Review

# Cancer Stem Cells: Biological Features and Targeted Therapeutics

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Advance in stem cells (SCs) has become significant by the isolation of the tissue-specific SCs in a tissue, because it is the beginning of using SC utility for regenerative medicine. Likewise in SCs, a small subpopulation of cancer cells, named cancer stem cells (CSCs), also have similar properties. These properties include indefinite self-renewal potential and sharing similar signaling pathways with normal SCs, because the originality of CSCs is from the mutation of normal SCs. Hierarchically, CSCs in solid tumors may organize from the normal SCs in the highest cellular hierarchy of these cancer cells. The functional assay techniques to assess the differentiation frequency of normal SCs are similarly used in CSCs to sustain tumor growth and recurrence after therapy. In this review, we discuss the different parallels between adult SCs and CSCs in solid cancer disease and applications toward targeted therapy in use of molecular level on CSCs.

**Key Words:** Stem Cells; Neoplastic Stem Cells; Genetic Heterogeneity; Molecular Targeted Therapy

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## INTRODUCTION

Stem Cells (SCs) biology has given a way toward the utility of regenerative medicine and methods in the isolation of tissue-specific SCs. Tissue-specific SCs are important for retaining tissue homeostasis and wound healing processes. These SCs have two basic functional characteristics: self-renewal and differentiation potentials in cell lineages. In early SC technology to assess these characteristics, they found out cell differentiations were not equivalent and some cells seemed to have more activity in proliferation at different differentiation stages. By further observations, they hypothesized that the existing proliferating undifferentiated cells might differentiate into self-renewal potent cells [1].

By virtue of the development in functional assays including *in vitro* clonogenic assays and lineage-tracing experiments, the differentiation of multilineage in tissue-specific SCs was suggested. This suggestion was primarily demonstrated by bone marrow cells transplanted in irradiated mice, which differentiated into different haematopoietic lineages. Later, there was an experiment using epi-

thelial cells that showed that keratinocytes stimulate the differentiation. These cells were engrafted into long-term renewal cells as functional tissue [2]. This is meaningful in the *in vitro* cultivation of SCs for maintaining differentiation potential. In a more advanced research, *in vivo* lineage tracing has been adapted to demonstrate the crucial role of SCs during tissue homeostasis and repair in their natural environment [3].

SCs have self-renewal ability, which is the most useful hematopoietic property of tissue regenerative applications. Perhaps the origin of cancer is from the transformation of normal SCs, cancer cells in a given tissue also do not have identical properties with each other like in SCs. There exists a small portion of cancer cells, which have rapid differentiation potential compared with others. Not like SCs, these cancer cells have few unique characteristics. They have potency to generate tumor, *in vivo* propagation, and potential to generate different self-renewly potent cells with variant phenotypes [4]. Taking the similar signaling pathways of the ability to self-renew in cancer cells, several rare cells with indefinite potential that drive tumorigenesis, are known as cancer stem

cells (CSCs). Their origin is yet to be discovered, though a series of hypotheses have been proposed in this regard.

The concept of CSCs derives from the fact that cancers are dysregulated tissue clones that continuously maintain propagation from a biologically distinct subpopulation of cells which are very uncommon [5]. This concept was recently recognized as a widely accepted rationale because of the expanded definition of hierarchies in normal tissue, multistep traits of oncogenesis, and improved methods of xenotransplantation that from primary human cancers in the immunodeficient mice model.

There are two concepts to define: heterogeneity and cancer cell proliferation. The first concept explains that the cancer cells are equivalent and a proportion of cancer cells proliferate and differentiate stochastically. Compared to the first concept, the second concept explains that cancer cells are hierarchically organized, that certain cells are involved in cancer growth and that undifferentiated progenitor cells have limited growth potential (Fig. 1). The similar model of tissue-specific SCs supports the second concept of CSCs that are responsible for the tumor growth and maintenance of cancer. Both these concepts are further extended to clonal evolution, in which cancer cells progressively accumulate genetic alterations such as base substitutions and small insertions/deletions. Some of which provide increased advantages of fitness and survival and allow the mutated cancer cells to outcompete other cancer cells [6].

In a recent study, evidence has shown that some cancer cells shared certain molecular mechanisms like in tissue-specific SCs [7]. An increased understanding of the molecular signaling events that regulate cellular hierarchy and stemness, and successes in de-

fining molecular signatures of CSCs have opened up new avenues that have accelerated the development of novel diagnostic and treatment strategies. Here, we highlight the recent advances in understanding of the pathogenesis in CSCs by comparing different techniques of functional assays to reveal genetic heterogeneity and proliferation hierarchy. Furthermore, we also discuss the implications for targeted therapy in cancer cells.

## HETEROGENEITY IN NORMAL TISSUE AND CANCER

Heterogeneous potential was initially introduced by the demonstration of epithelial tissues in the epidermis. Through the different culturing conditions on keratinocytes, the three types of clones have been identified and they had shown different clonogenic potentials of the epidermis. The holoclones consisted of undifferentiated cells including epidermal SCs and showed the highest proliferative capacity. On the other hand, the meroclones were in the transitional stage, and the paraclones had transit-amplifying progenitors that showed terminal differentiation [8]. Moreover, they showed holoclones presenting considerable proliferative potential *in vitro*, but most of these cells were quiescent *in vivo* [9]. Finally, the idea that these cells are multipotent SCs were derived from transplantation and isolation using microdissection and flow cytometry [10].

To look into the tissue heterogeneity, *in vitro* clonogenic assays were applied to study differentiation potentials of different cell populations at a single-cell level. The sphere-forming assays were adapted in SCs and CSCs to assess the multilineage differentiation and renewal capacities. But, they were restricted only in a fraction of cells from primary colon [11], breast carcinoma [12], and brain carcinoma [13]. Since only these tissues were able to form spheres, accurate quantification of the differentiation frequency is not granted in SCs in the *in vivo* system. Recently, to expand SCs and progenitor cells from different epithelium, the organoid culture method (three-dimensional, non-adherent condition) was applied [14].

The same strategies were applied on CSCs to explain the different growth potentials that contributed different cancer growth in the microenvironment such as xenotransplantation and *in vitro* [15]. The experiment in mouse glioma had shown that the group of cells which had low expression level of inhibitor of DNA binding 1 (ID1) generated secondary cancer more efficiently than the group of cell which had higher expression level of ID1 on transplantation, but the group of ID1$^{low}$ cells presented lower self- renewal
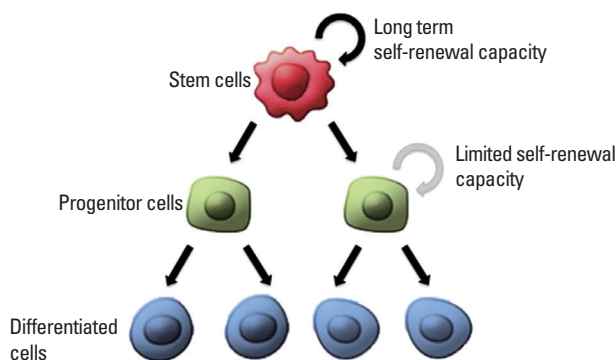


**Fig. 1.** Hierarchy in normal tissue and cancer. SCs have self-renewal and differentiation potential, but the capacity of self-renewal is not equivalent to differentiated generations. SCs present long-term renewal potential, and progenitor cells present restricted self-renewal capacity. These progenitor cells give rise to terminally differentiated cells. SCs, stem cells.

potential than ID1$^{hi}$ cells in a sphere-forming assay *in vitro* [16].

Serial transplantations of cancer cells on sphere form *in vitro* assess the self-renewal potential of CSCs. A large amount of isolated cells were required to maintain secondary cancer cell population, because most studies precluded the assessment of their self-renewal ability after transplantation. The gold-standard assay to this problem was solved by the genetic lineage-tracing approach (Fig. 2). It explains self-renewal and differentiation capacity of cancer initiation and cell development. In mouse genetic lineage-tracing experiments, transgenic mice expressed a reporter gene by drug-inducible Cre activation in a specific lineage. This experiment came out with different types of differentiated cells referred to the differentiation potential of SCs that the fraction of indicated cells persisted over time [17]. In mouse squamous cell carcinoma, the frequency of tumor propagating cells (TPCs) was increased in CD-34$^{hi}$ population but decreased in CD34$^{low}$ population during serial

transplantation, whereas there was no change in primary cancer [18]. This was also shown in mouse lung adenocarcinoma, the frequency of TPCs was enriched only in the spinocerebellar ataxia type 1+ cells. However, the spinocerebellar ataxia type 1– cells only gave rise to small secondary cancers that could not be serially grafted [19].

Although the development of the sphere-forming assays had a great role in finding the characteristics of CSCs *in vitro* on single cell levels, there are still unrevealed questions about what extent of the capacity of cancer cells growing as spheres directly correlated with sustained cancer growth *in vivo*.

## BIOLOGICAL AND MOLECULAR CHARACTERISTICS OF CSCs

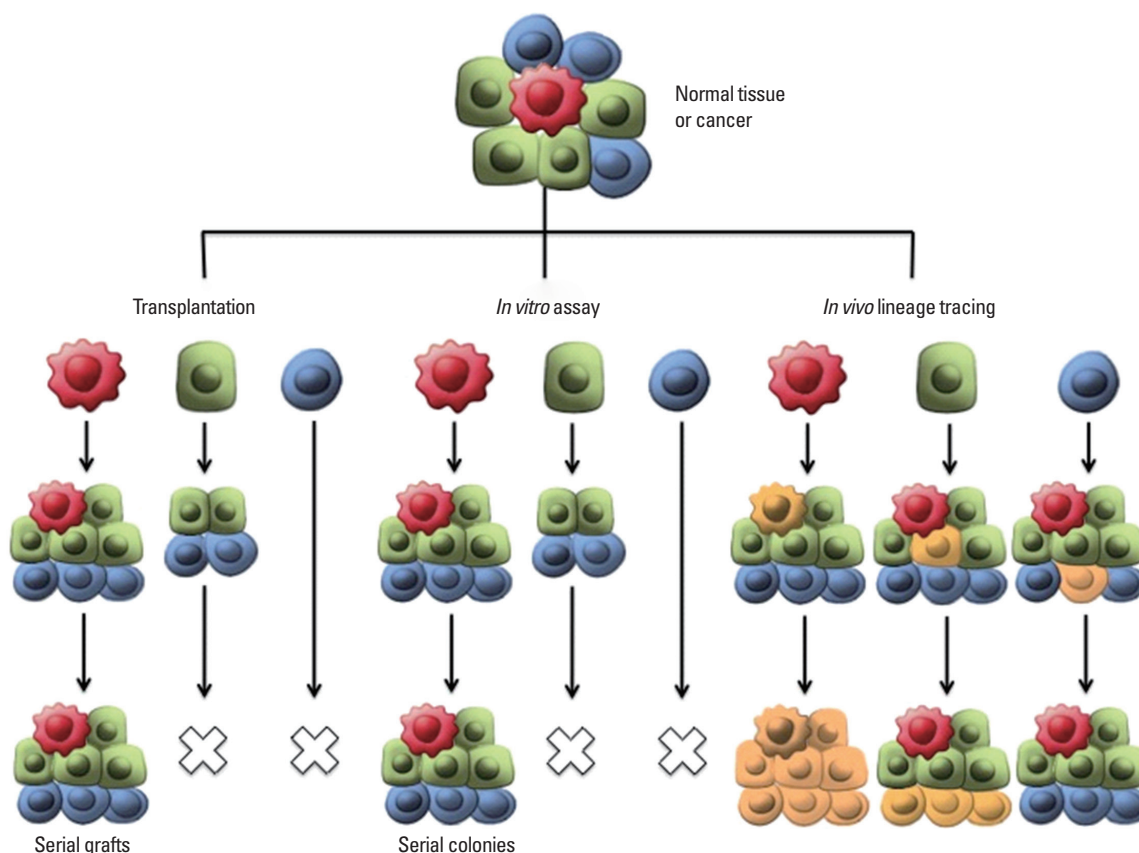Since normal SC has no universal molecular characteristics, we



**Fig. 2.** Functional assay experiments of normal Stem Cells and Cancer Stem Cells. Three techniques to assess differentiation and the potential of self-renewal in SCs and CSCs; transplantation, *in vitro* clonogenic assays, and *in vivo* lineage-tracing experiments. The SCs and CSCs can perform long-term renewal that were serially transplanted *in vivo*, serially colonized *in vitro*, and big subsets of colonies were created in lineage-tracing experiments (marked cells are shown in yellow). The progenitor cells would not be able to be serially transplanted on transplantation to secondary cancer. However progenitor cells form colonies only transiently in *in vitro* and small clones in *in vivo* lineage-tracing experiments. Differentiated cells will not give rise to any colonies. SCs, stem cells; CSCs, cardiac stem cells.

can use biological markers like LGR5 to identify different populations of SC in the specific tissues. The same aspects was applied to CSCs in that CD133 had been associated with TPCs in many different types of cancer tissues [20]. Using experimentally confirmed surface markers, CSCs' population can be assessed successfully. In liver cancer, cell markers like CD133, CD90, CD44, CD24, CD13, and epithelial cell adhesion molecule were used to identify the liver CSC, which had features of self-renewal potential and tumor growth [21].

Based on the signaling pathway of regulating normal SCs, when the WNT–β-catenin signaling pathway was abnormally activated with nuclear factor-κB, it was influenced in intestinal epithelium shown by the occurrence of colorectal cancers [22]. This is consistent with the idea that the signaling pathway of normal SCs can induce cancer initiation by reprogramming to a progenitor-like fate [23]. This suggests that the role of CSCs have similar features with tissue development on the activation of signaling networks

that leads to cancer development.

Clarifying the mechanism of cancer initiation in various types of cancer is necessary to augment molecular features of CSCs. Through experiments on transplanting CSCs into immunodeficient mice, these cells are defined to reform secondary cancer operationally. In colorectal cancers, when the WNT–β-catenin signaling pathway was mutated on transplantation, active β-catenin was over-regulated and it gave CSCs properties to LGR5+ cells by dedifferentiation on secondary cancer [24]. Similarly, in skin squamous cell carcinoma, CD34low cells gained CSCs potentials from the transplantation of CD34hi cells. However, the differentiation by CD34low cells in secondary cancer does not directly imply the efficiency of CD34hi cells isolated from the primary cancer [18]. In human breast cancers, the non-TPCs presented a great population of CD44hi CD24− marked cells in secondary caner, which means they have gained CSCs potentials. These results suggest that a transition of stochastic state is associated with increased the potential of
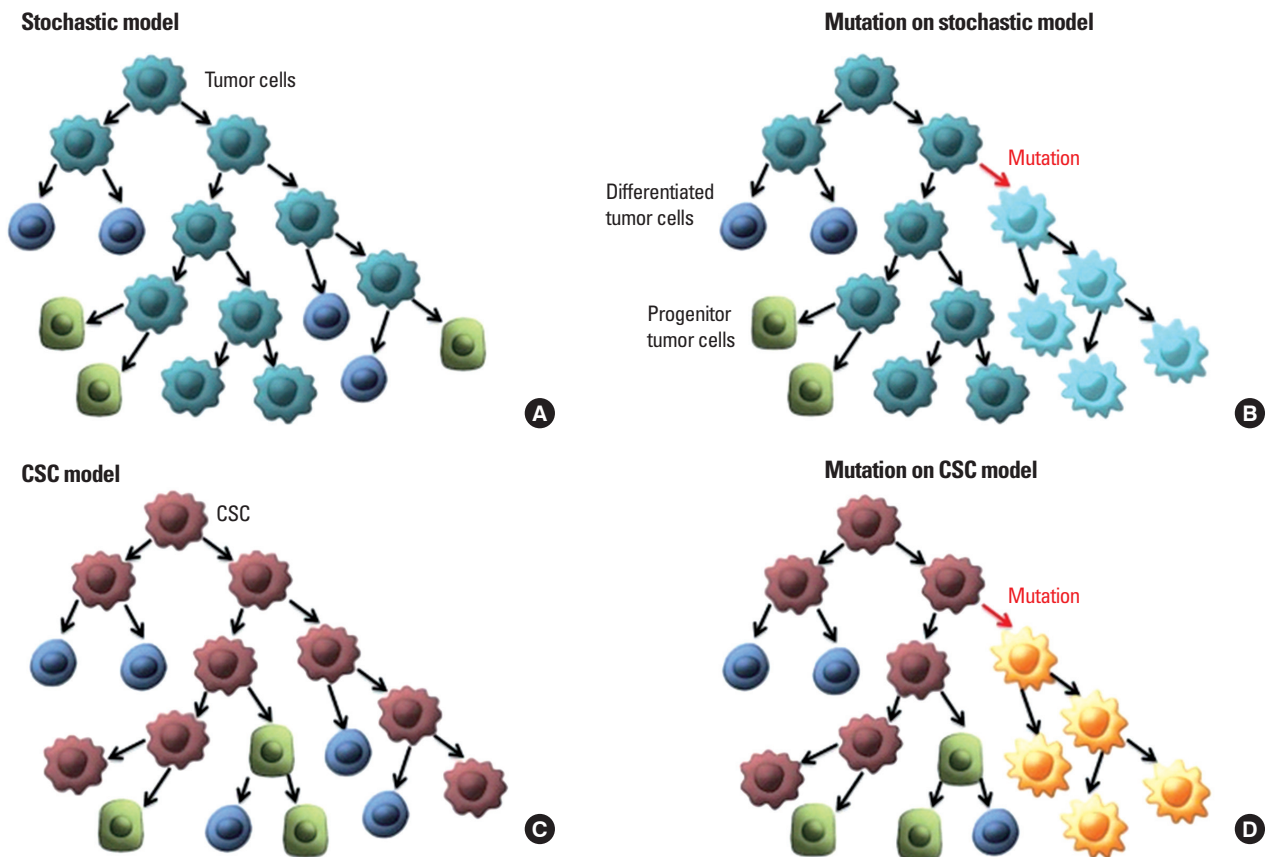


Fig. 3. The two different models of tumor growth. (A) In the stochastic model, all tumor cells can self-renew or differentiate stochastically. (C) In the CSC model, only the CSCs have the long-term self-renewal, and the committed progenitors perform limited differentiation potential. (B, D) In the clonal diversity, somatic mutations can increase tumor heterogeneity. CSCs, cardiac stem cells.

CSCs among different subpopulations [25]. To verify this suggestion, more research based on the transition of stochastic state occurring in primary tumors from different tissues will be needed (Fig. 3).

Not only cancer initiation, but cancer progression and metastasis are also important features in CSCs. Usually the metastasis by CSCs was achieved through producing mutants by the stochastic state transition-associated genes. For example, in colorectal cancer, the CD26+ cells induced liver metastasis more than CD26- cells, and long-term TPCs were able to perform metastases [26]. These cells have been known as a subset of CSCs. Other evidence of metastasis were also found in prostate cancer [27], breast cancer [28], and melanoma [29] by expression of CXCR4, which was expressed in pancreatic adenocarcinoma from CD133+ CSCs [30]. But this is still controversial as to whether CXCR4 is related to CSCs in other human metastatic cancers.

Therefore, in case that a study can determine the cellular hierarchy of cancer metastasis from CSCs, the cancer cells expressing certain CSCs markers like CXCR4 or CD26 could be potential therapeutic targets by pharmacological inhibition.

## MICROENVIRONMENT AND CSC NICHE

The microenvironment can reflect the heterogeneity of SCs and CSCs that might utilize the balance between self-renewal and differentiation features. This specific native environment is called a niche; the secretion of soluble molecules or cell-to-cell communication balances the heterogeneity of SCs. To identify the actual progenitor-like potential of SC, it is important to recognize that SCs present an important plasticity *in vivo*. Under SC depletion, these progenitor-like cells present a certain degree of plasticity that reorganizes the SC niche and clonial repopulation [31].

For cancer initiation and cancer growth, it is obvious that angiogenesis and secretion of growth factors from endothelial cells are very important. For this reason, the regulation of perivascular niche can be the main key to regulate CSCs. This has been shown in normal SC that specialized the composition of mesenchyme in their niche, such as neuronal SC with endothelial cells that secreted growth factor to utilize the self-renewal features [32]. However, the components of cancer niche remain generally unknown. One experiment showed CSCs were related to endothelial cells in glioblastoma by culturing CSCs *in vitro* that greatly utilized their self-renewal potentials. Which claimed that perivascular niche enhanc-

ed the formation of CSCs in glioblastoma [33]. Another similar experiment can be found in malignant brain cancer. They found that therapy using anti-vascular endothelial growth factor A (VEGFA) had reduced the population of CSCs [34]. Accordingly, the density of CSCs was also decreased in squamous cell carcinoma by inhibiting angiogenesis with VEGF receptor 2 antibodies. It has been recently reported that VEGFA stimulates the formation of perivascular niche that indirectly enhances the formation of CSCs [35].

There was an opposite view on the perivascular niche. One has reported that the hypoxic niche increased the clonogeneity of SC. In hematopoietic normal SCs, the leukemic SC population has been decreased under the absent condition of hypoxia-inducible factor (HIF) 1α *in vivo* [36]. This means the hypoxic niche promotes HIF to form an environment that is allows SCs to manipulate stemness in cancer cells. Moving on to hypoxia in CSCs, HIF1α activates the expression of CD133+ cells in pancreatic cancer and estrogen receptor (ER)+ cells that expand TPCs in breast cancer [37]. These results show that different CSCs responded differently to hypoxia conditions. Although the regulation of CSCs in two different models, the perivascular niche and hypoxic niche seem to have no connections, but one niche can transform to another by abnormal functionality.

Another study on the regulation of CSCs has found a close association between fibroblasts and colorectal cancers, and that myofibroblasts activated in WNT signaling stimulate the cell to cell communication between CSCs [38]. In liver cancer, usually the capacity of proliferation of the hepatic SCs is increased in chronic liver disease. The hepatocyte proliferation is induced by continuous cell regeneration but the proliferative capacity, which is affecting motility and proliferation, is not infinitely increased. In the CSC niche, the activation of hepatic progenitors is tightly regulated and related to other risks of hepatocellular carcinoma (HCC) like liver infection and inflammation [39]. HBV and HCV infection promote cell mutagenesis and genomic instability resulting DNA damage to induce HCC [40]. The HBV X gene study suggested that the HBV X gene promoted HCC by modulating p53 signaling pathways [41]. Similarly, inflammation might give rise to oncogenic events resulting in cell proliferations and genetic alterations. [42]. Therefore, accumulating the pathophysiological changes in a range of liver lineages would verify the development mechanism of liver CSC.

Further understandings of these molecular interactions and the cellular factors of the niche are needed to identify the genetic and epigenetic mechanism of cancer clonal evolution. Defining the

contribution of the establishment of CSCs would then allow extension into targeted therapy of CSCs on cancer propagation.

## CLINICAL APPLICATIONS ON GENETIC TARGETED THERAPY

The main focus of targeted therapy on CSCs is the capability of cancer relapse. Just like normal SCs, they are also resistant to chemotherapy, radiotherapy, and other therapies. After radiotherapy in xenografts using TPCs of glioma, one showed that CSCs rapidly repaired damaged-DNA and avoided apoptosis induced by ionizing radiation [43]. Another study also demonstrated in the colorectum CSCs initiated the activation of damaged-DNA checkpoints [44]. Also, radiotherapy in brain cancer was affective when blocking the activation of checkpoint kinases CHK1 and CHK2 from CSCs [43]. In breast cancer, because of high expression of the free radical-scavenging mechanism in SCs, the inhibition of scavenging machinery decreased its clonogenicity and could give affective radiotherapy [45].

In clinical applications, there are two ways to improve cancer treatment. One is the development of novel cancer diagnosis, and the other one is development of new strategies of targeted treatment. For example, gene expression profiling techniques by using microarray or next generation sequencing can help identify dysregulated genes in cancers that reveal tumorigenic, metastatic features, and/or predict patients' prognosis. As we already know, early diagnosis of cancer is very crucial and will have great effect on patient survival. However, conventional diagnosis on the cancer tissue has limitations on predicting the actual prognosis change after radical treatment. By applying gene expression profiling technologies, these limitations were resolved in liver cancer [46]. In the future, CSCs could be isolated very easily from circulating tumor cells in peripheral blood, and may provide diagnostic or prognostic impacts.

Generally, HCC patients are treated with transcatheter arterial chemoembolization (TACE) in the intermediated stage. But there is the problem of tumor cells relapsing from CD133+ CSCs after TACE. To prevent HCC recurrence, 5-FU or CD13 inhibitor are applied for targeting CD133+ CSCs [47]. By advanced examination, a study suggested that 5-FU inhibited the signaling pathway of PTEN, Akt, and ABCG2 to suppress the self-renewal ability of CD133+ liver CSCs [48]. The therapeutically beneficial model of targeted therapy to control cancer growth would directly eradicate CSCs. This therapeutic approach has been performed with patients in acute promyelocytic leukemia by all-*trans* retinoic acid controlling self-renewal and differentiation [49]. To enhance the targeted therapy of CSCs, it is important to identify the cellular factors that inhibit the growth of CSCs without affecting normal SCs. Recently, numerous methods to develop the library of these cellular level molecules have been found by screening techniques. Through classical chemotherapy in mouse glioblastoma, cancer-relapsing cells were identified and then the rate of cancer relapsing, targeting were decreased using genetic lineage ablation approach to these cells [50]. Therefore, more specific research is demanded on therapeutics focusing eradication of CSCs, and it may lead to a considerable clinical benefit.

## CONCLUSION

The transplantation of CSCs to immunodeficient mice is mainly focused on applying the highest clonogenic potential that have no effect on any medical therapy. The genetic and epigenetic pathways of clonal evolution in metastasis are still not clear on whether the population of cancer cells are independently formed or evolved in branching. To provide an essential insight to this remaining uncertainty, the following question is necessary to be answered on other clonal analyses. Does *in vivo* lineage ablation technique maintain the potential strength of CSCs compared to transplantation assays? Many studies have shown significant results by lineage-tracing experiments in mice CSCs. To verify these results on human cancer, it is important to develop new techniques like viral barcoding and inducible lineage tracing approach. The first step is to build mouse models that provide the same niche found in primary human cancers.

In the field of targeted therapeutics, the advancement of novel assay would play a great role in identifying genetic heterogeneity and cancer hierarchy of CSCs. By finding the irreversible transformation between normal SC into CSCs, an appropriate targeted therapy will be developed to prevent cancer initiation and relapse after targeted therapy.

## ACKNOWLEDGMENTS

# REFERENCES

1. Leblond C, Clermont Y, Nadler N. The pattern of stem cell renewal in three epithelia (esophagus, intestine and testis). Proc Can Cancer Conf 1967;7:3-30.
2. Gallico Gr, O'Connor NE, Compton CC, Kehinde O, Green H. Permanent coverage of large burn wounds with autologous cultured human epithelium. New Engl J Med 1984:448-51.
3. Van Keymeulen A, Blanpain C. Tracing epithelial stem cells during development, homeostasis, and repair. J Cell Biol 2012;197:575-84.
4. Biddle A, Liang X, Gammon L, Fazil B, Harper LJ, Emich H, et al. Cancer stem cells in squamous cell carcinoma switch between two distinct phenotypes that are preferentially migratory or proliferative. Cancer Res 2011; 71:5317-26.
5. Nguyen LV, Vanner R, Dirks P, Eaves CJ. Cancer stem cells: an evolving concept. Nat Rev Cancer 2012;12:133-43.
6. Greaves M, Maley CC. Clonal evolution in cancer. Nature 2012;481:306-13.
7. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature 2001;414:105-11.
8. Barrandon Y, Green H. Three clonal types of keratinocyte with different capacities for multiplication. Proc Natl Acad Sci U S A 1987;84:2302-6.
9. Cotsarelis G, Sun TT, Lavker RM. Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. Cell 1990;61:1329-37.
10. Blanpain C, Lowry WE, Geoghegan A, Polak L, Fuchs E. Self-renewal, multipotency, and the existence of two cell populations within an epithelial stem cell niche. Cell 2004;118:635-48.
11. Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C, et al. Identification and expansion of human colon-cancer-initiating cells. Nature 2007;445:111-5.
12. Ponti D, Costa A, Zaffaroni N, Pratesi G, Petrangolini G, Coradini D, et al. Isolation and in vitro propagation of tumorigenic breast cancer cells with stem/progenitor cell properties. Cancer Res 2005;65:5506-11.
13. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, et al. Identification of human brain tumour initiating cells. Nature 2004;432:396-401.
14. Jung P, Sato T, Merlos-Suárez A, Barriga FM, Iglesias M, Rossell D, et al. Isolation and in vitro expansion of human colonic stem cells. Nat Med 2011;17:1225-7.
15. 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;15:504-14.
16. Barrett LE, Granot Z, Coker C, Iavarone A, Hambardzumyan D, Holland EC, et al. Self-renewal does not predict tumor growth potential in mouse models of high-grade glioma. Cancer Cell 2012;21:11-24.
17. Visvader JE. Cells of origin in cancer. Nature 2011;469:314-22.
18. Lapouge G, Beck B, Nassar D, Dubois C, Dekoninck S, Blanpain C. Skin squamous cell carcinoma propagating cells increase with tumour progression and invasiveness. EMBO J 2012;31:4563-75.
19. Curtis SJ, Sinkevicius KW, Li D, Lau AN, Roach RR, Zamponi R, et al. Primary tumor genotype is an important determinant in identification of lung cancer propagating cells. Cell Stem Cell 2010;7:127-33.
20. Grosse-Gehling P, Fargeas CA, Dittfeld C, Garbe Y, Alison MR, Corbeil D, et al. CD133 as a biomarker for putative cancer stem cells in solid tumours: limitations, problems and challenges. J Pathol 2013;229:355-78.
21. Haraguchi N, Ishii H, Mimori K, Tanaka F, Ohkuma M, Kim HM, et al. CD13 is a therapeutic target in human liver cancer stem cells. J Clin Invest 2010;120:3326.
22. Schwitalla S, Fingerle AA, Cammareri P, Nebelsiek T, Göktuna SI, Ziegler PK, et al. Intestinal tumorigenesis initiated by dedifferentiation and acquisition of stem-cell-like properties. Cell 2013;152:25-38.
23. Reya T, Clevers H. Wnt signalling in stem cells and cancer. Nature 2005; 434:843-50.
24. Kemper K, Prasetyanti PR, De Lau W, Rodermond H, Clevers H, Medema JP. Monoclonal antibodies against Lgr5 identify human colorectal cancer stem cells. Stem Cells 2012;30:2378-86.
25. Gupta PB, Fillmore CM, Jiang G, Shapira SD, Tao K, Kuperwasser C, et al. Stochastic state transitions give rise to phenotypic equilibrium in populations of cancer cells. Cell 2011;146:633-44.
26. Pang R, Law WL, Chu AC, Poon JT, Lam CS, Chow AK, et al. A subpopulation of CD26+ cancer stem cells with metastatic capacity in human colorectal cancer. Cell Stem Cell 2010;6:603-15.
27. Taichman RS, Cooper C, Keller ET, Pienta KJ, Taichman NS, McCauley LK. Use of the stromal cell-derived factor-1/CXCR4 pathway in prostate cancer metastasis to bone. Cancer Res 2002;62:1832-7.
28. Li YM, Pan Y, Wei Y, Cheng X, Zhou BP, Tan M, et al. Upregulation of CXCR4 is essential for HER2-mediated tumor metastasis. Cancer Cell 2004;6:459-69.
29. Kim M, Koh YJ, Kim KE, Koh BI, Nam DH, Alitalo K, et al. CXCR4 signaling regulates metastasis of chemoresistant melanoma cells by a lymphatic metastatic niche. Cancer Res 2010;70:10411-21.
30. 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: 313-23.
31. Ito M, Kizawa K, Hamada K, Cotsarelis G. Hair follicle stem cells in the lower bulge form the secondary germ, a biochemically distinct but functionally equivalent progenitor cell population, at the termination of catagen. Differentiation 2004;72:548-57.
32. Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. Nature 2011;473:298-307.
33. Hamerlik P, Lathia JD, Rasmussen R, Wu Q, Bartkova J, Lee M, et al. Autocrine VEGF–VEGFR2–Neuropilin-1 signaling promotes glioma stem-like cell viability and tumor growth. J Exp Med 2012;209:507-20.
34. Calabrese C, Poppleton H, Kocak M, Hogg TL, Fuller C, Hamner B, et al. A perivascular niche for brain tumor stem cells. Cancer Cell 2007;11:69-82.
35. Beck B, Driessens G, Goossens S, Youssef KK, Kuchnio A, Caauwe A, et al. A vascular niche and a VEGF-Nrp1 loop regulate the initiation and stemness of skin tumours. Nature 2011;478:399-403.
36. Takubo K, Goda N, Yamada W, Iriuchishima H, Ikeda E, Kubota Y, et al. Regulation of the HIF-1α level is essential for hematopoietic stem cells. Cell Stem Cell 2010;7:391-402.
37. Soeda A, Park M, Lee D, Mintz A, Androutsellis-Theotokis A, McKay R, et al. Hypoxia promotes expansion of the CD133-positive glioma stem cells through activation of HIF-1α. Oncogene 2009;28:3949-59.
38. Malanchi I, Santamaria-Martínez A, Susanto E, Peng H, Lehr H-A, Delaloye J-F, et al. Interactions between cancer stem cells and their niche govern metastatic colonization. Nature 2012;481:85-9.
39. Clouston AD, Powell EE, Walsh MJ, Richardson MM, Demetris AJ, Jonsson JR. Fibrosis correlates with a ductular reaction in hepatitis C: roles of impaired replication, progenitor cells and steatosis. Hepatology 2005;41: 809-18.
40. Feitelson MA, Lee J. Hepatitis B virus integration, fragile sites, and hepatocarcinogenesis. Cancer Lett 2007;252:157-70.
41. Wang XW, Forrester K, Yeh H, Feitelson MA, Gu JR, Harris CC. Hepati-

tis B virus X protein inhibits p53 sequence-specific DNA binding, transcriptional activity, and association with transcription factor ERCC3. Proc Natl Acad Sci U S A 1994;91:2230-4.

42. Hussain S, Schwank J, Staib F, Wang X, Harris C. TP53 mutations and hepatocellular carcinoma: insights into the etiology and pathogenesis of liver cancer. Oncogene 2007;26:2166-76.

43. Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 2006;444:756-60.

44. Dylla SJ, Beviglia L, Park IK, Chartier C, Raval J, Ngan L, et al. Colorectal cancer stem cells are enriched in xenogeneic tumors following chemotherapy. PLoS One 2008;3:e2428.

45. 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;458:780-3.

46. Hoshida Y, Villanueva A, Kobayashi M, Peix J, Chiang DY, Camargo A, et al. Gene expression in fixed tissues and outcome in hepatocellular carcinoma. N Engl J Med 2008;359:1995-2004.

47. Ma S, Tang KH, Chan YP, Lee TK, Kwan PS, Castilho A, et al. miR-130b Promotes CD133+ liver tumor-initiating cell growth and self-renewal via tumor protein 53-induced nuclear protein 1. Cell Stem Cell 2010;7:694-707.

48. Ma S, Lee T, Zheng B, Chan K, Guan X. CD133&plus; HCC cancer stem cells confer chemoresistance by preferential expression of the Akt/PKB survival pathway. Oncogene 2008;27:1749-58.

49. de Thé H, Chen Z. Acute promyelocytic leukaemia: novel insights into the mechanisms of cure. Nat Rev Cancer 2010;10:775-83.

50. Chen J, Li Y, Yu T-S, McKay RM, Burns DK, Kernie SG, et al. A restricted cell population propagates glioblastoma growth after chemotherapy. Nature 2012;488:522-6.