus CSCs are defined as a small subset of cancer cells that comprise a pool of self-sustaining cells with the exclusive capacity to maintain tumor.[11]

As with normal somatic stem cells, the CSCs are able to self renew and give rise to heterogeneous population of stem cells. In addition to their replicative capacity, CSCs, like their somatic counterparts are also more resistant to the effects of cytotoxic chemotherapy and radiation damage.[24-29]

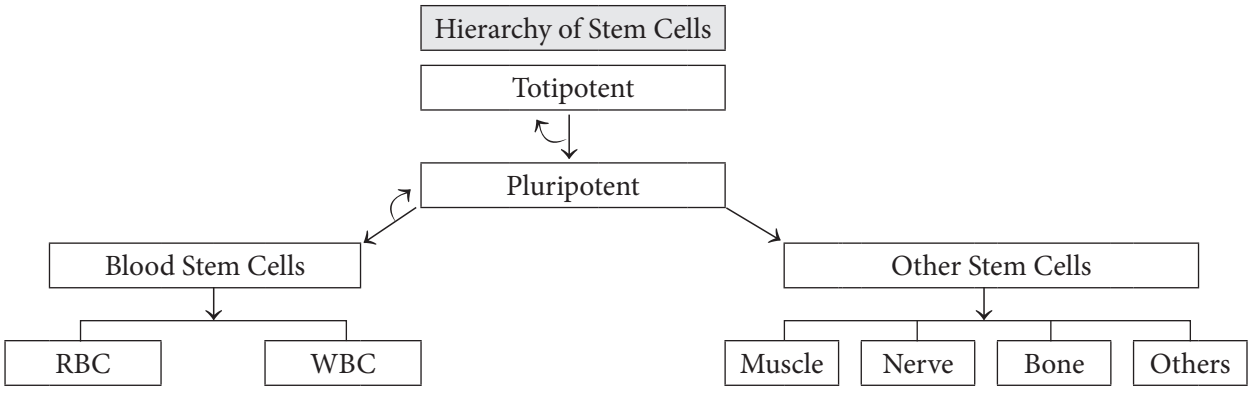

Figure 1: Schematic diagram showing hierarchy of somatic stem cells that depict the differentiating property of stem cells.

Two distinctive forms of CSCs has been proposed by Brabletz et al in 2005:[30] stationery CSCs (sCSCs) that reside in the epithelia and cannot disseminate and mobile/migrating CSCs (mCSCs) located in host-tumor interface and mediate metastasis. Both types of cells play role in differentiation and heterogeneity but, sCSC form the primary tumor and mCSC mediate metastasis. It is also note worthy that mCSC is derivative of sCSC by acquiring a transient epithelial-mesenchymal transition (EMT). But even the metastatic tumors derived from the mCSC may undergo a mesenchymal-epithelial transition (MET) at the site of metastasis which can be an answer to why EMT is often not evident at site of metastasis. Brabletz et al proposed that mCSC concept could underlie the heterogeneous morphology of primary tumor as well as the metastatic recapitulation of heterogeneity in differentiation and this hypothesis have been supported by recent studies of breast and pancreas.[8,30,31]

IDENTIFICATION OF CSCS

Historical perspectives

After the CSC hypothesis was proposed, numerous efforts have been made to demonstrate the presence of CSCs in various malignant lesions. Noteworthy advancement occurred in late 80s and early 90s when fluorescence activated cell sorting (FACS) could allow to isolate the normal hematopoietic stem cells by identifying the distinct cell surface marker profile.[32] Consequently, John Dick and colleagues became the first to experimentally define CSCs in hematopoietic malignancy in 1994.[33] A defined subpopulation of human acute myeloid leukemia (AML) cells (CD34hi and CD38low) when transplanted into immune-deficient mice was able to recapitulate AML that was phenotypically and pathologically similar to original patient's lesion. But in contrast, remaining cells population (CD34,low CD34,hi and CD38hi) failed to give rise to new lesion.

Approximately 15 years after identification of leukemic stem cells, in 2003, Al-Hajj et al[14] became the first to identify a CSC population in a solid tumor; breast cancer. Though Prince et al in 2007[1] published on identification of a subpopulation of cells with CSC properties in HNSCC by demonstration of membrane CD44+ and nuclear BMI1+, a subsequent study also demonstrated that 60% to 95% of normal epithelia express the CD44+1 which explained that a lot more studies and research needs to be done to clearly define the CSC concept and its implication in HNSCC.

Identification of stem cells in HNSCC

As suggested by the American Association for Cancer Research (AACR) workshop on cancer stem cells in 2006, the orthotopic xenograft assay is considered the golden standard for the identification of CSCs.[34] Such an assay allows reliable testing for main features of CSCs like self renewal and tumorigenicity. The cancer cells either from cell lines or tumor tissues are initially sorted by specific cell surface markers. The selected cell population is then injected into the experimental animals for tumorigenesis. If as low as 100 to 500 cells of the selective population can be shown to reproduce lesion on experimental animals, the featured cell surface markers can serve as CSC-specific biomarkers. There is no universal CSC marker for all types of cancers; CSC markers are more like tumor specific.

The most commonly used cell markers for solid tumors to date are CD34, CD133, CD44, CD29, CD31, and ALDH1. Among these, CD44 and ALDH1 have been implicated as important markers for head and neck squamous cell carcinomas (HNSCC) and elaborated here along with other identification parameter like SP cells and tumor sphere formation. The table below summarizes few molecular markers implicated in solid tumors (Table 1).

Table 1: Molecular markers implicated in solid tumors.

CSC markers	Tumor types	Minimum cell no. for tumor formation
CD44+/CD24-	Breast	200
CD44+	**Head and Neck**	5000
CD44+/CD24-/ESA+	Pancrease	100
ALDH1+	Breast	500
	Head and Neck	50
Side population	Prostate	100
CD133+	Brain	100
	Head and Neck	1000

CD44

This constitutes one of the most well recognized CSC markers and is a large cell surface glycoprotein involved in cell adhesion and migration. It is a known receptor for hyaluronic acid and interacts with other ligands such as matrix metalloproteases.[35,36] It was initially identified as a solid malignancy CSC marker in breast cancer,[1] later Prince et al demonstrated that CD44 expression could also be used to isolate a tumor subpopulation with increasing tumorigenicity in HNSCC.[1] They were able to show that as few as 5000 CD44+ HNSCC cells could reproduce a tumor when transplanted into flank of immune-compromised mice, where higher concentrations of CD44- cells failed to form the tumor. Also, these tumors could even reproduce the original tumor's cellular heterogeneity and could be seriously passaged, thus defining characteristics of CSCs.

But a question can be raised to whether CD44 expression alone is sufficient for isolation of a pure CSC population. This is because in breast cancer cells, as few as 100 CSCs injection into mammary pad fats of immune-compromised mice generated tumors[14] whereas it required about 5000 cells in case of HNSCC. Moreover, two-thirds of HNSCC samples were initially passaged through immune-compromised mice to generate a sufficient number of tumor cells for cell sorting and this has potential to alter native CSC expression pattern. Use of primary human tumor samples as well as utilizing a more natural host microenvironment through orthotopic xenograft model might reduce the number of cells needed to generate the tumor and consequently the expression of CD44 could be more efficiently documented as potential stem cell marker in HNSCC.

Aldehyde Dehydrogenase (ALDH)

ALDH is an intracellular enzyme normally present in liver that primarily functions to convert retino[l] to retinoic acid and oxidation of toxic aldehyde metabolites, like those formed during alcohol metabolism and with certain chemotherapeutics such as cyclophosphamide and cisplatin.[37,38] Chen et al in 2009 isolated ALDH1+ cells from HNSCC patients and shows that these HNSCC- ALDH1+ cells display radio-sensitivity and represent a reservoir for generating tumors. They also displayed the evidence of having EMT shifting and CSC properties in those ALDH1+ HNSCC cells.[39] Clay et al demonstrated that as few as 500 ALDH1+ cancer cells could give rise to new HNSCC when transplanted into immune-compromised mice, ten times fewer cells than isolation by CD44 positivity. Most of the ALDH1+ cells were also CD44+ suggesting that ALDH activity defines a subset of HNSCC CD44+ with increased tumorigenicity.

Side population (SP)

Side population (SP) cells are a subset of enriched progenitor cells exhibiting CSC- like phenotypes with a distinct low Hoechst 33342 dye staining pattern.[40,41] These cells have been identified and isolated from various solid tumors and exhibit stem cell property like markers expression, self-renewal and tumorigenicity.[40,42] They exhibit high levels of ATP binding cassette (ABC) transporter family member viz. MDR1 (multi drug resistance transporter1) and ABCG2, responsible for cellular efflux of dyes like Hoechst 33342 and some chemotherapeutic drugs.[43,44] The MDR1 that is implicated in the property of drug resistance is exhibited by these cells.[45] First successfully identified in 1996 in mouse bone marrow,[46] the characterization of SP in HNSCC was recently in 2010, which indicates that SP cells might be a potential driving force for causation of head and neck tumor formation and metastasis. It also showed that highly metastatic cell lines M3a2 and M4e contained more SP cells compared to the low metastatic parental HNSCC cell line 686LN.43 Further clarification on the characterization and research on therapeutic strategies against the SP cells yet remains to be elucidated.

Tumor sphere formation

The ability of the CSCs to form clonally derived collection of cells by the addition of growth factors to the CSCs in undifferentiated state and serum free condition is termed as tumor sphere formation.[15] This property of CSC, but not other cells has been used in neural tumors to identify population enriched for CSCs. Similarly, in HNSCC, these spheres have been shown to enrich for stem markers (CD44+ and others) as well as exhibit increased tumorigenicity in orthotopic grafts.[47]

WHY ARE CSCs RESISTANT TO CHEMOTHERAPY AND RADIOTHERAPY

As mentioned earlier, the isolated CSCs based on specific molecular markers showed the resistance to conventional chemotherapeutic and radio therapeutic strategies,[2-7] which further clarified the reason of failure in attaining considerable progress in cancer treatment by chemo and radiotherapy. The various

genetic and cellular adaptations in CSCs that defines the resistance to chemo-radiotherapy can be elaborated as follows:

Resistance to DNA damage within CSCs

The mutations that give rise to CSCs also serve to protect the stem cells from the DNA damaging effects of chemo-radiotherapy.[7] Though the exact mechanism underlying the resistance at the DNA level is not fully understood, it paves the way for further research so that ant-cancer drugs could be formulated with CSC-DNA damaging effects.

Resistance to drug penetration into CSCs

Normal somatic stem cells demonstrate a high expression of efflux transporters from ATP Binding Cassette (ABC) gene family.[48] This protects the genome more effectively against chemical mutagens. Since CSCs have been shown to express these drug efflux pumps as in normal somatic stem cells, the same might be the mechanism of resistance.

Resistance to apoptosis

The activation of Akt pathway[49,50] and over amplification of apoptosis inhibition proteins have also been proposed as one of the explanations of chemo-radio resistance. This was first demonstrated in chemo resistant hepatocellular carcinoma CSCs which was found to activate Akt/PKB and bcl-2 cell survival pathways that inhibited the apoptosis.[51]

Oxygen tension and CSCs

Oxygen, a radio sensitizer has an ability to form radiation induced free radicals that can damage DNA. Thus, keeping in consideration the reliability of radiotherapy on oxygen free radicals, it has long been postulated that areas of low oxygen tension within the tumor may prevent the radiation-induced damage. But later on, it was proven that CSCs reside along perivascular areas with abundance of oxygen. This also proves the fact why anti angiogenic drugs like bivacizumab could be effective; may be because it was CSC-targeted. But it can still be speculated that if radiotherapy is followed after anti-angiogenic therapy that already compromise the oxygen supply, this may confer to resistance of CSCs to radiation produced damage.[52]

THERAPEUTIC IMPLICATIONS AGAINST CSCs

Having proposed the role of CSCs in cancer initiation and propagation and having understood the mechanism of resistance to chemotherapy and radiotherapy, a lot researches have been done, more undergoing to explore the therapeutic implications against CSCs. Moreover, now it is known that the hedgehog signaling pathway, the Wnt/B- cantenin signaling pathway, the notch signaling pathway and also the PTEN- signal way/P13K/Akt and the p53 pathways in the recognition of stem cells is of utmost importance.[53] Reviews of major proposition of drugs that will potentially yield clinical values and meanings in recent future and function by acting against the signaling pathways are mentioned below and an update is illustrated in Table 2 (taken from http://clinicaltrials.gov/)(Table 2). Though these trials are not specifically targeted against HNSCC, it can be assumed to follow similar pattern of action and thus the implication, similar way the molecular markers were first identified in hematopoietic malignancy, then solid tumors like breast and then HNSCC.

Wnt Inhibitors: The role of notch and wnt/B-catenin[8] signaling have been examined, and both been implicated in development and progression of several types of leukemia.[54-56] So, Wnt inhibitors have been designed to therapeutically prevent this possibility

Table 2. Update on clinical trials for CSC molecular targets

Target	Drug	Cancer	Phase	Identifier	Sponsor
Wnt	Resveratrol	Colon	I. II	NCT00256334	University of California, Irvine
Notch	MK0752	**Breast**	**I**	NCT00106145	Nerck
		Pancreatic	I,II	NCT01141569	Cancer Research UK
	RO4929097	Renal Cell	II	NCT01141569	Univesity Health Network, Toronto
	PF-03084014	**Leukemia**	**I**	NCT00878189	Pfizer
Hedgehog	GDC-0449	Solid tumors	I	NCT00968981	Genentech
		Colorectal	II	NCT00636610	Genentech
	PF-04449913	**Hematologic**	**I**	NCT00953758	Pfizer
	BMS-833923	**Basal cell**	**I**	NCT00670189	Bristol-Myers Squibb
	LDE225	**Medulloblastoma**	**I**	NCT00880308	Novartis

and include ICG-001, fungal derivatives PKF 115-854 and CGP 049090, as well as monoclonal antibodies against Wnt-1 and Wnt-2.[57]

Notch inhibitors: The notch signaling pathways are found to be activated in both breast CSCs[58] and in endothelial cells[59] in response to radiation. Significant efforts to down regulate Notch signaling pathways are underway.[60] Currently available Notch signaling inhibitors include MK-0752, a gamma- secretase inhibitor that is in clinical trial for treatment of leukemia.[61]

Hedgehog inhibitors: The hedgehog signaling pathways inhibitors have shown to inhibit medulloblastoma growth in mice,[62] and at least three different hedgehog inhibitors have reached the phase I clinical trials.

Therapy against CSC marker CD133: The tumor initiating role of CD133 has been demonstrated in CNS cancer. Study of Wang et al recently demonstrated potential therapeutic use of targeting CD133 to direct therapy specifically towards CSCs. This can have a similar implication in targeting therapies against most prevalent molecular marker in HNSCC i.e. CD44+ and ALDH1+.

SUMMARY

Though insufficient, the advent of multidisciplinary approaches to cancer therapy including HNSCC has made some progresses in improving the quality of life of patients over few decades. With the new discoveries pertaining to CSCs, it is supposed to bring a positive revolution in the management protocol of HNSCC management. With the identification of various molecular markers to recognize CSCs in a tumor and recognition of signaling pathways that regulate tumorigenesis, an ample number of therapeutic agents have been proposed. Some agents are underway in manufacturing and some have already entered clinical trials. May be in recent future, we will be targeting the cancer stem cells directly so that the pitfalls deep rooted in chemo-radiotherapy and surgical therapy can be recovered and long term survival of patients with HNSCC be ensured. But not to be forgotten, whatever might be the progresses, the achievements in HNSCC are comparatively less as compared to other solid tumor counterparts. Thus, more researches to identify more distinct markers and signaling pathways as targets of head and neck cancer therapy still need to be done.

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JNDA

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):973-8.
2. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin. 2005;55(2):74-108.
3. Parkin D, Pisani P, Ferlay J. Global Cancer Statistics. CA: A Cancer Journal for Clinicians. 1999;49(1):33-64.
4. Jemal A, Murray T, Ward F. Cancer statistics, 2005. CA: A Cancer Journal for Clinicians. 2005;55(1):10-30.
5. Kamangar F, Dores GM, Anderson WF. Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world. J Clin Oncol. 2006;24(14):2137-50.
6. Khawaja MR, Mazahir S, Majeed A, Malik F, Merchant KA, Maqsood M, et al. Chewing of betel, areca and tobacco: perceptions and knowledge regarding their role in head and neck cancers in an urban squatter settlement in Pakistan. Asian Pac J Cancer Prev. 2006;7(1):95-100.
7. Vermorken JB, Remenar E, van Herpen C, Gorlia T, Mesia R, Degardin M, et al. Cisplatin, fluorouracil, and docetaxel in unresectable head and neck cancer. N Engl J Med. 2007;357(17):1695-704.
8. Bhaijee F, Pepper DJ, Pitman KT, Bell D. Cancer stem cells in head and neck squamous cell carcinoma: A review of current knowledge and future applications. Head Neck. 2012 Jun;34(6):894-9.
9. Haddad RI, Shin DM. Recent advances in head and neck cancer. N Engl J Med. 2008;359(11):1143-54.
10. Sayed SI, Dwivedi RC, Katna R, Garg A, Pathak KA, Nutting CM, et al. Implications of understanding cancer stem cell (CSC) biology in head and neck squamous cell cancer. Oral Oncol. 2011;47(4):237-43.

11. Chen ZG. The cancer stem cell concept in progression of head and neck cancer. J Oncol. 2009;2009:894064.
12. Krivtsov AV, Twomey D, Feng Z, Stubbs MC, Wang Y, Faber J, et al. Transformation from committed progenitor to leukaemia stem cell initiated by MLL-AF9. Nature. 2006;442(7104):818-22.
13. Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature. 1994;367(6464):645-8.
14. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A. 2003;100(7):3983-8.
15. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, et al. Identification of human brain tumour initiating cells. Nature. 2004;432(7015):396-401.
16. Collins AT, Berry PA, Hyde C, Stower MJ, Maitland NJ. Prospective identification of tumorigenic prostate cancer stem cells. Cancer Res. 2005;65(23):10946-51.
17. Eramo A, Lotti F, Sette G, Pilozzi E, Biffoni M, Di Virgilio A, et al. Identification and expansion of the tumorigenic lung cancer stem cell population. Cell Death Differ. 2008;15(3):504-14.
18. Dalerba P, Dylla SJ, Park IK, Liu R, Wang X, Cho RW, et al. Phenotypic characterization of human colorectal cancer stem cells. Proc Natl Acad Sci U S A. 2007;104(24):10158-63.
19. Hermann PC, Huber SL, Herrler T, Aicher A, Ellwart JW, Guba M, et al. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. Cell Stem Cell. 2007;1(3):313-23.
20. Yang ZF, Ho DW, Ng MN, Lau CK, Yu WC, Ngai P, et al. Significance of CD90+ cancer stem cells in human liver cancer. Cancer Cell. 2008;13(2):153-66.
21. Fang D, Nguyen TK, Leishear K, Finko R, Kulp AN, Hotz S, et al. A tumorigenic subpopulation with stem cell properties in melanomas. Cancer Res. 2005;65(20):9328-37.
22. Tan BT, Park CY, Ailles LE, Weissman IL. The cancer stem cell hypothesis: a work in progress. Lab Invest. 2006;86(12):1203-7.
23. Zhao C, Blum J, Chen A, Kwon HY, Jung SH, Cook JM, et al. Loss of beta-catenin impairs the renewal of normal and CML stem cells in vivo. Cancer Cell. 2007;12(6):528-41.
24. Al-Assar O, Muschel RJ, Mantoni TS, McKenna WG, Brunner TB. Radiation response of cancer stem-like cells from established human cell lines after sorting for surface markers. Int J Radiat Oncol Biol Phys. 2009;75(4):1216-25.
25. Bertolini G, Roz L, Perego P, Tortoreto M, Fontanella E, Gatti L, et al. Highly tumorigenic lung cancer CD133+ cells display stem-like features and are spared by cisplatin treatment. Proc Natl Acad Sci U S A. 2009;106(38):16281-6.
26. Chang CJ, Hsu CC, Yung MC, Chen KY, Tzao C, Wu WF, et al. Enhanced radiosensitivity and radiation-induced apoptosis in glioma CD133-positive cells by knockdown of SirT1 expression. Biochem Biophys Res Commun. 2009;380(2):236-42.
27. Creighton CJ, Li X, Landis M, Dixon JM, Neumeister VM, Sjolund A, et al. Residual breast cancers after conventional therapy display mesenchymal as well as tumor-initiating features. Proc Natl Acad Sci U S A. 2009;106(33):13820-5.
28. Diehn M, Clarke MF. Cancer stem cells and radiotherapy: new insights into tumor radioresistance. J Natl Cancer Inst. 2006;98(24):1755-7.
29. McCord AM, Jamal M, Williams ES, Camphausen K, Tofilon PJ. CD133+ glioblastoma stem-like cells are radiosensitive with a defective DNA damage response compared with established cell lines. Clin Cancer Res. 2009;15(16):5145-53.
30. Brabletz T, Jung A, Spaderna S, Hlubek F, Kirchner T. Opinion: migrating cancer stem cells - an integrated concept of malignant tumour progression. Nat Rev Cancer. 2005;5(9):744-9.
31. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell. 2008;133(4):704-15.
32. Spangrude GJ, Heimfeld S, Weissman IL. Purification and characterization of mouse hematopoietic stem cells. Science. 1988;241(4861):58-62.
33. Mack B, Gires O. CD44s and CD44v6 expression in head and neck epithelia. PLoS One. 2008;3(10):e3360.
34. Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CH, Jones DL, et al. Cancer stem cells--perspectives on current status and future directions: AACR Workshop on cancer stem cells. Cancer Res. 2006;66(19):9339-44.
35. Isacke CM, Yarwood H. The hyaluronan receptor, CD44. Int J Biochem Cell Biol. 2002;34(7):718-21.
36. Kajita M, Itoh Y, Chiba T, Mori H, Okada A, Kinoh H, et al. Membrane-type 1 matrix metalloproteinase cleaves CD44 and promotes cell migration. J Cell Biol. 2001;153(5):893-904.

37. Bosron WF, Lumeng L, Li TK. Genetic polymorphism of enzymes of alcohol metabolism and susceptibility to alcoholic liver disease. Mol Aspects Med. 1988;10(2):147-58.
38. 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 tumor antigen in squamous cell carcinoma of the head and neck. Cancer Res. 2007;67(21):10538-45.
39. 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 Biophys Res Commun. 2009;385(3):307-13.
40. Wu C, Alman BA. Side population cells in human cancers. Cancer Lett. 2008;268(1):1-9.
41. Challen GA, Little MH. A side order of stem cells: the SP phenotype. Stem Cells. 2006;24(1):3-12.
42. Haraguchi N, Inoue H, Tanaka F, Mimori K, Utsunomiya T, Sasaki A, et al. Cancer stem cells in human gastrointestinal cancers. Hum Cell. 2006;19(1):24-9.
43. Song J, Chang I, Chen Z, Kang M, Wang CY. Characterization of side populations in HNSCC: highly invasive, chemoresistant and abnormal Wnt signaling. PLoS One. 2010;5(7):e11456.
44. Moserle L, Ghisi M, Amadori A, Indraccolo S. Side population and cancer stem cells: therapeutic implications. Cancer Lett. 2010;288(1):1-9.
45. 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 tumor cells. Proc Natl Acad Sci U S A. 2004;101(39):14228-33.
46. Goodell MA, Brose K, Paradis G, Conner AS, Mulligan RC. Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. J Exp Med. 1996;183(4):1797-806.
47. Chiou SH, Yu CC, Huang CY, Lin SC, Liu CJ, Tsai TH, et al. Positive correlations of Oct-4 and Nanog in oral cancer stem-like cells and high-grade oral squamous cell carcinoma. Clin Cancer Res. 2008;14(13):4085-95.
48. Dean M. ABC transporters, drug resistance, and cancer stem cells. J Mammary Gland Biol Neoplasia. 2009;14(1):3-9.
49. Hambardzumyan D, Becher OJ, Rosenblum MK, Pandolfi PP, Manova-Todorova K, Holland EC. PI3K pathway regulates survival of cancer stem cells residing in the perivascular niche following radiation in medulloblastoma in vivo. Genes Dev. 2008;22(4):436-48.
50. Rao G, Pedone CA, Del Valle L, Reiss K, Holland EC, Fults DW. Sonic hedgehog and insulin-like growth factor signaling synergize to induce medulloblastoma formation from nestin-expressing neural progenitors in mice. Oncogene. 2004;23(36):6156-62.
51. Ma S, Lee TK, Zheng BJ, Chan KW, Guan XY. CD133+ HCC cancer stem cells confer chemoresistance by preferential expression of the Akt/PKB survival pathway. Oncogene. 2008;27(12):1749-58.
52. Diehn M, Cho RW, Lobo NA, Kalisky T, Dorie MJ, Kulp AN, et al. Association of reactive oxygen species levels and radioresistance in cancer stem cells. Nature. 2009;458(7239):780-3.
53. Mozet C, Wichmann G, Dietz A. [Translational approaches in cancer stem cell research]. HNO. 2011;59(9):859-65.
54. Barker N, Clevers H. Mining the Wnt pathway for cancer therapeutics. Nat Rev Drug Discov. 2006;5(12):997-1014.
55. Weerkamp F, van Dongen JJ, Staal FJ. Notch and Wnt signaling in T-lymphocyte development and acute lymphoblastic leukemia. Leukemia. 2006;20(7):1197-205.
56. Lu D, Zhao Y, Tawatao R, Cottam HB, Sen M, Leoni LM, et al. Activation of the Wnt signaling pathway in chronic lymphocytic leukemia. Proc Natl Acad Sci U S A. 2004;101(9):3118-23.
57. Pang RW, Poon RT. From molecular biology to targeted therapies for hepatocellular carcinoma: the future is now. Oncology. 2007;72 Suppl 1:30-44.
58. Phillips TM, McBride WH, Pajonk F. The response of CD24(-/low)/CD44+ breast cancer-initiating cells to radiation. J Natl Cancer Inst. 2006;98(24):1777-85.
59. Scharpfenecker M, Kruse JJ, Sprong D, Russell NS, Ten Dijke P, Stewart FA. Ionizing radiation shifts the PAI-1/ID-1 balance and activates notch signaling in endothelial cells. Int J Radiat Oncol Biol Phys. 2009;73(2):506-13.
60. Bolos V, Blanco M, Medina V, Aparicio G, Diaz-Prado S, Grande E. Notch signalling in cancer stem cells. Clin Transl Oncol. 2009;11(1):11-9.
61. Morrison R, Schleicher SM, Sun Y, Niermann KJ, Kim S, Spratt DE, et al. Targeting the mechanisms of resistance to chemotherapy and radiotherapy with the cancer stem cell hypothesis. J Oncol. 2011;2011:941876.
62. Berman DM, Karhadkar SS, Hallahan AR, Pritchard JI, Eberhart CG, Watkins DN, et al. Medulloblastoma growth inhibition by hedgehog pathway blockade. Science. 2002;297(5586):1559-61.