Cytometry PART A
Journal of the International Society for Advancement of Cytometry

ISAC
International Society for Advancement of Cytometry

The Cancer Stem Cell: Cell Type or Cell State?

Stemness. Cancer is often viewed as a caricature of normal developmental processes, but the extent to which it depends upon mechanisms central to embryonic multilineage differentiation, or adult stem cell mediated regeneration remains unknown. In embryogenesis, experiments from nuclear-somatic cell transfer to the creation of induced pluripotent stem cells (1) are consistent with the concept that stemness is a cell state and not a cell type. Behind the concept of cell type is the acquisition of specialized and fixed functionality based on a unidirectional differentiation paradigm. On the other hand, cell states, such as entry into cell cycle, are conditional and reversible. The fact that a definitive stem cell gene expression signature has remained elusive has received much attention and elicited a variety of explanations, including the hypothesis that stemness results from the arrest of a linear process of differentiation (2). Oct4, Sox2 and Nanog, three of four genes capable of inducing pluripotency in differentiated human cells, are consistently coexpressed in pluripotent stem cells. According to a theory proposed by Casanova, each factor promotes a given fate by repressing the alternative: Oct4 suppresses neural ectodermal differentiation and promotes mesendodermal differentiation, while Sox2 inhibits mesendodermal differentiation and promotes neural ectodermal differentiation. When coexpressed, they repress all germ-layer differentiation and, in so doing, promote the stem cell phenotype (3). Thus embryonic stem cells retain their *stemness* not because all differentiation pathways are open, but because they are closed.

The classical tissue maintenance/regeneration scheme, drawn from hematopoiesis, is a unidirectional paradigm in which resting self-replicating adult tissue stem cells are rarely called into cycle, giving rise to progenitor cells of high proliferative capacity, or a cascade of amplifying cells as in the erythroid series. The progeny of these lineage-committed progenitors differentiate into mature functional cell types with limited (monocytes, lymphocytes) or no (erythrocytes, polymorphonuclear leukocytes, platelets) proliferative capacity. Increasingly, examples have been noted in which mature functional cells appear to be conditionally differentiated: hepatocytes, airway cells and pancreatic islet cells appear to dedifferentiate to a transit-amplifying progenitor state under conditions that summon tissue repair, suggesting that, in these tissues as well, the capacity to self-renew and differentiate is a state rather than a discrete cell type (4).

The widespread use of the term *stem cell* to describe both the pluripotent cell responsible for embryogenesis, and the somatic cells responsible for tissue maintenance and repair has generated confusion in the literature, especially when applied to cells outside these two paradigms. Nevertheless, both usages refer to cells capable of self-renewal and differentiation. Further, maintenance of the stem cell phenotype is highly dependent on signals provided by surrounding cells. In embryogenesis, plasticity is a hallmark. In adult tissue stem cells, the capacity to give rise to multiple lineages is more restricted. In most instances cells described as stem cells are more resistant to toxic insults than their progeny. This is accomplished through a variety of mechanisms including phase I metabolism, conjugation and transport.

Differentiation, dysdifferentiation, transdifferentiation and dedifferentiation in cancer. The modern interpretation of the cancer stem cell hypothesis is drawn largely from analogies of clonogenic tumor cells to normal stem cells, both embryonic and adult, and is supported by phenotypic (surface marker), functional (metabolic enzymes and transporters), and clonogenic (self-renewal and tumorigenicity) data. The concept that stemness results from loss of differentiation signaling may be applied to cancer, both in its initiation and in its progression. Cancer is a disease of genetic alteration and epigenetic dysregulation, potentially explaining well-known cases of transdifferentiation (B cell blast crisis in chronic myeloid leukemia) and dysdifferentiation, the partial expression of a differentiation program or the promiscuous expression of lineage incompatible proteins. The epithelial to mesenchymal transition in epithelial cancers, a normal process in embryonic development, can be viewed as transdifferentiation, but also dedifferentiation as it is accompanied by the expression of CD44 and CD90, proteins associated with both epithelial and mesenchymal adult tissue stem cells. Dedifferentiation, in the sense of reacquisition of stem-like properties by a tumor cell with a mature phenotype, has been speculative (5), because it can only be definitively distinguished from clonal selection at the

Received 4 June 2012; Revision Received 30 August 2012; Accepted 31 August 2012

*Correspondence to: Vera S. Donnenberg, Associate Professor of Cardiothoracic Surgery and Pharmaceutical Sciences, 5117 Centre Avenue, Suite 2.42b Research Pavilion, Pittsburgh, Pennsylvania 15213, USA. E-mail: donnenbergvs@upmc.edu and Albert D. Donnenberg, Professor of Medicine, 5117 Centre Avenue, Suite 2.42e Research Pavilion, Pittsburgh, Pennsylvania 15213, USA. E-mail: donnenbergad@upmc.edu

Published online 18 October 2012 in Wiley Online Library (wileyonlinelibrary.com)

DOI: 10.1002/cyto.a.22208

single-cell level. A potential mechanism for dedifferentiation and reacquisition of stemness during tumor evolution is suggested by Wahl *et al*., who note that p53 mutation may not only result in loss of appropriate responses to DNA damage, it may also abrogate the role of p53 as a central regulator of differentiation, self-renewal and plasticity (6).

Tumor heterogeneity and the pedigree of intra-tumor subpopulations. Primary breast cancers are heterogeneous, containing a variety of neoplastic and reactive cell types that can be distinguished on the basis of morphology, protein expression and tumorigenicity in xenograft models. Genotyping 100 cells from a primary cancer and a concurrent liver metastasis from the same individual, Navin et al. have provided definitive evidence that aneuploid cells prevalent in the primary tumor have the same irreversible mutational signature as aneuploid cells prevalent in the liver metastasis, indicating descent without significant subsequent mutation (7). The genomic profiles of these aneuploid cells defined a single clonal lineage clearly distinct from all other cells tested. Cells outside the clonal lineage included apparently normal cells and occasional pseudodiploid cells with idiosyncratic chromosomal aberrations unrelated to the prevalent clonal lineage. On the face of it, these results are at odds with the cancer stem cell hypothesis, which predicts that critical mutations in the primary tumor's rare stem-like population would be conserved in the metastasis, with additional mutations unique to the metastatic lesion, having occurred subsequent to the migration and propagation of the cancer stem cell. The conservation of irreversible deletion mutations in the primary and metastatic lesions suggests a common descent or alternatively, that the most aggressive cells from the metastasis may have entered the circulation and re-seeded the primary lesion, as has been suggested by Kim et al. (8).

Dedifferentation as a feature of metastasis and relapse. How can these recent genetic studies be reconciled with mounting evidence from numerous laboratories in multiple cancers (9–12) that cells expressing markers associated with adult tissue stem cells have enhanced tumorigenicity and contribute to therapy resistance? Data published by the Donnenberg laboratory provides a possible clue: Unlike MDR+ stem/progenitor-like CD90+ low light scatter (small, resting) tumor cells which gave rise to tumors at low dose (50–100 cells/site) (13–15), more differentiated, high light scatter CD44+/CD90+ tumor cells were not tumorigenic at low cell dose unless coinjected with adipose-derived feeder cells (13). The xenograft tumors resulting from injection of more differentiated CD90+ MDR negative high light scatter tumor cells at higher cell number (13,200 per site) recapitulated the phenotypic heterogeneity of original patient tumors, containing mature epithelial tumor cells but also the MDR+/CD90+ small resting stem/progenitor phenotype that was absent in the sort purified injected cells (15). Since inocula of sort purified mature tumor cells are statistically unlikely to be contaminated by the far rarer stem/progenitor phenotype, the data suggested "dedifferentiation" of mature drug-sensitive tumor to a more stem-like, small resting ABCG2+ resistant state.

Hypothesizing a metastasis-associated change of state resulting in dedifferentiation of more prevalent "mature" tumor cells into a stem-like tumor phenotype is consistent with the cancer stem cell paradigm, the genomic data and the well-known tendency for recurrent cancer to become more aggressive as it becomes less differentiated. Given the relatively high prevalence of circulating and disseminated tumor cells and the variability of time to relapse, such dedifferentiation may be a rare event, requiring appropriate environment cues, cooperative interaction of different tumor cell types, or chance mutations.

Viewing stemness as a state that can be conditionally re-expressed when differentiation signaling pathways are blocked by gene deletions, environment, or epigenetic reprogramming may help us appreciate the important analogy between tumorigenicity and normal tissue renewal, without locking us into a one-way differentiation paradigm that views cancer stem cells as a unique cell type. Perhaps a new wrinkle is that dedifferentiation resulting from deletion of genes involved in lineage specification would preclude future differentiation. Referring to such an undifferentiated, self-renewing, self-protected cell as a stem cell is inconsistent with the primary definition of stemness and perhaps new terminology would be in order should the deletion hypothesis prove correct. As the most developed single cell technology, cytometry and particularly multidimensional cell sorting, provides critical tools for molecular and functional analysis of cancer cell states.

Acknowledgment

The authors would like to thank Drs. Dov Zipori and Gerald Schatten for their critical reading of this commentary.

Albert D. Donnenberg*
University of Pittsburgh Cancer Center, Pittsburgh, Pennsylvania 15213
McGowan Institute of Regenerative Medicine, Pittsburgh, Pennsylvania 15219
Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213

James B. Hicks
Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

Michael Wigler
Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

Vera S. Donnenberg*
University of Pittsburgh Cancer Center, Pittsburgh, Pennsylvania 15213
McGowan Institute of Regenerative Medicine, Pittsburgh, Pennsylvania 15219
Department of Cardiothoracic Surgery, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213

Literature Cited

1. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131:861–872.
2. Mikkers H, Frisen J. Deconstructing stemness. The EMBO J 2005;24:2715–2719.
3. Casanova J. Stemness as a cell default state. EMBO Rep 2012;13:396–397.
4. Zipori D. The stem state: Mesenchymal plasticity as a paradigm. Current Stem Cell Res Therapy 2006;1:95–102.
5. Sell S. Cellular origin of cancer: Dedifferentiation or stem cell maturation arrest? Environ Health Perspect 1993;101 (Suppl 5):15–26.
6. Spike BT, Wahl GM. p53, stem cells, and reprogramming. Genes Cancer 2011;2:404–419.
7. Navin N, Kendall J, Troge J, Andrews P, Rodgers L, McIndoo J, Cook K, Stepansky A, Levy D, Esposito D, et al. Tumour evolution inferred by single-cell sequencing. Nature 2011;472:90–94.
8. Kim M-Y, Oskarsson T, Acharyya S, Nguyen DX, Zhang XHF, Norton L, Massagué J. Tumor self-seeding by circulating cancer cells. Cell 2009;139:1315–1326.
9. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature 2001;414:105–111.
10. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci USA 2003;100:3983–3988.
11. Fabian A, Barok M, Vereb G, Szollosi J. Die hard: Are cancer stem cells the Bruce Willises of tumor biology? Cytometry A 2009;75A:67–74.
12. Donnenberg VS, Landreneau RJ, Donnenberg AD. Tumorigenic stem and progenitor cells: Implications for the therapeutic index of anti-cancer agents. J Control Release 2007;122:385–391.
13. Zimmerlin L, Donnenberg AD, Rubin JP, Basse P, Landreneau RJ, Donnenberg VS. Regenerative therapy and cancer: In vitro and in vivo studies of the interaction between adipose-derived stem cells and breast cancer cells from clinical isolates. Tissue Eng Part A 2011;17:93–106.
14. Donnenberg VS, Donnenberg AD, Zimmerlin L, Landreneau RJ, Bhargava R, Wetzel RA, Basse P, Brufsky AM. Localization of CD44 and CD90 positive cells to the invasive front of breast tumors. Cytometry B Clin Cytom 2010;78B:287–301.
15. Donnenberg VS, Luketich JD, Landreneau RJ, DeLoia JA, Basse P, Donnenberg AD. Tumorigenic epithelial stem cells and their normal counterparts. Ernst Schering Foundation Symp Proc 2006;5:245–263.