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An Overview of Concepts for Cancer Stem Cells

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Running title: Cancer stem cells overview

ABSTRACT

For many years, cancer research has focused on the adult stem cells present in malignant tumors. It is believed that current cancer treatments sometimes fail because they do not target these cells. According to classic models of carcinogenesis, these events can occur in any cell. In contrast, the cancer stem cell (CSC) hypothesis states that the preferential targets of oncogenic transformation are tissue stem cells or early progenitor cells that have acquired the potential for self-renewal. These tumor-initiating cells or CSCs, in turn, are characterized by their ability to undergo self-renewal, a process that drives tumorigenesis and differentiation, which contributes to the cellular heterogeneity of tumors. Herein, we discuss the definitions and properties of CSCs in the major human cancers.

BACKGROUND AND DEFINITIONS

Stem cells are found in many different somatic tissues and are important participants in their physiology (Fig. 1). Stem cells have 3 distinctive properties: (a) self-renewal; (b) the capability to develop into multiple lineages; and (c) the potential to extensively proliferate. The combination of these 3 properties makes stem cells unique. The attribute of self-renewal is especially notable because its subversion is highly relevant to oncogenesis and malignancy (1,57). Aberrantly increased self-renewal, in combination

with the intrinsic growth potential of stem cells, may account for much of what is considered a malignant phenotype.

CSCs are found in tumors or hematological cancers and possess characteristics associated with normal stem cells, specifically the ability to give rise to all cell types found in a particular cancer sample; therefore, these cells are tumorigenic, in contrast to other non-tumorigenic cancer cells. CSCs may generate tumors through the stem cell processes of self-renewal and differentiation into multiple cell types. Such cells are proposed to persist in tumors as a distinct population and cause relapse and metastasis. Therefore, the development of specific therapies targeted at CSCs could improve the survival and quality of life of cancer patients, especially in cases of patients with metastatic disease.

The first conclusive evidence for the existence of CSCs was reported in 1997. Bonnet and Dick (5) isolated a subpopulation of leukemic cells that expressed the CD34 surface marker, but lacked the CD38 marker. This $CD34^{+}/CD38^{-}$ subpopulation was capable of initiating tumors that were histologically similar to the donor in NOD/SCID mice (48).

In cancer research experiments, tumors can be established by the injection of tumor cells into an experimental animal. Disease progression is then followed over time, and novel drugs can be tested for their ability to inhibit tumor growth; however, efficient tumor formation requires the introduction of thousands or tens of thousands of cells.

Traditionally, this feature has been explained by poor methodology (i.e., the tumor cells lose their viability during transfer) or the critical importance of the tumor microenvironment (i.e., the particular biochemical surroundings of the injected cells). Supporters of the CSC paradigm argue that only a small fraction of the injected cells, the CSCs, have the potential to generate a tumor. In human acute myeloid leukemia (AML), the frequency of these cells is less than 1 in 10,000 (5).

Further evidence for this hypothesis has been provided by histological studies of tumors. Many tumors are heterogeneous and contain multiple cell types native to the host organ. Heterogeneity is commonly retained during the metastasis of tumors. This implies that the cell that produced them had the capacity to generate multiple cell types, i.e., it possessed multi-differentiation potential, a classical hallmark of stem cells (5).

The identification of leukemic stem cells prompted further research into other types of cancer, leading to the identification of CSCs in several solid tumors, including cancers of the brain (64), breast (2), lung (40), colon (53,55), ovary (72), pancreas (45), and prostate (42,47).

CANCER STEM CELLS IN THE HEMATOPOIETIC SYSTEM

The hematopoietic system is the best characterized somatic tissue with respect to

stem cell biology. Over the past several decades, many of the physical, biological, and developmental features of normal hematopoietic stem cells have been defined (41,63), and useful methods for studying stem cells in almost any context have been established (49). Hematopoietic cell cancers, such as leukemia, are clearly different from solid tumors, but certain aspects of hematopoietic stem cell biology are relevant to our understanding of the broad principles of CSC biology (57). CSCs have been unequivocally identified in various types of leukemia, and several biologic properties of these stem cells have direct implications for therapy (21,30,37,69).

CSCs are readily evident in chronic myelogenous leukemia (CML) (34) and acute myelogenous leukemia (AML) (5,43), and they have also been implicated in acute lymphoblastic leukemia (ALL) (8,13,16). CML stem cells have a well described stem-cell phenotype and a quiescent cell-cycle status. Similarly, AML stem cells are mostly quiescent (29,31,33,67), suggesting that conventional antiproliferative cytotoxic regimens are unlikely to be effective against them. AML stem cells have surface markers, such as the interleukin-3–receptor α chain, that are not present on normal stem cells (36). These markers may be useful for antibody-based (38) or other related therapeutic regimens (6,26). Early efforts have demonstrated the usefulness of antibodies against the CD33 antigen in the treatment of AML (32,44), and recent reports indicate that CD33 is expressed on some leukemic stem cells (66). These observations warrant the continued development of

immunotherapy strategies against stem cell-specific antigens.

CANCER STEM CELLS IN THE BRAIN

Brain tumors are the leading cause of cancer mortality in children and are difficult to treat despite advances in surgery and adjuvant therapy. Most current brain tumor research is focused on the molecular and cellular analysis of the tumor mass. Brain tumors are typically comprised of morphologically diverse cells that express a variety of neural lineage markers. Analysis of the basic morphology and phenotype of brain tumors has yielded a limited amount of knowledge with respect to the clinical behavior of the tumor, as brain tumors that share a similar morphology and phenotype can have a very different prognosis and response to treatment.

The determination of key cells in the tumor population that maintain the tumor will provide an insight into the mechanism of brain tumorigenesis and allow us to identify the cell of origin in the normal brain. There is overwhelming evidence in other malignancies, such as leukemia, that the clonal population of neoplastic cells exhibits marked heterogeneity with respect to proliferation and differentiation (5,61). Rare stem cells within the leukemic population possess extensive proliferative and self-renewal capacities that are not observed in the majority of leukemic cells. Fractionation and functional

analysis of leukemic stem cells indicated that they are necessary and sufficient to induce leukemia (5,43).

We have isolated normal neural stem cells in culture (as clonally derived neurospheres, each of which arise from a single stem cell) for the analysis of human pediatric brain tumors (62). Analysis of neurosphere cells were used to functionally characterize the populations of tumor cells. We purified and characterized a cell from primary human brain tumors with different phenotypes; this cell type had a marked capacity for proliferation, self-renewal, and differentiation. This cell represented a minority of the tumor cell population and was identified by the expression of the cell surface marker CD133. This $CD133^+$ cell, which we have termed the brain tumor stem cell (BTSC) (43), lacked the expression of neural differentiation markers and was necessary for the proliferation and self-renewal of the tumor in culture. This cell was also capable of differentiating *in vitro* into the cell types found in the tumor *in situ*. The marker phenotype of the BTSC was similar to that of normal neural stem cells, in that it expressed CD133 and nestin, and was the same in patients with the same pathological type of tumor and in patients with different pathological subtypes. This suggests that brain tumors with a very similar phenotype can be generated from BTSCs. Further cellular and molecular studies of BTSCs would be required for better understanding of brain tumor biology. Comparison of normal neural stem cells and BTSCs will aid in finding the normal brain cell that is the

source of the tumor.

CANCER STEM CELLS IN THE BREAST

Breast CSCs have been definitively identified by Al-Hajj et al. in specimens from patients with advanced stages of metastatic breast cancer. They demonstrated that cells with a specific cell-surface antigen profile ($CD44^+/CD24^-$) could successfully establish themselves as tumor xenografts (2). Recent studies have provided definitive evidence for the existence of CSC populations within breast cancer cell lines. Charafe-Jauffret et al. used 33 cell lines derived from human breast cancers and nontransformed breast cells to study the expression of the stem cell marker aldehyde dehydrogenase (ALDH) (9). ALDH is a detoxifying enzyme that oxidizes intracellular aldehydes, and it is thought to play a role in the differentiation of stem cells via the metabolism of retinal to retinoic acid (11). ALDH activity, as assessed by the fluorescent ALDEFLUOR assay, has been successfully used to isolate CSCs from multiple myeloma and acute myeloid leukemia as well as from brain tumors (10,15). ALDH activity can be used to isolate a subpopulation of cells that display stem cell properties from normal human breast tissue and breast carcinomas (27). The majority of breast cancer cell lines contain an ALDEFLUOR-positive population with a distinct molecular profile that displays CSC properties (9). These studies have important

implications for the interpretation of data using cell lines and suggest that these lines may be useful for elucidating the regulatory pathways of CSCs.

CANCER STEM CELLS IN THE LUNG

Bronchoalveolar stem cells (BASCs), which may be precursors of lung adenocarcinoma, have been identified using a mice model for lung cancer (40). BASCs were resistant to bronchiolar and alveolar damage and proliferated during epithelial cell renewal *in vivo*. BASCs exhibited self-renewal and were multipotent in clonal assays, highlighting their stem cell properties (3); furthermore, BASCs expanded in response to oncogenic K-ras *in vitro* and in lung tumor precursors *in vivo* (35). These data support the hypothesis that BASCs are a stem cell population that maintain the bronchiolar Clara cells and alveolar cells of the distal lung and that their transformed counterparts give rise to adenocarcinomas.

The K-ras, phosphatase and tensin homolog (Pten), phosphoinositide 3-kinase (PI3K), and cyclin-dependent kinase pathways have been implicated in the proliferation of these stem cells (70,71). The potential role of bronchoalveolar stem cells and other tumorigenic stem-cell populations in the development and prognosis of human lung cancer and its resistance to drugs is an important area for future investigation.

CANCER STEM CELLS IN THE COLON

Colon cancer is one of the best understood neoplasms from a genetic perspective (24,25,60), yet it remains the second most common cause of cancer-related death, indicating that some of its cancer cells are not eradicated by current therapies (54,56). It has not been established whether every colon cancer cell possesses the potential to initiate and sustain tumor growth, or whether the tumor is hierarchically organized so that only a subset of cells, i.e., CSCs, possess such a potential (22,39,69). O'Brien et al. (55) used renal capsule transplantation in non-obese diabetic (NOD)/severe combined immunodeficient (SCID) to identify a human colon cancer-initiating cell (CC-IC). Purification experiments established that all CC-ICs were $CD133^+$; the $CD133^-$ cells, that comprised the majority of the tumor, were unable to initiate tumor growth. CC-ICs within the $CD133^+$ population were able to maintain themselves, differentiate, and reestablish tumor heterogeneity after serial transplantation. The identification of CSCs in the colon, that are distinct from the bulk of tumor cells, provides strong support for the hierarchical organization of human colon cancer. Their existence suggests that for therapeutic strategies to be effective, they must target the CSCs.

CANCER STEM CELLS IN THE OVARY

In ovarian cancer, Bapat et al. (4) isolated 2 clones from patients' ascites that could organize anchorage-independent, spherical structures (spheroids) *in vitro*, similar to those observed in ascites *in vivo* (7). These clones formed xenografts in nude mice (with a histopathology similar to the parental human tumors), serially propagated in animals (4), and expressed the stem cell factor receptor CD117 (c-kit)—a well-known proto-oncoprotein (52). Another ovarian cancer study identified a subpopulation of dye-excluding cultured murine cells, representing membrane transporter-expressing putative stem cells, that were highly tumorigenic in mice when compared to dye-staining (i.e., non-effluxing) cells (65). To satisfy the CSCs' criterion of distinctive cell surface markers (18), the expression of CD117 (similar to the ascites study) and the hyaluronate receptor CD44 (also a marker for CSCs from several other solid tumors (14,19,45,59) was observed in these cultured murine cells.

CANCER STEM CELLS IN THE PANCREAS

Al-Hajj et al. (2) reported that a phenotypically distinct and relatively rare population of $CD44^+/CD24^-$/epithelial-specific antigen-positive (ESA^+) tumor-initiating cells was

responsible for the propagation of human metastatic breast cancer specimens in NOD/SCID mice. Li et al. identified a highly tumorigenic subpopulation of $CD44^+/CD24^+/ESA^+$ pancreatic cancer cells in a xenograft model of primary human pancreatic adenocarcinomas grown in immunocompromised mice (45). $CD44^+/CD24^+/ESA^+$ pancreatic cancer cells (0.2–0.8% of pancreatic cancer cells) had a 100-fold increased tumorigenic potential when compared to non-tumorigenic cancer cells. Fifty percent of animals injected with 100 $CD44^+/CD24^+/ESA^+$ cells formed tumors that were histologically indistinguishable from the human tumors from which they were derived. The enhanced ability of $CD44^+/CD24^+/ESA^+$ pancreatic cancer cells to form tumors was confirmed in an orthotopic pancreatic tail injection model. The $CD44^+/CD24^+/ESA^+$ pancreatic cancer cells possessed the stem cell properties of self-renewal, the ability to produce differentiated progeny, and the increased expression of the developmental signaling molecule sonic hedgehog (SHH). The identification of pancreatic CSCs and the elucidation of the signaling pathways that regulate their growth and survival may provide novel therapeutic approaches to treat pancreatic cancer, which is notoriously resistant to standard chemotherapy and radiation.

CANCER STEM CELLS IN THE PROSTATE

The cellular origins of prostate cancer are still undecided. It has been suggested that prostate cancers arise from terminally differentiated luminal cells (20,51) because the bulk population of tumor cells, in the most common form of prostate cancer, express luminal cell-specific markers (cytokeratins (CK) 8 and 18, androgen receptor (AR), prostate-specific antigen (PSA), and prostatic acid phosphatase (PAP)), but lack the expression of basal cell markers, such as p63. Moreover, cells that solely express basal cell markers, such as CK5 and CK14, are rarely observed. This has led to the speculation that prostate cancers are derived from intermediate progenitors that have acquired the ability for self-renewal (68).

Several lines of evidence support the hypothesis that prostate cancer stem cells arise from normal stem cells. Advanced prostate cancers are androgen-independent and basal cells can be phenotypically identified in the majority of metastases (46). Craft et al. showed that advanced androgen-independent tumors arise from the clonal expansion of AR^- cells that are present at a frequency of 1 per 10^5-10^6 AR^+ cells (17). Recent studies have found that only the most primitive cells ($\alpha 2\beta 1^{hi}$/$CD133^+$/$CD44^+$), which were phenotypically identical to normal prostate stem cells, could self-renew *in vitro* (14). Under differentiating conditions, AR^+/PAP^+/$CK18^+$ luminal cells could be identified in these cultures, suggesting that they were derived from the more primitive cell population. In support of this finding, the $CD44^+$ population from xenograft tumors and cell lines had

enhanced proliferative potential and tumor-initiating ability *in vivo* compared to $CD44^-$ cells (58). The $CD44^+$ cells were AR^- and expressed higher mRNA levels of "stemness" genes, such as *OCT3/4* and *BMI1*. By using clonally-derived human prostate cancer epithelial cells expressing human telomerase reverse transcriptase (hTERT), Gu et al. demonstrated that these lines could regenerate tumors in mice that resembled the original tumor (with respect to the Gleason score) in the patient (28). The tumors contained luminal, basal, and neuroendocrine cells, implying that the clone of origin could differentiate into the epithelial cell lineages of the prostate. In this case, the tumor-initiating cell was $AR^-/p63^-$ and expressed the stem cell genes *Oct-4, Nanog, Sox2, nestin, CD44, CD133,* and *c-kit*. Moreover, *Sca-1*-sorted cells, enriched for cells with prostate-regenerating activity, showed evidence of a basal and luminal lineage.

CONCLUSION

CSCs were first identified in acute myeloid leukemia as cells expressing the surface antigen phenotype $CD34^+/CD38-$, which have the capacity to reproduce the complete leukemic hierarchy following xenoengraftment (5). The epithelial linings of most tissue surfaces undergo continuous turnover and are organized according to a stem cell hierarchy; similar to the hematopoietic system (18,50). $CD24^-/CD44^+$ cells that could be serially

propagated in animals and recapitulate their original phenotype were isolated from human breast tumors (2). CSCs have also been identified in numerous other epithelial malignancies (melanoma, lung, head/neck, pancreas, prostate, and colon cancers (14,19,23,40,45,59). A consensus of 5 defining criteria has been established to affirm the existence of CSCs: (a) self-renewal, (b) restriction to a small minority of the total tumor population, (c) reproducible tumor phenotype, (d) multipotent differentiation into non-tumorigenic cells, and (e) expression of distinctive cell surface markers permitting consistent isolation (12,18).

Some types of cancer, when identified at an early stage, can be successfully treated; however, advanced cancers and a number of other cancer types, such as pancreatic cancer, still have dismal survival rates. Other cancers, such as head and neck cancers, are often resistant to current therapies, making less-invasive treatments more difficult. In addition, current chemotherapeutic regimens cause severe side effects because they target all rapidly dividing cells.

Existing cancer treatments have mostly been developed using animal models, where therapies that were able to promote tumor shrinkage were deemed effective. However, animals do not provide a complete model of human disease. Tumor relapse is exceptionally difficult to study in mice because they have a short life-span that does not usually exceed 2 years. The study of CSCs is still in its early stages, primarily taking place

in the laboratory. No standard treatments have yet been developed as a result of research on CSCs. The next step is to understand CSC activity and to identify drugs that target the stem cells without harming normal cells. Treatments that specifically target CSCs would cause fewer side effects for patients.

REFERENCES

1. Al-Hajj, M.; Clarke, M. F. Self-renewal and solid tumor stem cells. Oncogene 23(43):7274-7282; 2004.

2. Al-Hajj, M.; Wicha, M. S.; Benito-Hernandez, A.; Morrison, S. J.; Clarke, M. F. Prospective identification of tumorigenic breast cancer cells. Proc. Natl. Acad. Sci. USA 100(7):3983-3988; 2003.

3. Bannister, L. H. Respiratory system. In: Williams P. L.; Warwick R.; Dyson M.; Bannister L. H., eds. Gray's Anatomy. New York, NY: Churchill Livingstone; 1999:1666-1672.

4. Bapat, S. A.; Mali, A. M.; Koppikar, C. B.; Kurrey, N. K. Stem and progenitor-like cells contribute to the aggressive behavior of human epithelial ovarian cancer. Cancer Res. 65(8):3025-3029; 2005.

5. Bonnet, D.; Dick, J. E. Human acute myeloid leukemia is organized as a hierarchy

that originates from a primitive hematopoietic cell. Nat. Med. 3(7):730-737; 1997.

6. Bonnet, D.; Warren, E. H.; Greenberg, P. D.; Dick, J. E.; Riddell, S. R. CD8(+) minor histocompatibility antigen-specific cytotoxic T lymphocyte clones eliminate human acute myeloid leukemia stem cells. Proc. Natl. Acad. Sci. USA 96(15):8639-8644; 1999.

7. Burleson, K. M.; Casey, R. C.; Skubitz, K. M.; Pambuccian, S. E.; Oegema, T. R., Jr.; Skubitz, A. P. Ovarian carcinoma ascites spheroids adhere to extracellular matrix components and mesothelial cell monolayers. Gynecol. Oncol. 93(1):170-181; 2004.

8. Castor, A.; Nilsson, L.; Astrand-Grundström, I.; Buitenhuis, M.; Ramirez, C.; Anderson, K.; Strömbeck, B.; Garwicz, S.; Békássy, A. N.; Schmiegelow, K.; Lausen, B.; Hokland, P.; Lehmann, S.; Juliusson, G.; Johansson, B.; Jacobsen, S. E. Distinct patterns of hematopoietic stem cell involvement in acute lymphoblastic leukemia. Nat. Med. 11(6): 630-637; 2005.

9. Charafe-Jauffret, E.; Ginestier, C.; Iovino, F.; Wicinski, J.; Cervera, N.; Finetti, P.; Hur, M. H.; Diebel, M. E.; Monville, F.; Dutcher, J.; Brown, M.; Viens, P.; Xerri, L.; Bertucci, F.; Stassi, G.; Dontu, G.; Birnbaum, D.; Wicha, M. S. Breast cancer cell lines contain functional cancer stem cells with metastatic capacity and a distinct molecular signature. Cancer Res. 69(4):1302-1313; 2009.

10. Cheung, A. M.; Wan, T. S.; Leung, J. C.; Chan, L. Y.; Huang, H.; Kwong, Y. L.;

Liang, R.; Leung, A. Y. Aldehyde dehydrogenase activity in leukemic blasts defines a subgroup of acute myeloid leukemia with adverse prognosis and superior NOD/SCID engrafting potential. Leukemia 21(7):1423-1430; 2007.

11. Chute, J. P.; Muramoto, G. G.; Whitesides, J.; Colvin, M.; Safi, R.; Chao, N. J.; McDonnell, D. P. Inhibition of aldehyde dehydrogenase and retinoid signaling induces the expansion of human hematopoietic stem cells. Proc. Natl. Acad. Sci. USA 103(31):11707-11712; 2006.

12. Clarke, M. F.; Dick, J. E.; Dirks, P. B.; Eaves, C. J.; Jamieson, C. H.; Jones, D. L.; Visvader, J.; Weissman, I. L.; Wahl, G. M. Cancer stem cells-perspectives on current status and future directions: AACR workshop on cancer stem cells. Cancer Res. 66(19):9339-9344; 2006.

13. Cobaleda, C.; Gutierrez-Cianca, N.; Perez-Losada, J.; Flores, T.; García-Sanz, R.; González, M.; Sánchez-García, I. A primitive hematopoietic cell is the target for the leukemic transformation in human Philadelphia-positive acute lymphoblastic leukemia. Blood 95(3):1007-1013; 2000.

14. Collins, A. T.; Berry, P. A.; Hyde, C.; Stower, M. J.; Maitland, N. J. Prospective identification of tumorigenic prostate cancer stem cells. Cancer Res. 65(23):10946-10951; 2005.

15. Corti, S.; Locatelli, F.; Papadimitriou, D.; Donadoni, C.; Salani, S.; Del Bo, R.;

Strazzer, S.; Bresolin, N.; Comi, G. P. Identification of a primitive brain-derived neural stem cell population based on aldehyde dehydrogenase activity. Stem Cells 24(4):975-985; 2006.

16. Cox, C. V.; Evely, R. S.; Oakhill, A.; Pamphilon, D. H.; Goulden, N. J.; Blair, A. Characterization of acute lymphoblastic leukemia progenitor cells. Blood 104(9):2919-2925; 2004.

17. Craft, N.; Chhor, C.; Tran, C.; Belldegrun, A.; DeKernion, J.; Witte, O. N.; Said, J.; Reiter, R. E.; Sawyers, C. L. Evidence for clonal outgrowth of androgen-independent prostate cancer cells from androgen-dependent tumors through a two-step process. Cancer Res. 59(19):5030-5036; 1999.

18. Dalerba, P.; Cho, R. W.; Clarke, M. F. Cancer stem cells: models and concepts. Annu. Rev. Med. 58:267-284; 2007.

19. Dalerba, P.; Dylla, S, J.; Park, I. K.; Liu, R.; Wang, X.; Cho, R. W.; Hoey, T.; Gurney, A.; Huang, E. H.; Simeone, D. M.; Shelton, A. A.; Parmiani, G.; Castelli, C.; Clarke, M. F. Phenotypic characterization of human colorectal cancer stem cells. Proc. Natl. Acad. Sci. USA 104(24):10158-10163; 2007.

20. DeMarzo, A. M.; Meeker, A. K.; Epstein, J. I.; Coffey, D. S. Prostate stem cell compartments. Expression of the cell cycle inhibitor p27kip1 in normal, hyperplastic, and neoplastic cells. Am. J. Pathol. 153(3):911-919; 1998.

21. Dick, J. E. Acute myeloid leukemia stem cells. Ann. N. Y. Acad. Sci. 1044:1-5; 2005.

22. Dick, J. E. Breast cancer stem cells revealed. Proc. Natl. Acad. Sci. USA 100(7):3547-3549; 2003.

23. Fang, D.; Nguyen, T. K.; Leishear, K.; Finko, R.; Kulp, A. N.; Hotz, S.; Van, Belle, P. A.; Xu, X.; Elder, D. E.; Herlyn, M. A tumorigenic subpopulation with stem cell properties in melanomas. Cancer Res. 65(20):9328-9337; 2005.

24. Fearon, E. R.; Jones, P. A. Progressing toward a molecular description of colorectal cancer development. FASEB J. 6(10):2783-2790; 1992.

25. Fearon, E. R.; Vogelstein, B. A genetic model for colorectal tumorigenesis. Cell 61(5):759-767; 1990.

26. Feuring-Buske, M.; Frankel, A. E.; Alexander, R. L.; Gerhard, B.; Hogge, D. E. A diphtheria toxin-interleukin 3 fusion protein is cytotoxic to primitive acute myeloid leukemia progenitors but spares normal progenitors. Cancer Res. 62(6):1730-1736; 2002.

27. Ginestier, C.; Hur, M. H.; Charafe-Jauffret, E.; Monville, F.; Dutcher, J.; Brown, M.; Jacquemier, J.; Viens, P.; Kleer, C. G.; Liu, S.; Schott, A.; Hayes, D.; Birnbaum, D.; Wicha, M. S.; Dontu, G. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Cell 1(5):555-567; 2007.

28. Gu, G.; Yuan, J.; Wills, M. L.; Kasper, S. Prostate cancer cells with stem cell characteristics reconstitute the original human tumor in vivo. Cancer Res. 67(10):4807-4815; 2007.

29. Guan, Y.; Gerhard, B.; Hogge, D. E. Detection, isolation, and stimulation of quiescent primitive leukemic progenitor cells from patients with acute myeloid leukemia (AML). Blood 101(8):3142-3149; 2003.

30. Guzman, M. L.; Jordan, C. T. Considerations for targeting malignant stem cells in leukemia. Cancer Control 11(2):97-104; 2004.

31. Guzman, M. L.; Neering, S. J.; Upchurch, D.; Grimes, B.; Howard, D. S.; Rizzieri, D. A.; Luger, S. M.; Jordan, C. T. Nuclear factor-kappaB is constitutively activated in primitive human acute myelogenous leukemia cells. Blood 98(8):2301-2307; 2001.

32. Hamann, P. R.; Hinman, L. M.; Hollander, I.; Beyer, C. F.; Lindh, D.; Holcomb, R.; Hallett, W.; Tsou, H. R.; Upeslacis, J.; Shochat, D.; Mountain, A.; Flowers, D. A.; Bernstein, I. Gemtuzumab ozogamicin, a potent and selective anti-CD33 antibody-calicheamicin conjugate for treatment of acute myeloid leukemia. Bioconjug. Chem. 13(1):47-58; 2002.

33. Holyoake, T.; Jiang, X.; Eaves, C.; Eaves, A. Isolation of a highly quiescent subpopulation of primitive leukemic cells in chronic myeloid leukemia. Blood 94(6):2056-2064; 1999.

34. Holyoake, T. L.; Jiang, X.; Drummond, M. W.; Eaves, A. C.; Eaves, C. J. Elucidating critical mechanisms of deregulated stem cell turnover in the chronic phase of chronic myeloid leukemia. Leukemia 16(4):549-558; 2002.

35. Jackson, E. L.; Willis, N.; Mercer, K.; Bronson, R. T.; Crowley, D.; Montoya, R.; Jacks, T.; Tuveson, D. A. Analysis of lung tumor initiation and progression using conditional expression of oncogenic K-ras. Genes Dev. 15(24):3243-3248; 2001.

36. Jordan, C. T. Unique molecular and cellular features of acute myelogenous leukemia stem cells. Leukemia 16(4):559-562; 2002.

37. Jordan, C. T.; Guzman, M. L. Mechanisms controlling pathogenesis and survival of leukemic stem cells. Oncogene 23(43):7178-7187; 2004.

38. Jordan, C. T.; Upchurch, D.; Szilvassy, S. J.; Guzman, M. L.; Howard, D. S.; Pettigrew, A. L.; Meyerrose, T.; Rossi, R.; Grimes, B.; Rizzieri, D. A.; Luger, S. M.; Phillips, G. L. The interleukin-3 receptor alpha chain is a unique marker for human acute myelogenous leukemia stem cells. Leukemia 14:1777-1784; 2000.

39. Kawamoto, H.; Yuasa, T.; Kubota, Y.; Seita, M.; Sasamoto, H.; Shahid, J. M.; Hayashi, T.; Nakahara, H.; Hassan, R.; Iwamuro, M.; Kondo, E.; Nakaji, S.; Tanaka, N.; Kobayashi, N. Characteristics of CD133(+) Human Colon Cancer SW620 Cells. Cell Transplant. 2010 Jun 29. [Epub ahead of print]

40. Kim, C. F.; Jackson, E. L.; Woolfenden, A. E.; Lawrence, S.; Babar, I.; Vogel, S.;

Crowley, D.; Bronson, R. T.; Jacks, T. Identification of bronchioalveolar stem cells in normal lung and lung cancer. Cell 121(6):823-835; 2005.

41. Kondo, M.; Wagers, A. J.; Manz, M. G.; Prohaska, S. S.; Scherer, D. C.; Beilhack, G. F.; Shizuru, J. A.; Weissman, I. L. Biology of hematopoietic stem cells and progenitors: implications for clinical application. Annu. Rev. Immunol. 21:759-806; 2003.

42. Lang, S. H.; Frame, F.; Collins, A. Prostate cancer stem cells. J. Pathol. 217(2):299-306; 2009.

43. Lapidot, T.; Sirard, C.; Vormoor, J.; Murdoch, B.; Hoang, T.; Caceres-Cortes, J.; Minden, M.; Paterson, B.; Callgiuri, M. A.; Dick, J. E. A cell initiating human acute leukaemia after transplantation into SCID mice. Nature 367(6464):645-648; 1994.

44. Larson, R. A.; Sievers, E. L.; Stadtmauer, E. A.; Löwenberg, B.; Estey, E. H.; Dombret, H.; Theobald, M.; Voliotis, D.; Bennett, J. M.; Richie, M.; Leopold, L. H.; Berger, M. S.; Sherman, M. L.; Loken, M. R.; van Dongen, J. J.; Bernstein, I. D.; Appelbaum, F. R. Final report of the efficacy and safety of gemtuzumab ozogamicin (Mylotarg) in patients with CD33-positive acute myeloid leukemia in first recurrence. Cancer 104(7):1442-1452; 2005.

45. Li, C.; Heidt, D. G.; Dalerba, P.; Burant, C. F.; Zhang, L.; Adsay, V.; Wicha, M.; Clarke, M. F.; Simeone, D. M. Identification of pancreatic cancer stem cells. Cancer

Res. 67(3):1030-1037; 2007.

46. Liu, A. Y.; Nelson, P. S.; van den Engh G, Hood, L. Human prostate epithelial cell-type cDNA libraries and prostate expression patterns. Prostate 50(2):92-103; 2002.

47. Maitland, N. J.; Collins, A. T. Prostate cancer stem cells: a new target for therapy. J. Clin. Oncol. 26(17):2862-2870; 2008.

48. Matsui, W.; Huff, C. A.; Wang, Q.; Malehorn, M. T.; Barber, J.; Tanhehco, Y.; Smith, B. D.; Civin, C. I.; Jones, R. J. Characterization of clonogenic multiple myeloma cells. Blood 103(6):2332-2336; 2004.

49. Mazo, M.; Gavira, J. J.; Abizanda, G.; Moreno, C.; Ecay, M.; Soriano, M.; Aranda, P.; Collantes, M.; Alegría, E.; Merino, J.; Peñuelas, I.; García Verdugo, J. M.; Pelacho, B.; Prósper, F. Transplantation of mesenchymal stem cells exerts a greater long-term effect than bone marrow mononuclear cells in a chronic myocardial infarction model in rat. Cell Transplant. 19(3):313-328; 2010.

50. Miller, S. J.; Lavker, R. M.; Sun, T. T. Interpreting epithelial cancer biology in the context of stem cells: tumor properties and therapeutic implications. Biochim. Biophys. Acta 1756(1):25-52; 2005.

51. Nagle, R. B.; Ahmann, F. R.; McDaniel, K. M.; Paquin, M. L.; Clark, V. A.; Celniker, A. Cytokeratin characterisation of human prostatic carcinoma and its derived cell

lines. Cancer Res. 47(1):281-286; 1987.

52. Natali, P. G.; Nicotra, M. R.; Sures, I.; Santoro, E.; Bigotti, A.; Ullrich, A. Expression of c-kit receptor in normal and transformed human nonlymphoid tissues. Cancer Res. 52(22):6139-6143; 1992.

53. Navarro-Alvarez, N.; Kondo, E.; Kawamoto, H.; Hassan, W.; Yuasa, T.; Kubota, Y.; Seita, M.; Nakahara, H.; Hayashi, T.; Nishikawa, Y.; Hassan, R. A.; Javed, S. M.; Noguchi, H.; Matsumoto, S.; Nakaji, S.; Tanaka, N.; Kobayashi, N.; Soto-Gutierrez, A. Isolation and Propagation of a Human CD133-Negative Colon Tumor Derived Cell Line with Tumorigenic and Angiogenic Properties. Cell Transplant. 2010 Jun 29. [Epub ahead of print]

54. Nelson, H.; Petrelli, N.; Carlin, A.; Couture, J.; Fleshman, J.; Guillem, J.; Miedema, B.; Ota, D.; Sargent, D.; National Cancer Institute Expert Panel. Guidelines 2000 for colon and rectal cancer surgery. J. Natl. Cancer Inst. 93(8):583-596; 2001.

55. O'Brien, C. A.; Pollett, A.; Gallinger, S.; Dick, J. E. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature 445(7123):106-110; 2007.

56. O'Connell, J. B.; Maggard, M. A.; Ko, C. Y. Colon cancer survival rates with the new American Joint Committee on Cancer sixth edition staging. J. Natl. Cancer Inst. 96(19):1420-1425; 2004.

57. Pardal, R.; Clarke, M. F.; Morrison, S. J. Applying the principles of stem-cell biology to cancer. Nat. Rev. Cancer 3(12):895-902; 2003.

58. Patrawala, L.; Calhoun, T.; Schneider-Broussard, R.; Li, H.; Bhatia, B.; Tang, S.; Reilly, J. G.; Chandra, D.; Zhou, J.; Claypool, K.; Coghlan, L.; Tang, D. G. Highly purified $CD44^+$ prostate cancer cells from xenograft human tumors are enriched in tumorigenic and metastatic progenitor cells. Oncogene 25(12):1696-1708; 2006.

59. Prince, M. E.; Sivanandan, R.; Kaczorowski, A.; Wolf, G. T.; Kaplan, M. J.; Dalerba, P.; Weissman, I. L.; Clarke, M. F.; Ailles, L. E. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. Proc. Natl. Acad. Sci. USA 104(3):973-978; 2007.

60. Radtke, F.; Clevers, H. Self-renewal and cancer of the gut: two sides of a coin. Science 307(5717):1904-1909; 2005.

61. Reya, T.; Morrison, S. J.; Clarke, M. F.; Weissman, I. L. Stem cells, cancer, and cancer stem cells. Nature 415(6859):105-111; 2001.

62. Reynolds, B. A.; Weiss, S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 255(5052):1707-1710; 1992.

63. Shizuru, J. A.; Negrin, R. S.; Weissman, I. L. Hematopoietic stem and progenitor cells: clinical and preclinical regeneration of the hematolymphoid system. Annu. Rev. Med. 56:509-538; 2005.

64. Singh, S. K.; Clarke, I. D.; Terasaki, M.; Bonn, V. E.; Hawkins, C.; Squire, J.; Dirks, P. B. Identification of a cancer stem cell in human brain tumors. Cancer Res. 63(18):5821-5828; 2003.

65. Szotek, P. P.; Pieretti-Vanmarcke, R.; Masiakos, P. T.; Dinulescu, D. M.; Connolly, D.; Foster, R.; Dombkowski, D.; Preffer, F.; Maclaughlin, D. T.; Donahoe, P. K. Ovarian cancer side population defines cells with stem cell-like characteristics and Mullerian inhibiting substance responsiveness. Proc. Natl. Acad. Sci. USA 103(30):11154-11159; 2006.

66. Taussig, D. C.; Pearce, D. J.; Simpson, C.; Rohatiner, A. Z.; Lister, T. A.; Kelly, G.; Luongo, J. L.; Danet-Desnoyers, G. A.; Bonnet, D. Hematopoietic stem cells express multiple myeloid markers: implications for the origin and targeted therapy of acute myeloid leukemia. Blood 106(13):4086-4092; 2005.

67. Terpstra, W.; Ploemacher, R. E.; Prins, A.; van Lom, K.; Pouwels, K.; Wognum, A. W.; Wagemaker, G.; Löwenberg, B.; Wielenga, J. J. Fluorouracil selectively spares acute myeloid leukemia cells with long-term growth abilities in immunodeficient mice and in culture. Blood 88(6):1944-1950; 1996.

68. Van Leenders, G. J.; Schalken, J. A. Stem cell differentiation within the human prostate epithelium: implications for prostate carcinogenesis. BJU Int. 88 Suppl 2:35-42; discussion 49-50; 2001.

69. Wang, J. C.; Dick, J. E. Cancer stem cells: lessons from leukemia. Trends Cell Biol. 15(9):494-501; 2005.

70. Yanagi, S.; Kishimoto, H.; Kawahara, K.; Sasaki, T.; Sasaki, M.; Nishio, M.; Yajima, N.; Hamada, K.; Horie, Y.; Kubo, H.; Whitsett, J. A.; Mak, T. W.; Nakano, T.; Nakazato, M.; Suzuki, A. Pten controls lung morphogenesis, bronchioalveolar stem cells, and onset of lung adenocarcinomas in mice. J. Clin. Invest. 117(10):2929-2940; 2007.

71. Yang, Y.; Iwanaga, K.; Raso, M. G.; Wislez, M.; Hanna, A. E.; Wieder, E. D.; Molldrem, J. J.; Wistuba, I. I.; Powis, G.; Demayo, F. J.; Kim, C. F.; Kurie, J. M. Phosphatidylinositol 3-kinase mediates bronchioalveolar stem cell expansion in mouse models of oncogenic K-ras-induced lung cancer. PLoS ONE 3(5):e2220; 2008.

72. Zhang, S.; Balch, C.; Chan, M. W.; Lai, H. C.; Matei, D.; Schilder, J. M.; Yan, P. S.; Huang, T. H. Nephew, K. P. Identification and characterization of ovarian cancer-initiating cells from primary human tumors. Cancer Res. 68(11):4311-4320; 2008.

FIGURE LEGENDS

Figure 1. Stem cells physiology: For examples in Adult Somatic Tissues. (A) Hematopoietic Stem cells generate all types of mature blood cells. (B) Neural Stem cells generate Brain tissue. (C) Lung Stem cells generate Lung tissue.

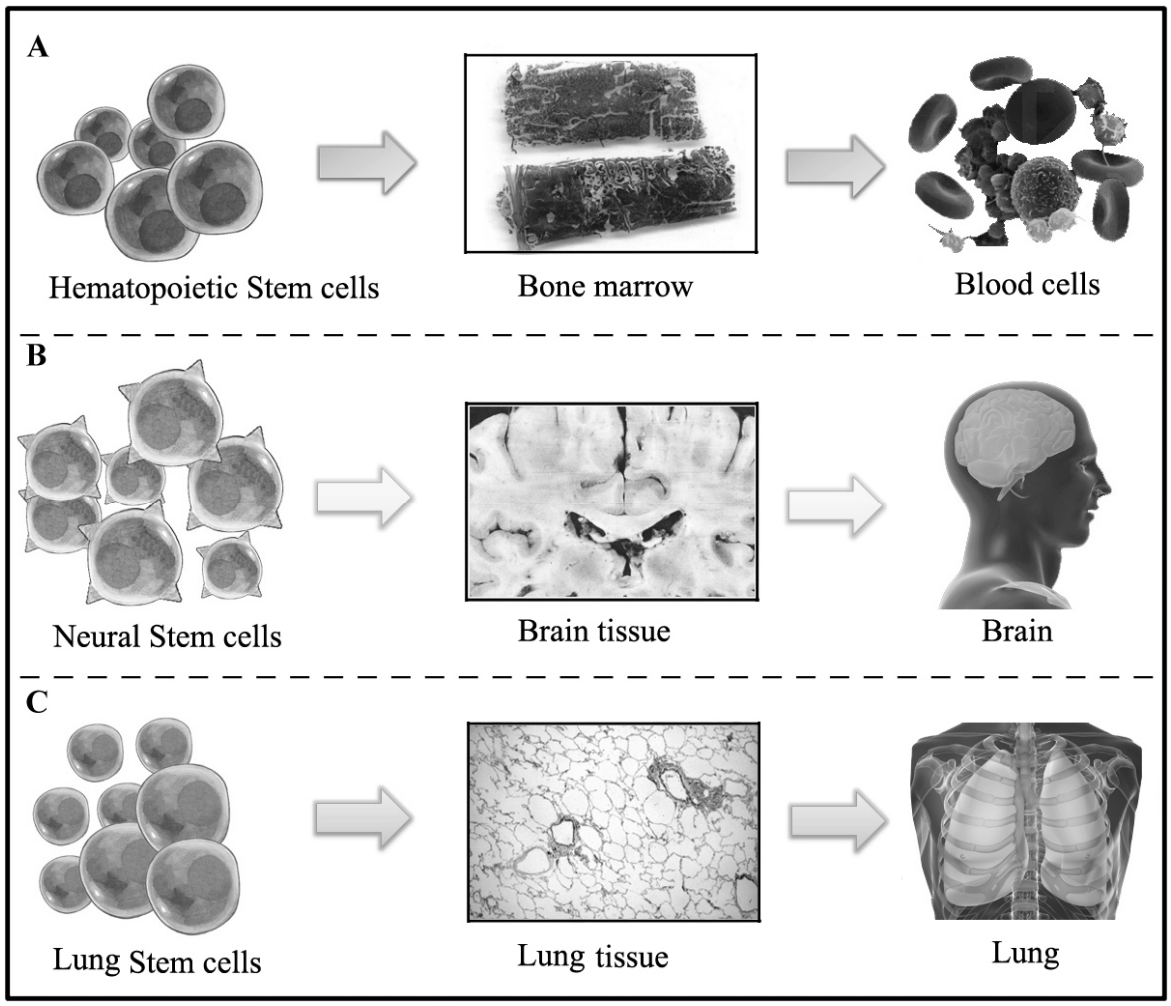
A
Hematopoietic Stem cells
Bone marrow
Blood cells
B
Neural Stem cells
Brain tissue
Brain
C
Lung Stem cells
Lung tissue
Lung

Figure 1.