

Cancer stem cells, a fuzzy evolving concept

A cell population or a cell property?

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Keywords: cancer, cancer hallmarks, stem cell, stemness, tumor

The cancer stem cells (CSC) hypothesis represents a pathological extrapolation of the physiological concept of embryonic and somatic stem cells. In its initial definition, it encompassed the hypothesis of a qualitatively distinct population of immortal cancer cells originating from somatic stem cells, which generate in xenotransplants by a deterministic irreversible process, the hierarchy of more differentiated finite lifespan derived cells, which constitute, themselves, the bulk of the cancer. These CSC would express specific biomarkers and gene expressions related to chemo- and radioresistance, stemness, epithelial–mesenchymal transition, etc.

No convincing congruence of several of these properties in one cell population has been demonstrated. The concept has greatly evolved with time and with different authors (“the plasticity of cancer stem cells”), leading to a minimal definition of cells generating a hierarchy of derived cells. In this article these concepts are analyzed. It is proposed that stemness is a property, more or less reversible, a hallmark of some cells at some time in a cancer cell population, as immortality, dormancy, chemo- or radioresistance, epithelial–mesenchymal transition etc. These phenotypic properties represent the result of independent, linked, or more or less congruent, genetic, epigenetic, or signaling programs.

The Cancer Stem Cell Concept

The concept of cancer stem cells (CSC) arose from the discovery that a majority of cells from some human leukemia (acute myeloid leukemia), at different stages of differentiation, originated from transformed undifferentiated pluripotent stem cell.^{1–3} This led to the hypothesis, then the theory, that as in the normal somatic stem cells (SSC) and their derived tissues, a small population of cells, the cancer stem cells (CSC) would reproduce ad infinitum and generate the very diverse, limited lifespan, multi-lineage differentiated majority of cells in a cancer, called the derived population cells (DC) (Fig. 1).^{1,4} This was the concept of an aberrant stem cell system, a system gone awry. In agreement with such a scheme, CSC were assumed to originate from somatic stem cells (SSC) and to represent a minor, qualitatively distinct,

eternal population, transforming deterministically and irreversibly in a limited lifespan, more or less differentiated hierarchy of derived cells that would constitute the bulk of phenotypically diverse cancer cell populations.^{1,3}

Of course clinicians, frustrated by the only partial and transient success of their therapies, liked the idea. If the concept was valid, they would have to deal with one well-defined, but difficult to identify and study, population of cells responsible for a cancer. This would allow them to solve the problem of a cancer with one therapy well aimed at these cells.^{3,5,6} For industry the definition of different causal populations of cells in different patients would also allow proposing combinations of diagnoses and treatments for each patient. The experimental support of the concept was essentially that in xenotransplant experiments in immunologically deficient mice, only a minor fraction of the injected cells would generate tumors: the cancer stem cells. In suspension *in vitro*, this cell minority would grow in spheroids. Biomarkers were found to allow some purification of the CSC by cell sorting.^{1,7}

Proposed Attributes of the Cancer Stem Cells

Over time quite diverse properties have been attributed to the postulated CSC. The origin of the CSC in the transformation of SSC seemed logical.³ In cell-shedding tissues, like the skin and mucosae, it makes sense, as only the SSC would have the time to accumulate the range of mutations necessary for cell transformation. However, if the first oncogenic event is to confer an increase *in loco* of the lifespan to the cells, this would not be necessary. In fact, in experiments in various models in which an oncogene was expressed all during the cells reproducing and differentiating sequence, the transformation took place at the stage of pluripotency as well as of unipotency and even later (for review, see ref. 8).

From the beginning of the CSC story, although, to our knowledge, it was not formally expressed, the concept of a minor population of cells, responsible for the progression and evolution of a cancer cast a doubt about the validity and interest of the work on epigenetics and transcription expression performed in bulk tumors, in which most of the cells would be degenerate or terminally differentiated. The fundamental results of such studies in our knowledge of the biology of cancer and on

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Submitted: 09/17/2013; Revised: 11/21/2013; Accepted: 11/21/2013
<http://dx.doi.org/10.4161/cc.27305>

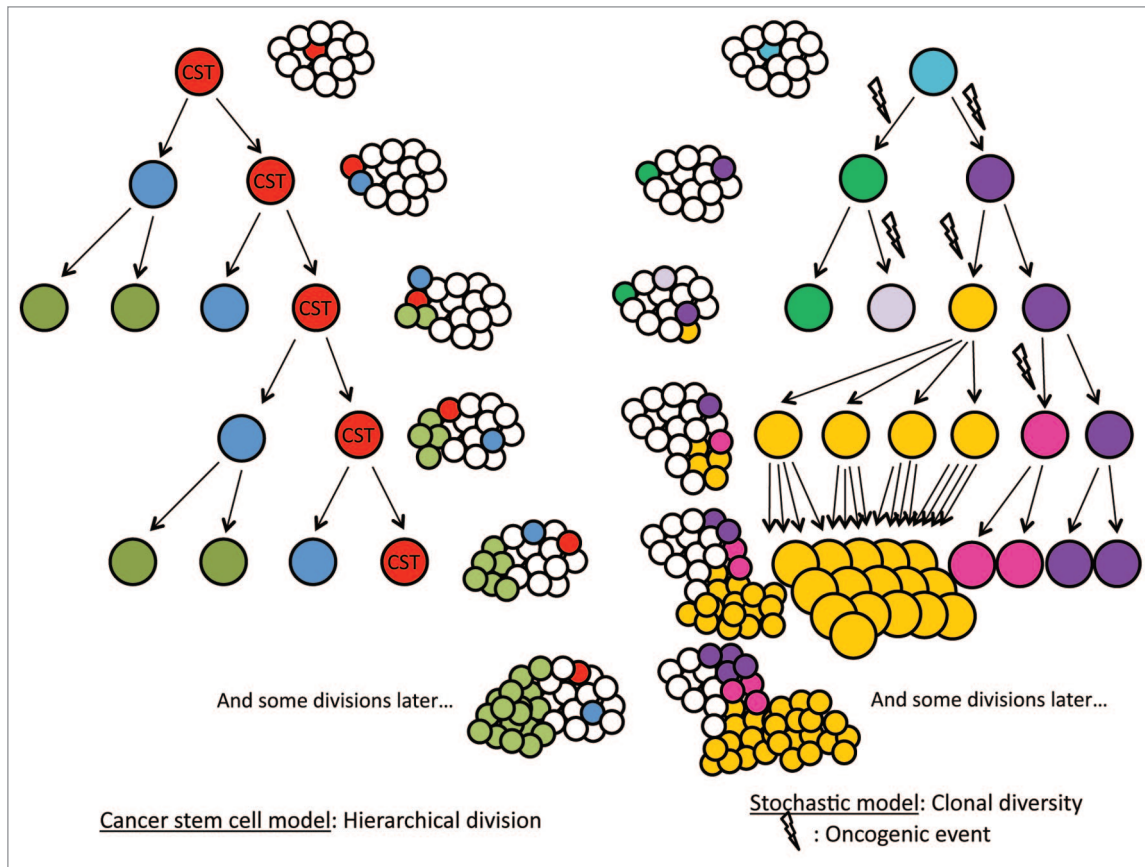


Figure 1. Cancer stem cell model compared with stochastic model.

prognostic diagnosis disagree with this concept:^{7,9-11} for instance gene expression allows to clarify and treat specifically breast and other cancers.^{9,12}

The notion of CSC as a qualitatively distinct population of cells has been implicit and sometimes expressed.¹ It is not supported by the various experimental tests applied to the cells (transplants, sphere formation in vitro, cell sorting with biomarkers, etc.) which always show overlaps with other cells (DC).⁷ On the other hand, if properly transplanted in fully immune-compromised mice, more than one-third of human melanoma cells generate tumors, i.e., would be CSC.¹³ Even in acute lymphoid leukemia, multiple subclones exist with a complex and nonlinear clonal architecture.¹⁴

Intratumor heterogeneity also bears against the concept of one population of cells generating all the cells in a cancer.¹⁵ This heterogeneity is both genetic and epigenetic and may also result from altered signaling.¹⁶ For example, the intratumor heterogeneity of human glioblastomas and other tumors suggests a multi-clonal evolution compatible with the stochastic but not the CSC model.¹⁷⁻²⁰

Another implied property of CSC is, as for SSC, the irreversibility of the transformation in more differentiated cells, DC. This property, in fact, is now questioned for SSC and embryonic stem cells (ESC), since the expression of just 4 transcription factors has now been shown to be sufficient to convert fully

differentiated cells to an ESC state, and progenitor cells spontaneously revert to SSC upon crypt cloning.^{21,22} As for CSC–DC cell interconvertibility, the constant conversion of a few CSC cells in many multiplying DC is certainly easier to demonstrate than a rare reversion of these numerous DC into a tiny minority of CSC. The irreversibility is not valid in the case of human melanoma²³ and is disproved by the regeneration over time in culture of a diversified cell population from purified DC²⁴ and by the generation of cancer cells with stemness properties spontaneously²⁵ following oncogene expression²⁶ in primary p53-null mouse cells or metastasis²⁷ or following EMT induction in cell lines.²⁸ Conversely, CSC, EMT, chemoresistance characteristics are acquired by lung cancer cell lines after treatment with anti-EGFR drugs.²⁹

Linked to the concepts of irreversibility and qualitative nature of CSC to DC transformation are the implicit or explicit assumptions that this transformation is deterministic. The deterministic character of the conversion was even related to the semiconservative character of DNA replication, an asymmetric cell division, of one CSC to one conserved CSC and one derived cell. This deterministic character opposes all the evidence in favor of stochastic mechanisms of cancer cell diversity.^{15,30} This concept is, to our knowledge, no longer expressed in the literature.

Chemoresistance and resistance to radiation have also been attributed to CSC.³¹⁻³³ Of course, again, if these properties

applied only to CSC, the therapeutic problem would also converge on this cell population. This property could be ascribed and certainly would apply to a dormant population. However if CSC multiply fast (vide infra), it should be related to another attribute. Indeed ABCG drug transporters and enhanced DNA repair have been observed in some cancer cells.^{1,7} However, again, the congruence of these properties with the other proposed CSC properties has not been demonstrated.

The analogy between SSC and CSC has even been extended to the concept of the niche.³⁴ Quiescent SSC are often sequestered in a safe, protected environment, the niche. A similar concept has sometimes been proposed for CSC. In fact, rapidly dividing supposed CSC are often found in preferential sites of the tumor, e.g., near capillaries.³⁵ However, it is a farfetched extrapolation to interpret these favorable spaces as preordained sites, the niche. It seems simpler, even if less spectacular, to assume that an oxygen-rich environment is more favorable for proliferation.

The use of biomarkers, allowing purifying, to some extent, CSC by cell sorting, has greatly contributed to the expansion of the field (e.g., CD44, CD133, ALD4). The panel of biomarkers used is different from one type of cancer to another.³⁴ However, the validity of these biomarkers is cast in doubt by one disturbing fact. Although the question was asked several times in reviews, sequential cell sorting purifying CSC having all the properties and biomarkers of CSC has, to our knowledge, never been reported.¹⁶ In fact, the fraction of cells expressing one biomarker in breast CSC was not expressing another biomarker.³⁶ With this and other methodologies, the separation of a proportion of cells by any criterion (whether seeding capacity, sphere formation, biomarker-based cell sorting, etc.) assumes that the property used is stable in tissue, i.e., cells in a negative phase of any fluctuating property (e.g., epigenetic, transcriptional, feedback linked control) will be missed. Of course this would not apply to a qualitatively irreversibly well-defined CSC population! In fact, any criterion used to define a cell population applies to those cells that answer it at the moment it is used. Finally, the specificity of these biomarkers has never been demonstrated. In fact some of them are good markers for normal tissue adjacent to a tumor.³⁷

In the CSC literature, there has always been an ambiguity with regard to the proliferative or quiescent state of the CSC cells.³⁰ In fact, the normal stem cell models have opposite characteristics: while ESC are strongly proliferative, SSC were, until recently, considered as rarely dividing cells.¹ This was even used, for some SSC, as a criterion to identify them by the remnants of their DNA labeling.³⁸ However, even for some SSC, it is now accepted that there may be 2 interconverting populations of SSC, one quiescent, one proliferating.³¹ The concept of quiescent CSC, while useful to explain the continued presence of cancer cells after therapy, does not fit in with other proposed properties of the supposed CSC: e.g., the spheroid formation assay, which allows purification to some extent CSC by selective growth, the SP cells which extrude drugs and which would soon disappear if they reproduced less than other cancer cells.^{39,40} Moreover, various supposed CSC were shown to be highly proliferative.^{30,36,41,42} This concept is even more incompatible with the existence of CSC in cell lines: they would soon disappear.⁷

Present State of the Literature

With the progressive abandonment of claimed SSC and ESC properties, the CSC concept has evolved toward the existence of very competitive cancer cells continuously generating a majority of cells, less active, degenerating, and with limited reproduction capacity and even survival potential.³⁴ This was the simple conclusion of Tubiana group 40 y ago.⁴³ It explained the important discrepancy between cell proliferation rates, evaluated by tritiated thymidine labeling of biopsies, and the measured *in vivo* tumor growth rate in breast cancers. This has been confirmed and visualized more directly by the more sophisticated method of linear tracing in experimental models in which the expression of an oncogene is induced at a chosen time and the consequences examined in the cell of origin of the tumor and in its successive descendants.⁴⁴⁻⁴⁶ The hierarchy of progressively more diversified tumor phenotypes, in fact, reproduces the well-known behavior of cells in developing metastases.⁴⁷ It is easily explained by the fact that most genetic (and probably epigenetic and others) events, when not neutral, lead to loss of competitiveness and much more rarely to an increase of competitiveness.^{48,49} In the case of cancer cells, as in ESC, the default state of the cell is differentiation, which may be due to loss of the stem cell program.^{3,50} In this framework, the CSC are just the fraction of most competitive cells in a cancer. It has been proposed to call them “stemoids”.³⁹ However this concept is opposite to the hypothesis of a dormant population of CSC.

The different characteristics of so-called CSC do not necessarily coexist in the same cells. In fact, in several cases, cells that exhibit the biochemical markers of CSC are precisely not those that have the functional characteristics of sphere formation and tumorigenicity.⁵¹ The progressive simplification and evolution of the definition of CSC has been called euphemistically “a paradigm shift” and described as “the plasticity of CSC”,⁵² “plasticity and clonal diversity of CSC”,⁵³ “stemness as a flexible quality of cancer cell”,^{27,54} etc. This constantly evolving definition has of course made the concept an elusive target for critical analysis.^{7,39,55} This conclusion is apparent, but not explicit, in other reviews.^{3,15,34,53,56-58} Reprogramming stemness with NANOG and LIN28 may even reverse back the cancer character of sarcoma cells⁵⁹ and, as shown more than 30 y ago, the oncogenic potential of teratocarcinoma cells after grafting in blastocysts was reversed. The question therefore is: why continuing to use a misleading terminology? Is stemness not rather a property or phenotype that cancer cells may have, lose, and even acquire, like the other hallmarks of cancer, rather than the definition of a distinct population of cells?^{15,16,60,61}

Necessity of the Use of a Well-Defined Terminology

In the present stage of uncertainty, the use of a precise well-defined terminology based on operational definitions would certainly clarify a literature confused by the use of the variously defined, constantly changing, fuzzy concept of CSC. The literature proposes such definitions: the cell of origin of a tumor (COT) is the one in which the original oncogenic event(s)

occurred.^{8,57,62} The tumor initiating cell (TIC) is the cell that first showed the signs of transformation and initiated the tumor. It may not necessarily be the cell of origin: for instance, in linear tracing experiments the expression of the oncogene begins at the time chosen by the experimenter, but the transformation may occur at a later stage of the cell evolution (e.g., pluripotent cell).⁴⁵ Cell of origin and TIC identity may bear on the phenotype of the resulting tumor.⁶³ The tumor propagating cell (TPC)^{7,64} or cancer repopulating cell⁵⁷ is the already committed cancer cell that is able to generate a tumor; for instance, in xenotransplants the cancer cell transplant does not initiate a cancer, it merely propagates it in a new site. Xenotransplants can be orthoptic (implanted at the site of the naturally occurring tumor) or ectopic (other sites). In situ and metastatic propagations should be distinguished.

“Cancer stemloids” would be the rapidly dividing, evolving cells of the cancer that generate the slower dividing, more differentiated “derived cells”.³⁹ Non-proliferating cancer cells that may redevelop in a cancer are dormant cells. This is a phenotype adopted for example by disseminated tumor cells, which at some time may explode in metastatic growth.⁶⁵ In some cases, this is conditioned by the vascular microenvironment.⁶⁶ “Biomarker characterized cells” (e.g., CD44⁺ CD24⁻ cells, CD133⁺ cells, aldehyde dehydrogenase-expressing cells ADH⁺, etc.) should be called by their operational property, e.g., CD133 cells etc. Any claim to the full congruence of such definitions and programs should be proved. Cancer cells expressing an embryonic stem cell program, essential for maintaining dedifferentiation, and thus repressing the expression of the various differentiation programs that constitute the hierarchical descendants of these cells, are just cells expressing the stemness ESC program or pluripotency. Such a program may totally or partially overlap with other programs developed at some time by cancer cells, such as the epithelial–mesenchymal transition (EMT).³⁰ The 3 programs stemcellness, EMT, and radioresistance can be induced together by EMT inducing transcription factors (e.g., ZEB) in some cell lines.^{67,68}

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Conclusion

Thus, stemness would be a hallmark or a phenotype of cancer, probably linked to dedifferentiation, characterizing in a cancer some cells at some time. There would not be a separate population of CSC, just as there is no population of cancer EMT cells! The stemness could be a functional (transplant, hierarchy, etc.) or/and a biochemical (expression of ESC inducing transcription factors) property or hallmark. In fact, the state of any given cell in a tumor results from the programs expressed, at the time of the investigation, by this cell. This is more or less reversible depending of the mechanisms involved, genetic, epigenetic, or resulting from a signaling equilibrium.¹⁶ Presumably, a large majority of tumor cells with a differentiated phenotype and a limited lifespan will never revert to active dedifferentiated phenotype.

Of course, if to treat a cancer one had to target one population of well-defined CSC, the aim of therapeutic research should be to find drugs for this target. This has been tried:⁶ salinomycin as the candidate CSC drugs. Our and other analyses bear against this approach.³⁹ On the other hand, if, as the evidence suggests, we have to deal with a constantly evolving diversified population of cells, the best therapies should then, as in the case of HIV, be simultaneous multi-target therapies: hit most of the cells at the same time, not allowing cells already resistant to one therapy to survive and permit some of them to acquire later resistance to another therapy.⁷

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors thank WELBIO, the Fonds National de la Recherche Scientifique (FNRS) and the “Plan Cancer” for the support of their research.

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